California Olive Committee

Final and Interim Research Reports

Compiled by
Louise Ferguson and Dan Flynn
UC Liaison Officers to the COC

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UC ANR
Relevant AES/CE Project No.: Y11-893

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year: 2011 – 2012 **Duration of Project: year 1 of 2**

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Title: Epidemiology and management of olive knot disease: redefining management strategies based on pathogen population dynamics.

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Carolyn DeBuse, MS, UCCE Farm Advisor, Yolo and Solano Counties.
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2011 Objectives:

I. Update UCCE resources on management of olive knot disease based on research findings.
II. Investigate survival potential of *P. savastanoi* in gall tissue.
III. Investigate potential of *P. savastanoi* to survive in xylem.
IV. Develop techniques for assessing epiphytic populations of *P. savastanoi*.
V. Efficacy of film-forming polymers as physical barriers to infection, as well as for enhancing persistence of copper on leaf surfaces.

2011 Progress to Date (January 2012):

I. In an effort to promulgate updated information on olive knot disease, I have published a UC ANR peer-reviewed Pest Note, and have developed a website pertaining to my work on olive knot. This website can be linked from my UCCE Tulare County website, as well as from the UC Fruit and Nuts Information Center website. As data are collected and analyzed, the summarized results will be routinely published on the website. Additionally, I've given talks on olive disease management at UC Davis Olive Center Symposia, as well as at our local UCCE Tulare County Olive Day.

II. In order to generate galls of equal age for commencement of a pathogen survival study, exposed leaf scars on mature 'Manzanillo' branches were inoculated in April 2011. Five branches were flagged on each of seven trees, and 10 pairs of leaf scars were exposed on flagged branches. Leaf scars were inoculated by misting with a 10^6 cfu/ml suspension of *P. savastanoi*. The inoculum cocktail was composed of 9 isolates of the pathogen, all collected from the UC Davis Ag Experiment Station (Solano Co., CA). Symptoms were not observed until October 2011. Quantification of bacterial populations in galls will be conducted in 2012.

III. In May and November 2011, symptomatic and asymptomatic twigs were sampled from *P. savastanoi*-infected "Manzanillo" trees in Arbuckle, CA to assess in planta populations of *P. savastanoi*. A pressure bomb was used to force fluid from the xylem of 12 inch branch sections. During the active growing season (May), approximately 38-40 bar pressure was needed to extract fluid from the xylem. However, we were not able to extract xylem fluid from twig samples collected in November 2011, presumably due to reduced water transport during the winter dormant season. Xylem fluid extracted in May 2011 as diluted and plated on both Kings B and PVF media. Twigs sampled in May 2011Using a pressure bomb, fluid was extracted from the xylem of 12 inch branch sections, and the xylem fluid was then diluted and plated on both Kings B and PVF media. Fluorescent colonies were found on sap from 4 out of 10 symptomatic branches; no fluorescent colonies were found in fluid from asymptomatic branches. Putative *P. savastanoi* colonies and nine non-fluorescent colonies were subcultured and stored for identification (PCR, genetic sequencing). Only one colony of *P. savastanoi* was recovered from xylem exudates, and the positive sample was from a symptomatic twig.

IV. Two techniques were utilized to assess epiphytic populations of *P. savastanoi* on olives: 1) leaf presses on agar media, and 2) leaf washes followed by dilution plating. In May and November 2011, foliage was collected from two mature 'Manzanillo' orchards within approximately 2 miles of each other. One orchard exhibited high disease pressure, and the other is asymptomatic (Nickels Soils Lab). In January 2012, the sampling was extended over a wide geographic area, with samples collected from 12 orchards ranging from Tulare Co to Tehama Co.

For the leaf-press technique, two leaves were selected from each of 10 trees at each of two sites. Each leaf was divided in half down the midvein, with one half of the leaf pressed on KB medium and the other half on PVF medium. Additionally, both the adaxial and abaxial leaf surfaces were pressed onto each agar medium, allowing for comparison of bacterial populations between the top and bottom of the leaf. There was no significant difference in bacterial populations on the ab- and ad- axial leaf surfaces. The total epiphytic bacterial populations were significantly higher at Nickels (asymptomatic for olive knot) than in the nearby commercial orchard (symptomatic for olive knot). Fluorescent colonies were subcultured and *P. savastanoi* identity was confirmed using diagnostic PCR. One *Psv* colony was detected on a leaf press sample from the heavily infested orchard. No *Psv* has been detected to date from samples collected at Nickels Soils Lab.

The leaf wash technique was employed to assess epiphytic pathogen populations in May and November 2011 and January 2012. With the expanded sampling in January 2012, five trees were flagged in each of 12 orchards with varying levels of disease for repeated sampling over

time. The canopy of each flagged tree was divided into 4 quadrants, and 25 leaves were sampled from each quadrant for a total of 100 leaves per tree. To assess bacterial populations over equal leaf surface areas, a single 1.2 cm diameter leaf disk was cut from each leaf, and the 100 leaf disks from a given tree were constantly shaken in phosphate buffer for 2 h at 200 rpm. The resulting bacterial suspension was dilution plated onto both KB and PVF media using a spiral plater. KB medium was utilized to quantify total bacterial populations on leaf surfaces, whereas PVF was utilized to assess fluorescent Pseudomonads, and specifically *Psv*. In May 2011, one colony of Psv was recovered from leaf wash samples at the symptomatic Arbuckle site; three fluorescent Pseudomonads were collected from the Nickels Soils Lab and have yet to be identified. In November 2011, *Psv* was not detected in epiphytic populations at either site. The results from the January 2012 sampling are not in yet as the cultures are still growing out.

