AES/CE MAR 84

Workgroup: Olive

Department: Plant Pathology/ UCR

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

Project Year: 2015 Anticipated Duration of Project: 2nd year of 3 years

Principal Investigators: J. E. Adaskaveg

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

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

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

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JUSTIFICATION/ BACKGROUND

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

Induction of olive knots on wounded, inoculated branches depends on inoculum concentration as well as olive cultivar (Penyalver, 2006). We are focusing on studies on cv. Manzanillo with minor trials on cv. Arbequina. Both are susceptible to Psv infection, but disease develops at higher incidence, severity, and speed on cv. Manzanillo. Based on these observations, we will determine the minimum threshold inoculum concentration for each cultivar for which knots develop. Management strategies may depend on keeping Psv populations below these threshold values.

Knot induction is usually localized to the initial entry point of Psv. Systemic movement of the pathogen has rarely been observed (Wilson and Magie, 1964). In spring 2014 evaluations of our fall 2013 trials in commercial and experimental olive plots we noticed apparent systemic movement of Psv which we never observed in any of our previous trials. Infections caused bark blistering and cracking as well as development of knots in proximity to and away from the initial point of inoculation, even on neighboring branches. In most severe cases, inoculated branches died. Potential causes of systemic movement have not been well characterized. Thus, one of our objectives is to determine environmental or other factors leading to these symptoms and whether the pathogen is migrating internally or externally on the host. In preliminary investigations, we have been able to reproduce environments in growth chamber studies that lead to systemic movement of the bacterium in olive plants. More detailed studies are planned in 2015. This information will contribute to knowledge on the epidemiology of the pathogen and possibly identify new management strategies.

Sanitation and prevention are the most successful strategies for management of olive knot. Any horticultural practice that promotes tree health, minimizes tree stress, and results in less leaf drop will reduce infections. Pruning and removal of knots during dry periods (i.e., summer and early fall) reduces inoculum and avoids re-infection at pruning sites. Because the bacteria may be carried on pruning shears, frequent disinfection of equipment is necessary. Painting galls with Gallex is an effective therapeutic treatment but is very labor intensive and impractical. Spray applications of copper-containing bactericides have been very effective in minimizing the disease, but they often may need to be repeated to protect new wounds. A minimum of two applications is usually necessary: one in the fall before the rainy season starts and one in the spring when most leaves have been shed. Additional applications may be needed during winter rains or spring/summer hail storms. New copper formulations have been developed to reduce the metallic copper equivalent while maintaining the efficacy of the treatment. Our evaluations of copper sensitivity in populations of the olive knot pathogen indicated a reduced sensitivity of all strains with several strains showing an increased tolerance to copper. These results demonstrate a potential risk towards resistance development of Psv to copper with its continual and often exclusive use. Although the combination of copper and mancozeb is highly toxic to strains of Psv less sensitive to copper, the EPA will not allow additional crops to be added to the mancozeb label. Thus, we initiated a search for other compounds that could be mixed with copper to increase its activity. We have identified amino-thiadiazole (ATD), a food-grade additive, as a synergistic compound that increases the activity of copper against copper sensitive and less sensitive strains. Field trials will be established in late 2014 and 2015. Because olive knot infections occur mostly during the rainy period, knowledge on the persistence of treatments is critical. Thus, we will determine the efficacy of copper and kasugamycin alone and in mixtures with materials that may increase its persistence by inoculating treated twig wounds selected times after application of simulated rain. Rainfall will be simulated using overhead irrigation in orchards at UC Davis.

We have been instrumental in the development of the new agricultural antibiotic kasugamycin (commercial name Kasumin) for several bacterial diseases of agronomic crops in the United States. Kasugamycin has high activity against *Erwinia* and *Pseudomonas* species and moderate activity against *Xanthomonas* species and other plant pathogenic bacteria. We found it to be the most promising new treatment for preventing olive knot in our field studies, including in a commercial application to inoculated branches. In this proposal, we plan to continue its evaluation. Kasugamycin is currently federally registered on pome fruit crops (e.g., apples and pears), whereas use on olives was approved as an "A" priority by IR-4 for the 2015 season. We will be involved with the IR-4 residue studies in the spring of 2015. Other products including antimicrobial peptides from commercial sources will also be evaluated in 2015. Several systemic acquired resistance (SAR) compounds (e.g., Actigard, Regalia, quinoxyfen - Quintec, and USF2018A) were also effective in our studies, but not equivalent to copper or kasugamycin. SAR treatments need to be applied several days in advance of favorable disease conditions (or inoculation) but they potentially have a longer lasting effect on plant health and may provide sustainable treatments that could be developed for an integrated approach with other treatments.

We have also been working on sanitation treatments as part of an integrated olive knot management program. We demonstrated that quaternary ammonia compounds, guanidine, and chlorhexidine were highly toxic against the olive knot pathogen in laboratory studies. Citrox, a natural product derived from citrus extracts, and the quaternary ammonia sanitizers were also highly effective in disinfecting hard surfaces that were contaminated with Psv. The quaternary ammonia sanitizers are volatile compounds that leave near zero residues and are not corrosive to equipment. Deccosan 321 has a federal label and is used in Florida for disinfesting field equipment to prevent the spread of the bacterial disease citrus canker. Deccosan 321 was submitted to the State of California as a Section 24c, Special Local Need, registration on harvesting and pruning equipment used by the olive industry. Registration is expected in the spring of 2015. We will test additional parameters that may affect the efficacy of the sanitizers such as inoculum concentration and post-inoculation treatment time. Field evaluations of the material as an equipment sanitizer will be accomplished once registration is approved. Additionally, we will evaluate a new non-phenolic based quaternary ammonium compound, KleenGrow, for use as a protective treatment directly on trees with initial efficacy trials supported by IR-4 and the registrant. Bactericides with different modes of action other than antibiotics are difficult to identify. If efficacy trials with KleenGrow are successful in reducing disease on inoculated wounds, we can nominate this product with a high priority for IR-4 residue studies in Sept. 2015.

RESEACH OBJECTIVES

1) Epidemiology – pathogen genetic variability, inoculum availability, threshold inoculum level for disease induction, systemic movement of Psv
   a. Monitor galls for production of inoculum over time
   b. Evaluate the effects of inoculum concentration on disease development
   c. Investigate environmental factors that may lead to systemic movement of Psv
   d. Track the systemic movement (endophytic or epiphytic) of Psv on the olive host using selective re-isolation techniques and microscopy
2) Evaluate populations of the pathogen for laboratory sensitivity to chemicals
   a. Population dynamics of copper-resistant in relation to copper-sensitive strains of Psv
3) Test the performance of an equipment sanitizer under field conditions once registration has occurred.
   a. Test the performance of the quaternary ammonium Deccosan 321 under field condition in comparison to chlorine
4) Field trials on the efficacy of bactericides and SAR compounds.
   a. Timing studies: Protective (pre-infection) vs. post-infection activity of treatments; proper timing and application of SAR compounds; effect of inoculum concentration on the efficacy of SAR compounds
   b. Develop copper activity-enhancing materials such as mancozeb, amino-thiadiazole (ATD), and dodine
   c. Determine the efficacy of a new, non-phenolic-based quaternary ammonium formulation (i.e., KleenGrow) for use as a protective treatment on olives
   d. Persistence of different copper formulations with and without the addition of lime, pinolene, or carnauba-based additives under simulated rain conditions

PLANS AND PROCEDURES

1) Epidemiology – pathogen variability, inoculum availability, threshold inoculum level for disease induction, systemic movement of Psv after infection

a) Pathogen variability. Newly isolated strains of Psv will be continued to be characterized for their genetic variability using repetitive primers in PCR reactions.

b) Monitor galls for production of inoculum over time. Previous studies will be repeated with olive galls attached to branches instead of detached galls. Galls will be drip-washed with a continuous low-volume flow of water, the runoff will be collected, and bacteria will be enumerated after different time durations. Age of galls in relation to inoculum production may also be studied in order to determine if galls eventually decline or if they continually produce Psv.

c) Evaluate the effect of inoculum concentration on disease development. Branches of cvs. Arbequina and Manzanillo olives will be wound-inoculated using different bacterial concentrations. Disease incidence and severity will be assessed on plants in the greenhouse and in the field.

d) Investigate environmental factors that may lead to systemic movement of Psv. Plants will be subjected to low temperatures (-5°C) for selected periods of time (4 to 12 h) after wounding and inoculation. Plants will then be transferred to the greenhouse and observed for disease development. Scanning electron microscopy (SEM) of frost-damaged olive twig sections will be performed to visualize bark injuries.

To track the movement of Psv and to determine if migration is systemically inside plant tissues or epiphytically, we will use selective re-isolation techniques and electron microscopy. Secondary wounds will be made at different distances from the initial inoculated wound. Development of knots and re-isolation of Psv from these wounds and from inside tissues distal to the original inoculation site will suggest systemic movement. Tissue samples will be taken after different incubation periods at different distances from the initial wound and examined using SEM. Inoculations may also be done with a copper-resistant strain to allow for discrimination of the inoculated isolate from potentially naturally present Psv strains (the majority of field strains are copper-sensitive).

