California Olive Committee

Final & Interim Research Reports 2010

Complied by

Marshall W. Johnson
UC Liaison Officer to the COC
mjohnson@uckac.edu

Cooperative Extension Specialist & Entomologist
Department of Entomology
University of California
Riverside, CA 92521

28 January 2011

California Olive Committee

Final Research Reports 2010

Complied by

Marshall W. Johnson
UC Liaison Officer to the COC
mjohnson@uckac.edu

Cooperative Extension Specialist & Entomologist
Department of Entomology
University of California
Riverside, CA 92521

28 January 2011

The final reports contained within provide findings and conclusions from research projects funded by the California Olive Committee for the year of 2009 and 2010. These reports were submitted on 5 January 2011. The 2009 projects were extended to 2010 with a no cost extension.

List of Project Reports **Page No.**

2009 – 2010 Projects

2010 Projects

Evaluation of Pathogenicity and Insect Transmission of *Xylella fastidiosa* Strains to Olive Plants

Project Leader: Rodrigo Krugner, USDA-ARS-SJVASC, 9611 S. Riverbend Ave, Parlier, CA 93648, Phone: 559-596-2887, FAX: 559-596-2921, E-mail: Rodrigo.Krugner@ars.usda.gov.

Cooperating Personnel:

Marshall W. Johnson, University of California, Riverside
Jianchi Chen, USDA-ARS, SJVASC, Parlier, CA

Reporting Period: 1 January 2009 to March 2010

ABSTRACT

Xylella fastidiosa (*Xf*) is a xylem-limited bacterium that causes a number of diseases in economically important crops in California and worldwide. Scorching symptoms in olive trees potentially caused by *Xf* infection have emerged as a potential disease threat to olive production areas in California. The role of *Xf* as the causal agent of "olive leaf scorch disease (OLSD)" was investigated. A total of 90 samples of olive plants showing branch dieback and leaf scorch symptoms were collected to determine if *Xf* is constantly associated with the disease. PCR and isolation detected *Xf* in 23 samples, from which three strains were isolated. Four-primer DNA analysis indicated that *Xf* in all positive samples have the A genotype, which is known to cause Almond and Oleander Leaf Scorch Diseases, but not Pierce's Disease in grapevines. Bacterial cells from laboratory cultures were needle-inoculated in healthy olive plants and monitored every two weeks over a one-year period for symptom development. Branch dieback and leaf scorch symptoms similar to those observed in the field were not consistent among inoculated plants. Thus, we were unable to determine if *Xf* is or is not the causal agent of OLSD.

INTRODUCTION

Xylella fastidiosa Wells et al. is a xylem-limited bacterium that causes a number of diseases in California such as almond leaf scorch (Mircetich et al. 1976, Davis et al. 1980), Pierce's Disease (PD) in grapes (Davis et al. 1978), alfalfa dwarf (Davis et al. 1978, Goheen et al. 1973, Hewitt et al. 1956), and many other diseases in the U.S. and worldwide such as phony peach disease (Davis et al. 1981, Wells et al. 1983), plum leaf scald (Raju et al. 1982), pear leaf scorch (Leu and Su 1993), and citrus variegated chlorosis (Chang et al. 1993). Work by Hutchins et al. (1953) and Boyhant et al. (1996) showed that *X. fastidiosa* can be transmitted by grafting and budding in peaches and plum. In California, *X. fastidiosa* strains are transmitted by several species of leafhoppers (Mircetich et al. 1976; Purcell 1980), including the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar), (Turner 1959, Purcell and Saunders 1999, Almeida and Purcell 2003a, Almeida and Purcell 2003b, Costa et al. 2000, Damsteegt et al. 2006, Costa et al. 2006).

There are a number of different strains of *X. fastidiosa*. Schaad et al. (2004) divided 26 strains into three sub-species groups: *X. fastidiosa* subsp. *fastidiosa*, *multiplex*, and *pauca*. However, Schuenzel et al. (2005) divided them into *X. fastidiosa* subsp. *fastidiosa*, *multiplex*, and *sandyi*. About 145 plant species have been identified in California as potential hosts of *X. fastidiosa* strains (Hewitt et al. 1949, Freitag 1951, Raju et al. 1980, 1983, Hopkins and Purcell

2002, Costa et al. 2004) and the importance of some of these plants as hosts and sources of pathogenic strains within and among plant species has been investigated (Hill and Purcell 1995, Purcell et al. 1999, Purcell and Saunders 1999, Costa et al. 2004, Baumgartner and Warren 2005, Chen et al. 2005, Groves et al. 2005, Wistrom and Purcell 2005, Hernandez-Martinez et al. 2006a, b, Shapland et al. 2006) as well as the ability of GWSS to transmit different strains of *X. fastidiosa* to different plant species.

GWSS was first found in southern California in 1990 (Sorensen and Gill 1996) and has now established in the San Joaquin Valley (SJV) (Kern, Tulare, and Fresno Counties) and most of southern California (CDFA 2006). Because it is a highly mobile (Blackmer et al. 2004, Blackmer et al. 2006, Coviella et al. 2006) and polyphagous leafhopper, with over 100 known hosts (Turner and Pollard 1959, Hoddle et al. 2003), it changed the epidemiology of diseases caused by *X. fastidiosa*. Hernandez-Martinez et al. (2007) isolated *X. fastidiosa* from olive plants in southern California, which they characterized as belonging to the *multiplex* sub-species group. Jerry Turney (Plant Pathologist, Los Angeles County Agricultural Commissioner's Office) has reported increasing occurrences of olive tree mortality in the Los Angeles area due to olive scorch (personal communication, 2008). In 2008, preliminary surveys were conducted by R. Krugner and M. W. Johnson to evaluate the association of *X. fastidiosa* with scorch symptoms in olive trees in southern California and southern SJV. *Xylella fastidiosa* was isolated from symptomatic plants and preliminary analysis revealed its phylogenetic relationship with genotypes A found in California. In recent years, leaf scorch symptoms in olive trees potentially caused by *X. fastidiosa* infection have emerged as a potential disease threat to olive production areas in California. However, the role of *X. fastidiosa* strains as the causal agent of "olive leaf scorch disease (OLSD)" has not been fully investigated. In addition, potential vectors of OLSD strains of *X. fastidiosa* have not been documented. Knowledge of the disease symptoms and genetic diversity of OLSD strains associated with OLSD in California will help in devising effective strategies for managing OLSD. Knowledge of which vector species transmit *X. fastidiosa* to olive plants, where they acquire the pathogen, when they move into orchards, and when they spread the pathogen to olive trees is critical to understanding and managing the spread of this disease.

The overall goal of this project is to increase our understanding of the pathogenicity of *X. fastidiosa* strains and accurately describe the symptoms in olive plants. Another focus will be to identify potential vectors of *X. fastidiosa* in olive orchards, to characterize temporal patterns of disease incidence within selected orchards and urban areas, and to determine the genetic structure of *X. fastidiosa* strains associated with the scorching symptoms. An ultimate goal of this research will be to provide fundamental, research-based information needed to manage this potential disease threat in Central Valley orchards. Results from these studies are anticipated to benefit olive growers and crop consultants and will be essential for properly timing any vector control measures and/or implementation of cultural practices.

OBJECTIVES

1. To determine the pathogenicity of *Xylella fastidiosa* strains in olive plants;

2. To evaluate the incidence and characterize *X. fastidiosa* in olive orchards and urban areas in the Central Valley, and

3. To evaluate the transmission efficiency of *X. fastidosa* in olive plants by glassy-winged sharpshooter.

PROCEDURES

Objective 1

Samples of olive plants showing leaf scorching and limb dieback symptoms were photographed and collected from olive orchards in the SJV (where GWSS is common) and urban areas throughout central, coastal, and southern California to determine if *X. fastidiosa* is constantly associated with the disease. Samples were screened in laboratory by PCR and culturing. *Xylella fastidiosa* isolates from infected plants were grown in pure culture and needle-inoculated on healthy olive plants. A total of 30 plants of each cultivar: Mission, Manzanillo, Sevillano, Arbequina, Arbozano, Koroneiki, and Barouni were inoculated in four different periods (March, May, July, and September 2009) with the Rolling Hills isolate and monitored for one year for symptom development. A second group of 30 plants (i.e., Manzanillo) were inoculated on October 2009 with the Fillmore isolate. Inoculated plants were kept in a greenhouse and monitored for *X. fastidiosa* establishment and multiplication by PCR, culturing, and symptom development.

Objective 2

Sites for sampling were determined by visual inspections in urban areas containing ornamental olive trees and in olive orchards with leaf scorch symptoms with the assistance of local University of California Farm Advisers and Cooperative Extension personnel. Samples of olive plants showing leaf scorch and limb dieback symptoms were screened for *X. fastidiosa* in the laboratory as described above. During the process of environmental adaptation such as host specialization and pathogenesis development, a bacterial genome could be subject to base mutation, sequence deletion and/or insertion, horizontal gene transfer, and genome rearrangement. Such genetic information could only be acquired through genomic comparison involving other strains from different sources. Randomly Amplified Polymorphic DNA (RAPD) analysis were conducted using primers OPA02, OPA03, OPA09, OPA11, and OPA18 to compare strains of *X. fastidiosa* obtained from symptomatic olive trees to the strains: Grape Temecula (G genotype), Dixon (A type), Almond M12 (A type), Almond M23 (G type), and Citrus CVC (A type).

Objective 3

Establishment and persistence of *X. fastidiosa* in olive plants will be evaluated in a series of inoculations using *X. fastidiosa*-inoculative insect vectors. Healthy olive tree cuttings or potted plants will be obtained from local nurseries and kept in an insect-free greenhouse at the USDA-ARS San Joaquin Valley Agricultural Sciences Center in Parlier until used in the experiments. Once infected olive plants are produced (see results of Objective 1), these diseased plants will serve as source plants for glassy-winged sharpshooters reared from laboratory colonies. Insects will be caged on source plants for a 96-h acquisition access period (AAP) and transferred in groups of five individuals per test plant for a 96-h IAP on healthy test plants. Inoculations will be conducted in a containment facility established at the California State University Campus in Fresno. Sets of non-inoculated plants will be used as a control. Control and inoculated plants will be kept in an insect-free environment for 12 months. The fate of bacterial inoculations will be evaluated by symptom development, PCR, ELISA, and culturing.

RESULTS AND DISCUSSION

Pathogenicity of *Xylella fastidiosa* strains in olive plants. Branch dieback and leaf scorch leaf symptoms (**Fig. 1**) have been observed among inoculated plants of all cultivars. Branch dieback is more common on cultivars Mission (10 plants) and Arbequina (5 plants). Although *X. fastidiosa* has been detected in inoculated plants by ELISA, we were unable to re-isolate the bacteria from symptomatic plants and thus, Koch's Postulates have not been fulfilled yet. A second *X. fastidiosa* isolate from Fillmore, which appeared to grow faster in laboratory media than the Rolling Hills isolate, was inoculated in Manzanillo plants on October 2009, but symptoms have not developed yet. In other studies involving *X. fastidiosa* inoculations, development of disease symptoms varied from 6 weeks (grapevines in greenhouse experiments) to 16 months (almonds under field condition) (Krugner, *personal unpublished data*). Recently, Hernandez-Martinez et al. (2009) described two new xylellae diseases: sweetgum dieback and leaf scorch of purple-leafed plum. Symptoms in sweetgum plants were first observed 9 months after inoculation. Thus, at this point we are unable to determine if *X. fastidiosa* is or is not the causal agent of OLSD. Inoculated plants will be monitored and other experiments will be conducted beyond the 15-month duration of the project funded by the COC.

Incidence and characterization of *X. fastidiosa* in olive trees. To date, a total of 90 samples of olive plants showing limb dieback (**Fig. 2A**) and leaf scorching (**Fig. 2B**) symptoms were collected from trees in urban areas in central, coastal, and southern California to determine if *X. fastidiosa* is constantly associated with the disease. A total of 23 samples were PCR positive for *Xf*, from which three strains were isolated (**Table 1**). Despite a relative high

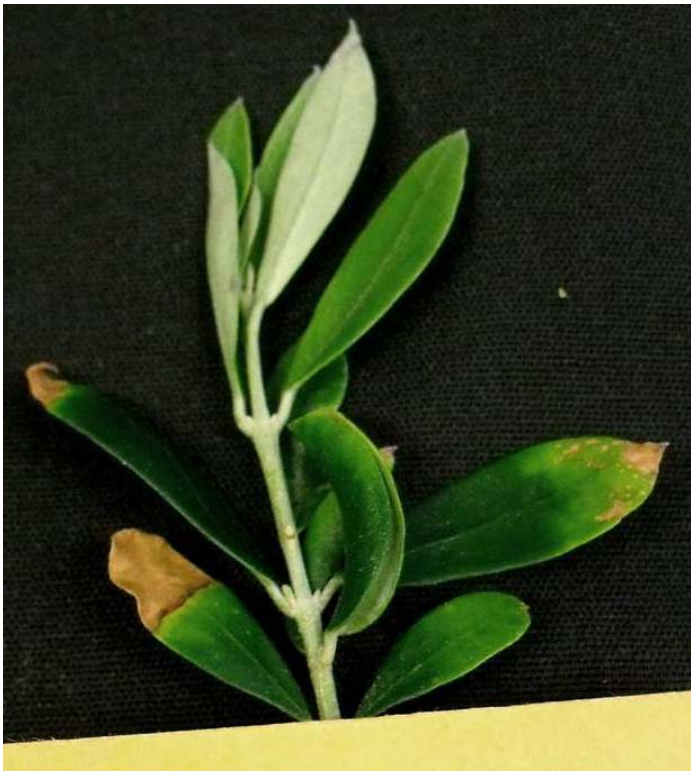

Fig. 1. Leaf scorch symptoms observed in an inoculated olive plant (c.v. Arbequina). *Xylella fastidiosa* has been detected.

Fig. 2. Branch dieback (A) and leaf scorch (B) symptoms observed in trees sampled for *Xylella.*

incidence of dieback symptoms in Bakersfield and Ducor, none of the samples collected in those areas were PCR positive for *X. fastidiosa,* which suggests that infections are limited to southern California. Interestingly, almost all olive trees showing leaf scorch or branch dieback symptoms in southern CA and Fillmore had high numbers of GWSS nymphs and/or adults or signs of previous infestation (e.g., old egg masses).

RAPD analysis indicated that *X. fastidiosa* in all positive samples belong to the A genotype group, which is known to cause Almond and Oleander Leaf Scorch Diseases, but not Pierce's Disease in grapevines. Results indicate that the olive strains (Fillmore and Rolling Hills) are closely related to the Almond M12 genotype. However, further genotype evaluations using

Table 1. Number of olive plant samples collected in California from August 2008 to August 2009 and laboratory screening results for *Xylella fastidiosa*.

Collection date	Location	Number of samples	PCR result (Positive/Total)	Isolation result
	Northern California – Yolo County			
May 09	Davis	4	0/4	0/4
	San Joaquin Valley – Kern and Tulare Counties			
August 08	Bakersfield	5	0/5	0/5
August 08	Porterville	3	0/3	0/3
August 09	Ducor	20	0/20	0/20
	Southern California – Orange County			
May 09	Newport Beach	8	2/8	0/8
May 09	Costa Mesa	7	1/7	0/7
	Southern California – Los Angeles County			
October 08	Rolling Hills	5	0/5	1/5
March 09	Rancho Bernardo	3	3/3	0/3
April 09	Rolling Hills	3	3/3	1/3
	Southern California – Ventura County			
August 09	Piru	5	2/5	0/5
August 09	Fillmore	7	1/7	1/7
August 09	Ventura	3	3/3	0/3
	Southern California – San Diego County			
May 09	Carlsbad	3	3/3	0/3
	Southern California – Riverside County			
May 09	Riverside	8	0/8	0/8
August 09	Riverside	6	5/6	0/6
	Total	**90**	**23**	**3**

more olive strains and biological studies *in planta* are needed to elucidate the pathogenicity of olive strains on other crops, and vice-versa.

CONCLUSIONS

Olive tree infections by *X. fastidiosa* appear to be limited to southern California and exclusively by Genotype A. The bacteria has been detected and isolated from trees showing branch dieback and leaf scorch symptoms, but *X. fastidiosa* inoculations in a controlled environment have not consistently reproduced symptoms observed in the field. Although *X. fastidiosa* was detected in inoculated plants showing scorching symptoms, we were unable to re-isolate the bacteria and finally describe this new potential disease. Therefore, a longer evaluation period is necessary to confirm or rule out *X. fastidiosa* as the causal agent of disease. Evaluations will continue throughout 2010 using the currently available isolates and new isolates obtained in further sample collections.

REFERENCES

Almeida, R. P. P., and A. H. Purcell. 2003a. *Homalodisca coagulata* (Hemiptera: Cicadellidae) transmission of *Xylella fastidiosa* to almond. Plant Dis. 87: 1255-1259.

Almeida, R. P. P., and A. H. Purcell. 2003b. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae). J. Econ. Entomol. 96: 264-271.

Baumgartner, K., and J. G. Warren. 2005. Persistence of *Xylella fastidiosa* in riparian hosts near northern California vineyards. Plant Dis. 89: 1097-1102.

Blackmer, J. L., J. R. Hagler, G. S. Simmons, and L. A. Cañas. 2006. Comparative dispersal of *Homalodisca coagulata* and *Homalodisca liturata* (Homoptera: Cicadellidae). Environ. Entomol. 33: 88-99.

Blackmer, J. L., J. R. Hagler, G. S. Simmons, and T. J. Henneberry. 2006. Dispersal of *Homalodisca vitripennis* (Homoptera: Cicadellidae) from a point release site in citrus. Environ. Entomol. 35: 1617-1625.

Boyhant, G. E., B. R. Abrahams, and J. D. Norton. 1996. Budding method affects transmission of *Xylella fastidiosa* in plum. Hortscience 31: 89-90.

CDFA. 2006. County agricultural commissioner's data, calendar year 2005. California Department of Food and Agriculture, Sacramento, CA, USA.

Chang, C. J., M. Garnier, L. Zreik, V. Rossetti, and J. M. Bové. 1993. Culture and serological detection of *Xylella fastidiosa*, the xylem-limited bacterium associated with citrus variegated chlorosis disease, pp. 294-300. *In* L. W. T. P. Moreno, J. V. da Graça (eds.), Proceedings, 12th Conf. Int. Org. Citrus Virol., New Delhi. Riverside, CA: Univ. Calif.

Chen, J., R. L. Groves, E. L. Civerolo, M. Viveros, M. Freeman, and Y. Zheng. 2005. Two *Xylella fastidiosa* genotypes associated with almond leaf scorch disease on the same location in California. Phytopathol. 95: 708-714.

Costa, H. S., M. S. Blua, J. A. Bethke, and R. A. Redak. 2000. Transmission of *Xylella fastidiosa* to oleander by the glassy-winged sharpshooter, *Homalodisca coagulata*. Hortscience 35: 1265-1267.

Costa, H. S., E. Raetz, T. R. Pinckard, C. Gispert, R. Hernadez-Martinez, C. K. Dumenyo, and D. A. Cooksey. 2004. Plant hosts of *Xylella fastidiosa* in and near southern California vineyards. Plant Dis. 88: 1255-1261.

Costa, H. S., A. Guzman, R. Hernandez-Martinez, C. Gispert, and D. A. Cooksey. 2006. Detection and differentiation of *Xylella fastidiosa* strains acquired and retained by glassy-winged sharpshooters (Hemiptera: Cicadellidae) using a mixture of strain-specific primer sets. J. Econ. Entomol. 99: 1058-1064.

Coviella, C. E., J. F. Garcia, D. R. Jeske, R. A. Redak, and R. F. Luck. 2006. Feasibility of tracking within-field movements of *Homalodisca coagulata* (Hemiptera: Cicadellidae) and estimating its densities using fluorescent dusts in mark-release-recapture experiments. J. Econ. Entomol. 99: 1051-1057.

Damsteegt, V. D., R. H. Brlansky, P. A. Phillips, and A. Roy. 2006. Transmission of *Xylella fastidiosa*, causal agent of citrus variegated chlorosis, by the glassy-winged sharpshooter, *Homalodisca coagulata*. Plant Dis. 90: 567-570.

Davis, M. J., A. H. Purcell, and S. V. Thompson. 1978. Pierce's disease of grapevines: isolation of the causal bacterium. Science 199: 75-77.

Davis, M. J., W. J. French, and N. W. Schaad. 1981. Axenic culture of the bacteria associated with phony disease of peach and plum leaf scald. Curr. Microbiol. 6: 309-314.

Freitag, J. H. 1951. Host range of Pierce's disease virus of grapes as determined by insect transmission. Phytopathol. 41: 920-934.

Goheen, A. C., G. Nyland, and N. K. Lowe. 1973. Association of a rickettsialike organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease grapevines. Phytophatol. 63: 341-345.

Groves, R. L., J. Chen, and E. L. Civerolo. 2005. Spatial analysis of almond leaf scorch disease in the San Joaquin Valley of California: factors affecting pathogen distribution and spread. Plant Dis. 89: 581-589.

Hernandez-Martinez, R., T. R. Pinckard, H. S. Costa, D. A. Cooksey, and F. P. Wong. 2006a. Discovery and characterization of *Xylella fastidiosa* strains in southern California causing mulberry leaf scorch. Plant Dis. 90: 1143-1149.

Hernandez-Martinez, R., H. S. Costa, C. K. Dumenyo, and D. A. Cooksey. 2006b. Differentiation of strains of *Xylella fastidiosa* infecting grape, almonds, and oleander using a multiprimer PCR assay. Plant Dis. 90: 1382-1388.

