CALIFORNIA OLIVE COMMITTEE
2565 Alluvial Ave. • Suite 152
Clovis, CA 93611
PHONE 559/456-9096 FAX 559/456-9099

AGENDA

Ripe Olive Research Subcommittee Meeting

Double Tree • Ball Room 4 & 5

Wednesday, November 7, 2018

12:00 p.m.

(Lunch to be provided at 12:00pm)

I. Call to Order

a. Roll call
b. Approval of 6-13-18 Research Subcommittee Minutes (action item)
c. Research Subcommittee chairman comments

II. Discussion and Review of 2018 Projects

a. Budget Status Update

III. Presentation of 2019 Proposals

IV. Approval of 2019 Budget (action item)

a. Closed Session

V. Approval of authority to the Executive Director and Chairman to approve No-Cost extensions. (action item)

VI. Approval of Authority for Inter-Item Transfers of the Research Budget (action item)

VII. Other Business

VIII. Adjournment

COC Subcommittees for 2017-2019

Executive Subcommittee:

Mike Silveira, G-1
Mark Hendrixson, G-2
Dennis Burreson, MUS
Julia Tinsley, 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
Vacant, BCF

Marketing Subcommittee:

Bill McFarland, MUS
Colleen Morris, BCF
Tim T. Carter, BCF
Ed Curiel, G-1
Tracey Wood, MUS
Julia Inestroza, G-2
Pat Ricchiuti, G-2
Scott Hamilton, MUS
Mark Hendrixson, G-2
Phil Quigley, BCF
Edward Garcia, G-1
Mike Silveira, G-1
Rick Benson, G-2
Pablo Nerey, G-1
Joan Whelan-Vanderhorst, G-2
Sergio Mendez, BCF
Vito DeLeonardis, G-2
Felix Musco, MUS

Inspection Subcommittee:

Julia Tinsley, BCF
Julia Inestroza, G-2
Dennis Burreson, MUS
Pablo Nerey, G-1
Rick Benson, G-2
Janet Edwards, MUS
Vacant, MUS
Chris Henderson, G-1
Vacant, BCF
Vacant, BCF
John Pieretti, MUS
Scott Hamilton, MUS
Jacob Peter, BCF
Mike Silveira, G-1
Edward Garcia, G-1
Carolina Burreson, G-1
John Patterson, G-2
Galen Pfeiffer, G-2
Joan Whelan-Vanderhorst, G-2

Research Subcommittee:

Dennis Burreson, MUS
Julia Tinsley, BCF
Carolina Burreson, G-1
Mike Silveira, G-1
Bert Quezada, G-2
Vito DeLeonardis, G-2
Chris Henderson, G-1
Vacant, BCF
Vacant, MUS
Phil Quigley, BCF
John Pieretti, MUS
Pablo Nerey, G-1
Ed Curiel, G-1
Pat Ricchiuti, G-2
Galen Pfeiffer, G-2
Jacob Peters, BCF
John Patterson, G-2
Janet Edwards, MUS

CALIFORNIA RIPE OLIVES
ENJOYED BY FAMILIES EVERYWHERE

CALIFORNIA OLIVE COMMITTEE
Research Subcommittee Meeting Minutes
Wednesday, June 13, 2018
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 Michael SILVEIRA at 9:58 a.m., and the following members were present:

Members	Affiliation:
Felix MUSCO	MUSCO
Bert QUEZADA	GROWER
John PIERETTI	MUSCO
Rick BENSON	GROWER
Jacob PETERS	BELL-CARTER
Mike SILVEIRA	GROWER
Dennis BURRESON	MUSCO
Carolina BURRESON	GROWER
Julia TINSLEY	BELL-CARTER
Ed CURIEL	GROWER
Vito DELEONARDIS	GROWER
Janet EDWARDS	MUSCO
Edward GARCIA	GROWER
Galen PFEIFFER	GROWER
Mark HENDRIXSON	GROWER
Tim CARTER	BELL-CARTER

Others Present:	
Alexander OTT	COC
Todd SANDERS	COC
Liza RAMON	COC
Elizabeth CARRANZA	COC
Peter SOMMERS	USDA
Adin HESTER	OLIVE GROWERS COUNCIL

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

MOVED BY Julia TINSLEY, duly seconded by Chris HENDERSON, and unanimously carried THAT the minutes of the November 10, 2016 Research Subcommittee meeting be approved. (Motion 6.20.17 #1)

II. DISCUSSION AND APPROVAL OF 2019 PRIORITIES

Each year the Research Subcommittee sets priorities of research they would like executed on their behalf for the following year. These efforts are to fund more specific and calculated research to enhance the benefits to the industry. Once the priorities are set they are provided to the University of California liaisons to request proposals from researchers. Proposals will be reviewed for funding in November by the subcommittee.

<u>List of Priorities:</u>
Management of foliar disease of olive (cont.)
Epidemiology and management of olive knot (cont.)
Standardization of Acrylamide analysis methods for table olives
Canopy Management, Tree Hedging and topping to Optimize Yield (cont.)
Northern Fly Trapping (cont.)
Southern Fly Trapping (cont.)
Evaluating alternative weed control products for registration
Trunk shaking, limb shaking, and abscission
Research analysis review on past and present technological studies in Spain & Israel
Differentiation of olive Cultivars using DNA and NMR-based fingerprinting (cont.)
Research review of historical COC projects
Evaluation of drone technology, satellite imagery and lidar to measure canopy density

MOVED BY Julia TINSLEY, duly seconded by Carolina BURRESON, and unanimously carried THAT the existing projects with six new projects be approved for 2019 priorities. (Motion 6.13.18 #2)

VII. ADJOURNMENT

Chairman Dennis BURRESON adjourned the meeting at 10:34 a.m.

I hereby certify that the above is full, true and correct copy of the minutes of the meeting held on June 13, 2018 in Modesto, California, by the Subcommittee.

June 14, 2018
Date: June 14, 2018

Liza Ramon
Liza Ramon, California Olive Committee

SUMMARY OF MOTIONS FOR JUNE 13, 2018

Motion 6.13.18 #1 **APPROVED**

MOVED BY Julia TINSLEY, duly seconded by Chris HENDERSON, and unanimously carried THAT the minutes of the November 10, 2016 Research Subcommittee meeting be approved.

Motion 6.13.18 #2 **APPROVED**

MOVED BY Julia TINSLEY, duly seconded by Carolina BURRESON, and unanimously carried THAT the existing projects with six new projects be approved for 2019 priorities.

*******FOR YOUR INFORMATION*******

FROM: RESEARCH SUBCOMMITTEE

SUBJECT: PROGRESS REPORTS for 2018

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.

2018 Research Projects

Updated 10/31/2018

Researcher	Project	Amount	Finalized MOU	Paid thus far	% Paid	No Cost Extension
Reza Ehsani	A new fruit removal head for an olive harvesting system	$ 45,741.00	1/5/2018	$ 27,444.60	60%	
Rich Rosecrance	Canopy Management, Tree Hedging and topping to Optimize Yield	$ 31,075.00	1/29/2018	$18,645.00	60%	
Carol Lovatt & Elizabeth Fichtner	Managing Alternate Bearing in olive with PGRs and Pruning	$ 20,698.00	1/9/2018	$12,418.80	60%	
Selina Wang	Evaluation of Several Promising Additives for Reducing Acrylamide in Black Ripe Table Olives	$ 53,280.00	1/31/2018	$ 31,968.00	60%	
Selina Wang	Differentiation of olive cultivars using DNA and NMR-based fingerprinting methods	$ 67,433.00	1/31/2018	$40,459.80	60%	
Jim Stewart	Southern Fly Trapping	$ 6,400.00	1/9/2018	$4,750.02	74%	
Ernie Simpson	Northern Fly Trapping	$ 6,500.00	1/11/2018	$4,100.00	63%	
J. E. Adaskaveg	Epimiology and management of olive knot caused by Pseudomonas savastanoi pv. Savastanoi	$ 16,650.00	3/23/2018	$9,990.00	60%	
Debra Keenan	Evaluation of new chemistries to control Olive Fruit Fly (contingency fund)	$ 25,000.00	1/29/2018	$15,000.00	60%	
J. E. Adaskaveg	Management of foliar diseases of olive (contingency fund	$ 15,000.00	4/3/2018	$ 9,000.00	60%	
	Contingeny Fund	$ 50,000.00		$40,000.00	80%	
	Total	**$ 337,777.00**			0%	

First Year Project Report

Project Title: A New Fruit Removal Head for an Olive Harvesting System

Project Leaders: Reza Ehsani (Professor, University of California, Merced, 5200 N. Lake Road, Merced, CA 95343, Phone: 209-228-3613, Email: rehsani@ucmerced.edu)

Cooperators: Louise Ferguson (CE Pomologist, Department of Plant Sciences, UC Davis, Phone: 559-737-3061, Email: lferguson@ucdavis.edu)

Problems and Significance:

Production acreage of table olives, California's signature crop, has significantly decreased in recent years due to the high cost of production and small margin of profit. Harvesting is a major cost of production for table olives. Currently, the majority of table olives are hand-harvested. Although some growers are using trunk shakers with some success, this method has not been widely utilized because older trees often have large or irregular-shaped trunks that cannot be harvested by trunk shakers. While trunk shakers work on smaller trees, growers are hesitant to remove and replace high yield producing older trees with younger trees. Mechanical harvesting, using contact canopy shakers, is the most promising method for harvesting table olives. Scientists at UC Davis have developed a prototype of a canopy shaker that has been tested and has shown some level of success. The UC Davis-designed canopy shaker is very similar to the canopy shaker used in harvesting process oranges in Florida. The proposed technique showed promising results, but it was relatively heavy and couldn't accommodate the shape of the tree very easily. Despite all past efforts, there is still a need for a cost-effective and efficient harvesting system to match the needs of existing table olive trees.

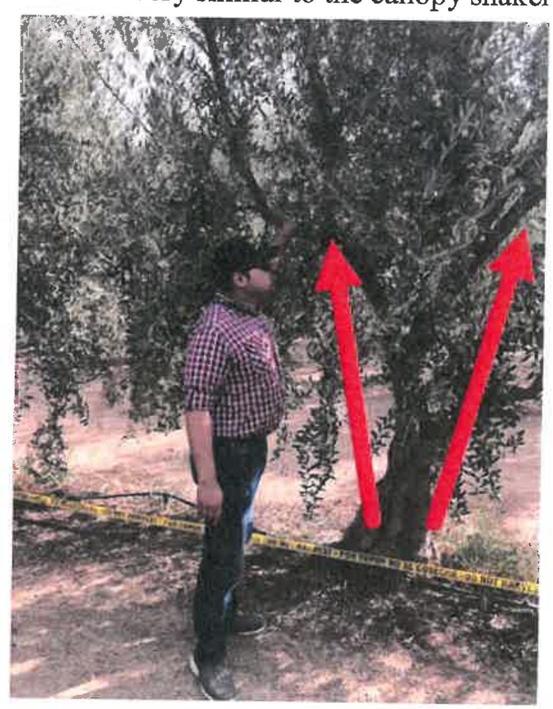

Figure 1. Evaluating trees, looking at small and large branches within the canopy.

Objectives:

The ultimate goal of this study is to develop a low-cost harvesting head for the table olive industry in California. The specific objectives were as follows:

- Reduce the harvesting cost for table olives.
- Develop a cost-effective fruit removal system for existing conventional olive orchards.
- Ensure the harvesting system is highly efficient and does not damage the trees or the fruit.

Experimental Procedures:

We visited and evaluated trees in a two table olive orchards and measured the canopy size height, and width of these trees (Figure 1) to design a properly sized harvesting system. To design a proper harvesting mechanism, we reviewed the current mechanical harvesting systems to identify their strength and weaknesses.

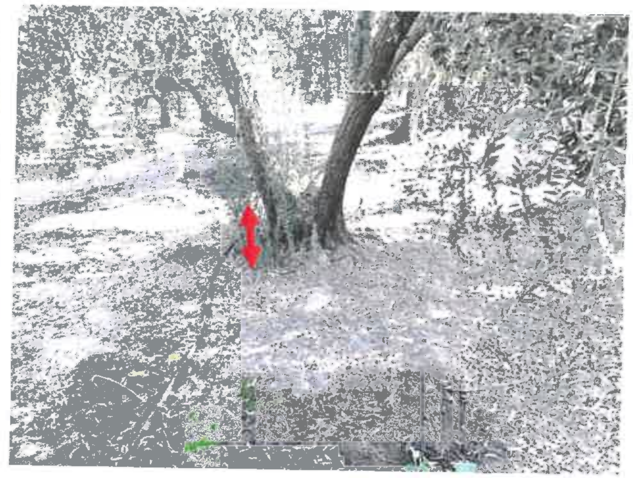

Figure 2. Not enough trunk length for trunk shaker to attach (left); Irregular trunk shape inhibiting use of trunk shaker (right)

Trunk shakers are one of the mechanical harvesting systems currently used for table olives. This type of harvester needs about 2–3 feet of clearance on the trunk to attach and subsequently shake a tree. This can be challenging, especially in conventional olive orchards where trees have not been trained for mechanical harvesting. Trees might have short trunks or an irregular tree shape, which would inhibit a shaker from attaching to these trees, as shown in Figure 2.

Figure 3. Vibration dampening as the vibration travels from thick trunk to large branches and small branches, causing olive fruit to detach.

Trunk shakers vibrates the tree trunk, and the vibration energy travels from the trunk to the large branches and small branches (Figure 3), causing olive fruit to detach. Because of its properties, olive trees tends to significantly damp the vibrational energy. Also, olive has a large detachment force to fruit weight ratio that separate it from most other fruits usually harvested by trunk shaker. This ratio can get to 200-400 with oil varieties and of 100-200 with table olive varieties (Ravetti, 2008). Due to this damping of energy, trunk shakers must shake trees very intensely to get a high fruit removal percentage this can be harmful to trunk and root of trees. On the other hand, we have canopy shakers which instead of vibrating tree trunk they tend to vibrate tree's canopy. This is a much safer approach for health of tress over time but there is no commercially available canopy shaker that is specifically designed to harvest olives.

Design Procedures:

We designed a canopy shaker specifically for table olive trees. This design has two major advantages over current solutions.

1- It's able to compress and squeeze tree canopy.
2- It's adjustable to a range of tree sizes and height.
3- It can be used to harvest fruit within the row.

Squeezing canopy while shaking it can improve harvest efficiency. This can reduce the amount of energy needed to harvest each tree resulting in safer harvest process for trees. Figure 4 shows a harvesting mechanism with tree wings that can squeeze canopy. It has four active harvester wheels that vibrate in a linear motion.

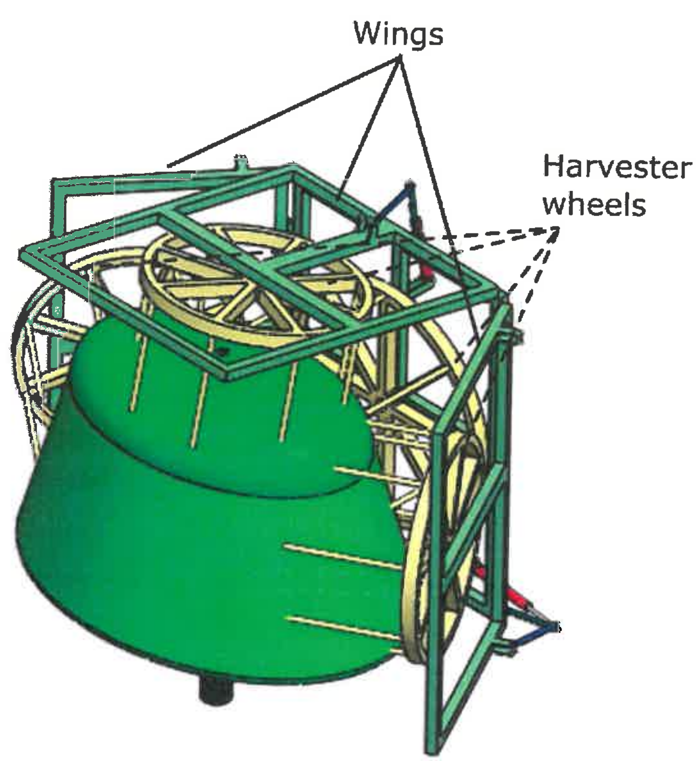

Figure 4 Proposed harvesting mechanism with tree wings and four harvester wheels (yellow).

We decided to build a simpler version with two wings and two harvesting wheels as shown in figure 5 for the first trial.

This design uses one linear hydraulic cylinder to open and close each wing (two in total). A slider-crank mechanism has been utilized to move harvesting wheels back and forth to generate shaking pattern. Table 1 shows the amplitude we can produce using this crank-slider mechanism. This mechanism uses one hydraulic motor on each wing. The crank is directly connected to hydraulic motor and harvesting wheel is mounted on a slider see figure 6.

Table 1. Range of amplitude that can be generated using the various crank radius

CRANK RADIUS (IN)	AMPLITUDE (IN)
1	2
1.25	2.5
1.5	3

The whole harvesting head attached to a Bobcat 337 which has a retractable boom. Using this machine, we were able to rise our harvesting head up to 12 feet to harvest trees up to 16 feet see Figure 7.

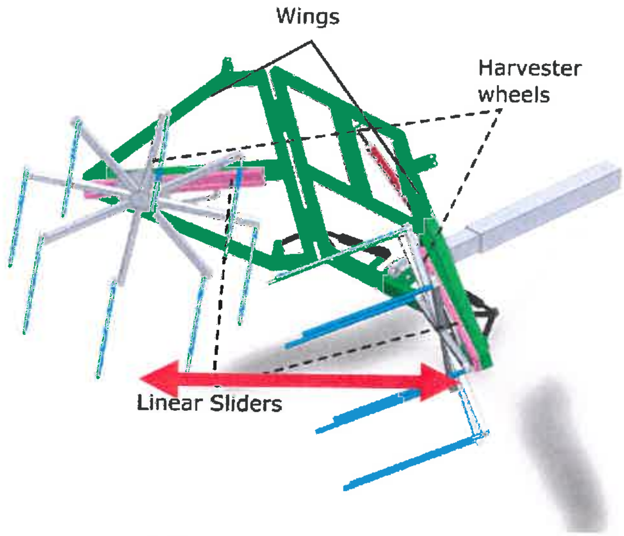

Figure 5 Final design modeled in Solid works

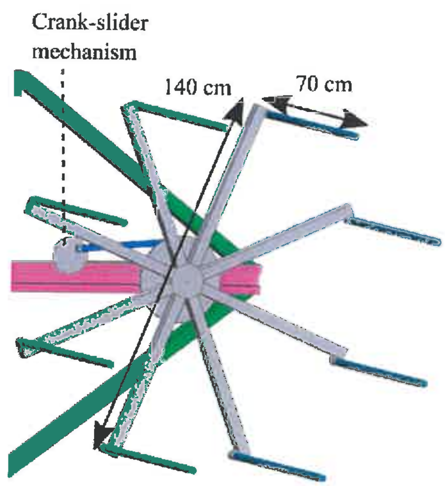

Figure 6. Crank – Slider mechanism is used to move the harvesting wheel back and forth (red arrow) to shake olive trees.

Figure 7. Head frame attached to the Bobcat 337. Harvesting head can rise up to 12 feet from ground and harvest trees up to 16 feet.

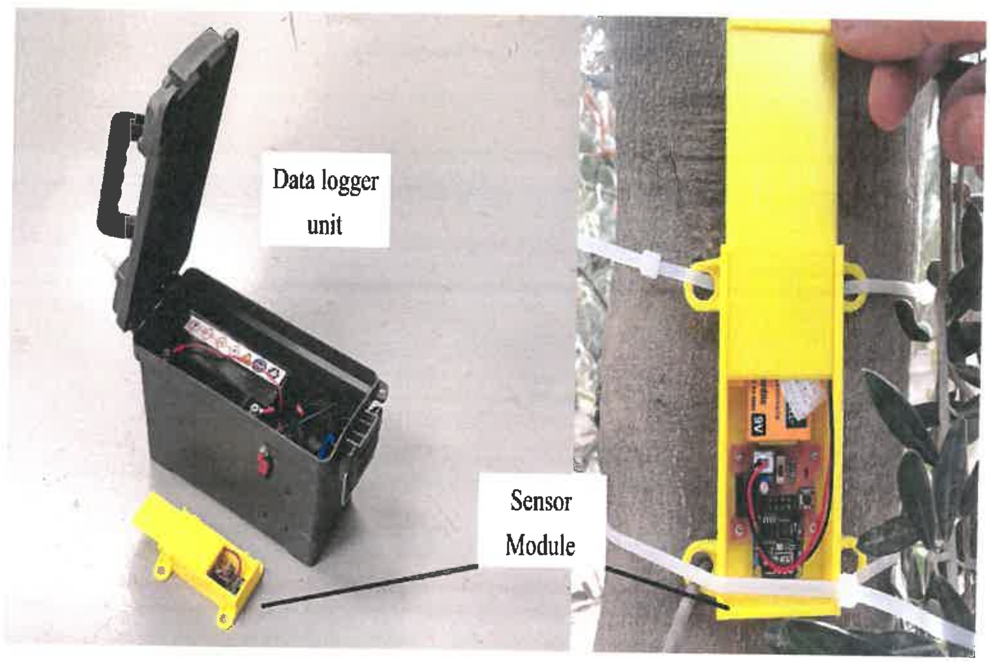

Figure 8. Wireless accelerometer sensor system contains multiple sensor modules (yellow boxes) and one data logger unit.

To measure and record vibration data from field experiments we have developed and built a truly wireless sensor system. Each sensor has a built-in accelerometer, wireless module and a battery. Also, we made a data logger unit that connect to all sensors and record data from all of them in one place, see Figure 8.

Results:

This harvesting machine was tested in an Olive orchard on Oct 1st, 2018. We attached 3 accelerometer sensors to each tree, one attached to tree trunk, one to the main branch and one to the secondary smaller branch to measure how vibration energy travels through a tree.

Using this sensor system, we could compare our canopy shaker harvester to a trunk shaker harvester which was available at the experimental site. Figure 9 shows the acceleration of each sensor of two harvesting machines.

From data that was collected from canopy shaker, we observed that the sensor on the small branch shows much higher vibration amplitude than the sensor on the big branch or the trunk sensor. This shows that that a much smaller amount of energy goes through tree trunk which confirms that this method would be much safer for tree compared to trunk shaker. Figure 9 (B) shows the results for the trunk shaker, it shows there is much higher vibration amplitude in trunk compared to small branches. This means the harvester machine should apply much more energy to a tree to get enough energy to detach olive fruit at small branches.

Table 2 shows shaking frequency produced by our canopy shaker and the trunk shaker.

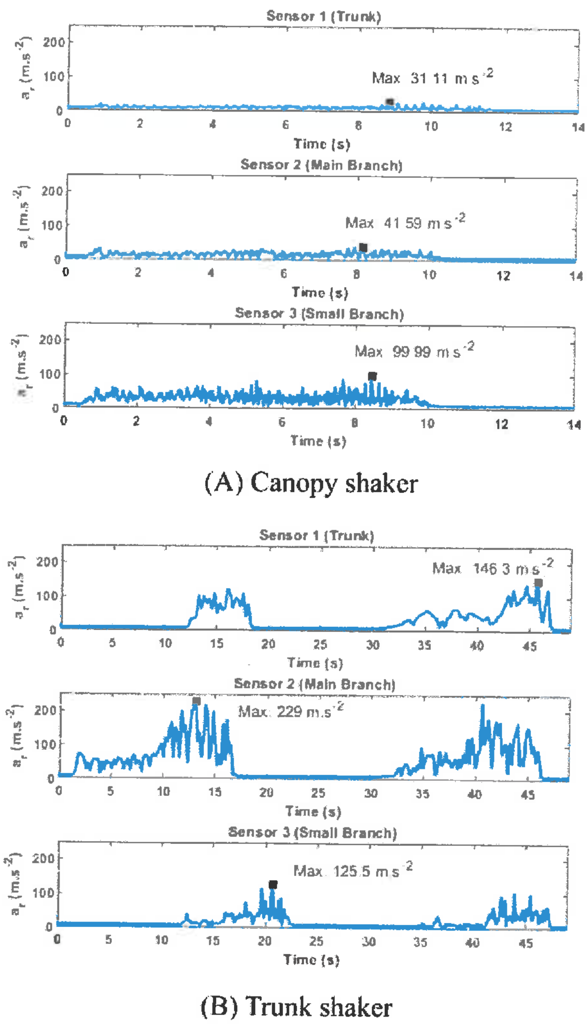

Figure 9. Vibration amplitude from our designed canopy shaker Vs. a Trunk shaker.

Canopy Shaker	Trunk Shaker
Frequency: 3.7 Hz	Frequency: 15.5 Hz
Max Amplitude 99.9 ms^{-2}	Max Amplitude 229 ms^{-2}

Table 2. Shows working frequency and maximum amplitude of these two harvesters.

Our harvesting machine was not quite successful in removing fruit due to lower shaking frequency and lower shaking amplitude. To improve our design, we need to modify our shaking system to increase the force acting on the fruit, this required us to redesign or shaking system as described below and we are hoping to make the system ready for another test this harvesting season.

Modification and Future Plans:

To increase the shaking frequency and amplitude, we designed a new shaking mechanism to generate higher shaking frequency. The current harvesting wheels will be attached to an off-center shaft connection and will be driven by the same hydraulic motor using chain.

We also needed to increase the number of shaking rods to increase contact area with the canopy and improves transmission of vibration energy to the tree canopy. We are adding eight more rods to each harvesting wheel as shown in figure 10. We are almost finished with manufacturing the component of the new system and soon we test this system.

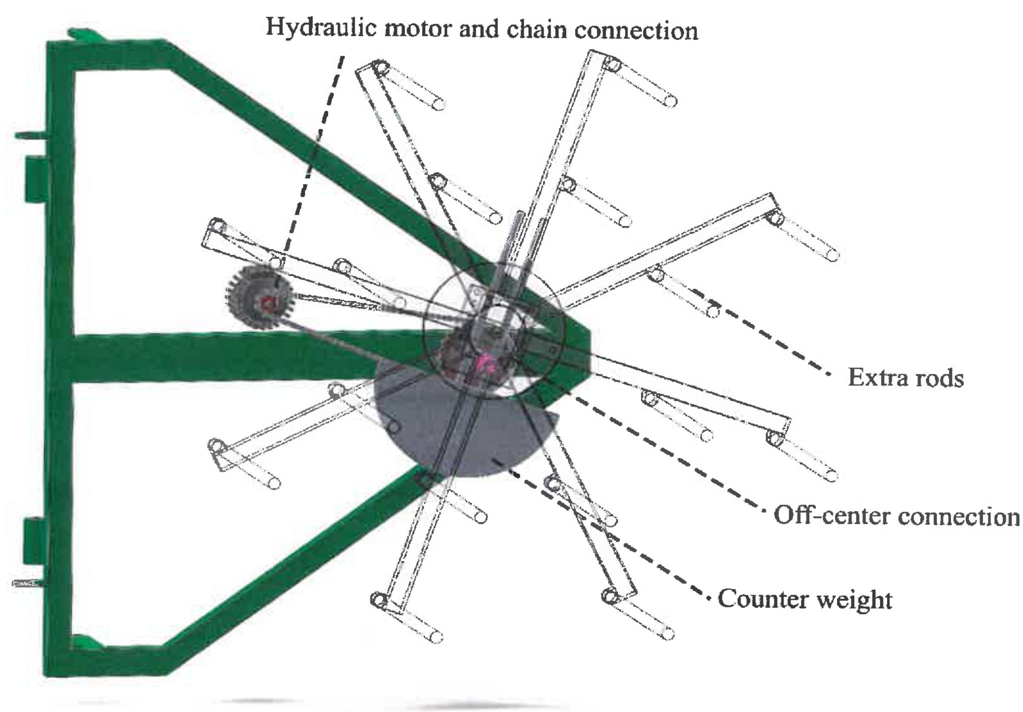

Figure 10. Modified design using an off-center connection allowing the harvesting wheel to generate circular shaking motion.

References:

Ravetti, Leandro, and Leandro Ravetti. Guide to efficient olive harvesting. Rural Industries Research and Development Corporation, 2008.

Canopy management, tree hedging and topping to optimize yield

Introduction and scope

Mechanical hedging and topping can be an important tool for improving harvest efficiencies by affecting return bloom, helping maintain trees in their allotted space and to reduce hand pruning and picking costs. Typically, hedging and topping result in smaller and more compact trees. Smaller trees will facilitate hand harvest by reducing the need for tall, cumbersome ladders and likely increase the number of bins harvested per hour. Picking crews have repeatedly indicated a preference for harvesting mechanically hedged and topped trees compared to traditionally pruned trees (Louise Ferguson, personal communication). In oil olive orchards, mechanical hedging has resulted in increased harvest efficiency and reduced alternate bearing (Charlie Garcia, California Olive Ranch, personal communication). However, timing of mechanical hedging is critical for optimal yields. Hedging too late in the season may not provide enough time for new shoots to grow and flower buds to initiate. Earlier work that we conducted on 'Arbequina' oil olives indicated that shoot growth that occurred after early July did not produce flowers the following year. Whether 'Manzanillo' olives will behave the same is unknown. Hedging too early in the season can cause extensive vegetative growth at the expense of fruit growth. Thus, finding 'the sweet spot' for the timing of mechanical hedging is important to maximize and help regulate yields.