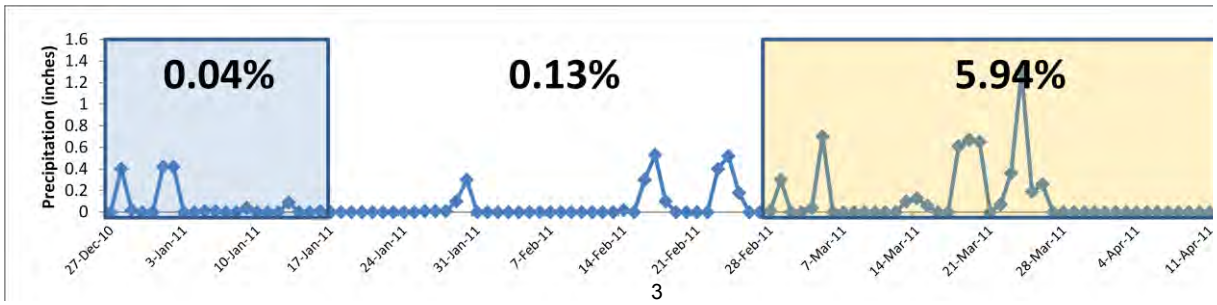
Results suggest that the leaf-press technique offers a "quick and easy" means of detecting fluorescent Pseudomonads on olive leaf surfaces; however, the leaf wash technique, though labor intensive, better allows quantification of populations. Consequently, for all future assessments of epiphyte populations we will only use the leaf wash technique.

V. Two experiments (potted plant and tagged branch/whole tree) were conducted in 2011 to elucidate the efficacy of film-forming polymers as physical barriers to infection, as well as tank additives to enhance persistence of copper on leaf surfaces. The potted plant experiment was repeated on December 23, 2011, and will proceed until November 2012.

For the potted plant experiment, olive seedlings were treated with a suite of polymer/copper treatments and exposed to natural inoculum from January through April 2011. A complete factorial treatment design comprising of two copper levels (no copper control; 4.2 g Kocide/L) and 4 polymer treatments (no polymer control; 1% VaporGard; 0.11% Nu Film P; 2.5% Anti-Stress 550) was utilized in both experiments. Spray treatments were conducted on December 24, 2010, January 16, 2011 and February 28, 2011. Twenty pairs of leaf scars were exposed prior to each spray application to test whether polymers created a physical barrier to infection and assess when leaf scars are most susceptible to infection. After each spray application, foliage was sampled to determine the influence of polymers on Cu persistence. Climatologic data (precipitation, temperature, solar radiation) was collected throughout the experiment.

On potted olives, disease incidence and severity were assessed in November 2011. The total number of galls formed per tree was assessed, as well as the number of infections of leaf scars exposed on Dec 24, Jan 16, and Feb 28. The majority of infections of leaf scars occurred during the spring rain events (>5%), whereas, less than 1% of leaf scars were infected between Dec 24, 2010 and Feb 28, 2011 (Fig 1).

Fig 1. Percent leaf scars infected during three time intervals over the winter 2010/2011 season. The majority of infections occurred in March, during spring rain events.



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Preliminary results demonstrate that Anti-Stress 550 may enhance Cu persistence on the leaf surface when applied prior to several consecutive days of incoming solar radiation. This polymer, however, did not enhance copper persistence if applied within 48 h of a rain event. Neither NuFilm P nor VaporGard had an influence on copper persistence, regardless of climatologic conditions surrounding application. Additionally, only Cu had a significant effect on disease incidence ($P \leq 0.01$) (Figure 2).

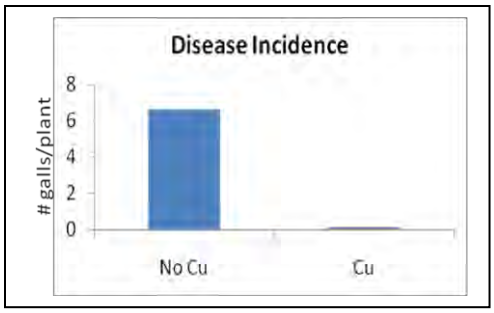
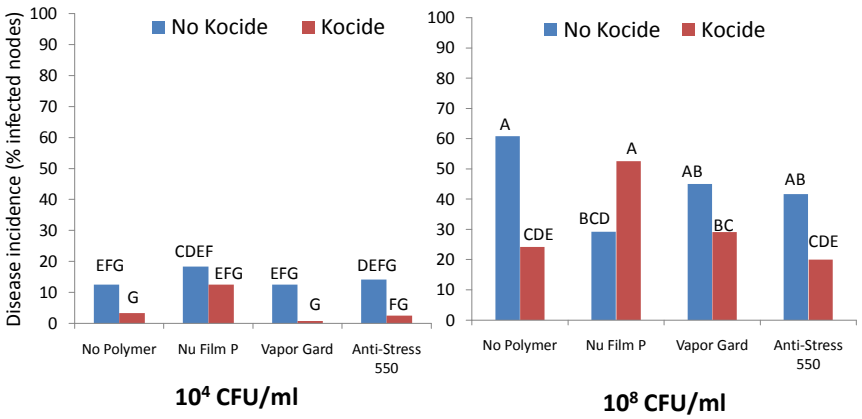


Fig 2. Disease incidence in potted plants was influenced by presence of Cu, but not by polymer coatings.