Hernandez-Martinez, R., K.A. de la Cerda, H. S. Costa, D. A. Cooksey, and F. P. Wong. 2007. Phylogenetic relationships of *Xylella fastidiosa* strains isolated from landscape ornamentals in Southern California. Phytopathology 97: 857-864.

Hernandez-Martinez, R., D. A. Cooksey, and F. P. Wong. 2009. Leaf scorch of purple-leafed plum and sweetgum dieback: two new diseases in Southern California caused by *Xylella fastidiosa* strains with different host ranges. Plant Disease 93: 1131-1138.

Hewitt, W. B., N. W. Frazier, J. H. Freitag, and A. J. Winkler. 1949. Pierce's disease investigations. Hilgardia 19: 207-264.

Hewitt, W. B., B. R. Houston, N. W. Frazier, and J. H. Freitag. 1956. Leafhopper transmission of the virus causing Pierce's disease of grape and dwarf of alfalfa. Phytopathol. 36: 117-128.

Hill, B. L., and A. H. Purcell. 1995. Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. Phytopathol. 85: 1368-1372.

Hoddle, M. S., S. V. Triapitsyn, and D. J. W. Morgan. 2003. Distribution and plant association records for *Homalodisca coagulata* (Hemiptera: Cicadellidae) in Florida. Fla. Entomol. 83: 89-91.

Hopkins, D. L., and A. H. Purcell. 2002. *Xylella fastidiosa*: Cause of Pierce's disease of grapevine and other emergent diseases. Plant Dis. 86: 1056-1066.

Hutchins, L. M., L. C. Cochran, W. F. Turner, and J. H. Weinberger. 1953. Transmission of phony disease virus from tops of certain affected peach and plum trees. Phytopathol 43: 691-696.

Leu, L. S., and C. C. Su. 1993. Isolation, cultivation, and pathogenicity of *Xylella fastidiosa*, the causal bacterium of pear leaf scorch disease in Taiwan. Plant Dis. 77: 642-646.

Mircetich, S. M., S. K. Lowe, W. J. Moller, and G. Nyland. 1976. Etiology of almond leaf scorch disease and transmission of the causal agent. Phytopathology 66: 17-24.

Purcell, A. H. 1980. Almond leaf scorch: leafhopper and spittlebug vectors. J. Econ. Entomol. 73: 834-838.

Purcell, A. H., and S. R. Saunders. 1999. Fate of Pierce's disease strains of *Xylella fastidiosa* in common riparian plants in California. Plant Dis. 83: 825-830.

Purcell, A. H., S. R. Saunders, M. Hendson, M. E. Grebus, and M. J. 1999. Causal role of *Xylella fastidiosa* in oleander leaf scorch disease. Phytopathol. 89: 53-58.

Raju, B. C., S. F. Nomé, D. M. Docampo, A. C. Goheen, G. Nyland, and S. K. Lowe. 1980. Alternative hosts of Pierce's disease of grapevines that occur adjacent to grape growing areas in California. Am. J. Enol. Vitic. 31: 144-148.

Raju, B. C., J. M. Wells, G. Nyland, R. H. Brlansky, and S. K. Lowe. 1982. Plum leaf scald: Isolation, culture, and pathogenicity of the causal agent. Phytopathol. 72: 1460-1466.

Schaad, N. W., E. Postnikova, L. George, M. Fatmi, and C. Chang. 2004. *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. *piercei*, subsp. nov., *X. fastidiosa* subsp. *multiplex*, subsp. nov., and *X. fastidiosa* subsp. *pauca*, subsp. nov. System. Appl. Microbiol. 27: 290-300.

Shapland, E. B., K. M. Daane, G. Y. Yokota, C. Wistrom, J. H. Connell, R. A. Duncan, and M. A. Viveros. 2006. Ground vegetation survey for *Xylella fastidiosa* in California almond orchards. Plant Dis. 90: 905-909.

Schuenzel, E. L., M. Scally, R. Stouthamer, and L. Nunney. 2005. A multigene phylogenetic study of clonal diversity and divergence in North American strains of the plant pathogen *Xylella fastidiosa*. Appl. Environ. Microbiol. 71: 3832-3839.Sisterson, M. S., J. Chen, M. A. Viveros, E. L. Civerolo, C. Ledbetter, and R. L. Groves. 2008. Effects of almond leaf scorch disease on almond yield: implications for management. Plant dis. (In Press).

Sorensen, J. T., and R. J. Gill. 1996. A range extension of *Homalodisca coagulata* (Say) (Hemiptera: Clypeorryncha: Cicadellidae) to southern California. Pan-Pac. Entomol. 72: 160-161.

Turner, W. F. 1959. Insect vectors of phony peach disease. Science 109: 87-88.

Turner, W. F., and H. N. Pollard. 1959. Life histories and behavior of five insect vectors of phony peach disease. U. S. Dep. Agric. Tech. Bull. 1188: 1-28.

Wells, J. M., B. C. Raju, and G. Nyland. 1983. Isolation, culture, and pathogenicity of the bacterium causing phony disease of peach. Phytopathol. 73: 859-862.

Wistrom, C., and A. H. Purcell. 2005. The fate of *Xylella fastidiosa* in vineyards weeds and other alternate hosts in California. Plant dis. 89: 994-999.

Appraising the Threat of Olive Psyllid to California Table Olives

Project Leaders:

Marshall W. Johnson, UC CE Specialist and Entomologist, Department of Entomology, UC Riverside, Kearney Agricultural Center, Parlier, CA 93648, (559) 646-6519, mjohnson@uckac.edu.

Kent M. Daane, UC CE Specialist, Division of Organisms and Environment (Dept. ESPM), UC Berkeley, Kearney Agricultural Center, Parlier, CA 93648, (559) 646-6522, daane@uckac.edu.

Kris Lynn-Patterson, UC Academic Coordinator, Geographic Informational Systems, UC Kearney Agricultural Center, 9240 South Riverbend Ave., Parlier, CA 93648, (559) 646-6592, krislynn@uckac.edu

Cooperating Personnel:

Bradford Hawkins, University of California, Irvine
Gary Bender, UCCE Farm Advisor, San Diego County
James Bethke, UCCE Farm Advisor, San Diego County
David Kellum, Entomologist, Office of the County Agric. Commissioner, San Diego County
Lynn LeBeck, Association of Natural Biocontrol Producers, Clovis
Hannah Nadel, University of California, Riverside
Charles Pickett, CDFA, Sacramento
Genet Tulgetske, University of California, Riverside
Christiane Weirauch, University of California, Riverside

Reporting period: 1 January 2009 to 31 December 2010

ABSTRACT

The olive psyllid, *Euphyllura olivina* (Costa), was discovered in July 2007 in the California counties of San Diego and Orange. Surveys of greater than 40 sites conducted in from 2008 to 2010 suggest that olive psyllid was well established along the coast and inland areas in Orange and San Diego Counties. New records of infestation were recorded near Fallbrook (San Diego County) and from Temecula to Murrieta (Riverside County). The Temecula infestations were the first records of the psyllid being found in Riverside County. A new infestation of the olive psyllid was reported and verified (by Charles Pickett, CDFA) in Carmel Valley (Monterey County).

In most locations with infestations, the rankings of the infestations did not appear to be damaging (ranks less than 3) to the olives present at the various sites. The psyllid infestations appeared to follow a seasonal trend similar to what has been reported in Europe and the Middle East. Population rankings in most of the infested sites that were continuously monitored remained low from October to March and then increased over the spring period and peaked in

June. As summer temperatures increased, population rankings decreased in August and hit their low point in December. The presence of continuing psyllid infestations in the Temecula area in spring 2010 suggests that the psyllid could potentially establish in the Central Valley. However, its ability to reach damaging levels during critical times (i.e., budding and flowering) is unknown. Based on what has been observed in southern California, the probability of the psyllid being a major problem in the San Joaquin and Sacramento Valleys is low. However, given the climatic trends of both valleys, it is probably more likely to better establish in the cooler Sacramento Valley. The olive psyllid will probably establish in cooler areas along the California coast where olives grow.

Natural enemies were rarely observed in association with the psyllid clusters. However, the inability to dissect the clusters and closely examine the psyllid individuals within (due to the lack of a laboratory setting where the clusters could be examined) reduced our ability to say how frequently natural enemies may have been associated with the psyllids. No parasitoids were found in association with the psyllid. However, a predatory insect, similar to the minute pirate bug (Hemiptera: Anthocoridae), was found infesting one shipment to UC Berkeley and is believed to have significantly reduced the numbers in the psyllid shipment.

INTRODUCTION

The olive psyllid, *Euphyllura olivina* (Costa), was discovered in July 2007 in the California counties of San Diego and Orange (CDFA Pest Exclusion Advisory No. 20-2007) and positively identified on 25 September 2007 (personal communication, Kevin Hoffman, CDFA). Psyllids are related to aphids, mealybugs, scales, leafhoppers, and sharpshooters. The life stages of *Euphyllura olivina* include an egg, 5 nymphal instars, and adults (both sexes). Olive psyllids are very small insects ranging from 0.015 inches (0.4 mm) to 0.06 inches (1.5 mm) in length as immatures and about 0.1 inches (2.5 mm) as adults. The life cycle is about 3 months long depending on temperature. Optimal growth conditions are between 68 and 77°F. Individual females may lay more than 1,000 eggs. Females start egg laying when new shoots appear on the olive tree host. Eggs are deposited on the top of twigs or among the leaves of minor shoots. Eggs hatch after 8 to 12 days. *Euphyllura olivina* has three host plants that it feeds upon and lays eggs on: a) olive; b) Russian olive (*Elaeagnus angustifolia* [oleaster]), and c) mock privet (*Phillyrea latifolia*). In Europe and the Middle East, there are typically three generations. The first generation becomes active in the spring as temperatures warm up. The second generation becomes inactive when temperatures exceed 81°F and active again when temperatures drop. Above 90°F survival may be limited.

Nymphs and adults feed by rupturing cells and ingesting sap, reducing nutrients that are essential for tree development and fruit production. Plant parts attacked include the buds, tender shoots, floral axes, inflorescences, and young fruits. Nymphs secrete a sticky wax (similar to mealybugs), which accumulates on the foliage and stimulates premature flower drop. The wax can actually protect the psyllid from some insecticides so it is best to control the first generation when buds and flowers are not present. When nymphal colonies are on the apical parts of young shoots, sap loss is not important. When colonies are on inflorescences, the level of sap loss is directly proportional to psyllid numbers. Yield reductions in some areas outside the USA have been as much as 40 to 60%. Population densities greater than 20 nymphs per inflorescences can cause such losses.

On their own power, olive psyllid nymphs and adults do not naturally disperse far (e.g., few yards) from infestation sites. Given this, it has been suggested that the insects may be

redistributed when pruning shears become contaminated with the sticky wax (including nymphs) and the same contaminated tools are used to prune several trees. If so, commercial landscaping companies and nurseries may unintentionally spread the psyllid among their clientele's olive trees in different locations. This may explain why infestations are highly clumped among olive trees within some areas. However, various psyllid species can be dispersed great distances by strong winds.

After seeing healthy *Euphyllura olivina* infestations in San Marcos, Oceanside, and Carlsbad in June 2008, the senior P.I. conducted surveys for olive psyllid in early July 2008 and early October 2008. Olive trees in residential (around apartment complexes, homes) and business areas (malls, shopping centers, parking lots, etc.) in Orange and San Diego Counties were randomly selected and inspected for psyllid colonies and the wax they produce. As of late 2008, the distribution appeared to include areas from Newport Beach and Irvine (Orange County) in the north to the city of San Diego in the south. Olives checked in the Palos Verdes area, Anaheim, Burbank, Santa Clarita, Riverside, Sun City, and Temecula were free of the insect. A monitoring program was initiated in 2009 and in February 2010, the project was approved for a 'no cost extension' which permitted its continuation to December 2010.

Although *Euphyllura olivina* is common in many olive growing areas in the Mediterranean and Middle East, little was known about its biology and distribution in California. Likewise, nothing has been reported on natural enemies (e.g., parasitoid wasps, lacewings, ladybugs) attacking the psyllid within infested areas of the state. It was unclear whether the distribution of *Euphyllura olivina* would expand beyond San Diego and Orange Counties. Its sensitivity to temperatures above 81°F suggested that it may not obtain high densities during the summer period in the Central Valley when temperatures are commonly above 95°. However, it may establish in the Central Valley and become a significant pest during the spring period before temperatures become high and buds and flowers are on the trees. This would be the time when it could impact olive production by reducing the numbers of fruit that complete development. Additionally, it may be able to impact the production of olive oil if it can expand its northern distribution along the cooler California coast, where significant amounts of oil are produced. Given the above information, the following objectives were proposed to provide knowledge that would 1) identify whether olive psyllid will be a threat to olives in the Central Valley; and 2) aid in the management of *Euphyllura olivina* within California.

OBJECTIVES

1) Determine and map the distribution of *Euphyllura olivina* over the next 12 months and quantify infestation levels within California;

2) Identify and quantify the impact of existing natural enemies (e.g., lacewings, ladybugs, parasitoids, etc.) that are attacking *Euphyllura olivina* stages in infested areas.

PROCEDURES

Objective 1. Prior to COC funding in 2009, initial surveys were conducted in July and October 2008 at more than 20 sites in the counties of San Diego, Orange, Los Angeles, and Riverside. Six surveys during March to December 2009 in the same counties were funded by the COC and included more than 40 sites that were revisited to document the population trends of the psyllid

at the various locations. Olive trees in residential (around apartment complexes, homes), business (malls, shopping centers, parking lots, etc.), and agricultural areas (near vineyards, roadside landscapes) were randomly selected and inspected for olive psyllid colonies and the wax they produce (the psyllids within the waxy mass were termed a "cluster"). At several sites the psyllid's presence (as indicated by waxy formations resembling cotton balls, see **Fig. 1**) was easily detected by the untrained eye. For each survey in 2009, between 326 and 624 trees were checked among the sites. At each site, each tree was given an infestation rating of 0 to 4 (0 = non-infested; 1 = 1 to 3 psyllid clusters; 2 = 4 to 10 psyllid clusters; 3 = light infestation with $1/10^{th}$ of tree infested; 4 = heavy infestation with more than $1/10^{th}$ of tree infested). In 2010, seven surveys were conducted in southern California, and up to 50 sites were checked. The same rating systems was used to quantify the infestations. From March to May 2010, efforts were made to discover if the distribution of the psyllid had increased. During this time, the temperatures were conducive to psyllid reproduction and development. From June to December 2010, surveys were limited to the same 10 sites were psyllids were commonly found with the goal of documenting their seasonal patterns.

In 2009 and 2010, attempts were also made to discover the psyllid along the California coast from Los Angeles to Monterey. Five sites were checked in the Monterey area in 2009. Twenty-two sites were checked from Los Angeles to San Juan Baustista in 2010.

Objective 2. The presence of natural enemies among clusters of immatures was noted during the survey. Occasionally, large numbers of infested shoots were held in containers to determine if any of the psyllids were parasitized.

RESULTS AND DISCUSSION

Objective 1

Southern California surveys conducted in 2009. More than 40 sites were surveyed in 2009. Psyllids were not found at 17 sites (**Table 1**) and were found at 25 sites (**Table 2**). The current olive psyllid distribution appears to include areas from Huntington Beach, Newport Beach, and Irvine (Orange County) in the north to the city of San Diego in the south and east to Escondido and Temecula (**Fig. 2**). To date (2010), olives checked in Palos Verdes, Anaheim, Burbank, Santa Clarita, Sylmar, Riverside, Sun City, Perris, and Hemet were free of the psyllid (**Table 1**).

The percentages of trees with olive psyllid within the infested areas varied among sites and the time of the surveys (i.e., March, April, June, August, October, & December 2009) (**Table 3**). For example, in June 2009, the psyllid was found on 61% of the trees within the infested areas. By December 2009, psyllids were only found on 6.6% of trees in infested areas. At the majority of sites, usually more than one tree would be infested even when infestations were very low (e.g., 1 to 3 clusters per tree) (**Table 3**). This suggests that once the psyllid becomes established in a location, the adults disperse to many trees and reproduce in the vicinity. However, only in a few localities (Carlsbad and San Diego) were the populations considered potentially damaging (infestation ratings were between 3 and 4). From October 2008 to June 2009, the percentage of infested trees increased at the checked sites and then percentages dropped in August and October 2009 following the summer period (**Fig. 3**). By December 2009, only 5 sites out of 24 previously infested sites displayed the characteristic waxy clusters (**Fig. 1**) produced by the immature psyllids. These five sites had mean rankings of 0.63, 0.18, 0.44, 0.13, and 1.00, which is basically equal to or less than three clusters per tree. The two most significant infestations in December 2009 were at Carlsbad (near downtown) and Irvine (near insection of I-405 and I-5

Fig. 1. Waxy secretions produced by olive psyllid immatures. Immature and adult individuals may be found underneath the waxy material. Each waxy mass was referred to as a "cluster".

freeways). In the vicinity of the Irvine infestation, small clusters of the immature psyllids could be found along with several adult psyllids on the foliage. In June 2009, the Carlsbad site had the highest infestation rankings (= mean of 4.0 for all trees present) of all sites monitored to date. When originally surveyed in 2008, *Euphyllura olivina* infestations were confined to an area within 15 miles of the coast in eastern San Diego and Orange Counties. In 2009, the survey area was expanded and the psyllid was found east of Fallbrook near I-15 (San Diego County) and at three locations around Temecula (Riverside County). These areas are about 30 miles from the coast.

Southern California surveys conducted in 2010. Surveys were continued and expanded in area to attempt to delineate the actual area infested by the psyllid in Southern California. Seven surveys were conducted from March to December (**Table 5**). In May 2010, over 50 sites were checked for the presence of the psyllid. Based on cluster ratings, May appears to be the optimal time for the psyllid immatures, and new infestations may be the easiest to find in May. The olive psyllid was found in two sites northwest of Temecula in Murietta and one site in Valley Center (33° 12" 47' N -117° 01" 59' W), east of Escondido. It was reported to be in Wildomar (33° 35" 37' N -117° 14" 49' W) and at Camp Pendleton (2 sites) by Dr. Genet Tulgetske, UC Riverside.

Over the survey period of 2009 - 2010, once the psyllid was discovered in a location, it was commonly found again in later surveys. There were two exceptions to this trend: Site #36 (Crystal Cove Promenade; 33° 34' 11" N -117° 49' 58" W) where the insect was common in 2009, but absent in 2010. This was odd because the climatic conditions were excellent for the pest at this site, which sat within 100 yards of the ocean between Newport Beach and Laguna Beach. Efforts to ascertain from the landscape manager if the trees had been treated with any insecticide were unsuccessful. It was also absent from Site 40 (Culver Plaza, Culver; 33° 41' 46" N -117° 47' 59" W), where intense pruning appeared to suppress the insect.

California Coastal Surveys in 2009 and 2010. Efforts were also made to find olive psyllid infestations along the coast from Los Angeles north to Monterey. Olive trees were checked in 2009 and 2010 at sites in Oxnard, Thousand Oaks, Santa Barbara, Montecito, Los Olivos, Santa Maria, San Lui Opispo, Carmel Valley, Monterey, Salinas, San Juan Bautista, and Casa del Fruita (**Table 6**). No olive psyllids were found, however, one grower reported an olive psyllid infestation on his trees in Carmel Valley (36° 31' 19" N -121° 50' 53" W). This report was verified by Dr. Charles Pickett, CDFA. Other sightings of the psyllid in San Meto and Santa Clara Counties were reported to the P.I.'s, and we will verify these as soon as we can. No reports have been made of the psyllid's presence in the Central Valley.

Fig. 2. Sample sites in southern California where olive psyllid was found infesting olive trees in urban and agricultural areas in the counties of San Diego, Orange, and Riverside.

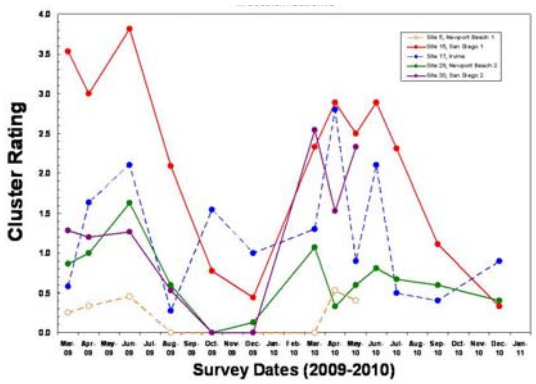

Fig. 3. Changes in mean ranking of psyllid cluster infestations at five survey sites in southern California from March 2009 to December 2010.

Table 1. Twenty-four locations where olive psyllid clusters were not found in Southern California during six and seven surveys in 2009 and 2010, respectively.

