California Olive Committee

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Complied by

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Release and Evaluation of Imported Natural Enemies of the Olive Fly

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ABSTRACT

The olive fruit fly (OLF), *Bactrocera oleae* (Rossi), has become widely established in California and now threatens the State's olive oil and table olive industries. Classical biological control is a part of a program to develop long-term management practices for this invasive pest. Currently, African parasitoid species – *Psyttalia lounsburyi* and *Psyttalia humilis* (often reported earlier as *P. concolor*) – have been approved for release (USDA-APHIS), and one parasitoid, *Psyttalia ponerophaga*, has a field release permit still pending. To improve the predictability as to which parasitoid species or subspecies may have a better chance of establishment and success in California, we conducted field release and evaluation of parasitoid performance and OLF levels, and studied key parasitoid biological traits (thermal performance, overwintering biology) that might determine their establishment in California. Field cage-releases demonstrated successful attacks on OLF by both *P. lounsburyi* and *P. humilis* throughout the year. *P. humilis* was more effective than *P. lounsburyi*, regardless of olive fruit size, seasons, and field locations. Open field releases of *P. humilis* in relatively large quantities (500-1000 females per release) in San Luis Obispo, California, resulted in consistent recoveries of the parasitoid within the same year, but not the following year. However, no parasitoids were recovered after field release of *P. lounsburyi* (300 females per release) in San Luis Obispo, and of *P. humilis* and *P. lounsburyi* (110-500 females) in Sonoma. The results suggest that future releases with large quantities of

one or both parasitoid species may be needed. Laboratory evaluations showed that suitable temperature ranges for development and survival were different between the two parasitoid species. *P. humilis* was more heat tolerant (range 14-32°C) while *P. lounsburyi* was more cold tolerant (range 10-30°C). The *P. lounsburyi* temperature range more closely matched that of OLF. The most suitable temperatures for reproduction were 26 -30°C for *P. humilis* and 18-22°C for *P. lounsburyi*; therefore, *P. humilis* more closely matched OLF's reproductive temperature range. Exposure to low temperatures (< 10°C) reduced the longevity and post-exposure fecundity of adult *P. humilis*. Both *P. humilis* and *P. lounsburyi* did not appear to enter diapause during the winter, and could oviposit (deposit eggs) from October to early November, and larvae developed during the winter months (Fresno County). The effect of cold storage on the development and survival of *P. ponerophaga* was also investigated. The emergence rate of this parasitoid after being stored for 1-3 months at 6°C averaged 17.2%, which was considerably higher than that of *P. humilis* (< 2.31% emergence). Other studies are ongoing.

INTRODUCTION

The olive fruit fly (OLF), *Bactrocera oleae* (Rossi), was first found in southern California in 1998, and has dispersed rapidly throughout the state. Commercial orchard managers at infested sites rely upon a broad-spectrum insecticide combined with highly attractive baits. The effectiveness of insecticide-based programs is, however, limited by abundant roadside and residential olive trees that act as reservoirs for the fly's reinvasion of treated orchards. Current research efforts, therefore, emphasis long-term management practices and biological control is a part of this program.

Because indigenous natural enemies are not effective enough to suppress OLF populations in California, classical biological – the importation and establishment of natural enemies from other regions – may offer some opportunity to economically suppress OLF populations. Classical biological control programs require a series of quarantine studies to evaluate prospective natural enemies for their potential impact as well as non-target risks to native or beneficial species. After quarantine studies are completed and field release permits granted, field studies should have controlled field release and rigorous monitoring and evaluation of the imported natural enemies, including an assessment of impact on the targeted pest.

Classical biocontrol programs for OLF were initiated in 2003. Foreign exploration was conducted by members of the USDA-ARS European Biological Control Laboratory (EBCL), the California Department of Food and Agriculture (CDFA), the University of California (UC), and cooperators in the Republic of South Africa, Namibia, Kenya, La Réunion, Canary Islands, Morocco, Pakistan, India, and China. Collections of parasitoids were made primarily from wild olives from south to northeast Africa, where a rich diversity of fruit fly parasitoids was found. The parasitoids reared from OLF included *Psyttalia lounsburyi* (Silvestri), *Psyttalia concolor* Silvestri, *Psyttalia humilis* Silvestri, *Psyttalia ponerophaga* (Silvestri), *Utetes africanus* (Silvestri), and *Bracon celer* Szépligeti (all Hym.: Braconidae). *P. lounsburyi* and *P. humilis* (commonly referred to as *P. concolor*) were among the most common species collected in Africa.

Parasitoids were sent to UC Berkeley's Insectary and Quarantine (UCB I&Q) Facility, where they were evaluated for their non-target risks and efficiency against OLF. In addition, a few more generalist parasitoids (those attack numerous other tephritids species and are successfully

used as biological control agents for other pest tephritids), were imported and evaluated. These included *Fopius arisanus* (Sonan), *Diachasmimorpha kraussii* (Fullaway), and *D. longicaudata* (Ashmead). Presently, *P. humilis* (as *P. concolor*) and *P. lounsburyi* have been approved for release in California, and a release permit for *P. ponerophaga* is pending.

Current efforts are focused on evaluation of these available parasitoid species. The selected parasitoids should not only be safe and effective under laboratory conditions, but also climatically adapted to different regions in California. Cultivated olives set fruit in summer and these fruit mature in late fall and early winter. Wild olives follow a similar pattern, but can be far more variable and mixed in their developmental time, in part because of many varieties, allowing for the presence of some ripe olives year round in their native environments. Therefore, a large gap in time occurs when host availability to parasitoids in California (e.g., between late winter to early summer) as compared with their wild counterparts in Africa, and this may limit the fly's and parasitoids' survivorship when susceptible olives are scarce on trees. Knowledge of how both the fly and parasitoids bridge this gap and how the parasitoid matches up with the fly in a set of climate-driven responses would aid in the selection of the best parasitoids for release. Important traits would include temperature performance, overwintering ability, and field survival.

We investigated these specific traits for the three currently available parasitoid species (*P. humilis*, *P. lounsburyi* and *P. ponerophaga*). Field-cage evaluation is a relatively rapid way to demonstrate the impact of different parasitoids on pest populations, and this information can be used to compare the relative effectiveness of different parasitoids under different field conditions. The laboratory studies on parasitoid and fly thermal performance will show how closely coupled a selected parasitoid is to the targeted pest. We also continued field release and monitoring of the two approved parasitoids. Rigorous post-release experiments and monitoring are essential for validating decisions and contributing to understanding of the factors which influence biological control success. Additionally, several other factors that may affect the rearing, as well the success and establishment of these parasitoids (e.g., fruit fly baiting, rearing host, interspecific competition, sex ratio, etc) were also investigated under partial funding of this program, but are not reported here.

OBJECTIVES

1. Evaluate performance of *P. humilis* and *P. lounsburyi* in open-field and caged releases;
2. Determine temperature effects on *P. humilis*, *P. lounsburyi* and *P. ponerophaga*; and
3. Study the overwintering biology of *P. humilis* and *P. lounsburyi*.

PROCEDURES

Objective 1

1.1. Parasitoid shipment in 2009

Two different populations of *P. humilis* that were originally collected from Namibia (synonym of *P.* nr. *concolor*) and Kenya (synonym of *P.* cf. *concolor*), and two population of *P. lounsburyi* originally collected from South Africa and Kenya were evaluated in this study. For

convenience, hereafter, we referred to these as: *P. humilis* (NA = Namibian population), *P. humilis* (KA = Kenyan population), *P. lounsburyi* (KA =Kenyan population), and *P. lounsburyi* (SA = South African population).

Four shipments of *P. humilis* (NA) and *P. lounsburyi* (KA), both reared on Mediterranean fruit fly (medfly) in Israel were received at the UCB I&Q Facility (**Table 1**). After processing, the material was divided among UC Kearney Agricultural Center (KAC), Parlier, California, CDFA (Dr. Charles Pickett), Sacramento, and the UCB I&Q Facility for cultures and field releases. One shipment of *P. lounsburyi* (SA) from EBCL, one shipment of *Fopius arisanus* from Hawaii, and two shipments of *P. ponerophaga* from Pakistan were also received and processed at the UCB I&Q Facility (**Table 1**). All USDA-APHIS protocols were followed for the importation into and release out of quarantine.

Additionally, about 3000 individuals of *P. humilis* (KA) from the USDA-APHIS-PPQ, MOSCAMED Parasitoid Rearing Facility at San Miguel Petapa, Guatemala, were received via the USDA-ARS San Joaquin Valley Agricultural Science Center (Dr. Victoria Y. Yokoyama) on a separate USDA-APHIS permit. For all parasitoid shipments, a few sampled females were freshly killed, placed in 95% alcohol, and sent to KAC (Matt Middleton) for a backup of DNA identification.

1.2. Cultures of insects

Laboratory colonies of *B. oleae* on olive fruit, and *P. humilis* (KA and NA) and *P. lounsburyi* (NA and SA) on OLF were established at the UCB I&Q Facility and KAC, respectively. Additionally, colonies of *P. ponerophaga* and *F. arisanus* were also maintained on OLF at the UCB I&Q Facility.

1.3. Field evaluation

Field releases were conducted in climatically-mild coastal and northern California sites where high OLF populations often occur. Because of large variations in tree fruit load, tree size, and levels of fly infestation among different release sites, the major aim of open field release was

Table 1. Parasitoids received and processed at the UCB I&Q Facility in 2009

Provider	Date received	Parasitoid species (population, number)
Israel (Yael Argov)	5 August	*Psyttalia lounsburyi* (NA, 2800♀, 700♂)
		Psyttalia humilis (KA, 3000♀, 2000♂)
	24 August	*Psyttalia lounsburyi* (NA, 1800♀, 1800♂)
		Psyttalia humilis (KA, 3600♀, 3600 ♂)
	14 September	*Psyttalia lounsburyi* (NA, 2010 ♀, 2010♂)
		Psyttalia humilis (KA, 2610♀, 2610♂)
	19 October	*Psyttalia lounsburyi* (NA, 3600♀, 750♂)
		Psyttalia humilis (KA, 3000♀, 1500♂)
EBCL (Arnaud Blanchet)	21 August	*Psyttalia lounsburyi* (SA, 191♀, 27♂)
Hawaii (Russell Messing)	5 November	*Fopius arisanus* (56♀, 20♂)
CABI-SA, Pakistan	9 November	*Psyttalia ponerophaga* (20)
(Abdul Rehman)	12 November	*Psyttalia ponerophaga* (75)

NA = Namibian population; KA = Kenyan population; SA = South African population. Sexes were not recorded for received *P. ponerophaga*.

to confirm the recovery of released parasitoids, rather than to quantitatively estimate parasitism rates. The number of parasitoids released also varied, depending on quantities received in each shipment and the number available from laboratory colonies at the time of the releases. For a quantitative measure of parasitism, caged releases were conducted in different locations and throughout different fruit production seasons.

1.3.1. Open field release

Open field releases were conducted in San Luis Obispo (SLO), and Sonoma, California. Non-commercial sites that were unsprayed and never harvested were selected to allow pre- or post-release sampling. The sites in SLO were (1) California Polytechnic State University (Cal Poly) campus; (2) Avila Beach (two private properties, each separated by about 1000 m); and (3) Broad Street (a private parking lot). The three locations were at least 5 miles apart, each site had more than 10 Mission-type trees that could be sampled. The selected release trees were bordered by other residential olive trees within 1-2 miles. About 500, 1000, and 1000 female *P. humilis* (NA) were released on 1 September (Bill's property), 22 October (Ann's property), and 4 November (Cal Poly), respectively (**Table 2**). About 300 female *P. lounsburyi* (KA) were released on 1 September at the Broad Street site.

In Sonoma, about 110 and 500 female *P. humilis* (NA) were released on 4 September and 27 October, and about 200 *P. lounsburyi* (KA) on 4 September were released at a private property with about 20 roadside mission type olive tress (**Table 2**).

Because open field releases of *P. humilis* (NA) were also conducted during the 2008 field season in the same Broad St. and Cal Poly sites, and in another site ("David's" property, that had 10 large Mission or Sevillano trees and was at least 10 miles away from all other sites in SLO), surveys of infested olives were conducted in these three sites prior to the 2009 releases to determine if any parasitoids could be recovered from the 2008 releases (**Table 2**). Pre- and post-release samplings were conducted for all field releases in 2009 (**Table 2**). Fruit were randomly collected from the trees that were used for the field releases. The collected fruit were placed in plastic containers (11 cm high × 11 cm diam.) covered with organdy cloth and held under room conditions at 20-25°C. Fly pupae were collected and held until the emergence of wasps or flies.

1.3.2. Field-cage test

To quantify field performance of *P. lounsburyi* and *P. humilis*, field-cage tests have been conducted since 2007 at different locations and throughout different seasons (**Table 3**). Three more field-cages tests were conducted in 2009 at KAC (**Table 3**). Each field-cage test consisted of 10 screen cages (45 cm long × 25 cm diam.). Each cage enclosed 50-80 fruit. The cages were established prior to the release of parasitoids; gravid female OLF were released into each cage to establish the fly infestation. After the OLF larvae developed to 2nd to 3rd instars, 15 female wasps were released into each cage, with water and honey provided for the wasps. After 1 to 3 weeks (exposure time varied to reflect the flies' developmental time and the parasitoids' longevity in the field), when the resulting flies and wasps were about to pupate, all fruit were collected and then held in the laboratory at KAC (24 ± 2 ºC, 40-60% RH) until adult flies or wasps emerged. Climatic data were taken from CIMIS weather station located at KAC.

Table 2 Open field release and recovery of two larval olive fruit fly parasitoids in 2009

Field release site	Field release		Pre-release sampling				Post-release sampling			
	Date	Female parasitoid released	Date	Fruit collected	Flies	Wasps	Date	Fruit collected	Flies	Wasps
Bill Property	1 Sept.	500 *P. humilis*	1 Sept.	161	40	0	8 Sept	248	3	0
							15 Sept	225	21	0
							22 Oct.	387	248	5
Broad Street *	1 Sept.	200 *P. lounsburyi*	22 July	114	22	0	22 Oct.	169	333	0
			12 Aug.	131	34	0				
			1 Sept.	140	70	0				
Anne Property	22 Oct.	1000 *P. humilis*	22 Oct.	100	130	0	25 Oct.	298	308	27
							29 Oct.	178	198	9
Cal Poly *	4 Nov.	1000 *P. humilis*	22 July	271	50	0	7 Nov.	102	107	19
			22 Oct.	294	361	0	29 Oct.	204	211	1
			21 Oct.	205	248	0				
David Property*			22 Oct.	336	233	0				
Napa*			17 Aug.	100	71	0				
Sonoma*	4 Sept.	110 *P. humilis*	17 Aug.	100	78	0	16 Sept.	200	82	0
		200 *P. lounsburyi*					27 Oct.	250	200	0
	27 Oct.	500 *P. humilis*					30 Oct.	250	310	0

SLO: San Luis Obispo, California. All *P. humilis* were Namibian population, and all *P. lounsburyi* were Kenyan population.
* Sites where *P. humilis* were also released in 2008 field season.

Table 3. Patterns of *B. oleae* parasitism by two larval parasitoids in field-cage tests

Parasitoid	Location	Set-up date	Ratio of host to parasitoid	Parasitism%	Temperature range (°C)
P. lounsburyi (SA)	KAC	02/09/07	4.2 ± 0.7a	26.2 ± 3.5a	5.5-19.2
	Lindcove	03/19/07	13.2 ± 1.7b	8.7 ± 1.1b	8.7-21.7
	Lindcove	05/04/07	5.0 ± 0.9a	19.2 ± 1.8a	10.2-28.9
	Lindcove	06/15/07	6.7 ± 0.4a	24.0 ± 2.0a	15.4-35.4
	KAC	10/04/07	4.5 ± 0.9a	27.4 ± 4.5a	8.5-22.5
	KAC	10/01/08	6.2 ± 1.7a	7.9 ± 2.9b	8.7-25.2
	SLO	10/22/08	4.2 ± 0.9a	6.2 ± 1.8b	10.0-25.1
	KAC	10/20/09	7.0 ± 1.0a	16.5 ± 4.1a	7.7-23.7
P. humilis (KA)	Lindcove	06/15/07	7.4 ± 0.5a	47.2 ± 7.6a	15.4-35.4
	KAC	10/04/07	4.3 ± 0.6b	42.5 ± 3.4a	8.5-22.5
	KAC	04/28/08	6.5 ± 1.1ab	49.0 ± 5.9a	9.9-26.8
	KAC	10/20/09	4.6 ± 0.7 b	38.4 ± 2.9a	7.7-23.7
P. humilis (NA)	KAC	10/01/08	6.2 ± 1.2a	60.3 ± 6.7a	8.7-25.2
	SLO	08/22/08	6.5 ± 0.6a	32.4 ± 5.7bc	12.9-27.0
	SLO	08/28/08	4.6 ± 0.9a	64.3 ± 9.7a	11.9-28.4
	SLO	10/07/08	10.3 ± 1.9b	42.5 ± 5.3b	10.1-26.2
	SLO	10/22/08	3.5 ± 0.7c	37.7 ± 4.6bc	10.0-25.1
	KAC	10/20/08	4.1 ± 0.7ac	43. 9± 5.4b	8.8-26.4
	KAC	11/12/08	3.3 ± 0.4c	31.3 ± 3.3c	6.1-17.0
	KAC	03/10/09	5.9 ± 0.5a	80.5 ± 3.9d	2.2-17.9

Data were compared within the same species or strains. Values (means ± SE) followed by different letters are significantly different (ANOVA, $P < 0.05$). SLO = San Luis Obispo, KAC = Kearney Agricultural Center.

Objective 2

Thermal performance

A series of laboratory experiments were conducted at nine different constant temperatures (low: 10, 12, 14°C; middle: 18, 22, 26 °C; high: 30, 32, 34 °C) to compare the effects of temperature on the development, survival, and reproduction of *P. lounsburyi* (KA), *P. humilis* (both populations), *P. ponerophaga*, as well as OLF. Tests with the temperatures of 12, 22, and 30°C were conducted at the UCB I&Q Facility, while other tests were carried out at KAC. All tests were performed in temperature cabinets (Percival Scientific Inc., Model I-36VL) in which the relative humidity was maintained around 50% and photophase ran from 0600 to 2000 hours. Procedures were similar for each parasitoid population tested.

Olive fruit were infested by exposing them to flies until each fruit contained 3-5 oviposition punctures (exposure time varied from 2 to 4 h). Infested fruit were then randomly distributed to different temperature treatments to measure the developmental time and survival of OLF. For

the measure of each parasitoid, infested fruit were first kept at 25°C until the fly eggs had developed into young third instars (9-10 d) and were then exposed to each parasitoid in its holding cage for 12-24 h. Exposed fruit were then randomly divided amongst the various temperature treatments.