In the second experiment, initiated in April 2011, exposed leaf scars on mature 'Manzanillo' trees were treated with the same compliment of polymer/copper treatments and then artificially inoculated with *P. savastanoi*. In April 2012, this experiment will be repeated, but an additional polymer treatment 'WiltPruf' will be included in the study. A randomized complete block design was utilized, with each of 12 trees serving as a block. Treatments were therefore "nested" on individual trees. On each tree, treatments were challenged with two inoculum levels of *P. savastanoi*, 10^4 and 10^8 cfu/ml. Disease incidence and severity ratings were completed in October 2011. Disease incidence was influenced by an interaction of polymer, presence of Cu, and inoculum level ($P \leq 0.045$) (Fig 3). In general, higher levels of disease severity were observed at the higher inoculum level and copper significantly reduced disease incidence. None of the three polymers protected leaf scars from infection at either inoculum level, nor did any polymers enhance the efficacy of Cu in reducing disease incidence. Interestingly, there is no clear influence of Cu, polymer, or inoculum level on disease severity. In general, this suggests that the size of the developing gall is not related to the amount of inoculum entering the tree.

Fig 3. Influence of Cu, Polymer, and inoculum level on disease incidence on potted plants.



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University of California
Division of Agricultural Sciences

FINAL RESEARCH REPORT: 2011 CROP YEAR

Project Year: 2011 – 2012 **Duration of Project: year 1 of 1**

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Title: Developing Mechanical Harvesting for Black Ripe Table Olives

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Summary of 2011 Original Objectives:

I. Improve (based on 2010 results) 2 methods of olive harvesting technology, canopy contact and trunk shaking, and evaluate at harvest in hand pruned and mechanically pruned orchards in two locations:
    a. Canopy contact harvester in a medium density (139 trees/acres) hedgerow orchard (Rocky Hill Ranch, Tulare County CA) and high-density (203 trees acre) hedgerow orchard (Nickels soils Laboratory, Colusa County, CA).
        i. Mechanically hedged (6 feet from the trunk on west side) a medium density (139 trees/acre) hedgerow orchard (Rocky Hill Ranch, Tulare County CA)
    b. Trunk shaking harvester in a high-density orchard (202 trees/acre) hedgerow orchard (Nickels soils Laboratory, Colusa County, CA).
        i. Mechanically topped at 12 feet and hedged 2 feet from trunk on one

side a medium density orchard (202 trees/acre) hedgerow orchard (Nickels soils Laboratory, Colusa County, CA).

II. Evaluated Effects of mechanical pruning on yield, quality and values of 'Manzanillo' olives at locations.
   a. Mechanically hedged (6 feet from the trunk on west side) a medium density (139 trees/acre) hedgerow orchard (Rocky Hill Ranch, Tulare County CA)
   b. Mechanically topped at 12 feet and hedged 2 feet from trunk on one side a medium density orchard (202 trees/acre) hedgerow orchard (Nickels soils Laboratory, Colusa County, CA).

III. Visit the Israeli and Spanish mechanical harvesting research and industry programs.

Summary of 2011 Results:

Due to 2011 being the third lowest crop since 1984 and the breakdown of the canopy contact harvester at the 139 tree/acre orchard (Tulare, CA) the 2011 mechanical harvesting research results are limited.

- The trunk-shaking harvester was not tested due to low crop.
- The canopy contact harvester was tested but due to a breakdown (in the 139 trees/acre orchard) and low crop (202 trees/acre orchard) analyzed results are extremely limited. However, some results are significant.
  - The canopy contact harvester had a 48% final efficiency in the partial row of the 139-tree/acre orchard before it broke down. These trees had a 0.98 ton/acre crop.
  - The canopy contact harvester had an average 83.8% final efficiency in a limited trial (1 replications of 4 trees) in a mechanically pruned hedgerow (202 tree/acre). These trees had a 0.98 ton/acre crop. With the corresponding hand pruned control the canopy contact harvester had 23.9% final efficiency with a 2.41ton/acre crop. This is very limited data but indicates what the potential efficiency of the canopy contact harvester in properly prepared trees.
  - A visual analysis demonstrated the unharvested fruit in the top 10% of the trees, particularly between the rounded edges of two trees in a row was the hardest to remove because it was not accessed. This indicates better removal will be achieved with properly prepared canopies, specifically hedgerows.
  - The canopy contact harvester preferentially removed larger fruit
  - When the relationship between fruit removal force and fruit size was analyzed by regression analysis there was no relationship. Thus the larger fruit was not removed because it was had a lower fruit removal force but because it was larger and heavier. This strongly suggests the controlling crop load with chemical thinning and crop accessibility by pruning is necessary for successful mechanical harvesting.
  - A rating of damage in the mechanically harvested trees found less than 1% broken. If no major branches were broken a hand-harvested tree could not be visually distinguished from a hand-harvested tree.