Site	Location	Latitude (deg, min, sec)	Longitude (deg, min, sec)	2009	2010
2	Hwy 1, Palos Verdes, Shopping Center	33 48 47 N	-118 22 05 W	X	X
3	Palos Verdes Blvd / Via Monte Doro	33 48 59 N	-118 23 08 W	X	X
11	Government Building on Silver Strand, Coronado Island	32 40 41 N	-117 10 23 W	X	X
18	California Adventure, Anaheim	33 48 23 N	-117 55 09 W	X	X
20	Nr. Woodbury College, Burbank	34 12 33 N	-118 20 38 W	X	X
21	On Via Gavola, Santa Clarita	34 23 41 N	-118 33 04 W	X	X
22	UCR Campus, Riverside	33 58 17 N	-117 19 45 W	X	X
23	Cocos Restaurant, Sun City	33 42 52 N	-117 11 30 W	X	X
33	PF Chang Restaurant, Riverside	33 54 26 N	-117 27 26 W	X	X
34	Sylmar, next to I-210	34 19 23 N	-118 27 56 W	X	X
36	Crystal Cove Promenade [a]	33 34 11 N	-117 49 58 W		X
38	The Marketplace, Irvine	33 43 31 N	-117 47 21 W	X	X
40	Culver Plaza, Culver [a]	33 41 46 N	-117 47 59 W		X
41	Olive View Lane, Sylmar	34 19 27 N	-118 26 59 W	X	X
42	Near Chicago & University, Riverside	33 58 22 N	-117 20 56 W		X
43	Murrietta Rd., Sun City	33 43 08 N	-117 12 23 W	X	X
44	Corner Menifee Rd, & Mapes Rd, Perris	33 45 28 N	-117 09 18 W	X	X
45	Hemet Valley Shopping area, Hemet	33 44 48 N	-116 58 47 W	X	X
46	Nr. Acacia Ave & Darmouth St, Hemet	33 44 37 N	-116 56 13 W		
47	Nr. corner Crest Dr & Dartmouth St, Hemet	33 43 26 N	- 116 56 12 W	X	X
50	Nr. corner Hemet Ave & Thornton Ave, Hemet	33 43 34 N	- 116 55 26 W	X	X
51	Rincon Springs	33 17 18 N	-116 57 03 W		X
53	Chase & Cajon Valley View Streets, El Cajon	32 46 46 N	-116 57 44 W		X
54	Trees by UCR Theatre on campus	33 58 18 N	-117 19 41 W		X
55	Wildomar Shopping Center	33 35 37 N	-117 14 49 W		X

[a] Psyllids were found in the 2009 surveys

Table 2. Twenty-five locations where olive psyllid clusters were found in Southern California during six surveys in 2009.

Site No.	Location	Latitude (deg, min, sec)	Longitude (deg, min, sec)
5	Hwy 1, Newport Beach restaurant	33 36 18 N	-117 52 28 W
6	McArthur Blvd, Newport Beach Library	33 36 27 N	-117 52 26 W
7	San Marcos insurance building	33 08 11 N	-117 11 14 W
8	San Marcos fish restaurant	33 08 08 N	-117 11 19 W
12	Nr. navy hospital across from Balboa Park, San Diego	32 43 50 N	-117 08 46 W
14	Fashion Mall parking lot, San Diego	32 46 01 N	-117 10 14 W
15	Fashion Mall next to building, San Diego	32 46 00 N	-117 10 11 W
16	East Escondido	33 07 55 N	-117 04 07 W
17	Irvine shopping center, intersection I-5 and I-405, Irvine	33 38 53 N	-117 44 25 W
24	Temecula Medical Center, Temecula	33 30 52 N	-117 09 34 W
25	Target Store along I-8, San Diego	32 46 01 N	-117 09 05 W
26	Carlsbad Outlet Stores, Paseo del Norte, Carlsbad	33 07 28 N	-117 19 13 W
27	Mission at Oceanside	33 13 57 N	-117 19 06 W
28	Bella Terra Mall, Huntington Beach	33 43 52 N	-117 59 30 W
29	Bison Bluffs Shopping Center, Newport Beach	33 38 17 N	-117 51 38 W
30	Presidio, San Diego	32 45 33 N	-117 11 40 W
31	Grande Ave, Carlsbad	33 09 48 N	-117 20 43 W
32	Edward Cinemas, San Marcos	33 08 02 N	-117 11 35 W
35	East of Fallbrook, E. Mission Rd & E. Live Oak Park Rd	33 23 27 N	-117 11 13 W
36	Crystal Cove Promenade	33 34 11 N	-117 49 58 W
37	Newport Coast Shopping Center	33 36 28 N	-117 49 39 W
39	Nr. corner Anteater & Bonita Canyon Dr, UC Irvine	33 38 01 N	-117 49 45 W
40	Culver Plaza, Culver	33 41 46 N	-117 47 59 W
48	Baron's Marketplace, Temecula	33 30 50 N	-117 06 11 W
49	Ponte Winery nr Temecula	33 32 13 N	- 117 03 04 W

Table 3. Results of six surveys for olive psyllid clusters during 2009.

Survey Period 2009	Total no. sites checked	Total no. trees checked	Mean no. trees checked per site	No. infested sites	Mean % of infested trees at infested sites	Highest mean cluster rating observed[b]
Mar. 29–April 4	26	341	13.1	16	54.0	3.71
April 24–27	27	365	13.5	18	59.4	4.00
June 4–7	40	624	15.6	23	61.0	4.00
August 8–9	38	575	15.1	17	33.2	2.36
October 13–17	38	526	13.5	4	12.5	1.67
December 5–7[a]	24	326	13.6	5	6.6	1.00

[a] Only previously infested sites were checked because we did not expect the psyllid distribution to expand in cold weather

[b] Infestation ratings were from 0 to 4 (0 = non-infested; 1 = 1 to 3 psyllid clusters; 2 = 4 to 10 psyllid clusters; 3 = light infestation with 1/10th of tree infested; 4 = heavy infestation with more than 1/10th of tree infested).

Table 4. Twenty-six locations where olive psyllid clusters were found during seven surveys in 2010.

Site No.	Location	Latitude (deg, min, sec)	Longitude (deg, min, sec)
5	Hwy 1, Newport Beach restaurant	33 36 18 N	-117 52 28 W
6	McArthur Blvd, Newport Beach Library	33 36 27 N	-117 52 26 W
7	San Marcos insurance building	33 08 11 N	-117 11 14 W
8	San Marcos fish restaurant	33 08 08 N	-117 11 19 W
9	Old Town, San Diego	32 45 14 N	-117 11 51 W
12	Nr. navy hospital across from Balboa Park, San Diego	32 43 50 N	-117 08 46 W
14	Fashion Mall parking lot, San Diego	32 46 01 N	-117 10 14 W
15	Fashion Mall next to building, San Diego	32 46 00 N	-117 10 11 W
16	East Escondido	33 07 55 N	-117 04 07 W
17	Irvine shopping center, intersection I-5 and I-405, Irvine	33 38 53 N	-117 44 25 W
24	Temecula Medical Center, Temecula	33 30 52 N	-117 09 34 W
25	Target Store along I-8, San Diego	32 46 01 N	-117 09 05 W
27	Mission at Oceanside	33 13 57 N	-117 19 06 W
28	Bella Terra Mall, Huntington Beach	33 43 52 N	-117 59 30 W
29	Bison Bluffs Shopping Center, Newport	33 38 17 N	-117 51 38 W
30	Presidio, San Diego	32 45 33 N	-117 11 40 W
31	Grande Ave, Carlsbad	33 09 48 N	-117 20 43 W
32	Edward Cinemas, San Marcos	33 08 02 N	-117 11 35 W
35	East of Fallbrook at corner E. Mission Rd and E. Live Oak Park Rd	33 23 27 N	-117 11 13 W
37	Newport Coast Shopping Center	33 36 28 N	-117 49 39 W
39	Nr. corner Anteater & Bonita Canyon Dr, UC Irvine	33 38 01 N	-117 49 45 W
48	Baron's Marketplace, Temecula	33 30 50 N	-117 06 11 W
49	Ponte Winery nr Temecula	33 32 13 N	- 117 03 04 W
52	Golf Course, Valley Center	33 12 47 N	-117 01 59 W
56	Murrieta Spectrum Center	33 33 23 N	-117 11 58 W
57	Murrieta	33 34 55 N	-117 10 39 W

Table 5. Results of seven surveys for olive psyllid clusters during 2010.

Survey Period 2010	Total no. sites checked	Total no. trees checked	Mean no. trees checked per site	No. infested sites	Mean % of infested trees at infested sites	Highest mean cluster rating observed[b]
March 18–20	25	339	13.6	12	50.3	2.93
April 17–19	20	290	14.5	18	52.4	3.14
May 20–24	51	639	12.5	21	79.2	3.29
June 21–23[a]	10	151	15.1	10	74.2	3.27
July 19–20[a]	10	151	15.1	10	48.3	2.13
September 13–15[a]	10	153	15.3	5	22.9	2.40
December 11–15[a]	10	152	15.2	6	17.8	0.90

[a] The June to December 2010 surveys were limited to ten documented, infested sites to provide information on seasonal trends.

[b] Infestation ratings were from 0 to 4 (0 = non-infested; 1 = 1 to 3 psyllid clusters; 2 = 4 to 10 psyllid clusters; 3 = light infestation with 1/10th of tree infested; 4 = heavy infestation with more than 1/10th of tree infested).

Table 6. Twenty-three locations where olive psyllid clusters were not found along the California coast north of the Palo Verdes peninsula during 2009 and 2010.

Location	Latitude (deg, min, sec)	Longitude (deg, min, sec)	2009	2010
Pacific Coast, Hwy 1	33 50 01 N	-118 23 06 W		23 June
Pacific Coast, Hwy 1	33 55 21 N	-118 23 43 W		23 June
Thousand Oaks	34 10 33 N	-118 50 45 W		23 June
Thousand Oaks	34 10 49 N	-118 52 36 W		23 June
Oxnard	34 13 10 N	-119 10 32 W		24 June
Santa Barbara	34 25 20 N	-119 41 48 W		24 June
Santa Barbara	34 25 25 N	-119 41 38 W		24 June
Montecito	34 25 41 N	-119 38 39 W		24 June
Montecito	34 26 32 N	-119 38 35 W		24 June
Los Olivos	34 40 02 N	-120 06 54 W		24 June
Santa Maria	34 56 02 N	-120 25 08 W		24 June
San Lui Opispo	35 17 08 N	-120 39 49 W		24 June
Carmel Valley	36 20 01 N	-121 45 19 W		31 May
Carmel Valley	36 31 51 N	-121 50 06 W		31 May
Carmel Valley	36 31 54 N	-121 50 28 W		31 May
Carmel Valley	36 32 32 N	-121 54 19 W		31 May
Carmel Valley	36 32 35 N	-121 53 51 W	28 May	31 May
Monterey	36 36 10 N	-121 53 34 W	28 May	
Salinas	36 39 23 N	-121 39 41 W	28 May	31 May
Salinas	36 40 17 N	-121 39 15 W	28 May	31 May
San Juan Bautista	36 50 29 N	-121 32 04 W		31 May
San Juan Bautista	36 50 45 N	-121 32 40 W		31 May
Casa del Fruita	36 59 24 N	-121 22 53 W	28 May	31 May

Objective 2

Over the two-year survey period very few natural enemies were seen in association with olive psyllid clusters. Green lacewing larvae, mealybug destroyer (*Cryptolaemus montrouzieri*), and some ladybugs were infrequently found associated with the olive psyllid. On three occasions, large numbers of psyllid-infested branches were collected from several heavily-infested sites and held in the laboratory (UC Irvine and UC Berkeley quarantine) to see if parasitoid natural enemies would emerge from the collected material. No parasitoids were found in association with the psyllid. However, a predatory insect, similar to the minute pirate bug (Hemiptera: Anthocoridae), was found infesting one shipment to Berkeley and is believed to have significantly reduced the numbers in the psyllid shipment.

A parasitoid, *Psyllaephagus* spp. (Hymenoptera: Encyrtidae), was collected in Spain by K. M. Daane, UC Berkeley, and C. Pickett, CDFA, and shipped to the quarantine facility at UC Berkeley in 2009 and 2010. This species was maintained in colony in the quarantine for several months, but was lost due to problems in establishing a continuing olive psyllid colony.

CONCLUSION(S)

Olive psyllid is well established along the coast and inland areas in Orange, San Diego, and Riverside Counties. New records of infestation were recorded near Fallbrook and Valley Center (San Diego County) and within and outside the area from Temecula to Murrieta (Riverside County). The Temecula infestations were the first records of the psyllid being found in Riverside County. In most locations, the mean rankings of the infestations (≤ 3) did not appear to be damaging to the olives present at the various sites. The psyllid infestations appeared to follow a seasonal trend similar to what has been reported in Europe and the Middle East. Rankings of populations in most of the infested sites that were monitored remained low from October to March and then increased over the spring period and peaked in June. As summer temperatures increased, density rankings decreased in August 2009 and hit their low point in December 2009. Similar trends were noted in 2010 when rankings peaked in June and declined in December. The existence of continuing psyllid infestations in Temecula and near Fallbrook from 2009 to 2010 should provide insights into the potential for the psyllid to establish in the San Joaquin Valley.

Currently, the distribution of the psyllid appears to be limited by summer temperatures (**Fig. 4**). The psyllid has been reported to be unable to survive temperatures above 90°F. The map below represents the frequency that maximum daily temperatures surpass 90°F in southern California. The dark red areas are where temperatures in August are common from 80 to 100 percent of the time (based on 10 years of climatic data). Note that the red dots indicate sites where olive pysllid infestations were found and tend to be more common in the cooler parts of the map. Yellow dots indicate the absence of psyllids in surveys. Yellow dots in the cooler areas probably represent areas to which the psyllid has not expanded its distribution.

Natural enemies were rarely observed in association with the psyllid clusters. However, the inability to dissect the clusters and closely examine the psyllid individuals within (due to the lack of a laboratory setting where the clusters could be examined) reduced our ability to say how common natural enemies may have been associated with the psyllids.

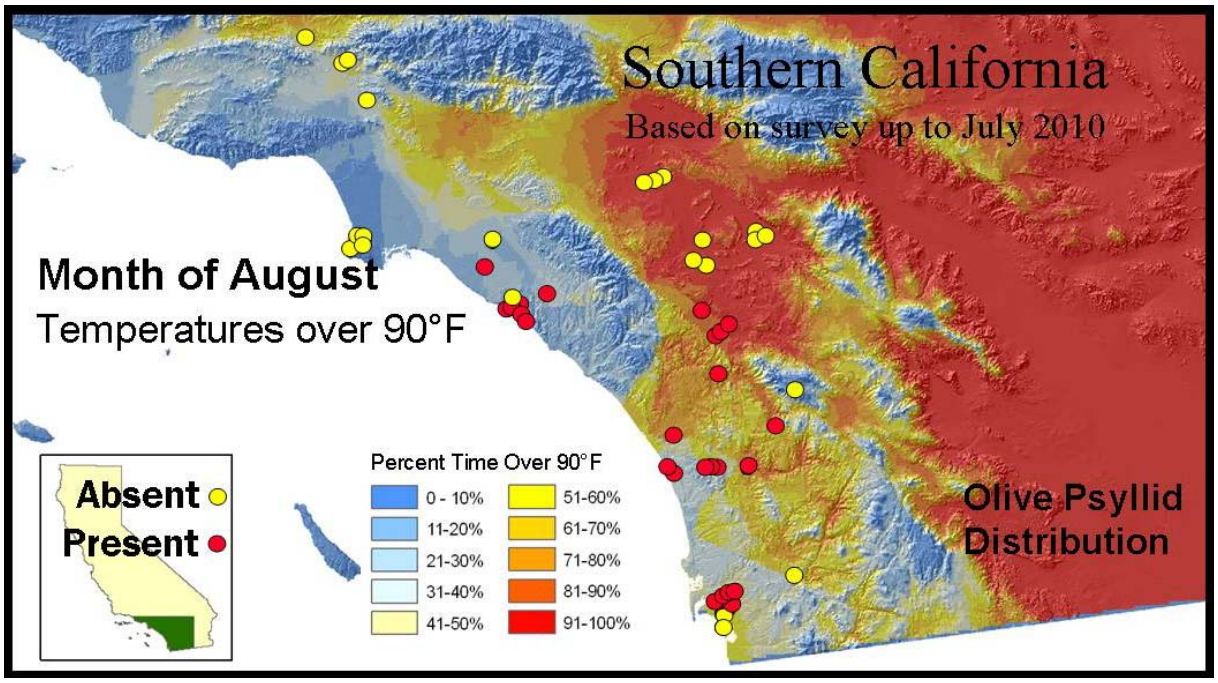

Fig. 4. Presence (red dots) and absence (yellow dots) of olive psyllid infestations based on 2 years of surveys in southern California. The various colors in the map represent the percent of time that maximum temperatures are above 90°F (based on 10 years of temperature data).

ACKNOWLEDGEMENTS

Special thanks to Lynn LeBeck who assisted with surveys in southern California and Brad Hawkins for providing space in his laboratory at UC Irvine. Thanks to Genet Tulgetske, who found new infestations in the Temecula and Camp Pendleton areas. Thanks to Charles Pickett for information on the infestation in Carmel Valley.

FUNDING SOURCE(S)

Funds used to support this project were provided by the California Olive Committee and from Hatch funds allocated to the senior P.I. by UC Riverside to support research on tree crops.

Biological & Cultural Control of Olive Fruit Fly in California — Utilization of Parasitoids from USDA-APHIS-PPQ, Guatemala & Cultural Control Methods

Project Leader: Victoria Y. Yokoyama, USDA-ARS-SJVASC, 9611 S. Riverbend Ave., Parlier, CA 93648, (559) 596-2751, vyokoyama@fresno.ars.usda.gov

Cooperating Personnel:

Pedro Rendón and Carlos Cáceres, USDA-APHIS-PPQ-CPHST, Guatemala City, Guatemala
Xingeng Wang, Associate Research Entomologist, UC Riverside & UC Berkeley
Marshall W. Johnson, Cooperative Extension Specialist & Entomologist, UC Riverside
Kent Daane, Cooperative Extension Specialist, UC Berkeley

Reporting period: 1 January 2009 to 30 June 2010.

ABSTRACT

The parasitoid *Psytallia humilis* (= *P*. cf. *concolor*) (Szépligeti) was reared on sterile Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), larvae at the USDA, APHIS, PPQ, Moscamed biological control laboratory in San Miguel Petapa, Guatemala, and shipped to the USDA, ARS, Parlier, for biological control of olive fruit fly in California. Parasitoids were released in olives infested with olive fruit fly in the Sacramento and San Joaquin Valleys, and North and Central Coast of California. Subsequent generations of parasitoids were recovered from olive fruit fly pupae in most locations, except when infested olives were scarce such as found during winter. Numbers of olive fruit fly were very low in the Central Valley except for Lodi based on adult trap captures and fruit collections, but higher numbers of the pest were found in foothill locations. Irradiation of the host at different doses did not affect survival of *P. humilis* in laboratory tests at different temperatures with and without water and honey for food. Life span was significantly increased by the presence of water and honey (for food), and cool temperature at 15°C versus 35°C. The parasitoid showed high dispersal capacity with continuous flight in different air temperatures and air speeds. Female and male parasitoid flight time ranged from 4-20 min and 3-14 min, and the longest flights ranged from 53-110 and 20-52 min, respectfully. An olive fruit fly colony has been reared on formulated diet for > 40 generations, but the larval stage was not acceptable for oviposition when exposed to parasitoids in mesh and waxed cages. The number of olive fruit fly larvae per fruit from trees with parasitoid releases versus without was 0.6 and 1.0, respectively, suggesting that the *P. humilis* reduced host numbers in Lodi.

INTRODUCTION

The parasitoid *Psytallia* cf. *concolor* (Szépligeti) has been imported from the USDA, APHIS, PPQ, Moscamed, biological control laboratory in San Miguel Petapa, Guatemala, for biological control of olive fruit fly, a major pest in olives. Recent genetic studies have shown that the parasitoid is distinct from related species, and the name was changed from *P*. cf. *concolor* to *P. humilis* (Yokoyama et al. 2010). Results from previous field releases show that the parasitoid reproduces in olive fruit fly in different regions of California as far south as San Diego, in many coastal areas, and as far north as Orland. To date, *P. humilis* has shown promise

for biological control of olive fruit fly by augmentative field releases with resultant high rates of parasitism in some locations. Although we have recovered the parasitoid in a new crop of olives following a release the previous year, we have not yet attained establishment in olive fruit fly. We propose to continue large-scale production of *P. humilis* and importation and release in California with emphasis in the canned olive growing areas of the Central Valley to help reduce pest infestations.

The Petapa biological control laboratory in Guatemala currently mass produces parasitoids for control of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and Mexican fruit fly, *Anastrepha ludens* (Loew), including *Psyttalia humilis* (= *P.* cf. *concolor*) from Kenya in 1997, which is the species that we have released in California; *P. concolor* from Kenya in 2001; *P. cosyrae* from Kenya in 2002; and *Fopius ceratitivorus* collected from Kenya in 2001. The latter two species are available and have been selected as candidates for testing on olive fruit fly. We have been successful in obtaining USDA-APHIS-PPQ permission to ship olive fruit fly and other potential non-target fruit fly species including the pests, walnut husk fly and cherry fruit fly; and the beneficial cape ivy fly and seedhead fly that are used for biological control of weeds to the quarantine laboratory in Petapa, but the permits from Guatemala to allow entry have been elusive.

The use of irradiated Mediterranean fruit fly to prevent contamination of parasitoid shipments with fertile hosts may affect the survival and vigor of the parasitoids produced by this method. We propose that we can evaluate the quality of imported parasitoids in laboratory studies. Longevity of parasitoids reared from hosts irradiated at different doses can be studied in incubator tests. Flight endurance as a measure of vigor between parasitoids reared from fertile and irradiated hosts can be determined in laboratory and wind tunnel tests.

A formulated diet and rearing methods developed in the laboratory of Dr. Marshall Johnson have been used in our lab to rear olive fruit fly in large quantities. Techniques to rear large numbers of parasitoids from these insects will be investigated. The ability to produce a domestic supply of parasitoids on demand will support the olive fruit fly biological control program. Furthermore, maintenance of this colony provides research material throughout the year to conduct studies of control techniques.

Olive fruit fly infestations in the San Joaquin and Sacramento Valleys have been exceptional low in comparison to coastal populations. The hot and arid inland valley climate has suppressed the development of high numbers of the pest. These conditions may be conducive to economical means of control including mass trapping to reduce the number of adults in orchards. We have discovered that the older larvae that leave the fruit have the capacity to disperse over long distances and techniques may be available to restrict such movement. The ability of adults emerging from pupae for movement through soil and litter on the orchard floor needs further investigation to develop methods to contain this active life stage.