The fruit were placed in groups of 20 to 25 fruit per plastic containers (11 cm diam., 10 cm high) with a screen cover, and spread over hardware cloth 2 cm above the bottom of the container to allow air flow and reduce the growth of mold. When fly or parasitoid emergence was ready to begin (after about 9 days) the containers were checked twice daily (AM and PM) and the number and sex of the emerged insects was recorded. After emergence was complete, dead pupae were also counted and then dissected to determine if they had been parasitized. There were 10 replicates for each temperature treatment and parasitoid population.

To determine the effect of temperature on reproduction, 2-wk old gravid female OLF were individually provided with 10 fruit. At the same time, 1-wk old gravid female parasitoids were individually provided with 10 infested fruit, each containing 3-5 young third instars, for 48 h in cylindrical acrylic cages (20 × 15 × 15 cm). The cage had two organdy screen holes for ventilation, and was held under respective temperature incubators. All exposed fruit (OLF and parasitoid) were transferred to a plastic container (11 cm diam., 10 cm high) and maintained at 25°C until the flies or wasps emerged. There were 20-30 replicates at each tested temperature, with each female fly or parasitoid considered a replicate.

Objective 3

Overwintering biology

Laboratory and field studies were conducted to investigate the effect of low temperature and cold storage on the parasitoids' fitness (development, survival and reproduction) and to determine the patterns of overwintering mortality and spring emergence patterns. The cold storage study may also help develop an efficient method for maintaining parasitoid colonies during periods when olive fruit for the fly colony are difficult to collect.

3.1. Low temperature effects on adult parasitoids

Newly emerged *P. humilis* (NA) were placed in cages (30 × 30 × 30 cm) and held at room temperature (24 ± 2 ºC, 16L: 8D h, 40-60% RH) for 1-2 d with food and water provided. The parasitoids were then exposed to different temperature treatments: (1) constant temperatures of 6, 8, or 10 °C (temperature cabinets); (2) outdoor winter conditions (November to February, outside of insectary building at KAC); and (3) suitable room temperature (24 ± 2 ºC). For each treatment, 100 adult (approximately similar numbers of males and females) were held in cages (30 × 30 × 30 cm), with water and honey provided. For the outdoor treatment, the cages were placed under a metal roof to reduce exposure to rain, which would damage the cages, and positioned slightly above the ground to protect the insects from foraging ants. The outdoor tests were started on 25 November 2008 and ended after all wasps were dead in February 2009. It was repeated in November 2009 (which is still in progress). All treatments were checked daily and parasitoid condition (alive or dead) was recorded.

To determine the effect of low temperature exposure on parasitoid reproduction, female *P. humilis* (NA) were exposed to one of three low temperatures (6, 8, 10 °C) for one of four

exposure periods (1, 2, 4, and 6 weeks), and then tested for their relative fecundity. For all treatments, adult parasitoids were provided access to hosts (6 infested fruit every 2 d) for a one week period in cylindrical acrylic cages (20 × 15 × 15 cm) at 24 ± 2 ºC. All exposed fruit were kept in separate containers until the flies or wasps emerged. All dead pupae were dissected to determine the presence or absence of parasitoid. Fifteen females were tested for each exposure time at each temperature treatment (15 replicates per treatment or 16 'temperature × exposure period' combination).

3.2. Cold storage effect on parasitoids

The aim of this experiment was to determine the best parasitoid developmental stage and temperature for cold storage of OLF parasitoids. The study factors were survival rate during cold storage and fitness of emerged adults after cold storage (i.e., developmental time, emergence rate, sex ratio, longevity and fecundity of emerged wasps). For the test of *P. humilis* (NA), the experiment consisted of five temperatures (4, 6, 8, 10 and 12°C), five parasitoid developmental stages (egg, young larva, old larva, pupa, and pharate adult, but only the pupal stage for 4 and 12 °C), and four periods of cold storage duration (0, 1, 2, and 3 months).

Parasitized fly puparia were collected and divided evenly amongst the treatments. After the parasitoids developed into egg (0-2 d), young larva (4-6 d), old larva (7-10 d), pupa (12-15 d), and pharate adult (> 17 d) at 24 ± 2ºC, they were placed in the corresponding temperature incubators for cold storage of the different duration periods. Following each storage period, exposed parasitoids were moved out of cabinet and held at 24 ± 2ºC to record the developmental time and sex of emerged parasitoids. To consider if storage conditions would also affect the longevity and fecundity of emerged parasitoids, newly emerged females and males were first kept together for 4-6 d to allow mating and then each female was provided with five infested olives every 2 d for one week.

The effect of cold storage effect on the survival and development of *P. ponerophaga* was conducted in quarantine at the UCB I&Q Facility. Parasitized OLF puparia were stored at 6°C for different periods of time ranged from 41 to 94 d, and then held at controlled room conditions (22 ± 2°C, 16L: 8D, 40% RH). In total, 33 exposures, each consisted of 35 to 359 parasitized puparia were tested.

3.3. Overwintering survival and spring emergence of parasitoids

Two field experiments were conducted in the winter of 2008 and 2009 to investigate the overwintering survival and emergence of OLF in the field. In 2008, the experiment was conducted at KAC with *P. humilis* (NA). Lab-parasitized fly puparia containing *P. humilis* eggs were placed in a groups of 20 to 30 individuals (4-5 replicates) in small plastic containers (150 ml), which were then hung on olive trees (1.5 m high). The initiation of exposure periods began on 1 October 2008 and was repeated every 5-7 d until 16 December 2008 (10 exposures in total). As a control, about half of parasitized puparia from each group of parasitoids were held at room temperature (24 ± 2 ºC). All field containers were checked weekly to record and remove emerged flies or wasps until March 2009 (after the expected fly / parasitoid emergence period should have been completed).

In 2009, *P. humilis* (NA) and *P. lounsburyi* (KA) were tested at KAC and Berkeley, using similar procedures for both parasitoid species and locations. In these trials, exposure periods

were initiated in early October, and repeated each week until late December (10 exposures in total). First, each plastic container (150 ml) was filled with about 50 g sandy soil (40% RH) (occupied 4 / 5 of the container), and a group of 20 to 50 pupae (the number of pupae per container varied, depending on availability) were then buried 1-2 cm below the soil surface. Second, a large wooden box (10 cm high by 50 cm width by 120 cm length) with a metal screen bottom was filled with sandy soil and buried into the field, so that the soil surface inside the box was about at the same level as the soil surface in the field. All containers (4-5 replicates per parasitoid species and each exposure date) were buried into the wooden box. All sides and the bottom of the container were needle-punctured, to allow water to drain but prevent ant entrance, and covered with a screen lid. The test consisted of four different treatments of soil humidity (natural, low at ca. RH25%, moderate at ca. RH 50%, and high at ca. RH75%) for each exposure date. The different humilities were controlled by applying a mist spray of water over the containers once each week. The wooden boxes of the three controlled humidity treatments were covered by plastic cloth 1.5 m over the boxes, which still allowed access of wind and sunshine, but protected the experimental area from heavy rains (that could occur occasionally during the winter). The air and soil (5 cm below surface) temperatures were recorded using a temperature data logger (HOBO, Onset Computers).

RESULTS AND DISCUSSION

1. Field evaluation of *P. humilis* and *P. lounsburyi*

In SLO, *P. humilis* were recovered in 2009, 1-3 months after the field release (**Table 2**). However, field samplings of infested olives in 2009 in all three sites, where *P. humilis* were released during the 2008 field seasons and recovered in 2008, did not recovered *P. humilis*. At this site, over 2000 *P. humilis* were released in November 2008. We noticed that the fruit load per tree was light and the levels of fly infestation were low in 2009 (< 1 larva per fruit, see **Table 2**) when compared to 2008 (2-3 larva per fruit). No *P. lounsburyi* were recovered from the Broad Street site after 300 females were released in 2009. In Sonoma and Napa, post-release surveys also did not recover any parasitoids in 2009. We suspect that the numbers of parasitoids released might be too low for *P. lounsburyi* to establish at the Sonoma release, and future release may need to consider releasing large quantities of parasitoids. These release sites will be continually monitored in 2010 for the recovery of released parasitoids.

Field-cage tests during the last three years have confirmed that both parasitoids could successfully attack larval *B. oleae* throughout the fruit seasons both in the Central Valley (Fresno), and central coast California (SLO). Parasitism levels ranged from 31.3 to 80.5% by *P. humilis*, and 6.2 to 27.4% by *P. lounsburyi* (**Table 3**). Within the same parasitoid species, parasitism of larval OLF was always higher on smaller fruit compared to larger fruit (**Fig. 1**). Overall, parasitism levels of larval OLF by *P. humilis* (both populations) were higher than *P. lounsburyi* (**Table 3**), regardless the fruit size (**Fig. 1**), test dates and locations (**Fig. 2**). However, many other factors might have affected the levels of parasitism on OLF by both parasitoids in the field-cage tests, and further detailed analyses on various possible effects (temperature, fruit size, host density etc.) on the parasitism are still in progress.

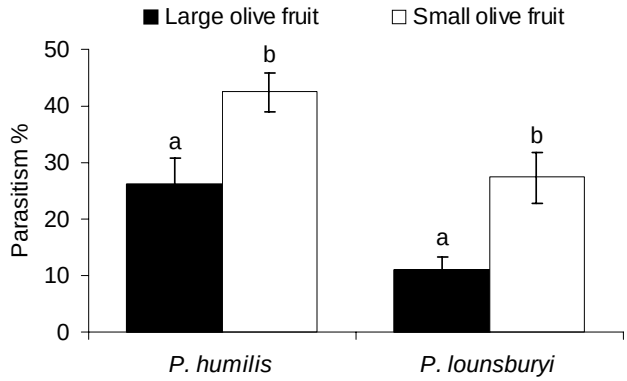


Fig. 1. Parasitism levels by *P. humilis* or *P. lounsburyi* on larval *B. oleae* in small (Mission) and large (Sevillano) olive fruit. Values (means ± SE) followed by different letters are significantly different (ANOVA, $P < 0.05$).

2. Thermal performance

The suitable temperature range for parasitoid development (egg to adult) was different between *P. humilis* and *P. lounsburyi* (**Fig. 3**). Among the tested temperatures, *P. humilis* failed to develop at 10, 12 and 34°C, but successfully developed from 14 to 32°C, while *P. lounsburyi* could develop from 10 to 30 °C, but failed to develop at 32°C (to date, only one female developed at 30°C and one male developed at 10°C). The relationships between temperature and developmental time were similar for the two different populations of *P. humilis* and for both parasitoid species within the temperature range of 14-30°C (**Fig. 3**). Overall, males developed faster than females for each species (**Fig. 3**).

OLF could successfully develop from 10 to 30°C, and failed to develop at 32 or 34°C. Dissections found that the majority of OLF eggs successfully hatched at 32°C, and they died during the young larval stages. Most eggs died at 34°C. Mature OLF larvae were also observed to successfully pupate at 32 or 34°C, but failed to develop into adults. The results suggest that young larvae were most sensitive to high temperatures. Because the survival of endoparasitic and koinobiont parasitoids rely on the survival of their host, both parasitoids have an advantage by attacking mature larvae, especially for *P. humilis*, which survived at 32°C. These results also suggest that *P. lounsburyi* is more cold tolerant than *P. humilis* and its temperature range for development appears to match up better with OLF.

Experiments at the two low temperatures (10 and 12°C) are still in progress. Our preliminary analyses showed that the most suitable temperatures for offspring survival (estimated based on the number of adult wasps reared per fruit among different temperatures) and reproduction (estimated based on the number of offspring produced over a period of 48 h) were 26-30°C for OLF and *P. humilis,* and 18-22°C for *P. lounsburyi*. Detailed analyses are still in progress. These data will be used to determine the key biological parameters (e.g., low and high thresholds) and develop temperature-driven relationships for development, survival, and reproduction for each insect.

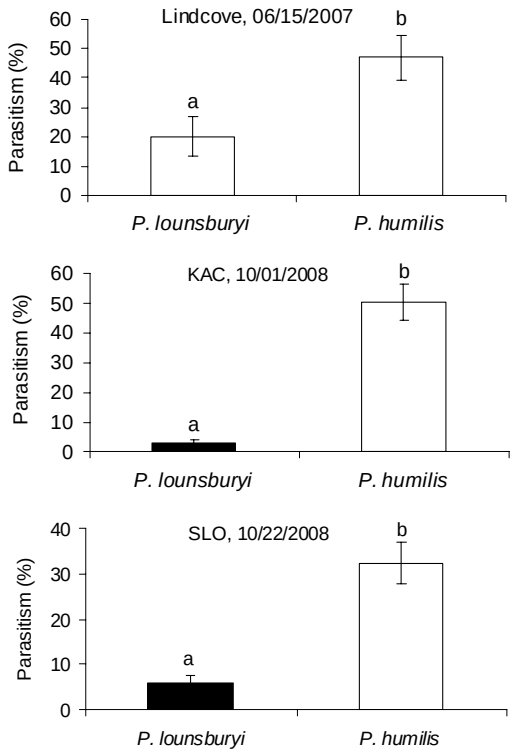


Fig. 2. Comparison of parasitism between *P. lounsburyi* and *P. humilis* on larval *B. oleae* at three different locations (SLO = San Luis Obispo, KAC = Kearney Agricultural Center). Values (means ± SE) followed by different letters are significantly different for each pair comparison (ANOVA, $P < 0.05$).

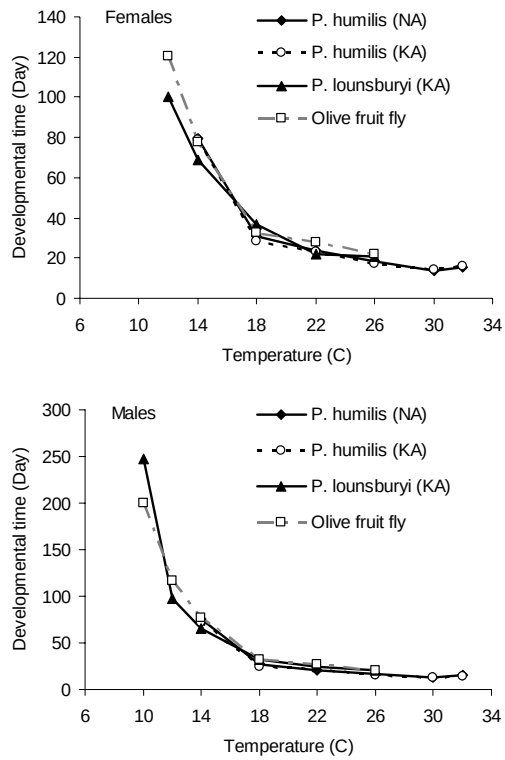


Fig. 3. Effect of temperature on the developmental time of *P. humilis*, *P. lounsburyi* and *B. oleae*. Top: females; bottom: males.

3. Overwintering biology

Exposure to constant low temperatures reduced the longevity of adult *P. humilis* (**Fig. 4**). The parasitoids survived for shorter periods at 6 and 8ºC than at 10ºC. On average, female *P. humilis* lived 22, 41, and 54 d at 6, 8, and 10°C, respectively. However, under ambient conditions (in the field) adult *P. humilis* survived as long as those held at room temperature during the winter trial. The longest lived female held at ambient temperatures died on 16 March 2009 (i.e., survived 109 days). At a constant temperature of 10ºC or < 10ºC, adult *P. humilis* were inactive and unable to feed on the provided honey. Although the mean winter temperature for most period of the field trial (i.e., November to February) was about 10ºC, the parasitoids were observed to readily feed on the honey and water during the warming period of sunny day. This might have helped them to sustain their life longer in the field. This also suggests that adult parasitoids may be able to survive the winter in the field. The 2009 field trial on adult parasitoid survival is still in progress.

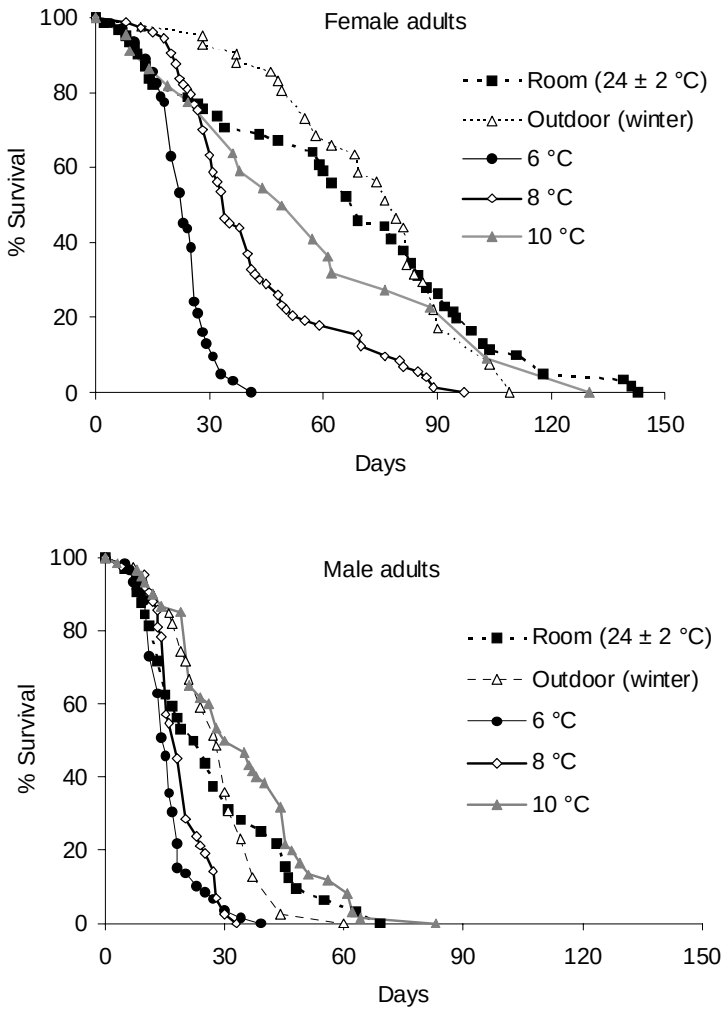


Fig. 4. Effects of low temperatures on the survival of adult *P. humilis*.

A one-week exposure to three low temperatures (6, 8 and 10ºC) did not appear to affect the parasitoid's post-exposure fecundity (**Fig. 5**). The fecundities between 8 and 10°C treatments were similar for all exposure durations, and were higher than those at 6ºC when the exposure periods were > 2 weeks. The parasitoid's fecundity decreased with increased exposure time to each low temperature (**Fig. 5**). After a 6-week exposure some parasitoids were unable to attack hosts.