Materials, methods and results

Nickels Trial

We initiated the trial at the Nickels Estate in Arbuckle in late April 2016 (Figure 1) as a randomized complete block design with 3 treatments and 4 replicates. The treatments were: a) 10 foot topping, b) 13 foot topping and c) no topping where the primary scaffolds were cut with loppers to outside laterals at approximately 13 feet. All trees were topped on April 25 followed by hand pruning on May 26 and 27 to thin out the tree canopy. We measured the time it took for 2 pruners to prune the 10 data trees in the center row of the 30 tree plots in all replicates of all treatments to estimate pruning costs. The 10 foot topping treatment removed significant amounts of wood and produced shorter statured trees. Trees were harvested on October 1, 2018 and samples were taken to Musco Olive to evaluate fruit size and crop value,

Pruning costs and crop yields are presented in Table 1. Trees that were topped at 10 feet resulted in lower pruning costs likely because the canopies were smaller and close to the ground than trees pruned at 13 foot. No significant differences ($p < 0.05$) were found between olive yields in 2016, 2017, or 2018; however, trees topped at 10 feet produced lower cumulative yields than trees topped at 13 feet and the non-topped control. Trees topped at either 10 or 13 feet produced a higher value crop (in terms of price per ton) than the control trees. Examination of the grading sheets indicated that topping increased the percentage of medium and large fruit compared with the untopped trees.

Hedging Timing Trials

Nielsen Trial

A major goal of these trials is to determine the most effective timing of canopy hedging to ensure return bloom and minimize excessive vegetative growth. Another important goal is to evaluate hedging effects on alternate bearing. In oil olive, hedging reduces the severe yield swings in alternate bearing trees (Charlie Garcia, California Olive Ranch, personal communication). The experiment was established as a randomized block design with 4 replicates in a 15 year-old orchard with and east west row orientation at Erik Nielsen's farm. Hedging was done on the south side of these east-west rows only. In this case, we defined severe hedging as approximately 6.5 feet from the trunk and moderate hedging as approximately 8.5 feet from the trunk. No hedging occurred in 2018, however, tree canopies were measured and yield ratings were determined.

Tree yields were very low in 2018 across all treatments, making it difficult to draw firm conclusions. In 2018, trees that were hedged in earlier in the year of 2017 yielded more than trees hedged later in the year (Table 2). Severe hedging in 2016 decreased yields in 2016 and 2017. In 2018, trees that produced poorly in 2017 improved significantly in 2018. Indeed, 40% of the variation in 2018 yields could be attributed to crop yields in 2017 (Figure 3). Moderate hedging, however, reduced these large swings in yield from one year to the next, thus reducing alternate bearing.

Burreson Trial

In Spring 2017, we initiated a trial on trees planted in 2009 at Heath Burrison's orchard (Figure 4). The orchard is a north-south planting with a 12 in row by 18 foot between row spacing. The trial was set up as a factorial design with four hedging dates and two canopy sides (east or west) and replicated 5 times. The 10-tree plots were hedged on February 23, April 3, May 1, and June 28, 2018. Trees tend to grow slightly more on west-facing canopies than on canopies facing east. Thus, we want to evaluate the effect of canopy orientation on yield and canopy growth.

In 2018, trees produced few flowers, even fewer fruit, and very low yields. Inflorescence ratings were conducted on May 21, 2018. Time of hedging the previous year did not affect inflorescence rating (data not shown). Trees were not harvested in 2018 because yields were so low. Yields were rated prior to when the trees would have been harvested using a scale of 0 to 10 where 0 represented no crop and 10 represented and extremely heavy crop. No differences in yield were noted in 2017 or 2018 (Table 3); however, there was a trend for hedging earlier in the season producing more crop.

Hedging early in the spring also increased canopy growth (Table 3). Trees hedged in February/March and April produced greater canopy diameters than from trees hedged in June. Hedging earlier in the season allowed more time for current year shoots to regrow. Hedging breaks apical dominance and can increase canopy regrowth. We evaluated the effect of the time of hedging on canopy growth on the cut and uncut side of the tree. Hedging in February/March produced a larger canopy in the cut vs the uncut side of the tree. This indicated that hedging stimulated growth to such an extent that after being cut, shoots grew farther out in the tree row than shoots from the uncut side of the tree. Hedging later in the season, however, did not provide enough time for the canopies to grow, resulting in smaller sized canopies in the cut vs uncut side of the tree.

Additional Activities

Light bar measurements at 3 different levels in the tree canopy have been taken over two weeks. Currently were are analyzing that data and correlating light levels with fruit size and weight. In addition, final canopy measurements will be taken in December to determine total annual growth for 2018.

'Manzanillo' olives have been collected from various olive orchards and are currently being dried. Following drying they will be ground and set for nutrient analyses at UC Davis. These data will be used to develop a nutrient removal calculator for 'Manzanillo' olives.

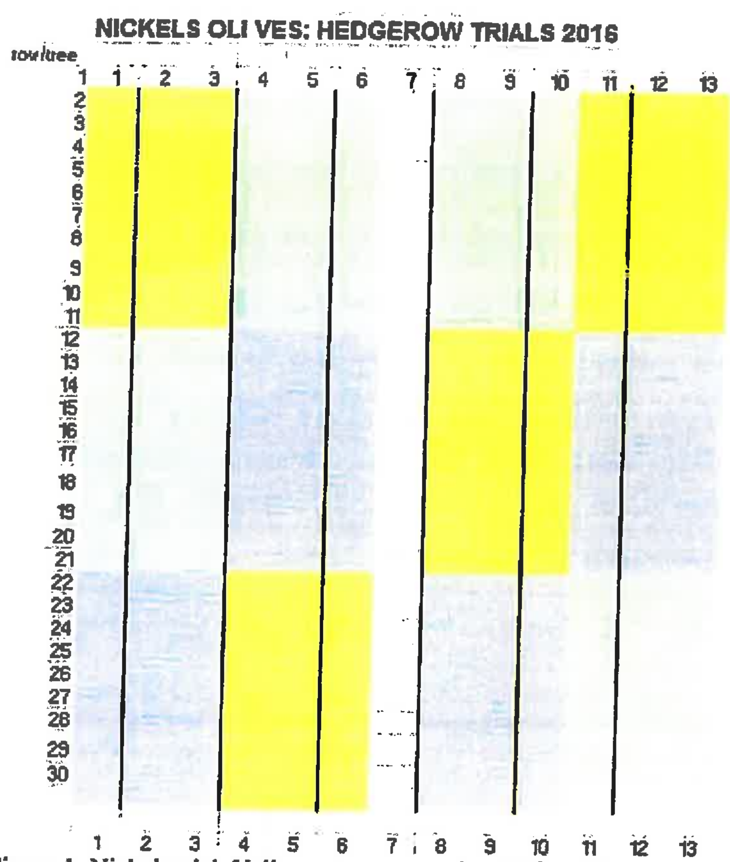

Figure 1. Nickels trial. Yellow = trees topped at 10 feet, Blue = trees topped at 13 feet, and Green = Hand pruned primaries cut to laterals at approximately 13 feet. All treatments were hand pruned to remove hedging and topping stubs and thin the canopy.

Figure 2. Nielsen trial in Orland, California. Colors correspond to the following hedging dates:

Black = 27-Apr -16	Blue = 15-Jul-16 Severe	Blue Pokadot= 24-May-16 Severe
Green = 24-May-16	Pink = 27-Apr-16 Severe	Orange = 15-Jul-16
Red/White = 1-Mar-17	Purple = 29-Mar-17	Yellow = 3-May=17
White = Control		

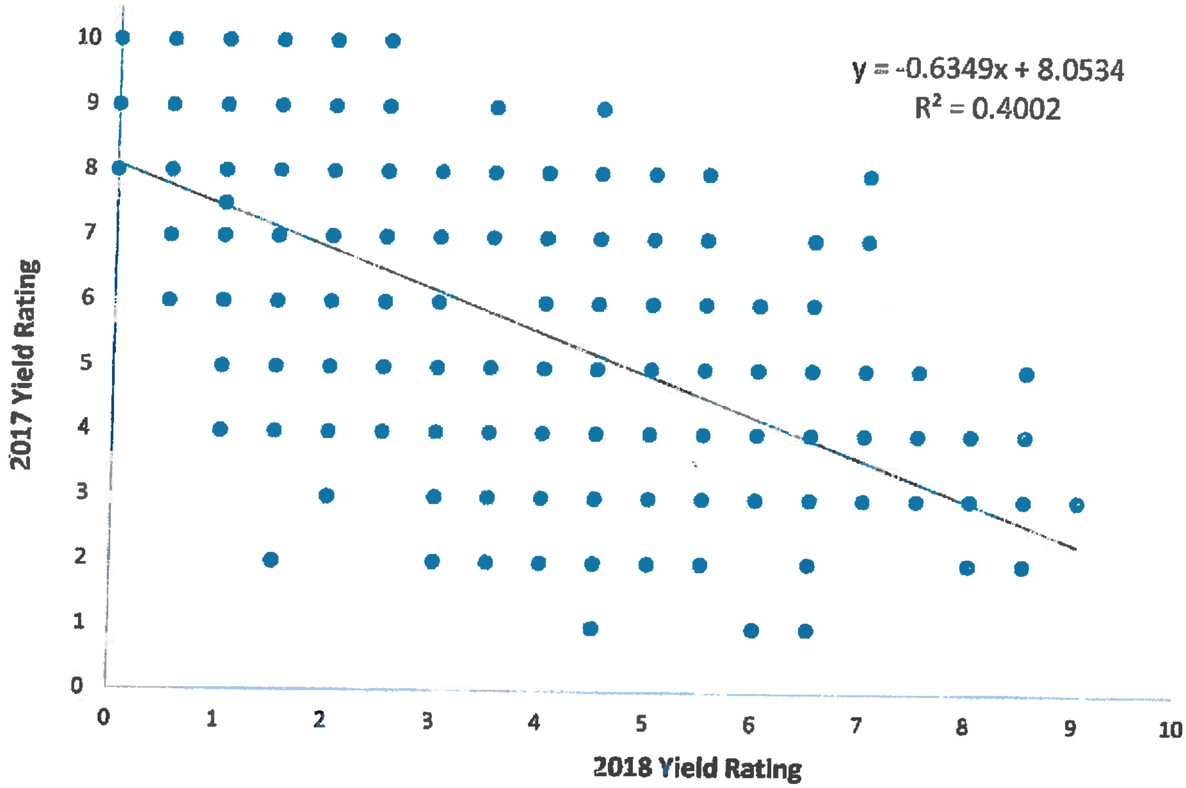

Figure 3. Relationship between tree yields in 2017 and the yields in 2018 at the Nielsen trial. Each dot represents an individual tree. Rating was based on a 0-10 scale, 0 = no crop and 10 = extremely heavy crop.

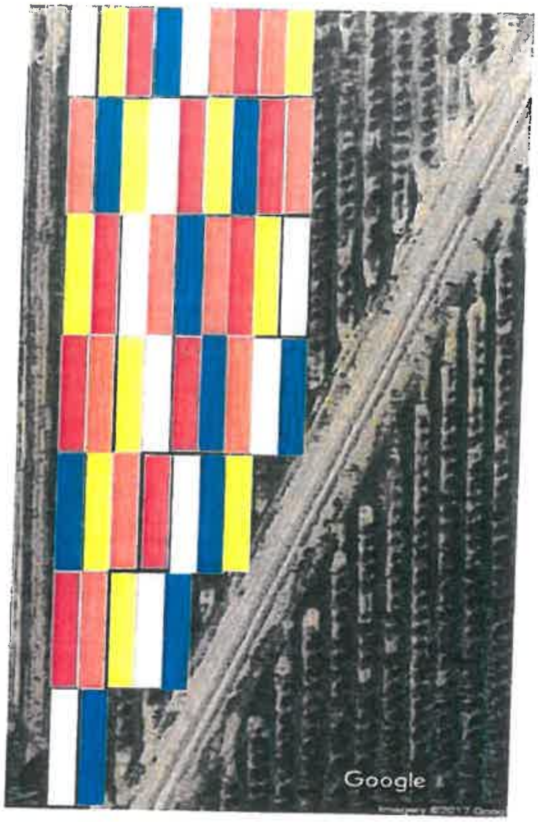

Figure 4. Hedging timing trial located at Heath Burreson's orchard, Orland, California. Trees hedged at the following times: Yellow = 8-Mar, Red = 5-Apr, Blue = 8-May, Orange = 8-Jun, and White = No Hedge Control. Experiment is set up as a factorial design with 5 hedging timings and 2 hedging positions (east and west) with 5 replicates.

Table 1. Relationship between topping height and pruning costs, 'Manzanillo' olive yields, fruit value, and return at Nickels Estate.

Treatment	Pruning Costs 2016* ($/a)	Pruning Costs 2017 ($/a)	Pruning Costs 2018 ($/a)	Pruning Cost Cumulative	Yields (t/a) 2016	Yields (t/a) 2017	Yields (t/a) 2018	Yields Cumulative (t/a) (2016 - 2018)	Average Value ($/t 2016-2018)
Topped at 10'	500 a**	237 a	219	956 a	2.01	3.78	2.03	7.82 b	1360 a
Topped at 13'	885 b	317 b	275	1477 b	3.57	5.27	2.49	11.33 a	1344 a
Control	930 b	304 b	320	1554 b	4.65	4.37	3.44	12.46 a	1310 b
P value	0.045	0.026	Ns	0.042	0.091	0.241	Ns	0.050	0.007

*pruning costs based on time needed to prune the trees multiplied by $11/hr. not sure that would be enough now, probably should be minimum wage plus 40% for overhead

**different letters in the same column indicate significance $p < 0.05$.

Table 2. Nielsen trial, Effects of hedging date and severity of hedging on 'Manzanillo' olive yields.

Hedging Date	Severity of Hedge*	Yield Rating 2016	Yield Rating 2017	Yield Rating 2018
No Hedge	NA	6.5 a	7.0 ab	3.6 b
24-May-16	Moderate	7.2 a	7.4 a	2.5 c
27-Apr-16	Moderate	7.1 a	7.1 ab	2.6 c
27-Apr-16	Severe	3.7 b	6.7 ab	2.4 c
15-Jul-16	Moderate	6.1 ab	6.3 ab	3.4 bc
15-Jul-16	Severe	5.5 ab	5.3 bc	3.6 b
1-Mar-17	Moderate		5.1 bc	4.1 ab
3-May-17	Moderate		4.1 c	4.2 ab
29-Mar-17	Moderate		4.3 c	3.8 ab
3-May-17	Moderate		5.1 bc	3.6 b
P value		.041	0.0001	.046

* Moderate = approximately 8.5 feet from trunk; Severe = approximately 6.5 feet from trunk.
Different letters in the same column indicate significance $p < 0.05$

Table 3. Burreson trial, Effects of hedging date on 'Manzanillo' olive yields and canopy growth.

Cut Date	**Yields (t/a)**	**Yields Ratings***	**Canopy Diameter (ft)**		**Growth in cut vs non-cut side of tree** (ft)**
	2017	2018	2017	2018	2017
Feb/Mar	8.9	1.5	9.2 ab	9.6 b	0.43 a
April	9.1	1.5	9.1 ab	8.9 bc	-1.1 b
May	7.9	1.3	9.0 ab	8.6 bc	-1.7 b
June	7.8	1.3	8.2 b	8.3 c	-2.1 b
Control	9.4	1.5	10.3 a	12. 4 a	
p-value	0.17	0.51	0.004	0.002	0.0006

*rating scale 0 to 10, 0 = no crop and 10 = extremely heavy crop.
**Difference in growth between cut and not cut side of the tree.
Different letters in the same column indicate significance $p < 0.05$

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: 2018

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

Project Leaders:

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Project Title: Managing Alternate Bearing in Olive with Plant Growth Regulators (PGRs) and Pruning

Cooperators:

Lindcove REC — 'Manzanillo' table olive orchard, Lindcove

Overview: To mitigate alternate bearing in 'Manzanillo' olive trees, it is necessary to increase the number of non-bearing shoots during the ON-crop year by removing inflorescences or fruit during the ON-crop year. This is due to the fact that only non-bearing shoots, which are in low number on ON-crop trees, produce a significant number of inflorescences the following spring (Fichtner and Lovatt, 2018; Fichtner et al., 2017). Return bloom is dramatically reduced on bearing shoots, the majority of shoots on ON-crop trees, due to the combined effects of the total fruit on the tree (whole tree effect of crop load) and individual fruit set on bearing shoots (localized effect of fruit) of 'Manzanillo' olive trees which (i) inhibit summer vegetative shoot growth and thereby reduce the number of nodes that can produce floral (inflorescence) buds the foillowing spring (Sibbett, 2000), (ii) reduce spring bud break, (iii) prevent floral gene transcription, and (iv) increase floral bud abscission (Fichtner and Lovatt, 2018; Fichtner et al., 2017). The overall goal is to even out the 2-year cumulative yield in an alternate bearing 'Manzanillo' orchard, which is currently distributed with ≥ 80% of the crop harvested in the ON-crop year and 20% or less harvested in the OFF-crop year to a more uniform distribution of 50% of the 2-year cumulative yield harvested in each year. Once achieved, the second objective is to maintain annual yield at 60% to 70% of the average yield for the ON-crop year in the orchard for *each year* of the 2-year cycle in order to increase annual and cumulative yield, maintain commercially valuable fruit size and to increase income over multiple, successive cropping years. Thus far, using NAA to reduce inflorescence number or pruning to reduce fruit number on one side of the tree starting in the ON-crop year and repeating the treatments on the opposite side of the tree the following year, we have achieved 2-year cumulative yields that are distributed ~60% in the putative ON-crop year and ~40% in the following putative OFF-crop year with no significant reduction in 2-year cumulative yield.

Objectives for 2018 (Year 1): The objective of this year's research was to test the efficacy of removing flowers or fruit on one side of an ON-crop tree one year and the other side of the tree the following year. Two approaches for crop reduction are being tested: (1) flower thinning with a foliar application of the plant growth regulator (PGR) naphthaleneacetic acid (NAA) (ALCO® Olive Stop™; AMVAC Corp., already registered for use on olive) applied at full bloom at the manufacture's suggested rate; and (2) fruit removal by pruning (mechanical hedging) one side of a second set of ON-crop 'Manzanillo' olive trees. (We had hope to compare the efficacy of NAA to that of a new product ACC, but Valent BioSciences was unable to provide ACC to include in the research in 2018.) By chemically thinning only half of the tree with NAA, the impact of over-thinning on yield if a heat wave occurs is reduced. In our experiment, pruning was delayed to after fruit set to enable growers to evaluate the crop set by their trees before deciding how much fruit to remove. This allows a grower to tailor the degree of pruning to accommodate years with a poor fruit set in spite of a heavy bloom. An added benefit of both treatments is that increasing the number of non-bearing shoots will improve the efficacy of PGR treatments that increase summer vegetative shoot growth and spring bud break to increase floral intensity following the production of the ON crop and PGR treatments designed to improve fruit set or size (Fichtner and Lovatt, 2018; Fichtner et al., 2017). All treatments were applied to 'Manzanillo' olive trees in a block, which included 'Barouni' olive trees as the pollenizer planted at a ratio of one to ten, at the Lindcove REC in Exeter, CA.

Progress during 2018: Bloom estimates determined prior to treatment applications in 2018 clearly distinguished ON-bloom trees from OFF-bloom trees (Table 1), which produced ON and OFF yields at harvest, respectively (Table 2). ON-crop trees that were subjected to NAA application or pruning on the west side of the tree in 2017 did not flower as intensely in 2018 as we had anticipated and thus, the 2018 bloom for these trees was significantly less than ON-crop control trees and equal to that of the OFF-crop control trees. Although pruning in 2017 appeared to have a more negative effect on return bloom than the 2017 NAA treatment, return yields for these two treatments in 2018 were equal (Table 2). At bloom, we suspected that pruning at the end of June (June 26) might have been too late for the trees to fully transition from vegetative to reproductive growth. Thus, when treating the opposite side of the tree in 2018, we pruned at the end of May (28 days after full bloom). However, now that we have the yield data, it is clear that this was not the case, but instead evidence of the greater variation in results obtained with pruning compared to the use of NAA that is starting to accumulate with multiple years of data. Average bloom estimate per tree was strongly correlated with total yield (kg/tree) at harvest (r = 0.80; $P < 0.0001$). This result, taken together with the fact that NAA and pruned trees produced yields that were not significantly different, indicates that NAA treatment does not have a negative effect on pistil viability (i.e., does not cause pistil abortion or increase the proportion of male flowers). If it did, one would expect NAA-treated trees to have significantly lower yields than pruned trees.

In spring of 2018, NAA was applied to the east side of the trees at full bloom and pruning was earlier this year at 28 days after full bloom (May 30). Estimates of crop load accurately distinguished ON- versus OFF-crop trees (Table 1). Estimated crop loads for trees treated with NAA or pruned in spring 2018 were intermediate to those of ON- and OFF-crop trees and these trees produced total yields at harvest that were intermediate to ON- and OFF-crop control trees (Table 2). In contrast, estimated crop loads were significantly lower for trees treated on one side in 2017 and then the other in 2018 compared to ON-crop control trees and ON-crop trees treated on one side of the tree in 2018 only and equal to OFF-crop trees ($P < 0.0001$). These results were reflected in the total yields at harvest and there was a strong correlation between average crop load estimate per tree and total yield (kg/tree) at harvest ($r = 0.83$; $P < 0.0001$).

In 2017, the first NAA and pruning treatments applied to the west side of ON-crop trees reduced the yield by 32% and 33%, respectively, resulting in yields intermediate to those of ON- and OFF-crop control trees in 2017 (Table 2). However, return yields were lower than anticipated and total yield was not significantly different from that of OFF-crop control trees in 2018. Yields of NAA-treated and pruned trees were 77% and 70% lower than the yield of ON-crop control trees in 2018. The results raise two questions: (1) should more flowers and fruit be removed from ON-crop trees or (2) is it better to leave the fruit contributed by the OFF-crop side of the tree in year 2. In other words, should trees be treated on one side of the tree every other year rather than annually. In both treatments, yields were 2.8- and 2.7-fold greater than that of OFF-crop control trees in 2017 but only 1.5- and 1.8-fold greater than the OFF-crop control trees in 2018, despite yields of the OFF-crop control trees being very similar in 2017 and 2018.

In 2018, we repeated the treatments with a new set of ON-crop trees. Consistent with crop estimates, the trees treated with NAA or pruned on one side in 2018 produced yields intermediate to the ON- and OFF-crop control trees (Table 2). Compared to the ON-crop control trees yield was reduced 31% by NAA but only 18% by pruning, making NAA treatment much more consistent than pruning. NAA-treated trees produced 3.1-fold more fruit than the OFF-crop control trees, whereas the pruned trees produced 3.75-fold more fruit, raising concern that the amount of fruit removed by pruning in 2018 might be too low.

Thus far, trees treated annually have 2-year cumulative yields that are 15-20% lower than the average for the two sets of ON/OFF control trees, but the differences are not statistically different; yield varies between the two sets of ON/OFF control trees by ~ 15% for the 2 years of the research. The goal is to shift the distribution of the yields in the ON/OFF cycles of an alternate bearing 'Manzanillo' olive orchard from 80%: 20% in the ON- versus OFF-crop years to 50%: 50% for each year of a 2-year cycle to improve fruit size and stabilize income. Thus far, the treatments have achieved a yield distribution of 64%: 36% for flower thinning using NAA and 59%: 41% for fruit removal by pruning for the 2 years of the alternate bearing ON/OFF cycle. This successfully improved fruit size. In each year of the research, average fruit size for OFF-crop trees was extra large, whereas the average fruit size for ON-crop control trees was medium (2017) or large (2018). In each year the NAA and pruning treatments were initiated on ON-crop trees, average fruit was large for both treatments (Table 2). Another benefit from evening out alternate bearing may be a greater proportion of green fruit at harvest. In 2017, OFF-crop control trees had the largest proportion of black or partially black fruit (54%) ($P < 0.0003$), whereas the proportion green olives on NAA-treated (81%) and pruned trees (75%) was equal to that of ON-crop control trees (90%) ($P < 0.0003$). In 2018, 93% to 100% of all fruit per tree were green, with OFF-crop control trees having the least green fruit ($P = 0.0012$). Moreover, for ON-crop trees treated with NAA or pruned annually for two years, the severity of alternate bearing has been reduced more than 20%. The alternate bearing index (ABI) for the ON/OFF control trees over the two years of the research is 0.75, whereas for NAA and pruned trees treated in both 2017 and 2018, it is 0.52. Note: ABI is calculated as (year 1 yield – year 2 yield)/(year 1 yield + year 2 yield), with yield as total kg/tree and the difference in yield between years 1 and 2 expressed as an absolute value. ABI = 0.0 means no alternate bearing; whereas ABI = 1.0 is complete alternate bearing, with crop one year and no crop the other year (Pearce and Dobersek-Urbanc, 1967).

This coming year, for trees treated in 2017 and 2018, we will continue to apply the foliar NAA treatment to reduce inflorescence number and the pruning treatment to reduce fruit number. The treatments will be on the opposite side of the trees from last year to determine if over the course of 3 years the treatments continue to equilibrate annual yield across years to reduce ABI and also to increase total annual yield and cumulative yield relative to ON/OFF control trees. In addition, we will compare the results of this strategy with a second strategy of applying the NAA or pruning treatments to one side of the tree and then the other side of the tree every other year, not annually, to compare the efficacy of this strategy to better increase yield following the ON-crop year and improve 2-year cumulative yield while evening out alternate bearing. We initiated the research for this strategy in 2018, thus the yield results of 2019 will tell us whether we need the yield of the OFF-crop side of the tree in year 2 to increase the 2-year cumulative yield and better even out yield distribution over the 2-year yield cycle.

Select References:

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Pearce, S.C. and S.Dobersek-Urbanc. 1967. The measurements of irregularity in growth and cropping. J. Hort. Sci. 42(3):295–305.

Sibbett, S. (2000). Alternate bearing in olive trees. California Olive Oil News. 3(12),1

Table 1. Effect of crop load and NAA and pruning treatments on the 2018 bloom and crop load estimates.

2017[y]	2018	Bloom[z] estimate (Before 2018 treatment)			Crop load estimate (After 2018 treatment)		
Treatment		West	East	Average	West	East	Average
OFF-crop control	ON-crop control	2.7 a[x]	2.6 a	2.7 a	2.7 a	2.5 a	2.6 a
OFF-crop	ON crop - NAA east (ON crop) side of tree @ full bloom	2.2 a	2.3 a	2.3 a	2.4 a	1.6 b	2.0 b
OFF-crop	ON crop - Pruned east (on-crop) side of tree @ 28 days after full bloom (May 30)	2.6 a	2.6 a	2.6 a	2.4 a	2.3 a	2.4 ab
ON-crop control	OFF-crop control	0.7 bc	0.4 b	0.6 b	0.9 c	0.7 c	0.8 c
ON crop - NAA west side of tree @ full (on crop) bloom	OFF crop - NAA east (OFF crop) side of tree @ full bloom	1.1 b	0.9 b	1.0 b	1.6 b	0.9 c	1.3 c
ON crop - Pruned west (on crop) side of tree @ end of June (June 28)	OFF crop - Pruned east (OFF crop) side of tree @ 28 days after full bloom (May 30)	0.6 c	0.9 b	0.8 b	0.8 c	0.9 c	0.9 c
P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[z] Bloom and crop load was evaluated on the following scale: 0, no inflorescence or no crop; 1, low floral intensity or low crop load; 2, medium floral intensity or medium crop load; and 3, high floral intensity or heavy crop load crop .

[y] All trees were topped in July 7, 2017 and May 30, 2018

[x] Mean values within a vertical column followed by different letters are significantly different at the specified *P* level by Fisher's Protected LSD test.

Table 2. Effect of crop load and NAA and pruning treatments on 2017 and 2018 yield and average fruit size and 2-year cumulative yield.