OBJECTIVES

1) Continue releases of the parasitoid *P. humilis* in different regions in California for biological control of olive fruit fly;

2) Evaluate other parasitoids that are mass-produced in Guatemala for importation;

3) Develop methods to rear a domestic supply of large numbers of parasitoids; and

4) Develop cultural control practices that further reduce pest populations in the inland valley regions.

PROCEDURES

Objective 1

The *P. humilis* used in field releases are reared on Mediterranean fruit fly at the Petapa biological control laboratory in Guatemala. Personnel are hired to rear, collect, and package the parasitoids for shipment to California. The parasitoids are flown under permit by DHL air freight from Guatemala City and enter the U.S. through Miami Customs and Border Protection and USDA, APHIS, PPQ inspection. The shipment arrives in Parlier, California, in two days with low parasitoid mortality in transit. The parasitoids are unpackaged in an isolated screened room and released into cages, fed honey, and provided with water. The insects are held for 1-2 days for observation and mating, and transported by automobile to release sites.

Olive fruit fly adult populations are monitored in infested olive trees with yellow sticky traps with spiroketal lures and ammonium carbonate baits. Larval populations are determined by larval, pupal, and adult emergence from pre-release collections of infested fruit at each site. Release sites are made available by individuals and industry cooperators primarily in the San Joaquin and Sacramento Valleys and the North and Central Coast Regions. The parasitoids are released from opened cages into trees with fruit infested with olive fruit fly. Parasitoid releases are made either once with a subsequent post-release evaluation or repetitively in locations with high olive fruit fly infestations and fruit that remains in the trees for an extended period. Temperature and humidity data are monitored with data loggers.

Post-release samples of infested fruit that have been exposed to the parasitoid are collected and maintained in the laboratory to determine the numbers of olive fruit fly and parasitoids that emerge. Rates of parasitism are calculated from previously published formulas and are based on the number hosts or parasitoids that emerge from pre-release and post-release fruit samples. The number of olive fruit fly larvae per fruit was compared in trees with and without parasitoid releases in Lodi.

Objective 2

Different species of parasitoids including *P. humilis*, *P. cosyrae*, and *Fopius ceratitivorus* that are produced in large numbers at the Petapa biological control laboratory in Guatemala will be tested for development in olive fruit fly and non-target fruit fly hosts including cape ivy fly, seedhead fly, walnut husk fly, and cherry fruit fly. Olive fruit fly and the other fruit fly species that are currently in culture in USDA, ARS, laboratories will be sent to the biological control laboratory in Petapa and tested in the quarantine facility using techniques previously described for host range tests. USDA, APHIS, PPQ, letters of no-permit required have been granted for all fruit fly species to allow shipment to Guatemala, and a proposal to allow entry into quarantine in the Petapa biological control laboratory has been submitted to the Guatemala Ministry of Agriculture and is pending.

Mediterranean fruit fly larvae were irradiated at 0, 40, 50, 60, and 70 Gray (Gy) and exposed to oviposition by *P. humilis* in the Petapa biological control laboratory in Guatemala. The parasitoids that emerged from the irradiated larvae were shipped to Parlier, CA, to determine longevity in cage and incubator tests. About 50 parasitoids reared from larvae from each irradiation dose were placed in a small plastic cage and exposed to either 15, 25, or 35°C and provided with either 1) no water and no honey for food, or 2) water alone, or 3) water and honey. Mortality in each cage was determined daily and each test was replicated three times.

The flight endurance of *P. humilis* in static and moving air was determined at different temperatures and air velocities. A 2.5 cm long, 70 denier nylon thread was glued to the ventral side of the abdomen of individual female and male parasitoids. The opposite end of the nylon thread was attached to a pinch clamp or to the top of a wind tunnel. Air was wafted across the parasitoid to initiate flying and the time in non-stop flight greater than 1 min was determined for individuals at air speeds of 0, 0.51, and 0.89 m per sec; temperatures from 21–25ºC; female parasitoid age of 3–15 d; and male parasitoid age of 5–13 d.

Objective 3

Olive fruit fly larvae can be produced in very large quantities on a laboratory diet with brewers yeast and soy protein as the main nutritive ingredients. Mature larvae emerge from the diet and pupate. The pupae are harvested and adults that emerge from the pupae are placed in ovipositional cages with waxed cones. The adults lay eggs in the cones and the eggs are harvested and placed in the diet to produce more larvae. Any life stage can be produced in very high numbers using this rearing procedure. Olive fruit fly produced from the colony on laboratory diet will be used in behavioral studies and laboratory and greenhouse tests to determine the efficacy of different control techniques.

Parasitoid adults will be placed in screened cages after mating and mature olive fruit fly larvae will be provided in small cloth cages for exposure to parasitoid oviposition. The parasitized larvae will be allowed to pupate and held in incubators until parasitoid adults emerge. The longevity and fecundity of parasitoids will be compared between those that emerge from hosts produced on diet versus olive fruit. The parasitoids will be used for release or maintenance of the parasitoid colony.

Objective 4

Our studies have shown that different styles of traps are effective in attracting olive fruit fly adults when combined with baits and lures. Color and color patterns that are acceptable to the adults, but not other insects will be evaluated in the greenhouse and field with and without baits and lures. These traps will be developed for economical acquisition and use and as a potential alternative to commercial traps. In addition, traps developed by other workers and used for tropical and subtropical fruit fly control will be investigated for attract-and-kill and mass trapping of olive fruit fly.

Preliminary studies have shown that mature olive fruit fly larvae and newly emerged, non-flying adults can travel long distances. Investigations will be continued to determine the full range of dispersal by these life stages and factors that may inhibit such behavior. Movement of larvae and newly emerged adults will be measured on temperature controlled resin surfaces and factors that may limit dispersal such as ground mulches will be tested in screened greenhouse cages.

RESULTS AND DISCUSSION

Objective 1

P. humilis was mass produced in the biological control laboratory in Petapa, Guatemala, and shipped to Parlier, CA. Thirteen shipments were received between September and December 2009 and the number of parasitoids in each shipment ranged from 2,930 to 21,922 individuals.

Ten of the shipments were used for field releases in olive trees infested with olive fruit fly and three shipments were used to determine the effect of rearing the parasitoids on irradiated host.

Parasitoid releases conducted in late 2008 through December 2009 were evaluated with pre-release and post-release samples of fruit in regions and locations as follows: Sacramento Valley at Orland and Oroville; North Coast at San Jose; San Joaquin Valley at Lemon Cove, Visalia, Grapevine, Bakersfield, and Lodi; and the Central Coast at San Luis Obispo, and Solvang. Weather data, including temperatures and relative humidities; olive fruit fly adults captured on yellow Pherocon AM traps with baits and lures; larval infestations in pre-release and post-release fruit samples, and calculated rates of parasitism will be reported in a comprehensive manuscript for publication when the evaluations have been completed. An interim summary of these parasitoid releases are shown in **Table 1**. Subsequent generations of the parasitoid were not recovered from olive fruit fly after winter releases in Orland, Oroville, San Jose, Lemon Cove, and Solvang primarily due to the scarcity of fruit in the trees and negligible olive fruit fly infestations during the winter. Parasitoids were released in Visalia and Porterville during the fall, but the progeny were not recovered from fruit samples at these locations because of very low infestations of olive fruit fly larvae. The number of olive fruit fly larvae per fruit from trees with versus without parasitoid releases was 0.6 and 1.0, respectively, suggesting that the *P. humilis* reduced host numbers in Lodi.

Parasitoid releases in 2009 emphasized olive fruit fly infestations on the east and south side of the Central Valley. Olive fruit fly numbers and rates of parasitism in these locations are under evaluation, but subsequent generations of the parasitoid were recovered in most post-release samples (**Table 1**). New locations of infestations were identified in the foothills and were related to slightly higher elevations where temperatures were cooler than in the Central Valley.

Parasitoid releases were conducted in the Central Coast locations in December, but discontinued in 2010 because fruit infested with olive fruit fly were limited. Prior to a 2009 parasitoid release in San Jose and Solvang, fruit infested with olive fruit fly were evaluated for new generations of parasitoids that may have resulted from previous 2008 releases, but over-wintering parasitoids were not recovered.

Yokoyama et al. (2010) reported the results of parasitism of olive fruit fly resulting from releases of *P. humilis* in 2008 through 2009.

Objective 2

Shipment of olive fruit fly and other fruit fly species to the quarantine facility for host testing at the Petapa biological control laboratory in Guatemala was cancelled due to technical difficulties. Further collaborations resulted in the application of USDA, APHIS, PPQ, permits to import two parasitoids, *Fopius ceratitivorus* and *P. corsyrae*, into the UC Berkeley quarantine facility for evaluation for biological control of olive fruit fly. APHIS, PPQ and the California Department of Food and Agriculture with the provision of full containment has expressed no issue with the importation of the two parasitoids. The egg parasitoid *F. ceratitivorous* was found to be established in association with olive fruit fly infestations in the Middle East and may have potential to adapt to olive fruit fly in California.

The effect of rearing parasitoids from irradiated Mediterranean fruit fly larvae is still under investigation. Our preliminary results in laboratory cage tests show that Mediterranean fruit fly larvae irradiated with doses of 40, 50, 60, and 70 Gy do not affect the quality of the parasitoid produced from the larvae (**Table 2**). Temperature is the most important factor that affects parasitoid survival. Under optimum conditions of providing the parasitoids with honey for food and water, the adults are short lived (7-16 d) at the highest temperature tested 35°C (95°F);

Table 1. 2009 locations, dates, and numbers of *Psyttalia humilis* adults released in different regions of California, and the occurrence of parasitoid progeny in olive fruit fly from post-release samples of fruit.

Region	Location	2009 Release date	No. released	2009 Post-release date	Parasitoid progeny recovered
Sacramento Valley	Orland	9 Jan.	15,768	30 Jan.	No
	Oroville	10 Jan.	8,078	30 Jan.	No
		22 Nov.	<5,240	28 Nov.	Yes
North Coast	San Jose	18 Dec. ('08)	18,677	10 Feb.	No
		17 Sept.	<11,136	24 Sep.	Yes
San Joaquin Valley	Lemon Cove	30 Oct. ('08)	4,330	9 Jan.	No
	Visalia	16 Sep.	<5,568	25 Sep.	No
	Grapevine	5 Oct.	<6,144	9 Oct.	Yes
	Bakersfield	9 Oct.	<1,192	15 Oct.	Yes
	Lodi	10 Oct.	<1,788	23 Oct.	No
		27 Nov.	<4,523	4 Dec.	Yes
		4 Dec.	<9,533	14 Dec.	No
	Porterville	26 Oct.	<4,984	3 Nov.	No
Central Coast	Solvang	25 Jan.	13,510	3 Feb.	No
		21 Dec.	<21,922	30 Dec.	Yes
	San Luis Obispo	11 Dec.	<11,273	18 Dec.	Yes
		18 Dec.	<21,922	31 Dec.	Yes

survived the longest (> 83 d) at the lowest temperature tested 15°C (59°F); and lived for 31 to >76 d at moderate temperatures 25°C (77°F). In the absence of water and honey for food, the parasitoids were much shorter lived than when receiving full provisions at all temperatures tested. At 15°C, the parasitoids could survive about 1 wk without water and food and about 2 wk with water. Irradiation of Mediterranean fruit fly larvae for production of *P. humilis* for biological control of olive fruit fly in California will help ensure that the fertile host will not occur in shipments.

Average flight endurance of the parasitoid *P. humilis* ranged from 4-21 and 7-14 minutes for females and males, respectively under different environmental conditions (**Table 3**); and the longest flights were about 75 and 39 minutes for the females and males, respectively (**Fig. 1**). The results show that the parasitoids are capable of flight over long distances that are favorable for dispersal among olive fruit fly infestations in different areas.

The results of the parasitoid flight tests conducted in this study were published by Yokoyama et al. (2010).

Table 2. Longevity (range in days of 3 replicate tests) of the parasitoid, *Psyttalia humilis*, when reared from Mediterranean fruit fly larvae irradiated at different doses and provided without honey for food, water only, or honey and water and held at different temperatures.

Temp., °C	Provisions	Irradiation dose, Gy				
		0	40	50	60	70
15 (59°F)	None	0-11	8-15	9-11	11	9-12
	Water	10-15	11-15	11-12	13-15	13-15
	Water + Honey	>55-69	>55-69	>55-69	>55-69	>55-69
25 (77°F)	None	3	3	3	3	3
	Water	4-12	3-5	3-4	4-5	3-4
	Water + Honey	46- >69	31- >62	40- >62	35- >62	39- >62
35 (95°F)	None	1-2	2	2	2	2-3
	Water	2-3	2-3	2-3	2-3	2-3
	Water + Honey	8-16	7-11	9-14	9-13	9-14

Table 3. Mean (± SEM) minutes of continuous flight time for female and male *Psyttalia humilis* at different air speeds and temperatures.

Sex	Air speed (m per sec) / temperature treatments			
	0 / 24 ºC	0.51 / 25 ºC	0.51 / 21 ºC	0.89 / 22 ºC
Female	4.06 ±1.04	5.70 ± 0.73	14.98 ± 5.27	20.54 ± 8.11
Male	6.58 ± 1.94	2.94 ± 0.61	5.66 ± 1.54	13.69 ± 5.02

Objective 3

The parasitoid was not stimulated to lay eggs in mature olive fruit fly larvae when the larvae were exposed to the females in diet or in different devices with domed shapes to simulate olive fruit.

The olive fruit fly colony reared on formulated diet has been maintained through more than 40 generations. The colony will be used for collaborative research with the USDA, ARS, Albany, CA, for irradiation studies to develop a sterile insect technique for olive fruit fly. The insects from the colony are currently used for behavioral studies and to determine the efficacy of control methods in the laboratory and greenhouse when the wild population is unavailable.

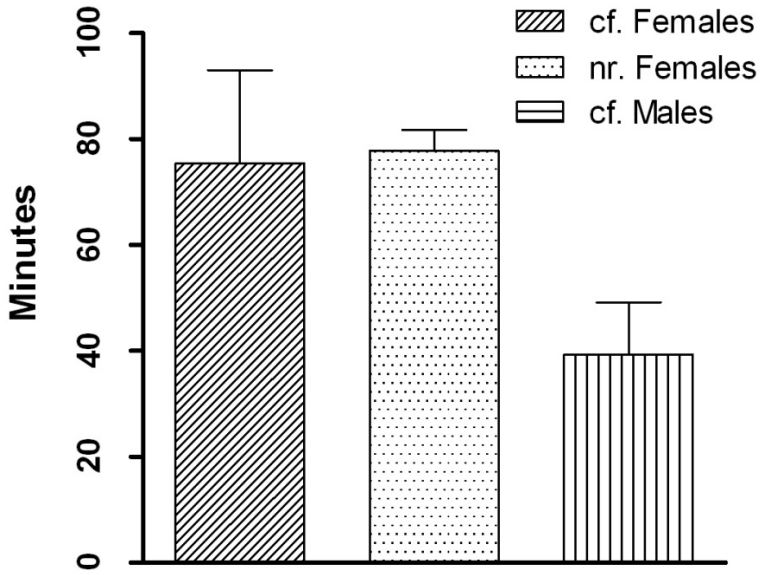

Fig. 1. Comparative flight endurance of females and males of *Psyttalia humilis* originating from Kenya (cf) or Tunisia (nr).

Objective 4

A large yellow pan trap was obtained from the USDA, ARS, Hilo, HI, for evaluation for control of olive fruit fly. The trap was developed in Hawaii and shown to be effective in reducing numbers of tropical fruit fly species and cherry fruit fly. A second yellow cone trap manufactured and used in Asia for fruit fly control was also acquired through the ARS, Hilo lab for testing on olive fruit fly. Lures and baits are attached as insect attractants and GF-120 is applied to the underside of the trap as an insecticide.

The distance that olive fruit fly adults can travel without flight was at least 20 feet in laboratory pipe tests. The test device has been extended to 25 feet in aluminum channels. The adult that has just emerged from the pupal stage was found in these tests to be highly active and capable of walking long distances prior to flight. The dispersal capacity of the third instar and the non-flying, newly emerged adult is under investigation in relation to containment by ground mulches.

CONCLUSIONS

The parasitoid *Psyttalia humilis* was successfully mass-produced on sterile Mediterranean fruit fly in Guatemala imported into California for biological control of olive fruit fly. Irradiation of the host did not affect parasitoid survival after importation, but the parasitoid required water and food for honey and mild temperatures for longevity. Subsequent generations of the parasitoid were recovered in most release locations except when fruit and olive fruit fly larvae were scarce such as in the winter or in the Central Valley where summer temperatures are not optimum for the host. Female parasitoids were found to fly as long as 110 minutes without stopping, thereby showing a strong capacity to disperse among pest populations. Newly emerged olive fruit fly adults showed the ability to travel distances without flight which would enhance the distribution of the pest in olive orchards.

FUNDING SOURCES

External funding was received from the California Olive Committee. Internal funding was provided by the USDA-ARS-SJVASC, Parlier, CA; USDA-APHIS-PPQ-CPHST, Guatemala; and the Moscamed Program, USA-Mexico-Guatemala

REFERENCES

Yokoyama, V. Y. and G. T. Miller. 2004. Quarantine Strategies for olive fruit fly (Diptera: Tephritidae): Low-temperature storage, brine, and host relations. J. Econ. Entomol. 97: 1249-1253.

Yokoyama, V. Y., G. T. Miller, and J. Sivinski. 2004. Quarantine control strategies for olive fruit fly in California, pp. 241-244. Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance, 6-10 May 2002, Stellenbosch, South Africa..

Yokoyama, V. Y., G. T. Miller, J. Stewart-Leslie, R. E. Rice, and P. A. Phillips. 2006. Olive fruit fly (Diptera: Tephritidae) seasonal populations in relation to region, trap type, season, and availability of fruit. J. Econ. Entomol. 99: 2072-2079.

Yokoyama, V. Y. and G. T. Miller. 2007. Olive fruit fly biology and cultural control practices in California. IOBC/WPRS Bull. 30: 277-285.

Yokoyama, V. Y., P. A. Rendón, and J. Sivinski. 2008. Biological control of olive fruit fly (Diptera: Tephritidae) by releases of *Psyttalia* cf. *concolor* (Hymenoptera: Braconidae) in California, parasitoid longevity in presence of the host, and host status of walnut husk fly, pp. 157-164. Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance, 10-15 September 2006, Salvador, Bahia, Brazil.

Yokoyama, V. Y., P. Rendón, and J. Sivinski. 2008. *Psyttalia* cf. *concolor* (Hymenoptera: Braconidae) for biological control of olive fruit fly (Diptera: Tephritidae) in California. Environ. Entomol. 37: 764-773.

Yokoyama, V. Y. 2011. Olive fruit fly (Diptera: Tephritidae) longevity and development in laboratory and greenhouse tests and in olive fruit in California. J. Econ. Entomol. (in review).

Yokoyama, V. Y., P. A. Rendón, X. G. Wang, S. B. Opp, M. W. Johnson, & K. M. Daane. 2011. Response of *Psyttalia humilis* (Hymenoptera: Braconidae) to olive fruit fly (Diptera: Tephritidae) and conditions in California olive orchards. Environ. Entomol. 40: (in print).

Yokoyama, V. Y., C. E. Cáceres, L. P. S. Kuenen, X. G-Wang, P. A. Rendón, M. W. Johnson, and K. M. Daane. 2010. Field releases, reproduction, and flight endurance of an olive fruit fly parasitoid, *Psyttalia humilis* (Hymenoptera: Braconidae), reared on irradiated Mediterranean fruit fly. Biological Control 54: 90-99.

Formation of Acrylamide in California-Style Black Ripe Olive

Project Leader: **Alyson E. Mitchell**, Department of Food Science and Technology, Robert Mondovi Institute of Wine and Food Science, University of California, Davis, CA 95616, (530) 752-7926, aemitchell@ucdavis.edu

Cooperating Personnel:

Dan Flynn, UC Davis Olive Center, Robert Mondavi Institute for Wine and Food Science, University of California, Davis

Suthawan Charoenprasert, Graduate Student, Department of Food Science and Technology, Robert Mondovi Institute of Wine and Food Science, University of California, Davis

Reporting period: 1 January 2009 to 31 December 2010

ABSTRACT

California-style black ripe olive processing methods result in the formation of acrylamide. Acrylamide is classified as a probable human carcinogen (IARC 1994) and is listed as a Proposition 65 (Safe Drinking Water and Toxic Enforcement Act of 1986) chemical in California. The finding of high levels (μg to mg kg-1) of acrylamide in foods cooked at elevated temperatures by the Swedish National Food Authority in 2002 led to worldwide concern and recommendations by the WHO for food manufactures to mitigate levels in food products. Acrylamide is typically found in heat-treated foods that contain high levels of the amino acid asparagine, and reducing sugars such as glucose. These precursors react together through Maillard chemistry to form acrylamide. California black ripe olives contain relatively high concentrations of acrylamide (200-2000 ng/g) compared to other carbohydrate rich foods such as french fries (100-1300 ng/g) and roasted nuts (<500 ng/g) (USDA 2006), yet have low levels of the typical precursors that are found in these foods. To date, little is known regarding the formation mechanism of acrylamide in olives

During our first year of research funded by the California Olive Committee (2009-2010) we developed analytical methods and laboratory scale processing models to evaluate the influence of California black-ripe olive processing methods on acrylamide formation. Our results indicate that acrylamide levels in California black-ripe olives (386-467 ng/g) are much higher than that in canned green-ripe, Spanish-style, and Greek-style table olives. According to the results from our laboratory scale processing models, California black-ripe olives contain acrylamide levels twice higher than non-oxidized olives and ferrous gluconate treatment does not affect acrylamide formation. We also determined that the precursors of acrylamide are formed during the lye treatment step, when air oxidation is involved, and converted to acrylamide during sterilization. Based upon these findings, we have developed the hypothesis that acrylamide precursors are being formed through the oxidation of lipids during lye processing and that subsequent pasteurization results in the conversion of these precursors to acrylamide. We believe that the levels of acrylamide can be decreased through the addition of appropriate antioxidants during critical processing steps. Addition of antioxidants during the right stage of processing will: decrease the degradation of the naturally occurring olive polyphenolics and decrease the

oxidative formation of acrylamide precursors (e.g., lipids) and ultimately acrylamide in processed olives. We also would like to investigate if phenolic antioxidants obtained from olive waste materials (e.g., leaves, stems) are an economical source of antioxidants (e.g., hydroxytyrosol, the major antioxidant in olives). The use of these materials to control oxidative formation of acrylamide would have the added benefit of reducing processor waste and improving industry sustainability.