The cold-storage tests showed that not a single *P. humilis* developed after being stored at 4°C for 1 month, at 6 and 8°C for > 2 months, or at 10 and 12°C for > 3 months (**Table 4**). Regardless of storage temperature, not a single egg developed after being exposed for > 1 month. Percentage emergence of post-stored parasitoids increased with increased storage temperature (**Table 4**). Mature larvae, pupae or pre-adults appear to be the most suitable stages for the cold storage. About 48% of emerged adults from the pre-adult treatments (mostly from 10°C treatment) had deformed wings, and those deformed parasitoids only lived 3.5 ± 0.55 d (n =16). On average (n =15), females developed after being stored at 10°C survived 54.6 ± 8.5 d with food and water, and reproduced 2.4 ± 0.49 offspring within one week, while normal females

Table 4. Emergence rate of *P. humilis* after being stored for different periods of time at different temperatures

		One month			Two month			Three months	
Temp.	Stage	N	% Emergence	% Female	N	% Emergence	% Female	N	% Emergence
4 °C	Pupa	80	0		80	0		80	0
6 °C	Egg	161	0		104	0		77	0
	Young larva	86	0		64	0		56	0
	Old larva	142	2.31	100	99	0		207	0
	Pupa	139	0.76	100	100	0		204	0
	Pre-adult	92	0	0	39	0		107	0
8 °C	Egg	126	0		110	0		80	0
	Young larva	55	0		64	0		56	0
	Old larva	158	5.63	37.5	110	0		110	0
	Pupa	136	4.76	100	102	0		204	0
	Pre-adult	91	1.15	100	50	0		101	0
10 °C	Egg	105	0		114	0		82	0
	Young larva	85	8.62	40.0	59	4.55	50.0	56	0
	Old larva	160	8.86	57.1	103	0		205	0
	Pupa	143	11.19	80.0	103	0		204	0
	Pre-adult	85	13.1	66.7	101	13.8		163	0
12 °C	Pupa	140	7.9	33.3	140	10.0	41.2	280	0
CK*	Egg	128	75.82	49.3					
	Young larva	125	77.78	50.0					
	Old larva	129	54.39	56.5					
	Pupa	163	83.89	56.8					
	Pre-adult	37	72.97	37.1					

* Under room condition (24 ± 2 °C).

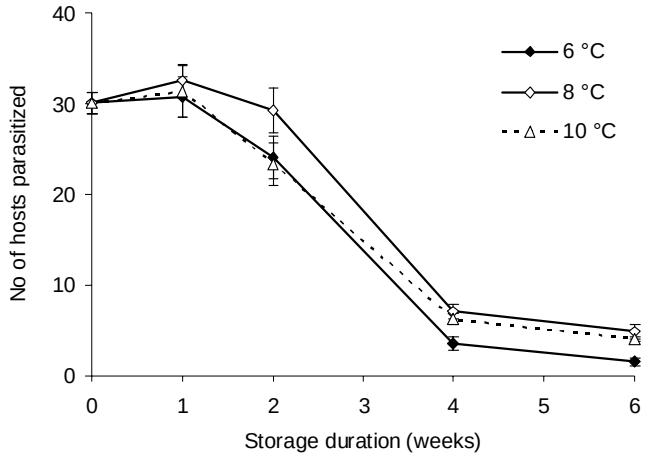


Fig. 5. Effects of cold storage on the post-storage fecundity of *P. humilis*.

(directly reared at 24 ± 2 ºC) survived 65.5 ± 4.9 d (n= 30), and produced 15.3 ± 1.06 offspring under the same conditions. However, developmental times from various immature developmental stages to adults were similar to those parasitoids held continuously at 24 ± 2ºC. However, when *P. ponerophaga* parasitized OLF puparia were stored at 6°C for 1-3 months, the emergence rate average was 17.2%; this was considerably higher than that of *P. humilis* (2.31%) after being stored at the same temperature. Post-storage developmental time increased while post-storage emergence rate decreased with increasing duration of cold storage (**Fig. 6**). Males developed faster than females.

When lab-parasitized fly pupae containing *P. humilis* eggs were hung on olive trees in the field in 2008, the parasitoid eggs laid in early October developed into adults within 2008 (developmental time ranged 30-50 d), but those laid in late October developed in January or February 2009 (> 60 d) (**Table 5**). No parasitoids emerged from the exposures later than 27 October 2008, but unparasitized fly puparia developed into adults in early March 2009. Mortality of parasitized pupae was very high when compared to the control treatment. Most pupae apparently were dead due to the dehydration.

In 2009, when lab-parasitized fly puparia were buried into soil, emerged parasitoids were collected from the first four exposures (from 15 October to 3 November at KAC, from 14 October to 5 November at Berkeley), and the overall emergence patterns for both locations and both species were similar. For example, the parasitoids from the first burial on 15 October at KAC started emergence on 17 November and the cumulative number of emerged wasps peaked by 31 December while the parasitoids from the fourth burial on 3 November at KAC started emergence on 3 December and the cumulative number of emerged wasps increased over the next weeks (**Fig. 7**). Overall, the survival rate from the middle humidity soil treatment was higher than the other humidity treatments (**Fig. 7**). No parasitoid has yet emerged from the burial treatments that were initiated after 3 November at KAC or after 5 November at Berkeley (as of 10 January 2010) and these experiments are still in progress.

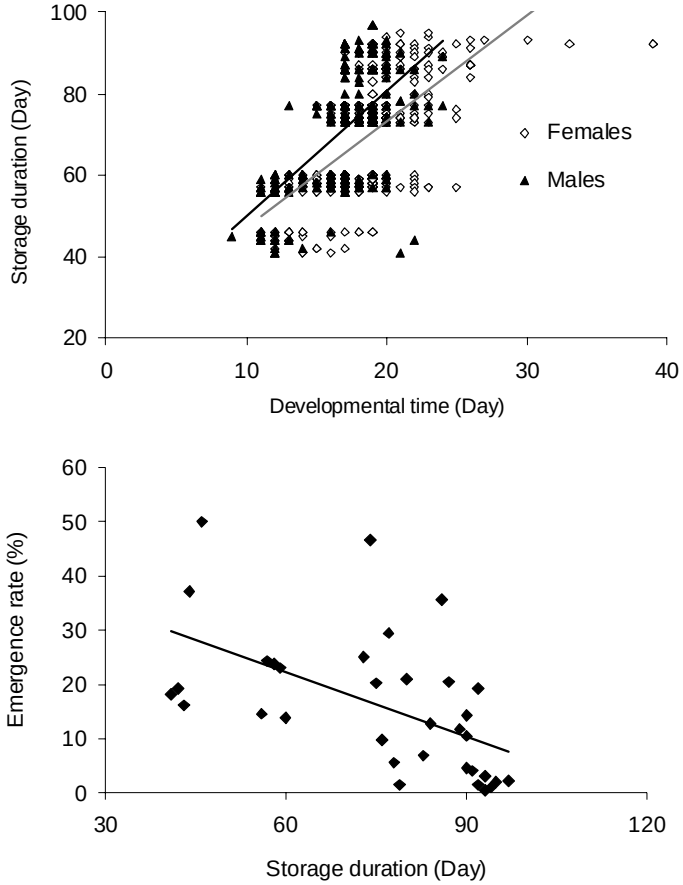
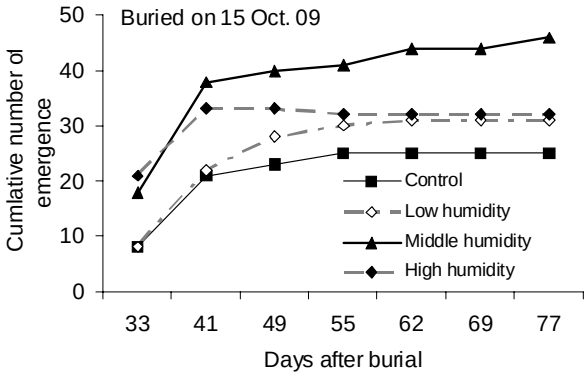


Fig. 6. Effects of cold storage (6 °C) on the developmental time (top) and emergence rate (bottom) of *P. ponerophaga.*

Table 5. Survival and emergence of *P. humilis* in outdoor during 2008 winter seasons

Set-up date	Outdoor (winter)			Inside room (24 ± 2 °C)		
	No	No. wasps emerged	Mortality %	No	No. wasps emerged	Mortality %
10/01/08	52	34	34.6	149	81	44.5
10/07/08	52	14	80.9	50	25	50
10/13/08	54	6	92.1	43	22	51.2
10/20/08	50	1	96.7	47	11	76.1
10/27/08	50	0	100	50	10	79.6
11/03/08	58	0	100	47	25	46.8
11/10/08	50	0	100	55	10	81.5
11/17/08	54	0	100	71	36	33.3
11/25/08	84	0	100	73	18	65.2
12/16/08	48	0	100	44	11	71.1



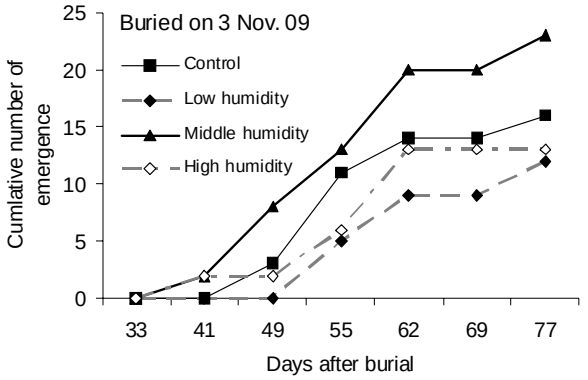


Fig. 7. Emergence patterns of overwintering fruit fly parasitoids in the field (*P. humilis* as examples, parasitized fly pupae were buried on 15 Oct. and 3 Nov. 2009).

CONCLUSIONS

Biological control can be a practical, safe, and economically effective means of fruit fly control, and its importance continues to grow in regions where pesticide use is less desirable (e.g., sustainable agriculture) or more restricted (e.g., urban trees). Field evaluation has demonstrated that the introduced parasitoids, *P. humilis* and *P. lounsburyi*, can attack OLF under field conditions in both coastal and interior regions of California. This is especially true for small fruit cultivars, as discussed in earlier reports, due to their short ovipositors. Laboratory studies showed that *P. lounsburyi* seems to be more closely coupled with its host in terms of its suitable temperature range for development and survival. Both *P. humilis* and *P. lounsburyi* did not appear to enter diapause during the winter in California. Thus, cold-tolerance could be a critical trait that would determine their overwintering survival and ultimately establishment of the two parasitoids. Detailed studies on their overwintering biology as well as more field releases with large quantities of both parasitoids are needed in the future.

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This information shows the importance of commodity support to help engage State and Federal funding for California farmers.

Implement Diagnosis, Epidemiology, and Control Methods for Fungal Pathogens Causing Stem Canker and Branch Dieback of Olive Trees in California

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Reporting Period: 1 January 2009 to 31 December 2008

ABSTRACT

Branch dieback of olive trees (*Olea europaea* L.) caused by perennial cankers is a major concern throughout the main olive-producing regions in the Mediterranean regions of Spain, Portugal, Italy, and Greece. However, the importance of this disease to the olive industry in California has not yet been evaluated. Therefore, the goal of this project is to determine the incidence of Branch dieback of olive trees in California and identify the major fungal pathogens associated with perennial canker in both mature orchards and young plantings of olive trees in the State. In order to accomplish the first objective of this project, field surveys were conducted throughout the main olive-production regions in California from October 2008 to September 2009 including Tehama, Glenn, Butte, Napa, Sonoma, Yolo, Solano, Sacramento, Merced, Madera, Fresno, Tulare, Santa Barbara, Ventura, and Riverside counties. Branch dieback symptoms of olive trees were characterized by perennial cankers that extend through the stems and continue through the main branch. Twig dieback was also observed associated with branches affected by cankers. Cankers were found to develop from pruning wounds, mechanical injuries, and natural openings on the main branches causing eventual death of the productive tree parts. Additionally, cankers were also associated with olive knot galls caused by the bacterium *Pseudomonas syringae* pv. *savastanoi*. Cankers often were observed to colonize the main trunk causing the slow decline and collapse of the whole tree. In all, over 680 samples of cankered twigs, branches, and trunks were collected from 56 different olive orchards representing the most important cultivars planted in California. Olive branch dieback was observed in all orchards surveyed in California. However, incidence of the disease varied with location and age of the trees.

Results from canker isolations showed species in the family Botryosphaeriaceae, Valsaceae, and Diatrypaceae to be the most predominant fungi associated with branch cankers of olive trees

in California. Botryosphaeriaceae species, which were isolated from 36% of the total of samples collected, were the most prevalent fungi isolated from perennial olive cankers and were found in all counties surveyed. In order of importance, Botryosphaeriaceae species were followed by Diatrypaceae species (9%), Valsaceae species (8.6%), and Basidiomycetes species (3%). Morphological studies along with DNA analyses of three genes including the internal transcribe spacer region (ITS1-5.8S-ITS2), a partial sequence of the beta-tubulin gene (BT2), and a part of the translation elongation factor 1-α gene (EF1-α), allowed us to identify nine Botryosphaeriaceae species, four Diatrypaceae species, five Valsaceae species, and two Basidiomycota species. Moreover, *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*, the causal agents of Esca and Young vine decline of grapevines, were also identified from necrotic wood of olive trees. In addition, several mitosporic fungi such as *Aspergillus*, *Alternaria*, and *Epicoccum* were isolated from cankers, but these were considered to be saprophytes.

In order to determine the importance of these fungi in olive tree health in California, pathogenicity tests in both Manzanillo and Sevillano cultivars are currently underway at the Nickels Soil Research Station in Colusa County.

INTRODUCTION

California's olive production, with over 30,000 acres and over 140,000 tons of olives in 2007, represents 99% of the total olive production in the United States (CDFA 2007). Olives, with a revenue of $86 million in 2007, have become an important and significant increasing crop in California.

The European olive (*Olea europaea* L.) is a long-lived, drought resistant, evergreen tree that suffers from both fungal and bacterial major diseases, threatening tree longevity and olive production. Major olive diseases known to occur in California include Armillaria root rot caused by *Armillaria mellea*, Olive leaf spot caused by *Cercospora cladospoporioides*, Verticillum wilt caused by *Verticillum dahliae*, Peacock spot caused by *Spilocaea oleaginea*, and Olive knot caused by *Pseudomonas syringae* pv. *savastanoi*. However, in the past years stem canker and branch dieback of olive trees have been of major concern to farmers in the main olive-producing areas not only in California, but worldwide because their direct impact in tree health and olive production. Fungal cankers and branch dieback are known to cause important yield reduction. In addition, cankers increase production costs as a result of cultural and chemical preventive measures as well as removing diseased wood from the tree after infection. Generally, little research has been conducted regarding fungi affecting stem and branch of olive trees in California. Though, in the past few years, severe limb dieback on both young and mature olive trees from the main production areas has been reported to our laboratory from both farm advisers and growers.

A few wood rotting and wood decay fungi have been reported to cause stem cankers and branch dieback of olive causing important economic losses to the olive industry. For example, *Cytospora oleina* and *Eutypa lata* have been shown as the main canker and branch dieback causing agents of olive trees in Greece (Rumbos 1988, Rumbos 1993). Additionally, *Phialophora parasitica* was associated with twig dieback and wilting of olive trees in Greece (Thanassoulopoulos and Thanassoulopoulos 1984). Recent reports have also shown *Neofusicoccum ribis* and *Neofusicoccum luteum* to cause important branch dieback and stem canker disease of olive in Spain and New Zealand, respectively (Romero et al. 2005, Taylor et al. 2001). *Botryosphaeria dothidea* has been reported to cause stem cankers and fruit rot of olives in Portugal (Phillips et al. 2005). Furthermore, a recent study has shown Botryosphaeriaceae

species as the main fungal pathogens causing branch dieback and drupe rot of olives in southern Italy (Lazzizera et al. 2008). However, the presence and importance of these fungal pathogens to the olive industry in California has not been evaluated. Although *Eutypa lata* and several Botryosphaeriaceae species are known as the most important canker causing agents on grapevines and other woody perennials in California (Moller and Kasimatis 1978, Úrbez-Torres et al. 2006, Úrbez-Torres and Gubler 2009), the actual importance and contribution of these pathogens to the major wood canker and limb dieback observed in California olives remains unknown. The lack of information on the fungal pathogens associated with perennial stem cankers and branch dieback in olive orchards render difficult all attempts to control disease development. Also, this confusion may mislead farmers towards the application of inappropriate, unnecessary and costly methods to control disease development.

The goal of this project is to identify the major fungal pathogens associated with canker diseases in both mature orchards and young plantings of olive trees in California. Methodology for identification of pathogens will include molecular based techniques as well as pathogenicity tests. Project achievement will provide disease and pathogen identification tools. Continuing projects will allow for testing of control methods suitable for use in organic farming.

OBJECTIVES

1) Identification and characterization of the current population of fungal pathogens associated with stem perennial cankers and branch dieback of olive trees in California;

2) Understand the epidemiology of olive canker pathogens: inoculum sources and dispersal patterns, as essential for IPM control strategy design; and

3) With the anticipation of continuing funding for the following year 2009 we will develop and implement chemical, biological and organically acceptable control methods for fungi involved in branch dieback of olive.

PROCEDURES

Objective 1

Field Surveys. Field surveys are critical in order to obtain a complete description of the occurrence and distribution of fungal species associated with stem cankers in olive trees in California. Monitoring orchards has become necessary to establish a thorough knowledge and comprehension of the main causal agent of olive branch dieback. This will constitute a first step towards carefully determining the importance that fungal species have in olive health in California. Field surveys will be conducted throughout the main olive-growing areas of California. Orchards of different ages among the predominant olive cultivars of California will be surveyed. Over ten samples showing stem cankers, twig dieback, and declined branches from declining olive trees will be collected in each orchard. Plant parts will be examined in the laboratory and isolations made.

Fungal isolation. Olive samples will be surface disinfested in 10% sodium hypochlorite for 10 minutes. After air drying, the surface tissue will be cut away to expose canker margins. Small pieces of tissue from margins of the cankers will be placed on Petri dishes containing 4% potato

dextrose agar (PDA) amended with 100 ppm of tetracycline in an attempt to recover the various fungal pathogens. Cultures will be incubated at room temperature until fungal colonies are observed. Pure cultures of the fungal species isolated from cankers will be obtained by excising a hyphal tip from colony margins emerging from the tissue pieces onto fresh plates of PDA-tet.