- Definite improvements for the canopy contact harvester were defined by the engineer/operator, Dr. John Miles of Biological and Agricultural Engineering.
    - In both the moderate hedgerow orchard (13' X 26' @ 139 trees/acre) and the medium density (12' X 18' @ 202 trees/acre) orchard olives were removed if contacted by the head using 300 RPM and a 5-inch stroke. A minute per tree, 30 seconds per side, was required to cover a tree.
    - Removal was better in topped and hedged trees.
    - In both orchards a larger, 6 foot, and repositioned (on the arm) head would reach more fruit and not break as many branches through damage was minimal and confined to the few 45 – 90* angle branches we did not remove (< 1%) before harvest.
    - A shorter, repositioned, telescoping arm to hold the head would allow better visibility for the operator.
    - The visual rating of removal demonstrated the highest olives, particularly those high and between two tree canopies within the row were the most difficult to remove, or if removed, the required force resulted in their flying over the catch frame. This strongly suggests lowering the canopies by topping trees to a lower height will increase harvester efficiency.
    - A true catch frame, with coverage on both sides and trunk closure is required to get good data.
    - Dr. Miles estimates a commercial canopy contact harvester mounted on a Bobcat with track tread would cost about 50,000.00. However, a catch frame needs to be developed
- The mechanical hedging and topping trials demonstrated the ability to produce an equal or better crop than hand pruned trees and significantly decrease alternate bearing, and increase mechanical harvester efficiency.
    - A 13' x 26' (139 trees/acres) spaced orchard mechanically topped and hedged annually since 2008 yielded a total 16.11 tons and annual 4.03 tons/acre average versus the cumulative 13.3 tons and annual 3.3 tons/acre average produced by the hand pruned control. This is a total of 3.1 tons per acre more over 4 years and average annual 0.73 tons per acre more than the hand pruned control. This strongly suggests that in addition to developing a better tree for mechanical harvesting and produce a crop equal tot hat of hand pruned trees mechanical topping and hedging has the ability to control alternate bearing. In 2010 trials the mechanically pruned trees harvested with an 8.0% significantly better (than hand pruned trees) efficiency.
- In depth visits to the Israeli and Spanish mechanical harvesting research programs demonstrated that neither has any abscission compounds that are not ethylene releasing compounds with/without monopotassium phosphate. Dr. Arnon Dag of Rehovet Institute, Tel Aviv will share his 2011 analyzed results when completed
- In Israel they are evaluating the same trunk shaking harvesters we have, often without catch frames, generally onto tarps.
- Dr. Sergio Castro Garcia of the Rural engineering Department of the University of Cordoba has a large program evaluating eleven different trunk-shaking harvesters

and an Oxbo canopy contact shaker for which he has developed a catch frame. I will be observing the Oxbo on February 13-15, 2012. All their data is information is available to us. And we are both cooperators on one another's research trials.

- The industries in Israel and Spain are investigating immediately post harvest in field liquid treatments to delay bruising on green processed olives.

2011 Experiments: Materials and Methods, Results and Conclusions

I. Evaluation of Canopy Contact Harvester in Two Locations

This was third lowest crop in the olive industry's history. Due to this and harvester equipment failures there were significant modifications in the planned experiments at both experimental plots.

1. The canopy contact harvester was tested:
    a. On a partial single row in the mechanically pruned 139/tree per acre hedgerow orchard (equipment failure).
    b. On a limited sample in the medium density 202/tree per acre hedgerow orchard (lack of crop).
2. The trunk-shaking harvester was not tested in any orchard (lack of crop).

I. Harvesters tested:

a. Canopy Contact Harvester Evaluations: Mechanically Pruned Hedgerow (139/trees per acre).

Materials and Methods:

Rocky Hill Ranch, Exeter, Tulare County: Manager, Mark Pascoe
Block 17 W
17-year-old 'Manzanillo' orchard with 'Sevillano' pollinators (irregularly placed)
13, 83 tree, rows spaced at 13' X 26' (139 trees per acre)

Pruning Treatments: June 6, 2011

Split plot with 6 hand-pruned and 6 mechanically pruned rows (replications)
Treatments: (Laux Pruning)

I. Mechanical hedged 6' from trunk on the western row side and skirted 4' from ground.
II. Hand pruned control; 6 eastern rows. All tree skirted 4' from ground (for harvester catch frame).
III. Crop was not thinned with NAA.

Data Collection: October 6 -7, 2011.

1) Fruit removal force and individual fruit weight was determined on 100 individual fruits/row.
2) Hand harvested 10 trees for hand harvest fruit quality controls from all 12 rows.
3) Mechanically harvested first border row and 49 of 73 trees in 1st of 6 mechanically pruned rows (then harvester broke down).

4) Balance of all rows was hand harvested to determine the effects of the mechanical pruning treatments.
5) Fruit removal force was determined on 100 olives in the single partial row that was mechanically harvested
6) In the single mechanically harvested partial row the location in the tree of branches with significant remaining fruit was mapped.
7) The partial row that was mechanically harvested was hand-gleaned.
8) Bins from hand harvest control rows, single machine harvested row and its hand gleaning harvest were:
   a) Field weighed and a representative 20" sample removed from each for Bell Carter.
   b) Bins delivered and reweighed at Musco Exeter receiving station.
   c) Received a COC grade and adjusted value per ton at both Musco and Bell receiving.

Results:

a. Evaluation of Canopy Contact Harvester

(139/trees per acre mechanically pruned hedgerow orchard)

The canopy contact harvester broke down 49 trees into the first 83 tree mechanically pruned row which was carrying an 8.04 tons/acre crop. As a result there are no statistics on final removal efficiency, or fruit quality for the canopy contact mechanical harvester in this orchard. However, the calculated final efficiency for this limited sample was 48%. This was probably a result of the harvester functioning poorly before it broke down.

Fruit removal force was done on this single row before and after mechanical harvesting. The data is given in Table 1 and Fig.1 below. Fruit remaining past mechanical harvesting had a higher fruit removal force. However, the data, due to lack of replication is not analyzable and only serves as an indicator.

Table 2 and Fig. 2 give the range of fruit removal forces in the 6 hand pruned and mechanically pruned rows. Interestingly, the mechanically hedged and topped trees had a significantly lower fruit removal force. This is an interesting result because the hand-pruned trees had a significantly lower crop load with significantly more extra large fruit.

The mechanically pruned trees had a significantly higher crop load and significantly higher percentages of large, medium and small fruit, Tables 3 and 4. It is very unfortunate the harvester broke down, as this would have been a good test of whether fruit removal force or fruit size is a greater factor in final mechanical harvesting efficiency.

Table 5 demonstrates that even though there was no significant difference in the adjusted price per ton, there was a significant increase in adjusted total crop value because the mechanically pruned trees had significantly higher yields.