INTRODUCTION

Olives contain high concentrations of oil accounting for 9.5-20.1% of total fruit weight and have low amounts of carbohydrate (1.3-3.2%) and protein (1.1-1.4%). Most lipids present in olives are triglycerides. Oleic acid, a monounsaturated fatty acid, is the major fatty acid in olives. It can comprise up to 80% of total fatty acids. Aspartic acid, glutamic acid, and arginine are the most abundant amino acids. They account for 30% of total amino acids in olives (Fernandez et al. 1997). Olives also contain high levels of phenolic compounds. Oleuropein, a phenolic secoiridoid glycoside, which is responsible for the bitterness in olives, is the most abundant (Eskin and Tamir 2006). The other phenolics found in olives include hydroxytyrosol, tyrosol, luteolin, and rutin (Romeo et al. 2002; Vinha 2005).

California black ripe olive processing methods consist of lye treatment and air oxidation followed by washing, neutralization with carbon dioxide, ferrous gluconate treatment, canning and sterilization. Olives that can not be processed right after the harvest are preserved in a brine solution containing acetic acid to prevent the growth of spoilage organisms. California black ripe olive processing methods remove the bitterness of olives via the alkali hydrolysis of oleuropein that occurs during lye treatment (Fernandez et al., 1997). Air injected during lye treatment oxidizes hydroxytyrosol, a product formed from the hydrolysis of oleuropein, and is responsible for the black pigments associated with these olives. These pigments are unstable. Therefore ferrous gluconate is used to fix the black color (Marsilio et al., 2001).

Hydroxytyrosol is the main bioactive phenolic compound in table olives. It has been shown to reduce the risk of cardiovascular disease and cancer. Unfortunately, the California black ripe processing methods result in olives with low levels of hydroxytyrosol as it becomes oxidized during color fixation by ferrous ions (ferrous gluconate).

The mechanism of acrylamide formation has been widely studied. Glucose and asparagine are well known to be the predominant precursors in carbohydrate rich foods. They react together and form acrylamide through Maillard reaction (Yaylayan et al. 2003; Stadler et al. 2004). Formation of acrylamide from lipids is another possibility. Ehling et al. (2005) investigated the formation of acrylamide in model systems where lipids were mixed with asparagine and heated at 180 °C for 30 minutes. High levels of acrylamide formed when heating the mixtures with lipids having a high degree of unsaturation, which is susceptible to oxidation. It is thought that the aldehydes and ketones produced through the oxidation of lipids may be responsible for the production of acrylamide. Another possible pathway for acrylamide formation via lipids, involves the reaction of acrolein and acrylic acid with ammonia. Acrylic acid can be formed through oxidation of acrolein, a product of thermal degradation and/or oxidation of free fatty acids or glycerol (Skog and Alexandra 2006). Yasuhara et al. (2003) heated model systems containing acrolein and ammonia at various temperatures for 30 minutes and found that acrylamide formed at room temperature and reached its maximum level (753 µg/g of ammonia) at a temperature of 180°C. In another model system containing acrylic acid and ammonia (heated at 180°C for 30 minutes) acrylamide formed at very high levels (190,000 µg/g of ammonia).

Formation of acrylamide during California black ripe olive processing was studied by Casado and Montaño in 2008. Olive samples were collected before and after sterilization. Sterilized olives contained acrylamide ranging from 3.3 to 19.0 µmol per 1 kilogram of fresh olives. Asparagine content in olives before sterilization ranges from 0.4 to 1.5 µmol per 1 kilogram of fresh olives, which is lower than concentration of acrylamide found in the final products. Moreover, there is no correlation between concentration of glucose and amino acids (both total and individual amino acids) with acrylamide formation in black olives. Therefore, it appears that asparagine and glucose are not the main precursors of acrylamide in California black ripe olives.

Most experiments for studying formation of acrylamide from lipid have been designed based on frying conditions. The California black ripe olive processing method consists of many different processing steps that involve the use of several chemicals. Various reactions can occur during the process. The mechanism of acrylamide formation in California black ripe olives may differ from those in other foods. Although California black ripe olives contain acrylamide at high concentration, little information on formation of this compound has been reported.

OBJECTIVES

Our first proposal was brought to the COC in 2009 as a three-year project with funding contingent upon the progress of our research. This project has three main objectives:

1. Evaluate the phytochemical composition of California-style olives (Year 1). Develop analytical methods and laboratory scale processing models to evaluate (a) the absence of air oxidation, (b) ferrous gluconate treatment, and (c) sterilization on California-style olives. Completed and described in our first annual report;

2. Understand the mechanism of the formation of acrylamide in California-style olives (Year 2). This research is in progress and is described herein;

3. Reduce the concentration of acrylamide in California-style olives through re-addition of natural olive antioxidants (e.g., hydroxytyrosol) into processing olives (Year 3). This includes evaluating olive pomace and waste stream materials (e.g., leaves, stems, etc) as economical sources of hydroxytyrosol and develop methods for its extraction from these materials.

PROCEDURES

Laboratory Scale Processing Models

We developed a laboratory scale processing model mimicking California black ripe olive processing. For medium Manzanilla olives, 30 fruits are required per one sample. During lye treatment, olives are soaked into 1% sodium hydroxide for 4 hours. Then olives are rinsed with fresh water before they are placed in water for 20 hours to remove sodium hydroxide residual. During the lye treatment and washing steps, air is injected into surrounding medium to oxidize olives. These processes are repeated for 4 days to allow sodium hydroxide to reach the pit. A solution of 1% phenolphthalein in iso-propanol is used to verify penetration of sodium hydroxide. After lye treatment, olives are treated with 0.125% ferrous gluconate solution for 4 hours to fix color. Carbon dioxide gas is injected into the solution to neutralize olives and phenolphthalein solution is used to verify a pH of olives. Then olives are rinsed and put into

fresh water for 30 minutes to remove residual ferrous gluconate. Air is injected during the washing step. Olives are packed in a glass flask and filled with 2% sodium chloride and sterilized in an autoclave at a temperature of 127^{o}C for 30 minutes.

Extraction Methods for Acrylamide

Samples consisting of 30 g of olives were extracted with 60 mL of water. A 1 mL sample of 4 ng/g of D3-acrylamide, an internal standard, was added to samples. The mixture was homogenized and 15 ml of hexane was added to remove oil and other non-polar compounds. The mixture was centrifuged at 4000 rpm for 30 minutes. The aqueous layer was collected. The solution was cleaned up with a Strata-X-C (200 mg, 3 mL) cartridge. Acrylamide was eluted with 1 ml of mobile phase (0.1% formic acid in water/methanol, 90:10, v/v). The eluted solution was collected and analyzed by LC-(ESI+)MS/MS method.

LC-(ESI+)MS/MS Methods for Acrylamide Determination

The quantification of acrylamide was performed using LC-(ESI+)MS/MS using a Prodigy ODS column (100A0, 4.6 mm x 250 mm, 5 μm). The mobile phase was the mixture of 0.1% formic acid in water/methanol (90:10, v/v). The LC-(ESI+)MS/MS was optimized using a capillary voltage of 3.0 kV, a cone voltage of 20V, in (+) mode ESI at a source temperature of 140°C and using a desolvation gas temperature of 400°C, 550L/hr desolvation gas flow rate. The collision energy for multiple reaction monitoring (MRM) was set at 12eV for both acrylamide and D3-acrylamide. Transition ions monitored for quantification were m/z 72 > 55 for acrylamide and m/z 75 >58 for D3- acrylamide. The limit of detection (LOD) of this method is 3.0 ng/g (wet basis) and the limit of quantification (LOQ) is 10.1 ng/g (wet basis).

RESULTS AND DISCUSSION

Acrylamide in fresh olives and table olives including Spanish-style, Greek-style, California-style, and canned green ripe table olives were extracted and quantified. Concentrations of acrylamide in each olive sample are shown (**Table 1)**. High levels of acrylamide were found in California black ripe olives followed by canned green ripe olives. Acrylamide levels in fresh, Spanish-style, and Greek-style table olives were lower than the limit of quantification (LOQ). These four types of table olives are processed with completely different processing methods. Spanish and Greek methods preserve olives by using additives or pasteurization, while olives processed with the other two methods need to be sterilized at higher temperature and longer time. The canned green ripe processing method differs from the California black ripe olive processing method because it does not have air oxidation or ferrous gluconate treatment and only fresh olives are used in canned green ripe processing. The results indicated that brine storage, air oxidation, ferrous gluconate treatment, and high temperature used during sterilization may play an important role in formation of acrylamide.

To confirm the results above, frozen olives were processed using the lab scale processing model. California black ripe olive processing method was compared to modified methods (e.g., the absence of air oxidation, ferrous gluconate treatment, and sterilization). As shown in **Table 2**, acrylamide levels in California black ripe olives are twice as great as non-oxidized olives and that ferrous gluconate treatment did not affect acrylamide levels. California black ripe olives contained twice the level of acrylamide than olives processed in the absence of air oxidation and ferrous gluconate treatment. Acrylamide in non-sterilized olives was also much lower than that

Table 1. Acrylamide levels in fresh, Spanish-style, Greek-style, canned green ripe and California black ripe olives

Olives	Acrylamide in olive (ng/g) (wet basis)		
	Sample1	Sample2	Sample3
Fresh olives (Manzanilla)	<LOD	<LOD	<LOD
Spanish-style table olives	<LOD	<LOD	<LOD
Greek-style table olives	<LOD	<LOQ	<LOD
California black ripe olives	467.0 ± 5.0	386.0 ± 4.8	405.0 ± 3.0
Canned green ripe	90.7 ± 0.7	102.0 ± 1.2	63.5 ± 0.5

Results are mean values of two measurements

Table 2. Effect of air oxidation, ferrous gluconate treatment and sterilization on concentration of acrylamide in olives.

Olive samples	Acrylamide in olive (ng/g) (wet basis)
California-style black ripe olives	1178.1 ± 51.0
Olives processed with modified method lacking of air oxidation	596.6 ± 44.9
Olives processed with modified method lacking of ferrous gluconate treatment	1164.2 ± 27.0
Olives processed with modified method lacking of air oxidation & ferrous gluconate treatment	607.3 ± 21.1
Olives processed with modified method lacking of sterilization	52.4 ± 10.1

in California black ripe olives. Taken together, our results indicated that the precursors of acrylamide are formed during lye treatment when air oxidation is involved and finally converted into acrylamide during sterilization. We are testing the effect of brine storage on formation of acrylamide. Olives harvested in 2010 will be used in this experiment. Olives stored in a brine solution with different periods of time will be sampled and processed with California black ripe olive processing method. Acrylamide in the end products will be measured and compared.

Based upon our data from Year 1 funding, we have identified the following as possible pathway(s) for the formation of acrylamide in California black ripe olives (**Fig. 1**). We developed the hypothesis that acrylamide precursors arise through oxidation of lipids prior to sterilization and that subsequent pasteurization results in the formation of acrylamide.

CONCLUSIONS

Concentration of acrylamide in California black ripe olives is much higher than that in the other table olives including canned green ripe, Spanish-style, and Greek-style table olives. Air oxidation and high temperature used in the California black ripe olive processing method have the great impact on formation of acrylamide. Our results from the first year of funding indicate that acrylamide precursors are formed during lye treatment and converted into acrylamide during sterilization. Therefore, with a second year of funding we propose to test our hypothesis and importantly, develop a mechanistic understanding for the formation of acrylamide. This understanding will allow for the potential mitigation of acrylamide levels in California-style olives. We will also begin our investigations of waste materials (e.g., leaves) as economical

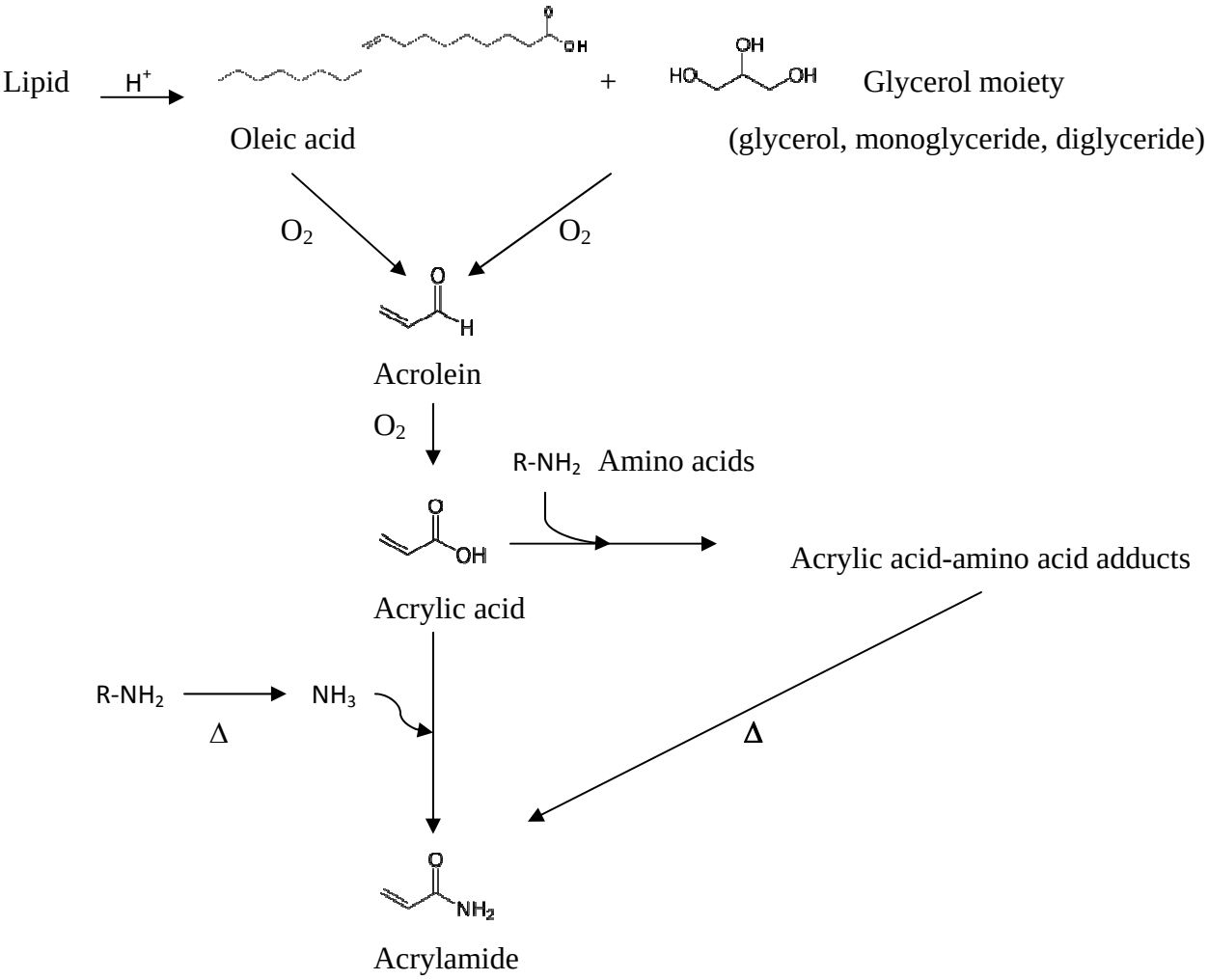

Fig. 1. The proposed pathway for the formation of acrylamide in California black ripe olives

sources of phenolic antioxidants that we can use to improve nutritional and functional value of California-style olives.

Recovery of Olive Oil from Olive Processor Wastewater

Project Leader: W. D. Ristenpart, 3202 Robert Mondavi Institute, Dept. of Food Science & Technology, Davis, CA; 3012 Bainer Hall, Dept. of Chemical Engineering & Materials Science, Davis, CA UC Davis, Shields Ave., Davis, CA 95616, (530) 752-8780, wdristenpart@ucdavis.edu

Reporting period: 1 January 2010 – 31 December 2010

ABSTRACT

The main goal of this research project was to determine the feasibility of using electrohydrodynamic (EHD) effects to induce aggregation and coalescence of emulsified olive oil droplets in wastewater generated during production of California black ripe olives. Preliminary experiments with 'clean' salt water and emulsified oil were very promising (cf. Vigo and Ristenpart, *Langmuir* 2010), and the COC allocated $10,000 to fund an undergraduate summer intern to perform lab experiments with actual samples of olive processor wastewater. Two main results were obtained. First, conventional means were used to separate the emulsified oil from wastewater to procure a sample large enough for sensory analysis. The aggressively unpleasant odor of the purified oil prevented more detailed sensory analysis, but did confirm that any separated oil would require further refining, deodorizing, and bleaching. Second, the preliminary EHD experiments revealed that the high concentration of lye (NaOH) in the wastewater was interfering with the EHD flow that causes coalescence, for reasons that remain unknown. This observation has catalyzed ongoing investigations in my lab to understand the effect of salt type, but given the widespread use of lye in California olive processing facilities we concluded it was unlikely that EHD effects would be useful at this time. The project was concluded early; only $7,518 of the original $10,000 allocation was spent.

INTRODUCTION

Olive processing facilities generate enormous amounts of wastewater. For example, during production the Musco Family Olive Company generates approximately 500,000 gallons of wastewater per day. This wastewater, including all of the trace organic materials, is currently discarded at great cost.

Because the wastewater was used to process olives, a fraction of the wastewater, approximately 2%, is emulsified olive oil. Although 2% might sound small, the scale of production indicates that a fortune in olive oil is being lost each day:

(500,000 gallons wastewater / day) x (2% olive oil) = 10,000 gallons oil per day
(10,000 gallons oil / day) x ($10 per gallon) = **$100,000 per day**

The challenge, however, is that the emulsified olive oil drops are very small (typically microns in size) and therefore difficult to separate. Conventional separation techniques, such as centrifugation or distillation, are economically prohibitive. A different and less expensive approach is necessary.

The main goal of the proposed research was to test a separation technique based on electric fields (**Fig. 1**). Specifically, research over the past 10 years has shown that low-voltage AC

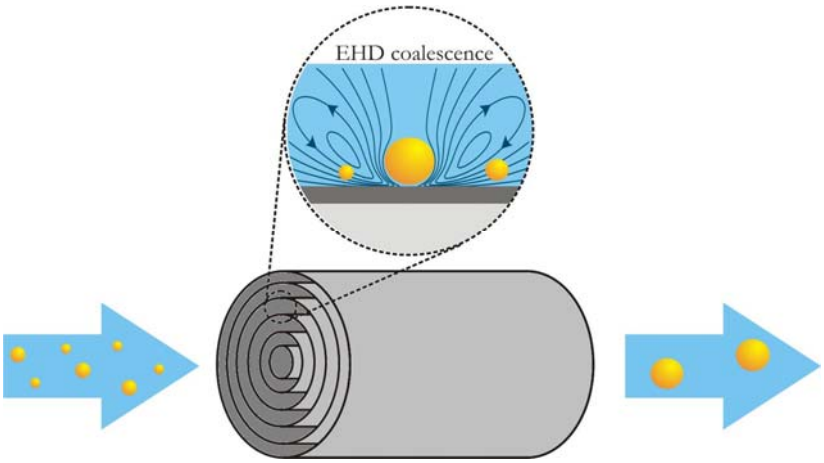

Fig. 1. Schematic of the proposed EHD separation apparatus. A series of concentric electrodes causes droplets of olive oil to coalesce, simplifying their separation from wastewater.

fields (approximately 2 V) cause solid particles to aggregate near electrodes. A type of electrically generated fluid flow known as electrohydrodynamic (EHD) flow is generated around each particle, causing the particles to aggregate. Rigid particles simply form clusters, but the project leader hypothesized that liquid droplets would come together and coalesce. If coalescence occurs, the EHD approach could be used to drastically increase the average size of the oil drops in the wastewater and make separation feasible.

Preliminary funding was generously provided by Musco Family Olive Company to test whether EHD flows would cause olive oil droplets to coalesce. Preliminary experiments performed with extra virgin olive oil suspended in clean salt water (~1 mM sodium chloride) had demonstrated that olive oil droplets indeed aggregate and coalesce near planar electrodes for applied electric fields near 4 V 100 Hz (see **Fig 2**). Full details of the preliminary experiments are now available in the article: Vigo and Ristenpart, "Aggregation and Coalescence of Oil Droplets in Water via Electrohydrodyamic Flows," *Langmuir* **26**, 10703-10707 (2010).

The COC kindly provided $10,000 as seed funding to pay for an undergraduate student to determine whether similar aggregation and coalescence will occur for emulsified olive oil droplets in actual wastewater produced by olive processors. Part of the funding was intended to pay for preliminary sensory analysis of the recovered oil, to be overseen by Dan Flynn at the UC Davis Olive Center.