Fungal identification. Fungal species will be identified based on morphology, color, spore shape, density, and conidial production colonies. However, fungal species are often difficult to identify based only on morphological characters. Thus, by combining morphology characters with molecular work we can develop a reliable technique to identify and separate different fungal species. Fungal DNA from the different species will be obtained using an AquaPure Genomic DNA Kit (Bio-Rad Laboratories; Hercules, CA). Fungal species will be identified based on the PCR amplification of the 5.8S nuclear ribosomal DNA region (ITS1 and ITS2) using primers ITS4 and ITS5. A portion of the beta-tubuline gene (BT2) will also be amplified using primers Bt2a and Bt2b. Additionally, a portion of the elongation factor 1-α gene (EF1-α) will be amplified using primers EFF and EFR. Consensus sequences will be obtained using Sequencher™ (version 4.1.2). Sequences will be aligned by using Clustal W multiple alignments program (10) and will be edited manually. Phylogenetic analyses will be performed with PAUP (version 4.0b10) (11). DNA sequences obtained from the different fungal species from olive trees from California will be compared with olive and other hosts sequences from previous studies available in GenBank and from our collection. Morphological results, as well as ITS, beta-tubuline, and elongation factor data will be used to obtain precise fungal species identification.

Pathogenicity tests. In order to determine which fungal species isolated from cankers of olive trees are important pathogens and to differentiate those from saprophytes, secondary colonizers or weak parasites, pathogenicity tests will be assessed. *In vivo* pathogenicity tests will be conducted in the University of California Nickels Soil Field Station in Arbuckle, Colusa County. Olive branches from Manzanillo and Sevillano cultivars will be inoculated under controlled conditions with the most prevalent fungi isolated from olive cankers from field surveys. Inoculum of the potential pathogenic fungi (mycelium colonized agar plugs) will be introduced in the olive stem and branches through a fresh wound. Fungal treatments will be compared to control treatments (non-colonized agar plugs). Pathogenicity will be assessed by the extent of vascular discolorations and necroses observed in inoculated wood and by the percentage of recovery of the pathogen from these necroses.

Objective 2

No information is available about the epidemiology and mode of inoculum dispersal for fungal pathogens causing stem canker and branch dieback of olives. Consequently, spore traps will be used to determine what environmental conditions favor the release of fungal inoculum. Furthermore, spore trapping studies should reveal yearly or seasonal patterns of spore release and eventually determine periods of low risk of infection. Spore sampling devices will be placed in orchards throughout the main olive-producing counties. Spores will be trapped using microscope slides covered with a thin layer of Vaseline and will be placed on olive branches. Spore traps will be collected and changed weekly and the quantity of inoculum will be estimated weekly. Results will be presented as number of spores over weekly time intervals and will be superimposed with records of precipitations and temperatures obtained from local weather stations. Furthermore, visual surveys to investigate the occurrence and type of fungal fruiting bodies in diseased olive orchards will be conducted. Also, we will investigate the presence of fungal fruiting bodies in

other cultivated perennial crops, ornamental or native plant hosts in the vicinity of olive orchards. Wood samples from within and nearby diseased olive orchards will be collected and investigated for the presence of fertile fruiting bodies. Spore producing structures will be isolated and identified in the laboratory and specimens will be compared with isolates obtained from cankers from olive surveys. This work will help in deciding for appropriate sanitation practices, time periods for pruning, and application of wound protecting agents in order to reduce the risk of infection by canker pathogens.

Objective 3

Compounds and agents to be used will include organic compounds, biological and wetting agents against the major pathogenic fungi associated with olive canker disease. Compounds to be tested will be the boron-based products biopaste and bioshield that have shown efficiency to reduce infection by *E. lata* in grapevine (Rolshausen et *al.*, 2005). Bioshield consisted of a mixture of boric acid (5% wt/vol) and a spore suspension of *C. herbarum* that had been challenged until the fungus could grow and survive at this boron concentration (10,000 spores per µl). Biopaste consisted of a mixture of boric acid (5% wt/vol) and a commercial paste (latex paint) used on trees as wound dressing and grafting seal (Doc Farwell's, Wenatchee, WA). Biocontrol agents to be tested include *Cladosporium* and *Trichoderma* spp. Biocontrol agents will be applied on the surface of pruning to act as wound colonizer, prior to the inoculation of the various wood pathogens identified. Results will be estimated 10 to 12 months after inoculation.

Pentra-Bark is a superior nonionic wetting agent designed for water based herbicide application in agriculture and forestry operations. Pentra-Bark is being used in combination with systemic fungicides in the treatment of Sudden Oak Death and canker disease control in grapevines. It is designed for fast-spreading, uniform distribution and absorption of spray on leaf and stem surfaces. Accordingly, Pentra-bark will be tested in mixtures with various fungicides for efficiency in spray application and to evaluate the control of pruning wound invading canker disease pathogens. Fungicides to be tested with Pentra-Bark will include: Enable 2F, Rally 40W, Thiram 65 WP and Topsin M (70 WP). All treatments will be amended with Pentra-Bark (Agrichem Manufacturing Industries, Queensland, Australia) and Freeway (United Agri Products, Colorado, USA), both at a high label rate of 5 ml per liter to ensure maximum penetration of the cork cambium. An untreated control will be reserved for statistical comparison.

RESULTS AND DISCUSSION

Objective 1

Field Surveys. To date, field surveys have been conducted in 15 different counties including Tehama, Glenn, Butte, Napa, Sonoma, Yolo, Solano, Sacramento, Merced, Madera, Fresno, Tulare, Santa Barbara, Ventura, and Riverside (**Fig. 1**). Field surveys conducted up to date have showed the significance of branch dieback of olive trees in California. Olive dieback was observed in all counties surveyed and in 54 out of 56 orchards visited (**Table 1**). Branch dieback of olive trees was more prominent in orchards older than 20 years-old no matter the cultivar surveyed (Manzanillo, Sevillano, Mission, Ascolano, and Barouni). Affected trees were characterized by abundant dead twigs (**Fig. 2a**) and dieback of stems and main branches (**Fig. 2b**). These cankers often extend through the main branches (**Fig. 2d**) until they reach the trunk causing dieback and eventual dead of the tree (**Fig. 2c**). Wedge-shape cankers were the most

Fig. 1. Olive-growing regions surveyed from October 2008 to September 2009.

common vascular symptom observed when affected branches were sectioned (**Fig. 2e**). However, other vascular symptoms such as dark wood streaking and/or dark brown tissue were observed in contrast to the yellowish green of healthy tissue. Cankers were also observed in young trees affecting the crown of the tree (**Fig. 2f**). Although cankers were found to develop mostly from pruning wounds done in the main branches they were also observed to develop from olive knot galls (**Fig. 2h**). Olive trees are pruned back when severe dieback affects main branches and trunks causing severe economic losses to growers (**Fig. 2g**).

Fungal identification. Results from canker isolations along with morphological studies showed species in the family Botryosphaeriaceae, Valsaceae, and Diatrypaceae to be the most predominant fungi associated with branch cankers of olive trees in California.

Table 1. Olive twig and branch dieback in California and incidence of the dominant fungal species isolated from dead twigs and branch and trunk cankers.

			Number (%) of samples yielding					
Olive region	**Orchards** [a]	**Samples** [b]	**Botryo-sphaeriaceae**	**Diatrypaceae**	**Valsaceae**	**Mixed fungi** [c]	**Basidio-mycetes**	**Others** [d]
Tehama County	3	35	7 (20%)	1 (3%)	-	7 (20%)	-	20 (57%)
Glenn County	5	48	7 (15%)	3 (6%)	-	3 (6%)	-	35 (73%)
Butte County	6	73	22 (30%)	7 (9.5%)	-	5 (7%)	-	39 (53%)
Napa County	5	59	6 (10%)	14 (24%)	10 (17%)	9 (15%)	11 (18.5%)	9 (16%)
Sonoma County	3	52	15 (29%)	13 (25%)	-	2 (4%)	8 (15.4%)	14 (27%)
Yolo County	2	28	20 (70%)	1 (3.5%)	-	3 (10%)	-	4 (14%)
Solano County	2	25	18 (72%)	2 (8%)	-	-	-	5 (20%)
Sacramento	3	43	14 (33%)	1 (2%)	-	1 (2%)	-	27 (63%)
County	5	65	24 (37%)	-	6 (9%)	2 (3%)	-	33 (51%)
Merced County	6	60	32 (53%)	-	4 (6.5%)	4 (6.5%)	-	20 (33%)
Madera County	1	10	2 (20%)	-	3 (30%)	2 (20%)	-	3 (30%)
Fresno County	4	40	22 (55%)	-	1 (2.5%)	5 (12.5%)	-	12 (30%)
Tulare County	2	23	11 (48%)	-	4 (17.4%)	2 (8.7%)	-	6 (26%)
Santa Barbara	5	58	15 (26%)	8 (13.8%)	14 (24.1%)	3 (5.1%)	-	18 (31%)
Ventura	4	64	29 (45%)	11 (17.2%)	15 (23.4%)	2 (3.1%)	-	7 (11%)
Riverside								
TOTAL	**56**	**683**	**244 (36%)**	**61 (9%)**	**57 (8.6%)**	**50 (7.6%)**	**19 (3%)**	**252 (37%)**

[a] Total number of surveyed olive orchards in this study.
[b] Total number of olive samples collected in this study.
[c] Number of samples from which Botryosphaeriaceae, Diatrypaceae, and/or Valsaceae were isolated together.
[d] In this category are included all mitosporic fungi such as species of *Aspergillus*, *Alternaria*, *Epicoccum*, *Nigrospora*, *Penicillum*, etc.

Fig. 2. Symptoms of branch dieback of olive trees in California.

Morphologically, the Botryosphaeriaceae fungal group was characterized by a dark green fast-growing mycelium on PDA-tet (**Fig. 3a,c**). With age, most of these cultures developed single or grouped, black, globose fruiting bodies (pycnidia) from which different type of spores (conidia) were identified. Two main spore types including pigmented (**Fig. 3b**) and hyaline (**Fig. 3d**) conidia were observed from the cultures studied. Based on comparison with previously identified isolates from grapevines from California, Botryosphaeriaceae fungal cultures with pigmented conidia were tentatively divided in different species including *Lasiodiplodia theobromae*, *Diplodia seriata*, *Diplodia mutila*, and *Dothiorella* species. Fungal cultures with hyaline conidia were tentatively divided in *Botryosphaeria dothidea* and at least 3 different *Neofusicoccum* species.

The Diatrypaceae fungal group was characterized by having white to white-cream flocculent slow-growing mycelium on PDA-tet (**Fig. 3e**). With age, a light-brown to dark-brown coloration developed at the centre of the colony on the reverse. Some cultures were exhibited a much slower growth with irregular and lobate colony margins. Conidia that developed from fruiting bodies in the plates were filiform and mostly curved in shape (**Fig. 3f**). These characters were consistent with the description of species in the Diatrypaceae family; however, identification to species level was not possible based only on colony and conidia morphology.

The Valsaceae fungal group was characterized by having white to light grey, slow-growing mycelium (**Fig. 3g**). Fungal colonies were slightly raised and developed prominent growth rings with margins becoming black with age. Colonies produced dark, eustromatic pycnidia over time. A mucilaginous light-cream cirrhi was observed from pycnidia (**Fig. 3h**). Cirrhi contained filiform and mostly curved. All these morphological characteristics were consistent with the description of *Phomopsis* and/or *Diaporthe* species; however, identification to species level was not possible based only on colony and conidia morphology.

Phylogenetic studies. Identification using morphology was not always possible because isolates collected in their anamorphic stage did not show enough morphological variation characters for identification to species level. Therefore, several isolates from the most prevalent fungal species were selected to conduct molecular studies. DNA analyses of the internal transcribe spacer region (ITS1-5.8S-ITS2), a partial sequence of the beta-tubulin gene (BT2), and a part of the translation elongation factor 1-α gene (EF1-α), allowed us to identify over 25 different fungi associated with perennial cankers of olive trees in California (**Table 2**).

ITS, BT2, and EF1-α sequences of Botryosphaeriaceae species from olive trees from California were aligned with GenBank ITS, BT2, and EF1-α sequences of Botryosphaeriaceae species from different hosts including the epitype sequences. After alignment, a partition homogeneity test showed a value of $P = 0.74$, indicating that ITS, BT2, and EF1-α dataset were congruent ($P>0.05$) and could be combined in a single Phylogenetic analyses. The combined dataset consisted of 1272 characters, of which 642 were constant, 331 were parsimony uninformative, and 299 were parsimony informative. ITS, BT2, and EF1-α phylogenetic analysis showed seven well-separated clades (bootstrap values > 90), which correspond with seven different Botryosphaeriaceae species including *Neofusicoccum mediterraneum*, *Neofusicoccum australe*, *Neofusicoccum vitifusiforme*, *Botryosphaeria dothidea*, *Diplodia mutila*, *Diplodia seriata*, *and Lasiodiplodia theobromae* (**Fig. 4**). *Pseudofusicoccum stromaticum* isolate CBS117448 was used as out-group in the analysis. Additionally, a separate phylogenetic analyses of the ITS and BT2 sequences of the previously identified *Dothiorella* group showed 4 putative *Dothiorella* species (*Dothiorella* sp. I, *Dothiorella* sp. II, *Dothiorella* sp. III, and *Dothiorella* sp. IV) associated with olive cankers in California. Further studies including EF1-α sequences from these species are currently underway to determine whether or not these fungi

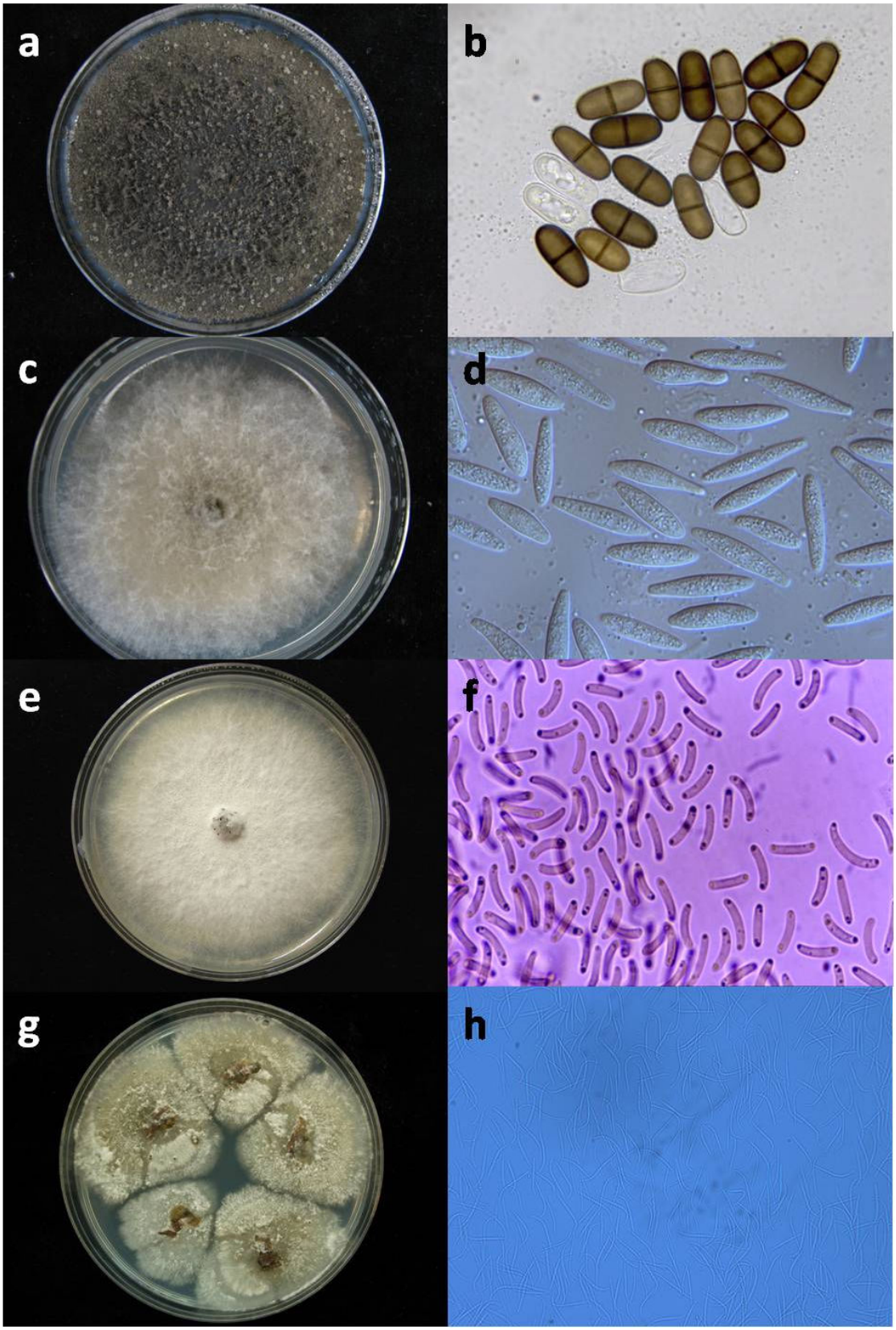

Fig. 3. Characteristic colony and spore morphological features of the most common fungi isolated from olive perennial cankers from twig and branch dieback.