2017[z]	2018	2017		2018		2-yr cumulative yield (kg/tree)
Treatment		Yield (kg/tree)	Fruit size (g/fruit)	Yield (kg/tree)	Fruit size (g/fruit)	
Off-crop control	On-crop control	25.3 c[y]	5.5 a	121.5 a	4.4 d	146.9 a
Off-crop	On crop - NAA east side of tree @ full bloom	20.3 c	5.6 a	83.6 b	4.9 c	103.8 a
Off-crop	On crop - Pruned east side of tree @ 28 days after full bloom (May 30)	15.4 c	5.5 a	100.5 ab	4.3 d	115.9 a
On-crop control	Off-crop control	103.9 a	4.1 c	26.8 c	6.1 a	130.7 a
On crop - NAA west side of tree @ full bloom	NAA east (off crop) side of tree @ full bloom	71.3 b	4.9 b	39.6 c	5.5 b	110.9 a
On crop - Pruned west side of tree @ end of June (June 26)	Pruned east (off crop) side @ 28 days after full bloom (May 30)	69.3 b	4.7 b	47.5 c	5.6 ab	116.9 a
P-value		< 0.0001	<0.0001	< 0.0001	<0.0001	0.1182

[z] All trees were topped in July 7, 2017 and May 30, 2018.

[y] Mean values within a vertical column followed by different letters are significantly different at the specified *P* level by Fisher's Protected LSD test.

Evaluation of Several Promising Additives for Reducing Acrylamide in Black Ripe Table Olives

Objective:

To evaluate whether the addition of amino acid or phenolic additives prior to sterilization can reduce acrylamide content in black ripe table olives.

Progress:

The first step in the project was developing a reliable, accurate method for measuring acrylamide in black ripe olives. We developed a simple extraction method for acrylamide with liquid chromatography-UV detection. However, the processors raised concerns about the consistency of data between our liquid chromatography (LC) method and the gas chromatography (GC) method which was being used by a private lab. To investigate this discrepancy, we compared multiple methods for acrylamide analysis including:

- LC-UV detection
- LC-mass spectrometry
- GC-mass spectrometry (which includes sample derivatization)
- LC-UV detection with sample derivatization.

The instrument detection limits for brominated acrylamide were much higher than non-brominated acrylamide (Table 1). Brominated samples had to be concentrated before analysis to achieve a method detection limit comparable with non-brominated samples. LC-MS/MS had the greatest sensitivity overall.

To assess method accuracy and precision, we performed spike recovery experiments, where a known amount of acrylamide was added to the olive pulp before extraction. Samples were fortified in duplicate with 200 µg/kg and 600 µg/kg of acrylamide. Results are shown in Table 2. According to the AOAC, acceptable recovery for analytes in this concentration range is between 80-110%, and the acceptable limit for relative standard deviation (RSD) is below 15%. Recoveries at the low level could not be calculated for the LC-UV methods since acrylamide was not quantifiable in the unfortified samples. All other methods had acceptable recoveries at both the low and high level with the exception of the high spike in GC-MS. The GC-MS conditions were still being optimized during these trials and we suspect that caused an error in the quantification, which would be eliminated if the trial was repeated. All samples were below the acceptable limit for RSD, with LC-MS/MS having the lowest variability.

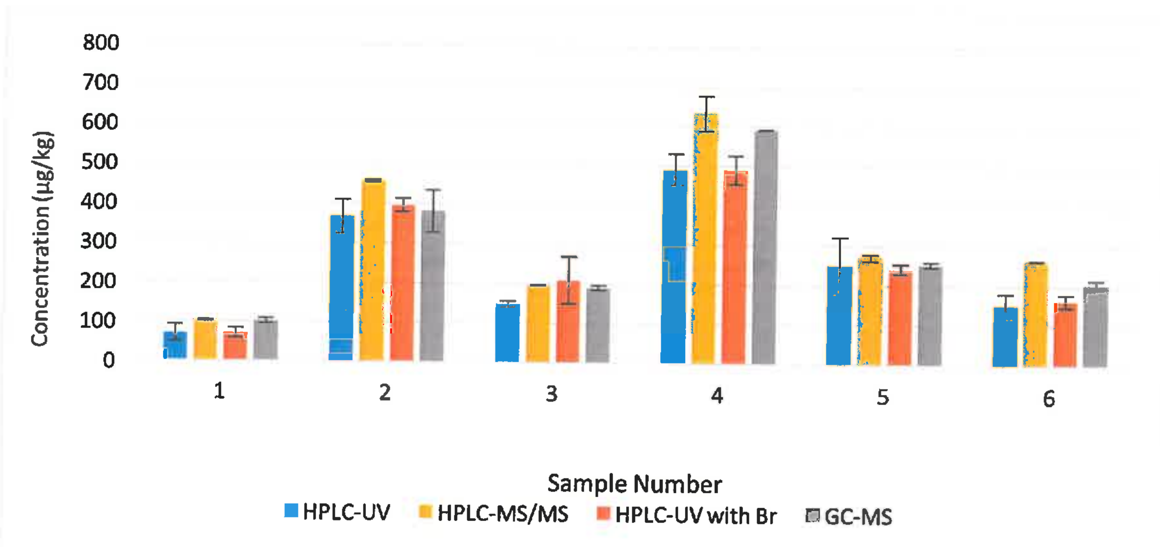
	Instrumental limit (µg/mL)	Method limit (µg/kg)
LC-UV	0.025	71
LC-MS/MS	0.01	28
LC-UV with derivatization	0.20	76
GC-MS with derivatization	0.75	62

Table 1. Limits of quantification for each detector and method

	Low spike (200 µg/kg)		High spike (600 µg/kg)	
	Recovery	Relative Standard Deviation	Recovery	Relative Standard Deviation
LC-UV	na[3]	14%	95%	5%
LC-MS/MS	110%	1%	110%	0.2%
LC-UV with derivatization	na	6%	90%	3%
GC-MS with derivatization	101%	2%	156%	1%

Table 2. Recoveries and relative standard deviations of acrylamide added at two different spike levels
According to the AOAC, acceptable recovery range is 80-110% and acceptable relative standard deviation is ≤ 15%.
[1]na (not applicable) = The unfortified sample was below the quantification limit and recovery could not be calculated.

We then analyzed six different commercial black ripe samples using all four of the developed methods in order to assess the consistency of data. Although there was some variability between methods, values were generally within the same range and the trends between samples were consistent (Figure 1). LC-MS/MS had the lowest standard deviation, followed by GC-MS.



Based the results, LC-MS/MS was identified as the best method in terms of precision, sensitivity, selectivity and ease. However, we are in the process of conducting a more in-depth comparison and validation of these different methods. The results will be published, and can hopefully be used as a starting point for developing a standard analytical method for the table olive industry.

Future work:

In previous trials in our lab, certain additives like cysteine and n-acetyl cysteine have shown great promise in reducing acrylamide *in vivo*. Other published studies have also shown that proline, sarcosine and arginine inhibited acrylamide formation in an olive model system. With the help of processors, we plan to test the effectiveness of these amino acids and other additives at the industrial scale. Different concentrations of additives will be tested in order to assess acrylamide reduction, as well as changes in the quality or sensory profile of the olives.

Differentiation of olive cultivars using DNA and NMR-based fingerprinting methods

Objective:

To use DNA and NMR-based fingerprinting methods and fatty acid profiles (FAP) for differentiating cultivars of processed olives. Color-coded loading plots will be generated from supervised multivariate statistical analysis methods to indicate the significance of specific metabolites in different cultivars and regions.

Materials:

Canned olives of known cultivars were collected from processors. Sample information is listed in the table below:

Cultivar	Origin	Quantity
Manzanilla	Domestic	1 gallon-sized can; 17 retail-sized cans
	Mexico	1 gallon-sized can
	Spain	1 retail-sized can
Manzanilla Fina	Domestic	9 retail-sized cans
Sevillano	Domestic	1 gallon-sized can, 10 retail-sized cans
Gordal	Spain	5 retail-sized cans
Hojiblanca	Spain	3 retail-sized cans
Barouni	Domestic	1 gallon-sized can
Mission	Domestic	1 gallon-sized can
Obliza	Mexico	1 gallon-sized can
Chalkidiki	Greece	1 retail-sized can

Individual olives within the same can will be treated as biological replicates, as they very likely originated from different trees.

Progress:

DNA-based differentiation. A method for extracting DNA from processed California-style olives is being developed. Extraction of DNA from olive fruit is a novel and difficult task due to the high content of lipids and secondary metabolites like phenolic compounds. Commercial kits were first tried, but extraction was unsuccessful. Instead, we are currently experimenting with a cetyl trimethylammonium bromide (CTAB) extraction method. Small amounts of DNA were recovered upon initial trials, but yield can be greatly improved through method optimization. The following techniques are being assessed:

- Freezing and crushing fruit into fine powder under liquid nitrogen
- Utilizing zirconimum beads and a tissue lyser during sample homogenization
- Removing lipids using a phenol:chloroform wash
- Removing polysaccharides using a salt:chloroform wash

NMR fingerprinting: Method development is being performed in collaboration with Dr. Emmanuel Hatzakis at the Ohio State University (OSU). Canned olives of two different cultivars were frozen over dry ice and crushed into a powder using a mortar and pestle. Samples were shipped to Dr. Hatzakis' lab for trials, where they are currently assessing whether polar or nonpolar compounds will be more effective for differentiating the cultivars. Dr. Hatzakis is also comparing different NMR spectroscopy methods including liquid ^{1}H NMR and ^{13}C NMR, and solid-state High Resolution-Magic Angle Spinning (HR-MAS) NMR. Conditions that provide the most robust and sensitive differentiation will be optimized.

Future work:

DNA-based differentiation: Through a literature search, we will identify specific markers (biallelic single nucleotide polymorphisms, or SNPs) that vary between olive cultivars. DNA primer sequences that target these SNPs will be designed. We will then extract DNA from canned olives of different cultivars/origins using the previously-developed method. We will test the performance of these SNPs on the extracted DNA to determine whether cultivars can be effectively differentiated. Ultimately, we aim to produce a 48-SNP panel that can be used as a tool to reliably identify cultivar for the industry.

NMR fingerprinting: We plan to use the previously-optimized method to analyze at least 30 olives from each cultivar. Data pre-processing will be performed using AMIX software and a model for differentiating cultivars will be constructed using partial least squares discriminant analysis (PLS-DA). Thirty olives of various known cultivars will then be analyzed in order to test the prediction ability of our model.

Fatty acid profiles: The fatty acid profiles of 30 olives from each cultivar will be determined using gas chromatography-flame ionization detection (GC-FID) following the IOC official method (COI/T.20/Doc. no. 24-2001). As with NMR analyses, PLS-DA will be used construct a model to differentiate between cultivars and the FAP of 30 olives of various known cultivars will be used to test the prediction ability of the model.

Southern Region Olive Fruit Fly Project

Sponsored by: California Olive Committee, Nutrien Ag Solutions & Ag IPM Consultants

2018 Total OLFF for the Week Ending

	Apr.06		Apr. 13		Apr.20		Apr. 27		May.04		May. 11		May.19		May. 25		Jun.01		Jun. 08		Jun.15		Jun. 22		Jun.29		Jul. 06		TOT/YR	
Block	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Woodlake	set	set	86	18	27	6	15	6	14	5	9	6	3	2	4	3	29	8	9	5	19	11	4	3	1	0	0	0	220	73
Ivanhoe	set	set	40	8	11	2	18	4	16	5	17	6	6	4	0	1	9	3	3	2	9	9	1	1	0	0	1	0	131	45
Exeter	set	set	73	13	40	13	48	10	12	3	13	7	20	8	5	1	8	3	3	1	8	3	0	1	0	0	0	0	230	63
South Exeter	set	set	7	0	3	0	9	4	4	2	9	4	4	0	0	1	2	1	3	1	0	0	1	1	2	1	3	3	47	18
Tonyville	set	set	195	41	72	22	10	21	90	15	28	9	32	9	41	14	26	21	18	6	16	4	5	2	6	1	0	0	539	165
W. Lindsay	set	set	31	8	16	9	23	8	14	3	12	6	11	3	5	1	19	27	4	1	21	6	19	9	2	0	11	2	188	83
Strathmore	set	set	106	39	33	10	31	14	12	4	19	0	14	4	8	3	36	15	4	3	5	4	7	2	3	1	19	4	297	103
Porterville	set	set	32	6	14	3	11	5	9	3	12	3	3	2	12	3	33	13	9	4	4	0	1	0	9	7	1	0	150	49
Terra Bella	set	set	16	5	13	6	32	4	4	2	28	17	53	10	22	5	68	9	26	18	27	20	39	7	8	6	4	1	340	110
Total			586	138	229	71	197	76	175	42	147	58	146	42	97	32	230	100	79	41	109	57	77	26	31	16	39	10	2142	709
City of Visalia	set	set	7	4	13	1	49	9	1	1	17	7	21	7	8	1	10	2	13	4	29	11	5	5	5	2	6	1	197	55

	Jul.13		Jul. 20		Jul.27		Aug. 03		Aug.10		Aug. 17		Aug.24		Aug. 31		Sep.07		Sep. 14		Sep.21		Sep. 28		Oct.05		Oct. 12		TOT/YR	
Block	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Woodlake	2	0	1	0	4	2	2	1	3	3	1	5	1	4	4	4	2	1	5	4	5	0	3	0	7	1	0	0	260	98
Ivanhoe	22	6	10	3	5	2	6	1	0	3	3	1	0	1	2	2	3	1	7	3	14	1	2	1	6	2	3	1	214	73
Exeter	2	1	0	0	0	0	0	0	0	1	0	0	0	0	2	1	0	0	4	4	2	0	0	0	1	1	1	0	242	71
South Exeter	19	3	8	4	2	1	0	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	2	1	80	29
Tonyville	8	2	2	2	1	0	3	0	0	0	4	0	0	0	3	2	0	0	1	0	5	0	1	0	2	1	0	1	569	173
W. Lindsay	10	5	10	5	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	213	95
Strathmore	6	2	4	1	51	5	10	1	4	1	2	0	2	1	2	0	1	0	2	1	2	0	1	0	2	1	1	2	387	118
Porterville	2	1	1	0	1	0	0	0	0	0	2	1	0	1	1	1	0	0	1	0	0	1	2	1	0	0	3	2	163	57
Terra Bella	7	2	3	0	0	0	1	1	0	0	3	1	2	1	0	0	1	0	3	1	1	0	1	1	1	1	3	2	366	120
Total	78	22	39	15	68	11	22	5	7	8	15	8	5	8	14	10	8	2	23	14	30	2	11	3	19	7	13	10	2494	834
City of Visalia	35	10	11	2	3	1	2	1	2	1	3	0	0	1	0	0	0	0	1	1	2	0	0	0	0	1	1	0	256	73

	Oct. 19		Oct. 26		Nov. 02		Nov. 09		Nov. 16		Nov. 23		Nov. 30																TOT/YR	
Block	M	F	M	F	M	F	M	F	M	F	M	F	M	F															M	F
Woodlake	2	0	1	8																									263	106
Ivanhoe	10	3	0	4																									224	80
Exeter	3	1	0	4																									245	76
South Exeter	1	0	0	0																									81	29
Tonyville	0	0	2	0																									571	173
W. Lindsay	2	0	2	0																									217	95
Strathmore	3	1	2	0																									392	119
Porterville	3	1	0	1																									166	59
Terra Bella	2	1	2	5																									370	126
Total	26	7	9	22																									2529	863
City of Visalia	5	0	0	0																									261	73

Southern Region Olive Fruit Fly Project
2018

Date	Block	1		2		SUBTOT	
		M	F	M	F	M	F
Oct.25	Woodlake	1	7	0	1	1	8
Oct.25	Ivanhoe	0	1	0	3	0	4
Oct.25	Exeter	0	0	0	4	0	4
Oct.25	South Exeter	0	0	0	0	0	0
Oct.25	Tonyville	0	0	2	0	2	0
Oct.25	W. Lindsay	0	0	2	0	2	0
Oct.25	Strathmore	1	0	1	0	2	0
Oct.25	Porterville	0	1	0	0	0	1
Oct.25	Terra Bella	1	0	1	5	2	5
	TOTAL					9	22
Oct.24	City of Visalia	0	0	0	0	0	0

UCCE Glenn County - Olive Fruit Fly Populations
for Glenn and Tehama County

	2-Apr		9-Apr		17-Apr		23-Apr		30-Apr		7-May		14-May		21-May		29-May		4-Jun		11-Jun		18-Jun		25-Jun		TOT/YTD	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Orland 1 Glenn County Fairgrounds	3	3	5	3	6	2	24	8	14	16	3	3	6	5	66	30	50	34	32	22	69	22	91	36	42	19	411	203
Orland 2 Road 200 & Road E	0	1	4	6	1	1	67	26	110	37	81	34	173	51	155	90	106	63	26	12	33	15	18	15	21	10	795	361
Orland 3 SE Orland N & 16	56	47	26	18	9	5	29	8	10	4	6	4	10	3	4	0	11	5	3	1	20	13	11	4	4	2	199	114
Orland 4 NE Orland Rd 12 & N	0	0	0	0	1	0	0	1	2	0	4	0	0	1	0	0	0	0	0	0	3	1	1	0	4	3	15	6
Orland 5 Rd 21 & M	4	0	7	1	6	2	5	0	20	8	6	4	16	10	27	13	39	15	15	3	13	17	4	0	0	0	162	73
Orland 6 Hwy 99W & Rd 18	33	15	46	17	24	6	84	32	67	24	45	22	21	14	20	9	8	3	34	24	46	18	44	35	4	5	476	224
Corning 1 Northbound I-5 Reststop	1	0	0	1	3	1	3	1	6	2	8	3	14	4	20	9	74	31	106	24	269	79	242	74	130	43	876	272
Corning 2 Fig Lane & Houghton	1	2	7	3	3	0	5	6	4	0	2	1	17	7	8	7	42	23	8	3	9	7	8	3	15	11	129	73
Corning 3 Barham & Sampson	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	6	3	6	2	25	17	27	12	25	16	91	50
Corning 4 Sac River - Kopta Rd	2	3	0	2	3	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	3	3	1	1	0	12	10
Corning 5 Viola Ave & Orchard Ave	3	1	1	0	0	0	1	1	1	0	0	0	2	0	2	0	17	1	5	1	9	7	4	2	0	1	45	14
Corning 6 Dora Ave & Marguerite Ave	0	0	0	0	-	-	0	0	0	0	0	0	12	3	10	3	37	12	6	8	5	3	5	3	5	9	80	41
Total	103	72	96	51	56	17	218	83	236	91	155	71	272	98	312	161	390	190	242	101	502	202	458	185	251	119	3291	1441

	2-Jul		9-Jul		16-Jul		23-Jul		30-Jul		6-Aug		13-Aug		20-Aug		27-Aug		4-Sep		10-Sep		17-Sep		24-Sep		TOT/YTD	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Orland 1 Glenn County Fairgrounds	23	12	10	13	4	3	1	1	0	1	0	0	0	1	1	0	0	1	9	16	4	10	4	2	4	1	471	264
Orland 2 Road 200 & Road E	25	12	7	7	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	4	4	0	4	832	391
Orland 3 SE Orland N & 16	20	6	5	0	1	1	0	0	0	0	0	0	0	1	1	0	2	0	0	1	7	1	13	3	4	4	252	131
Orland 4 NE Orland Rd 12 & N	1	1	4	4	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	5	9	9	1	35	24
Orland 5 Rd 21 & M	0	0	4	3	0	1	0	0	0	0	0	0	0	0	2	2	0	0	4	3	8	2	25	13	34	20	239	117
Orland 6 Hwy 99W & Rd 18	13	16	9	16	16	10	1	1	1	2	1	0	0	0	0	0	2	0	2	4	5	1	1	3	7	5	534	282
Corning 1 Northbound I-5 Reststop	142	52	136	50	34	11	1	0	1	1	1	1	0	0	0	0	0	0	1	0	0	0	3	0	4	2	1199	389
Corning 2 Fig Lane & Houghton	15	6	16	6	11	5	0	0	1	0	0	1	3	0	0	0	1	1	0	0	2	1	3	2	8	8	189	103
Corning 3 Barham & Sampson	33	23	18	12	1	0	1	2	0	0	0	0	3	1	0	0	2	0	0	0	2	0	3	0	2	6	156	94
Corning 4 Sac River - Kopta Rd	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	1	3	0	18	13
Corning 5 Viola Ave & Orchard Ave	3	0	6	5	1	0	0	0	0	0	0	2	0	1	0	0	0	2	0	0	0	0	0	0	4	8	59	32
Corning 6 Dora Ave & Marguerite Ave	8	2	4	2	1	0	1	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	3	2	3	6	102	55
Total	283	131	219	118	70	31	6	5	3	5	2	4	6	5	4	3	8	5	20	26	28	17	64	39	82	65	4086	1895

	1-Oct		8-Oct		15-Oct		23-Oct																				TOT/YTD	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Orland 1 Glenn County Fairgrounds	3	4	8	12	161	101	302	137																			945	518
Orland 2 Road 200 & Road E	6	2	13	13	71	48	90	71																			1012	525
Orland 3 SE Orland N & 16	9	2	23	16	131	38	99	53																			514	240
Orland 4 NE Orland Rd 12 & N	2	1	2	1	7	5	13	5																			59	36
Orland 5 Rd 21 & M	32	18	35	11	152	76	158	63																			616	285
Orland 6 Hwy 99W & Rd 18	13	11	11	10	131	68	132	93																			821	464
Corning 1 Northbound I-5 Reststop	0	2	-	-	2	1	3	2																			1204	394
Corning 2 Fig Lane & Houghton	10	1	10	1	56	40	32	29																			297	174
Corning 3 Barham & Sampson	2	1	1	1	21	9	6	13																			186	118
Corning 4 Sac River - Kopta Rd	2	2	3	1	23	14	29	20																			75	50
Corning 5 Viola Ave & Orchard Ave	0	2	0	1	6	10	16	23																			81	68
Corning 6 Dora Ave & Marguerite Ave	6	2	9	1	26	18	17	15																			160	91
Total	85	48	115	68	787	428	897	524	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5970	2963

ANNUAL RESEARCH REPORT
California Olive Board and California Olive Oil Commission
November 2018

Project Year: 2018 Anticipated Duration of Project: 2^{nd} of 3 years

Principal Investigators: J. E. Adaskaveg

Cooperating: D. Thompson, K. Nguyen, and H. Förster

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

Keywords: Bactericides, copper enhancing compounds, antimicrobial natural products, biological controls

JUSTIFICATION/ BACKGROUND

Olive knot caused by the bacterium *Pseudomonas savastanoi* pv. *savastanoi* (*Psv*) is a serious disease of olives (*Olea europaea*) worldwide (8). The pathogen enters through wounds causing outgrowths (knots, tumors, galls) on branches and infrequently on leaves and fruit. Olive knot is one of the most economically important diseases of olives as infection may lead to tree defoliation, dieback, and reduced tree vigor, which ultimately lowers fruit yield and quality (6). *Psv* can survive epiphytically on olives but the main sources of inoculum are bacteria living within knots (7). Large quantities of bacterial ooze can be exuded upon wetting knots. This exudate is disseminated by rain, wind, insects, birds, as well as human activity. The opportunistic pathogen takes advantage of wounds caused by natural leaf abscission (4), frost, and hail, as well as cultural practices such as pruning and harvesting. These latter practices also lead to direct mechanical damage of the knots, exposing and spreading inoculum to healthy tissue. After entering its woody host, the pathogen actively induces knot formation through the production of indoleacetic acid (IAA) and cytokinins (2). 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 low temperatures (-5° C) and thus, wetness is the main limiting factor for the disease. None of the currently grown olive cultivars is resistant to the pathogen (5).

Management of olive knot is difficult, and growers rely on applications of copper-based bactericides as the only effective foliar treatment. Manual application of cresol- and xylenol-based compounds (Gallex) to knots can eliminate the knot pathogen but is unfeasible on a commercial scale. Copper has been extensively used in olive production for many years for the control of diseases such as peacock spot and olive knot. Reliance on a single active ingredient has led to our detection of copper resistance in *Psv* strains from a commercial olive orchard. The incidence of copper resistance is currently very low, accounting for only 2% of the total strains collected in different olive growing regions of California. When resistant strains were inoculated to Arbequina and Manzanillo olive wounds, application of copper provided reduced or no control as compared to inoculation with a sensitive strain. Copper-resistant strains caused less disease on leaf scars as compared to Cu-sensitive strains, but still resulted in a high incidence of disease over a range of inoculum concentrations. Therefore, there is a potential risk of copper resistance spreading with continued and sole use of copper. This necessitates the development of new bactericides or copper-activity-enhancing materials to overcome resistance. The latter strategy has proven to be effective for walnut blight management where copper resistance in *Xanthomonas arboricola* pv. *juglandis* is common and copper-mancozeb mixtures have provided exceptional control. Mancozeb can no longer be registered on new crops but other copper-enhancing alternatives can be evaluated. Salicylidene benzoylhydrazone (SBH) was recently discovered to display synergism when combined with copper against *Alternaria solani* causing early blight of tomatoes. We performed preliminary tests with a derivative of this molecule with promising results with several genera of phytopathogenic bacteria including *Psv*. Low concentrations of metallic copper combined with SBH were highly inhibitory in vitro against a copper-resistant *Psv* strain while copper or SBH by themselves at the same concentrations were not effective. Field trials in 2017 on managing olive knot, SBH-copper, however, did not improve performance of copper. Other derivatives of SBH will be supplied by Dow AgroSciences, and these will be tested in 2018.

Other potential bactericides have also been made available to us by agrochemical registrants in 2017. These include a nanoparticle zinc product called Zinkicide and experimental inhibitors of type III secretion systems in plant pathogenic bacteria. The latter compounds are novel in their mode of action. They act on the mechanism that delivers bacterial proteins into the host cells that are necessary for *Pseudomonas* species to cause disease. Currently, we are testing Zinkicide and three experimental type III secretion system inhibitors.

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* (1) 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 extensive field studies, including in a commercial application to inoculated branches. Kasugamycin is currently federally registered and in 2018 received California registration on pome fruit, cherry, and walnut crops, whereas registration on olives, peaches, and almonds is pending for late 2019. Kasumin – olive is still in the IR-4 program with the final report and submission to the EPA pending in fourth quarter 2018. Kasugamycin would greatly complement current copper sprays and could be used in rotation or mixtures with copper. Oxytetracycline was also submitted to IR-4 and is in the field trial phase of the IR-4 program for establishing tolerances. We will conduct additional studies with oxytetracycline to potentially improve its efficacy by using registrant-recommended adjuvants. New antibiotic registrations, however, find little acceptance with regulatory agencies, and we are currently in discussion with EPA to develop a science-based approach on the use of antibiotics in plant agriculture.

In addition to developing conventional chemical compounds, research on alternative materials such as biopesticides and food additives may provide new modes of action for managing olive knot. Biopesticides such as Serenade contain the gram-positive bacterium *Bacillus subtilis* (strain QST 713) that produces various compounds that are antagonistic against a broad range of fungal and bacterial organisms. In our efficacy trials, Serenade and Serenade-copper mixtures, however, were not effective at recommended rates.

Table 1. Chemicals used for evaluation as potential bactericides against olive knot

Treatments

No.	*Treatment*	*Tradename/experimental name*	*Chemical class/use*
1	Lactic acid	Lactic acid	Organic acid
2	Citric acid	Citric acid	Organic acid
3	Zinc	Zinkicide	Zinc nanoparticle
4	Zinc thiadiazole	ZTD	Zinc thiadiazole
5	Oxytetracycline	Fireline	Antibiotic (tetracycline)
6	Kasugamycin	Kasumin	Antibiotic (aminoglycoside)
7	Copper hydroxide	ChampION++	Copper
8	TS-28 10% SC 5 mM	TS-28	Type III secretion system inhibitor
9	TS-108 20% SC 5 mM	TS-108	Type III secretion system inhibitor
10	TS-153 10% SC 5 mM	TS-153	Type III secretion system inhibitor
11	Nisin	Niprosin	Food preservative
12	Epsilon-poly-L-lysine	e-Polylysine	Food preservative
13	Nisin Alginate	Nisin Alginate	Food preservative
14	Epsilon-poly-L-lysine Alginate	e-poly-L-lysine Alginate	Food preservative
15	DAS1	DAS1	Derivative of salicylidene benzoylhydrazone

Adjuvants

No.	*Treatment*	*Tradename*	*Chemical/use*
A	Adjuvant	Tactic	Surfactant/sticking agent
B	Adjuvant	Nu-Film P	Spreader/sticker agent
C	Adjuvant	Regulaid	Nonionic surfactant
D	UV stabilizer	---	Zinc oxide

Several food additives that are considered 'generally recognized as safe' (GRAS) have antimicrobial properties. They are often naturally produced molecules of gram-positive *Streptomyces* species. Although these compounds are typically applied to food products as preservatives, they may have potential for controlling plant diseases when applied as a foliar treatment. Integration of these alternative materials with conventional treatments may improve disease control, reduce the risk of resistance development, and provide olive growers with more resources for managing olive knot. In 2017, we evaluated nisin, Ɛ-poly-L-lysine, and lactic acid and all showed similar efficacy to copper in reducing olive knot on leaf scars, but not on lateral wounds. This information is still valuable because rotational programs could be developed with different modes of actions for different phases of the disease, i.e., leaf scars or lateral wounds occurring during leaf drop or harvest and pruning, respectively. These materials are registerable for conventional and possibly organic treatments.