2) Evaluate populations of the pathogen for laboratory sensitivity to chemicals

a) In vitro sensitivity studies. Newly acquired Psv strains will be tested for their in vitro sensitivity to chemicals including copper and kasugamycin. We will also evaluate potential copper-enhancing materials such as mancozeb, dodine, and thiadiazoles in mixtures with fixed and non-fixed copper formulations. These in vitro tests have the goal to identify treatments for locations with copper tolerance.

b) Population dynamics of copper-resistant in relation to copper-sensitive strains of Psv. Olive orchards where copper-resistant strains were obtained previously will be extensively re-sampled to determine the distribution and incidence of copper-resistance. Copper treatments in these orchards may induce shifts to a higher incidence of copper-resistance. Evaluation of copper resistance will also be evaluated after application of other treatments such as kasugamycin and copper mixtures with copper enhancing products.

3) Test the performance of a quaternary ammonium compound as an equipment sanitizer under field conditions.

Pending registration approval of Deccosan 321, field evaluations will be done in orchards with high levels of olive knot. Mechanical harvesters will harvest from diseased rows and then treated with Deccosan 321 and rinsed with water. The equipment will be sampled before and after sanitation, and populations of Psv will be enumerated. Alternatively, a healthy row of olives will be pruned, then the pruning equipment will be contaminated with Psv and sanitized and rinsed with water. A new healthy row will then be pruned and efficacy of the sanitation treatments will be determined based on olive knot development. Comparative studies with chlorine may also be done.

4) Evaluate the efficacy of protective treatments such as new copper formulations, antibiotics (Kasumin, Mycoshield), dodine, SAR compounds (acibenzolar-S-methyl - Actigard, PM-1, quinoxyfen, ProAlexin, Regalia), and combination treatments

a. Timing studies: Protective (pre-infection) vs. post-infection activity of treatments; proper timing and application of SAR compounds; effect of inoculum concentration on SAR compound efficacy. Previous studies with SAR compounds revealed that they were inconsistent in their efficacy. Different treatment timings before inoculation and different inoculum concentrations will be evaluated. The goal is to integrate SAR compounds with other chemical treatments to possibly provide prolonged protection against olive knot. Chemical treatments may be able to reduce Psv populations to lower levels that are sensitive to host defense activation by SAR compounds.

b. Develop copper activity-enhancing materials such as mancozeb, thiadiazole, and dodine. The detection of copper tolerance in Psv stresses the need to develop improved copper treatments and copper alternatives. In addition to copper hydroxide-kasugamycin mixtures that in previous selected trials performed better than either compound alone we will evaluate other copper activity enhancing materials such as mancozeb, dodine, and thiadiazoles. Olive branches will be wounded, treated, and then inoculated with a copper-tolerant Psv strain. These compounds will also be evaluated in vitro where media amended with a fixed copper concentration are further amended with potential copper-enhancing compounds using the spiral gradient dilution method. Bacterial suspensions will then be radially streaked onto the plate.

c. Determine the efficacy of a quaternary ammonium formulation for use as a protective treatment. Field trials will test the performance of a new quaternary ammonium compound (KleenGrow) as a protective treatment against Psv. KleenGrow has beneficial properties for use as a protective treatment on plant surfaces (wounds) because it does not evaporate, is effective as a biocide at low concentrations, and safer than other quaternary ammonia compounds. KleenGrow has been shown to be effective on a number of plant fungal and bacterial pathogens so its potential use on olive knot needs investigation. Cv. Manzanillo and Arbequina olive twigs will be wounded followed by treatment and inoculation or inoculation and treatment. Efficacy of treatments will be based on incidence of knots that develop at the point of inoculation.

d. Persistence of different copper formulations with and without the addition of lime, pinolene, or wax based additives under simulated rain conditions. Addition of the adjuvant NuFilm-P to kasugamycin in past studies failed to give adequate control in simulated rain studies. The addition of lime to copper (Kocide 3000) increased the persistence of copper under simulated rain conditions but reduced its efficacy. We will evaluate several other adjuvants (e.g., lime, Vaporgard - pinolene, Washgard - a carnauba wax) to

be mixed with different copper formulations or kasugamycin. Olive branches will be wounded, treated, simulated rain will be applied for selected times, and wounds will be inoculated with Psv.

References

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Budget Request:

Budget Year: 2015 Funding Source: Olive Board of California

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

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

J.E. Adaskaveg Date: Sept. 30, 2014

Originator's Signature (PI)

Katherine Borkovich Date: Sept. 30, 2014

Dept. Chair

(Riverside Campus)

Liaison Officer _____ Date: _____

Workgroup/Department: Olive / Plant Sciences, UC Davis

University of California

Division of Agricultural Sciences

CONCEPT PROJECT PLAN / RESEARCH GRANT PROPOSAL

Project Year: 2015-2016 Anticipated Duration of Project: 1 year

Project Leader: Judy Jernstedt, Professor Location: 212 Hunt Hall

Mailing Address: Plant Sciences, Mail Stop 1, UC Davis, Davis, CA 95616

Phone: 530-752-7166 FAX: 530-752-4361 E-mail: jjernstedt@ucdavis.edu

Project Title: Cell wall composition of stylar tissue: Where no pollen tube fears to go (continuation of 2014-2015 Research Priority: PGRs, with Focus on Pistils)

Cooperating Personnel: **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

Keywords: cell walls, immunocytochemistry, pistils, style, stigma, pollen, pollen tube, fruit set

Commodity(s): olives Relevant AES/CE/Hatch Project No.: CA-D*-PLS-6410-H

Problem and its Significance:

The research proposed is a continuation of the project begun with 2014-2015 funding, namely, the investigation of structural and developmental aspects of pollen tube growth in the style. The focus of our work this year has been pistils, a main component of the 2014-2015 PGRs research priority. The “big-picture” problem under investigation is that 'Manzanillo' olives often fail to set fruit in high temperatures, but cross pollination with 'Sevillano' pollen seems to reduce this failure. Researchers in Croatia reported that the limitation to fruit set is not ovule viability or the stigmatic surface. They suggested that the problem is the style. Their results may be relevant to California olives, but this is not certain since the Croatian scientists investigated ‘Oblica’ olives. The research under way in my lab focuses on ‘Manzanillo’ olives, with the goal of obtaining structural and developmental data to assess the pathway of pollen tube growth and

transmission through the style and into the ovary. Cell walls are the contact point between the pollen tube and stylar tissue, so our emphasis will be characterization of style cell wall composition and changes through development.

Failure to set fruit under high temperatures has increasingly become a problem for California olives and will likely persist if the trend of ten or more years of record high temperatures continues. If we (researchers, growers, and processors) knew more about this phenomenon and the biology involved, the industry, including olive breeders, will be better prepared to devise possible solutions.

Objectives: To evaluate microscopically the comparative localization of pectins in the styles of "Manzanillo" olive flowers (hand self-pollinated, unpollinated, and open pollinated). Pectins have been implicated in pollen tube growth processes and may function in pollen tube adhesion and guidance.

Plans and Procedures:

Plant Material: Established trees with verified varietal identity in the UCD orchards will be the source of material, with backup material available from the USDA National Clonal Germplasm Repository, Wolfskill, Winters, CA. Trees will receive irrigation and fertilization as in commercial orchards. Climatic data will be obtained from the UCD-LAWR weather station.

Pollination: When approximately 50% of the Manzanillo flowers are open, selected newly opened flowers will be self-pollinated (Treatment A), emasculated and covered to prevent stray pollen (Treatment B), or tagged but otherwise unmanipulated (Treatment C). The goal is 100 flowers for each treatment, spread over several days.

Sample collection: Beginning on the day of pollination and daily for the first week post-pollination, flowers will be detached, placed in plastic bags with most paper towels, and transported in a cooler to the lab. In the lab, outer floral parts (sepals, petals and stamens) will be removed. The pistil still attached to the pedicel will be fixed (chemically killed), dehydrated, and embedded for sectioning. Half the isolated pistils will be fixed in 2.5% glutaraldehyde, rinsed in phosphate buffer, dehydrated, and embedded in Technovit resin for sectioning at a thickness of 2 microns. The other half will be fixed in 4% paraformaldehyde, rinsed in PEM buffer, dehydrated, and embedded in LR White resin for sectioning at 1 micron thickness.

Staining and microscopy: To understand cell wall composition of styles, two approaches will be employed. Technovit resin sections will be stained with PAS (periodic acid-Schiff's) for carbohydrates and with PAS-toluidine blue O for general anatomical and histochemical examination. Other resin sections will be stained with 0.07% calcofluor for cellulose. LR White sections will be used for immunocytochemistry to examine the location and extent of specific cell wall pectins (methyl-esterified and non-esterified). Antibodies to these specific cell wall carbohydrates will bind to them and these will then be tagged with secondary antibodies bearing fluorescent labels. Sections will be observed by epifluorescence microscopy using appropriate filter sets. Images will be captured using a MicroFire digital camera.

Analysis: Sections will be examined for tissue integrity (vs. senescence), location and extent of transmitting tissue (stigma, style and ovary), and location and extent of positive immunostaining for cell wall components. Specific comparisons will be made between pollinated and unpollinated pistils and early and late time points post-anthesis.