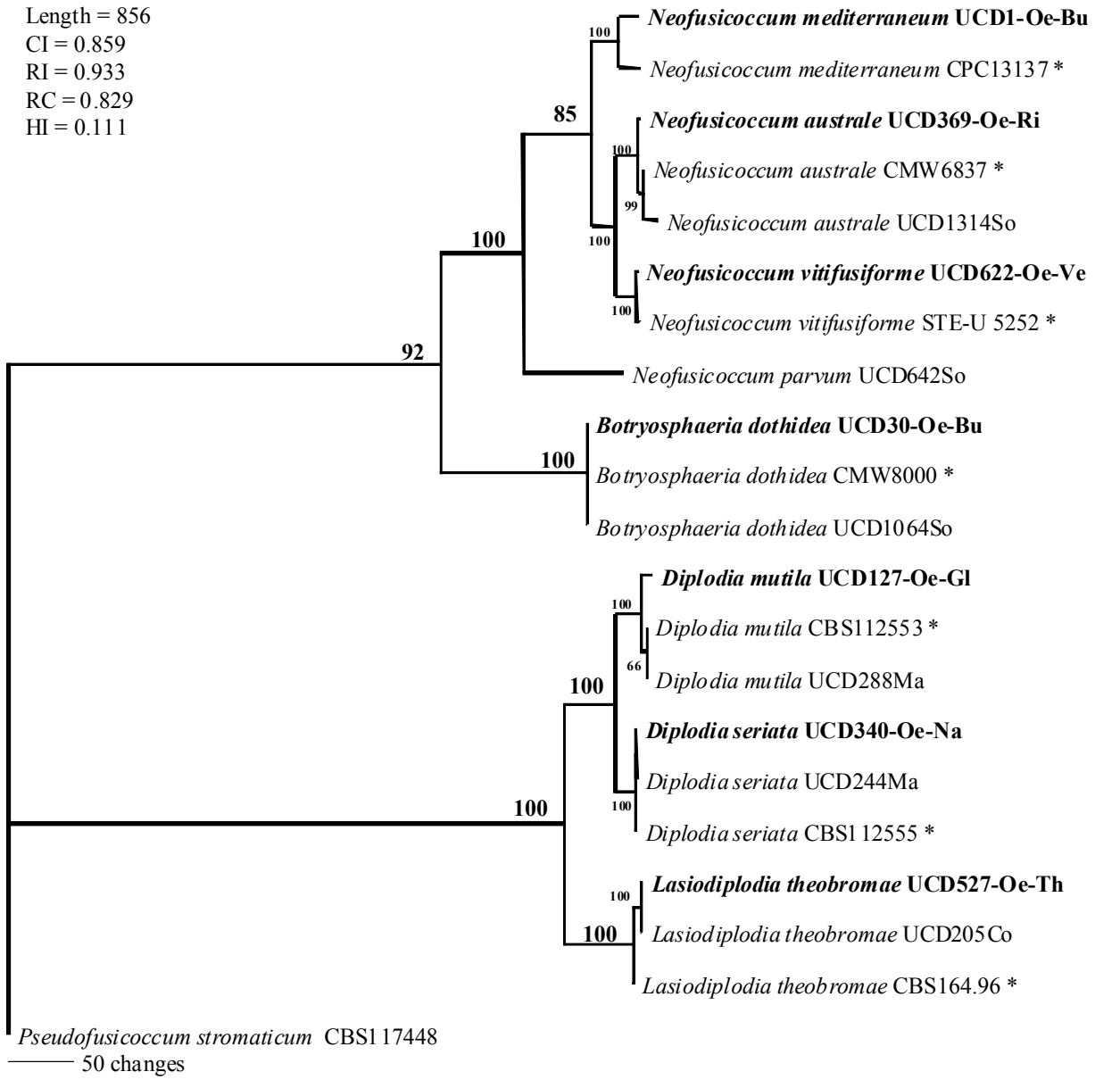

Fig. 4. One of the most parsimonious trees with bootstrap value using 1000 replicates generated in PAUP 4.0b10 from the ITS, BT2, and EF1-α region of Botryosphaeriaceae species. Bold isolates represent isolates from olive trees from California. * represents epitype isolates.

represent novel species in California (**Fig. 5**). *Pseudofusicoccum stromaticum* isolate CBS117448 was used as out-group in the analysis.

ITS and BT2 sequences of Diatrypaceae species from olive trees from California were aligned with GenBank ITS and BT2 of Diatrypaceae species from different hosts including the epitype sequences. After alignment, a partition homogeneity test showed a value of $P = 0.9$, indicating that ITS and BT2 dataset were congruent ($P>0.05$) and could be combined in a single phylogenetic analyses. The combined dataset consisted of 976 characters, of which 535 were constant, 203 were parsimony uninformative, and 238 were parsimony informative. ITS and BT2 phylogenetic analysis showed four well-separated clades (bootstrap values > 90), which corresponded with four different Diatrypaceae species including *Diatrype stigma*, *Diatrype oregonensis*, *Eutypa lata*, and a *Libertella* sp. (**Fig. 6**). *Botryosphaeria dothidea* isolate CMW8000 was used as out-group in the analysis. Further studies including EF1-α sequences from these species are currently underway to determine the identity of the *Libertella* sp.

ITS sequences of Valsaceae species from olive trees from California were aligned with GenBank ITS sequences of Valsaceae species from different hosts including the epitype sequences. The dataset consisted of 550 characters, of which 367 were constant, 95 were parsimony uninformative, and 88 were parsimony informative. ITS phylogenetic analysis showed four well-separated clades (bootstrap values > 80), which corresponded with four different Valsaceae species including *Diaporthe viticola* and three putative *Phomopsis* species (*Phomopsis* sp., *Phomopsis* sp. II, and *Phomopsis* sp. III) (**Fig. 7**). *Botryosphaeria dothidea* isolate CMW8000 was used as out-group in the analysis. Further studies including BT2 and EF1-α sequences from these species are currently underway to determine whether or not these *Phomopsis* species represent novel fungal species in California.

ITS, BT2, EF1-α showed the presence of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*, the main fungal pathogens causing Esca and Young vine decline of grapevines, associated with necrotic wood of olive in California. ITS sequence analyses also allowed us to identify two Basidiomycota species including *Trametes versicolor* and *Schizophyllum commune* associated with branch dieback of olive trees. Furthermore, ITS sequences of an unknown fungal isolate showed 96% homology with isolates previously identified as *Cytospora* sp. Further studies including BT2 and EF1-α sequences from this species are currently underway to determine whether or not *Cytospora* is the correct identification. Finally, five different mitosporic fungi were identified including *Aspergillus* sp., *Alternaria citri*, *Alternaria alternata*, *Epicoccum nigrum*, and *Nigrospora oryzae*, but these were considered to be saprophytes.

Fungal incidence and distribution. Botryosphaeriaceae species, isolated from 36% of the total of samples collected, were the most prevalent fungi isolated from perennial olive cankers and were found in all counties surveyed. In order of importance, Botryosphaeriaceae species were followed by Diatrypaceae species (9%), Valsaceae species (8.6%), and Basidiomycetes species (3%) (**Table 1**). Regarding the different Botryosphaeriaceae species identified in this study, *Neofusicoccum mediterraneum*, *Botryosphaeria dothidea*, *Diplodia mutila*, and *Dothiorella* species were the most abundant; and *Lasiodiplodia theobromae*, *Diplodia seriata*, *Neofusicoccum australe*, and *Neofusicoccum vitifusiforme* were the least abundant. Botryosphaeriaceae species were found in all olive-growing areas surveyed; however, species distribution varied throughout the State. *Dothiorella* species were the most wide spread Botryosphaeriaceae species in California and were isolated from perennial cankers in seven different counties including Butte, Yolo, Solano, Sacramento, Santa Barbara, Merced, Madera, Fresno, and Tulare. *Neofusicoccum mediterraneum* was the second most spread Botryosphaeriaceae species and was isolated from cankers in Butte, Yolo, Solano, Sacramento,

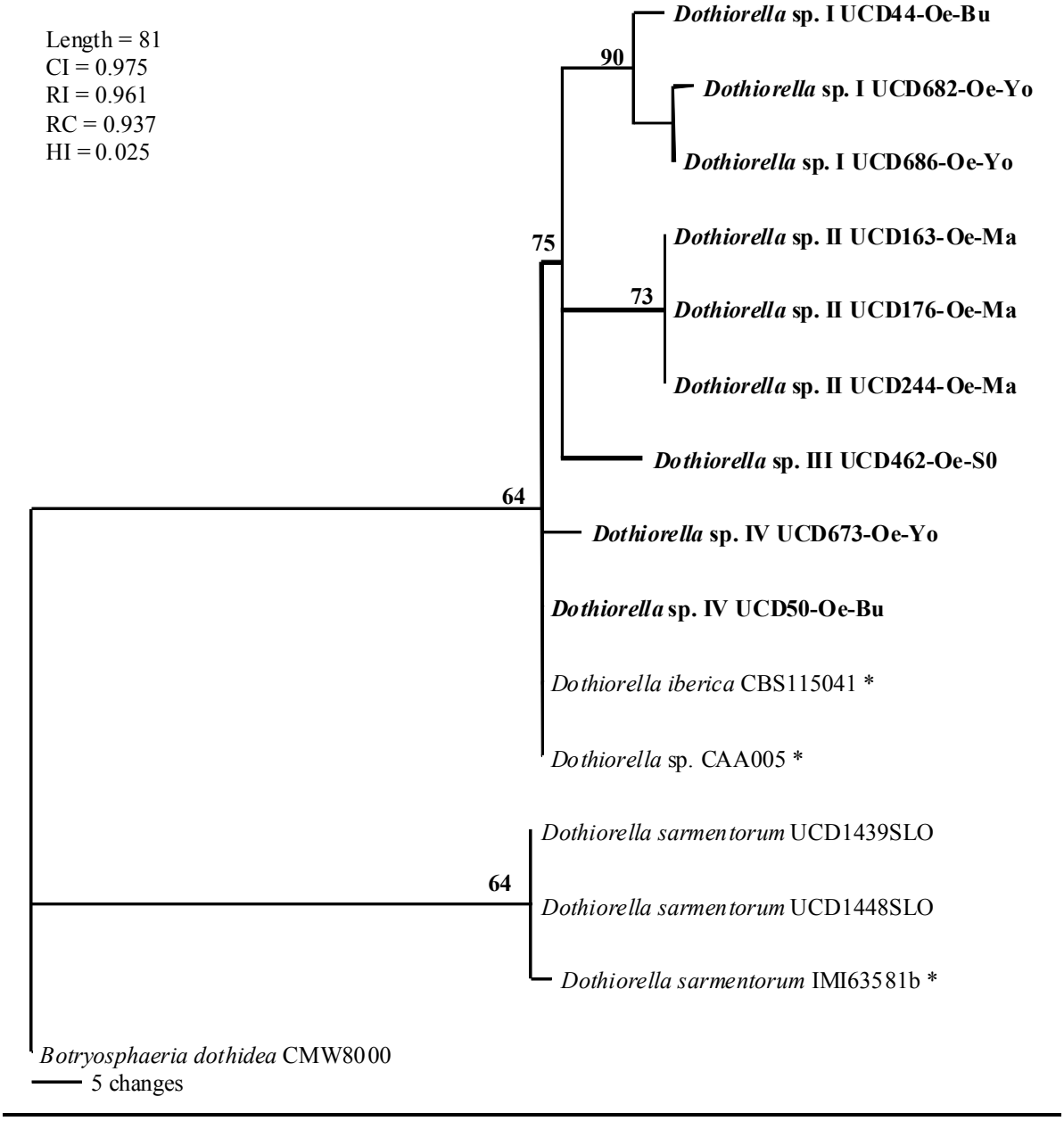

Fig. 5. One of the most parsimonious trees with bootstrap value using 1000 replicates generated in PAUP 4.0b10 from the ITS and BT2 region of *Dothiorella* species. Bold isolates represent isolates from olive trees from California. * represents epitype isolates.

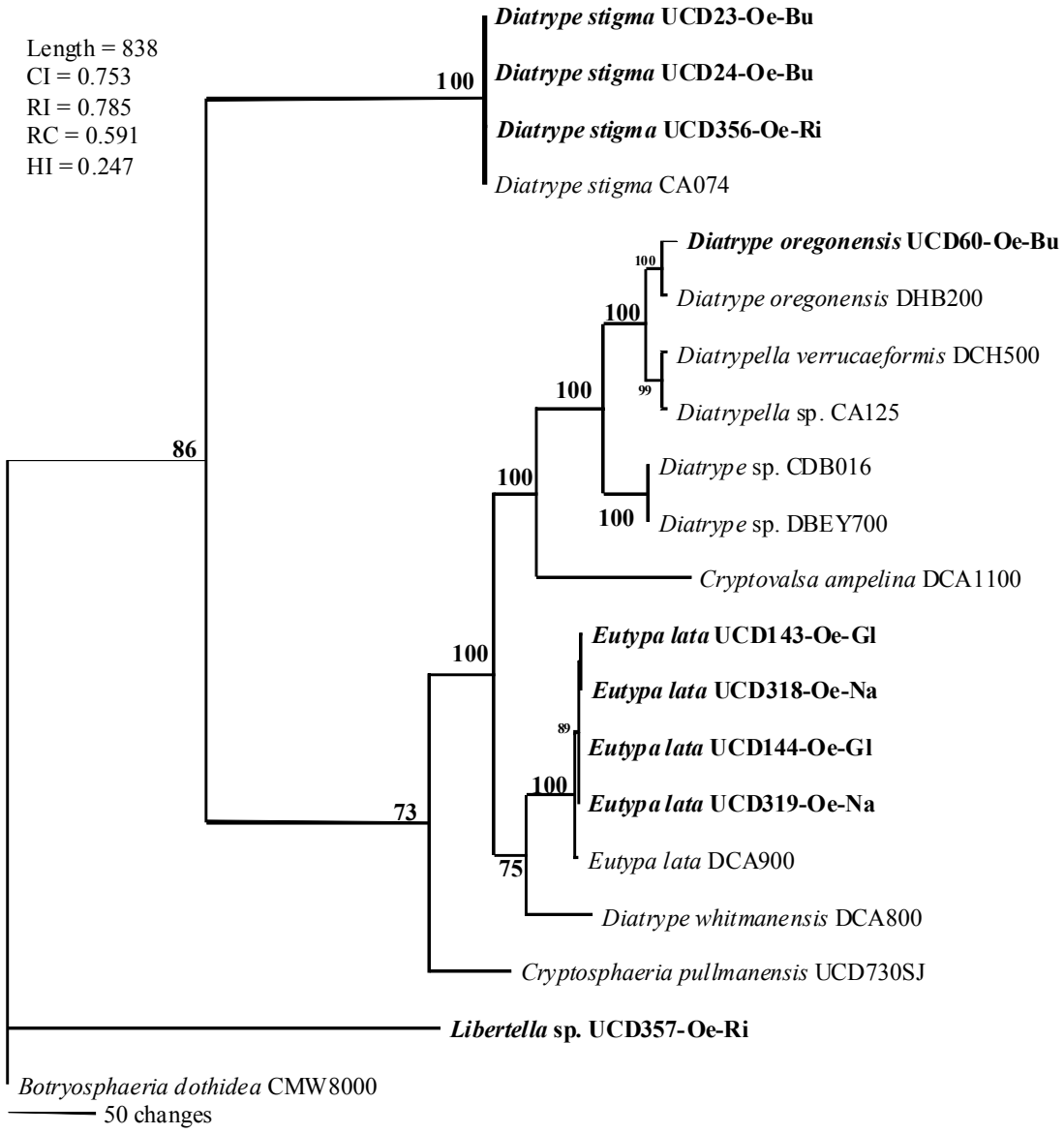

Fig. 6. One of the most parsimonious trees with bootstrap value using 1000 replicates generated in PAUP 4.0b10 from the ITS and BT2 region of Diatrypaceae species. Bold isolates represent isolates from olive trees from California.

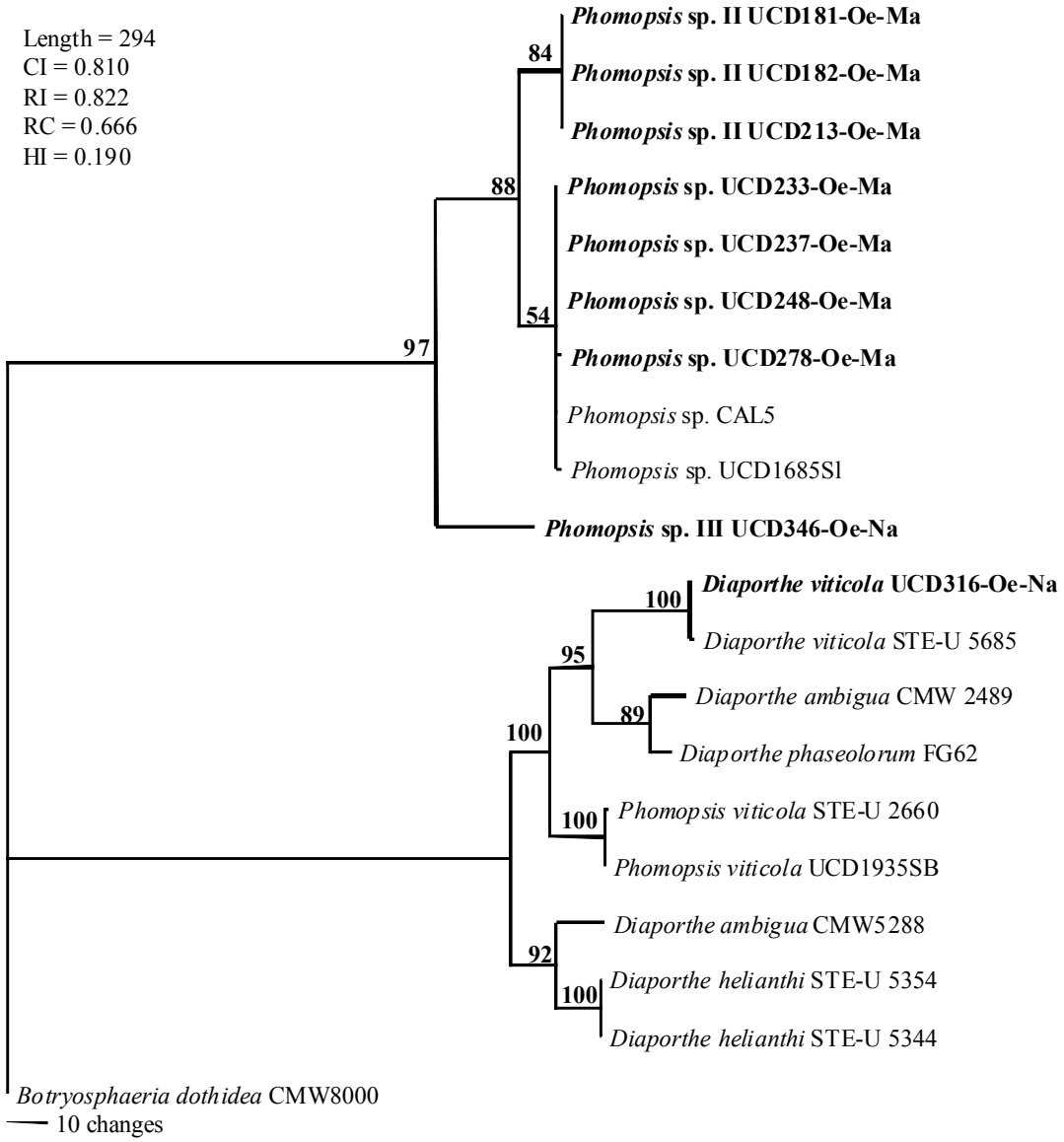

Fig. 7. One of the most parsimonious trees with bootstrap value using 1000 replicates generated in PAUP 4.0b10 from the ITS region of Valsaceae species. Bold isolates represent isolates from olive trees from California. * represents epitype isolates.

Merced, Santa Barbara, and Ventura Counties. *Diplodia mutila* was isolated in Glenn, Tehama, Napa, and Sonoma Counties. *Botryosphaeria dothidea* was isolated in Butte and Sonoma Counties, and *Neofusicoccum australe*, *Neofusicoccum vitifusiforme*, *Diplodia seriata*, and *Lasiodiplodia theobromae* were only found in Riverside, Ventura, Napa, and Tehama, respectively.

Diatrypaceae species identified in this study were mostly found in olive orchards of Butte, Glenn, and Napa Counties. However, a *Libertella* sp. was isolated in olive-orchards of Riverside County in southern California. Among all Diatrypaceae species identified, *Eutypa lata* and *Libertella* sp. were the most and least abundant, respectively.