RESEARCH OBJECTIVES

1) **Develop new bactericides and potential enhancers of copper activity against *Psv***
   a) In-vitro sensitivity of *Psv* to Zinkicide, Type III secretion system inhibitors, and copper mixtures with new SBH derivatives (using selected ratios).
   b) Efficacy of new bactericides in comparison with kasugamycin for the management of olive knot caused by copper-sensitive and -resistant strains of *Psv* in field studies.
      i) Zinkicide
      ii) Potential enhancers of copper activity - new SBH derivatives.
      iii) Type III secretion system inhibitors
      iv) Oxytetracycline formulations in combination with adjuvants recommended by the registrant.
2) **Evaluate several food additives and a sanitizer for the control of olive knot**
   a) Determine the efficacy of the GRAS food additives nisin, Ɛ-poly-L-lysine, and the GRAS sanitizers lactic and citric acid in field studies for the management of olive knot.
3) **Continue to support the registration of the antibiotics kasugamycin and oxytetracycline** - UV blockers and stabilizers and EPA policy.

PLANS AND PROCEDURES

1) Develop new bactericides and potential enhancers of copper activity against *Psv*.

1a. In vitro sensitivity of two *Psv* strains against selected food additives and ZTD. The toxicity of nisin, Ɛ-poly-L-lysine, and a zinc thiadiazole (ZTD) to a copper-sensitive and a -resistant strain of *Psv* was evaluated in a direct exposure assay. Bacterial suspensions were mixed with solutions of each of the toxicant as selected concentrations and incubated for 60 s. For the control, water was used instead of the toxicant. Aliquots were then diluted 1:100 with sterile distilled water, and plated onto King's medium B. The number of colonies on each plate were enumerated after 2 days of growth.

1b. Evaluation of bactericides and experimental treatments in field studies. Zinkicide, copper-SBH mixtures, Type III secretion system inhibitors, oxytetracycline, and other treatments (Table 1) were tested in the field on Arbequina and Manzanillo olives at UC Davis or in a commercial planting. Lateral wounds on 1-2-year-old twigs were made using a scalpel and removing the bark to expose cambial tissue. Leaf scars were made by pulling leaves off the same twigs. In addition, wounds from natural leaf drop were used in some studies. Treatments were sprayed onto wounds before inoculation with a suspension of copper-sensitive or -resistant *Psv* strains. Oxytetracycline was used in combination with recommended adjuvants because it is especially vulnerable to UV-degradation. Treatments were compared to Kasumin and copper. The efficacy of treatments was assessed as the percent incidence of knots forming on treated, inoculated wounds as compared to wounds that were treated with water and inoculated (i.e., controls).

2) Evaluate several food additives for the control of olive knot. Field tests were conducted on Arbequina and Manzanillo olives to evaluate the efficacy of nisin, Ɛ-poly-L-lysine, and lactic acid against *Psv*. The same wounding, treatment application, inoculation, and evaluation procedures were used as described above.

3) Continue to support the registration of the antibiotics kasugamycin and oxytetracycline. An inter-commodity and industry group continued to work with the Minor Crop Farmer Alliance to recommend an EPA policy change towards the use of antibiotics in plant agriculture. Specifically, a suggested new internal EPA Guidance Document (GD) for use of antibiotics in plant agriculture was developed based on science and submitted to EPA. Historically, EPA GD 152 for registration of antibiotics in animal husbandry is used for all requests in agriculture. Additionally, we will continue to work with a USDA working group to address CODEX initiatives for establishing policies on all antibiotic use in agriculture including animal and plant uses.

RESULTS AND DISCUSSION

1a. In vitro sensitivity of two *Psv* strains against selected food additives and ZTD.
Direct contact assays demonstrated a high in vitro toxicity of Ɛ-poly-L-lysine against copper-sensitive and -resistant strains of *Psv* (Fig. 1). Incubation in 10 ppm Ɛ-poly-L-lysine for 60 sec completely inhibited growth of both strains. In contrast, a concentration of 1000 ppm of nisin was required to completely inhibit growth of the sensitive strain, and growth of the resistant strain was reduced by 2.5 log. ZTD was not effective in these studies. Thus, Ɛ-poly-L-lysine and possibly nisin were identified as the most promising treatments for managing olive knot. These compounds are antimicrobials for processed food use and have not been formulated for agricultural use.

1b and 2). Evaluation of bactericides, food additives, and other experimental treatments in field studies.
In contrast to the high toxicity of **Ɛ-poly-L-lysine** in in vitro studies, this compound showed only low to no activity in field studies on the management of olive knot (Fig. 2A, B). An attempt was made to make the compound more persistent, and we prepared an alginate formulation that was described in the literature to provide environmental stability. Additionally, we added zinc oxide to this formulation to increase UV stability. Still, this formulation was also not effective (Figs. 3, 4). Thus, in our future studies we will explore other methods to make Ɛ-poly-L-lysine more effective in the field. The other food preservative, **Nisin**, did not show efficacy in reducing knot development (Fig. 2). The nisin-alginate-zinc oxide formulation sometimes improved efficacy (Fig. 4), but not at other times (Figs. 3, 4). The third food preservative, **lactic acid**, was tested only in one trial, and a moderate reduction in knot development was observed (Fig. 2A, B). Thus, this compound deserves further evaluation, possibly including it in mixtures. **Citric acid**, however, was not effective (Fig. 2A, B).

Three **type III secretion system inhibitors** were evaluated in four studies using copper-sensitive and/or -resistant strains of the pathogen (Figs. 3, 4, 5, 6). TS-108 and TS-153 were moderately effective in one study on Manzanillo olive (Fig 6A), TS-108 and TS-153 were moderately effective in another study (Fig. 5), but in other cases, they were not effective. These inhibitors were provided to us by other researchers, and possibly other inhibitor formulations could be more effective.

The **zinc nanoparticle product Zinkicide** was evaluated in two studies and showed good to very good efficacy on lateral wounds (Figs. 5, 6). This product, however, has currently no registrant that is willing to cover the expenses for a registration. Furthermore, nanoparticle products, may be difficult to obtain EPA approval due to the number of toxicity and environmental testing required. **Zinc thiadiazole** was not effective in reducing olive knot development (Figs. 3, 4, 5, 6) and also did not improve copper activity (Figs. 3, 4) in preventing or reducing olive knot.

The **antibiotic oxytetracycline (FireLine)** was evaluated by itself or in combination with the recommended adjuvant Tactic. Results were variable with no efficacy when used by itself (Fig. 2A, B) or with Tactic (Figs. 2B, 3) or resulting in good efficacy using Tactic on lateral wounds (Figs. 2A, 4). Thus, this antibiotic still has potential considering its high in vitro toxicity. The other antibiotic, **kasugamycin (Kasumin)**, was very effective (Figs. 2A, B, 6A) or highly effective (Fig. 5) on lateral wounds and sometimes also showed good efficacy on artificial leaf scar wounds (Fig. 5). Statistically, Kasumin was mostly similar in efficacy to copper. Adding adjuvants like Tactic (Figs. 2A, B) or Nufilm (Figs. 3, 4) did not improve efficacy of Kasumin. Copper (ChampION) was highly effective (Figs. 2A, 3, 4, 5, 6) on lateral and leaf scar wounds when used by itself, but efficacy was reduced when inoculations were done with a copper-resistant strain of *Psv* (Fig. 2B) The addition of **DAS-1, a potential enhancer of copper activity**, did not improve the performance of copper (Figs. 3, 4). **Copper-Kasumin mixtures** were similarly effective as either compound by itself or more effective than either copper alone (Figs. 3, 4). This mixture will provide an excellent strategy for the most effective olive knot management and to prevent or delay the spread of resistance against each component.

One study was done treating inoculated natural leaf scars with ChampION, Kasumin, or a ChampION-Kasumin mixture. In contrast to the studies discussed above with artificial leaf scar injuries where leaves were detached by pulling off, the three treatments were highly effective in protecting natural leaf scars from infection by a copper-resistant strain, and Kasumin completely prevented knot development (Fig. 7). Although these natural leaf scar assays are very laborious to perform (every leaf scar has to be inoculated and treated separately in 20 to 25 replications), these assays need to be repeated and also be used for some of the other treatments under evaluation (e.g., Ɛ-poly-L-lysine, nisin, oxytetracycline).

3) Continue to support the registration of the antibiotics kasugamycin and oxytetracycline. Kasugamycin was first federally registered on pome fruits, and in 2018, it received California registration on pome fruit, cherry, and walnut crops. Registration on olives, peaches, and almonds is pending for late 2019. The petition for Kasumin on olive was still in the IR-4 program this summer and the final report and submission to the EPA are pending in fourth quarter of 2018. This was due to complications with extractions of kasugamycin from large amounts of oil in some olive samples that caused delays. Working with IR-4 and Arysta, we had additional procedures for alternative extraction procedures sent to the GLP residue lab to resolve the difficulties.

I also attended the IR-4 meeting and met with EPA representatives. I re-emphasized the need for a separate guidance document to GD152 that is specific for registration of bactericides for plant agriculture. Currently, GD152 is for all agriculture, but more importantly, was developed for registration of antibiotics for animal agriculture by USDA and FDA. With the extensive change-over in personnel at EPA, these meetings are critical in moving forward on these issues.

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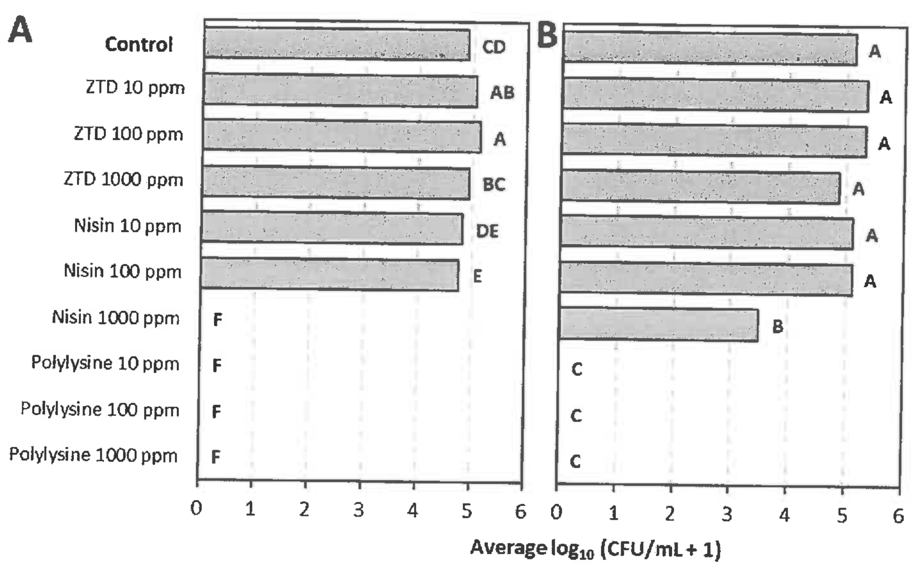

Fig. 1. In-vitro toxicity of selected antimicrobials against *Pseudomonas savastanoi* pv. *savastanoi* strain **(A)** O1-26 (copper-sensitive) or **(B)** O1-113 (copper-resistant), in a direct exposure assay. Bacterial suspensions were exposed to the antimicrobials for 60 s and then plated onto agar media.

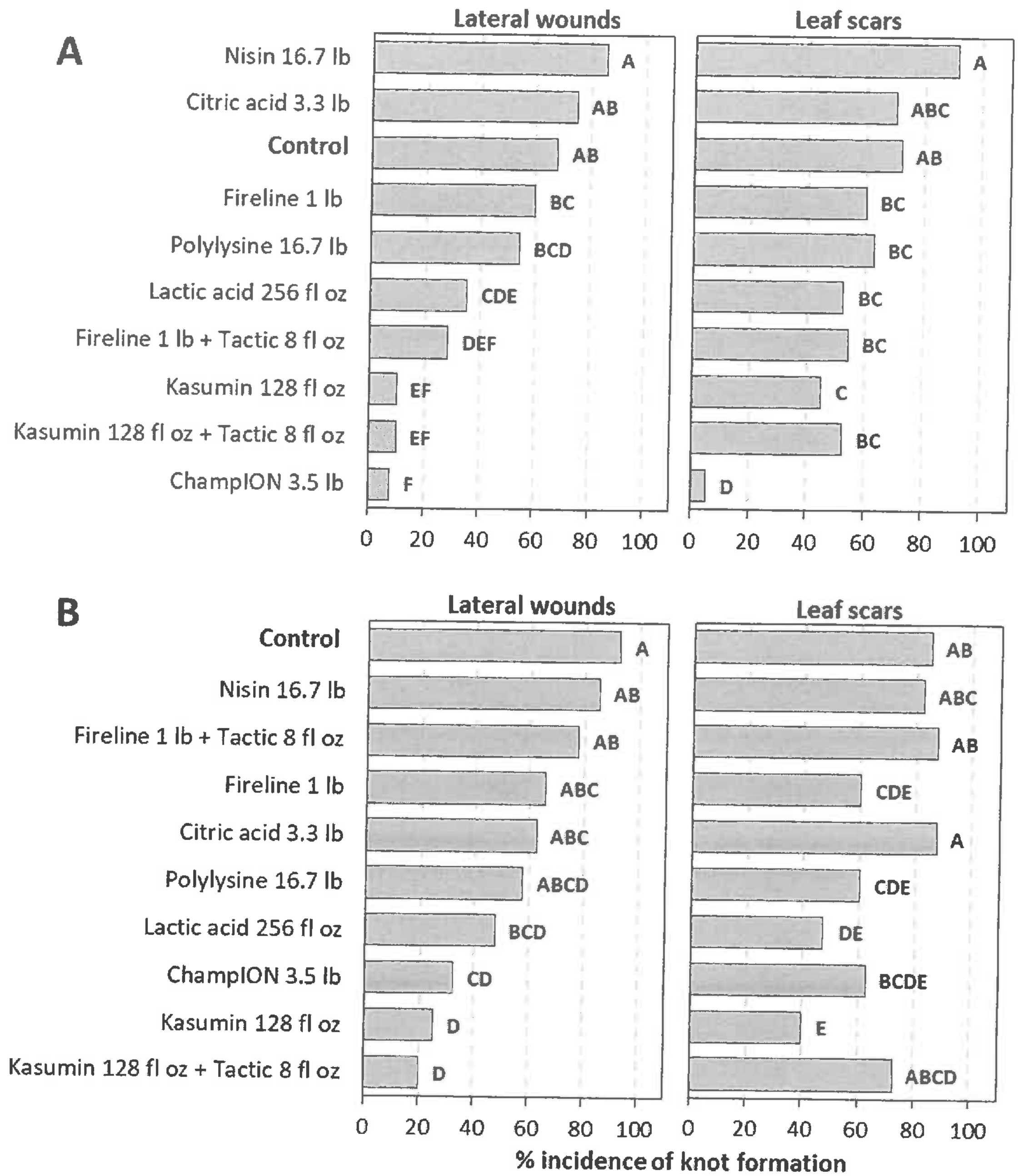

Fig. 2. Field trials on the management of olive knot initiated fall on 'Manzanillo' olives at UC Davis. Treatments were spray-applied to wounds until runoff and allowed to dry. Treatment rates are calculated per acre. Wounds were then spray-inoculated with a **(A)** copper-sensitive, or **(B)** copper-resistant *Pseudomonas savastanoi* pv. *savastanoi* strain.

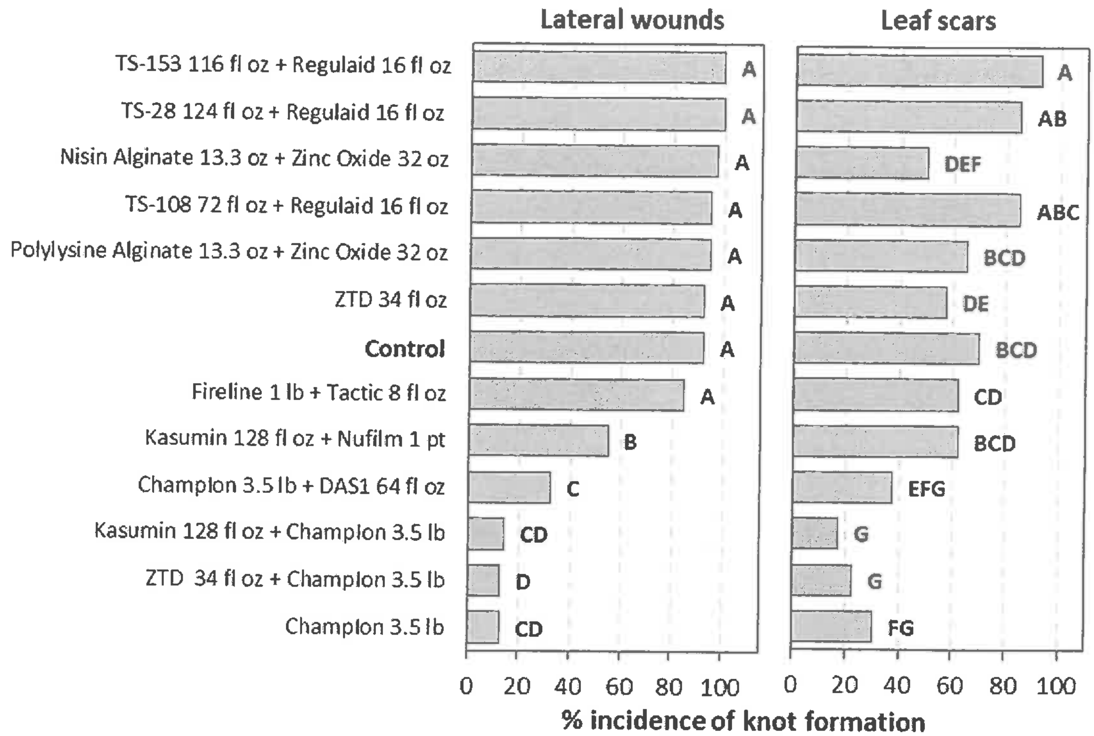

Fig. 3. Field trials on the management of olive knot of 'Manzanillo' olives initiated spring 2018 at UC Davis. Treatments were spray-applied to wounds until runoff and allowed to dry. Treatment rates are calculated per acre. Wounds were then inoculated with a copper-sensitive *Pseudomonas savastanoi* pv. *savastanoi* strain (O1-26).

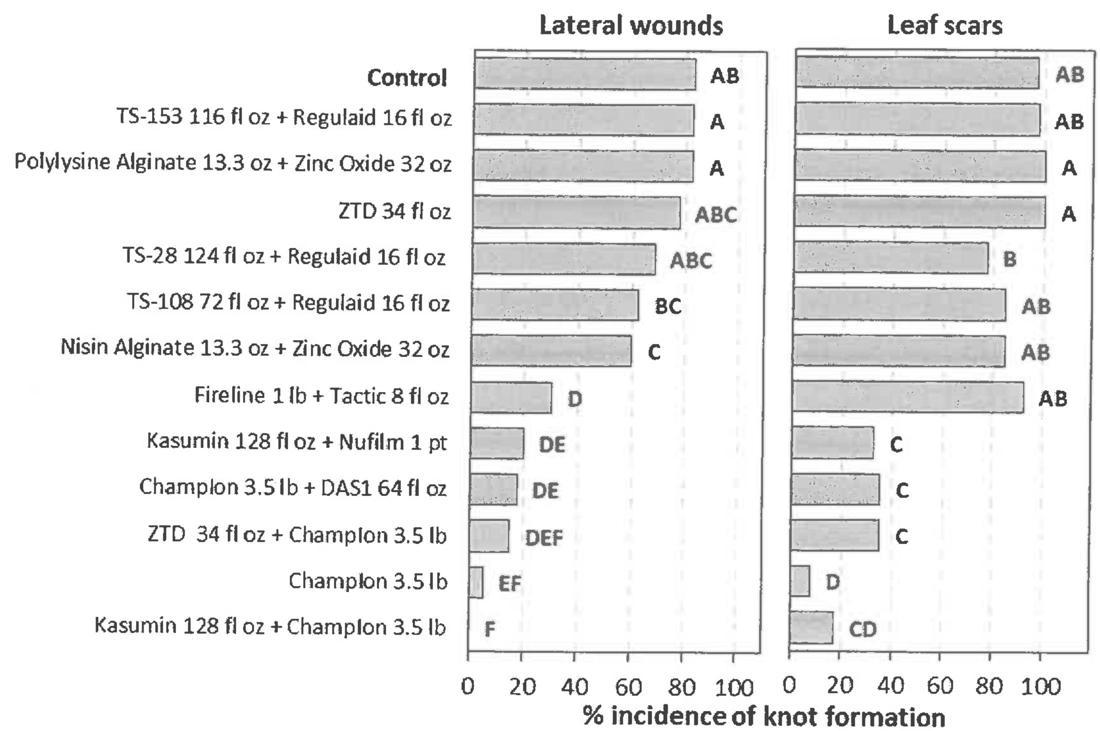

Fig. 4. Field trials on the management of olive knot initiated spring 2018 in a commercial 'Arbequina' orchard. Treatments were spray-applied to wounds until runoff and allowed to dry. Treatment rates are calculated per acre. Wounds were then inoculated with a copper-sensitive *Pseudomonas savastanoi* pv. *savastanoi* strain (O1-26).

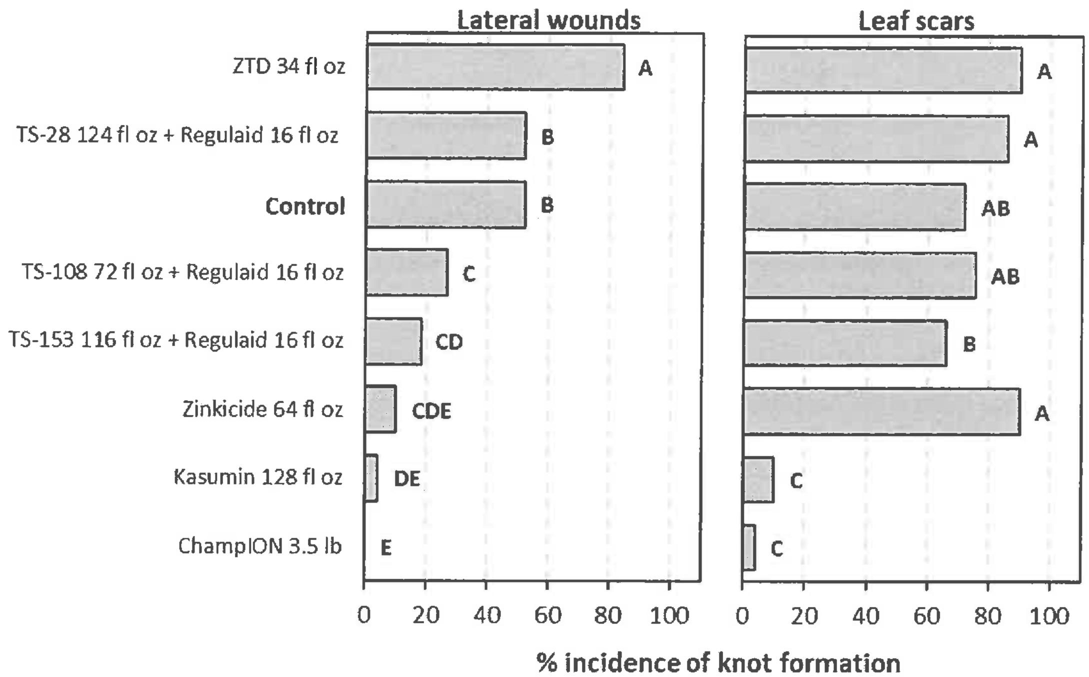

Fig. 5. Field trials on the management of olive knot initiated fall 2017 on 'Arbequina' olives at UC Davis. Treatments were spray-applied to wounds until runoff and allowed to dry. Treatment rates are calculated per acre. Wounds were then spray-inoculated with a copper-sensitive (O1-23) of *Pseudomonas savastanoi* pv. *savastanoi* strain.

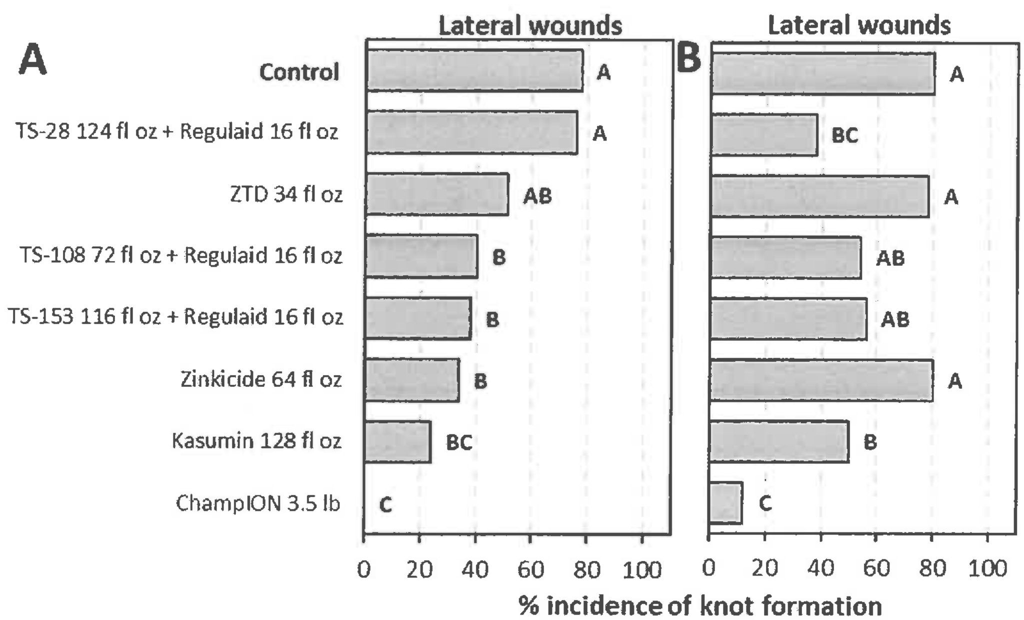

Fig. 6. Field trials on the management of olive knot of 'Manzanillo' olives initiated fall 2018 at UC Davis. Treatments were spray-applied to wounds until runoff and allowed to dry. Wounds were then inoculated with a **(A)** copper-sensitive (O1-23), or **(B)** copper-resistant *Pseudomonas savastanoi* pv. *savastanoi* strain (O1-113).

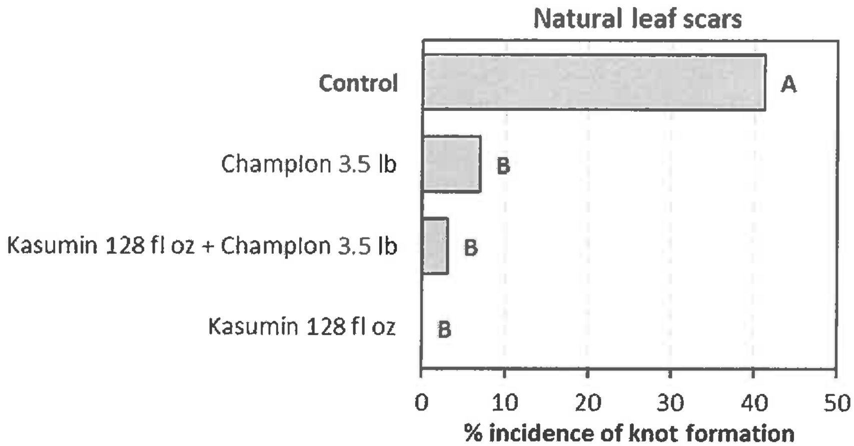

Fig. 7. Field trial on the management of olive knot initiated May 2018 on 'Arbequina' olive at UC Davis. Natural leaf scars were made by removing yellow leaves that readily abscised when gently pulled. Treatments were spray-applied until runoff, allowed to dry, and spray-inoculated with a copper-resistant *Pseudomonas savastanoi* pv. *savastanoi* strain (O1-113). Leaf scars were evaluated for knot development after approximately 3 months.

Research 2000

Olive fruit fly control. Efficacy program to combat resistance.