Interpretation: Treatment A (manually self-pollinated Manzanillo flowers) will document the normal course of olive pollen tube growth in this cultivar in these growing conditions. Treatment B (emasculated and covered Manzanillo flowers) will document changes in the pistil when pollination and fertilization fail, to provide characteristics which might aid in recognizing and assessing early stages of pollination failure in Treatment A. Treatment C is included to assess the stages of natural, unmanipulated self-pollination and fertilization in Manzanillo olives. Comparison of Treatments A (self-pollinated) and C (unmanipulated) will allow assessment of artificial pollination versus natural self-pollination in terms of pistil condition and pollen tube growth through the style and into the ovary to the ovule. Failure or comparative irregularities at any of these stages will contribute to a mechanistic understanding of olive fruit set.

BUDGET REQUEST

Budget Year: 2014-2015

Funding Source: California Olive Committee (COC)

Salaries and Benefits:		
Postdocs/RAs		0
SRAs		0
Lab/Field Assistance (Undergraduate Student Assistants)		$14,000
Subtotal	Sub2	$14,000
Employee benefits: (1.3%)	Sub6	$ 182
	TOTAL	$14,182
Supplies and Expenses (chemicals, stains, slides, coverslips, confocal microscope time, SEM consumables, SEM beam time)	Sub3	$ 8,000
Equipment	Sub4	0
Travel	Sub5	300
	TOTAL	$ 8,300
UCD Overhead (11 %)		2,473.02
Total Budget		**$ 24,995.02**

Department account number:

Judy Jernstedt (signed) Date: 3 Oct. 2014

Originator's Signature

COOPERATIVE EXTENSION

County Director: _____ Date _____

Program Director: _____ Date _____

AGRICULTURAL EXPERIMENT STATION

Department Chair: _____ Date _____

UC COC LIAISON OFFICER: _____ Date _____

California Olive Committee

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Department: UC Agricultural and Natural Resources

Project Year: 2015-2016 Anticipated Duration of Project: One year

Project Title: Evaluation of Magnet^{TM} OL attract-and-kill device for efficacy against olive fruit fly and potential alternative to GF-120.

Project Leaders:

Dr. Dani Lightle, UC Farm Advisor, Glenn, Butte, and Tehama Counties, P.O. Box 697, 821 E. South St., Orland, CA 95963. 530-865-1153 (office), 530-936-7728 (cell), dmlightle@ucanr.edu

Dr. Emily J. Symmes, UC Area IPM Advisor, Sacramento Valley, 2279-B Del Oro Ave., Oroville, CA 95965. 530-538-7201 (office), ejsymmes@ucanr.edu

Cooperating Ranches:
Jeff Aguiar, Orland, CA, 530-865-2551
Gary Alves, Willows, CA, 530-624-5494

Commodity: Olive Relevant AES/CE Project No: n/a

Current Funding Request: $11,592

Problem and its Significance:

Recent increases in olive fruit fly population numbers have raised questions about possible alternative control measures to GF-120. One alternate control strategy, known as "attract and kill," consists of olive fly attraction to an insecticide coated surface that causes mortality after physical contact with the device. A variety of attract-and-kill devices are available on the market in Europe, where the control approach has been used for area-wide pest management (Petacchi et al. 2003), as well as individual orchards (El Sayad et al. 2009). In Europe, research has shown that devices may be used alone when fly densities are low, or along-side a reduced number of insecticide applications when fly densities are high.

A control device, the Magnet™ OL attract-and-kill "trap" (Suterra, LLC, Bend, OR), is available for purchase by growers and pest management districts, and a second attract-and-kill device (also manufactured by Suterra) is in the late research and development stage. The Magnet OL is the only attract and kill device registered in olives in the United States. Magnet OL uses a combination of olive fly sex pheromone and food attractant lures in combination with a surface treated with a pyrethroid (lambda-cyhalothrin). While Magnet OL has shown efficacy in trials in Europe (Ianotta et al. 2010), **neither device has been tested in independent controlled trials under California growing conditions**. Additionally, manufacturer recommendations for device density are 40 to 60 per acre, resulting in an **up-front cost to growers of $110 to $160 per acre, plus labor**.

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

Magnet OL devices are currently being used by the Glenn and Tehama County Pest Management Districts, as well as some growers in the Sacramento Valley and Coastal growing regions. Additionally, inquiries have been made to UCCE advisors about how Magnet OL devices can best be used, indicating growing interest in Magnet OL devices in the region. Some growers have also raised concerns that the device becomes less effective after three to four months than when first placed in the field. Knowledge of how Magnet OL or the experimental device (as a possible Magnet OL replacement) performs under California growing conditions is imperative for growers to make an informed decision before making the investment. In recognition of the need for regional data, **Suterra, LLC has offered to provide support for this study in**

addition to the COC funds requested in this proposal (*see letter of support*). This proposed study will evaluate the performance of the Magnet OL attract-and-kill device in northern California olive groves.

Objectives:

Objective 1. Evaluate the efficacy of Magnet OL and experimental (*tentative, pending R&D status*) attract-and-kill devices in reducing olive fly populations and damage in Manzanilla and Sevillano field plots in a key olive growing region of California.

Hypothesis 1: Field plots treated with attract-and-kill devices will have comparable olive fly numbers in reference traps and similar olive fly damage in harvested fruit compared to a GF-120 treated reference plot.

Objective 2. Determine the performance of the insecticide used in the attract-and-kill device under various field conditions.

Hypothesis 2: Exposure to differing conditions (sun, shade) will cause the insecticide to break down at different rates, thereby influencing fly mortality.

Plans and Procedures

Objective 1: Efficacy of attract-and-kill devices for olive fly control.

Experimental design: This study will be replicated in three olive groves in located in the Sacramento Valley. The following treatments will be evaluated in 7 to 10 acre plots:

- Treatment I: Magnet OL devices (sex pheromone and food attractant lures in combination with lambda-cyhalothrin treated surface) distributed uniformly throughout the plot at a manufacturer's recommended rate of 40 to 60 devices / acre.
- Treatment II: Experimental attract-and-kill device (sex pheromone and food attractant lures in combination with insecticide treated surface) distributed uniformly throughout the plot at the manufacturer's recommended rate of 40 to 60 devices / acre. *This treatment is tentative*, depending on R&D status.
- Treatment III: Standard GF-120 treated plot with no lures or control devices. Frequency of GF-120 application will be based on standard decision-making practices within that orchard.

Devices and lures will be deployed in field plots when olive fly is increasing in McPhail reference traps baited with Torula yeast (roughly May 2015), as this is the manufacturer's

recommended time frame for hanging Magnet OL in the field. A subset of devices in treatments I and II will be equipped with a thin band of translucent material covered with Tanglefoot to estimate the numbers of olive flies and non-target species (particularly natural enemies common in olive systems) landing on the devices.

Data collection and analysis: Efficacy of the devices will be determined by (1) monitoring olive fly densities using reference traps distributed throughout each treatment plot, (2) estimating the number of olive flies and non-target species landing on the devices, and (3) assessing the number of stings at harvest.

1) Olive fly populations in each plot will be monitored using reference traps (McPhail traps baited with Torula yeast pellets) at a rate of 10-15 reference traps / plot. Traps will be checked weekly and the number of male and female olive flies counted and compared among treatments using standard statistical methods.
2) Olive flies and other species trapped in the sticky material will be counted weekly. Estimates of the numbers of olive flies and non-target species landing on the devices will be extrapolated based on these data.
3) Harvest samples will be collected (minimum of 2200 Manzanillas or 1000 Sevillanos per acre within the treatment plot) and visually examined for olive fly stings. The number of fruit with sting(s) will be compared among treatments using standard statistical methods.

Objective 2: Performance of insecticide in Magnet OL devices.

Experimental design: This lab experiment will be replicated five times with the following treatments:

- Treatment I: Device placed outdoors with full sun exposure.
- Treatment II: Device placed outdoors in full shade (no sun exposure).
- Treatment III: Device placed inside the laboratory with stable temperatures, protected from high heat and/or UV exposure, and kept dry.
- Treatment IV: Control devices kept in original packaging and stored according to manufacturer's recommendations until comparison.

Devices will be placed in their respective treatment conditions at the same time as deployment of devices in Objective 1 and bioassays conducted in the laboratory six months after deployment.

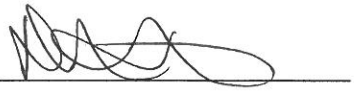
Data collection and analysis: Bioassays using field-collected olive flies will be used to determine the performance of the insecticide for each treatment. Flies will be exposed to the insecticide-treated surfaces and mortality and time until death and compared among treatments using standard statistical methods.