Valsaceae species were also widely distributed throughout California olive-growing regions and *Phomopsis* species were isolated from olive cankers in Napa, Sonoma, Merced, Madera, Fresno, Tulare, Santa Barbara, Ventura, and Riverside. Finally, the Basidiomycota species *Trametes versicolor* and *Schizophyllum commune* were only isolated from olive cankers from Sonoma and Napa Counties.

Pathogenicity tests. *In vivo* pathogenicity tests are currently underway at the University of California Nickels Soil Field Station in Arbuckle, Colusa County. Olive branches from Manzanillo and Sevillano cultivars were inoculated with at least one isolate of each identified fungus isolated from olive cankers from field surveys. Ten branches were inoculated per fungal species in each cultivar. Inoculum of the potential pathogenic fungi (mycelium colonized agar plugs) was introduced in the olive stem and branches through a fresh wound. Fungal treatments will be compared to control treatments (non-colonized agar plugs). Pathogenicity will be assessed by the extent of vascular discolorations and necroses observed in inoculated wood and by the percentage of recovery of the pathogen from these necroses. Pathogenicity test results will be available by June 2010.

Objective 2

This objective will be accomplished with anticipation of continuing funding.

Objective 3

This objective will be accomplished with anticipation of continuing funding.

CONCLUSIONS

The importance of olive branch dieback has remained overlooked in California. However, research conducted in our laboratory in the past two years has showed the importance of perennial cankers and consequent dieback of olive trees in all olive-growing regions in California. Furthermore, our research has successfully demonstrated that perennial cankers of olives are complex and more than one fungal genus contributes to the dieback observed in the field. We have identified a range of new fungal pathogens of olive trees in California, including the destructive Botryosphaeriaceae and several fungi in the family Diatrypaceae, and Valsaceae families, previously unknown from California olive orchards. Consequently, we have significantly increased the understanding of olive fungal pathogens contributing to an enhanced insight into olive canker disease etiology. We are currently conducting pathogenicity tests in

order to determine the importance of all these fungi as well as which ones pose the greatest threat to olive health and survival in California. Research in our laboratory has also shown the geographical distribution of the different fungi as well as high disease pressure areas throughout the State. Additionally, we have considerably improved olive canker diseases diagnostic techniques based in our data collection, fungal repository, and DNA database collection. Until recently, removal of cankered wood (surgery) was the only available disease management for olive growers. Although effective, surgery economical costs have significantly increased in the past few years and disease control alternatives are needed. Consequently, more work is being done to better understand the biology and epidemiology of fungal pathogens causing branch dieback of olive trees, which will allow us to implement chemical, biological, and organically acceptable control methods for fungi involved in branch dieback of olive.

FUNDING SOURCE

This work was funded by the California Olive Committee

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In Preparation

Úrbez-Torres, J. R. and Gubler W. D. Identification, distribution, and pathogenicity of the fungal species causing twig and branch dieback of olive trees (*Olea europaea* L.) in California.

Development and Performance Evaluation of the Hutcheson Mechanical Olive Harvester

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Project Period: 10 February 2009 through 3 November 2009

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 off the tree
- At minimum replace the daily output of a 50 man hand crew
- Harvest olives with quality equal to or better than the hand crew

The current machine was built to wrap around one 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.

OBJECTIVES

The following improvements resolve the deficiencies found during the brief test period in October 2008:

1) Improve functions of current picking arm, both hydraulically and mechanically;
2) Improve padding and metal covering to avoid damage to falling fruit; and
3) Put to use under harvest conditions in a commercial orchard, per Louise Ferguson's trial specifications.

PROCEDURES

Objective 1

a. Relocate hydraulic cylinder mounts and joint locations to increase collapse of arm.
b. Re-do knuckle at end of arm that mounts to the picking head for greater flexibility and maneuverability into tree.
c. Replace arm swing cylinder with hydraulic motor, chain, and sprocket for increased movement around perimeter of tree.
d. Adjust hydraulic control valves for smoother and more coordinated movement of arm and picking head.

Objective 2

a. Wrap picking arm and mast mount with canvas type material that is flexible enough to not hinder movement yet tough enough to withstand constant flexing of arm and grabbing of tree branches.
b. Attach Fruit Foam materials to stationary surfaces where olives will land after being stripped from tree.

Objective 3

***Location*: Cilker Orchards**

***Experimental Design*: Twelve (12) total rows (96 trees/row) will be randomly assigned to:**

Treatment I. Hand Picking
Treatment II. Mechanical Picking

***Experimental Procedures*:**

1. In June the six trees assigned to mechanical harvest were skirted to four feet.
2. In October both sets of experimental trees were to be harvested:

- **Hand Harvest:**
- the 6 hand harvested rows were harvested by hand, and the total row weight taken.
- the bins from each lot were combined into a single lot
- the bins were graded as a single lot at the receiving station

- **Machine Harvest:**
- The 6 machine harvested rows were to be harvested and the total row weight taken.
- The time to harvest the six rows was to be recorded to determine harvest time per tree.
- the bins were to be combined into a single lot.
- the bins were to 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 equation will determine the efficiency of the mechanical harvester:

$$\frac{\textbf{Weight Machine Harvested}}{\textbf{(Weight of machine harvest + hand gleaned fruit weight)}}$$

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; and 3) the effect of machine harvest on fruit quality.

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

RESULTS

As described in the objectives above, I completed mechanical and hydraulic improvements to the picking arm, added padding to exposed metal areas, and prepared our test plot at Cilker Orchards per Louise Ferguson's specifications.

The improved picking arm allowed much better access to the perimeter of the tree as well as areas between trees. The added padding increased the safety to falling olives. In March, trees were hedged during normal pruning and in August, six rows were skirted to a height of 4 feet. This greatly improved the ability of the catching frame to surround the bottom of the tree and catch harvested fruit.

On 4, 11, and 18 October 2009, the harvester entered the orchard for trial runs. It was apparent that the picking head exhibited limitations. As the picking head entered the perimeter of the trees, the fruiting branches wrapped around the drum so tightly that they broke. In one case, a 3 inch diameter scaffold was pulled from the tree. After some modifications to the head, I tried again with the same result. The machine harvested some fruit, and the fruit appeared to be in good condition. However, the tree damage caused me to curtail further harvesting.

CONCLUSIONS

On 19 October, Louise Ferguson, Uriel Rosa, Elizabeth Fichtner, and Steve Sibbett arrived to observe. Uriel suggested running the pick head in reverse and at a different angle. This helped somewhat, but we still had a problem with branch wrapping. We discussed welding some end plates on the drum and stopping the rotation using the head in a rake motion. The other suggestion was to change from a rotating drum with 4 rows of fingers to a single rake or comb type mechanism. During all of these trials not enough fruit was harvested to make any efficiency or quality evaluations. As such, I would like to extend this project for one year to correct these new problems while using Uriel's suggestions and trying some other ideas, (i.e., shorter picking fingers and oscillating fingers in a comb configuration).

In January 2010, I will implement Uriel's suggestions and my other ideas while harvesting the Navadillo olives that we use for oil.

FUNDING SOURCES

Funds to support this project were provided by Art and Justine Hutcheson and the California Olive Committee.

Olive Fly Trapping Results for Glenn and Tehama Counties for 2009

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Reporting period: April 2009 to November 2009

ABSTRACT

Ten sites were monitored weekly from April to November in Glenn (5) and Tehama Counties using plastic McPhail traps. These results were posted weekly on the UCCE Glenn County website (http:/ceglenn.ucdavis.edu.) and made available to the California Olive Committee (COC) for further distribution. Six of the sites have been monitored since 2006 and 4 since 2008. We began catching flies on April 10th 2009. Trap catches peaked between May 22nd and June 26th. With the onset of high temperatures in early July, trap catches in all sites declined to near 0 and remained low the rest of the season. Total trap catches for the 10 sites declined in 2009 compared to 2008 going from 443 to 211. This was due mostly to a decline in catches in one orchard near Orland that was added in 2008 which went from 250 to 31. However, total trap catches in the 6 sites monitored since 2006 stabilized in 2009 after having steadily declined since 2006. Total catches in those sites were 1583 in 2006, 801 in 2007, 110 in 2008 and 135 in 2009. Orchards in the Orland area had an average of 10 flies caught per trap for the season compared to16 flies per trap per season in the Corning area. Unsprayed orchards averaged 16 flies per trap for the season compared to 11.3 for the sprayed orchards.

INTRODUCTION

Trapping to monitor Olive Fly populations in individual orchards is recommended. This will allow growers and PCAs to follow trends in their orchards and help evaluate spray program efficacy. Having an idea of area-wide population trends will help growers and PCAs interpret results from their orchards. As part of a project funded by the California Olive Committee we have been monitoring OLF population in Glenn and Tehama Counties since 2006. The program and results from 2009 are described below.

OBJECTIVES

1) Provide timely information to area growers regarding area wide olive fruit fly population trends; and

2) Begin to develop a historical perspective of olive fruit fly populations for the area.

PROCEDURES

Starting on 27 April 2009, plastic McPhail traps using Torula yeast dissolved in water as the bait were hung in olive trees. Earlier work in Glenn and Butte Counties had shown that these traps catch more flies than the commonly used yellow panel trap. Traps were placed in the same ten locations as 2008, five in Glenn County (Orland area) and five in Tehama County (Corning area). In response to a concern about the limited number of sites and to get more unsprayed sites, additional traps were placed in 4 unsprayed sites (2 in Glenn County and 2 in Tehama County) in 2008. All trapping sites remained the same in 2009. In 2009, five of the 10 sites were believed to have been unsprayed. Flies were counted and the traps were serviced weekly. The results and field observations were posted on the Glenn County UC website (http://ceglenn.ucdavis.edu) and reported to the COC for further distribution. Trapping results were reported as male and female flies for individual traps and combined and averaged by site for a graphic presentation of the data (**Fig. 1**). Trapping and reporting continued through October 30, 2009.

RESULTS AND DISCUSSION

We began catching flies on 10 April 2009 (**Fig. 1**). Trap catches peaked between 22 May 22 and 26 June. With the onset of high temperatures in early July, trap catches in all sites declined to near 0 and remained low the rest of the season. Total trap catches for the 10 sites declined in 2009 compared to 2008 going from 443 to 211 flies. This was due mostly to a decline in catches in one orchard near Orland that was added in 2008 which went from 250 to 31. However, total trap catches in the six sites monitored since 2006 stabilized in 2009 after having steadily declined since 2006. Total catches in those sites were 1,583 in 2006, 801 in 2007, 110 in 2008 and 135 in 2009. Orchards in the Orland area had an average of 10 flies caught per trap for the season compared to 16 flies per trap per season in the Corning area. Unsprayed orchards averaged 16 flies per trap for the season compared to 11.3 flies for the sprayed orchards.

Overall total trap catches were relatively low compared to previous years. It is interesting to note that the six sites monitored since 2006 which had been decreasing steadily increased slightly in 2009. The orchard which had been the “hot spot” in 2008 had an 8-fold reduction in trap catches. Similar results have been observed on two other occasions in the history of this trapping program where orchards which had been “hot spots” had major reductions in trap catches in the second year of monitoring. In the Orland area, the Glenn County Pest Management District has identified an area that had much higher trap catches during the peak catch period.

In 2010 we plan to use the same sites as 2009 to continue developing an historical perspective and will add at least one site in the identified “hot spot” in the Orland area. We will also have yellow panel sticky traps at the sites to check earlier results which indicated higher catches for the Plastic McPhail traps. We will begin posting the results by the end of March 2010.

CONCLUSIONS

As more information becomes available on the effect of environment (temperature and food and water availability) on olive fly populations, it may better explain what is observed in the

traps and allow us to use trapping information as part of the information used to determine when and if sprays are necessary.

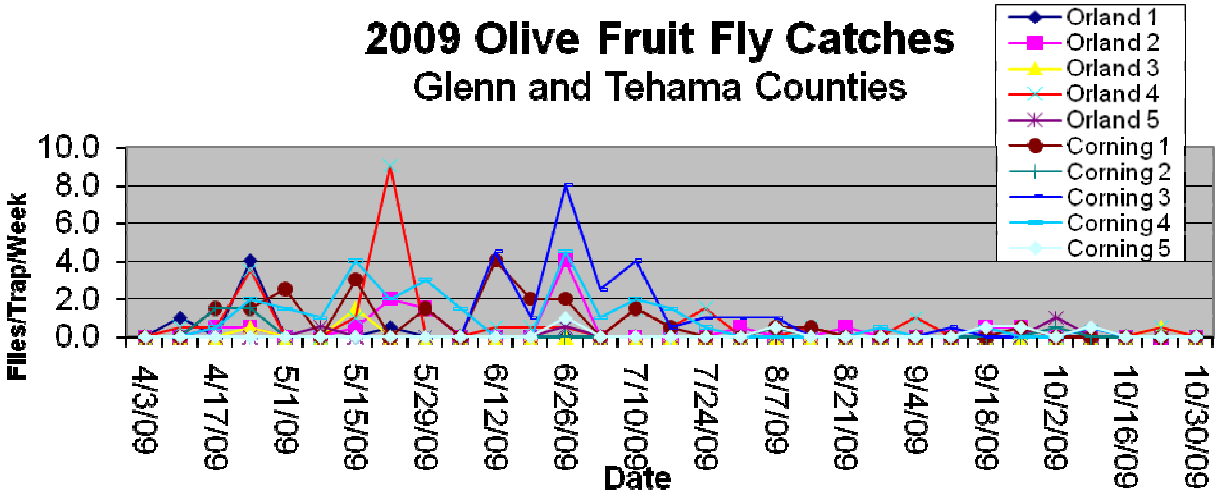

Fig. 1. Olive Fly trap catches for 2009 in selected orchards in Glenn and Tehama Counties.

FUNDING SOURCES

Funds were provided by the California Olive Committee ($5,500).

2009 Southern San Joaquin Valley Olive Fruit Fly Monitoring Project - Final Report

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Reporting Period: 1 April – 26 November 2009

INTRODUCTION

The monitoring of Olive Fruit Fly (OLFF) in commercial olive groves in the Southern San Joaquin Valley started in 2001. Initially the project started with 10 groves located in Madera, Fresno, Tulare and Kern Counties. Monitoring was accomplished by using five yellow panel traps with ammonium carbonate bait plus pheromone and five yellow panel traps with bait only per location. In order to save time and expense while maintaining a meaningful program, the locations were cut to eight locations in Tulare County and one location in Kern County in 2006. In addition, yellow panel traps were reduced to two per location and both contained the bait plus the pheromone lure. Starting in 2007, all nine locations were in Tulare County. Traps were monitored weekly and results were sent via the Internet or fax to the California Olive Committee and others that requested the information. Traps were deployed during the first week of April and ended the last week of November. OLFF is currently the most significant insect pest in commercial Olive groves. Black Scale is a pest that can be a problem in only certain years (especially years with cooler than normal late spring and early summer temperatures). Olive Scale is currently under excellent biological control. Ivy and Greedy Scales rarely require treatment in this area.

OBJECTIVES

The objective of this project was continuing the monitoring program of adult OLFF in commercial olive groves in the Southern San Joaquin Valley. Detection and seasonal monitoring of OLFF and the accurate timing of control measures, primarily bait sprays, was the goal of this project. In addition, monitoring continued to give growers information on the general OLFF population. This information was specific for only the groves being monitored and was available to growers to aid in making OLF management decisions in their respective production areas.

PROCEDURES

Nine sites in commercial olive groves were set up with traps. The locations were Ivanhoe, Woodlake, Exeter, South Exeter, Tonyville, West Lindsay, Strathmore, Porterville, and Terra Bella. All of these sites are in Tulare County where a high percentage of the commercial olives are located in the Southern San Joaquin Valley. All traps were in place by the first week of April and the program ended the last week of November. Traps were serviced and OLFF males and females counted weekly. Reports detailing the number of OLFF found at each location were submitted on a weekly basis. Two yellow panel traps with ammonium carbonate bait and male pheromone were used per block.

RESULTS AND DISCUSSION

The trap results indicated that the general population of OLFF was below last year's level and also the 8 year average (**Fig. 1**) (except for a few times mostly in the spring). From 2001 though 2006, the Bakersfield location caught the most OLFF while the Terra Bella location caught the second most OLFF (**Fig. 2**). Beginning in 2007, the Bakersfield location was not trapped due to the grove being removed; the Terra Bella location caught the most flies that year and also in 2009. In 2008, the Tonyville location caught the most flies with the Terra Bella location catching the second most numbers. The lowest numbers of flies were caught at South Exeter during 2009. In Mid June, a trap was placed in an olive tree in the City of Visalia (Many Thanks to Steve Sibbett and his assistant). The interesting finding was even though very few OLFF were trapped during the remainder of the season, infested fruit were found. As a side note, so far there has been no noticeable increase in Olive Scale populations due to the possible interference of bait treatments (GF-120) on the scale parasites in the Southern San Joaquin Valley.

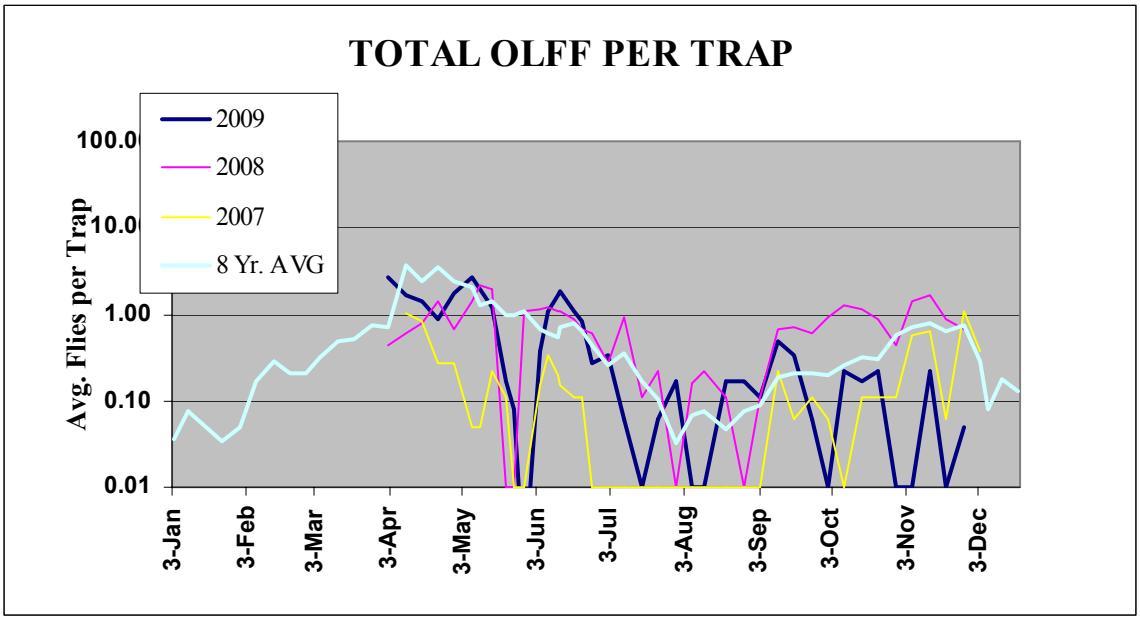

Fig. 1. Eight year average of total olive fruit flies (OLFF) caught per yellow sticky trap during the trapping season compared with totals trapped in 2007, 2008, and 2009.