Visual evaluations of the trees after mechanical harvesting demonstrated the fruit hardest to remove was high I the canopy and between trees in the tree row. This strongly suggests a hedgerow canopy would harvest more efficiently. A result strongly supported by our

2010 trials in which the mechanically pruned and mechanically trees in this orchard had a final 8% higher harvest efficiency than the mechanically harvested hand pruned trees.

Finally, the mechanically harvested trees had less than 1% of the trees with branch breakage.

Fruit Removal Force Before and After Mechanical Harvesting

When FRF Measured	*Mean*	*Std. Dev.*	*Std Err*	*Minimum*	*Maximum*
Before machine harvest	555	114.1	20.8	330	771.0
After machine harvest	690	152.0	27.8	371	1021

Table 1. This table shows a comparison of fruit removal force (g force) before and after machine harvest of mechanically pruned treatment (single replicate row = 2), and therefore no statistical analysis.

Effect of Mechanical Harvesting on Fruit Removal Force

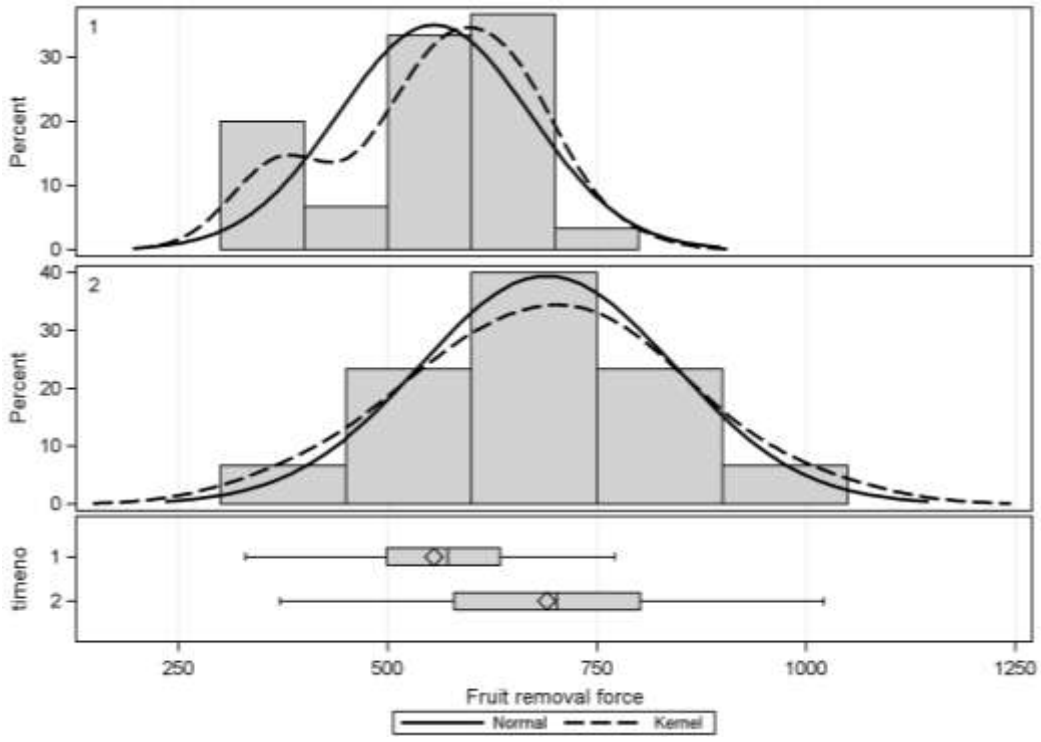


Fig. 1. This figure gives the distribution of fruit removal force (FRF) (g force) before (top of graph) and after (bottom of graph) machine harvest of mechanically pruned treatment (single replicate row = 2). The variances of the 2 populations are unequal and that of the 'before MH' group illustrates 2 population extremes; a group of fruit that had a much lower FRF than the rest of the population subsampled. This data was not replicated and as a result no analysis for significance can be done

Fruit Removal Force: by Row Replicate.

Rocky Hill Pruning trial: Fruit removal force, by replicate row, before machine harvest, 2011		
rep	*Pruning treatment*	Fruit removal force (g/cm^2)
1	*Hand pruned*	687
	Hedge/Top	555
2	*Hand pruned*	696
	Hedge/Top	645
3	*Hand pruned*	712
	Hedge/Top	574
4	*Hand pruned*	685
	Hedge/Top	602
5	*Hand pruned*	776
	Hedge/Top	676
6	*Hand pruned*	672
	Hedge/Top	708
Hand pruned		705 a***
Hedge and Topped		638 b

Table 2. This table shows the fruit removal force by row replicate before mechanical harvesting to demonstrate the range of variability in the replicates. It also demonstrates the mechanically pruned rows had significantly higher fruit removal forces. Unfortunately, due to the harvester breaking down the after harvesting fruit removal force was not obtained on this set of replicated treatments.. The means separation was by a Mixed Model regression, with 'pruning treatment' as a fixed effect and 'replicate row' as a random effect.