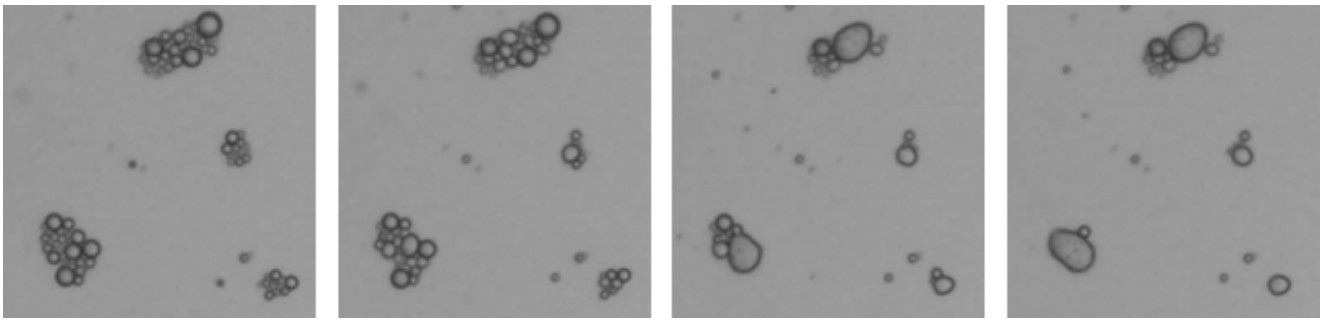

Fig. 2. Optical microscopy images of olive oil droplets (average size 10 μm) coalescing next to an electrode with a 4 V, 60 Hz applied electric field. Images are 5 seconds apart. A lower electric field strength (2 V) first brought them together (not shown), then a jump in the field strength to 4 V caused them to coalesce. Preliminary experiments funded by Musco Family Olive Company; see Vigo and Ristenpart, *Langmuir* (2010) for details.

OBJECTIVES

There were two main objectives for the research.

1) **Perform sensory analysis of oil recovered from wastewater.** Based on feedback received from members of the COC during the 2010 meeting, one of the key priorities was to determine the quality of any oil recovered from the wastewater. In other words, would the oil be palatable? Or would it require extensive post-processing after separation to obtain food-grade quality?

2) **Test EHD flow with wastewater.** The preliminary experiments were performed on olive oil emulsions prepared with pure oil and pure salt water. In contrast, the actual wastewater is "messy," since it contains approximately 5% solid colloids (e.g., small chunks of pits), and material properties like the pH and salinity can change from hour to hour during production. A key step was to determine how efficiently EHD flow causes the oil drops to coalesce for actual wastewater, and to test how variability in the wastewater affects the coalescence efficiency.

PROCEDURES

Objective 1. *Sensory analysis.*

The undergraduate student (Brian Viceral) and I traveled to a California black ripe olive processor, and procured several gallons of wastewater from the effluent of a pit scrubber (which had been identified as one of the 'oiliest' wastewater streams in the facility). The samples were transported back to Davis, and the student then placed some of the samples in 50 mL test tubes for use in a conventional centrifuge. Sixteen tubes were centrifuged at a time, with a maximum speed of 3740 RPM, for up to 15 minutes. A representative test tube following the centrifugation is shown in **Fig. 3**. Four discrete layers were observed: heavy solids at the bottom (mostly pit debris and dirt), then a large aqueous layer, a layer of 'light' solids (mostly olive flesh), and finally a very thin layer of olive oil on top. The fraction of olive oil recovered after a single centrifugation was very small; the approximate volumes of each layer were as follows:

Olive oil:	0.5 mL	(1%)
Heavy solids:	0.5 mL	(1%)
Light solids:	1.0 mL	(2%)
Aqueous:	48 mL	(96%)

Because the yield was so low, and because we suspected more oil might be comingled with the light solid layer, the top two layers from each tube were collected, placed into new tubes, and then separated again. (Note that because only 1.5 mL were collected into each tube, 32 tubes needed to be centrifuged to fill a single 50 mL tube with just the oil/light solids layers). A second centrifugation of this material again yielded four layers that were qualitatively similar, albeit with different ratios: 2 mL oil, 8 mL light,

Fig. 3. A sample of wastewater following a single centrifugation.

39.5 aqueous, and 0.5 mL heavy solids. The observed ratios suggested that little accessible oil is available in the light solid layer after centrifugation at this speed. After the second centrifugation, just the oily layers were collected, and these were repeatedly centrifuged to remove as much trace water and solids as possible. The oil was centrifuged again in 1 mL microtubes for 5 minutes at 7000 RPM. This was done to remove the small amount of water from the oil to make it as pure as possible. As a final step, the collected oil was run through a vacuum filtration apparatus (filter size 0.22 μm) to remove the remaining light solids and any bacteria present in the oil. The final yield after the entire process was approximately 10 mL of pure oil, which was intended for sensory analysis.

Preliminary sensory analysis was based entirely on visual and olfactory qualities. Visually, the oil had a gray greenish tinge - presumably due to the prolonged contact with the black ripe olives. Because of this green off-color, it did not 'look' like consumer-grade olive oil. More troubling, the purified oil smelled, in a word, “terrible.” I had several non-experts smell it; representative assessments included:

- "It smells like a dumpster full of rotting olives that's been left in the sun too long..."
- "It smells like a porta-potty that somebody gave birth in..."
- "Wow, this smells like #$%*..."

Dan Flynn also smelled it, and agreed that the aggressively bad odor meant that further sensory analysis was impractical (i.e., nobody in their right mind would put it in their mouth). The primary conclusion is that any oil recovered from the wastewater streams would require subsequent processing (i.e., refining, bleaching and deodorizing) to become a potentially food-grade product.

Objective 2. *EHD Experiments.*

A systematic series of electric field experiments was performed with the untreated (uncentrifuged) wastewater samples from the pit scrubber, using the same procedure as in the preliminary experiments (cf. Vigo and Ristenpart, *Langmuir* 2010). Electric fields with amplitudes between 0 – 5 volts and frequencies between 50 and 500 Hz were systematically tested to provide quantitative information about the corresponding rates of aggregation and coalescence for real wastewater (**Fig. 4**). The short summary is that no aggregation or coalescence was observed with the wastewater samples at the same field strengths and frequencies where tremendous coalescence was observed with extra virgin olive oil in salt water. No noticeable motion induced by the electric field was observed. Aggregation and coalescence were only observed at impractically low frequencies (< 10 Hz), where electrochemical reactions and electrolysis cause undesirable side effects (e.g., degradation of the electrode and bubble generation).

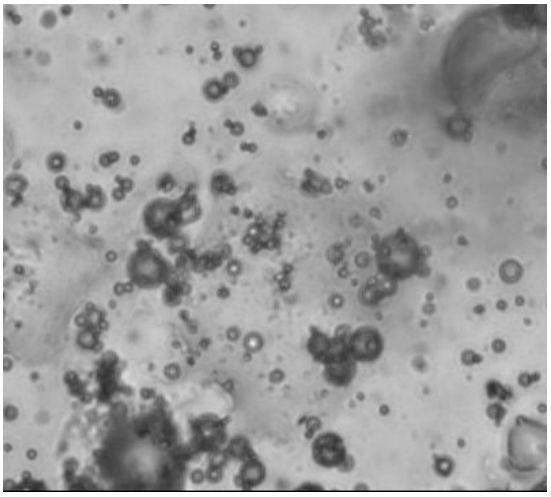

Fig. 4. Optical micrograph of the raw wastewater sample, showing the presence of emulsified oil and other solid particulates. (Grayscale, average obect size ~ 10 μm.)

To understand why aggregation was observed with extra virgin olive oil, but not the wastewater olive oil, we tried taking some of the purified wastewater olive oil and re-emulsifying it in the same salt water solution that we used with the extra virgin olive oil (1 mM sodium chloride). Surprisingly, the wastewater oil was observed to aggregate by EHD flow in the sodium chloride solution. This result indicated that the problem was not the quality of the oil; rather, something about the aqueous phase of the waste water was inhibiting the EHD flow responsible for the aggregation.

Because the original experiments used simple salt water (NaCl), the main implication was that some other chemical species in the wastewater was responsible for inhibiting EHD flow. Because sodium hydroxide is heavily used in the California black ripe process, we began a systematic investigation of the effect of NaOH concentration on the aggregation behavior. Model colloids were placed in a series of different electrolytes and the rate of aggregation at a constant field strength was measured; the results are tabulated in **Fig. 5**. The results indicate that NaOH indeed has a powerful inhibitory effect on EHD flow. Scientifically, this result is fascinating: nobody had previously tested for EHD flow in the presence of NaOH, so the reason for this inhibitory effect is unclear. Other members of my group are now working on understanding the underlying mechanism of the NaOH inhibition. Practically speaking for the olive processors, however, the main conclusions is that it seems unlikely that EHD flow will be useful for recovering olive oil from the wastewater streams as long as NaOH is used during the processing.

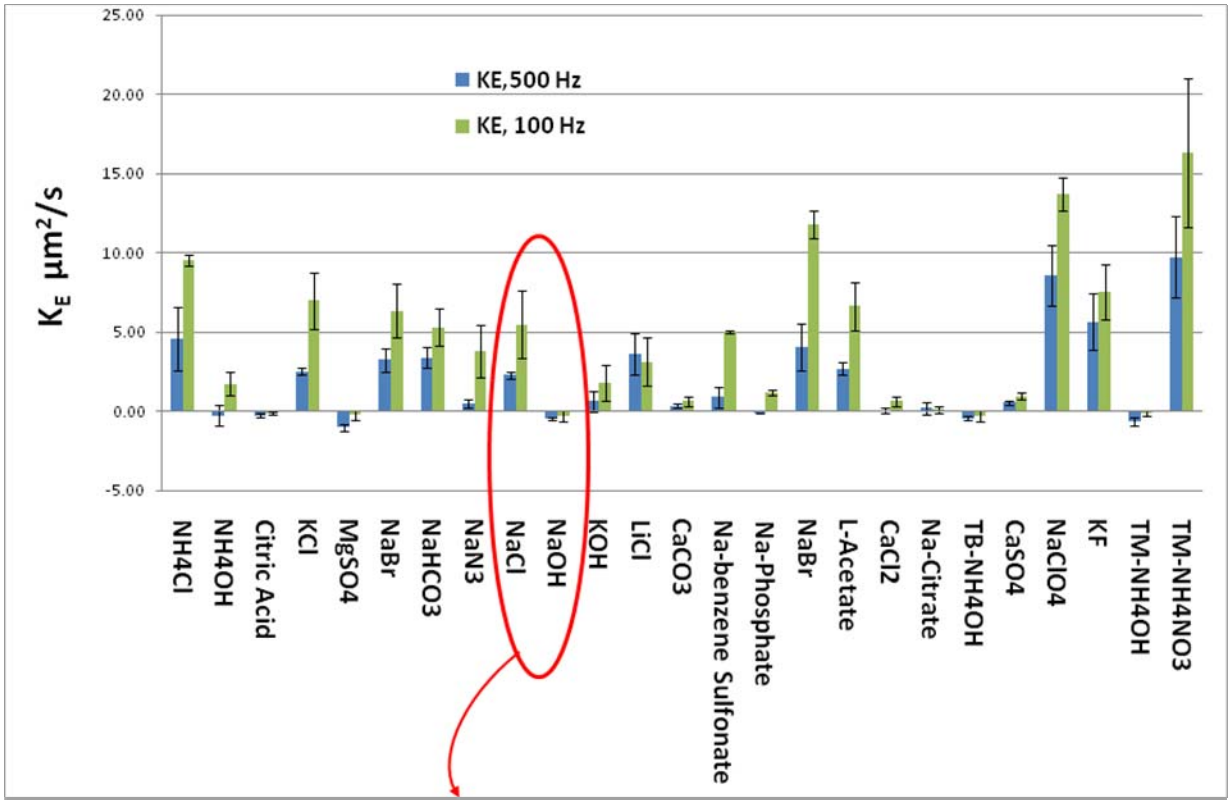

Fig. 5. The rate of aggregation of PS colloidal particles (2 µm diameter) via EHD flow as a function of electrolyte type for two different applied frequencies and at fixed field strength (5 V). Brian Viceral did the highlighted experiments (with COC funding), the other electrolytes were another student in my lab. It is unknown at this point why some electrolytes cause rapid aggregation (large values of KE) and why others impede aggregation (low or negative values of KE).

CONCLUSIONS

The research yielded two main conclusions:

1) Oil recovered from the wastewater streams will require subsequent refining, bleaching, and deodorizing, unless the oil is intended for non-consumer uses (e.g., biofuels).

2) The EHD approach for separation of the oil does not work because sodium hyroxide, for unknown reasons, prevents aggregation. Further experiments with olive processor wastewater should wait until a more fundamental understanding of the role of electrolyte type on EHD flow is obtained.

FUNDING SOURCE

Expenditures in the amount of $7,517.94 were made during the course of this research, approximately 25% less than the original allocation of $10,000 from the COC.

Improvement and Development of the DSE Mechanical Olive Harvester

Project Leader: Dave Smith, PO Box 639, Exeter, CA 93221; dsedave@yahoo.com

Cooperating Personnel:

Don Forester, Labco
Steven Splawn, Splawn Belting
Christopher Reese, Reese Hydraulics
Phil Scott, Agright

Reporting period: 1 January 2010 to 31 December 2010

INTRODUCTION

We are all aware of the expense of hand harvest labor which is estimated to be approximately 50% of an olive grower's total cost. Limited number of manual laborers available for harvest results in higher costs and in low yield years some acreage not being harvested. With this in mind, an efficient mechanical olive harvester is a goal worth pursuing.

In 2009 we continued to improve the harvester picking, fruit retrieval, and trash removal. These improvements are reflected in our graded packouts which were equal to hand harvested in quality and value.

At this point we have exhausted every design change suggested for the triple head style. Unfortunately there is no way to remove the gap between heads, which allows a band of unharvested fruit to remain on the tree. It is time to move forward with the dual head concept to eliminate these problems.

OBJECTIVES

Proceed to improve performance of the DSE Mechanical Olive Harvester by incorporating design changes resulting from field testing and in-depth research into concept function and execution.

PROCEDURES

Fabricate and install two custom built picking heads. The lower head to be constructed with a concave rod design to conform to existing olive trees with traditional pruning. The upper head built and installed on an angle allowing picking rods to intersect with lower head rods thus eliminating the gap. This style of harvester head allows the machine to harvest young trees as well as more mature olive trees.

A two joystick control verses three will be easier for the operator to manage and direct heads into the tree.

Install updated catcher paddles allowing harvester to operate in closer proximity to tree trunk.

Fabricate and install tilt mechanism on catcher assembly, which allows driver to maneuver under lower branches. This feature also aids in turning and road ability.

Elevate joystick controls operator approximately 4 feet to obtain better visibility during harvest operation.

RESULTS AND DISCUSSION

With all the modifications to the DSE Mechanical Olive Harvester completed, the following results were noted.

The contoured heads with additional reach have indeed improved access to fruit and increased the percentage of its removal.

Modifying the triple joystick control to the dual design did allow the operator improved control and focused concentration.

The catcher paddles with breakaway support enabled the harvester to stay in contact with the tree trunk while walking around any obstacle. At the same time maintaining a forward nonstop motion. Fruit falling on the paddles was guided to the main system delivery belt.

The hydraulic tilt assembly allowed for faster, smoother turns at row ends and ease of transport.

Elevated platform control station did afford operator greater visibility to direct picking heads for optimum fruit removal.

We do not have the results of the field testing at the Rocky Hill site. We do however have an evaluation of the DSE Mechanical Harvester that took place on our ranch under actual harvest conditions. We harvested twenty-eight rows, which was approximately 22 acres of mature olive trees planted at 100 trees to the acre. These olive trees were conventionally pruned. The machine harvested fruit was delivered to the Musco receiving station in Lindsay where it underwent the mandatory USDA grading and weighing process. Post machine harvest, the same trees and acreage were hand gleaned.

The results were:

Machine harvested fruit resulted in 91.60 tons
Gleaned hand harvested fruit resulted in 31.94 tons

Machine efficiency was 74%.
Hand gleaned was 26%.

CONCLUSION(S)

The harvester efficiency has greatly improved from past years when the average removal rate was approximately 64%.

The improvements to the harvester for 2010, concave picking heads, updated catcher paddles, hydraulic tilt and elevated control station proved to be extremely beneficial as indicated in the enhanced fruit removal.

FUNDING SOURCE(S)

Funding for the 2010 DSE Mechanical Olive Harvester development was provided by the California Olive Committee.

Development of Biological and Cultural Control of Olive Fruit Fly in the Central Valley of California

Project Leader: Victoria Y. Yokoyama, USDA-ARS-SJVASC, 9611 S. Riverbend Ave., Parlier, CA 93648, (559) 596-2751, victoria.yokoyama@ars.usda.gov

Cooperators:

Pedro Rendón and Carlos Cáceres, USDA-APHIS-PPQ-CPHST, Guatemala City, Guatemala.
Xingeng Wang, Associate Research Entomologist, UC Riverside & Berkeley
Marshall Johnson, Cooperative Extension Specialist & Entomologist, UC Riverside
Kent Daane, Cooperative Extension Specialist, UC Berkeley
Roger Vargas and Jamie Piñero, USDA-ARS-PBARC, Hilo, HI

Reporting period: 1 January 2010 to 31 December 2010.

ABSTRACT

The eastern side of the Central Valley of California where olives are grown for canning was surveyed for olive fruit fly, *Bactrocera oleae* (Rossi), infestations. The pest was found for the first time in unusually high numbers in Merced. The parasitic wasp, *Psyttalia humilis* (Silvestri), was imported from the USDA-APHIS-PPQ Moscamed biological control laboratory in Guatemala and released for biological control of olive fruit fly infestations in Oroville, Woodland, Lodi, Merced, Exeter, Bakersfield, and Grapevine. Subsequent F_1 progeny of the released adults were reared from olive fruit fly pupae showing the capacity of the parasitoid to adapt to the same environmental conditions as the fruit fly host in California. Two other parasitoids, *Fopius ceratitivorus* and *P. cosyrae*, were imported into the quarantine facility at Univ. of Calif. Berkeley and only *Psyttalia cosyrae* developed successfully in olive fruit fly larvae. Olive fruit fly adults from field infestations in central California were used to rejuvenate a laboratory colony that had been reared through 48 generations. Pre-flight olive fruit fly adults, newly emerged from pupae, escaped from the bottom to the top of 22-24 inch vertical columns of sand that weighed 41-45 lbs, or traveled horizontally to the opposite end of 25 ft pipes. Over solid surfaces, the third instar, prepupal larvae crawled an average of 79 ft at an approximate speed of 1 ft per hr; pre-flight adult males walked an average distance of 49 ft at a speed of 118 ft per hr; and females walked about 32 ft at a speed of 104 ft per hr. A yellow corrugated plastic, pan trap baited with olive fruit fly male spiroketal lure and ammonium bicarbonate female bait with GF-120 sprayed on the underside is currently under investigation for control of olive fruit fly in an olive orchard.

INTRODUCTION

Biological control using imported parasitoids can be used to reduce olive fruit fly, *Bactrocera oleae* (Rossi), infestations in olives. In 2008-2009, we mass produced the olive fruit fly larval parasitoid, *Psyttalia humilis* (= *P.* cf. *concolor*) (Silvestri) (**Fig. 1**), at the USDA-APHIS-PPQ, Moscamed, laboratory in San Miguel Petapa, Guatemala. We imported and released the parasitoid in infested olive groves in five regions and nine counties in California. To date, augmentative field releases of *P. humilis* have resulted in high rates of parasitism in some locations. In 2010, we released the parasitoid in the Central Valley of California with

emphasis on regions where olives are grown for canning and oil and determined the ability of the parasitoid to reproduce in olive fruit fly wherever infestations were found.

An egg parasitoid, *Fopius ceratitivorus*, and a larval parasitoid, *Psyttalia cosyrae*, are mass produced at the Moscamed biological control laboratory in Guatemala, and international workers have reported parasitism of olive fruit fly by *F. ceratitivorus*. Importation and further testing were conducted in the quarantine facility at University of California at Berkeley to determine the suitability of these parasitoids for use against olive fruit fly in California in addition to *P. humilis*.

We have reared and maintained large numbers of olive fruit fly on the formulated diet developed in the laboratory of Dr. Marshall Johnson. This colony is used to supply insects to investigators working on the development of the sterile insect technique, sorting mechanisms to remove damaged fruit, and attractants that can be used for mass trapping. In 2010, after many generations of being produced on a laboratory diet, we evaluated the need to change the colony to a new population by using insects collected from olives in California.

We determined the dispersal capacity of the immature stages of olive fruit fly, and begun evaluation of distances travelled by the newly emerged, pre-flight adults. These investigations help identify cultural practices that can be used in olive orchards that may reduce pest numbers by restricting mobility and dispersal of these unique life stages.

The low olive fruit fly infestations in the Central Valley of California are conducive to using trapping devices as a method to reduce the number of adults in orchards. A new type of trap, an inverted yellow pan with GF-120, was provided by Drs. Jaime Pinero and Roger Vargas, USDA-ARS, Hilo, HI. This trap was shown to be effective in reducing infestations of subtropical fruit flies, and will be evaluated on olive fruit fly. A similar device was needed for testing on olive fruit fly.

OBJECTIVES

The objectives were to: 1) continue releases of the parasitoid *P. humilis* in locations with olive fruit fly infested olives in the Central Valley; 2) import and evaluate *F. ceratitivorus* and *P. corsyrae* against olive fruit fly in the Berkeley quarantine facility; 3) maintain a colony of olive fruit fly to support research to develop control techniques; and 4) develop cultural control techniques that further reduce pest populations.