Budget Request

Budget Year: 2015

Funding Source: COC

Salaries and Benefits:		$0
Postdocs/ RA's:		$0
SRA's:		$0
Lab & Field Assistance:		
1 assistant for 26 weeks, 12 hrs / week @ $14.57/ hr		$4,546
Employee benefits:		
Assessed @ 52.7%		$2,397
	Sub1	**$6,943**
Supplies and Expenses:		
Suterra Magnet OL devices		$250
Monitoring supplies		$600
Lab bioassay supplies		$150
Equipment:		$0
	Sub2	**$1,000**
Travel:		
Travel to/from field sites @ $0.56/ mile		$2,500
	Sub3	**$2,500**
UC Overhead at 11%		$1,149
Total		**$11,592**

Originator's Signature:  Date 10/31/14

COOPERATIVE EXTENSION

County Director: Beth Kauk Date 10-31-14

Program Director: N/A Date _____

AGRICULTURAL EXPERIMENT STATION

Department Chair: N/A Date _____

UC COC LIAISON OFFICER: _____ Date _____

Olive Workgroup / Department of Botany and Plant Sciences, UCR

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year: 2015

Anticipated Duration of Project: 1 remaining year of this 2-year project. We have harvested the Year 1 OFF- and ON-crop control trees and ON-crop trees treated with PGRs. Now we need the harvest for the year following treatment in order to determine the efficacy of the PGR treatments as foliar sprays to increase yield in the putative OFF-crop year.

Project Leaders: Carol Lovatt and Elizabeth Fichtner

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

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

Location: Lindcove Research and Education Center, Exeter

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

Cooperating Personnel:
Lindcove Research and Education Center, Exeter

Keywords: Alternate bearing, ON-crop trees/year, OFF-crop trees/year, correlative inhibition, vegetative shoot extension growth, bud break, staminate flowers, perfect flowers, inhibition of floral development, plant growth regulators, fruit removal

Commodity(s): Table Olive **Relevant AES/CE Project No.:** 4556H

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

There is a recurring need to mitigate the problem of alternate bearing. Alternate bearing is initiated by external factors (freeze; lack of chilling; low or high temperatures at bloom,

pollination or fruit set; diseases etc) that cause poor flowering or pollination or excessive flower and fruit drop, resulting in an OFF-crop that is typically followed by an ON-crop, depending on how long it takes the trees to recover from the stress causing the loss of yield. Conversely, optimal conditions during bloom and fruit set such that normal crop thinning fails to take place result in an ON-crop, which is followed by an OFF-crop. Since climate is a factor initiating AB, the need for a corrective strategy reoccurs.

Summary of research results to date. The results of our earlier COC-funded research have provided the following information about alternate bearing in olive, confirming the report of Sibbett (2000) and providing significant new information.

- Summer vegetative shoot growth is inhibited in a manner directly related to total fruit number (crop load) (Sibbett 2000).
- Inhibition of summer vegetative shoot extension growth reduces the number nodes (sites) that can bear inflorescences the next spring (Sibbett 2000).
- Nonbearing shoots that do not set fruit in spring on OFF-crop trees produce the greatest amount of summer shoot extension growth and the greatest number of inflorescences the next spring.
- Fruit exert both a localized effect on the shoots that set them and a whole tree effect attributable to the total number of fruit on the tree.
- Bearing shoots, which are the majority of the shoots on ON-crop trees, produce the least amount of summer vegetative shoot extension growth and fewest inflorescences the following spring due to the combined inhibitory effects of fruit set on the shoots (localized effect) and all the fruit on the ON-crop tree (crop load effect).
- Nonbearing shoots, which are few on ON-crop trees, are subject only to the inhibitory effect of the On-crop on the tree and produce an intermediate amount of summer vegetative shoot growth and inflorescences at return bloom compared to nonbearing shoots on OFF-crop trees and bearing shoots on ON-crop trees (Table 1). **This is an important fact:** *For ON-crop trees, the return bloom and crop is produced by buds on shoots that did not set fruit; shoots that set fruit contribute little return bloom. Buds on nonbearing and bearing shoots on ON-crop trees are physiologically different.*
- Our results also suggest that fruit inhibit spring bud break in a manner related to crop load. Spring PGR treatments increased inflorescence number to a greater degree on nonbearing shoots than bearing shoots on ON-crop trees.
- Our results identified PGR treatments that significantly increase the number of inflorescences produced by nonbearing shoots on ON-crop trees 2-fold compared to nonbearing shoots on untreated ON-crop control trees and 40% greater than nonbearing shoots on untreated OFF-crop control trees. These same PGRs increased the number of inflorescences produced by bearing shoots on ON-crop trees 5-fold compared to bearing shoots on untreated ON-crop control trees. However, this degree of flowering was still only 1/3 to 1/4 of the maximum flowering attained by nonbearing shoots on OFF-crop control trees. **This is an important fact:** *It confirms that buds on nonbearing and bearing shoots on ON-crop trees are physiologically different.*
- Results of the fruit removal experiment that is part of this project (fruit are removed monthly through harvest October) revealed that the greatest amount of summer vegetative shoot extension growth occurs between June and July. Removing the fruit progressively later reduces shoot growth. **This is an important fact:** *In our current experiment, PGR treatments designed to stimulate vegetative shoot growth were applied in early June, earlier than we previously applied them, with a second application in early July.*
- Progressively later fruit removal reduced the number of inflorescences that developed per shoot, with bearing shoots producing significantly fewer inflorescences than nonbearing

shoots in each case. **The results provided two important facts:** *Fruit must be removed from ON-crop trees by mid-August to avoid a significant reduction in the number of inflorescences produced by nonbearing shoots on ON-crop trees. September fruit removal is too late to significantly increase bloom.*

- For nonbearing shoots on 2013 OFF-crop trees, 52%, 35% and 7% of buds at the first 8 node pairs, second 8 node pairs and basal 5 node pairs produced inflorescences in spring 2014 – the ON bloom. Surprisingly, 43%, 50% and 59% of the buds along the shoot remained inactive, respectively. In contrast, for bearing shoots on 2013 ON-crop trees, 75%, 76% and 71% of the floral buds had abscised from these shoot sections, respectively, one month before the 2014 bloom. For nonbearing shoots on the 2013 ON-crop trees, 89%, 67% and 63% of the floral buds along the shoot remained inactive (dormant) at spring bloom 2014, respectively. **The results provided three important facts:** *Bud abscission on bearing shoots is the main cause of low return bloom following the ON-crop year. The only source of return bloom following the ON-crop year is from nonbearing shoots; thus, it is critical to know if the buds on these shoots are viable floral buds that are merely inactive (dormant due the accumulation of hormones). PGR treatments must not only increase summer vegetative shoot growth during the ON-crop year, they must also increase floral bud retention and overcome floral bud dormancy to increase spring bud break and bloom.*
- By September 13, 2014, new growth on bearing shoots of OFF- and ON-crop trees had 50% fewer nodes to bear potential floral buds for next spring's bloom than nonbearing shoots on OFF- or ON-crop trees. Shoot extension growth of bearing shoots on OFF- and ON-crop trees stopped in June. For the new growth on nonbearing and bearing shoots on OFF- or ON-crop trees, 90% of the nodes developed buds and 98% of those buds were still on the tree through September 13, 2014. **The results provided one important fact:** *Floral buds do not abscise in summer, but abscise at a later time still to be identified. Identifying when floral buds abscise is critical to developing a corrective strategy.*

Objectives for Year 2: Objective (1) - to determine whether buds on bearing shoots on ON-crop trees are viable floral buds only inhibited from undergoing bud break or become nonviable floral buds due to inhibition of floral development by quantifying the expression of key genes in the floral development pathway. Objective (2) to test the ability PGR treatments applied as foliar sprays to break the AB cycle by increasing vegetative shoot length (node number) during the ON-crop year, increasing floral bud retention and increasing spring bud break to increase floral intensity and yield the year following the ON-crop to produce high back to back yields. ***NOTE:*** We applied the first part of the PGR treatments in summer 2014, the ON-crop year, and will apply the second part in February 2015. The final results are obtained with the harvest of 2015.

Plans and Procedures: *To meet Objective 1 of Year 2,* we will complete research quantifying floral gene expression to determine whether buds on bearing and nonbearing shoots on ON-crop trees are viable (floral gene expression is normal relative to nonbearing shoots on OFF-crop trees) or are not viable (floral gene expression is inhibited relative to nonbearing shoots on OFF-crop trees). We have a student who is conducting this research for his MS thesis. He has his own personal support. The buds necessary to complete this research have been collected. Samples have all been ground. All primer pairs necessary to complete the research are available and we have confirmed their validity by DNA sequencing of the PCR products and also by quantitative real time polymerase chain reaction (qRT-PCR) analyses of total RNA extracted from leaves, buds and flowers. Little genetic information is available for olive. We had to create degenerate primers. We are able to follow the expression (accumulation of mRNA) of genes that promote flowering [*FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS* (*SOC1*)] and floral organ identity genes *PISTILLATA* (*PI*) and *AGAMOUS* (*AG*), the final gene in the pathway required for carpel formation. The expression of olive actin and