Row Replicates of Fruit Removal Force

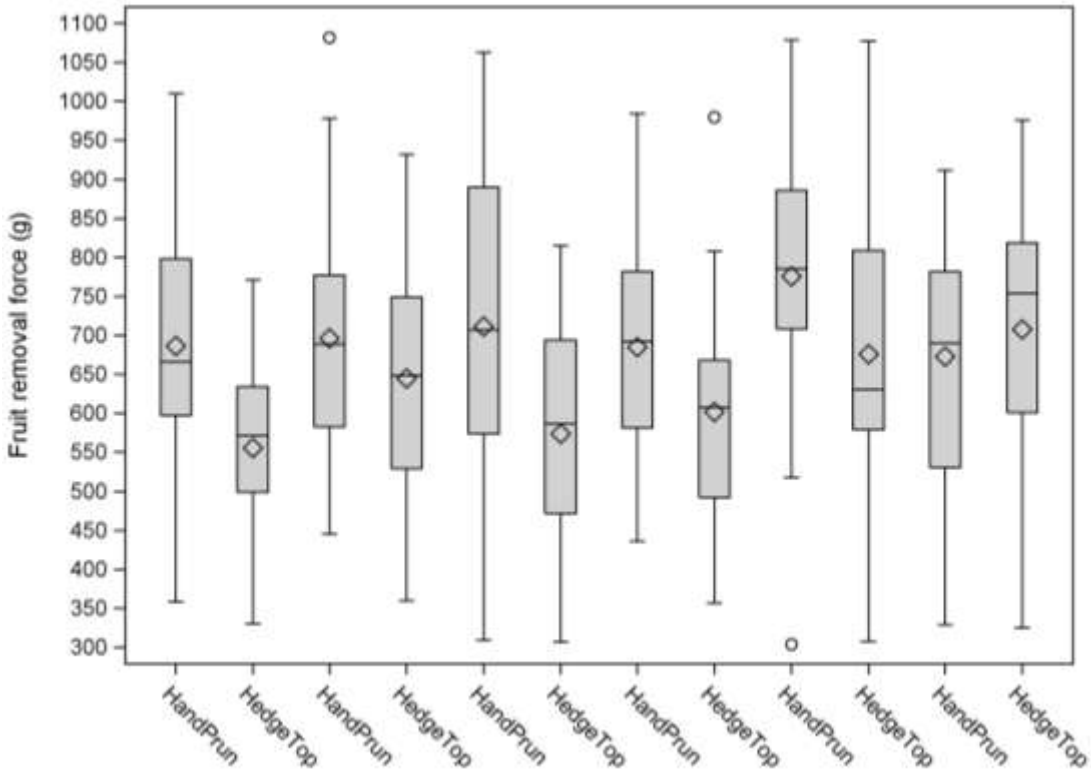


Fig. 2. Boxplot distribution of fruit removal force by treatment and replicate row prior to machine harvest demonstrates the range of variability in fruit removal force. The circles represent outliners. It also demonstrates the mechanical pruning had no effect on fruit removal force even though it did have a significant effect on the distribution of fruit sizes. Further supporting the finding that fruit removal efficiency is more a function of fruit weight than it is of fruit removal force

Effects of Mechanical Pruning on Yield: 2011

Pruning method	*Yield (lb./tree)*		
	First 10 trees	*Remainder of row (73 trees)*	*83 tree-row*
Hand-pruned	47.4 b[x]	38.7 b	39.8 b
Machine-pruned	117.8 a**	102.8 a**	104.6 a**
[x]Significant differences between means within a column, 5% level. Significance within columns by LS Means = ***,*,*, NS (0.001, 0.01, 0.05, respectively).			

Table 3. This table demonstrates this fourth year of mechanical pruning significantly increased yield in the mechanically pruned trees.

Effect of Pruning Method on Fruit Size: Processor A

Pruning method	*Percentage of sample*								
	Extra large	*Large*	*Medium*	*Small*	*Petite*	*Sub-petite*	*Undersized*	*Cull*	*Trash*
Hand	59.4 A[x]	27.4 B	6.0 B	2.1 B	1.1 B	0.8 B	0.8	4.0 B	0.9
Machine	31.8 B	41.7 A	19.2 A	8.2 A	4.4 A	2.1 A	1.3	1.9 A	1.5
Significance of pruning method	***	***	***	***	**	*	NS	*	*
Significance of replicate	NS	NS	NS	NS	NS	NS	NS	NS	*
[x]*Significant difference between means by T-test, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).*									

Table 4. This table demonstrates the significant differences in fruit size between the mechanically pruned and hand pruned control trees. The higher percentage of Extra Large fruit in the hand-pruned samples is probably due to much lighter yield. The larger percentages of fruit in 'Large' and 'Medium' grades for the significantly more heavily cropped machine-pruned samples are probably due to a shift of fruit into the 'Extra large' grade in the hand-pruned samples. Despite the higher percentage of these largest fruit in the hand-pruned trees, the overall adjusted total crop value for the machine-pruned trees warrants this method of pruning in this trial; see Table 5 below.

Effect of Mechanical Pruning on Crop Value: Processor A

Pruning method	*Adjusted total crop value($)*	*Adjusted price per ton ($)*	*% Cannable fruit*
Hand	874.96 B[x]	1139.97	94.4
Machine	1808.61 A	1048.64	95.8
Significance of pruning method	**	NS	NS
Significance of replicate	NS	NS	NS

[x]*Significant difference between means by LS Means, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).*

Table 5. The table demonstrates that even though the mechanical pruning did not have a significant effect on adjusted price per ton and canning percentage, due to yield, see Table 4, there was a significant effect on adjusted total crop value per acre.

Conclusions:

Due to a harvester breakdown the data obtained evaluating the performance of the canopy contact harvester in this 139 tree/acre 'Manzanillo' orchard with both mechanically pruned and hand pruned subplots was very limited. The major finding was that the fruit left on the tree after mechanical harvest had a significantly higher fruit removal force.

b. **Canopy Contact Harvester Evaluation on Mechanically and Hand Pruned Hedgerow (202 trees pre acre)**
   i. **Nickles:** little viable data was obtained due to an almost complete crop failure and inability to get replicated data for analysis.