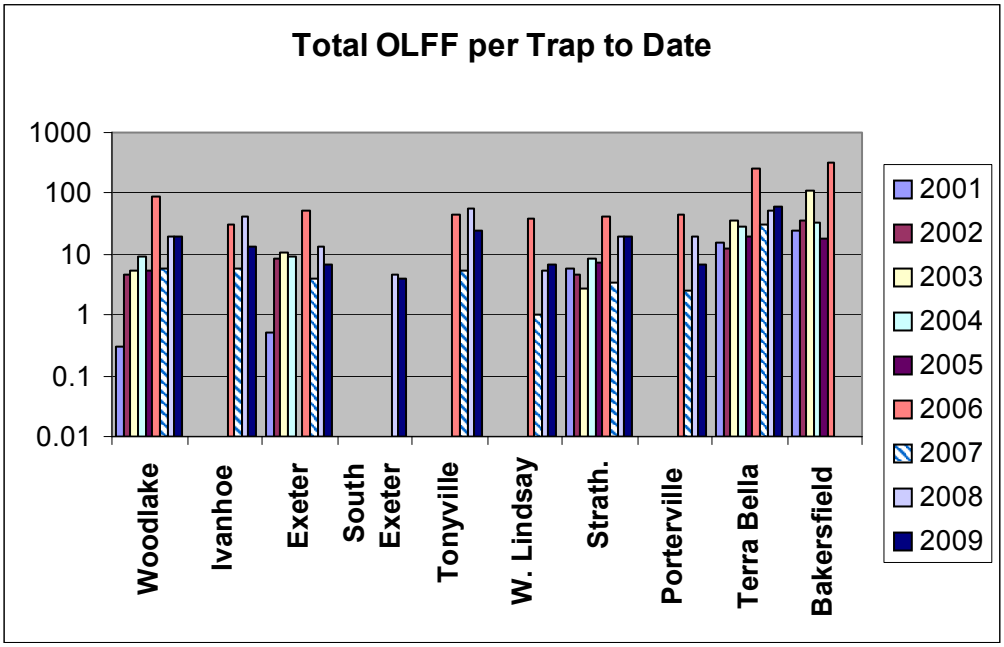

Fig. 2. Total numbers of olive fruit flies (OLFF) caught per trap (Y axis) in each survey location for the years 2001 to 2009.

FUNDING SOURCES

A total of $15,600 supported this work with one third of the total each coming from the California Olive Committee; Leffingwell Ag Sales Co., Inc.; and Ag IPM Consultants, Inc.

Development of (a) Canopy Shaking and (b) High Frequency Trunk Shaking Machines for Mechanical Harvesting of California Table Olives

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Reporting period: 10 February to 31 December 2009

ABSTRACT

Manual harvest costs for olives in California account for more than 50% of a grower's gross return. Decreasing harvest cost by implementing mechanical harvest with increased fruit removal efficiency without significantly injuring fruit is a promising way to increase the profit margin in olive production.

Three mechanical harvesting concepts were analyzed in 2009 to harvest table olives: an ENE precise control trunk shaker with innovative padding design; a Noli high frequency orbital shaker imported from Spain with kevlar padding design; and an AgRight over-the-row canopy shaker harvester equipped with padded finger rods, conveyors and catching frame.

Field tests performed with the two trunk shakers and the canopy shaker harvester have produced high quality fruit compared to hand harvested fruit, and acceptable to the processors. Through design, and extensive experimentation during the off season, we have developed padding and shaking technology to avoid bark damage.

Properly designed and implemented tree trunk and canopy shaker technologies seem to fulfill the needs of the table olive harvesting industry. However, further improvements are required because fruit removal efficiency was below 70%. An 80% removal efficiency has been calculated by Dr. Karen Klonsky, UC Davis Agricultural Economist, as the minimum requirement of the table olive industry for economical operation. Besides that, adequate tree pruning and training need to be implemented for optimum performance of the trunk and canopy shakers.

The over-the-row canopy shaker harvester was properly padded to avoid fruit injury, but was undersized for the size of the trees currently grown at Nickels ranch. Proper matching of tree dimensions and harvester is essential for the operation of this equipment. Field performance was not a constraint this year, and may be improved if tree dimensions are adequate and we continue working on machine design.

Adoption of side-by-side canopy shakers with an open center set up will avoid the current height limitation of the tested over-the-row harvester, and still maintain all desirable features of the canopy contact principle without excessively increasing machine size.

INTRODUCTION

Manual harvest costs for olives in California account for more than 50% of a grower's gross return. Decreasing harvest cost by implementing mechanical harvest with increased fruit removal efficiency without significantly injuring fruit is a promising way to increase the profit margin in olive production. Three mechanical harvesting concepts were analyzed in 2009 to harvest table olives: 1) an ENE precise control trunk shaker equipped with multi-directional Terry Tompkins head and innovative trunk padding design; 2) a Noli high frequency orbital shaker imported from Spain and unique kevlar fill trunk padding design, mounted on a ENE boom chassis;, and 3) an AgRight over-the-row canopy harvester equipped with automatic canopy following twin drums, with padded finger rods, conveyors and catching frame.

OBJECTIVES

The overall objective of this proposal was to design, fabricate, and test two trunk and one over-the-row canopy shaker based on our collaborative work and knowledge of harvesting table olives in California. This project was intended to adapt existing equipment for harvesting high quality table olives in California while reducing high labor picking costs. More specifically, the goals of this proposal were to improve, implement, and evaluate the design of the following available harvest equipment:

a) ENE multi-directional precise control trunk shaker, equipped with reprogrammable shake pattern capabilities, retrofitted with a parallel linkage Terry Tompkins shaker head and innovative fill and rug pads assembly;

b) Noli, orbital shaker head imported from Spain, equipped with a single hydraulic motor capable of running at high frequency, and retrofitted with a unique fill kevlar and rug pads design; and

c) Olivia, an AgRight over-the-row modified pomegranate harvester, equipped with automatic canopy following canopy contacting twin drums, specially padded finger rods, conveyors, hard surfaces to mitigate fruit injury, and a UC Davis GPS-based yield monitoring system to record machine performance in the field.

PROCEDURES

Field testing of the harvest equipment was performed on twelve rows of Manzanillos and one row of Sevillanos at Nickels ranch in coordination with Louise Ferguson's mechanical harvest proposal evaluations. Please refer to Louise Fergusons's report "*Developing Mechanical Harvesting for California Black Ripe Processed Table Olives: 2007-2010: Year 3/4 Progress Report*" for additional and complementary information. Here we describe our project procedures and aspects that required redesign of this equipment. Following is a description of the redesigned harvest equipment adopted during these trials.

ENE Precise control shaker. A shaker head was acquired from Terry Tompkins, Gold Country Hydraulics, and retrofitted onto an ENE trunk shaker unit. A receiver was also used on field operations to complete the catch-shake setup. After intensive engineering development and testing during the off season, an innovative computer-controlled servo system was assembled and tested at the Nickels Ranch. Among other features, this system can be reprogrammed to produce different excitations and patterns produced by the two distinct vibrating motors. Acceleration and deceleration of the motors are smooth during start and stop of the vibration. The adjustment of eccentric masses, possibility of programming and easily selecting numerous vibrating patterns, and adjusting vibrating time made this unit quite versatile.

In order to avoid bark damage, among numerous tested materials a combination of fill pads and double layer rugs produced excellent results and were adopted as the final padding system. The clamping pressure was adjusted for optimum performance and to avoid bark damage. **Fig. 1** shows the ENE precise control shaker equipped with reprogrammable shaking pattern capabilities and a fill pad and rugs system. Further investigations can be performed with the use of this computer controlled unit.

Noli shaker head. A high frequency, hydraulically controlled (not computer controlled) shaker head, manufactured by Noli industries, was imported from Spain for these trials. The shaker head was retrofitted to a boom chassis supplied by ENE. A fill pad made of our especially developed Kevlar pads created a large contact area and allowed adequate transmission of

Fig. 1. The ENE precise control shaker is equipped with reprogrammable shaking pattern capabilities (left) and a fill pad and rugs system (right; clamping on the tree).

vibration to the trees (**Fig. 2**). The kevlar pads were developed in the USA and similar versions have been instrumented and thoroughly tested in Spain by Dr. Sergio Castro. After extensive tests at ENE orchards, we added two rugs between the trunk and the fill pads, similarly used on the ENE precise control shaker pad design, to protect the delicate wet bark of Californian well-irrigated olive trees during trunk vibration. The hydraulic pressure of the clamping cylinder was limited to 800 psi for optimum performance and to avoid bark damage. Actual average contact area and bark pressure can still be evaluated during this study. Our initial tests at Nickels Ranch produced bark damage because we did not have adequate rug pads installed nor appropriate clamping pressure set on the hydraulic circuit. After proper adjustment of clamping pressure and introduction of double rugs between the fill kevlar pads and trunk the bark damage was avoided, even though we only had trees sitting on wet soils during the trials.

Olivia (AgRight). a modified pomegranate harvester, supplied by Dave Loquaci, was adapted to harvest small size table olive trees during the harvest trials at the Nickels ranch on 30 September (**Fig 3**). The size of the trees the machine would be able to harvest is presented on the diagram shown in **Fig. 4**. The height of the harvester was considered sufficient to bend "flexible" branches at the top and side of the trees without causing any damage to the tree.

The harvester required padding modifications to be table olive ready, thus avoiding excessive injury to the fruit. The shaking rod, catch frame, and other required hard surfaces were padded to reduce fruit damage. The tips of padded rods were equipped with plastic end caps to protect the rod rubber pads against mechanical failure (**Fig. 5**). Two symmetric drums are mounted side-by-side to vibrate the entire tree canopy in a single pass as the machine moves over the tree row.

Here we briefly describe the instrumentation used to measure Olivia's field performance. Our GPS-based olive yield monitoring system, developed by UC Davis (**Fig. 6**), was installed on top of the harvester to record all machine movements and later calculate machine field performance based on harvester ground speed and measured bin weights. A bin scale was not installed on the harvester for the 2009 trials. Instead, the bin weights were recorded on bins dropped on the ground by using multiple bins. Bin weight information data were also included in the analysis.

Fig. 2. A high frequency, hydraulically controlled shaker head, manufactured by Noli industries, imported from Spain, is retrofitted to a boom chassis supplied by ENE. A fill pad made of especially developed Kevlar pads created a large contact area and allowed adequate transmission of vibration to the trees (right).

Fig. 3. Overview of Olivia, an AgRight modified pomegranate harvester, belonging to Dave Loquaci, adapted to harvest small size table olive trees during the harvest trials performed at the Nickels ranch on Sep 30, 2009. **Left** - picture shows the front of the harvester before trees were pruned. **Right** - picture shows the rear of the harvester after trees were topped and selectively pruned to avoid excessive damage to the trees.

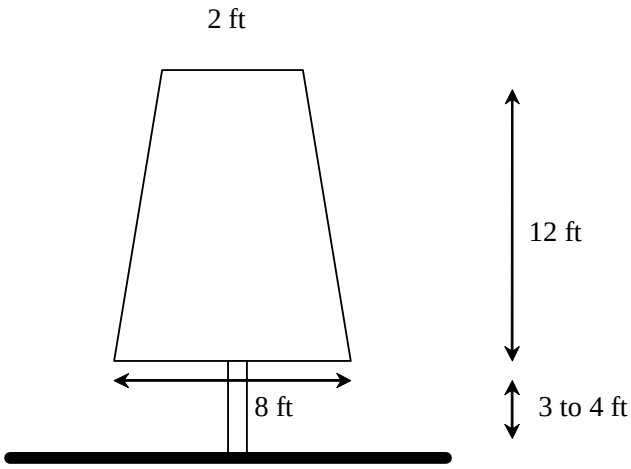

Fig. 4. Diagram of olive tree dimensions originally intended to be harvested by Olivia, the AgRight pomegranate harvester adapted to work on table olives at the Nickels ranch in the 2009 harvest season.

Harvester ground speed and location data were recorded by running the olive monitoring system continuously during the tests. GPS positioning, velocity and time information were recorded and later analyzed along with bin weight information. Instrumentation (accelerometers) used to evaluate the level of vibration and transmission of vibration from the shaker to the trunk for the Noli shaker is shown in **Fig. 7**.

Fig. 5. Top view of Olivia canopy shaker finger rods padded to reduce fruit damage and protect against mechanical failure due to finger interactions with canopy.

Fig. 6. A GPS-based olive yield monitoring system developed by UC Davis was installed on top of the AgRight harvester to record all machine movements and later calculate machine field performance. The picture on the left shows the instrumentation and on the right the GPS antenna mounted on top of the harvester.

Fig. 7. Set up shows accelerometers mounted on the tree (right), on the Noli shaker head (left) that were used to measure vibration levels produced during the olive trunk shaker tests and the transmissibility of vibration from the shaker to the trees.

RESULTS AND DISCUSSION

Samples were collected from nine Manzanillo rows to characterize the fruit (**Fig. 8**). The average fruit size and variability of nine samples collected during the tests for the Manzanillo rows was as follows: average olive weight was 5.7g with a standard deviation of 0.44g, and c.v. of 7.7% .

The results of the field tests performed for all equipment are described as follows.

ENE - Precise Control Shaker. We have developed a precise control shaker with collaborations of UCD and ENE. Extensive hours were spent on improving this design. However, we satisfactorily achieved two major goals. First, and very important, we developed a padding system that successfully avoids bark damage. This is especially important, because the trees we harvested were standing on well irrigated soils. Second, new shake patterns were programmed into the computer that controls the two unbalanced rotating masses. Suitable patterns have been tested and implemented by reprogramming the shaking vibration with a laptop computer. Developed shaking patterns are easily selected in the field. Around twelve different olive shaking patterns were created. Terry Tompkins has also collaborated with our group to help developing effective shaking patterns. Particular patterns showed the best results. As an example, Terry I and Terry II patterns were created. Terry II seems to present good results for harvesting olive trees. Additional features include easy adjustment and repeatability of total shake time, smooth start and stop of the head and combinations of patterns during the same shake period. Smooth start and stop of the vibration combined with fill pads and rugs along with use of appropriate clamping pressure helped controlling bark damage. A study needs to be performed to associate bark water content, strength, and soil irrigation status to completely reduce bark damage. Although bark damage was specifically quantified in this study, as a general result, after extensive experimentation and the implementation padding solutions were implemented on the two shaker heads, ENE and Noli (following section), bark damage was controlled. It is worth mentioning that the developed equipment required only off the shelf components and the further implementation of this technology is promising.

Results of tree gleaning and yield presented in Louise Ferguson's report indicated that this shaker running a Terry II shaker patterns produced 65% of fruit removal efficiency, 96% of cannable fruits and the adjusted value per ton was $1,147, while the hand harvested crop (i.e., 100% of harvested fruits) produced 97% cannable fruit and the adjusted value per ton was $1,179. Thus fruit quality is good for this shaker, but removal efficiency needs to be improved.

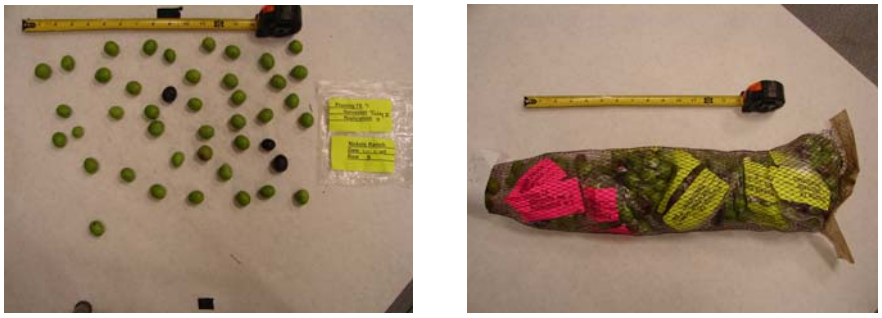

Fig. 8. Samples of Manzanillo fruits from nine rows harvested with trunk shakers at Nickels ranch during the harvest trials.

Noli Shaker. UCD and ENE visited Spain in March 2009 in a collaborative effort with Dr. Sergio Castro from the University of Cordoba. We visited Noli, a company that manufactures orbital shakers for use in olive harvesting in Spain. Following our trip we successfully imported this unit from Spain and retrofitted it onto an OMC nut harvest chassis. Fruit removal was similar for these two shakers during these trials, around 65% to 70%. Our goal would be to reach 80% efficiency for this operation to be economically feasible, according to studies reported on Louise Ferguson's report as well.

Bark damage was largely controlled with this trunk shaker even though we shook trees with a high level of bark water content. After clamping pressure was reduced to 800 psi on the hydraulic systems of the ENE boom equipment, kevlar fill pads, which provide a large contact area, and various layers of rugs were used then bark damage was controlled. Results of tree gleaning and weighing presented in Louise Ferguson's report indicated that the Noli shaker produced 70% of fruit removal efficiency, 94% of cannable fruits and the adjusted value per ton was $1147. Thus, fruit quality is good for this shaker, but removal efficiency needs to be improved.

Results of the instrumentation used to determine the level of acceleration of the trunk and the transmission from the trunk to the shaker are reported in **Table 1**.

Table 1. Results of transmission and acceleration tests performed on four instrumented Manzanillo trees and a Noli shaker at Nickels ranch on 10/7/09.

Tree number	Maximum excited frequency, Hz	Location of accelerometer	Acceleration, g, 0-peak		Vibration transmissibility	
			Parallel to boom line	Perpendi-cular to boom line	Parallel to boom line	Perpendi-cular to boom line
1	23	Shaker arm	23.7	21.7	0.7	1.2
		Tree trunk	16.1	26.4		
2	23	Shaker arm	22.6	23.8	0.5	1.0
		Tree trunk	11.0	24.9		
3	22	Shaker arm	23.0	21.5	0.5	1.2
		Tree trunk	10.5	25.5		
4	22	Shaker arm	22.6	20.8	0.6	1.2
		Tree trunk	13.1	24.4		

The transmissibility obtained on the direction perpendicular to the boom line, ranging from 1 to 1.2, seems to be adequate for proper vibration transmission from the shaker to the tree trunk. However, the transmissibility on the direction parallel to the boom should be improved for a better removal efficiency performance. The design of the padding system seems to be adequate since we have controlled bark damage. Further improvements can be made by redesigning the clamping arm systems and by maintaining a similar type of padding system used this year. Studies can be made to verify the efficiency of applying the vibration at orthogonal directions by using same shaker head.