PROCEDURES

Objective 1a. Parasitoid Rearing

A USDA-APHIS-PPQ permit is in place to import and release *P. humilis* (**Fig. 1**) in California. Permit applications were approved to import *P. cosyrae* and *F. ceratitivorus* from Guatemala into containment at the UC Berkeley quarantine facility. The USDA-APHIS-PPQ, Moscamed biological control laboratory, in San Miguel Petapa, Guatemala, reared the larval parasitoids *P. humilis* and *P. corsyrae* on Mediterranean fruit fly. Mediterranean fruit fly larvae were produced in the Moscamed rearing facility in El Pino, Guatemala. The larvae were irradiated with 40 Gy to prevent the emergence of fertile adults. The irradiated larvae were delivered to the Petapa biological control laboratory, and exposed in cages to oviposition by *P. humilis* or *P. coryrae*. The parasitized larvae were placed on rearing media until pupation. The Mediterranean fruit fly pupae containing developing parasitoids were harvested and held in cages until the parasitoid adults emerged. The parasitoids were collected, chilled, placed in waxed

Fig. 1. *Psyttalia humilis* (= *P.* cf. *concolor*) imported from APHIS-PPQ, Moscamed Biological Control Laboratory, Guatemala (Photo courtesy of Peggy Greb, USDA-ARS).

cups with honey for food and water, packed in shipping cartons, and sent by air freight to California. *P. humilis* was shipped to the USDA-ARS, Parlier, CA, and *P. cosyrae* was shipped to the U. C. Berkeley quarantine facility.

The Petapa biological control laboratory mass reared the egg parasitoid *F. ceratitivorus* from Mexican fruit fly produced in the adjacent rearing facility. Punctured apples were exposed to Mexican fruit fly adults for oviposition. Apples infested with Mexican fruit fly eggs in the punctures were exposed to *F. ceratitivorus* adults. Following oviposition by female parasitoids, the apples were sectioned and placed over fruit fly diet for development of the parasitized larvae. After pupation, the parasitized Mexican fruit fly pupae were harvested and placed in large cages until parasitoids emerged. The adult parasitoids were collected, placed in shipping cups and containers, and sent to the UC Berkeley quarantine facility.

Objective 1b. Parasitoid Field Releases

In 2010, suitable parasitoid release sites where olives were known to be infested with olive fruit fly were identified in the Central Valley of California by surveys and through contact with UC Farm Advisors, county departments of agriculture, pest control advisors, and growers. The San Joaquin Valley foothill areas were surveyed for olive fruit fly infestations by driving roads at elevations of about 700 feet or more, and by searching for potential sites using Google Earth. Olive trees suspected of supporting the pest were monitored with yellow sticky traps. Fruit samples are collected and returned to the lab to determine the presence of immature stages in the fruit.

The larval parasitoid *P. humilis* was shipped to Parlier, CA, for release in locations in the Central Valley where olives were found to be infested with olive fruit fly (**Fig. 2**). Environmental conditions and olive fruit fly populations were monitored in release locations with data loggers, adult traps, and larval fruit samples. Rates of parasitism were based on pre-release and post-release olive fruit fly larval infestations in fruit samples and recovery of subsequent generations of parasitoids from olive fruit fly pupae that had emerged from fruit exposed to parasitoids. Multiple releases were conducted at locations with high rates of

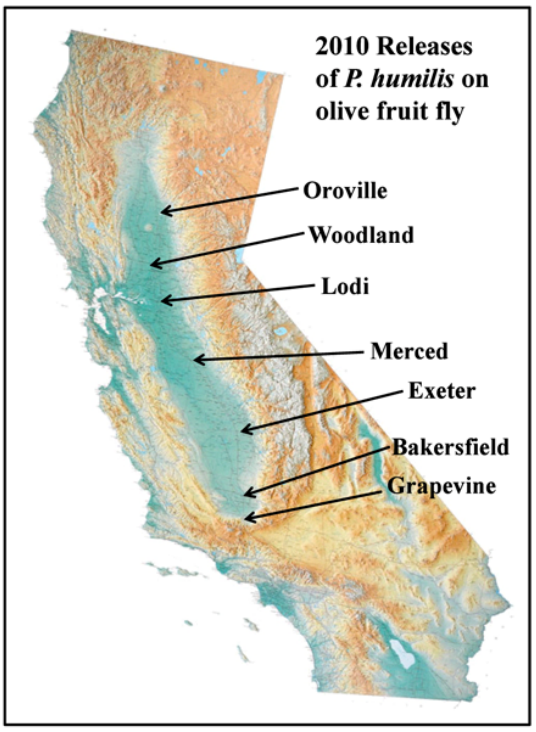

Fig. 2. Locations in the Central Valley of California where parasitoids were released.

parasitism. Olive fruit fly infestations in nearby trees were compared with infestations in trees exposed to parasitoids to evaluate the level of control achieved by the introduction of parasitoids.

Objective 2. Evaluate New Parasitoids in Quarantine

Two fruit fly parasitoids, *Fopius ceratitivorus* and *Psyttalia cosyrae*, both initially originated from Kenya and mass-produced at the USDA-APHIS-PPQ, MOSCAMED Parasitoid Rearing Facility at San Miguel Petapa, Guatemala, were introduced into the University of California Berkeley's quarantine facility for the evaluation of their potential as biological control agents against the olive fruit fly in California. Three shipments of each parasitoid species were sent to the quarantine facility. Experiments were conducted to determine if both parasitoids could attack and develop from various stages of olive fruit fly (i.e., host location and acceptance).

All parasitoids used for the tests were about one week old and mated. Olives were infested with the flies and the infested olives containing 2-3 oviposition marks were kept in the controlled quarantine room (23 ± 2ºC) for 4 (1st instar), 6 (2nd instar), 8 (young 3rd instar), and 10 days (old 3rd instar). Four infested fruit of either stage were exposed to one female wasp in a small cage (15 x 15 x 20 cm) for 24 h. Half of the exposed fruit were dissected 48 h later to determine if the hosts were parasitized and had the parasitoid eggs developed, while the other half of the exposed fruit were reared to determine the number of emerged flies or wasps.

Objective 3. Maintain Olive Fruit Fly on a Laboratory Diet

Olive fruit fly was reared and maintained on a laboratory diet that was previously modified from a Greek diet. Procedures were developed to maximize economical production of larvae. Presently, the colony has been maintained by the USDA-ARS in Parlier for 48 generations. The laboratory diet consists of a brewer's yeast based formulation with non-nutritive filler, and other

ingredients including soy protein and preservatives added. The diet is placed in trays and sprayed with a solution of olive fruit fly eggs. The infested diet is placed in cloth covered plastic boxes in a temperature and humidity controlled rearing room until larvae develop to the pupal stage. The pupae are harvested, held under low temperature storage, and placed into ovipositional cages for adult emergence. The females lay eggs through a waxed cone in the ovipostional cage and the eggs are harvested with water from the inside of the cone and used to start a new generation on diet.

Laboratory reared olive fruit fly was supplied to researchers requesting different life stages. The USDA-ARS laboratory in Albany will be provided with olive fruit infested with the olive fruit fly larval stages to develop a fruit sorter using x-ray technology. The puparial stage will be supplied to these same workers who are developing an insect sterilization technique.

Olive fruit fly adult males from infested olives collected from San Jose, San Juan Bautista, and Lodi were paired with laboratory-reared females to develop a new colony. Egg laying devices made from netting placed over bent plastic coated wire, shaped into domes, and coated with bees wax were tested for female oviposition as an alternative to cones.

Objective 4. Behavior and Cultural Control of Olive Fruit Fly

Experiments were conducted to determine distances traveled by pre-flight olive fruit fly adults that emerge from pupae. These adults are incapable of flight until their wings are spread through the vascular system and the cuticle hardens. Olive fruit fly pupae that were 1-3 d-old were placed at the bottom of a vertical PVC pipe 6 inches diameter by 36 inches high (**Fig 3**). The pupae were covered with sand to depths ranging from 8-26 inches. The weight of the sand column above the insects was calculated for each depth. The number of adults that emerged at the top of the sand column was reported as the percentage of the number that emerged in columns without sand. The number of adults at different depths in the sand was observed through plugged portholes in the column. Olive fruit fly pupae were placed in one end of a 25 ft pipe made of 0.75 inch PVC and covered with a foam insulated jacket (**Fig. 4**). The pipe was

Fig. 3. Vertical 6 in diameter x 36 in tall pipes filled with sand to determine upward distances traveled by newly emerged pre-flight olive fruit fly adults.

Fig. 4. Horizontal insulated 1 inch diameter x 25 ft long pipes used to determine distances traveled by newly emerged pre-flight olive fruit fly adults.

placed in an aluminum U-channel and aligned with a laser light. The percentage of adults that travelled through the pipe to the opposite end was compared to the number of adults emerging from pupae in petri dishes. The distance and duration of travel by newly emerged adults was evaluated through direct observation in the laboratory at about 23°C by tracing and measuring the track made by the adult on a solid surface covered with paper. Similar track records for the newly emerged third instar larvae are also reported.

A novel yellow pan trap (**Fig. 5**) with bait spray will be tested to determine the potential to reduce olive fruit fly numbers in a non-harvested olive orchard infested with olive fruit fly in San Luis Obispo. The traps were constructed from yellow corrugated plastic circles 3/8 inch thick and 16 inch diameter with a two inch wide collar around the outside edge. The olive fruit fly spiroketal lure for males and ammonium carbonate bait packets to attract females will be added. The traps will be tested in field studies to determine the effect on infestations by comparing numbers of larvae in fruit samples from trees with and without the traps.

Fig. 5. Yellow corrugated plastic pan trap with olive fruit fly lure, female bait and GF 120 on underside.

5. Analysis of data

Experiments were replicated three or more times and results are reported as the mean ± standard error of the mean. Data were analyzed by means comparisons among the tests or by *t*-tests between the tests. Results are reported in tables or figures. Findings are expected to be submitted to peer review journals for publication.

RESULTS AND DISCUSSION

1. Parasitoid Releases for Biological Control of Olive Fruit Fly

We have found reservoirs of olive fruit fly on the eastern edge of the Central Valley where climatic conditions are favorable for the adults. Foothill reservoirs of the pest are suspected to be sources of infestations in the Central Valley where numbers of olive fruit fly are low due to the hot and dry conditions. Central Valley locations where olive fruit fly has been previously found include Grapevine, Bakersfield, Porterville, Lodi, Woodland, and Oroville. In 2010, we found light and medium infestations in Exeter and Merced, respectively (**Table 1**). The olive fruit fly infestation in Exeter was at an altitude of > 700 ft. However, the infestation in Merced was on the valley floor where weather conditions would have been expected to mitigate pest numbers.

The parasitoid *Psyttalia humilis*, imported from Guatemala, was released for biological control of olive fruit fly in the locations shown in **Fig. 1**, and subsequent F_1 progeny of the released adults were recovered from olive fruit fly pupae that were exposed as larvae to the parasitoid except at Oroville, which is still under evaluation. The complete analyzed data showing olive fruit fly adults in traps and larval numbers in fruit, rates of parasitism, and environmental data for each location will be reported in a scientific manuscript.

We have been receiving parasitoids that were reared from irradiated or sterile Mediterranean fruit fly. Parasitoids reared from irradiated Mediterranean fruit fly reduces the risk of accidental contamination of shipments with a fertile host. However, the irradiated host may not be as suitable for the parasitoid development. Data concerning the longevity of imported parasitoids reared from irradiated Mediterranean fruit fly that was developed in 2009 and through the spring of 2010 is under analysis.

We have shown that our parasitoid, *P. humilis*, is an effective natural enemy of olive fruit fly and can reduce olive fruit fly larval infestations when released in infested trees. The parasitoid can survive and reproduce under the same environmental conditions that support olive fruit fly development. The parasitoid is only limited by the absence of an over-wintering stage that would allow it be become permanently established in infested olive orchards.

2. Evaluate New Parasitoids for Biological Control of Olive Fruit Fly

Shipments of *F. ceratitivorus* and *P. cosyrae* were received by the UC Berkeley quarantine laboratory on October 6, 20, and November 18 and both parasitoids were tested for parasitism of olive fruit fly larvae. Only 3 host larvae were parasitized by *F. ceratitivorus* among 150 dissected hosts that were exposed to the parasitoid (the parasitoid larvae were 2nd or 1st instars) and only 3 adult wasps (1 male and 2 females) emerged from about 2000 exposed olives to *F. ceratitivorus*. This suggests that *F. ceratitivorus* is not an effective parasitoid on olive fruit fly larvae although it could develop from this new host.

Both dissection and rearing of exposed hosts showed that *P. cosyrae* readily accepted and developed from olive fruit fly larvae, but clearly preferred the third larval instars (**Table 2**). At quarantine room conditions, the developmental time from egg to adult was 22.7 ± 0.24 days (n = 135) for males and 25.8 ± 0.24 days (n= 90) for females.

Table 1. Locations, olive fruit fly (OLF) infestations in fruit, dates and numbers of *Psyttalia humilis* adults released in different regions of the Central Valley of California in 2010, and the recovery of parasitoid progeny from OLF in post-release samples of fruit.

Location:	OLF larval infestion level:	Release date	≈ No. released	Post-release recovery date	Parasitoid progeny recovered
Grapevine	Medium	5 Nov.	12,000	19 Nov. 2010	Yes
Bakersfield	Medium	29 Oct.	6,000	8 Nov. 2010	Yes
	Medium	15 Nov.	6,000	26 Nov. 2010	Yes
Exeter	Light	2 Dec.	12,000	6 Dec. 2010	Yes
Merced	Medium	1 Nov.	6,000	12 Nov. 2010	Yes
Lodi	Heavy	12 Nov.	6,000	22 Nov. 2010	Yes
Woodland	Light	10 Dec.	6,000	17 Dec. 2010	Yes
Oroville	Light	18 Dec.	6,000	2 Jan. 2011	Under evaluation

Table 2. Host location and acceptance of olive fruit fly larvae by *P. cosyrae*.

Host age (days)	N	Dissection		Rearing	
		Flies / fruit	Wasps / fruit	Flies / fruit	Wasps / fruit
4	14	1.47 ± 0.21a	0 a	1.80 ± 0.19 a	0 a
6	12	2.45 ± 0.25 ab	0 a	2.45 ± 0.31 a	0 a
8	12	2.70 ± 0.37a	0.25 ± 0.25 ab	2.25 ± 0.35 a	0 a
10	12	1.71 ± 0.28 ab	0.67 ± 0.22 b	1.37 ± 0.38 a	1.08 ± 0.31 b
Statistics		$F_{3,46} = 4.67$, $P = 0.006$	$F_{3,46} = 3.77$, $P = 0.016$	$F_{3,46} = 2.26$, $P = 0.091$	$F_{3,46} = 12.80$, $P < 0.001$

Further experiments to compare the relative efficiency between *P. cosyrae* and either of the two known olive fruit fly parasitoids, *P. humilis* or *P. ponerophaga*, are still in progress. Rates of parasitism will be compared among the parasitoid species to determine the best candidates for biological control of olive fruit fly. If parasitism of olive fruit fly is successful, other non-host fruit fly species will be tested as potential hosts including the crop pests (i.e., walnut husk fly, cherry fruit fly, apple maggot) and the beneficial seedhead fly and cape ivy fly that are used for biological control of weeds. The effect on non-target fruit fly species will help determine the possibility of obtaining a USDA-APHIS-PPQ/CDFA permit for further field cage studies and potential releases on olive fruit fly in California.

3. Olive Fruit Fly Laboratory Colony

Olive fruit fly adults that have been reared from infested olives collected from different locations in California have been incorporated into the rearing process for the laboratory colony. Techniques are being tested to economically increase numbers including new methods to collect eggs. The adults from field collections have shown a low level of adaptability to egg laying in artificial devices versus olive fruit, which has limited production. The Moscamed biological control laboratory in Guatemala plans to start an olive fruit fly colony from a Greek culture from the IAEA laboratory in Seibersdorf, Austria. The Moscamed laboratory may have the ability to rear parasitoids on olive fruit fly instead of Mediterranean fruit fly.

Parasitoids and olive fruit fly pupae are periodically delivered to the facilities of the University of California at Davis and Riverside for basic research investigations. The USDA-ARS, Albany, CA, has completed the construction of an irradiator and will begin testing the effect of irradiation on olive fruit fly for potential development of a sterile insect technique program.

4. Behavioral Studies

About 20% of olive fruit fly adults emerging from pupae at the bottom of sand columns escaped to the top in 8-20 inch tall pipes (**Fig. 6**). The tests have not yet been completed, but some adults escaped to the top of 22-24 inch columns of sand that weighed 41-45 lbs. Only a small fraction of the weight of the sand column would actually be transferred as pressure on the newly emerged adult, and the pressure would diminish as the insect moved to the surface. The results show that the pre-flight stage of olive fruit fly that emerges from the pupal stage is

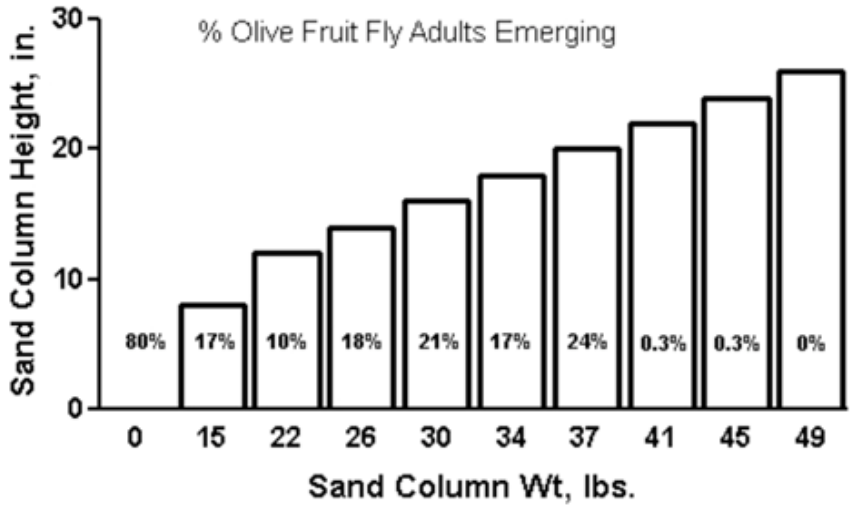

Fig. 6. Percentage of olive fruit fly adults emerging from pupae at the bottom and escaping to the top of different heights and weights of sand in columns.

capable of vertical movement through 2 ft of sand. The results suggest that olive fruit fly adults could emerge from the ground in olive orchards if the soil was granular or contained interstitial spaces for upward movement and that less permeable soils such as clay may present a barrier for vertical migration.

About 80 % of the pre-flight olive fruit fly adults that emerged from pupae traveled to the opposite end of 25 ft horizontal pipes. The flightless adults are capable of walking long distances across an orchard floor and could cross orchard inter rows. The third instar larvae (i.e., prepupal larvae) that leave the fruit to pupate were found to crawl an average of 79 ft at an approximate speed of 1 ft per hr (**Table 3**) over a solid surface. The larvae would normally pupate in a protected niche in the orchard, but could exit the fruit from which it was feeding, and leave the area beneath the tree canopy. The track of the newly emerged pre-flight adult male over a solid surface showed an average distance of 49 ft walked at a speed of 118 ft per hr. The females walked less than the males at a distance of about 32 ft at a speed of 104 ft per hr. The pre-flight stage of the newly emerged adult has great mobility and ability to disperse among trees on the floor of an olive orchard. Orchard maintenance practices such as disking may not be effective in destroying the larval or pupal stages especially if they are below the depth of the

Table 3. Mean distance traveled on solid surfaces by prepupal olive fruit fly larvae and newly developed non-flying adults after emergence from the pupae

Life Stage	Sex	Distance travelled, ft	Time travelled, hr	Ft/hr
Prepupal Larvae[1]		78.5	6.60	12
New Adult	Male	42.9	0.36	118
	Female	32.2	0.31	104

[1] Third instar

blades. The visible movement of the adults or larvae on the orchard floor could be detected by predators such as birds and these stages may be vulnerable for biological control by ants (Orsini et al. 2007) and ground beetles. These findings will be used to determine methods to control the late instar larvae that leave the fruit and the pre-flight adult stages to prevent flight and movement within olive orchards. Ground covers and mechanical procedures that can be used in the cultivation of olives may limit dispersal and survival of such life stages.

5. Cultural Control Techniques

The effectiveness of a yellow pan trap baited with olive fruit fly male spiroketal lure and ammonium bicarbonate female bait with GF120 sprayed on the underside is currently under investigation (**Fig. 5**). Based on previous trap studies, the yellow color of the pan attracts olive fruit fly adults. The circular outer rim of the pan simulates the curvature needed to induce olive fruit fly females to oviposit eggs in the laboratory colony. The pan trap is constructed from thick corrugated plastic that provides insulation. The underside provides a shaded area that is attractive to olive fruit fly adults during the summer and the GF-120 is protected from weathering. Olive groves and windrows on the eastern side of the Central Valley and a non-harvested orchard in San Luis Obispo with olive fruit fly infestations for the past 3-4 years will be used for study sites. Numbers of olive fruit fly adults in trees with traps versus trees without traps will be evaluated using fruit cutting and inspection for larvae as the criteria for infestation levels.

CONCLUSIONS

Olive fruit fly was found in olives at cooler elevations along the eastern edge of the California Central Valley near orchards where olives are grown for canning. The parasitoid *Psyttalia humilis* was imported from Guatemala and released in these infested trees. The parasitoid readily adapted to the new environment and successfully reproduced in olive fruit fly larvae showing biological control could be used to reduce pest numbers in these reservoirs. The pre-flight adults and crawling prepupal larvae of olive fruit fly were shown to travel long distances and may disperse on the ground throughout olive orchards. A corrugated yellow pan trap may provide attract-and-kill qualities for olive fruit fly control.