ubiquitin serve as reference genes to normalize the data. He is nearly done but we need to do some additional sampling to fully understand the results of the inactive buds on nonbearing shoots on ON-tree. *To meet Objective 2 of Year 2,* we are testing the ability of the four best PGR treatments (identified in our branch-injection research) as foliar sprays applied in summer and spring to increase inflorescence number and yield following the ON-crop year. The goal is to produce strong blooms and good yields back to back. The PGR treatments being tested are: The following PGR treatments were applied during the first week of June and again the first week of July (to increase shoot growth as node number, floral bud number and floral bud retention) and will be applied again in February (to increase spring bud break): (1) ON-crop control trees; (2) ON-crop trees treated with 6-BA + low-biuret urea; (3) ON-crop trees treated with cytokinin X (a natural product) + low-buiret urea; (4) OFF-crop trees treated with cytokinin X; and (5) OFF-crop control trees. The PGRs were applied at 0.9 g/tree, low-biuret urea at 0.18 kg N per tree. In our previous proof-of-concept research in two different orchards, 6-BA and cytokinin X were as effective when used alone as when they were combined with an auxin-transport inhibitor in significantly increasing the number of inflorescences produced by nonbearing shoots on ON-crop trees the following spring; the increase in flowering by bearing shoots on ON-crop trees was increased but not significantly. We are testing the cytokinins with low-biuret urea for three reasons: (1) to supply N to support PGR-stimulated shoot growth; (2) 6-BA plus urea increases floral bud retention 2.5- to 3-fold during the ON-crop year of pistachio and increases yield to the same degree (this use is on the Valent BioSciences 6-BA label; if successful, it should be easy to add olive to the label); and (3) to increase the potential for either cyokinin to be available to olive growers sooner, we used them alone without the complication of a second hormone, the auxin-transport inhibitor. All foliar-applied PGR treatments, including the ON- and OFF-crop control trees, were replicated on 15 individual trees in a randomized complete block design. At bloom in 2015, we will quantify the number of floral shoots on five shoots with and without fruit on untreated ON- and OFF-crop control trees and PGR-treated trees at the Lindcove REC. During bloom in 2015, we will also sample flowers to determine treatment effects on the number of perfect vs. staminate flowers. Results obtained the branch-injected PGRs provided evidence that several PGR treatments increased the number of perfect flowers. This needs to be confirmed. Taken together, these data will help to identify the best PGR treatment/s and the optimal time to spray the foliage, and determine whether or not fruit thinning will be required in the future to mitigate AB in olive.

Expected outcomes: If olive responds to these PGR treatments like ‘Nules’ Clementine mandarin and ‘Hass’ avocado, we anticipate that foliar-applied PGR treatments applied in summer plus spring will increase fruit size of the olives on ON-crop trees (and possibly yield, which occurs with avocado but not citrus) and increase yield and the yield of large size fruit in the following year, such that 2-year cumulative total yield and 2-year cumulative yield of large size fruit is greater than that of untreated ON- and OFF-crop control trees. We anticipate that the results will indicate that trees should be treated annually.

Literature Cited:

Dag, A., A Bustan, A. Avni, I. Tzipori, S. Lavee and J. Riov. Timing of fruit removal affects concurrent vegetative growth and subsequent return bloom and yield of olive (*Olea europaea* L.). Scientia Hort. 123:469-472.

Sibbett, S. 2000. Alternate bearing in olive trees. California Olive Oil News. Vol. 3, Issue 12.

BUDGET REQUEST – Lovatt and Fichtner
Budget Year: 1 February 2015 – 31 December 2015

Funding Source: California Olive Committee

Salaries and Benefits:

Postdocs/RA's

Toan Khuong- Assistant Specialist @ $ 4,362/mo. variable time equivalent to 100% x 1 mo. 4,362
(Under my supervision, assists in treatment applications, bloom data collection, data sheets, monthly data management, monthly data entry, and monthly statistical analyses of the data.)

SRA's and Lab/Field Assistance

Lab Assistant I @ $15.07/hr. x 300 man-hrs. 4,521
(Preparation of treatment solutions, treatment applications, monthly data collection on the fate of buds, bloom data collection, harvest, and fruit size determination.)

Graduate Student Researcher (GSR); Student has his own personal support. 0
(Under my supervision, the GSR will conduct the research on floral gene expression to determine if floral buds are viable but inhibited from undergoing bud break or become nonviable due to inhibition of floral development (floral gene expression) for his MS thesis to be completed in December 2014.)

Subtotal	Sub2	8,883
Employee benefits:		
TK = $4,362 x 76.11%	3,320	
Lab Asst I = $4,521 x 2.76%	125	
GSR = 0	0	
	Sub6	3,445
	TOTAL	12,328
Supplies and Expenses	Sub3	6,720

Recharge commitment to the Lindcove REC: use of olive grove, water, fertilizer, and harvest etc calculated by Anita Hunt at Lindcove for 2014-2015.

Equipment	Sub4	0
Travel	Sub5	4,218

7 roundtrips to Exeter
(520 mi x 7 = 3,640 mi x $0.6014 = $2,189;
UCR vehicle rental 15 days x $47.268/day = $709
$10/person/night per Lindcove Trailer x 3 people x 8 nights (all trips are 2 days; bloom count is 3 days) = $240
Meals @ $45/1.5 days/person/trip x 3 people x 8 days (all trips are 1.5 days; bloom count is 3 days) = $1,080

SUBCONTRACT

Sub7 10,091

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

Department account number: AO1082 TOTAL 33,357

Carol Lovatt

Originator's Signature Date 10/14/2014

COOPERATIVE EXTENSION

County Director: _____ Date _____

Program Director: _____ Date _____

AGRICULTURAL EXPERIMENT STATION

Department Chair: Michael L Roose Date 10/17/14

UC COC LIAISON OFFICER: _____ Date _____

BUDGET SUBCONTRACT

Budget Year: 1 February 2015 – 31 December 2015

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

Funding Source:

Salaries and Benefits:

Lab Assistant I and Student Assistant I

(Y. Martinez, Student Assistant I and B. Dougherty, Lab Assistant I, hourly rate of $10.75 and $14.60, respectively. Core benefit rate of 5.5% as limited term employees. Total hours=300 hrs.

		8,023
Lab Helper from the Lindcove REC (40 hrs x $13.45/hr, includes EB)		538
Subtotal	Sub2	8,561
Employee benefits:	Sub6	0
	TOTAL	8,561
Supplies and Expenses	Sub3	200
Field supplies: bags, ribbons, digital calipers		
Equipment	Sub4	0
Travel		
(Mileage @ $0.55/mile travel to UCCE grower meeting in Butte Co.	Sub5	$330
	SUBTOTAL	9,091
UC ANR Overhead @11%	Sub6	1,000

$9,184 x 11% =

SUBCONTRACT TOTAL $10,091

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

Elizabeth J. Fichtner

Date 10/16/2014

Originator's Signature

COOPERATIVE EXTENSION

County Director: _____ Date 10-16-14

Program Director: _____ Date _____

AGRICULTURAL EXPERIMENT STATION

Department Chair: _____ Date _____

UC COC LIAISON OFFICER: _____ Date _____

Ern's Pest Control

Project Plan/ Research Grant Proposal

Project Year: 2015

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

Commodity: Olive

Problem and its Significance:

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

Objectives:

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

Plans and Procedures:

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

Budget Request

Budget Year: 2015

Funding Source: California Olive Committee
Salaries_____$4700
Supplies and Expenses: Trapping Supplies_____$ 300
Travel 2545 mi. @ $.55/mi._____$1400
This may vary due to fuel prices

Total_____$6400

Originator's Signature_____

Ernie Simpson

FLEISHMANHILLARD

2015 RESEARCH RECOMMENDATION

Presented December 1, 2014

PROGRAM:

NUTRITION RESEARCH ASSESSMENT

- Nutritional and health benefits assessment of research concerning olives (non-COC funded)
- Work with USDA on evaluation of research and nutrition messaging

Budget: $12,000

CALIFORNIA OLIVE COMMITTEE

FINAL 2013 YEAR RESEARCH REPORT: ONGOING PROJECT
UPDATED 7/09/2014

Workgroup/Department: Olive / Plant Sciences, UC Davis

Project Year 2015 Anticipated Duration of Project: 3/10 year

Project Title:
Propagating Dwarfing Olive Rootstocks and Establishing a Long Term Orchard

Project Leaders:

Louise Ferguson, Extension Specialist, Department of Plant Sciences, 2037 Wickson Hall, Mail Stop II, UC Davis, 1 Shields Ave., Davis CA 95616, (530) 752-0507 [Office], (559) 737-3061 [Cell], LFerguson@ucdavis.edu
Dr. John Preece, Curator, USDA National Clonal Germplasm Repository, Davis CA. John.Preece@usda.ars.gov

Cooperators:
Dr. Tziano Caruso, University of Palermo, Palermo, Italy: Tziano.Caruso@unipa.it
Dr. Guiilana Marino, University of Palermo, Palermo, Italy: giulia.marino@unipa.it
Mr. James M. Jackson: Principal Superintendent, Plant Sciences Field Facility, UC Davis CA JMJackson@ucdavis.edu: (530)-753-2173 and (530)-681-2279

Commodity: Olive Relevant AES/CE Project No.

Year Initiated: 2013 Current Funding Request**: 13,245.00**

Problems and Significance:
To facilitate mechanical harvesting the newest table olive orchards are planted in hedgerows and are mechanically pruned to keep the trees small.

Hedgerow plantings and mechanical harvesting could be facilitated if Manzanillo scions could be grafted onto dwarfing rootstocks that kept them small.

The olives with slow growth and therefore potential for use dwarfing rootstocks are: Nikitskaya, *Olea cuspidate* Verticillium resistant Oblonga Seedling and Dwarf D.

We proposed to propagate these rootstocks, establish an orchard on the UC campus in coordination with the National Clonal Germplasm Repository and test them for their dwarfing potential with 'Manzanillo' to produce a tree that is more amenable to mechanical harvesting.