Trial Comments

Trt No.	Type	Treatment Name	Rate	Rate Unit	Appl Code	Spray Volume	Volume Unit	Mix Size	Mix Unit
1	CHK	Untreated							
2	INSE	GF120	1	fl oz/item	ABCDEF				
3	INSE	Danitol	16	fl oz/a	AB	100	GAL/AC	20	GAL
4	INSE	Harvanta	16	fl oz/a	AD	100	GAL/AC	20	GAL
	INSE	GF120	1	fl oz/item	BCEF				
5	INSE	Harvanta	24	fl oz/a	AD	100	GAL/AC	20	GAL
	INSE	GF120	1	fl oz/item	BCEF				
6	INSE	Asail	8	fl oz/a	AD	100	GAL/AC	20	GAL
	INSE	GF120	1	fl oz/item	BDEF				
7	INSE	Avaunt	4	oz wt/a	AD	100	GAL/AC	20	GAL
	INSE	GF120	1	fl oz/item	BCEF				
8	INSE	Avaunt	5	oz wt/a	AD	100	GAL/AC	20	GAL
	INSE	GF120	1	fl oz/item	BCEF				
9	INSE	Minecto Pro	12	fl oz/a	AD				
	INSE	GF120	1	fl oz/item	BCEF				
10	INSE	Sivanto	14	fl oz/a	AD				
	INSE	GF120	1	fl oz/item	BCEF				
11	INSE	Lorsban	4	fl oz/a	AD				
	INSE	GF120	1	fl oz/item	BCEF				
12	INSE	GF120	1	fl oz/item	ACE				
	INSE	Harvanta	16	fl oz/a	B	100	GAL/AC	20	GAL
	INSE	Asail	8	fl oz/a	D	100	GAL/AC	20	GAL
	INSE	Danitol	16	fl oz/a	F	100	GAL/AC	20	GAL

Replications: 6, Untreated treatments: 1, Design: Randomized Complete Block (RCB), Treatment units: US standard, Treated 'Plot' experimental unit size Width: 18 feet, Treated 'Plot' experimental unit size Length: 18 feet, Application volume: 100 GAL/AC, Mix size: 4.686 GAL, Overage: 5%, Format definitions: G-All7.def, G-All7.frm

Product quantities required for listed treatments and applications of trials included in this table:

Amount*	Unit	Treatment Name	Form Conc	Form Unit	Form Type	Lot Code

* Adjusted for multiple applications in treatment list.

General Trial Information

Study Director: California Olive Committee
Investigator: Debra Keenan

Trial Location

City: Orland **Country:** USA United States
State/Prov.: California
Postal Code: 95963

Directions:

4553 County Road RR site 1
Orland, CA 95963
Site 2 Henning
Conducted Under GLP: No
Conducted Under GEP: No

Keywords: efficacy of olive fruit fly control

Objectives:

Determine the best control measures for Olive Fruit Fly. Search new insecticides for potential control. Determine if the materials cause injury to the crop. Also determine if the insecticides have any control of olive scale which is becoming a bigger pest in the area.
Conduct trials in Olive growing regions. Conduct 4-5 trials.

Contacts

Study Director: California Olive Committee

Investigator: Debra Keenan
Organization: Research 2000

Cooperator/Landowner

Cooperator: Silveira site 1 **Role:** FALDOW

Henning
site 2

Crop Description			
Crop 1:OLVEU	Olea europaea	Olive	
	BBCH Scale:BPER		

Research 2000

Olive fruit fly control. Efficacy program to combat resistance.

Trial ID:OFFsilv Location:Orland Trial Year:2018
Protocol ID:Olive FF OC Investigator:Debra Keenan
Project ID:olivesilv Study Director:California Olive Committee
Sponsor Contact:Research 2000

Pest Description

Pest 1 Type:I Bactrocera oleae
Common Name:olive fruit fly

Site and Design

Treated Plot Width:18 FT **Site Type:**ORCHAR orchard
Treated Plot Length:18 FT
Treated Plot Area:324 FT2 **Treatments:**12
Replications:6 **Study Design:**RACOBL Randomized Complete Block (RCB)

Application Description Silv site 1

	A	B	C	D	E	F
Application Date:	May-24-2018	May-30-2018	Jun-20-2018	Jun-28-2018	Jul-6-2018	Sep-18-2018
Appl. Start Time:	8:00 AM	7:00 AM	9:00 AM	8:00 AM	7:30 AM	8:00 AM
Appl. Stop Time:	9:30 AM	8:30 AM	10:30 AM	9:30 AM	9:00 AM	9:30 AM
Application Method:	backpack	backpack	backpack	backpack	backpack	backpack
Application Placement:	GF type	GF type	GF type	GF type	GF type	GF type
Applied By:	D KEENAN	D KEENAN	D KEENAN	D KEENAN	D KEENAN	D KEENAN
Air Temperature, Unit:	68 F	66 F	71 F	72 F	72 F	61 F
% Relative Humidity:	20	30	10	20	10	20
Wind Velocity, Unit:	1 MPH	0 MPH	1 MPH	2 MPH	0 MPH	1 MPH
Wind Direction:	SE	NA	SE	SE	NA	SE
Dew Presence (Y/N):	N no	N no	N no	N no	N no	N no
Soil Temperature, Unit:	61 F	61 F	63 F	67 F	66 F	62 F
Soil Moisture:	DAMP	DAMP	DAMP	DAMP	DRY	DRY

Application Description Henning site 2

	A	B	C	D	E	F
Application Date:	Apr-19-2018	May-10-2018	May-21-2018	May-30-2018	Jun-11-2018	Sep-12-2018
Appl. Start Time:	7:00 AM	8:00 AM	7:00 AM	8:00 AM	11:00 AM	7:00 AM
Appl. Stop Time:	10:00 AM	9:30 AM	8:30 AM	9:30 AM	12:30 PM	8:30 AM
Application Method:	HANDGUN	GF 120	GF 120	GF 120	GF 120	GF 120
Application Timing:	PEST	PEST	PEST	PEST	PEST	PEST
Application Placement:	CANOPY	GF 120	GF 120	GF 120	GF 120	GF 120
Applied By:	D KEENAN	D KEENAN	D KEENAN	D KEENAN	D KEENAN	D KEENAN
Air Temperature, Unit:	65 F	66 F	68 F	71 F	82 F	6230 F
% Relative Humidity:	20	10	20	30	10	20
Wind Velocity, Unit:	1 MPH	0 MPH	1 MPH	1 MPH	1 MPH	1 MPH
Wind Direction:	SE	NA	SE	SE	SE	SE
Dew Presence (Y/N):	N no	N no	N no	N no	N no	N no
Soil Temperature, Unit:	59 F	60 F	61 F	62 F	66 F	60 F
Soil Moisture:	DAMP	DRY	DRY	DAMP	DRY	DRY

Crop Stage At Each Application

	A	B	C	D	E	F
Crop 1 Code, BBCH Scale:	OLVEU BPER	OLVEU BPER	OLVEU BPER	OLVEU BPER	OLVEU BPER	OLVEU BPER

Pest Stage At Each Application

	A	B	C	D	E	F
Pest 1 Code, Type, Scale:	I	I	I	I	I	I

Trt No **Treatment Application Comment** silv site 1
NOTE GF 120 ALSO SPRAYED ON 8-21-2018 AND 9-12-2018
NOTE ALL TREATMENTS WERE GF 120 THE TRAPS DID NOT WARRENT A CONVENTIONAL APPLICATION

Application Equipment henning site 2

	A	B	C	D	E	F
Appl. Equipment:	REARS	BACKPACK	BACKPACK	BACKPACK	BACKPACK	BACKPACK
Equipment Type:	HANDGU	GF 120	GF 120	GF 120	GF 120	GF 120
Operation Pressure, Unit:	300 PSI					
Nozzle Type:	DISC	HOLLOW	HOLLOW	HOLLOW	HOLLOW	HOLLOW
Nozzle Size:	D6					
Carrier:	WATER	WATER	WATER	WATER	WATER	WATER
Spray Volume, Unit:	100 GAL/AC	1 GAL/AC	1 GAL/AC	1 GAL/AC	1 GAL/AC	1 GAL/AC
Mix Size, Unit:	4.686 GAL	1 GAL	1 GAL	1 GAL	1 GAL	1 GAL

Trt No	**Treatment Application Comment** Henning site 2
	GF 120 ALSO APPLIED ON 6-25-2018, 7-6-2018, 8-22-2018, 9-1-2018,

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

University of California
Division of Agricultural Sciences
ANNUAL RESEARCH PROGRESS REPORT
California Olive Committee/ Olive Oil Commission of California
Project Year: 2018

Project Leader:
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Riverside, CA 92521
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jim.adaskaveg@ucr.edu

Title: Evaluation of new fungicides for control of olive leaf spot (peacock spot)

Introduction. Olive leaf spot or peacock spot, caused by the fungus *Fusicladium oleagineum* (syn. *Spilocea oleaginea, Venturia oleaginea*), occurs sporadically on olive trees in California. In years with favorable environmental conditions, an orchard may lose 9 to 15% of its leaves and 10 to 20% of fruiting twigs if the disease is not managed. Excessive leaf loss can also result in more olive knot because leaf scars are sites for bacterial infection. Symptoms most commonly develop on the leaf blade but are also found on petioles, fruit, and fruit peduncles (stems). At first, lesions are inconspicuous, superficial, sooty blotches. Later, they become dark green to black and circular, and form conidia. Spots are surrounded by yellow halos. Lesions resemble the spots on the tail of a peacock, and hence the name peacock spot. With numerous lesions, the leaf becomes chlorotic and falls.

Leaves in the lower canopy where the humidity is higher are more severely affected, resulting in greater defoliation. Defoliated twigs often die later in the summer. Leaf infections occur on the upper surface and seldom expand beyond the epidermal layer. Once the leaf drops, however, the fungus colonizes the internal tissues forming a dense mass of stromatic tissue. The sexual state of the pathogen has not been observed. Olive cultivars vary in their susceptibility. Mission is the most susceptible followed by Manzanillo and to a lesser extent Sevillano, but all cultivars including oil cultivars such as Arbequina and Arbosana may be affected by peacock spot.

Leaf drop occurs mostly in late spring and summer. Infected leaves remaining on the tree start sporulating along the margins of lesions in the fall. Rainfall and wind-driven rain are the main dissemination methods; whereas wind alone is not effective in detaching and disseminating conidia. In California, lesions start forming in the fall and winter, but most disease develops in the spring. Rainfall is essential for infections to occur regardless of the season. Temperature is important but often is not limiting the development of the pathogen. High temperatures are more limiting to spore germination and mycelial growth than low temperatures. The optimum temperature for growth of the fungus is 21°C, but growth can occur at 6 to 28°C. The minimum duration of leaf wetness for spore germination is 48 h at 16°C, 24 h at 20°C, or 36 h at 24°C. The incubation period for symptom development is 12 to 19 days over a temperature range of 10°C to 25°C.

Currently available chemicals for managing the disease are copper and lime sulfur. Copper is commonly used as Bordeaux mixtures or as fixed coppers to prevent phytotoxicity. Lime sulfur can also eradicate the fungus in leaf tissue. Other fungicides such as zineb are effective but no longer available in the United States. With more regulations concerning the use of copper (new copper limits for agricultural uses for all registered crops) and lime sulfur, alternative fungicides are needed that are highly efficacious and persist for extended time periods to prevent infections over the winter and spring when rainfall results in infection periods. Therefore, we initiated field studies to evaluate alternatives including older and newer fungicides. Currently, timing of fungicide treatments in California includes a postharvest fall application before winter rains begin, and again in early spring if wet conditions continue. These timings coincide with copper treatments for olive knot management. Some researchers have indicated that spring treatments are less effective, but this needs to be further substantiated.

OBJECTIVES

1. Evaluate the performance of new and older fungicides in field trials.
    a) Dithicarbamates (ziram), chlorinated hydrocarbons (chlorothalonil), and phthalimides (captan) (FRAC codes M3, M4, M5), DMIs (FRAC 3), SDHIs (FRAC 7), QoIs (FRAC 11), dodine (FRAC U12), polyoxins (FRAC 19), or mixtures such as FRAC 3/11, FRAC 3/7, FRAC 7/11, and FRAC 3/19.
2. Evaluate application timing of selected treatments.
    a) Fall, spring, or fall <u>and</u> spring.
3. Evaluate new fungicides for their in vitro activity.
    a) Determine the in vitro activity of selected fungicides that are effective in field trials.

MATERIALS AND METHODS

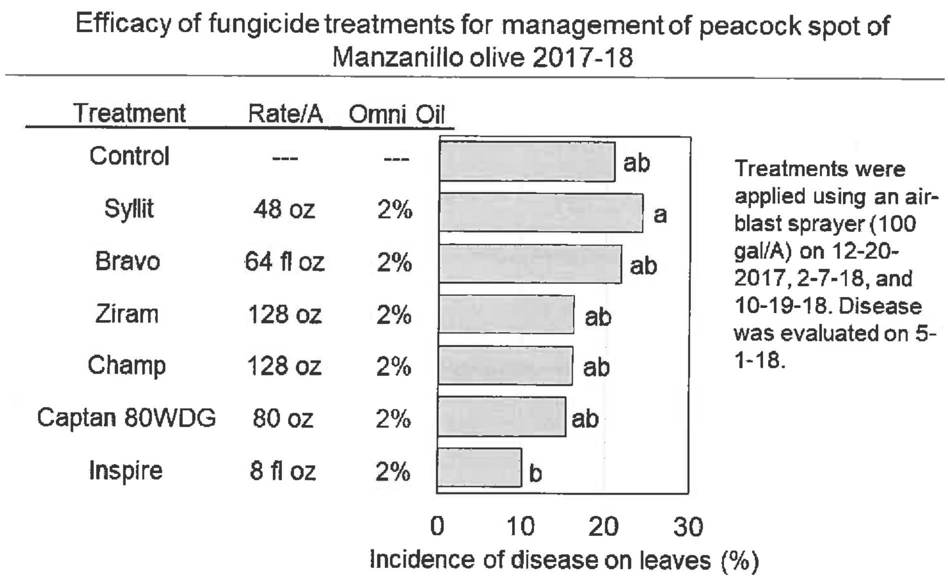
Evaluate the performance of new and older fungicides in field trials. In studies in two commercial Ascolano or Manzanillo olive orchards where the disease is known to occur, fungicides including ziram (FRAC M3), copper (Champ, FRAC M1), captan (FRAC M4), chlorothalonil (Bravo, FRAC M5), difenoconazole (Inspire, FRAC 3), polyoxin-D (Ph-D, FRAC 19), and dodine (Syllit, FRAC U12) were applied in combination with 2% Omni Oil (in the first two applications) using an air-blast sprayer (100 gal/A) on Dec. 20, 2017, Feb. 7, 2018, and Oct. 19, 2018. There were four replications for each treatment in a randomized complete block design. Disease incidence was evaluated on 5-1-18. For this, eight 15- to 25-cm-long terminal twigs were collected from two sides of the tree, and the number of diseased and healthy leaves was counted. Data were analyzed statistically using ANOVA and mean separation procedures of SAS 9.4.

Evaluate application timing of selected treatments. A field trial on Arbequina olive was established with applications of selected fungicides on 2-8-18 (in combination with 2% Omni Oil) or 10-19-18. Disease will be evaluated in the spring of 2019.

Submission of fungicides to the IR-4 program. Based on the results of our efficacy trials on peacock spot (see below) and in coordination with the research project on the management of the newly described Neofabraea and Phlyctema diseases of olive in California (project leader is F. Trouillas, funded by OOCC), fungicides were identified that are potentially registerable, have different modes of action, have low resistance potential, and are efficacious against the three diseases. Additionally, registrants of each fungicide were contacted for approval for the proposed usage on olive and proposed labels were prepared. Subsequently, an IR-4 nomination was made based on the proposed usage (rates, timing, etc.) and IPM compatibility. An emergency registration was also requested and coordinated with OOCC. The PI of this COC project traveled to the IR-4 Food Use Workshop in St. Louis, MO, in September 2018 to defend the nominations.

RESULTS AND DISCUSSION

Evaluate the performance of new and older fungicides in field trials. Among the two trial sites where fungicides were applied for evaluation of their efficacy against peacock spot, the disease only developed in the orchard with cv. Manzanillo that is considered more susceptible to the disease. Typical symptoms were present on leaves. On untreated control trees, 21% of the leaves were diseased. Among treatments evaluated in this trial, only Inspire significantly reduced the disease (10.1% incidence) compared to the control (Fig. 1). A numerical reduction in incidence was observed for Ziram, Captan, and Champ. These results are encouraging because application timings in the first year of the study were done late (Dec. 20, 2017) due to the approval of this project in early December of 2017. At this time, major infection periods likely had already occurred, and protective treatments no longer could inhibit disease development. The efficacy of Inspire in these late applications can be attributed to its local systemic and post-infection activity. Because most infections of the peacock spot pathogen occur in the fall, the late-winter applications in early February 2018 may not have been as effective. Applications at these trial sites are being continued, and in 2018, a fall application was done in mid-October. Disease will be evaluated again in the spring of 2019. Thus, in these 1.5- to 2-year-long trials, the pathogen will go through its complete disease cycle, and with two annual applications, the efficacy of these treatments can be most accurately established.



Evaluate application timing of selected treatments. A field trial was established with spring and fall timings, and results are pending in spring of 2019.

Evaluate new fungicides for their in vitro activity. Fungal isolations from diseased olive leaves were attempted but were not successful when done in the springtime. The pathogen is difficult to isolate because it is very slow growing and easily overgrown by contaminating micro-organisms. We will repeat our isolations in the fall of 2018 when sporulation typically occurs on leaves. Isolates will then be evaluated for their in vitro sensitivities to selected fungicides.

Submission of fungicides to the IR-4 program. Three fungicides were nominated to the IR-4 program in 2018: ziram (Ziram 76WDG), difenoconazole/cyprodinil (Inspire Super), and thiophanate-methyl (Topsin-M). Ziram and Inspire Super were approved for residue trials at the

National Food Use Workshop in Sept. for registration on olives. Strong support was provided based on the after-harvest and winter season usage with expected zero to limit-of-detection residues on the crop in the following harvest season. Ziram is a FRAC Code M3 whereas Inspire Super is a FRAC Code 3/9. Thus, integration of multi-site modes of action for both products was also established as an effective anti-resistance strategy. Topsin-M was not accepted due to a low probability of registration because of the EPA Re-registration Eligibility Decision concerning its human safety and the potential for selection of resistance in the *F. oleaginum* pathogen population.

2019 RESEARCH PROPOSALS FOR THE CALIFORNIA OLIVE COMMITTEE

TOPIC	LEADERS	AMOUNT
Canopy management, tree hedging and topping to optimize yield	Rich Rosecrance	$31,075
Evaluation of new chemistries to control Olive Fruit Fly	Debra Keenan	$25,000
Managing Alternate Bearing in Olive with PGRs and Pruning	Carol Lovatt Elizabeth Fichtner	$21,570
Development of a standard analytical method for acrylamide in California-style black ripe olives	Selina Wang	$75,241
Impact of processing variables on acrylamide formation in black ripe olives	Selina Wang	$83,768
Control of overwintering olive fruit fly using insect pathogenic fungi	Frank Zalom Joanna Fisher	$19,678
Epidemiology and management of olive knot caused by Pseudomonas savastanoi pv. savastanoi	J. E. Adaskaveg	$31,650
Management of foliar diseases of olive (peacock spot)	J. E. Adaskaveg	$15,000
Southern San Joaquin Valley Olive Fruit Fly Monitoring Project	Jim Stewart	$6,400
Sacramento Valley Olive Fruit Monitoring Project	Ernie Simpson	$6,500
Total		$315,882
* budget estimate; actual budget pending on results		

CALIFORNIA OLIVE COMMITTEE

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Workgroup/Department: Olive / Plant Sciences College of Agriculture, CSU Chico

Project Year 2019 Anticipated Duration of Project: 4 of 4 years

Project Title: Canopy management, tree hedging and topping to optimize yield

Project Leaders:

Rich Rosecrance, Professor, California State University, Chico. College of Agriculture, 400 West First Street, Chico, CA 95929-0310: rrosecrance@csuchico.edu

William H. Krueger: Glenn County Farm Advisor (Emeritus): whkrueger@ucanr.edu

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

Daniele Lightle: Glenn County Farm Advisor: DLightle@ucanr.edu

Cooperating Ranches and People:

Erik Nielsen Enterprises Inc. 4453 Co Rd O, Orland, CA 95963

Dennis Burreson, Musco Olives, 17950 Via Nicolo, Tracy, California 95377

Commodity: Olive Relevant AES/CE Project No.

Year Initiated: 2016 Current Funding Request: **31,075.00**

Problems and Significance:

Mechanical Hedging

Mechanical hedging and topping can be important tool in improving harvest efficiencies by affecting return bloom, helping to maintain trees in their allotted space and reducing hand pruning costs. Typically, hedging and topping result in smaller and more compact trees. Smaller trees will facilitate hand harvest by obviating the need for tall, cumbersome ladders and likely increasing the number of bins harvested per hour. Picking crews have repeatedly commented that they prefer to harvest from mechanically hedged and topped trees than from traditionally pruned trees (Louise Ferguson, personal communication). In oil olive orchards, mechanical hedging has resulted in increased harvest efficiency and reduced alternate bearing (Charlie

Garcia, California Olive Ranch, personal communication). However, timing of mechanical hedging is critical for optimal yields. Hedging too late in the season may not provide enough time for new shoots to grow and flower buds to initiate. Earlier work that we conducted on 'Arbequina' oil olives indicated that shoot growth that occurred after early July did not produce flowers the following year. Whether 'Manzanillo' olives will behave the same is unknown. Hedging too early in the season can cause extensive vegetative growth at the expense of fruit growth. Thus, finding 'the sweet spot' for the timing of mechanical hedging is important to maximize and help regulate yields.

Mechanical Topping

Unlike hedging, mechanical topping does not reliably produce a crop on shoots that grow in response to the topping. Our trials have demonstrated that topping produced vigorous growth with limited fruit and resulted in two problems: 1) The limited fruit in the upper canopy ripened sooner than the rest of the crop, producing overripe fruit that decreased grade and value by 25%, and 2) Vigorous vegetative growth that can shade fruitwood and decrease yields, even when the tree is topped every other year. The solution appears to be to top the tree annually with a gabled cut to eliminate this overly vigorous growth and overripe fruit.

Optimizing Tree Light Interception at different tree heights, and latitudes

A program that evaluates light interception at different tree heights, row spacings, and latitudes has been developed by David Connor in Spain. This program was developed to help determine optimal tree spacing and height to maximize light interception at different latitudes. This program will assist us in our hedging and topping treatment to increase light interception and yield.

Fruit Nutrient Removal Calculator

Significant quantities of nitrogen, phosphorus and potassium are removed by harvested portions of fruit crops. Thus, the nutrient removal rate is an important consideration for making fertilizer recommendations. Inadequate fertilization and/or nutrient imbalance can prevent growers from achieving desired fruit yields and quality. Recently, we developed a macro- and micro-nutrient removal calculator for 'Arbequina' oil olives (Figure 1). Oil olives, however, are smaller and have a greater pit to flesh ratio than 'Manzanilla' table olives, which influence fruit nutrient content. An online fruit nutrient removal calculator needs to be developed for table olives.

Progress to Date:

Nickels Soil Lab

We initiated the trial at the Nickels Estate in Arbuckle in late April 2016 as a randomized complete block design with 3 treatments and 4 replicates. The treatments were: a) 10 foot topping, b) 13 foot topping and c) no topping where the primary scaffolds were cut with loppers to outside laterals at approximately 13 feet. All trees were topped on April 25 followed by hand pruning on May 26 and 27 to thin out the tree canopy. We measured the time it took for 2 pruners to prune the 10 data trees in the center row of the 30 tree plots in all replicates of all treatments to estimate pruning costs. The 10 foot topping treatment removed significant amounts of wood and produced shorter statured trees. Trees were harvested on October 1, 2018 and samples were taken to Musco Olive to evaluate fruit size and crop value,

Pruning costs and crop yields are presented in Table 1. Trees that were topped at 10 feet resulted in lower pruning costs likely because the canopies were smaller and close to the ground than trees pruned at 13 foot. No significant differences ($p < 0.05$) were found between olive yields in 2016, 2017, or 2018; however, trees topped at 10 feet produced lower cumulative yields than trees topped at 13 feet and the non-topped control. Trees topped at either 10 or 13 feet produced a higher value crop (in terms of price per ton) than the control trees. Examination of the grading sheets indicated that topping increased the percentage of medium and large fruit compared with the untopped trees. We will be evaluating how topping affect pruning costs, return bloom, and yields in 2019

Nielsen Trial

No hedging occurred in 2018, however, tree canopies were measured and yield ratings were determined. Tree yields were very low in 2018 across all treatments, making it difficult to draw firm conclusions. In 2018, trees that were hedged in earlier in the year of 2017 yielded more than trees hedged later in the year (Table 2). Severe hedging in 2016 decreased yields in 2016 and 2017. In 2018, trees that produced poorly in 2017 improved significantly in 2018. Indeed, 40% of the variation in 2018 yields could be attributed to crop yields in 2017 (data not shown). Moderate hedging, however, reduced these large swings in yield from one year to the next, thus reducing alternate bearing. It will be interesting to see how 2016 hedging treatments affects yields in 2019, which should be a heavily cropping year.

Burrison Trial

In Spring 2017, we initiated a trial on trees planted in 2009 at Heath Burrison's orchard. The orchard is a north-south planting with a 12 in row by 18 foot between row spacing. The trial was set up as a factorial design with four hedging dates and two canopy sides (east or west) and replicated 5 times. The 10-tree plots were hedged on February 23, April 3, May 1, and June 28, 2018. Trees tend to grow slightly more on west-facing canopies than on canopies facing east. Thus, we want to evaluate the effect of canopy orientation on yield and canopy growth.

In 2018, trees produced few flowers, even fewer fruit, and very low yields. Inflorescence ratings were conducted on May 21, 2018. Time of hedging the previous year did not affect inflorescence rating (data not shown). Trees were not harvested in 2018 because yields were so low. Yields were rated prior to when the trees would have been harvested using a scale of 0 to 10 where 0 represented no crop and 10 represented and extremely heavy crop. No differences in yield were noted in 2017 or 2018 (Table 3); however, there was a trend for hedging earlier in the season producing more crop.

Hedging early in the spring also increased canopy growth (Table 3). Trees hedged in February/March and April produced greater canopy diameters than from trees hedged in June. Hedging earlier in the season allowed more time for current year shoots to regrow. Hedging breaks apical dominance and can increase canopy regrowth. We evaluated the effect of the time of hedging on canopy growth on the cut and uncut side of the tree. Hedging in February/March produced a larger canopy in the cut vs the uncut side of the tree. This indicated that hedging stimulated growth to such an extent that after being cut, shoots grew farther out in the tree row than shoots from the uncut side of the tree. Hedging later in the season, however, did not provide enough time for the canopies to grow, resulting in smaller sized canopies in the cut vs uncut side of the tree.

Olive Nutrient Removal Calculator

At fruit maturity, fruit samples were collected from eight orchards up and down the state. Fruits dried, grown, and analyzed for maco- and mico-nutrients at Dellavalle Labs, Fresno, California. We will use these data and data collected in 2017, 2018, and 2019 to develop a nutrient removal calculator.

Objectives:

We propose to:

1. Investigate the effects of timing of mechanical hedging on return bloom, yield on mature trees. The objective is to determine the optimal timing of hedging for hedgerow plantings for generating a 5-ton or more per acre annual average crop.
2. Compare the effects of a mechanical pruning program that incorporates annual topping at two different tree heights to controlling the tree height. All of the treatments would receive an every other row middle hedging. The objective is to determine the optimal hedgerow height for generating a 5-ton per acre annual average crop that can be produced with mechanical pruning. This data could then be used to evaluate the program for determining optimum tree height for hedgerow plantings.
3. Develop a web-based fruit nutrient removal calculator for 'Manzanillo' table olives

Experimental Procedures:

Experiment 1: Mechanical Hedging (Erik Nielsen's and Heath Burreson's orchard)

Hypothesis: optimal timing or mechanical hedging will not decrease yield and will facilitate mechanical harvesting.

Overall Objective: to determine the optimal timing of mechanical hedging for table olive productivity and fruit quality.

2017 Objectives:

I. Hedge Trees Monthly
II. Evaluate effect of pruning treatments on shoot growth, and return bloom and quality: perfect versus imperfect flowers.
III. Evaluate effect of pruning treatments on yield and fruit quality.
IV. Determine optimal timing of hedging treatment to facilitate high quality fruit production and return bloom.

Materials and methods:

Experimental Design:
Randomized complete block of four replications.

- Treatments: Evaluate timing by hedging the south side of the tree at monthly intervals starting in April and ending in August. Twelve trees from 4 tree rows will be hedged each month.
    - Hedging will aim to remove about 50 percent of the new growth
    - middle 10 trees of each treatment will be the data trees

- Data Collection:
- 100 fruiting and 100 non-fruiting branches will be tagged after hedging treatment
- Shoot growth will be measured at the end of the seasons
- At bloom the following season, flowering intensity (inflorescences per branch) will be determined from the tagged branches
- Following bloom, fruit set will be determined
- Measure fruit removal and yields following mechanical trunk shaking in the hedged trees.