FUNDING SOURCES

We are grateful for external funding for this project from the California Olive Committee and to the numerous cooperators who have allowed us to use their olive groves. Internal funding was provided by the USDA-ARS-SJVASC, Parlier, CA; USDA-APHIS-PPQ-CPHST, Guatemala; and the Moscamed Program, USA-Mexico-Guatemala. USDA-ARS-SJVASC workers who assisted with this project are Sean Pelham and Julie Gagnon.

REFERENCES

Piñero, J. C., R. F. L. Mau, G. T. McQuate, and R. I. Vargas. 2009. Novel bait stations for attract-and-kill of pestiferous fruit flies. Entomologia Experimentalis et Applicata 133: 208-216.

Burrack, H.J., Connell, J.H., Zalom, F.G. 2008b. Comparison of olive fruit fly (*Bactrocera oleae* (Gmelin)) (Diptera: Tephritidae) captures in several commercial traps in California. International Journal of Pest Management 54, 227-234.

Daane, K.M., Johnson, M.W. 2010. Olive fruit fly: Managing an ancient pest in modern times. Annual Review of Entomology 55, 155-169.

Hepdurgun, B., Turanli, T., Zümreoğlu, A. 2009. Parasitism rate and sex ratio of *Psyttalia* (=*Opius*) *concolor* (Hymenoptera: Braconidae) reared on irradiated *Ceratitis capitata* larvae (Diptera: Tephritidae). Biocontrol Science and Technology 19, 1-9.

Johnson, M.W., Zalom, F.G., Van Steenwyk, R., Vossen, P., Devarenne, A.K., Daane, K.M., Krueger, W.H., Connell, J.H., Yokoyama, V., Bisabri, B., Caprile, J., Nelson, J. 2006. Olive fruit fly management guidelines for 2006b. University of California Plant Protection Quarterly 16, 1-7.

Messing, R.H., Klungness, L.M., Purcell, M., Wong, T.T.Y. 1993. Quality control parameters of mass-reared Opiine parasitoids used in augmentative biological control of tephritid fruit flies in Hawaii. Biological Control 3, 140-147.

Orsini, MM, Daane KM, Sime KR, Nelson EH. 2007. Mortality of olive fruit fly pupae in California. *Biocontrol Sci. Technol.* 17:797–807

Rugman-Jones, P.F., Wharton, R., van Noort, T., Stouthamer, R. 2009. Molecular differentiation of the *Psyttalia concolor* (Szepligeti) species complex (Hymenoptera: Braconidae) associated with olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), in Africa. Biological Control 49, 17-26.

Tzanakakis, M.E. 1989. Small scale rearing: *Dacus oleae*. In: Robinson, A.S., Hooper, G., (Eds.), World Crop Pests: Fruit Flies - Their Biology, Natural Enemies and Control, Vol. 3B. Elsevier, Amsterdam. pp. 105-118.

Wharton, R.A., Trostle, M.K., Messing, R.H., Copeland, R.S., Kimani-Njogu, S.W., Lux, S., Overholt, W.A., Mohamed, S., Sivinski, J. 2000. Parasitoids of medfly, *Ceratitis capitata*, and related tephritids in Kenyan coffee: a predominantly koinobiont assemblage. Bulletin of Entomological Research 90, 517-526.

Wang, X. G., M. W. Johnson, K. M. Daane, and V. Y. Yokoyama. 2009. Larger olive fruit size reduces the efficiency of *Psyttalia concolor*, as a parasitoid of the olive fruit fly. Biol. Contr. 49: 45-51.

Wang, X. G., M. W. Johnson, V. Y.Yokoyama, C. H. Pickett, and K. M. Daane. 2011. Comparative evaluation of two exotic olive fruit fly parasitoids under varying abiotic conditions. BioControl. (accepted October 28, 2010).

Yokoyama, V. Y. and G. T. Miller. 2004. Quarantine Strategies for olive fruit fly (Diptera: Tephritidae): Low-temperature storage, brine, and host relations. J. Econ. Entomol. 97: 1249-1253.

Yokoyama, V. Y., G. T. Miller, and J. Sivinski. 2004. Quarantine control strategies for olive fruit fly in California, pp. 241-244. Proceedings of the 6th International Symposium, 6-10 May 2002, Stellenbosch, South Africa,.

Yokoyama, V. Y., G. T. Miller, J. Stewart-Leslie, R. E. Rice, and P. A. Phillips. 2006. Olive fruit fly (Diptera: Tephritidae) seasonal populations in relation to region, trap type, season, and availability of fruit. J. Econ. Entomol. 99: 2072-2079.

Yokoyama, V. Y. and G. T. Miller. 2007. Olive fruit fly biology and cultural control practices in California. IOBC/WPRS Bull. 30: 277-285.

Yokoyama, V. Y., P. A. Rendón, and J. Sivinski. 2008. Biological control of olive fruit fly (Diptera: Tephritidae) by releases of *Psyttalia* cf. *concolor* (Hymenoptera: Braconidae) in California, parasitoid longevity in presence of the host, and host status of walnut husk fly, pp. 157-164. Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance, 10-15 September 2006, Salvador, Bahia, Brazil.

Yokoyama, V. Y., P. Rendón, and J. Sivinski. 2008. *Psyttalia* cf. *concolor* (Hymenoptera: Braconidae) for biological control of olive fruit fly (Diptera: Tephritidae) in California. Environ. Entomol. 37: 764-773.

Yokoyama, V. Y. 2011. Olive fruit fly (Diptera: Tephritidae) longevity and development in laboratory and greenhouse tests and in olive fruit in California. J. Econ. Entomol. (in review).

Yokoyama, V. Y., P. A. Rendón, X. Wang, S. B. Opp, M. W. Johnson, & K. M. Daane. 2011. Response of *Psyttalia humilis* (Hymenoptera: Braconidae) to olive fruit fly (Diptera: Tephritidae) and conditions in California olive orchards. Environ. Entomol. 40: (in print).

Yokoyama, V. Y., C. E. Cáceres, L. P. S. Kuenen, X. G. Wang, P. A. Rendón, M. W. Johnson, and K. M. Daane. 2010. Field performance and fitness of an olive fruit fly parasitoid, *Psyttalia humilis* (Hymenoptera: Braconidae), mass reared on irradiated Medfly. Biological Control 54: 90-99.

California Olive Committee

Interim Research Reports 2010

Complied by

Marshall W. Johnson
UC Liaison Officer to the COC
mjohnson@uckac.edu

Cooperative Extension Specialist & Entomologist
Department of Entomology
University of California
Riverside, CA 92521

28 January 2011

The interim reports contained within provide findings and conclusions from research projects funded by the California Olive Committee for the year of 2010. These projects were approved for a no-cost extension and will terminate in 2011. These reports were submitted on 5 January 2011.

List of Project Reports **Page No.**

Developing Mechanical Harvesting for California Black Ripe Processed Table Olives: 2007-2010: Year 4/4 Interim Report

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

Jean-Xavier Guinard, Professor, Department of Food Science and Technology, UC Davis, JXGuinard@ucdavis.edu

Uriel Rosa, Associate Professor, Department of Bioagricultural and Mechanical Engineering, UC Davis, UARosa@ucdavis.edu

Jacqueline Burns, Professor, Department of Horticulture, University of Florida, JKBU@UFL.edu

Cooperating Personnel:

Soh Min Lee, PhD Candidate, Food Science and Technology, UC Davis
Sergio Castro Garcia, Visiting Scientist from University of Cordoba, Spain
Kitren Glozer, Associate Project Scientist, UC Davis
William H. Krueger, UCCE Farm Advisor, Glenn County
Elizabeth J. Ficthner, UCCE Farm Advisor, Tulare County
Neil O'Connell, UCCE Farm Advisor, Tulare County
Mari-Paz Suarez, Visiting Scientist, University of Seville, Spain
John Ferguson, Volunteer
Peter Kaleko, Volunteer
Skander Slama, Volunteer

Industry Cooperators

Ranch Cooperators	Harvester Cooperators	Processor Cooperators
Rocky Hill Ranch	DSE	Bell Carter Olives
Erick Nielsen Ranch	Erick Nielsen Inc.	Musco Family Olive Company

Reporting Period: 1 January to 31 January 2010 (year 4/4)

PROJECT SUMMARY AND MAJOR CONCLUSIONS

This is the interim report for the fourth and final year of a project to develop economically feasible mechanical harvesting for California black ripe 'Manzanillo' table olives. Harvests have been completed. The engineering analysis of the operating parameters of each harvesting technology evaluated will be completed before the presentation of the final 2011 proposal presentation (16 February 2011). The sensory and consumer evaluations of the harvested fruit will be completed in spring and summer of 2011 and a full final report submitted by September 31, 2011.

The objectives of this project were defined by, in order, the most limiting factors to mechanical harvesting; 1) fruit damage; 2) effective fruit removal technology; and 3) developing an economically viable harvester and defining its operating parameters. At this point, fruit damage has largely been eliminated and both canopy contact and trunk shaking technologies have been demonstrated to be effective fruit removal technologies. However, in practice both harvesting technologies are limited by tree canopy shape and by their respective platforms.

To achieve these objectives we focused on evaluating canopy contact and trunk shaking harvest technologies, evaluating the processed product, preparing trees with young orchard development and mature tree mechanical pruning, and evaluating potential abscission agents.

The major cumulative research results thus far are: 1) Canopy contact and trunk shaking harvesting technology can produce processed 'Manzanillo' olives that neither trained sensory or consumer panels could distinguish**;** 2) Canopy contact harvesting is over 90% efficient if the olives are accessible; 3) Mechanically hedged and topped trees were harvested significantly more efficiently by a canopy contact head than hand pruned control trees in 2010; 4) Ten year old 'Manzanillo' orchards trained with or without a trellis, in a 12x18 foot spacing, into a 12 foot tall, 6 foot wide tree skirted at 3 feet had statistically the same yields as conventionally trained trees**;** 5) Sixteen year old 'Manzanillo' trees spaced 13 X 26 feet and mechanically hedged 6 feet from the trunk on one side in 2008, the other side in 2009, and topped 12 feet and skirted at 3 feet both years, and not mechanically pruned in 2010, had significantly lower yields in 2010 than hand pruned control trees, but not in 2008 and 2009; and 6) Evaluating the most viable potential abscission compounds produced insignificant and inconsistent results.

T**he overall conclusion** that can be synthesized from these results is that both canopy contact and trunk shaking harvesting technologies can achieve economically viable mechanical harvesting if the fruit is accessible to the machine. To demonstrate this, the canopy contact and trunk shaking technologies must be improved through engineering, tested on effective platforms and evaluated on properly trained trees. This approach will require an engineer to head the project with cooperating horticulturists to prepare the trees in coordination with the engineer.

2010 RESEARCH RESULTS

In 2010 this project simultaneously focused on two major objectives: **1) improving the harvesting technology;** and **2) increasing harvester efficiency by adapting current orchards with mechanical pruning and developing new hedgerow orchards**. The preliminary data below gives the final efficiencies of two canopy contact heads, one in mechanically hedged and topped trees, and one trunk shaker.

The 49 – 62% final efficiency for the Canopy Contact I head was expected (**Table 1**). This was the range of harvest efficiencies demonstrated by this head in 2008 and no significant engineering improvements have been added to this head since then. In 2008, we concluded that we had learned as much as possible from this head in this configuration and should not evaluate it again unless the head had been improved. Our 2010 data confirms this; the overall efficiency of the head has not improved, remaining below 64%.

The Canopy Contact I head was 7.08% more efficient in mechanically hedged and topped trees having a statistically significant 57.35% average final efficiency in mechanically pruned rows versus 50.27% efficiency in conventionally pruned rows. However, these mechanically topped and hedged trees also had a statistically significant 19.4% lower yield per acre. As harvesting technology efficiency improves, we can determine if the significant decrease in harvest cost will offset the gross return losses generated by mechanical topping and hedging to

Table 1. Comparison of harvester technology final removal efficiency.

Harvest Technology	Final Removal Efficiency Range (%)	Efficiency Average (%)	Hand Pruned Trees *	Mechanically Hedged and Topped Trees**
Canopy Contact I	49 – 62%	55%	***57% a #***	***50% b #***
Canopy Contact II+	41 – 62%	49%		
Trunk Shaking +	31 – 49%	43%		

- *3, 83 tree rows: Rocky Hill Ranch, Tulare County (Average FRF = 0.586 kg)
- ** 6, 83 tree rows, Rocky Hill Ranch, Tulare County (Average FRF = 0.597 kg)
- # Values within ***these two cells*** are significantly different per T-test @ $p \leq 0.0001$
- + 3, 8 tree sets in hedgerow orchard: Nickels Soils Estate, Colusa County (Average FRF = 0.657 kg)

produce an equal or greater net return. Our results, and observations, strongly indicate the hedgerow configuration generated by the annual 12 foot topping, every other or third year mechanical hedging 6 feet from the trunk, and 4 foot skirting will be necessary to produce the flat "wall" of hanging olive shoots that make the fruit most accessible to the canopy contact head.

The preliminary 2010 data (**Table 2)** confirms the 2008 data for fruit quality. Both canopy contact heads and trunk shaking can produce a high percentage of cannable olives with low cull percentages equal to those of hand-harvested controls (HHC). The percentage of culls is a better method of evaluating harvest technology effects on fruit quality than the total adjusted price per ton as the latter can be strongly affected by fruit size percentages. The four cells on the far right of **Table 2** with values in ***bold italics*** highlight that there was a significant difference in cull percentages between the hand and machine harvested fruit between the two processors; indicated by A and B before the value in the table cells.

Now that both canopy contact heads and trunk shaking harvesting technologies can produce cannable olives with low cull percentages, the major emphasis should be on improving the harvesting technologies. The primary way of doing this is engineering. These two harvesting technologies need to be developed into effective harvesters that can maximize the efficiencies of the harvesting technology. The secondary method of improving the efficiency of these harvesters should be to improve mature tree pruning and young tree training.

Therefore the next step in this harvesting project should focus primarily on engineering and be directed by an engineer, preferably in cooperation with a commercial harvester fabricator.

Table 2. Effect of harvesting technology on % canning fruit and culls.

Harvester	% Canning*	% Canning* HHC	% Culls*+	% Culls*+ HHC
Canopy Contact I**	A: 95% B: 85%	A: 95% B: 92%	***A: 0.98% b*** ***B: 4.56% a***	***A: 0.81% b*** ***B: 0.88% b***
Canopy Contact II#	A: 93%	A: 94%	A: 0.71% B: 6.91%	A: 1.45% B. 0.66%
Trunk Shaker#	A: 95.6%	A: 87%	A: 0.69% B: 2.91%	A: 0.93% B: 2.00%

- *Olives were delivered to two processors on the same day
- **Replicated trial of 9 mechanically harvested and 12 HHC, 83 tree, rows; Rocky Hill Orchards, Tulare County.
- # 3, 8 tree, replications for each harvesting technology; Nickels Estate, Colusa County
- + Values in **two highlighted cells** followed by different letters (a,b) are significantly different at $p = \leq 0,0001$.

The horticultural improvements in tree pruning and training should be done in close cooperation with the PI engineer, based on what was learned through observations in 2010, and interactively during the harvesting evaluations. Effects on long-term tree health, primarily the effects mechanical harvesting on olive knot, can also be evaluated within this project.

If the sensory and consumer testing of 2010 fruit is consistent with the results obtained in 2008 (i.e., neither sensory or consumer panels could determine the difference between hand and mechanically harvested olives), then processed fruit sensory and consumer evaluations should be unnecessary in future trials.

In conclusion, this four-year project has successfully achieved some of its major objectives. First, the primary limiting factor to mechanical harvesting, final processed fruit quality, has been eliminated. Second, the best two potential harvesting technologies, canopy contact heads and trunk shakers have been identified and refined. For the latter, trunk shakers, the problem of trunk damage has also been eliminated. Third, how to improve harvesting efficiency through tree training and mechanical pruning, and how this will affect net return is being determined.

What is now needed is a stronger effort directed towards engineering improvement of the harvesting technology, including an effective harvesting platform, and a closer coordination of the engineering with preharvest and in-harvest season tree training and pruning. As a secondary objective, the long-term effects of mechanical pruning and harvesting can be coordinated with the already submitted olive knot proposal. Therefore an agricultural engineer, cooperating with the UCCE Specialists, and Tulare and Glenn county Farm Advisors, should direct this next stage of developing mechanical harvesting for California Black Ripe table olives. Final harvester development would probably be accelerated if the olive harvester fabrication industry could be involved in developing the final harvester that incorporated the picking technologies we have developed.

Development and Performance Evaluation of the Hutcheson Mechanical Olive Harvester

Project Leaders:

Art Hutcheson, 1425 W. Marinette Avenue, Exeter, CA, 93221, (559) 909-1909, oldirondad@verizon.net

Cooperating Personnel:

Louise Ferguson, University of California, Davis

Reporting Period: 1 January to 31 December 2010

ABSTRACT

This is the second year of trying to develop an economically feasible olive harvester. During these two years several things have been learned about adapting mechanization to harvesting traditionally spaced and shaped olive trees. First, rotating mechanisms tend to bind up with branches causing excessive tree damage. Second, tree shape does not always lend itself to easy access of olives by large awkward machinery, which in turn results in more tree damage and low yield numbers. In order to be successful in Year 3 of this project, we will need to implement a combination of harvester design improvements and adapting tree shapes to the limitations of the harvester.

INTRODUCTION

The number one problem as we in the olive industry all know is the high cost of hand harvesting canning olives. This cost varies from year to year depending on crop size, labor availability, and the quality of that labor. In many other crops, machine harvesting has been implemented to solve this problem. As we try to infuse mechanical harvesting into the olive industry, we have to be cognizant of the fact that the olives are tender; and any machine made to harvest the crop must do so in a way that produces the quality equal to or better than hand harvesting. It also must remove at least 95% of the fruit and not damage the tree any worse than the hand crews.

The goals I started with were to build a machine that was economical for the average grower, smaller in design than the current Korvan type machine yet able to do full size trees, able to mimic a hand picker in its harvesting action rather than knocking the fruit from the tree, at minimum replace the daily output of a 50-man hand crew, and harvest olives with quality equal to or better than the hand crew.

My harvester was built to wrap around one half of a tree at a time. An operator then moves the picking head over one side of the tree cleaning it completely before moving on to the next tree. Two men are required to operate the harvester.

During a brief test period in October 2009, I observed several deficiencies in the design of the picking head. This new project is a continuing effort to fully evaluate my harvester in field conditions that did not happen this past season due to problems with the head and arm design.

OBJECTIVES

The following objectives are needed to resolve the deficiencies found in during the test period:

1) Improve functions of current rotary picking head;
2) Construction of an alternate picking head;
3) Put both to use under harvest conditions in a commercial orchard

PROCEDURES

The following procedures are necessary to achieve the above objectives:

Objective 1. Improve functions of current picking head
- a. weld end plates to picking drum to reduce limb wrapping
- b. reduce length of picking fingers
- c. evaluate the merits of reduced rows of picking fingers
- d. evaluate alternate picking motions and rotations

Objective 2. Construction of an alternate picking head
- a. build single row comb with oscillating fingers
- b. build single row comb with stationary fingers

Objective 3. Put both to use under harvest conditions in a commercial orchard
- a. test the machine efficiency with field tests in January on oil varieties and in October under harvest conditions

Location: Cilker Orchards, Exeter, California

Experimental Design: 12 (trees/row) total rows will be randomly assigned to
Treatment I. Hand Picking
Treatment II. Mechanical Picking

Experimental Procedures: In June the six tree rows assigned to mechanical harvest will be skirted to four feet.
In October both sets of experimental trees will be harvested:
The 6 hand harvested rows will be harvested by hand, and the total row weight taken.
- the bins from each lot will be combined into a single lot:
- the bins will be graded as a single lot at the receiving station

The 6 machine harvested rows will be harvested and the total row weight taken.
The time to harvest the six rows will be recorded to determine harvest time per tree.
- the bins will be combined into a single lot.
- the bins will be graded as a single lot at the receiving station.

If significant fruit remains on the ground after the machine harvest it will be collected and weighed.

The machine harvested trees will be hand harvested and weighed.

The following calculation will determine the efficiency of the mechanical harvester:

$$\frac{\text{Weight Machine Harvested}}{(\text{Weight of machine harvest} + \text{hand gleaned fruit weight})} = \text{harvester efficiency}$$

The receiving station grade of the two, six row sets will be statistically analyzed to determine the effect of harvesting on fruit quality and grade.

The analyzed data should give:

1. The harvest efficiency of the mechanical harvester.
2. The harvest time per tree.
3. The effect of machine harvest on fruit quality.

This experiment can be done with different tree spacing or tree heights.

RESULTS AND DISCUSSION

As described in the objectives above, the current rotary head was altered by adding round end plates to eliminate branch wrapping and 12" fingers were used instead of the 18" ones. Both single row comb styles were also built, one with back and forth oscillating fingers and another with scissor action fingers. Our test plot at Cilker Orchards was prepared to Louise Ferguson's specifications last year and was still in good shape for this year's trial.

On 7 and 14 November 2010, the harvester entered the orchard to do some trial runs and it was apparent that the picking arm itself has some problems. It just does not seem agile enough to work over the perimeter of the tree as I had planned.

CONCLUSION

After doing my dry runs I determined my harvester was still not ready to do a legitimate trial that would yield any measurable results without further modifications. The rotary head appears not to have any favorable qualities for olive harvesting. The two comb style showed a lot of promise but the limitations of the picking arm need to be resolved first. With the receipt of a no cost extension for the 2011 season, I will be addressing these limitations in order to fully evaluate the abilities of my harvester in October and November of 2011.

FUNDING SOURCES

Art and Justine Hutcheson and the California Olive Committee