Progress to November 7th 2014:

Progress through 11/07/2014:

This application for initial funding was for two purposes:

I. Propagation and grafting of the rootstocks with 'Manzanillo' scions.

a. Dr. John Preece supervised the development of specific propagation techniques for 112 each of the following olive cultivars to be used as dwarfing rootstocks; Nikitskaya, *Olea cuspidate,* Verticillium Resistant Oblonga and Dwarf D. Dr. Pilar Rallo, partially supported by 2013 season funding, participated in this work.

II. **Established a 4-acre, split plot 'Manzanillo' table olive orchard in at the UC Davis Plant Sciences Field Facility.** The orchard was planted at 8 X 10 feet in the southern half and 10 X 15 feet in the northern half of the split plot block. The 13 tree sets of four different dwarfing rootstocks, and self grafted and ungrafted 'Manzanillos' planted at both tree spacings were replicated four times. Each block was surrounded by 'Sevillano' pollinizers; Attachment I: Field 3556 Plot Map

III. Lacking and died in the field since planting: will be replaced in spring 2014.

a. 47 perimeter 'Sevillano' pollinizers, 45 not delivered and 2 died.
b. 1 'Manzanillo' (died)
c. 3 'Oblonga' (died)
d. 5 'Nitskya' (died)
e. 40 *O. cuspidate* (not propagated) and 2 (died)
f. 63 'Dwarf D' (not propagated) and 9 (died)

Fig. 1. The four acre dwarfing rootstock trial on August 9th, 2014, three months after planting on the north side of Hutchinson Road on the University of California Davis campus.

Objectives: 2015

I. **Propagate and plant missing 47 missing border row Sevillano pollinizers.**
II. **Establish a three-wire trellis and begin training.**

The UC staff will install the system: installation and includes materials and labor
The system will be installed between February and June 2015. The trellis system is designed to support a 16-foot anchored end post pole with metal stakes at each tree (already installed) and 3, 16-foot posts within the row (every 10 trees) Three permanent wires (11 gauge) will be secured to the end posts and attached to the metal tree stakes at 1, 2 and 3 m.

III. **Graft the Manzanillo scions to the rootstocks in winter/spring 2014 - 2015.**
IV. **Begin initial growth measurements after grafting.**

BUDGET REQUEST: 2015

BUDGET REQUEST – Part I

Budget Year: 2014 - 2015 Funding Source: COC

Continuing Orchard Establishment Costs:		
Equipment Supplies & Expenses:		
Olive rootstock propagation costs		1,500.00
Orchard Maintenance Costs:		
700.00 per acre X 4 acres		2,800.00
Additional Sevillano trees:		
50 @ 6.00 each plus transport @ 500.00		800.00
Installation of Trellis: Materials and Labor:		
2,000 per acre X 4 acres		8,000.00
	Sub 1	**13,100.00**
Travel and Extension Costs:		
Sub 2		**13,100.00**
UC Overhead @ 11%:		**1441.00**
Sub 3		**13245.00**
TOTAL BUDGET		**13,245.00**

UNIVERSITY OF CALIFORNIA

Originator's Signature — Date: 11/06/2014

Agricultural Experiment Station

Department Chair — Date

Liaison Officer — Date

Scope of Work

Dr. Louise Ferguson:
Responsible for overall coordination of the project and directing orchard establishment in cooperation with UC Plant Sciences Field Crew. Collecting and analyzing data and writing up research reports.

Dr. John Preece:
Responsible for directing propagation of rootstocks, and being Co-PI for data collection, analysis and report writing.

James Jackson and Richard Pelzer:
Responsible for maintaining the orchard and field operations.

CALIFORNIA OLIVE COMMITTEE

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Workgroup/Department: Olive / Plant Sciences, UC Davis

Project Year 2015 Anticipated Duration of Project: 7 years

Project Title: Determining Optimal Hedgerow Canopy Height for Yield and Fruit Quality

Project Leaders:

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

Daniele Lightle: Glenn County Farm Advisor: DLightle@UCANR.Edu

Cooperating Ranches:
Nickels Soils Laboratory; Arbuckle: Stan Cutter; CutterFarms@Frontier.net

Commodity: Olive Relevant AES/CE Project No.

Year Initiated: 2013 Current Funding Request**: 31,871.00**

Problems and Significance:

It has been experimentally demonstrated that both trunk shaking and canopy contact harvesters are more efficient in orchards prepared with mechanical pruning. However, mechanical topping is proving unreliable. Unlike hedging it does not reliably produce crop the year after topping. For example, late winter topping, due to greater vegetative growth, will not reliably produce a crop the following year. Our trials have demonstrated topping produced vigorous growth with limited fruit and resulted in two problems. First, 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%. Second, even when the tree is topped every other year the growth is so strong the tree is too tall for efficient harvesting by both the canopy contact harvesters and trunk shaking harvesters. The solution appears to be to top the tree annually with a gabled cut to eliminate this overly vigorous growth and overripe fruit.

We propose to investigate the effects of a mechanical pruning program that incorporates of annual topping at three different tree heights with 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 and harvesting. Once it is demonstrated which height is optimal at this latitude; we can then extrapolate the light interception at different latitudes and design orchards for maximum light interception and therefor production, at other latitudes.

Materials and Methods:

Experimental Plot: (map attached)
Nickels Estate: 2 acre Manzanillo orchard established in 2002.

Hypothesis: mechanically topping hedgerow olive orchards will not decrease yield and will produce more uniformly ripened olives for mechanical harvesting.

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.

2012 Objectives:

I. Apply thee different tree height pruning treatments
II. Install sunlight exposure monitoring cameras
III. Evaluate effect of pruning treatments on bloom quality: perfect versus imperfect.
IV. Evaluate effect of pruning treatments on yield and fruit quality in upper and lower canopy at harvest.
V. 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 8,10 and 12 feet in February 2015
    - middle row of each treatment will be the data row
    - alternate row side hedging treatments established in 2011 will be continued:
        - scheduled for west side hedging in 2012
- Data Collection:
- Wingspan 24 hour monitoring cameras will be positioned at the northern end of 10-tree sets to monitor the E-W sun exposure of the canopies on both sides of the row.
- At bloom the canopy will be evenly divided into upper and lower quadrants
    - 5 shoots per tree will be collected at full bloom and evaluated for perfect vs. imperfect flowers
- A late season midday light interception measurement will be done to determine the percentage of light each treatment is intercepting.
- At harvest the upped and lower tree quadrants from the west and east sides of each 10-tree set will hand harvested separately and submitted for grade separately.
- Data Analysis:
    - The following relationships will be evaluated statistically for the east and west, upper and lower quadrants, within the three pruning treatments:
        - Effect of pruning treatment on ratio of perfect to imperfect flowers
        - 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.

As the 2015 olive shoots grew under 2014 sunlight and cropping conditions, and dormant chill, we do not expect strong light exposure results in 2015. However we do expect to see stronger correlations starting in 2016.

Desired Results:

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.

BUDGET REQUEST – Part I

Budget Year: 2015-2016 Funding Source: COC

Salaries & Benefits:		
Daniele Lightele: Glenn County Farm Advisor: Technical Support		12,000.00
Maintain cameras, data collection and entry, harvest support.		
	Sub 1	**12,000.00**
Sub 2		
Equipment Supplies & Expenses:		
24 Wingstop Time-Lapse Cameras @ 150.00/camera + peripherals		3,6000
http://www.wingscapes.com/wingscapes-timelapsecam		
(monitor sun exposure of the trees throughout the day)		
Pruning and Harvesting Costs: (based on previous year's cost)		
Mechanical (ENE Inc.), and hand pruning, brush shredding: Nickels Estate		2,500.00
Hand harvest and hauling at Nickels Estate: Contracted through Nickels		7,500.00
Miscellaneous harvest supplies: water, gloves, tarps, buckets		1,000.00
Total pruning and harvesting costs:		**11,000.00**
Sub 3		**14,600.00**
Experimental Travel Costs:		
Travel support for plot set-up, data collection, harvesting.		**2,112.00**
(8 months X 4 RT/month @ 120 miles/trip X .55/mile)		
	Sub 4	**28,712.00**
UC Overhead @ 11%	**Sub 5**	**3,159.00**
TOTAL BUDGET		**31,871.00**

UNIVERSITY OF CALIFORNIA

Originator's Signature — Date: 11/08/2015

Agricultural Experiment Station

Department Chair — Date

Liaison Officer — Date

Scope of Work

Dr. Daniele Lightele:
Responsible for overall coordination of the project, applying pruning treatments, executing harvest trials, data collection and analysis and writing final report.

Dr. Louise Ferguson: Responsible for directing mechanical pruning treatment in Nickels trial and co-coordinator of harvesting trial in Colusa County.

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

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year: 2015 Anticipated Duration of Project: one year

Project Leader: C. H. Pickett Location: UC Riverside quarantine
Mailing Address: CDFA, 3288 Meadowview Rd., Sacramento, CA 95832
Phone: 916.262-2053 FAX:916.262-2059 E-mail:cpickett@cdfa.ca.gov

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

Cooperating Personnel: Mr. John M. Jones and Dr. Mark Hoddle

Keywords: olive psyllid, biological control
Commodity(s): olive fruit Relevant AES/CE Project No.:

Problem and its Significance:

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

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

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

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

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

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

Objectives:

1. Complete host specificity testing of *P. euphyllurae*.
2. Survey California for the northern spread of olive psyllid.
3. Obtain a field release permit for the above parasitoid and release it into southern California.