- Data Analysis:
    - The following relationships will be evaluated statistically for the trial:
        - Effect of time of hedging on shoot growth in both fruiting and non-fruiting shoots.
        - Effect of time of hedging on flowering the next year from fruiting and non-fruiting shoots
        - Effect of time of hedging on fruit set the next year from fruiting and non-fruiting shoots
        - Evaluate the effects of the treatments of fruit removal and yields following mechanical trunk shaking.

Experiment 2: Mechanical Topping

Materials and Methods:
Experimental Plot: Nickels Estate - 2 acre 'Manzanillo' orchard established in 2002.

Hypothesis: mechanically topping hedgerow olive orchards will not decrease yield and will reduce hand harvesting costs by producing shorter statured trees.

Overall Objective: to determine the optimal row height for table olive productivity and fruit quality at a 12 X 18' orchard spacing (202 trees/acre) and develop the formulas for applying this information to different latitudes and orchard spacing.

2017 Objectives:

- **V.** Apply two different tree height pruning treatments and compare to controlling tree height with hand pruning
- **VI.** Install sunlight exposure monitoring cameras
- **VII.** Evaluate effect of pruning treatments on bloom quality: perfect versus imperfect.
- **VIII.** Evaluate effect of pruning treatments on yield and fruit quality in upper and lower canopy at harvest.
- **IX.** Correlate hours of sunlight exposure with fruit yield and quality.

Materials and methods:

Experimental Design:

Randomized complete block of four replications: map attached

- Treatments: three pruning treatments of three, 10 tree rows
  - topped at 10 and 13 feet in February 2017 and compared to pruning to lateral branches at 13 feet using thinning cuts
  - middle row of each treatment will be the data row
  - alternate row side hedging treatments will be applied

- Data Collection:
- Five photosynthetically active radiation (PAR) monitors will be positioned on a 20' pole and installed along a transect from the trunk to the top of the tree. Measurements will be taken every 5 minutes and compared with the full sun measurement. Fruit size at each position will be determined.
- A late-season mid-day light interception measurement will be done to determine the percentage of light each treatment is intercepting.
- Trees will be harvested and fruit quality will be assessed from samples taken from the upper and lower tree canopy.
- Yields will be compared with the MatLab program that predicts optimal tree size and spacing to maximize light interception.

- Data Analysis:
  - The following relationships will be evaluated statistically for the east and west sides, within the three pruning treatments:
    - Effect of pruning treatment on intensity of inflorescence and shoot growth
    - Effect of pruning treatment on total yield and fruit quality; size and color
    - Correlation of each of the above parameters with total hours of light exposure through the season from bud swelling through harvest.

Olive Nutrient Removal Calculator

At fruit maturity, fruit samples will be collected from eight orchards up and down the state. In 2018, we collected fruit samples during an off-year. We will compare crop nutrient removal levels from fruit during on- and off-years. Fruits will be dried, grown, and analyzed for maco- and mico-nutrients. We will use these data to develop a nutrient removal calculator. Growers will input their olive yield and this web-based tool will determine the amount of macro- and micro-nutrients removed in the harvested crop, similar to what is shown in Figure 1.

Anticipated Outcomes:

Hedging and Topping Treatments

The goal of these experiments are to determine the most effective timing of canopy hedging and topping height to ensure return bloom, maximize yields, and minimize excessive vegetative growth. We anticipate that hedging and topping treatments can producer similar yields to hand-pruned trees with lower labor costs. We also anticipate the hedging and topping will significantly reduce alternate bearing.

Light Measurements in topped, hedged, and control trees

The goal of these experiments are to determine how canopy management with mechanical topping and hedging affects total hours of canopy light exposure and therefore flower production, fruit yield and quality. The ultimate goal is to demonstrate how to calculate the optimal tree height for moderate density orchards at different latitudes.

Olive Nutrient Removal Calculator

The 'Manzanillo' nutrient removal calculator will estimate nutrient removal of macro- and micro-nutrients. Removal data for the 'Manzanillo' will be incorporated into the calculator found at http://www.csuchico.edu/~rrosecrance/Model/OliveCalculator/OliveCalculator.html

BUDGET REQUEST –

Budget Year: 2019-2020 Funding Source: COC

Personnel:

Rich Rosecrance, California State University, Chico, professor. data collection and entry, harvest support. (~87 hrs @ $69/hr)	6,100.00
Student (summer and fall; 375 hours at $12/hr)	4,500.00
Fringe @ 8.8%	932.00
Independent Contractor - Bill Krueger: Glenn County Farm Advisor (emeritus): Technical Support - data collection and entry, harvest support.	6125.00

Sub 1	**17,657.00**
Equipment Supplies & Expenses:	
Light measurement, timelapse cameras, field scale equipment	3,600.00
Sub 2	**3,600.00**
Pruning and Harvesting Costs: (based on previous year's cost)	
Hand pruning, brush shredding: Nickels Estate	1,500.00
Mechanical harvest (ENE Inc.) at Nickels Estate:	1,500.00
Hand harvest at Nickels Estate (post mechanical harvest)	1,000.00
Nutrient Analyses (18 samples x $56/sample)	1,000.00
Miscellaneous harvest supplies: water, gloves, tarps, buckets	1,000.00
Sub 3	**6,000.00**
Experimental Travel Costs:	
Travel support for plot set-up, data collection, harvesting. (8 months X 4 RT/month @ 120 miles/trip X .55/mile)	2,338.20
Sub 4	**2,338.20**
Facilities and Admin @ 5%	**1479.80**
TOTAL BUDGET	**31,075.00**

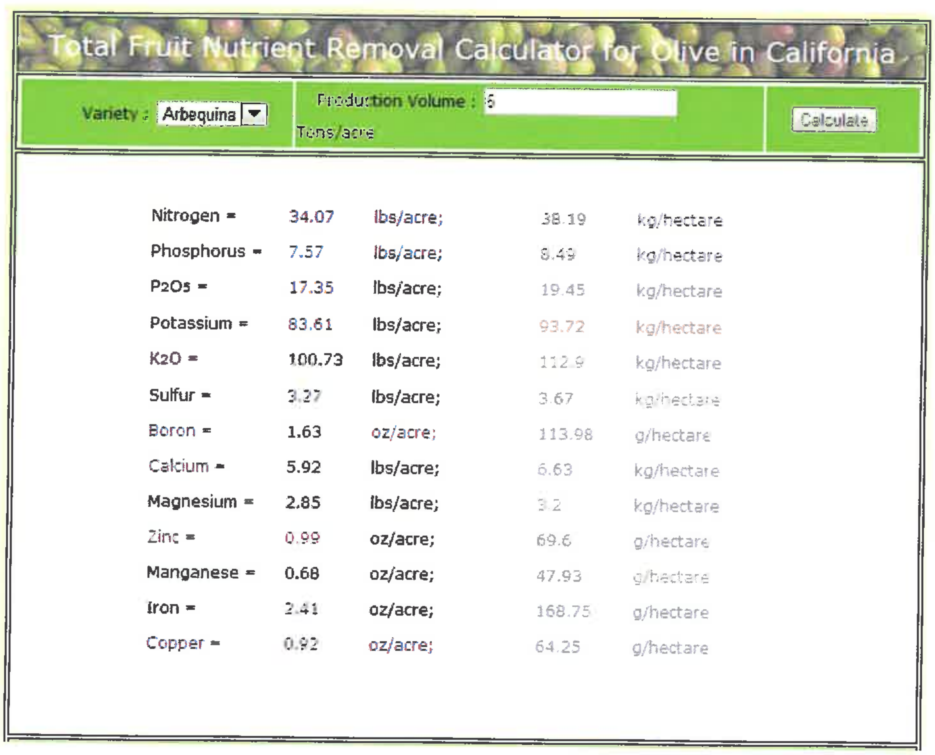
Scope of Work

Dr. Richard Rosecrance:
Responsible for overall coordination of the project, applying pruning treatments, executing harvest trials, developing fruit nutrient calculator, data collection and analysis and writing final report.

Bill Krueger, Louise Ferguson, and Dani Lightle: Responsible for assisting in the mechanical pruning treatment in Orland and Nickels trial and co-coordinator of harvesting the trials.

External Contractors: contracts to be secured after funding.

Pruning Contract at Nickels Soils Laboratory: Colusa, California
Hillary Nielsen Porter
ENE Inc.
4453 County Road O
Orland CA 95963
ENE@EneInc.com
Office: 800-844-9409
FAX: 530-865-4845



Nitrogen =	34.07	lbs/acre;	38.19	kg/hectare
Phosphorus =	7.57	lbs/acre;	8.49	kg/hectare
P2O5 =	17.35	lbs/acre;	19.45	kg/hectare
Potassium =	83.61	lbs/acre;	93.72	kg/hectare
K2O =	100.73	lbs/acre;	112.9	kg/hectare
Sulfur =	3.27	lbs/acre;	3.67	kg/hectare
Boron =	1.63	oz/acre;	113.98	g/hectare
Calcium =	5.92	lbs/acre;	6.63	kg/hectare
Magnesium =	2.85	lbs/acre;	3.2	kg/hectare
Zinc =	0.99	oz/acre;	69.6	g/hectare
Manganese =	0.68	oz/acre;	47.93	g/hectare
Iron =	2.41	oz/acre;	168.75	g/hectare
Copper =	0.92	oz/acre;	64.25	g/hectare

Figure 1. Nutrient removal calculator for 'Arequina', 'Arbosana', and 'Koroneiki' olive oil cultivars. Data will be collected to include 'Manzanillo' in the fruit nutrient removal calculator.

Table 1. Relationship between topping height and pruning costs, 'Manzanillo' olive yields, fruit value, and return at Nickels Estate.

Treatment	Pruning Costs 2016* ($/a)	Pruning Costs 2017 ($/a)	Pruning Costs 2018 ($/a)	Pruning Cost Cumulative	Yields (t/a) 2016	Yields (t/a) 2017	Yields (t/a) 2018	Yields Cumulative (t/a) (2016 - 2018)	Average Value ($/t 2016-2018)
Topped at 10'	500 a**	237 a	219	956 a	2.01	3.78	2.03	7.82 b	1360 a
Topped at 13'	885 b	317 b	275	1477 b	3.57	5.27	2.49	11.33 a	1344 a
Control	930 b	304 b	320	1554 b	4.65	4.37	3.44	12.46 a	1310 b
P value	0.045	0.026	Ns	0.042	0.091	0.241	Ns	0.050	0.007

*pruning costs based on time needed to prune the trees multiplied by $11/hr. not sure that would be enough now, probably should be minimum wage plus 40% for overhead
**different letters in the same column indicate significance $p < 0.05$.

Table 2. Nielsen trial, Effects of hedging date and severity of hedging on 'Manzanillo' olive yields.

Hedging Date	Severity of Hedge*	Yield Rating 2016	Yield Rating 2017	Yield Rating 2018
No Hedge	NA	6.5 a	7.0 ab	3.6 b
24-May-16	Moderate	7.2 a	7.4 a	2.5 c
27-Apr-16	Moderate	7.1 a	7.1 ab	2.6 c
27-Apr-16	Severe	3.7 b	6.7 ab	2.4 c
15-Jul-16	Moderate	6.1 ab	6.3 ab	3.4 bc
15-Jul-16	Severe	5.5 ab	5.3 bc	3.6 b
1-Mar-17	Moderate		5.1 bc	4.1 ab
3-May-17	Moderate		4.1 c	4.2 ab
29-Mar-17	Moderate		4.3 c	3.8 ab
3-May-17	Moderate		5.1 bc	3.6 b
P value		.041	0.0001	.046

* Moderate = approximately 8.5 feet from trunk; Severe = approximately 6.5 feet from trunk.
Different letters in the same column indicate significance $p < 0.05$

Table 3. Burreson trial, Effects of hedging date on 'Manzanillo' olive yields and canopy growth.

Cut Date	Yields (t/a)	Yields Ratings*	Canopy Diameter (ft)		Growth in cut vs non-cut side of tree** (ft)
	2017	2018	2017	2018	2017
Feb/Mar	8.9	1.5	9.2 ab	9.6 b	0.43 a
April	9.1	1.5	9.1 ab	8.9 bc	-1.1 b
May	7.9	1.3	9.0 ab	8.6 bc	-1.7 b
June	7.8	1.3	8.2 b	8.3 c	-2.1 b
Control	9.4	1.5	10.3 a	12. 4 a	
p-value	0.17	0.51	0.004	0.002	0.0006

*rating scale 0 to 10, 0 = no crop and 10 = extremely heavy crop.
**Difference in growth between cut and not cut side of the tree.
Different letters in the same column indicate significance $p < 0.05$

PRIMARY PI SIGNATURE PAGE: UNIVERSITY OF CALIFORNIA

Originator's Signature	Date
Department Chair/County Director	Date
Liaison Officer	Date

Project Year: 2019

Anticipated Duration of Project: 2-3 years

Principle Investigator: Debra Keenan, Research 2000

Project Title: Evaluation of new chemistries to control Olive Fruit Fly

Justification Background

The Olive Fruit Fly, *Bactrocera oleae*, has become a serious pest in olives. It was first seen in 1998 in Los Angeles. It quickly spread to the olive growing regions and has become a pest. The olive fruit fly causes a huge economic threat to the olive growers in the state. The larvae feed on the inside of the fruit. The larvae destroy the pulp and allow entry of secondary pests. The fruit rots and can cause the quality of the oil to degrade and cause the fruit to drop. Feeding damage can cause premature fruit drop and reduce fruit quality for both table olive and olive oil production. Large numbers of rotting fruit on the ground can create an unwelcome mess, especially in landscaped situations.

GF-120 NF Naturalyte Fruit Fly Bait, an organically acceptable product containing the biologically produced insecticide spinosad, recently has received registration for use on olives in California. GF-120 attracts olive fruit fly adults, which feed on the bait, and causes adult mortality. GF-120 is concentrated and needs to be diluted with water at 1:1.5 to 1:4 (GF-120 NF: water) before application. Follow label instructions for methods of dilution. GF-120 applications should commence when olive fruit fly adults are captured on the monitoring traps or at least 2 to 3 weeks before pit hardening. Repeat applications every 7 days until harvest when flies are captured on monitoring traps. GF-120 should be applied at a 2.5 to 7.5 ounce dilute spray per tree using a 1:1.5 dilution or at a 5 to 15 ounce dilute spray per tree using a 1:4 dilution with very large droplet size. Droplets should be 5 millimeters or more in size and uniformly dispersed around the tree. Other materials will be applied according to the label.

Data to Collect: Scout for adult flies that emerge from March to May and attack olives remaining on trees from the previous season. During early summer (June) as temperatures and day length increase and few mature fruit remain on trees, female flies do not lay eggs. Although few olives are present from the previous crop to host the egg laying, the adults remain active, and they may disperse to new locations such as citrus orchards or vineyards. By late June to the beginning of July as the new olive crop develops, females begin to lay eggs and are attracted to the fruit. Although eggs may be laid in small fruit, the larvae do not successfully develop until the ripening fruit grows to sufficient size. Eggs are laid just under the fruit's skin, often creating a dimple or brown spot. Will observe for the dimple or brown spot. The use of baited traps will be used to determine presence of the pest.

Research Objectives

1. Trap for the presence of the fruit fly. The most efficient trap for monitoring the olive fruit fly is the glass or plastic McPhail-type trap baited with torula yeast lures. Yellow sticky traps baited with sex-pheromone lures (attractive to male flies) and/or ammonium carbonate, ammonium bicarbonate food bait (attractive to both sexes) also are commonly used to monitor olive fruit fly populations, but these generally are less efficient than McPhail traps.
2. Apply materials to the olives for control of the olive fruit fly. Find new chemistries to control the pest. This will aid in resistance management.

Research 2000

Olive fruit fly control. Efficacy program to combat resistance.

Protocol Olive ID:FF OC
Trial ID:OFF-OC
Project ID:

Location:

Trial Year: 2019 (year 2)

By:Debra Keenan Research 2000

Study Director: COC

Trt	Treatment	Rate	Appl	Volume	Mix unit
1	Untreated				
2	GF120	1 fl oz/item	ABCDEF		
3	Danitol	16 fl oz/a	AB	100 GAL/AC	20 gallons
4	Harvanta FB GF 120	16 fl oz/a 1 fl oz	ABCDEF	100 GAL/AC	20 gallons
5	Harvanta FB GF 120	24 fl oz/a 1 fl oz	ABCDEF	100 GAL/AC	20 gallons
6	Assail FB GF 120	8 fl oz/a 1 fl oz	ABCDEF	100 GAL/AC	20 gallons
7	Avaunt FB GF 120	4 oz/a 1 fl oz	ABCDEF	100 GAL/AC	20 gallons
8	Avuant FB GF 120	5fl oz/a 1 fl oz	ABCDEF	100 GAL/AC	20 gallons
9	Minecto Pro FB GF 120	12 fl oz/a 1 fl oz	ABCDEF	100 GAL/AC	20 gallons
10	Sivanto FB GF 120	14 fl oz/a 1 fl oz	AB	100 GAL/AC	20 gallons
11	Lorsban FB GF 120	4 fl oz/a 1 fl oz	ABCDEF	100 GAL/AC	20 gallons
12	GF120 Harvanta Asail Danitol	1 fl oz/item 16 fl oz/a 8 fl oz/a 16 fl oz/a	ACE B D F	 100 GAL/AC 100 GAL/AC 100 GAL/AC	 20 gallons 20 gallons 20 gallons

3. Find alternatives to the current program. This will allow for resistance management and allow for the olive growers to have more tools to control the olive fruit fly.

4. Provide efficacy data to support registration of new products for the control of olive fruit fly.
5. Updated treatment list from 2018, program will have GF 120 applications if flies are not present.

Benefits to the industry.

Management of Olive Fruit Fly is very labor intensive. Currently growers do not have a lot of tools for the control of this pest. Researching new tools and new chemistries will help the growers. New tools will give them more ways to control this pest. The olive fruit fly poses a severe economic threat for the state's commercial olive growers. By finding new ways to control the pest industry may be able to register these products. New tools will allow for control of the pest and resistance management. The most effective strategy to combat insecticide resistance is to do everything possible to prevent it occurring in the first place. Crop specialists recommend Insect Resistant Management programs as one part of a larger IPM approach covering three basic components: monitoring pest complexes in the field for changes in population density, focusing on economic injury levels and integrating multiple control strategies.

Budget Request

Item	Total budget
Set up, spray, and evaluate the list of materials in the proposal – site 1 with crop purchase	$ 12,500.00
Set up, spray, and evaluate the list of materials in the proposal – site 2 with crop purchase	$ 12,500.00
Total	$ 25,000.00

The costs include the data to be compiled in a format that is acceptable to Department of Pesticide Regulation. Also will help determine which insecticides that should be targeted for registration.

This is the second of three years. In the following years the materials that are favorable in the screening will be looked at in greater depth.

Principle Investigator: Debra Keenan 10-12-2018

CALIFORNIA OLIVE COMMITTEE

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year: 2019 Anticipated Period of Performance: 1 year; this is the second year of the necessary 2 crop-years to assess treatment effects on the cumulative yield of alternate bearing olive trees

Project Title: Managing Alternate Bearing in Olive with Plant Growth Regulators (PGRs) and Pruning

Project Leaders: Elizabeth Fichtner and Carol Lovatt

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

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

Cooperators: Kurt Schmidt, Lindcove Research and Education Center, 22963 Carson Avenue, Exeter, CA 93221; Phone: 559-592-2408, ext. 153; Email: krschmidt@ucanr.edu

Commodity: Olive Relevant AES/CE Project No.: 4556-H

Year Initiated: 2018 Anticipated Duration of Project: 2 crop years 2019 request: $21,570

Problems and Previous Research Accomplishments: ***Problem.*** 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, as well as forest species (where it is called "masting"). For tree fruit crops, alternating high and low yields cause significant economic problems. In ON-years, trees produce a large number of small size fruit with reduced commercial value. In OFF-years, trees produce large fruit, but in some cases a significant proportion of the fruit are too large and have reduced economic value, further exacerbating the problem that there are too few commercially valuable fruit in OFF-crop years to provide growers with a good income. For olive, the ON-crop takes longer to mature, attain size and accumulate oil. The delayed harvest further reduces floral intensity the following spring. It is important to note that the lack of fruit in the OFF-crop year, if more or less industry-wide, has a negative economic impact on every step in the production chain from farm to consumer, including orchard management, harvesting, packinghouse operation, manufacture of value-added products, marketing, and consumer prices, which jeopardizes the stability and sustainability of tree-crop commodity-based industries such as olive. Since the major factor initiating AB is an extreme climate event (excessively high or low temperature, excessive winter rain causing soil hypoxia etc.) that ultimately reduces yield and initiates AB, there is a reoccurring need for a management strategy to mitigate the severity of AB. ***Previous Research Accomplishments.*** Our research confirmed that the ON crop of fruit inhibits summer vegetative shoot growth, reducing the number of nodes that can bear floral buds the following spring (Sibbett, 2000) and was the first to document that the ON crop causes bud abscission of existing putative floral buds through harvest, inhibits the transcription of genes necessary for flower development even after harvest, and reduces spring bud break the year following the ON-crop (Fichtner and Lovatt, 2018; Fichtner et al., 2017). For ON-crop trees, the severity of these effects is greater for bearing shoots, the majority of shoots on ON-crop trees, than non-bearing shoots. Further, plant growth regulator (PGR) treatments, which increase return bloom on non-bearing shoots, are not effective on bearing shoots. Only non-bearing shoots on ON-crop trees, which are in the minority, contribute a significant number of inflorescences to spring bloom following the ON-crop year (Fichtner and Lovatt, 2018; Fichtner et al., 2017). Taken together, these results provide evidence that increasing the number of non-bearing shoots on ON-crop trees is required to increase return bloom and yield the following year. ***Current accomplishments.*** Our current research is testing the efficacy of a PGR chemical flower thinning treatment (naphthaleneacetic acid [NAA], ALCO® Olive Stop™; AMVAC Corp., already registered for use on olive) at full bloom and mechanical pruning (hedging) after fruit set. Each treatment is applied to one side of the tree one year and the other side of the tree the next year, with topping each year. The goal is to equilibrate yield at a production level equaling 60% to 70% of the ON-crop yield annually to produce a greater 2-year cumulative yield than results from the ON-/ OFF-crop cycle of alternate bearing trees over the same 2-year period. Thus, the first objective is to even out the 2-year cumulative yield of alternate bearing 'Manzanillo' trees, which presently produce 80% of the 2-year yield in the ON-crop year and only 20% in the OFF-crop year. Our goal is for 50% of the 2-year cumulative yield to be produced each year of the 2-year cycle. Once achieved, the second objective is to maintain annual yield at 60% to 70% of the average yield for the ON-crop year in the orchard in order to increase overall yield, maintain commercially valuable fruit size, increase the percentage of green fruit, and to increase income over multiple successive cropping years. Thus far, using NAA to reduce inflorescence number or pruning to reduce fruit number on one side of the tree starting in the ON-crop year and repeating the treatment on the opposite side of the tree the following year, we have achieved 2-year cumulative yields that are distributed ~60% in the ON-crop year and ~40% in the following OFF-crop year, with no significant reduction in 2-year cumulative yield. Our results this year identified the potential to further increase yield in year 2, the year following the ON-crop year, by keeping the contribution of the OFF-crop side of the NAA-treated and pruned trees and delaying the treatment of the second side of the tree an additional year, i.e., by applying the treatments every other year, instead of annually. Thus, in 2019, we will also test the efficacy of pruning one side of the tree in one year and then the other side of the tree every other year rather than annually, again initiating the treatment in the ON-crop year.

2019 Objectives and Anticipated Outcomes: ***2019 Objectives.*** The research proposed for 2019 has three goals: Goal 1- to even out AB so there is a good crop annually by switching crop production from one side of the tree to the other side of the tree annually or every other year; Goal 2 - to sustain production each year at a level equal to 60% to 70% of the average ON-crop yield for an orchard, which will improve fruit size and the proportion of green fruit (both aspects of fruit quality are crop load dependent), so growers have a stable and good income annually; and Goal 3 - to provide growers with a means to mitigate AB when it reoccurs. These goals will be achieved using 'Manzanillo' olive trees in a block, which includes 'Barouni' olive trees as the pollenizer planted at a ratio of one to ten, at the Lindcove REC in Exeter, CA, to meet three objectives. All trees are topped to maintain uniform tree height. ***Objective 1*** – (a) To reduce crop load (total number of fruit per tree) by removing inflorescences with a foliar application of the plant growth regulator (PGR) NAA (ALCO® Olive Stop™; AMVAC Corp., already registered for use on olive) applied at full bloom at the manufacture's suggested rate; and (b) removing fruit by pruning (mechanical hedging) one side of a second set of ON-crop 'Manzanillo' olive trees.

Thereafter, the treatments are used to remove inflorescences or fruit on alternating sides of the tree annually. ***Objective 2*** – To apply the treatments to the second side of the tree every other year to obtain the crop contributed by the OFF-side of the tree in order to further increase total yield in the year following the ON-crop (year 2) (this objective was initiated in 2018, so in 2019 we will be able to assess the contribution the OFF-crop side of the tree to total yield in year 2 and thus to 2-year cumulative yield). ***Objective 3*** – To compare the efficacy of using NAA to remove inflorescences versus pruning (hedging) to remove fruit and to compare applying each treatment to the other side of the tree annually versus every other year in order to provide growers with a strategy to mitigate alternate bearing each time it is initiated. The final strategy will be one that succeeds in achieving annual yields that are significantly greater than OFF-crop yields and equal to 60% to 70% of the average ON-crop yield for an orchard with the two year cumulative yield distributed equally in each year of a 2-year cycle. **Please note the following benefits of the proposed treatments**. (1) By chemically thinning only half of the tree with NAA in any given year, the impact of over-thinning on yield if a heat wave occurs is reduced. (2) In our experiment, pruning is delayed to after fruit set to enable growers to evaluate the crop set by their trees before deciding how much fruit to remove. This allows a grower to tailor the degree of pruning to accommodate years with a poor fruit set in spite of a heavy bloom. (3) Reducing crop load on only one side of the tree reduces the negative effect of any subsequent adverse effects on fruit set and final yield over treating both sides of the tree in a given year. (4) Since the treatments increase the number of non-bearing shoots per tree, they will improve the efficacy of PGR treatments that increase summer vegetative shoot growth and spring bud break to increase floral intensity following the production of the ON crop and also the efficacy of PGR treatments designed to improve fruit set or size or delay fruit blackening (Fichtner and Lovatt, 2018; Fichtner et al., 2017). (5) In addition to shifting fruit into more commercially valuable size categories, evening out alternate bearing will ensure a greater proportion of green fruit (reduced proportion of black and partially black fruit) at harvest, which in some years is significant (2017). Both fruit size and percent green fruit are crop load-dependent, with OFF-crop trees producing fruit that are too large and have a greater proportion of black or partially black fruit.

Anticipated Outcomes.

- We will have data on the impact of different degrees of fruit thinning on only half of the tree on total yield, which will enable us to estimate the impact that over-thinning due to a heat wave would have on final yield, i.e., we will have some indication of the risk associated with the application of chemical thinning agents to only half of the tree.
- We will learn whether NAA inflorescence removal supports better summer vegetative shoot growth and return bloom and return yield than fruit removal by pruning (hedging).
- We will learn whether delaying pruning (hedging) to after fruit set to give growers the opportunity to evaluate their potential crop load to make a decision to prune or not to prune or how severely to prune, which is especially important in years with poor fruit set in spite of good blooms, is efficacious or has negative consequences.
- Specifically, we will learn whether pruning at the end of June (versus May) is effective or too late for stimulating summer vegetative shoot growth to increase return bloom and yield.
- Through these comparisons, potential benefits can be verified, e.g., fruit removal in June before pit hardening increases floral bud retention and flowering, or potential problems can be identified, e.g., June pruning causes loss of carbohydrates resulting in poor shoot growth, small fruit size or too much shoot growth, leading to competition and small fruit size (these potential problems have not encountered thus far with the late June pruning).

- We will have data establishing the year-to-year variability in yield encountered using NAA to remove inflorescences versus pruning to remove fruit.
- We will have data to support or refute that reducing crop load on one side of the tree starting in the ON-crop year using the PGR thinning agent NAA or pruning (hedging) increases yield in the following OFF-crop year sufficiently to even out AB and provide growers with a good annual income the following year.
- The data will document whether reducing crop load on alternate sides of the tree should be done each year or every other year to sustain good yields and grower income annually.
- The harvest data will quantify the effect that removing fruit on one side of the tree and then other side annually or every other year has on average fruit size, fruit size distribution (pack out), proportion of black versus green fruit, and crop value.
- The data will document the potential efficacy of the treatments to reduce the severity of alternate bearing (Alternate Bearing Index; ABI) in 'Manzanilo' olive orchards in California
- We anticipate that one or more of the strategies being tested will successfully mitigate alternate bearing and increase 2-year cumulative yield and grower income and thus, will be a strategy that can be successfully employed starting with the ON-crop each time alternate bearing is initiated by an adverse climate event or cultural problem and results in an OFF crop, which is then followed by and ON crop.