Mechanical Pruning Trial with Canopy Contact Harvester:
Nickels soils Laboratory, Arbuckle, Colusa County, California: Stan Cutter, Manager
'Manzanillo' orchard with center Sevillano pollinator row planted 2002
Olive Hedgerow Block: 13, 31 tree, rows of spaced at 12' X 18' (202 trees per acre)

Original Experimental Design:
Split plot with 4, 3 row, replications split into 15 tree hand-pruned and 15 mechanically pruned halves. However, only one experimental row had enough crop for harvest and was divided into 2, 4-tree replications, within the row.

Treatments: (July 6th, 2011 with ENE Inc.)

- Mechanical topped 12' from ground, west side hedged 2' from trunk and skirted 4' from ground for mechanically pruned treatment.
  - All branches between 180 and 90* degrees extending directly into the row were removed.
- Hand pruned control simulated mechanical pruning but was done by hand.

- Trees were not thinned with NAA

Data Collection: October 10 - 12th, 2011.
Only one central Sevillano pollinator row and one Manzanillo row with the mechanically pruned and hand-pruned treatments had sufficient crop for harvesting. The two rows were subdivided to generate replications. However, the crop was so low the results are suspect as they do not represent a normal crop.

1) Fruit removal force and individual fruit weight was determined on 100 individual fruits/row for both cultivar rows.
2) Hand harvested controls were obtained from both variety rows for hand harvest fruit quality controls.
3) Both rows were mechanically harvested.
4) Fruit removal force was determined on 100 olives in the single partial row that was mechanically harvested.
5) Both rows were hand harvested to determine the final harvester efficiency and effects of the mechanical pruning treatments.
6) Bins from hand harvest control rows, single machine harvested row and its hand gleaning harvest were:
   a) Field weighed and a representative 20" sample removed from each for Bell Carter.
   b) Bins delivered and reweighed at Musco Exeter receiving station.
   c) Received a COC grade and adjusted value per ton at both Musco and Bell receiving.

Harvester % efficiency for both machine and hand pruned treatments will be calculated as follows:

$$\frac{\text{Weight of machine harvested olives}}{\text{Weight of machine harvested + hand gleaned olives}}$$

Fruit quality and value of the machine and hand harvested olives will be based on:
Yield per tree/or acre
Adjusted price per ton; based on fruit grading (size and quality)
Total canning percentage

Post processing of harvested samples is no longer necessary as the two years' evaluations of processed fruits (2008/9, 2010/11) have demonstrated mechanical harvesting can produce mechanically harvested processed olives that neither sensory or consumer panels can distinguish from hand harvested olives. Therefore, the receiving station grades can serve as indictors for the processed quality

Results: 2011

Evaluation of Canopy contact harvester in 203/trees per acre mechanically pruned hedgerow orchard:

I. Sevillano Pollinator Row (30 trees)

This Sevillano pollinizer row, though not in the original experimental design, was harvested because it was available. The pull force and fruit size before and after harvest were evaluated, the final percentage efficiency of the canopy contact harvester calculated and the effect of harvest method on fruit quality were determined.

Pull force of the olives left on the tree after harvest was significantly higher but there was no significant difference in individual fruit weight before and after harvest. These results size and removal force probably due to the very small range in fruit size overall.

Effect of Mechanical Harvesting on Fruit Removal Force and Fruit Weight

Sevillanos		
Sample timing	*Fruit weight (g)*	*Fruit removal force (g/cm^2)*
Before machine harvest	9.0 A	473 B***
After machine harvest	10.0 A	678 A

Table 6. This T-test result demonstrates no difference in fruit weight before and after machine harvest. There was a significant difference (0.1%) in fruit removal force before and after machine harvest with fruit removal force significantly higher in those fruit left on the tree after machine harvest