Plans and Procedures:

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

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

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

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

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

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

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

Host Testing.

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

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

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

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

Releases.

Upon completion of studies, a field release permit will be requested from the USDA APHIS. Once a permit is obtained, parasitoids will be released during spring months onto trees infested with olive psyllid in southern California. Past surveys of infested trees in southern California have found no native parasitoids or predators feeding on these psyllids. Release trees will be monitored for establishment and impact of released parasitoids for as long as funding is available.

State Survey

A statewide survey of olive trees will be conducted spring 2015 to determine how far olive psyllid has spread within southern California, and northward along the coast. The psyllid will most likely infest coastal areas first, following a pattern observed in the southern infested counties. A coastal transect will follow the California Spanish Missions, including olive trees found at Missions and other areas with known populations of trees. Up to fifteen trees will be examined at each Mission location for the presence of olive psyllid. Any psyllids found on trees will be placed in 75% alcohol and returned to Sacramento for verification of identification.

References

Aversenq, S., C. Gratraud, and C. Pinatel. 2005. Ravageurs et auxiliaires des oliviers, synthèse de trois ans d'observations dans le Sud-Est de la France. Phytoma 586: 32-36.

Garcia-Mercet, R. 1921. Fauna Iberica. Himenópteros: Fam. Encirtidos. Junta para la ampliación de estudios e investigaciones cientificas. Instituto Nacional de Ciencias. Madrid, Spain.

Hoddle, M. S. and R. Pandey. 2014. Host range testing of *Tamarixia radiata* (Hymenoptera: Eulophidae) sourced from the Punjab of Pakistan for classical biological control of *Diaphorina citri* (Hemiptera: Liviidae: Euphyllurinae: Diaphorini) in California. Journal Economic Entomology 107: 125-136.

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Percy, D. M., A. Rung, and M.S. Hoddle. 2012. An annotated checklist of the psyllids of California (Hemiptera: Psylloidea). Zootaxa 3193: 1-27.

Tzanakakis, M. E. 2006. Insects and Mites Feeding on Olive, Distribution, Importance, Habits, Seasonal Development and Dormancy. Koninklijke Brill NV, Leiden, The Netherlands. 182 pp.

BUDGET REQUEST

Budget Year: 2015

Funding Source:

Salaries and Benefits:			_____
Postdocs/RA's			_____
SRA's			
Lab/Field Assistance (12 months)			$30,393
Subtotal		Sub2	$30,393
Employee benefits		Sub6	$3,411
		TOTAL	$33,804
Supplies and Expenses		Sub3	_____0
Equipment		Sub4	_____0
Travel		Sub5	$1500
	Total		$35,304

Department account number:

_____ Date_____

Originator's Signature

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project year: 2015 Anticipated Duration of the project: April –November 2015

Project Leader: Jim Stewart Location: Tulare County
Mailing Address: PO Box 1095, Exeter CA 93221
Phone: (559) 730-6243 FAX: (559) 592-4105 E-mail: jsagipmc@verizon.net

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

Cooperating personnel: Bert Quezada, Doug Bigham

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

PROBLEM AND ITS SIGNIFICANCE:

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

OBJECTIVES:

The objective of this project would be to continue the monitoring program of adult OLFF in commercial olive groves in the Southern San Joaquin Valley. Detection and seasonal monitoring of OLFF and the accurate timing of control measures, primarily bait sprays, would be the goal of this project. Correlation of fly collections with fruit susceptibility to infestation would indicate to growers when initial bait treatments should be applied. In addition, monitoring would continue to give growers information on the general OLFF population. This information would 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 same nine sites used in the years 2007 to 2014 in commercial olive groves will be set up with traps in April of 2015. The locations will be Ivanhoe, Woodlake, Exeter, South Exeter, Tonyville, West Lindsay, Strathmore, Porterville and Terra Bella. In addition, a site in the city of Visalia would also be monitored. All of these sites are in Tulare County where a high percent of the commercial olives are located in the Southern San Joaquin Valley. Some of the sites have been monitored starting in 2001. All traps will be in place by the first week of April and the program will end the last week of November. Two yellow panel traps with ammonium carbonate bait and male pheromone will be used per site. Traps will be serviced and OLFF counted weekly. Reports detailing the number of OLFF found at each location will be submitted to the California Olive Committee and interested parties within 24 hours on a weekly basis.

BUDGET REQUEST

Budget year: April 1, 2015-December 1, 2015

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

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

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

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

James R. Stewart 10/9/14

Bert Quezada 10/09/14

James R. Stewart
Project leader
AgIPM Consultants, Inc
PO Box 1095, Exeter CA 93221
Phone: (559) 730-6243
Fax: (559) 592-4105

Bert Quezada
Senior Pest Control Advisor
AgIPM Consultants, Inc
PO Box 1095, Exeter CA 93221
Phone: (559) 936-0102
Fax: (559) 592-4105

Table World Trade Proposal

Schramm, Williams & Associates Inc.

The report's information will include the following:

- 2013 table olive exports and imports for the last five years according to HTS numbers with a focus on major
  exporting, importing and transship countries, (a few developing countries may only have 2012 trade statistics),
- a narrative,
- major 2014 Gain reports, and;
- importing countries' applied and bound tariffs.

We will complete the report by February 15, 2015 or earlier. The report cost will be $15,000.

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year: 2015-2016 Anticipated Duration of Project: November 2014 – December 2015

Project Leader: Drs. Selina Wang, Roberto Avena, and Mendel Friedman

Location: UC Davis, ARS-USDA, Albany, CA

Mailing Address:
University of California
Olive Center
Food Science & Tech
1136 RMI North Bldg
Davis, CA 95616
ATTN: Selina Wang

Phone: 530-752-0612 FAX: 530-752-7080 E-mail: scwang@ucdavis.edu

Project Title:

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

Cooperating Personnel: Alice Tang (UC Davis) & Dr. Ivana Sedej (ARS-USDA)

Keywords: acrylamide reduction, black ripe table olives, flavonoids, sulfur amino acids

Commodity: olives

Problem and its Significance:

Acrylamide has been identified as a probable carcinogen by the National Toxicology Program and the International Agency for Research on Cancer. High levels of acrylamide have been found in black ripe table olives by researchers (Table 1). In a survey conducted by the US Food and Drug Administration (FDA), high amounts of acrylamide were found in black ripe table olives in US market (375-1925 μg/kg). Casado and Montaño screened 11 black ripe table olives obtained in Spain and found the levels of the acrylamide ranged from 176 to 1,578 μg/kg of olive pulp. Similarly, in our lab, we also found 288-1,192 μg/kg acrylamide in seven Spanish black ripe table olives samples.

Research Group	Acrylamide level (µg/kg)
US Food and Drug Administration	375-1,925
Casado and Montaño	176-1,578
UC Davis Olive Center Laboratory	288– 1,192

Table 1. Acrylamide levels found in recent research in black ripe table olives.

Seeking a quick and cost-effective solution to reducing acrylamide in black-ripe processing, the UC Davis Olive Center and California table olive processors in 2014 evaluated sodium bisulfate ($NaHSO_3$) -- Casado had found that addition of 25 mM $NaHSO_3$ reduced acrylamide by 37% without any impact on sensory qualities. Unfortunately, the Casado method proved ineffective when deployed at an industrial scale – in fact, UC Davis found that the amount of $NaHSO_3$ (0, 10, and 25 mM) actually increased the amount of acrylamide in black ripe table olives at 15 and 30 min sterilization times at 240°F. This disagreement may be due to the timing of the addition of $NaHSO_3$ (i.e. $NaHSO_3$ was added during canning not processing). Moreover, because sulfites are allergens, the addition of $NaHSO_3$ may limit commercial applications for canned olives.

Objective:

The objective of this proposal is to expedite the evaluation of promising approaches to reduce acrylamide in black-ripe olive processing by determining if the sterilization conditions and additional processing parameters (length of brine storage and lye treatment) and GRAS (generally accepted as safe) compounds such as sulfur containing amino acids and flavonoids (polyphenolic compounds) added at different steps in processing can reduce substantially the acrylamide levels in canned black ripe table olives.

Plans and Procedures:

The failure of sodium bisulfate to reduce acrylamide makes it necessary to methodically evaluate, with the cooperation of two USDA scientists (Dr. Avena is a Food Engineer who has vast amount of experiences with canning processes including table olives and Dr. Friedman who is a world expert on acrylamide and has written many peer-reviewed articles on the topic), additional promising approaches to achieving an expedited solution to reducing acrylamide in black-ripe processing:

- **Lye processing time.** Lye treatment was found to significantly decrease the levels of acrylamide in the final products (Chaopraesert and Mitchell). It would be valuable to

evaluate whether prolonged lye treatment will reduce acrylamide in the finished products.