Select References:

Chao, Y.Y. 2014. Alternate Bearing in Olive (*Olea europaea* L.). MS Thesis. University of California, Riverside, CA.

Fichtner, E., Lovatt, C.J. 2018. Alternate bearing in olive. Acta Hortic. 1199:103-108. doi:10.17660/ActaHortic.2018.1199.17

Fichtner, E.J., Y.Y. Chao, L. Ferguson, J.S. Verreynne, L. Tang and C.J. Lovatt. 2017. Repeating cycles of ON and OFF yields in alternate bearing olive, pistachio and citrus trees — *Different mechanisms, common solutions*. Acta Hort. (in review)

Pearce, S.C. and S.Dobersek-Urbanc. 1967. The measurements of irregularity in growth and cropping. J. Hort. Sci. 42(3):295–305.

Sibbett, S. (2000). Alternate bearing in olive trees. California Olive Oil News. 3(12),1

BUDGET REQUEST: (Carol J. Lovatt)

Budget Year: 2019 Funding Source: COC

Labor:	**(Line 1)**	**$6,287**
Salary: T Khuong @ $60,300/yr x 5% = $3,015; Lab Asst. 1 @ $16.80/hr. x 100 hr. = $1,680	$A	$4,695
Benefits: TK= $3,015 x 51% = $ 1,538 Lab Asst. 1 = $1,680 x 3.2% = $54	$B	$1,592
Subtotal 1	**Line 1 subtotal:**	**$6,287**
Supplies, Equipment:	**(Line 2)**	**$5,253**
Supplies: *(be specific. Examples include tape, tags, buckets, traps, safety, chemicals, etc)*	$C	$0
Equipment: (*be specific. Examples include balances, meters, devices, etc*)	$D	$0
Individual contractors: Recharge to Lindcove REC – use of olive orchard, irrigation, weeding, pruning, pest control, application of PGRs = $5,253 (actual under new rates; includes harvest)	$E	$5,253
Subtotal 2	**Line 2 subtotal**	**$11,540**
Travel:	**(Line 3)**	**$2,487**
Vehicle Use: 5 roundtrips to Exeter (520 mi x 5 = 2,600 mi x $0.6014/mi = $1,564; UCR vehicle Rental 10 days x $47.268/day = $473; $90/day per diem (Lindcove REC trailer, plus meals) for 1 person x 5 trips (1.5 days each) = $450	$F	$2,487
Meeting attendance: (*be specific. anticipated travel to meetings such as COC meetings, professional society meetings*)	$G	$0
Subtotal 3	**Line 3 subtotal**	**$14,027**
Subcontracts: Elizabeth Fichtner	**(Line 4)**	**$6,000**
Collaborator A: Elizabeth Fichtner	$H	$6,000
Subtotal 4	**Line 4 subtotal**	**$20,027**
UCR Total	**(Line 5)**	**$14,027**
UCR Overhead on $14,027 @ 11% IDC	**(Line 6)**	**$1,543**
(Total to primary PI – Carol Lovatt**)**	**(Line 7)**	**$15,570**
TOTAL BUDGET REQUEST	**Line 4+Line 7**	**$21,570**

PRIMARY PI SIGNATURE PAGE: UNIVERSITY OF CALIFORNIA

Carol J Lovatt 10/25/2018

Originator's Signature Date

Patricia Springer 10/25/2018

Department Chair/County Director Date

Liaison Officer Date

SUBCONTRACT BUDGET REQUEST: (*Elizabeth Fichtner*)

Budget Year: 2019

Funding Source: COC

Labor:	**(Line 1)**	**$4855.61**
Salary *(Junior Specialist at 7% FTE)*		$3495.76
Benefits *(38.9%)*		$1359.85
Sub 1		**$4855.61**
Supplies, Equipment:	**(Line 2)**	**$200.00**
Supplies: *(be specific. general field supplies (flagging tape, pruners, buckets, gloves. etc)*		$200
Sub 2		**$5055.61**
Travel:	**(Line 3)**	**$349.80**
Vehicle Use: (*Mileage from Tulare, CA to/from Modesto for COC meetings; 280 miles round trip @ $0.535/mile. Request partial funds ($200) toward attendance of Pomology Conference in Davis in March 2019.This is approximately 1/3 of the cost of attending the meeting; other costs would be contributed by walnut and pistachio accounts to share costs across main commodities that I serve.*		*$349.80*
Sub 3	**(Line 4)**	**$5405.41**
UCD/ANR/UCR Overhead @ 11%	**(Line 5)**	**$594.59**
Sub 4 (Total Subcontract)	**(Line 6)**	**$6000.00**

(Add Line 6 to primary PI budget in subcontract section 'H' and 'I')

SUBCONTRACT SIGNATURE PAGE: UNIVERSITY OF CALIFORNIA

Elizabeth [signature]	10/25/18
Originator's Signature	Date
[signature]	10/25/18
Acting Department Chair/County Director	Date
Liaison Officer	Date

Development of a standard analytical method for acrylamide in California-style black ripe olives

Problem and Significance:

Currently there is not a standard analytical method that has been approved by an independent standards-developing organization for measuring acrylamide in black-ripe olives. The absence of such a method has led to disparities in lab results and has contributed to confusion as to the accurate levels of acrylamide in black-ripe olives.

After acrylamide was discovered in foods in 2002, there were multiple studies in which different labs used their own analytical techniques to measure acrylamide in the same food samples. One study found that, depending on the sample, 10-14 out of 48 labs reported acrylamide values outside of the acceptable range (two z-scores from the median).[1] Even the range of statistically-acceptable values can be large; 836-1590 µg/kg was found acceptable for crispbread, 50-460 µg/kg for cocoa, 327-736 µg/kg for butter biscuit, and 3550-11020 µg/kg for contaminated mashed potatoes.[2,3] The labs in these studies used many different extraction and detection methods, which caused large variations in the results. The complexity of the matrix also affected the standard deviation of values between labs.[4] The results of these studies clearly demonstrated the need to develop standard analytical methods to measure acrylamide in different types of food products. Since then, there has been a lot of work in developing validated methods and certified reference materials for specific foods, primarily carbohydrate-based products.[8-10]

Analysis of acrylamide in foods can be a challenging task. Acrylamide is a polar compound and often gets extracted with other matrix components like salts and sugars. It also has a low molecular weight which, along with the presence of matrix interferences, reduces the sensitivity of mass spectrometry methods. Additionally, when analytes are present in low concentrations, it reduces the accuracy and repeatability of measurements. In black ripe olives, acrylamide is typically found at levels of 1 ppm or lower. Therefore, it is important that the method selected is accurate, sensitive and tailored to the type of food products.

The table olive industry would greatly benefit from a standard analytical method to ensure that values received by laboratories about their products are accurate, precise, reproducible and sensitive. Only one method has been developed specifically for analyzing acrylamide in black ripe olives. This method uses gas chromatography-mass spectrometry (GC-MS) with sample bromination.[5] Other liquid chromatography-mass spectrometry (LC-MS) methods without sample bromination have been used in olives, but have not been validated specifically for this matrix.[6,7] During previous studies, there have been inconsistencies in data between our lab (using LC methods) and another lab (using a GC method). It is unknown whether this discrepancy results from a difference in extraction methods, the use of sample bromination or the type of detector. To assess this problem, we will develop a study in which olive samples are extracted using the same method, and then analyzed with four different detection methods:

- Liquid chromatography with UV detection (LC-UV)
- Liquid chromatography with mass spectrometry detection (LC-MS/MS)
- Liquid chromatography with UV detection following sample bromination (LC-UV/Br)
- Gas chromatography with mass spectrometry detection following sample bromination (GC-MS/Br)

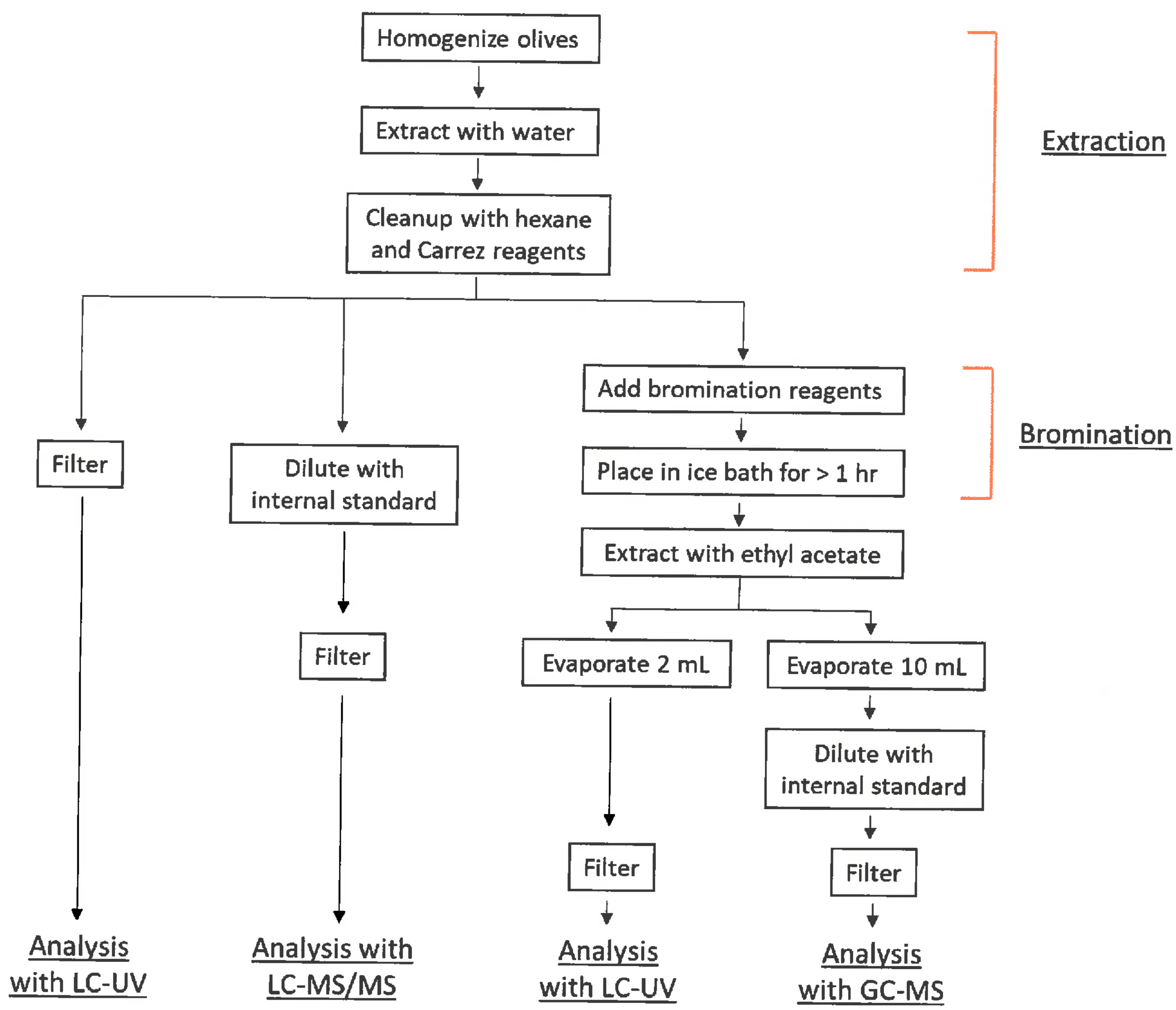
We will develop, optimize and validate each of these methods. We will then analyze commercial samples using all four methods. The design of this study should allow us to determine whether sample bromination or the type of detector have an effect on the accuracy and consistency of data. It will also allow us to determine which method is best suited for analyzing acrylamide in black ripe olives and should be the framework for a standard analytical method.

Objectives:

The goal of this project is to compare different extraction and quantification methods for acrylamide analysis in black-ripe olives, in terms of accuracy, precision, reproducibility and sensitivity, and then to identify the method that best meets these goals. We will also assess whether samples analyzed by different methods achieve comparable results.

Experimental Procedures:

The following is a flow chart of the four methods that will be compared:



Instrumentation. LC-UV analysis will be performed using an Agilent 1290 Infinity II LC system with a diode array detector. A Waters Alliance 2695 Separation Module coupled to a Quattro Micro API Mass Spectrometer operating in positive electrospray ionization (ESI) mode will be used for LC-MS/MS analysis. GC-MS analysis will be performed using a Varian 450 GC coupled to a Varian 220 MS operating in positive electron impact (EI) mode.

Method validation. Spike recovery experiments will be performed, where a known amount of acrylamide is added to the olive before extraction. A high and low level of added acrylamide will be assessed. Accuracy will be calculated as the percent recovery of acrylamide and precision will be calculated as the relative standard deviation of the samples. The sensitivity (limits of detection and quantification) of the different instruments and methods will be compared. We will also purchase certified reference materials with known concentration of acrylamide to validate our results. In addition, at least ten black ripe olive samples will be analyzed using all four methods and the consistency of the data will be evaluated. In order to assess method reproducibility, the same commercial sample will be analyzed on three different days.

Anticipated Outcome:

This study will determine the most accurate and robust method for acrylamide analysis in black ripe olives. We will communicate the results with processors and work with the AOAC (Association of Official Analytical Chemists) to develop an official standard analytical method for the table olive industry to use.

Select References:

[1] Wenzl, T.; de la Calle, B.; Gatermann, R.; Hoenicke, K.; Ulberth, F.; Anklam, E. Evaluation of the results from an inter-laboratory comparison study of the determination of acrylamide in crispbread and butter cookies. *Anal. Bioanal. Chem.* **2004**, *379*, 449–457.

[2] Clarke, D. B.; Kelly, J.; Wilson, L. A. Assessment of performance of laboratories in determining acrylamide in crispbread. *J. AOAC Int.* **2002**, *85* (6), 1370–1373.

[3] Fauhl, C.; Klaffke, H.; Mathar, W.; Palavinskas, R.; Wittkowski, R. *Acrylamide Interlaboratory Study*; Berlin, DE, 2002.

[4] Report of the Analytical Working Group. JIFSAN Acrylamide in Foods Workshop, Chicago, IL, April 14-15, 2004

[5] Casado, F. J.; Montaño, A. Influence of Processing Conditions on Acrylamide Content in Black Ripe Olives. *J. Agric. Food Chem.* **2008**, *56*, 2021–2027.

[6] Charoenprasert, S.; Mitchell, A. Influence of California-Style Black Ripe Olive Processing on the Formation of Acrylamide. *J. Agric. Food Chem.* **2014**, *62*, 8716–8721.

[7] Javier, F.; Montaño, A.; Carle, R. Contribution of peptides and polyphenols from olive water to acrylamide formation in sterilized table olives. *LWT - Food Sci. Technol.* **2014**, *59*, 376–382.

[8] Zhang, Y.; Zhang, G.; Zhang, Y. Occurrence and analytical methods of acrylamide in heat-treated foods Review and recent developments. *J. Chromatogr. A* **2005**, *1075*, 1–21.

[9] Elbashir, A. A.; Omar, M. M. A.; Ibrahim, W. A. W.; Schmitz, O. J.; Aboul-Enein, H. Y. Acrylamide Analysis in Food by Liquid Chromatographic and Gas Chromatographic Methods. *Crit. Rev. Anal. Chem.* **2014**, *44* (2), 107–141.

[10] Koch, M.; Bremser, W.; Koeppen, R.; Siegel, D.; Toepfer, A.; Nehls, I. Development of Two Certified Reference Materials for Acrylamide Determination in Foods. *J. Agric. Food Chem.* **2009**, *57*, 8202–8207.

BUDGET REQUEST (PI: Selina Wang)

Budget year: 2018-2019

Personnel (salaries and benefits): $42,485

- Graduate student GSR- 50%@$55,452: $28,564
- GSR Fees: $13,921

Supplies (instrumental and chemical supplies): $25,000

- Lab consumables for extractions, LC-UV, LC-MS/MS and GC-MS

Travel: $300

- Sample collections and meetings with the processors

Subtotal: $67,785

University overhead 11%: $7,456

TOTAL BUDGET REQUEST: $75,241

Impact of processing variables on acrylamide formation in black ripe olives

Problem and Significance:

Acrylamide is a toxin and probable carcinogen that has been found in canned black ripe olives.[1,2] It is known that acrylamide forms in olives during sterilization, although the mechanism and precursors involved are not well understood. At UC Davis, we have analyzed over 70 black ripe olive products from domestic processors and have found a very wide range of acrylamide levels (70 µg/kg – 1955 µg/kg). Our findings suggest that acrylamide formation is highly variable and dependent upon processing conditions. Both domestic processors follow standard methods for sterilization, using the same cook time and temperature. Therefore, other processing factors must be responsible for these variable levels.

Literature shows that processing factors that seem most likely to impact acrylamide formation are (1) calcium chloride, (2) storage brine acidity, and (3) oil content of the fruit. Calcium chloride is used in the storage brine to keep olives firm prior to processing. Previous studies found that calcium chloride increased acrylamide in both processed black ripe olives[3] and sterilized olive extract.[4] A hypothesis could be that calcium chloride increases olive firmness causing the fruit to retain more precursors to acrylamide during processing. However, the second study only used olive extract, which suggests that calcium may have another mechanism for promoting acrylamide formation. pH is also an essential factor and the highest amount of acrylamide forms around pH 6-7.[3,4] Differences in the acidity of storage brine could affect the pH of processed olives, which would then affect the amount of acrylamide that forms during sterilization. Storing olives in brine for a longer time before processing has been linked to decreased levels of acrylamide.[3] It is possible that acrylamide precursors degrade or diffuse out of the fruit during storage. Acrylamide formation may also be dependent on the cultivar of the olive. A previous study found that Manzanilla olives had higher acrylamide than Hojiblanca or Cacereña.[5] In our analyses, we have also observed that Manzanilla olives had higher acrylamide on average than Sevillano or Barouni. This may be related to differences in the fruit size, oil content or amount of acrylamide precursors between cultivars.

Bell Carter Foods Inc. and Musco Family Olive Co. are conducting joint experiments to try to isolate the effects of these and other processing conditions on acrylamide formation. The following specific variables will be tested:

- Calcium chloride levels added during storage and processing
- Sodium benzoate content, general solute content and acidity of storage brine
- Storage time of olives before processing
- Olive size and style (pitted vs. sliced)
- Can size
- Still vs. continuous retort

UC Davis will be a collaborator with Bell Carter Foods Inc. and Musco Family Olive Co.. We will measure acrylamide in processed olives in triplicates from these trials (total number of sample TBD). We will also measure pH, oil content and calcium levels of the olives. We will assist with synthesizing and interpreting data to identify trends between processing conditions and acrylamide concentrations.

Objective:

The objective of this collaborative study is to identify possible processing factors responsible for the formation of acrylamide in canned black ripe olives and to provide recommendations on how acrylamide levels can be reduced through modifications in processing.

Experimental Procedures:

Acrylamide analysis. Acrylamide in cooked olives will be measured using a previously validated method in our lab. Briefly, olives will be crushed and extracted with water using sonication and stirring. The extract will be cleaned-up using hexane (to remove lipids), Carrez reagents (to remove proteins and polysaccharides) and filtration (to remove solid particulates). Analysis will be performed with liquid chromatography-tandem mass spectrometry using a Waters Alliance 2695 Separation Module coupled to a Quattro Micro API Mass Spectrometer.

pH. Canned olives will be drained and the brine collected. The olives will be crushed and strained through cheesecloth. The pH of this olive extract and the brine will be measured using a pH meter.

Oil content. Oil content of raw olives will be measured using a Soxhlet extractor. Hexane will be repeatedly passed over ground, dried olive paste for 6 hours to extract all of the oil, which will then be measured by weight.

Calcium. Calcium levels in the raw, brine-stored, processed and cooked fruit will be measured using flame atomic absorption spectroscopy (FAAS) in the Chemistry Department at UC Davis.

Anticipated Outcome:

Olives from these processing trials will be tested for acrylamide, pH, oil content, and calcium. The resulting data will indicate the extent that there are relationships between specific processing variables and acrylamide content, leading to recommendations on how to modify the current commercial processing procedures to reduce the level of acrylamide in canned black ripe olives.

Select References:

[1] International Agency for Research on Cancer. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*; World Health Organization (WHO): **1994**; p 389. It is available on the website http://monographs.iarc.fr/ENG/Monographs/vol60/volume60.pdf.

[2] National Toxicology Program. *Toxicology and carcinogenesis studies of acrylamide*; U.S. Department of Health and Human Services: **2012**. It is available on the website http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr575_508.pdf.

[3] Charoenprasert, S., Mitchell, A. E. The influence of California-style black ripe olive processing on the formation of acrylamide. *Journal of Agriculture and Food Chemistry* **2014**, 62(34), 8716-8721.

[4] Casado, F. J., Sánchez, A. H., Montaño, A. Reduction of acrylamide content of ripe olives by selected additives. *Food Chem* **2010**, 119(1), 161–166.

[5] Casado, F. J.; Montaño, A. Influence of processing conditions on acrylamide content in black ripe olives. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 2021-2027.

BUDGET REQUEST (PI: Selina Wang)

Budget year: 2018-2019

Personnel (salaries and benefits): $48,166

- Graduate student GSR- 60% @$55,452 = $34,277
- GSR fees $13,889

Supplies (instrumental and chemical supplies): $27,000

- Lab consumables for extractions, LC-MS/MS, Soxhlet extractor and Flame AA.

Travel: $300

- Sample collections and meetings with the processors

Subtotal: $75,466

University overhead 11%: $8,302

TOTAL BUDGET REQUEST: $83,768

University of California
Entomology and Nematology

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project year 2019-20. Anticipated duration of project 1 year

Project leader Frank Zalom and Joanna Fisher   Location UC Davis

Cooperating Personnel Danielle M Lightle (Orchard Systems Advisor) and Emily Symmes (Area IPM Advisor) UC Cooperative Extension

Project Title Control of overwintering olive fruit fly using insect pathogenic fungi

Keywords Olive fruit fly, pest management, insecticide resistance management, overwintering population, insect pathogenic fungi

Commodity Olive   Relevant COC Project No. Click here to enter text.
Budget requested for 2019-2020:

Overview

Olive fruit fly, *Bactrocera oleae,* is the most important insect pest of California table olives. Until recently, its management has primarily relied on a single product, the spinosad bait GF-120, for which resistance has been documented. Additional control strategies are needed. Particularly useful would be those that target the overwintering generation. We propose using fungal insect pathogens to control overwintering olive fruit fly populations. If successful, this control strategy would require minimal input (1-2 sprays per year) and utilize commercially available products. A similar strategy for olive fruit fly control has been tested in Spain and has shown promising results. We want to see if the approach might be effective under California climatic conditions and with products that are already registered in California.

The problem and proposed solutions

The olive fruit fly (OLF), can cause significant crop loss and damage in California table olive production systems and there is zero tolerance for larvae in table olives (Burrack, 2011). Feeding of the OLF larvae in the developing olive destroys the pulp, can cause premature fruit drop, and leads to fruit rot due to the entry of bacteria and fungi (Zalom et al., 2009). Until recently, its management has relied almost exclusively on the use of a single product, GF-120, a bait containing the insecticide spinosad. GF-120 must be applied multiple times during the growing season to keep populations below economically damaging levels. However, spinosad resistance in California olive fruit fly populations was first reported in 2007 (Kakani et al. 2010). Continued use of GF-120 bait could lead to the development of widespread resistance and eventually the loss of olive fruit fly control in many CA olive growing regions. The pyrethroid insecticide fenpropathrin (Danitol) has been registered more recently for olive fruit fly control. However, pyrethroids are well known to disrupt scale and mite biological control in other systems where they are used, leading to secondary pest outbreaks (Cobourn et al., 2014). Overuse of pyrethroids frequently leads to the development of resist pest populations, and this has already been documented in some European olive growing regions (Pavlidi, 2018). We believe the management strategy we are proposing has the potential to provide growers with another control option that could be used to help address current issues with insecticide resistance to GF-120 and disruption by pyrethroid insecticides.

The fungi *Metarhizium brunneum* and *Beauveria bassiana* are insect-specific pathogens and commercial insecticide formulations containing these fungi are available. We propose testing the use of insect pathogens to control overwintering OLF larvae and pupae in the fall and potentially emerging adults in the spring. We would evaluate Met52, a *M. brunneum* based product, and two *B. bassiana* based products that contain different strains of the fungus, BioCeres and Mycotrol. The fungi would be applied to the soil prior to larval drop in the fall and a second time prior to adult emergence in the spring. A similar strategy has been tested in Spain, to control olive fruit fly, using *Metarhizium brunneum* (Yousef et al., 2017). When the fungi were applied twice per year it significantly reduced the number of OLF emerging in the Spring and being caught in traps by 50-70%. We will test the *M. brunneum* and the two *B. bassiana*-based products to determine which fungal strain has the highest virulence for controlling OLF. These products were chosen because they are either already registered in California for use on olives (Mycotrol) or already registered for other subtropical tree crops thus only minor label extensions would be needed for full registration. For our proposed study, we can easily obtain experimental use permits for Met52 and BioCeres. If our experiments indicated that one or both, of these products are effective, we would request a label extension which we are highly optimistic would be granted since the products are already registered on subtropical crops, they are biologicals and in this instance are not even being applied when olive fruit are present before harvest.

Emergence of overwintering OLF in Spring directly contribute to the population that will affect fruit later in the season. The use of insect pathogenic fungi to reduce the number of overwintering OLF has the potential to significantly reduce the size of the next season's OLF population, saving growers money and reducing the amount of in season pesticide that is needed. This control method does not rely on OLF attraction to a bait or trap. Instead insects are targeted when and where their location is known. The majority of OLF larvae pupate within the first 3 cm of the soil surface (Dimou et al., 2003). It is likely that pupating larvae will contact the fungi in the soil since it was found that more than 50% of *M. brunneum* and 87% of *B. bassiana* remained in the first 5 cm of the soil profile regardless of soil type (Storey and Gardner, 1988, Yousef et al., 2018). Finally, utilizing entomopathogenic fungi requires minimal labor or equipment since the products can be applied with commonly available ground spray booms, further reducing growers' management costs. The fungi, *M. brunneum* and *B. bassiana*, are endemic to the soil and depending on soil conditions, can persist in soil from weeks to months (Yousef et al., 2017; Zimmerman et al., 2017). Due to the ability of these fungi to persist in the soil only 1-2 spray applications per year will likely be needed and use of the fungi could significantly reduce the number of GF-120 and pyrethroid applications that are needed to achieve additional control if needed during the growing season.

Three objectives will be addressed through this research: (1) determine the virulence and application rate of *M. brunneum* and *B. bassiana* based products needed to kill OLF, (2) evaluate capacity of applied fungi to persist in CA olive orchards, and (3) assess efficacy of using insect fungal pathogens to control overwintering OLF under CA field conditions.

<u>Objectives and anticipated outcomes</u>

(1) Determine the virulence and application rate needed to kill olive fruit fly, B. oleae, larvae and pupae using commercially available insect pathogenic fungi.

Outcome: Efficacy of Met52, BioCeres and Mycotrol in controlling olive fruit fly larvae and pupae. Determine how long it takes the fungi to kill larvae and pupae and the optimal application rate.

(2) *Evaluate the capacity of insect pathogenic fungi to persist in olive orchards under California field conditions*

Outcome: Determine how long commercially available insect pathogenic fungi persist in the soil in olive orchards. Use this information to determine the number of sprays needed to control overwintering olive fruit fly.

(3) *Assess the efficacy of using insect pathogenic fungi for the control of overwintering olive fruit fly larvae and pupae using field cage trials.*

Outcome: Efficacy of using insect pathogenic fungi to control overwintering olive fruit fly larvae and pupae. Assess use of fungal sprays to reduce spring adult emergence.