These stationary tests show the maximum shaker frequency was below 30 Hz, because the engine was running at a maximum of 1800 rpm. During the actual shaker harvest tests the engine run at higher rpm, thus increasing the oil flow to the hydraulic motor of the shaker head and its maximum frequency. However, these tests were not performed with accelerometers while harvesting due to slow down of the operation. The engine ran at 2200 rpm, and could reach even 2500 rpm (not recommended).

The accelerometer levels reached at least by one direction of the shaker trunk should be sufficient to remove olive fruits if transmissibility to fruit location is adequate (i.e., hard wood and adequate structure is present on the shaking path from trunk to fruit). This can be subject of future studies.

AgRight – Olivia. Two rows at Nickel ranch were harvested by Olivia. The map of the harvested area is shown in **Fig. 9**. The yield monitor logged GPS data: time, latitude, longitude, ground speed and we measured average yield of every few harvested trees in separated bins (efficiency results reported by Louise's report).

Every row of the orchard had 30 trees. The Sevillanos row (row #7 in red rectangle, **Fig. 9**) was harvested from North to South. After the machine stopped and the orchard was pruned to reduce tree size allowing the harvester to run continuously to the end of the row, data to produce a field performance map was recorded (**Fig. 10)**. The average speed of the harvester was computed as 0.37 mph on this segment.

The Manzanillo row (row #6 in blue rectangle, **Fig. 9**) was sub-divided into four blocks. The harvester speed for each of these blocks is presented in **Fig. 11**. The quality of the fruit observed from this unit confirms that the canopy shaker principle is adequate to harvest good quality fruit (**Fig. 12**) when the harvester is properly padded. A recorded ground speed around 0.4 mph was much higher than compared with previous years, but can also be further increased. More tests would be necessary to obtain full field performance of this harvester. Note that the entire tree was harvested on a single pass at this speed, as opposed to previous years.

While full fruit quality analysis will be available in 2010, visual inspection shows fruit quality seems to be high, similar to last year results. Louise Fergusons's report "*Developing Mechanical Harvesting for California Black Ripe Processed Table Olives: 2007-2010: Year 3/4 Progress Report*", indicates there will be a complete analysis of fruit quality performed on samples currently being processed.

Olivia was undersized for the current size of the Nickels trees in 2009. Our recommendations for Nickels ranch are to use open canopy shake harvesters, perhaps two side by side to harvest the trees as a pair of machines harvest the same tree, or aggressively reduce tree size such as in super-high density orchards. However, this super high density solution would work better for a new orchard, probably not at Nickels at this point; other issues of using super high density would also need to be addressed.

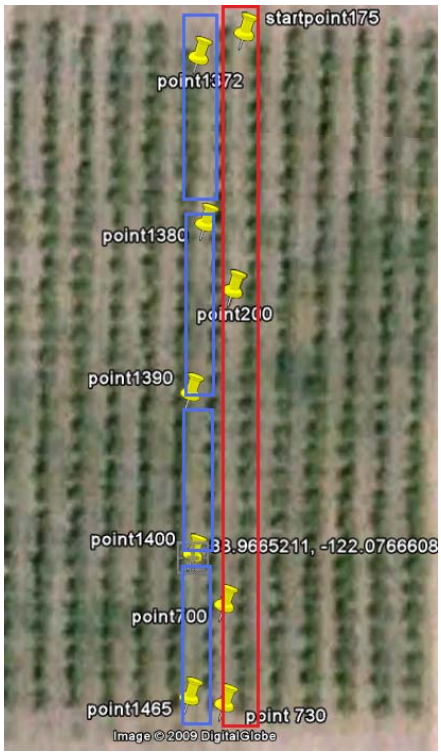

Fig. 9. This aerial map shows the first row, red rectangle, harvested first and the four partial rows, blue rectangles, subsequently harvested by Olivia on 30 September at Nickels ranch. The row marked by the red rectangle was of Sevillanos and all the others were Manzanillos.

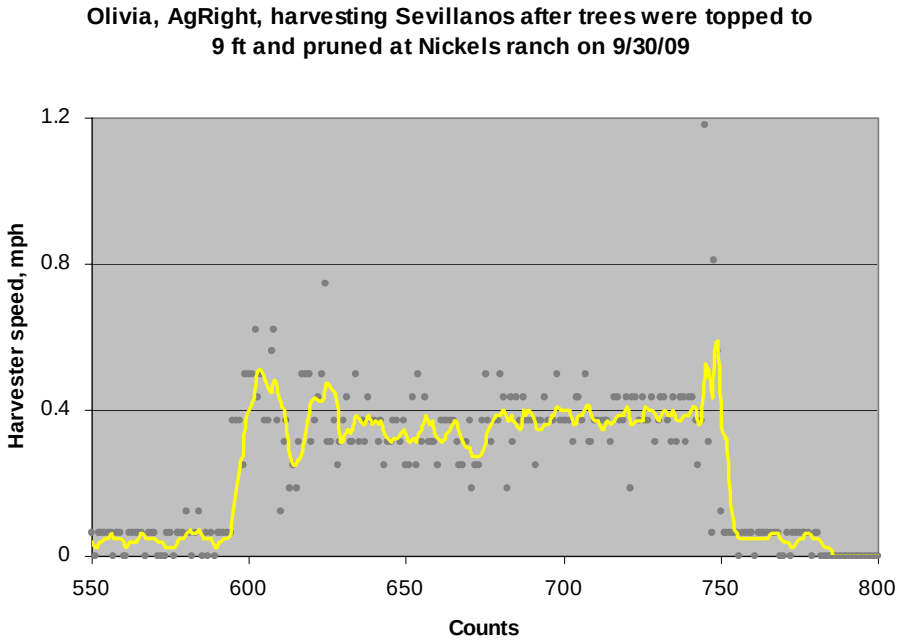

Fig. 10. Ground speed performance map of Olivia harvesting Sevillano trees after the trees were pruned, topped and hedged, to avoid tree damage.

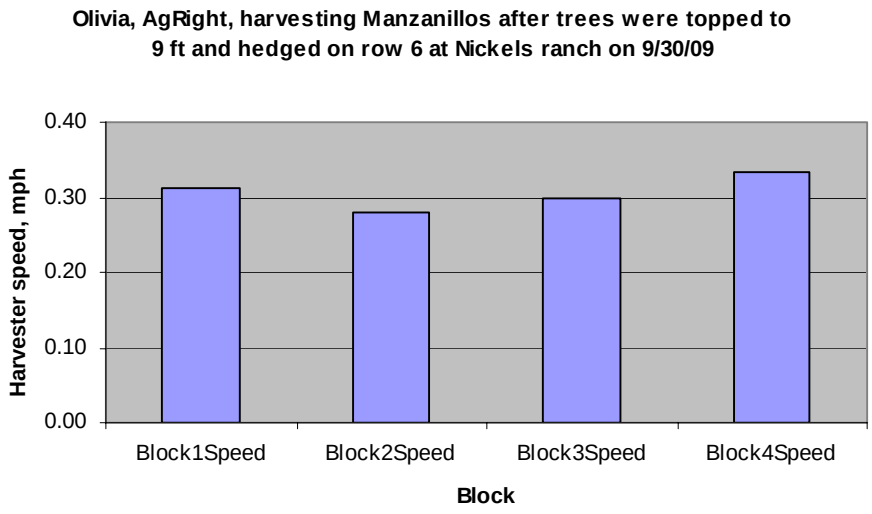

Fig. 11. Ground speed performance map for Olivia harvesting Manzanillo trees after the trees were pruned, topped and hedged, to avoid tree damage. The harvested row was divided into four blocks.

Fig. 12. Fruit harvested by Olivia seems to show high quality in agreement with results obtained in previous years by other type of canopy shaker.

Other Considerations

Trunk shakers. Adequate pruning needs to be developed for optimum performance of the trunk shakers. Quality of fruit harvested during the 2009 trials has been evaluated. Visual inspection and preliminary results indicate fruit quality is very high. Typical required shaking time may be around 15 to 20 seconds per tree. Pruning and shaker head studies can further improve fruit removal efficiency. It was also noted that trunk shakers may leave fruit on concentrated spots usually on flexible hangers outside the tree canopy which could be picked with other mechanical methods. Follow up studies could be conducted on this concept.

Rakes can be used to improve removal, similarly to how trees are harvested with trunk shakers in Europe. Unbalanced trees might create difficult to obtain full fruit removal. Balanced trees might help improve removal, thus balanced pruning may also help. Other accessories could help picking fruit left on concentrated spots of the tree, such as powered rakes or small canopy shakers. However, continuing improvements, with help of research and field testing, on trunk shaker technology might improve removal efficiency over the years.

Canopy shaker. For over-the-row harvesters, tree size has to match the maximum harvester dimensional capabilities to avoid tree damage. Although the canopy contact harvester showed average harvest speeds of 0.3 to 0.4 mph, it seems that this ground speed can be further increased. Properly pruned trees and of adequate size, such as trees of super high density orchards are required for this harvester. Otherwise tree damage would become a restriction.

CONCLUSIONS

Properly designed and implemented tree trunk and canopy shaker technologies seem to fill the needs of the table olive harvesting industry. However, further improvements are required because fruit removal efficiency was below 70%. An 80% removal efficiency seems to fulfill the minimum requirements of the table olive industry for economical operation.

We have successfully designed, implemented, and tested three harvesting concepts that can produce high quality fruit. However, adequate tree pruning and training need to be implemented for optimum performance of the trunk and canopy shakers.

Damage to tree trunk caused by trunk shakers was avoided by the development of innovative and simple padding systems implemented in different versions for both tested shakers. This is especially important because the trees we harvest are well irrigated, which creates a soft and weak tree bark.

The over-the-row canopy shaker harvester was properly padded to avoid fruit injury, but was undersized for the size of the trees currently grown at Nickels ranch. Proper matching of tree dimensions and harvester is essential for the operation of this equipment. Field performance was not a constraint this year, and may be improved if tree dimensions are adequate and we continue working on machine design.

Adoption of side-by-side canopy shakers with an open center set up will avoid the current height limitation of the tested over-the-row harvester, and still maintain all desirable features of the canopy contact principle without excessively increasing machine size.

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Continued Development of Mechanical Harvesting Technologies for Canning Olives

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ABSTRACT

This research focused on developing economical mechanical harvesting technology for table olives. Two distinctly different mechanical olive harvesters were developed. The first harvester developed by researchers at California State University, Chico, utilized pulsed air technology to remove the olives without making physical contact with the tree canopy. The second harvester, which was developed cooperatively with Bill Krueger, is a wheel rake harvester, designed specifically for narrow canopy hedgerow plantings such as have been developed at the Nickels Soils Laboratory. Preliminary field trials of the harvesters were conducted in the Fall harvest season, 2009, to determine their effectiveness and the future direction of this research. The preliminary field trials for the two machines produced promising results in terms of fruit removal efficiencies and post harvest tree and machine damage. The wheel rake harvester had a fruit removal efficiency of about 70% and the pulsed air harvester efficiency varied from 74% to 91%. The variation was largely due to the type of olives harvested.

INTRODUCTION

The California Olive Industry is faced with intense global competition from international olive producers and processors with access to cheap labor. Consequently, the industry is attempting to mechanize harvesting to reduce labor costs. Current mechanical olive harvesters suffer from several potential problems including low fruit removal efficiency, excessive fruit and tree damage, and/or poor machine reliability, which when combined with high initial and operating costs, make them uneconomical.

The research team at Chico State has developed a novel field scale olive harvester based on pulsed-air technology (see **Fig. 1**). The pulsed air harvester consists of two large centrifugal fans direct coupled to 300 horsepower diesel engines with a rotary air valve at each fan outlet. The engine speed determines the air velocity at the fan outlet while the rotary air valve determines the

Fig. 1. Pulsed-Air Harvester.

air pulse frequency. The fans pivot about their central axis which automatically directs the air pulse to different parts of the tree canopy. The operator can adjust both the intensity and frequency of the air pulse to maximize fruit removal efficiency. It has been determined that the size and shape of the nozzle at the air outlet of the rotary air valve, has a dramatic effect on harvester performance.

Previous preliminary field trials conducted at California State University, Chico (CSUC), and at the the UC Cooperative Extension test plot at the Nickels Soils Laboratory in Arbuckle, CA, demonstrated the pulsed-air harvester's potential to remove canning olives. The key feature of the pulsed-air harvester is that it does not physically contact the tree canopy. Therefore, there is minimal damage to the tree and none to the harvester during the harvesting operation. The pulsed-air harvester allows the operator to control the intensity, frequency, and duration of the air pulse directed into the tree canopy.

The other prototype harvester, as seen in **Fig. 2**, was developed in collaboration with Bill Krueger, U.C. Cooperative Extension, Glenn County, during the 2008/2009 academic year, and was also field tested during the Fall 2009 harvest season. The hedge row harvester consists of a wheel rake that can be moved with a back-hoe like arm to engage the tree canopy at differing orientations with the direction of rotation of the wheel reversible to rake either up or down the tree canopy. Research has focused primarily on developing hand and finger configurations that will maximize fruit removal while minimizing damage to the fruit, trees, and the machine. This prototype machine harvests the portion of the tree canopy that it physically contacts and multiple passes (typically three) are required to fully harvest the tree canopy. It is envisioned that multiple wheels will be necessary to completely harvest each side of the tree in a single pass. This machine physically contacts the olive canopy and was designed for narrow canopy hedgerow plantings that allow the picking head access to the fruit. The platform, which was donated to Chico State by Porter Welding of Orland, CA, allows the research team to test multiple, different picking head configurations and technologies. Many prototype hands and fingers were designed, constructed, and field tested. The final configuration consisted of six torsion spring-loaded hands mounted on a 5-foot diameter hay rake wheel with plates that utilized six crossing 0.5 inch diameter Delron™ fingers as shown in **Fig. 3**.

Fig. 2. 2008-09 Hedge Row Harvester (CSUC-UC Coop. Ext)

Fig. 3. Delron™ fingers

Both harvesters were tested in controlled field trials to determine their harvesting efficiency. This efficiency was evaluated in terms of fruit removal efficiency, machine capacity, and tree and fruit damage. This final report will focus on the results of the field trials.

OBJECTIVES

The objective of this research project was to develop economical mechanical harvesting technologies for table olives. The research team, consisting of faculty and students at CSUC, and Bill Krueger of U.C. Cooperative Extension, Glenn County, developed and field-tested two distinctly different mechanical olive harvesters. The first harvester utilizes pulsed air technology to remove the olives without making physical contact with the tree canopy. The second harvester, which was developed cooperatively with Bill Krueger, is a wheel rake harvester designed specifically for narrow hedgerow plantings. Field trials of the two harvesters were conducted during the harvest season of 2009 to determine the effectiveness of each harvester.

PROCEDURES

Although the major goal of this research project was the actual development of prototype mechanical olive harvesters, this report will focus on the procedures and results of the field trials conducted during the 2009 harvest season to establish the effectiveness of each harvester. The results of the field trials will help determine what modifications can be made to increase each harvester's effectiveness in harvesting table olives.

Field evaluation of the performance of both the pulsed air harvesting system and/or the hedgerow harvester was conducted to determine following parameters: Fruit removal efficiency; Tree and machine damage; and Economics of the harvesters.

Fruit removal efficiency was measured in two ways: 1. **Tagged fruit method**. On a minimum of three trees, a minimum of 14 fruiting branches were tagged with neon-colored zip ties in various locations throughout the tree canopy (e.g. lower, middle, and top). The number of olives on the branch past where the tag was attached was counted and recorded. After mechanical harvest, the remaining olives were counted for each tagged branch. The harvest efficiency was determined by dividing the difference between the pre- and post-harvest count by the preharvest count. 2. **Complete fruit removal method**. Mechanically harvested olives were collected by a catch frame or on tarps that were placed on the ground. The collected olives were weighed for a select number of trees to determine the weight of the fruit removed. The trees were then hand-harvested and the fruit was weighed to determine the amount of fruit left on the tree after mechanical harvesting. The fruit removal efficiency was then calculated as the weight of the machine harvested olives divided by the total sum of the collected olives (machine and hand harvested). On average, the two methods were found to give reasonably similar results.

Evaluation of fruit quality proved difficult to accomplish because, in some cases, the variety or timing was not conducive to a commercial evaluation of fruit quality. Our evaluation was confined to visual evaluation or, in one case, a fruit value grade sheet from a commercial processor was obtained. Tree and machine damage were evaluated qualitatively in the field in comparison to hand harvesting. The economics of the prototype harvesters is currently being evaluated according to standard industry procedures.

RESULTS

Field tests were conducted during the harvest season of 2009. The Wheel Rake harvester was tested at Nickels Soil Laboratory in Arbuckle on 3 October 2009 and 4 October 2009 where Manzanillas were harvested. The Pulsed Air harvester was tested at Genoa Farms in Corning on 26 October 2009 where Ascollanos were harvested and at the University Farm on 11 November 2009 on several varieties that were ripe (black) at the time of harvest.

The Wheel Rake Harvester had an average of 69.7% removal efficiency using the tagged fruit method and 56% removal efficiency using the complete fruit removal method on five trees. It is believed that difficulty in catching all of the mechanically removed fruit resulted in the lower removal percentage for the complete fruit removal method. Visual appearance of the mechanically harvested fruit appeared similar to fruit harvested at the same time with trunk shakers. COC grade sheets showed no difference in fruit value for the wheel rake harvested fruit compared to the hand harvested fruit.

The pulsed air harvester had an 85% removal efficiency on Ascolano trees in Corning, using the complete fruit removal method with three passes on a single tree. Using the tagged fruit method with a single pass on five trees, harvest efficiency was 79% after tagged shoots lost during the harvest were removed from the count. On 11 November 2009, the harvest of ripe (black) fruit at the CSUC farm, using only the complete removal method on three trees resulted in a harvest efficiency of 91% with a single pass.

CONCLUSIONS

In the absence of olive quality data, one conclusion that can be made is that the wheel rake harvester and the pulsed air harvester have demonstrated the potential for high fruit removal efficiency and warrant further development. It is believed that the efficiency of the wheel rake harvester can be improved by pruning the trees to eliminate the problematic areas of the tree that became apparent during the trial. It is also believed that harvester efficiency can be improved by continuing to work on the machine. Potential improvements would include enhancement of the ability of the picking head to laterally move in and out to conform to the tree canopy. It is important to note that approximately 500 acres of olives are being planted in Glenn and Tehama Counties patterned after the trees at Nickels so there will be orchards trained in this manner in the future.

It is believed that harvest efficiency of the pulsed air harvester could be improved by dropping the lowest point from which the pulsed air is delivered to increase air movement through the canopy. It was observed that fruit lower in the canopy was often more difficult to harvest.

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