- **Storage time.** Given that acrylamide levels dramatically increase in the first month of storage and gradually declined as the storage time increased under lab conditions (Chaopraesert and Mitchell), it would be useful to evaluate the effect of brine-storage time on acrylamide at industrial scale.
- **Sterilization time and temperature.** It has been shown that acrylamide in canned olives is primarily formed during sterilization, and that sterilization time and temperature can significantly influence the formation of acrylamide. Lowering the sterilization temperature and shortening the sterilization time could reduce the formation of acrylamide, while continuing to achieve appropriate process bacterial lethality.
- **Addition of antioxidative compounds.** Antioxidative polyphenolic compounds (flavonoids and anthocyanins) have been shown to be strong inhibitors of acrylamide formation (Cheng et al., 2009, 2015). We will, therefore, evaluate the potential of such food compounds to prevent acrylamide formation during black-ripe olive processing.
- **Addition of sulfur-containing amino acids.** Another class of compounds that are reported to inhibit non-enzymatic food browning (Maillard reaction) that is involved in the mechanism of acrylamide formation are SH-containing amino acids, including cysteine, N-acetylcysteine, cysteine methyl ester, and reduced glutathione (Friedman and Levin, 2008). These compounds will also be evaluated for black-ripe olive processing.

We propose two phases to reduce the acrylamide in black ripe table olives: Phase I is to adjust the sterilization time and temperature, and Phase II is to adjust lye treatment and brine storage length and adaptation of selective food additives. The Phase I will be conducted first, as it is the easiest modification to make, and does not need the additives or require approval from regulatory agencies. If Phase I is successful at eliminating acrylamide, phase II will not be necessary.

Phase I – Sterilization time and temperature

1. Adjustment of sterilization time and temperature

Chemical reactions in general can be reduced 50% after a 10 °C (18 °F) temperature drop. A practical possibility to achieve a significant reduction in acrylamide concentration in canned black ripe table olives could be to sterilize at lower temperatures, such as 230°F or 240°F, while increasing processing time to achieve equivalent sterilization effect. Using a steam-heated rotary retort at the UC Davis FST pilot plant, we will collect heat penetration data during sterilization of conventional processed canned black ripe table olives from Manzanilla variety with wireless data loggers inside 401x411 can size (1.187 lb) to establish equivalent lethality values to assure a safe *Clostridium. botulinum* spores reduction at different thermal processing conditions. Thermal processing requirements for different retort temperatures and intermittent

convection heating mode will be applied according to Stumbo, Purohit and Ramakrishnan. The research approach for phase I will be to use 230-260 °F as retort temperatures and, agitated retort to promote convection heating to speed up heat transfer and reduce processing time.

2. Determination of acrylamide concentration and quality attributes in the samples in Phase I (Part 1)

Samples of canned black ripe table olives sterilized by optimized heat processing method in Phase I (Part 1) will be analyzed for acrylamide concentration and quality attributes (instrumental texture and color analysis, and sensory evaluation) in the UC Davis Olive Center Laboratory.

A method to determine the acrylamide concentration in black ripe table olives was successfully developed and optimized by the UC Davis Olive Center Laboratory. This method is faster and cheaper than the standard method. Black ripe table olives (20 g) will be shaken off the brine and crushed in the mortar with a pestle. Then 2 g of sample will be placed in a centrifuge tube and spiked with 0.5 µg d_3-acrylamide as internal standard. Water (4 mL) will then be added to the centrifuge tube. After 10 min of shaking, hexane (1 mL) will then be added, followed by another shaking for 10 min. The samples will be then centrifuged at 8,000 rpm for 10 min to separate the aqueous and hexane layers. The separated aqueous layer (lower layer) will be vacuum filtered using a 125 mL Buchner funnel. Then nitrogen will be blown on top of the filtrate to completely evaporate the hexane.

A Sep-Pak C_{18} cartridge will be activated by methanol (2 mL) followed by water (2 mL). All of the filtrate (about 4 mL) will then be loaded on the cartridge and passed through the cartridge without vacuum (about 1.5 mL/min). The filtrate will be collected and evaporated to less than 1 mL and water to which water will be added to make up to exactly 1 mL. Acrylamide determination will be performed by LC-MS/MS.

The quantification of acrylamide and d_3-acrylamide will be performed on a Sciex API 2000 triple-quadruple MS system. The samples will be separated using a Hypersil-Keystone Hypercarb column (50×2.1 mm i.d., particle size 5µ). The mobile phase will be isocratic methanol/water (80:20, v/v) at 200 µL/min for a total run time of 5 min. The column will be operated at room temperature. The retention time of acrylamide and d_3-acrylamide is 1.56 min. The mass spectrum data will be acquired with positive ion atmospheric pressure ionization (APCI) utilizing the multiple-reaction monitoring (MRM) mode. Transitions for acrylamide and d3-acrylamide were monitored at 72/55 and 75/58, respectively.

Phase II – Other methods

1. Adjustment of other processing parameters and use of additives

Phase II (Part 1) will be completed in the Bell Carter (or at the FST UC Davis) pilot plant. A control will be conducted following the original parameters of the processing procedure

without brine storage. We will work with the canners on obtaining information on the ratio of olives and filling liquid as well as its formulation and current thermal processing applied. Sterilization will be done using optimized processing method determined in phase I.

- **Lye treatment variation:** To test the effect of lye treatment on acrylamide, the lye treatment time will be doubled for each lye-treatment-washing cycle. The length of washing time will be adjusted until the desired pH is reached.
- **Brine storage variation:** The effect of brine storage on acrylamide will be evaluated by storing the olive fruits in a tank for up to 2 month. The olive fruits will be taken for further processing every two weeks. Stirring will be applied before the fruits are taken from the tank. The storage solution cannot include $CaCl_2$. All other parameters are the same as the control.
- **Sulfur-containing amino acids variation:** Sulfur amino acids, L-cysteine and its derivative (N-acetyl-L-cysteine) will be added into the tanks at each washing/air oxidation steps and right before sterilization, individually. The concentration of L-cysteine and its derivative to be added are 5, 10 and 15 mM (Claeys, 2005). All other parameters will remain the same as the control. **Flavonoids and other polyphenolics variation:** Flavonoids (luteolin and naringenin), apple skin polyphenolics, grape seed or grape skin extracts, all available commercially will be added into the tanks at each washing/air oxidation steps and right before sterilization, individually. A small-scale trial will be conducted at current thermal processing conditions prior to the pilot plant trial to find out the optimum concentration of the flavonoids and other polyphenols. The concentrations of luteolin to be evaluated are 10^{-7}, 10^{-6} and 10^{-5} mM (Cheng, 2015). The concentrations of naringenin to be evaluated are 20, 25 and 30 mM (Cheng, 2009). The amount of apple skin polyphenols and grape seed or skin extracts to be tested are 0.2% (w/w), 0.5% (w/w) and 1.0% (w/w) for both (Acar, Gokman, 2009, Oral, 2014). After the discovery of the optimum concentration of the flavonoids and other polyphenols, the ^{p}ilot ^{p}lant test will be initiated. All other parameters will remain the same as the control. Sterilization will be done using optimized heat processing method determined in phase I.

For all the samples described above, the pH needs to be adjusted to 8.16 prior to sterilization.

2. Determination of acrylamide concentration and quality attributes in samples in Phase II Part 1.

All the samples in Phase II (Part 2) will be evaluated for the acrylamide concentration and quality attributes (instrumental texture and color analysis, and sensory evaluation) in UC Davis Olive Center Laboratory. The method to determine acrylamide concentration was described in the Phase I (Part 2).

Proximate Composition of Treated Olives

We will determine the composition of the treated olives by HPLC to find out whether the above-mentioned treatments affected the composition of the olives.

Expected Outcome:

All the samples processed in both phases will be tested for acrylamide levels. The results from phase I will determine if adjustment of sterilization temperature and time affect the formation of acrylamide; phase II will determine if the lengths of lye treatment and brine storage, and addition of sulfur-containing amino acids, flavonoids and other polyphenolics affect the formation of acrylamide. The data of phase I will be quantified and analyzed before proceeding with phase II. With the knowledge obtained from this project, we will be able to make the recommendation on how to best adjust the current commercial processing procedures to reduce the level of acrylamide in canned black ripe olives.

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BUDGET REQUEST

Budget Year: 2015-2016

Funding Source:

Phase I – Sterilization time and temperature

Postdocs/RA's:	one graduate student (15% at $50,000)	$7,500
	one postdoc (10% at $65,000)	$6,500
UC Davis Olive Center		$2,500
Supplies and Expenses		$6,000
Ecklund/Datatrace heat penetration equipment*		$15,000
Travel		$500
	SUBTOTAL	$38,000

Phase II – Other methods

Postdocs/RA's:	one graduate student (15% at $50,000)	$7,500
	one postdoc (10% at $65,000)	$6,500
UC Davis Olive Center		$2,500
Supplies and Expenses		$9,000
Ecklund/Datatrace heat penetration equipment*		$0 (already purchased from phase I)
Travel		$500
	SUBTOTAL	$26,000

Phase I + Phase II = $**64,000**

* We tried to get a loaner heat penetration equipment from a can supplier, however, they did not have one that would work for the retort that we have at UC Davis. Ecklund/Datatrace offers university discount which allows us to order the wireless thermocouple, software and other accessories at the cost listed above.