Plans and Procedures:

Activities	Timeline
Obj. 1: ***Determine the virulence and application rate needed to kill OLF larvae and pupae using commercially available insect pathogenic fungi.*** Establish time to death and the lethal dose of Met 52, BioCeres and Mycotrol against OLF larvae and pupae in a laboratory assay. Test each product at several application rates. Spray soil with fungi, allow larvae to drop into soil and determine time to death.	**September-November 2019**
Obj. 2: ***Evaluate the capacity of insect pathogenic fungi to persist in olive orchards under California field conditions*** Spray plots on a grower's farm and in an untreated UC Davis experimental olive orchard with the product identified in Obj. 1. Take soil samples monthly and extract and quantify living fungi using standard insect pathology methods (plate soil on media selective for *M. brunneum* and *B. bassiana*).	**October 2019-May 2020**
Obj. 3. ***Assess the efficacy of using insect pathogenic fungi to control overwintering OLF larvae and pupae using field cage trials.*** Conduct a cage field trial on a grower's farm and in an untreated UC Davis experimental olive orchard. Place cages beneath tree canopies. Spray soil surface with the product and at the rate identified in Obj.1. Treat control plots with water. Following spray, place infested olives on soil surface in cages and allow larvae to drop into soil to burrow and pupate. At several time points following treatment, dig up soil under a subset of the cages and determine larval and pupal mortality. Assess the efficacy of 1 vs. 2 sprays by spraying the soil under a subset of cages a second time, in the Spring, prior to adult emergence. Monitor cages for adult emergence and assess survival of overwintering OLF.	**October 2019-May 2020**
Disseminate research findings. Research findings will be disseminated through grower meetings, talks, trade journals and peer-reviewed journal articles	**Fall 2020-ongoing**

BUDGET REQUEST Budget Year 2019-20

Funding Source

Salaries and Benefits			
Student Assistant	20 hr/wk for 32 wks @$12/hr		$ 7,680
Employee benefits (1.5%)	1.5%		$ 115
Subtotal		**Sub 1**	**$ 7,795**
Supplies and Expenses			
Rearing Supplies			$ 600
Fungal persistence assay supplies	soil corers, plastic bags, vials, petri dish turntable, spreaders		$ 576
Media (fungal persistence assay)	216 plates @ $2.73/ plate		$ 590
Field cages	144 @ $30/cage		$ 4,320
Growth chamber maintenance			$ 400
Subtotal		**Sub 2**	**$ 6,486**
Travel	**(1 mi @ 54.5 cents/mi)**		
Travel to field sites	2 sites, 25 trips=5475 mi		$ 2,984
Collect infested fruit	2 trips = 216 mi		$ 118
Travel to extension meetings	2 trips = 633 mi		$ 345
Subtotal		**Sub 3**	**$ 3,447**
Subtotal			$ 17,728
Indirect Costs	11% of budgeted costs	**Sub 4**	**$ 1,950**
Cumulative Total			**$ 19,678**

Department account number ________

Originator's Signature [signature] Date 10/19/2018

[signature] Date 10/19/2018

Entomology and Nematology Department Chair [signature] Date 10/22/2018

Liaison Officer ______________ Date

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AES/CE MAR 84

Workgroup: Olive
Department: Plant Pathology/UCR

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

Project Year: 2019 Anticipated Duration of Project: 3rd of 4 years

Principal Investigators: J. E. Adaskaveg

Cooperating: D. Thompson, H. Förster, and K. Nguyen

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

Keywords: Bactericides, copper enhancing compounds, antimicrobial natural products, biological controls

JUSTIFICATION/ BACKGROUND

Pseudomonas savastanoi pv. *savastanoi* (*Psv*), the causal agent of olive knot, is a serious disease of olives (*Olea europaea*) throughout all olive growing regions of the world (8). The pathogen enters through wounds causing outgrowths (knots, tumors, galls) predominately on trunks, branches and twigs. Olive knot is one of the most economically important diseases of olives. Infection may lead to tree defoliation, dieback, and reduced vigor, which ultimately lowers fruit yield and quality (6). *Psv* can survive epiphytically on olives but the main source of inoculum is bacteria living within knots (7). Large quantities of bacterial ooze can be exuded when knots become wet. This exudate is disseminated by rain, wind, insects, birds, as well as human activity. The opportunistic pathogen takes advantage of wounds caused by natural leaf abscission (4), frost, and hail, as well as cultural practices such as pruning and harvesting. These latter practices also lead to direct mechanical damage of the knots, exposing and spreading inoculum to healthy tissue. After entering its woody host, the pathogen induces knot formation through the production of indoleacetic acid (IAA) and cytokinins (2). 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 low temperatures (-5° C) and thus, wetness is the main limiting factor for the disease. None of the currently grown olive cultivars is resistant to the pathogen (5).

Control of olive knot is difficult, and growers rely on applications of copper-based bactericides as the only effective foliar treatment. Manual application of cresol- and xylenol-based compounds (Gallex) to individual knots can eliminate the pathogen but is unfeasible on a commercial scale due phytotoxicity when applied as an air-blast foliar treatment. Copper has been extensively used in olive production for many years for the control of diseases such as peacock spot and olive knot. Reliance on a single active ingredient has led to our detection of copper resistance in *Psv* strains from a commercial olive orchard. Still, the incidence of copper resistance is currently very low, accounting for only 2% of the total strains collected in different olive growing regions of California. When resistant strains were inoculated to Arbequina and Manzanillo olive wounds, application of copper provided reduced or no control as compared to inoculation with a sensitive strain. Copper-resistant strains caused less disease on leaf scars as compared to Cu-sensitive strains, but still resulted in a high incidence of disease especially at higher inoculum concentrations. Therefore, there is a potential risk of copper resistance spreading with the continued and sole use of copper. This necessitates the development of new bactericides or copper-activity-enhancing materials to overcome resistance. The latter strategy has proven to be effective for walnut blight management where copper resistance in *Xanthomonas arboricola* pv. *juglandis* is common and copper-mancozeb mixtures have provided exceptional control for many years. Mancozeb can no longer be registered on new crops but other copper-enhancing alternatives can be evaluated. Salicylidene benzoylhydrazone (SBH) was recently discovered to display synergism when combined with copper against *Alternaria solani* causing early blight of tomatoes. We performed preliminary tests with this molecule with promising results using several genera of phytopathogenic bacteria including *Psv*. Low concentrations of metallic copper combined with SBH were highly inhibitory in vitro against a copper-resistant *Psv* strain while copper or SBH by themselves at the same concentrations were not effective. In field trials in 2018 on managing olive knot, however, a SBH derivative (DAS-1) did not improve the performance of copper. If additional derivatives of SBH are supplied by Dow AgroSciences, these will be tested in 2019.

Other potential bactericides were made available to us by agrochemical registrants in 2018. These include experimental inhibitors of type III secretion systems in plant pathogenic bacteria. These compounds are novel in their mode of action. They act on the mechanism that delivers bacterial proteins into the host cells that are vital for *Pseudomonas* species to cause disease. We tested three experimental type III secretion system inhibitors in the field but they performed poorly as compared to copper and kasugamycin treatments. Additional inhibitors may become available for 2019 studies.

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* (1) 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 extensive field studies. Although we previously reported that kasugamycin had reduced efficacy when treating leaf scars that were made by the removal of green healthy leaves, we discovered that kasugamycin was much more effective in controlling olive knot on naturally formed leaf scars and thus, would be a beneficial treatment during spring leaf drop. Kasugamycin was first federally registered on pome fruits. In 2018, it received California registration on pome, cherry, and walnut crops. Registration on olives, peaches, and almonds is pending for late 2019. The petition for Kasumin on olive was still in the IR-4 program this summer and the final report and submission to the EPA are pending in the fourth quarter of 2018. Kasugamycin would greatly complement current copper sprays and could be used in rotation or mixtures with copper. Oxytetracycline was also submitted to IR-4 and is in the field trial phase of the IR-4 program for establishing tolerances. We will conduct additional studies with oxytetracycline to potentially improve its efficacy by using selected UV-protecting adjuvants. New antibiotic registrations find little acceptance with regulatory agencies, and we are currently in discussion with EPA to develop a science-based approach on the use of antibiotics in plant agriculture.

In addition to developing conventional chemical compounds, we continued our research of food additives as possible new modes of action for managing olive knot. Several food additives that are considered 'generally recognized as safe' (GRAS) have antimicrobial properties. They are often naturally produced molecules of gram-positive *Streptomyces* species. Although these compounds are typically applied to food products as preservatives, they have potential for controlling plant diseases when applied as a foliar treatment. Integration of these alternative materials with conventional treatments may improve disease control, reduce the risk of resistance development, and provide olive growers with more resources for managing olive knot. In 2018, we evaluated nisin, Ɛ-poly-L-lysine, and two organic acids in field trials but disease control was not comparable to that of copper or kasugamycin treatments. In laboratory assays, however, low rates of organic acids and Ɛ-poly-L-lysine, and higher rates of nisin were toxic to Psv when bacterial cells were exposed for a short period of time demonstrating their effectiveness. Therefore, improvement in field performance will be necessary and is ongoing in this project. Potential strategies for optimizing these compounds may include the addition of adjuvants or buffering agents (9), or the use of these materials in mixed treatments with conventional products. This information is still valuable because rotational programs could be developed with different modes of actions for different phases of the disease, i.e., leaf scars or lateral wounds occurring during leaf drop or harvest and pruning, respectively. These materials are registerable as conventional and possibly organic treatments.

RESEARCH OBJECTIVES

1) **Evaluate new bactericides, potential enhancers of copper activity, food additives, GRAS sanitizers, and other experimentals against *Psv***
   a) Laboratory in-vitro sensitivity studies: copper mixtures with new SBH derivatives as they become available; nisin, Ɛ-poly-L-lysine, and the GRAS sanitizers lactic and citric acid alone or combination and in mixtures with selected adjuvants (see below).
   b) Field efficacy studies with new bactericides in comparison with kasugamycin for the management of olive knot caused by copper-sensitive and -resistant strains of *Psv*.
      i) Potential enhancers of copper activity - new SBH derivatives.
      ii) Type III secretion system inhibitors (as they become available)
      iii) Oxytetracycline formulations in combination with selected UV-protecting adjuvants.

iv) Nisin and Ɛ-poly-L-lysine alone, in combination with each other, or in mixtures with antimicrobial acids (e.g., lactic, citric, and other acids), chelators (e.g., EDTA), sodium diacetate, buffers (to neutralize acidic carbohydrates), as well as emulsifiers (e.g., dextran) and proprietary fatty acids.

3) Continue to support the registration of the antibiotics kasugamycin and oxytetracycline

a) Administrative support to EPA and other regulatory agencies about registration concerns for kasugamycin and other bactericides

b) Optimizing the efficacy of oxytetracycline under field conditions (UV blockers and stabilizers) as it goes through the registration process at IR-4.

PLANS AND PROCEDURES

1) Evaluate new bactericides, potential enhancers of copper activity, food additives, GRAS sanitizers, and other experimentals against *Psv.*

a. To evaluate the in vitro toxicity of SBH, nisin, Ɛ-poly-L-lysine, and the GRAS sanitizers lactic and citric acid alone or combination with each other and selected adjuvants (see below), the spiral gradient endpoint (SGE) method will be used where bacterial strains are exposed to a bactericide concentration gradient on a single agar plate. To evaluate SBH and other derivatives as they become available as new potential enhancers of copper activity against *Psv*, a dilution plate method will be combined with the SGE method. Agar media will be amended with fixed concentrations of copper. Subsequently, SBH and derivatives of SBH will be applied to the plates in radial concentration gradients using a spiral plater. Suspensions of *Psv* strains will be streaked radially onto the amended media. This will allow the determination of minimal inhibitory values for *Psv* at different ratios of copper and SBH derivatives. These data will then be used to calculate appropriate field rates.

b. Copper-SBH mixtures, Type III secretion system inhibitors, and oxytetracycline will be tested in the field on Arbequina and Manzanillo olives at UC Davis. Treatments will be compared to Kasumin and copper by themselves. We also plan to use combinations of different compounds (e.g., nisin + Ɛ-poly-L-lysine, nisin + lactic acid, etc.) and adjuvants to determine if the field efficacy can be optimized. Adjuvants will include chelators (e.g., EDTA), antimicrobial acids (e.g., lactic, citric, and other acids), chelators (e.g., EDTA), sodium diacetate, buffers (to neutralize acidic carbohydrates), as well as emulsifiers (e.g., dextran) and proprietary fatty acids. Plants will be wounded with lateral and leaf scar wounds. Lateral wounds on 1-2-year-old twigs will be made using a scalpel by removing the bark and exposing cambial tissue. Leaf scars will be made by pulling leaves off the same twigs. In addition, wounds from natural leaf drop will be used. Treatments will be sprayed onto wounds, allowed to air-dry, and inoculations will be done with a suspension of copper-sensitive or -resistant *Psv* strains. SBH derivatives will be applied using rates based on the laboratory tests. Oxytetracycline will be used in combination with new UV-protecting adjuvants because it is especially vulnerable to UV-degradation. The efficacy of treatments will be assessed as the percent incidence of knots forming on treated, inoculated wounds as compared to wounds that are treated with water and inoculated (i.e., controls).

2) Continue to support the registration of the antibiotics kasugamycin and oxytetracycline. An inter-commodity and industry group will continue to work with the Minor Crop Farmer Alliance to recommend an EPA policy change towards the use of antibiotics in plant agriculture. Specifically, a new internal EPA Guidance Document (GD) for use of antibiotics in plant agriculture needs to be developed based on science. Historically, EPA GD 152 for registration of antibiotics in animal husbandry is used for all requests in agriculture. Additionally, we will continue to work with a USDA working group to address CODEX initiatives for establishing policies on all antibiotic use in agriculture including animal and plant uses.

Benefits to the industry

For management of olive knot, in addition to cultural methods, sanitation practices, and the labor-intensive Gallex, only copper materials and the natural product Regalia are currently available. We obtained improved performance of copper when applications were made within 24 h of wounding events (e.g., harvesting, pruning, hail storms, freezing) as compared to later applications, and with high labeled rates of copper. In our previous research, we showed that copper resistance is currently only found locally where copper has been used for many years. Because copper-resistant strains of *Psv* were found to be virulent and likely competitive, and because they were not genetically clonal, there is a risk of further spread of copper resistance. Therefore, alternatives are needed for a sustainable and effective management program for many years ahead. We initiated the registration of the new agricultural antibiotic kasugamycin that was registered in 2014 on pome fruits, and in 2018 on pome fruits, cherry, and walnuts in California. The olive registration is pending in late 2019 together with almonds and peaches. Oxytetracycline is still in the registration process through the IR-4 program. Kasugamycin showed high activity against olive knot especially in mixtures with copper. Mancozeb as a mix partner with copper was considered by us and the industry, but EPA has denied any new registrations. In 2018, we evaluated new copper activity-enhancing compounds that, however, were not effective; therefore, possible additional derivatives of SBH will be evaluated in 2019 in cooperation with a US registrant (Dow AgroSciences). Working with Brandt Corp., we also tested Zinkicide, a nanoparticle zinc product in mixed treatments with copper and found it to have moderate activity. Thus, we will continue to explore and evaluate potential bactericide products that can be registered under current regulatory policies. Compounds that inhibit the bacterial Type III secretion system and prevent infection are critical to the survival of *Psv.* We also aim to optimize the natural GRAS products nisin, ε-poly-L-lysine, and the sanitizers lactic and citric acid in combination with adjuvants such as sodium diacetate, buffers (to neutralize acidic carbohydrates), and emulsifiers (e.g., dextran) to enhance the antimicrobial activity and persistence in managing olive knot. The registration of several materials for olive knot management will allow the implementation of anti-resistance strategies and will prevent over-use of any single mode of action bactericide. Still, integrated practices will be critical for the successful management of the disease. Any bactericide treatment will be most effective when pathogen population levels are at a minimum and the host is less susceptible.

References

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Budget Request:
Funding Source: California Olive Commission and California Olive Oil Commission

Budget Request with UC indirect costs:

Budget Year: 2019 Funding Source*:	**OOC**	**COC**	**Total Budget**
Salaries and Benefits: Post-Docs/RAs	7,000	7,000	14,000
Lab/Field Ass't	1,000	1,000	2,000
Subtotal	8,000	8,000	16,000
Employees' Benefits**	4,500	4,500	9,000
Subtotal	12,500	12,500	25,000
Supplies and Expenses	0	0	0
University Land and Orchard charges	1,000	1,000	2,000
Operating Expenses/Equipment Travel	0	0	0
Travel	1,500	1,500	3,000
Direct Cost Totals **Total**	**$15,000**	**$15,000**	**$30,000**
Off Campus IDC @ 11%		1,650	1,650
Total Budget Requested	**$15,000**	**$16,650**	**$31,650**

J. E. Adaskaveg

Originator's Signature (PI) Date: Oct. 30, 2018

Katherine Borkovich

Dept. Chair (Riverside Campus) Date: Oct. 30, 2018

Liaison Officer ______________________ Date:__________

AES/CE MAR 84

Workgroup: Olive
Department: Plant Pathology/ UCR

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

Project Year: 2019 Anticipated Duration of Project: 2nd year of 3 years

Principal Investigators: J. E. Adaskaveg

Cooperating: H. Förster, D. Thompson (UC Riverside)

Project Title: Management of foliar diseases of olive – A. Olive knot (see previous submission) and B. Evaluation of new fungicides for control of olive leaf spot (Supplemental Proposal)

Keywords: Chemical and biological control

JUSTIFICATION/ BACKGROUND

Olive leaf spot or peacock spot, caused by the fungus *Fusicladium oleagineum* (syn. *Spilocea oleaginea, Venturia oleaginea*), is a sporadic disease of olive trees in California. In years with favorable environmental conditions, an orchard may lose 9 to 15% of its leaves and 10 to 20% of the fruiting twigs if the disease is not managed. Excessive leaf loss can also result in more olive knot because leaf scars are sites for bacterial infection. Symptoms most commonly develop on the leaf blade but are also found on petioles, fruit, and fruit peduncles (stems). At first, lesions are inconspicuous, superficial, sooty blotches. Later they become dark green to black circular spots containing mycelium and conidia (Fig. 1), and spots are surrounded by yellow halos. These lesions resemble the spot on the tail of a peacock, and hence the name peacock spot. With numerous lesions, the leaf becomes chlorotic and falls.

Leaves in the lower canopy are more severely affected where the humidity is higher, resulting in greater defoliation. Defoliated twigs often die later in the summer. Leaf infections occur on the upper surface and seldom penetrate beyond the epidermal layer. Once the leaf drops, however, the fungus colonizes the internal leaf tissues forming a dense mass of stromatic tissue. The sexual state of the pathogen has not been observed. Olive cultivars vary in their susceptibility. Mission is the most susceptible followed by Manzanillo and to a lesser extent Sevillano, but all cultivars are generally susceptible.

Leaf drop occurs mostly in late spring and summer. Infected leaves remaining on the tree start sporulating along the margins of lesions in the fall. Rainfall and wind-driven rain are the main dissemination methods; whereas wind alone is not effective in detaching and disseminating conidia. In California, lesions start forming in the fall and winter, but most disease develops in the spring. Rainfall is essential for infections to occur regardless of the season. Temperature is important but often is not limiting the development of the pathogen. High temperatures are more limiting to spore germination and mycelial growth than low temperatures. The optimum temperature for growth of the fungus is 21°C, but growth can occur at 6 to 28°C. The minimum duration of leaf wetness for spore germination is 48 h at 16°C, 24 h at 20°C, or 36 h at 24°C. The incubation period is 12 to 19 days over a temperature range of 10°C to 25°C.

Currently available chemicals for managing the disease are copper and lime sulfur. Bordeaux mixtures or fixed coppers are commonly used to prevent copper injury. Lime sulfur can also eradicate the fungus in leaf tissue but lime sulfur is difficult to work with and requires extra protective equipment for workers. Other fungicides such as zineb are effective but no longer available. Timing of fungicide treatments in California include a postharvest application and an early spring application. Others, however, have indicated that spring treatments are less effective. Use of copper treatments at these time periods corresponds with olive knot management timings. With more regulations concerning the use of copper (new copper limits for agricultural uses) and lime sulfur, alternative fungicides are needed that are highly efficacious and persist for extended time periods to prevent infections over the winter and spring when rainfall results in infection periods. In 2018, Ziram and Inspire Super were approved for residue trials at the IR-4 National Food Use Workshop in September for registration on olives. Strong support was provided based on the after-harvest and winter season usage with expected zero to limit-of-detection residues on the crop in the following harvest season. Ziram is a FRAC Code M3, whereas Inspire Super is a FRAC Code 3/9. Thus, integration of multi-site modes of action for both products was also established as an effective anti-resistance strategy. Research on these and other fungicides needs to continue to identify potential products.

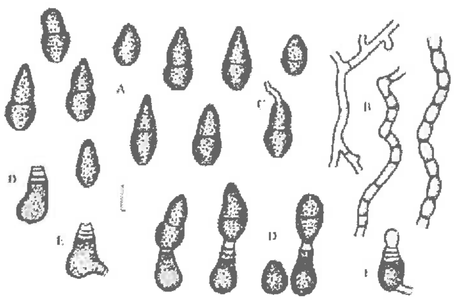

Fig. 1. *Fusicladium oleagineum.* A - conidia. B - superficial septate hyphae, C - germinating conidium, D - conidiogenous cells with several conspicuous annellations, E – percurrent proliferating conidiogenous cell, F - conidiogenous cell arising from a hypha. Scale = 10 µm.

OBJECTIVES

1. Evaluate the performance of new and older fungicides in field trials.
    a) Dithiocarbamates (ziram), chlorinated hydrocarbons (chlorothalonil), and phthalimides (captan) (FRAC Groups – M3, M4, M5), DMIs (FG 3), polyoxins (FG 19), or mixtures such as FG 3/9, and FG 3 + 19.
    b) Evaluate proprietary fatty acids to improve performance of fungicides (pesticides).
2. Evaluate application timing of selected treatments.
    a) Fall, spring, or fall and spring.
3. Evaluate new fungicides for their in vitro activity.
    a) Determine the in vitro activity of selected fungicides that are effective in field trials.

PLANS AND PROCEDURES

1. *a,b. Evaluate the performance of new and older fungicides in field trials.* In studies in a commercial olive orchard where the disease is known to occur and in an experimental orchard at UC Davis, fungicides including ziram (FG M3), captan (FG M4), chlorothalonil (FG M5), difenoconazole (FG 3), and polyoxin-D (FG 19), or mixtures such as difenoconazole/cyprodinil (FG 3/9) and difenoconazole+polyoxin D (FG 3+19) will be applied using an air-blast sprayer. Additionally, proprietary fatty acids that improve pesticide performance will also be evaluated in combination with selected treatments (e.g., Inspire Super, polyoxin-D, etc.). There will be four replications for each treatment in a randomized complete block design. Disease incidence and severity will be evaluated in late spring. Data will be analyzed statistically using ANOVA procedures and mean separation procedures of SAS 9.4.

2. *Evaluate application timing of selected treatments.* In field studies, selected fungicides will be applied at different timings to compare fall vs. spring or fall + spring timings. There will be four replications for each treatment in a randomized complete block design for a factorial experiment. Disease incidence and severity will be evaluated in late spring. Data will be analyzed statistically using ANOVA procedures and mean separation procedures of SAS 9.4.

3. *Evaluate new fungicides for their in vitro activity.* Isolates of the pathogen will be obtained from several locations. To evaluate the in vitro toxicity of selected new fungicides with efficacy in field trials, the SGE method will be used. Agar media will be amended with fungicides in radial concentration gradients using a spiral plater. Suspensions of spores or mycelial strips will be placed radially onto the amended media. This will allow the determination of EC_{50} values for each fungicide and isolate using a computer program.

BENEFITS TO THE INDUSTRY

Little information is available on the management of peacock spot although the disease is widely distributed and causes sporadic losses in olive growing regions of California. Chemical management is currently based on the use of copper and lime sulfur, two materials that are increasingly being restricted by regulatory agencies at the state and federal levels. Thus, the evaluation of the efficacy and timing of new and older fungicides is needed to provide the industry with alternative treatments for peacock spot management. With Ziram and Inspire Super (difenoconazole+cyprodinil) accepted into the IR-4 program in September 2018, registrations are planned, and efficacy data needs to be expanded. Due to the small acreage of olive production in California, registration of any new material will be limited to registrants that are willing to cooperate and to registration processes though the IR-4 program. UPI (ziram) and Syngenta (Inspire Super) were very agreeable to our proposals for their respective products on olive. Still, the registration process will take several years to complete.

REFERENCES

1. Teviotdale, B. 2005. Diseases of olive. Pages 119-122 in: Olive Production Manual, 2nd Edition, G. S. Sibbett and L. Ferguson, technical editors. University of California ANR, Publication No. 3353.
2. Ogawa, J. M., and English, H. 1991. Diseases of Temperate Zone Tree Fruit and Nut Crops. University of California, DANR, Publication No. 3345.

Budget Request: (Supplemental to the Olive Knot Proposal)

Budget Year: 2019

Funding Source: California Olive Commission

Salaries and Benefits:	Post-Docs/RAs		9,000
	Lab/Field Ass't		0
	Subtotal		9,000
	Employees' Benefits		5,000
		Subtotal	14,000
Supplies and Expenses			0
Equipment and University Land and Orchard charges			0
Operating Expenses/Equipment Travel (Davis Campus only)			0
Travel (include $500 for each Farm Advisor travel costs)			1,000
Department Account No. ________		**Total**	**15,000**

James E. Adaskaveg

____________________ Date: Oct. 30, 2019

Originator's Signature (PI)

Katherine Borkovich

Dept. Chair, Kathy Borkovich ____________________ Date: Oct. 30, 2019
(Riverside Campus)

Liaison Officer ____________________ Date:________

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project year: 2019 Anticipated Duration of the project: April –November 2019

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, Laura Doskocil

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 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 PROCEEDURES:

. 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 March 29, 2019. The traps will be read and reported by April 5, 2019 and continue on a weekly basis. 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 male and female Olive Fruit Fly found at each location will be submitted to the California Olive Committee and interested parties within 24 hours on a weekly basis during the project. The program will end November 15, 2019.

BUDGET REQUEST

Budget year: April 1, 2019-December 1, 2018

Funding Source: California Olive Committee
Nutrien Ag Solutions
Ag IPM Consultants, Inc.

Salaries and benefits:		$15,600.00
Supplies: Traps, bait and pheromone		1,200.00
Travel: Mileage to trap sites		2,400.00
Equipment:		0.00
	TOTAL	$19,200.00

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

Total funding from the California Olive Committee would be: $6,400.00

James R. Stewart
Project leader
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Bert Quezada
Senior Pest Control Advisor
Ag IPM Consultants, Inc
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Ern's Pest Control

Project Plan/ Research Grant Proposal

Project Year: 2019

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 will be reported via email to the COC for further distribution. Trapping results will be reported as male and female flies for individual traps and combined by site. Trapping and reporting will be continued through October or until table olive harvest has concluded.

Budget Request

Budget Year: 2019

Funding Source: California Olive Committee

Salaries	$4735
Supplies and Expenses: Trapping Supplies	$ 365
Travel 2545 mi.	$1800

This may vary due to fuel prices

Total	$6500

Originator's Signature______________________________

Ernie Simpson

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

FROM: RESEARCH SUBCOMMITTEE

SUBJECT: 2019 RESEARCH PROJECT

RECOMMENDATION: THAT the Subcommittee approve research project for 2019.

BACKGROUND: Each year the Research Subcommittee approves various research projects funded by the Full Committee. The Subcommittee must determine which proposed projects to recommend to the Full Committee for funding. An estimated budget of $315,882 is proposed based on the submitted projects.

2019 RESEARCH PROPOSALS FOR THE CALIFORNIA OLIVE COMMITTEE

TOPIC	LEADERS	AMOUNT
Canopy management, tree hedging and topping to optimize yield	Rich Rosecrance	$31,075
Evaluation of new chemistries to control Olive Fruit Fly	Debra Keenan	$25,000
Managing Alternate Bearing in Olive with PGRs and Pruning	Carol Lovatt Elizabeth Fichtner	$21,570
Development of a standard analytical method for acrylamide in California-style black ripe olives	Selina Wang	$75,241
Impact of processing variables on acrylamide formation in black ripe olives	Selina Wang	$83,768
Control of overwintering olive fruit fly using insect pathogenic fungi	Frank Zalom Joanna Fisher	$19,678
Epidemiology and management of olive knot caused by Pseudomonas savastanoi pv. savastanoi	J. E. Adaskaveg	$31,650
Management of foliar diseases of olive (peacock spot)	J. E. Adaskaveg	$15,000
Southern San Joaquin Valley Olive Fruit Fly Monitoring Project	Jim Stewart	$6,400
Sacramento Valley Olive Fruit Monitoring Project	Ernie Simpson	$6,500
Total		$315,882
* budget estimate; actual budget pending on results		

FISCAL IMPACT: Unknown

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

FROM: RESEARCH SUBCOMMITTEE

SUBJECT: NO-COST EXTENSIONS

RECOMMENTATION: Each year, researchers will request a no-cost extension should their program run past the fiscal year. THAT the Committee grant authority to the Executive Director in conjunction with the Chairman to approve requests for no-cost extensions.

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

FROM: RESEARCH SUBCOMMITTEE

SUBJECT: INTER-ITEM TRANSFERS OF THE RESEARCH BUDGET

RECOMMENDATION: THAT the Committee grant authority to the Executive Director and Chairman for inter-item transfers of the Research Budget.