Effect of Mechanical Harvesting on Fruit Removal Force and Fruit Weight

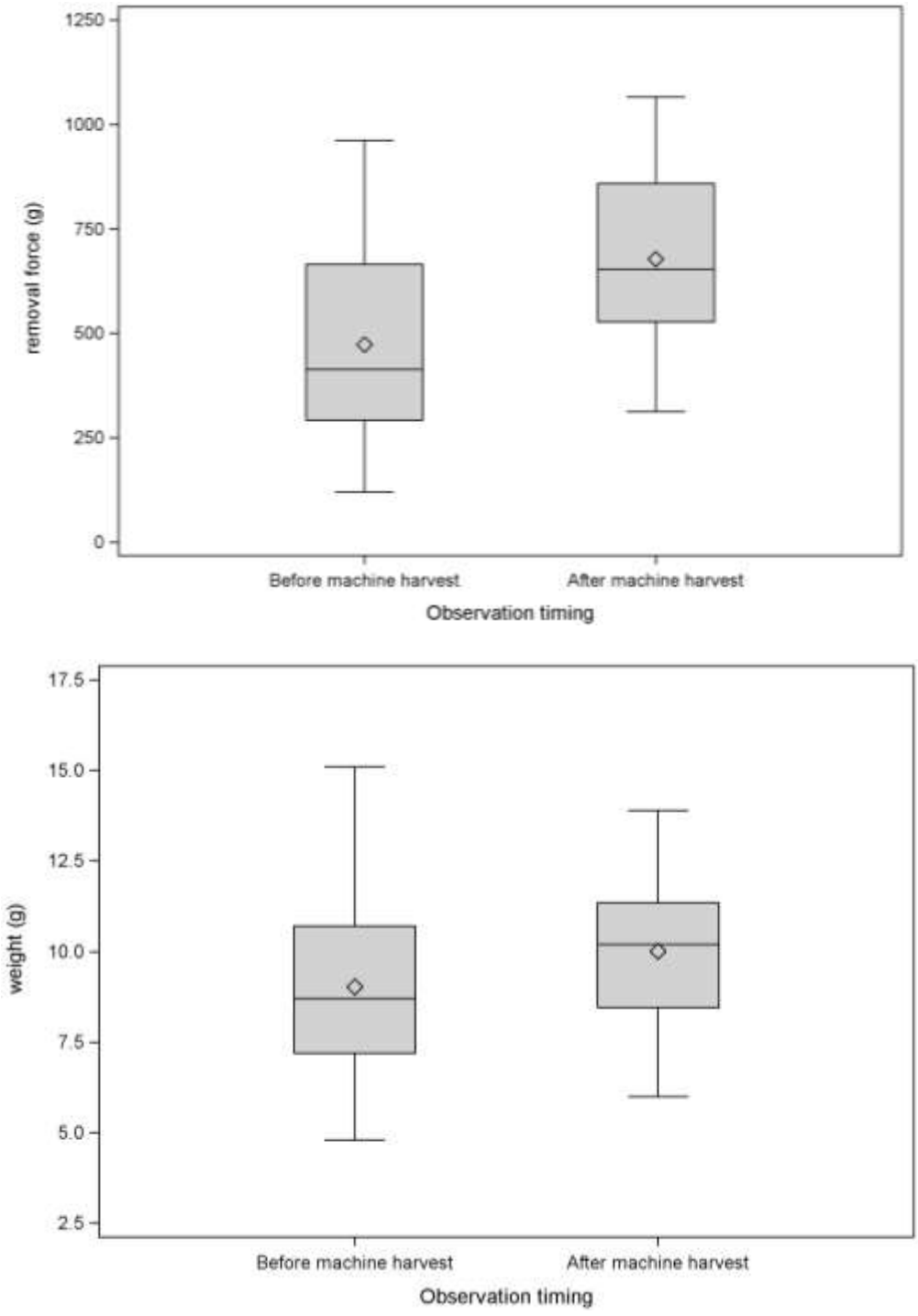


Fig. 3. These two figures demonstrate the significant difference in FRF before and after mechanical harvesting of the Sevillano row, top graph, but no difference in fruit weight before and after mechanical harvesting, bottom half.

In the entire Sevillano row the machine harvested total weight was 83.5 lb. and the and-gleaned total weight was 222 lb. The calculated final harvester efficiency was 27.3%. This

data should not be taken as an indication of the harvester efficiency as the very low crop makes all the data non-representative and therefore unreliable.

Effect of Harvest Method on Fruit Quality (grade) and Defects: Two Processors (A,B)

	% of sample			
Grade	**Processor A**		**Processor B**	
Harvest method	**Hand**	**Machine**	**Hand**	**Machine**
Super colossal	10.98	16.09	9.73	3.17
Colossal	32.44	41.09	28.64	32.08
Jumbo	35.85	23.27	26.88	16.73
Ex. Large "C"	9.27	11.14	6.83	5.59
Ex Large "L"	4.39	3.71	3.08	2.54
Large	2.20	2.23	2.31	2.06
Undersize	2.68	1.49	1.58	0.79
Cull (defects)	1.71	0.74	20.68	36.92
Trash	0.49	0.25	0.27	0.12
	Defects = Culls (above)			
Bell-Carter evaluation	**% Cullage**			
Wrinkled	0		0.6	
Deformed	7.00		5.7	
Bruised	49.4		62.4	
Fly damage	43.5		31.3	
Defects: Processor A evaluation (excluding fly damage and overripe)	mutilated, wrinkled		mutilated, wrinkled	
	Processor A		**Processor B**	
Crop valuation	**Hand**	**Machine**	**Hand**	**Machine**
Adjusted total crop value ($)	137.51	159.11	473.01	466.49
Adjusted price per ton ($)	670.78	787.70	413.10	337.34
% Cannable fruit	95.1	96.0	96.7	97.3

Table 7. This table gives the effects of the canopy contact harvester versus a hand-harvested control on Sevillano fruit quality; no statistics could be done as there was no replication. The most significant aspect of this comparison is the difference in olive grade and value between Processor A and B though both had similar % cannable fruit.

Conclusions: evaluation of a canopy contact harvester with the Sevillano cultivar. There was no difference in fruit size before and after mechanical harvesting however there was a significant difference (0.1% level) in fruit removal force of the olives left on the tree after harvest. This differed from the results with Manzanillo in which olives left on the tree

were significantly lighter and fruit weight had a positive correlation with increasing fruit weight and size.

This higher fruit removal force and prototype status of the harvester and the inability to conduct practice trials as there was no fruit contributed to the low 27.3 % final harvest efficiency.

The harvest method effects on fruit quality (grade and defects) and crop value couldn't be evaluated statistically due to non-replicated conditions. In some values there were greater differences by processor than by harvest method. Total cullage was much higher in Processor B's than Processor A's evaluation, even when fly damaged fruit were not considered. Processor B included a relatively high percentage of bruised fruit in their sample that was not found by the Processor A evaluation. Despite this fact, however, the % cannable fruit was quite high and consistent by processor and by harvest method.

Manzanillo: Canopy Contact Harvester Evaluation

The experimental design for the 'Manzanillo' cultivar consisted of a split plot with 5, 2 row, replications. In the split plot half of each row was mechanically topped at 12' and hedged 2' from the trunk on the west side. The other half of each row was hand pruned to simulate the mechanical pruning but was not as severe.

The next four sections discuss the split-plot pruning method X harvest method effects on:

1. Pull force before and after mechanical harvesting
2. Fruit size before and after mechanical harvesting
3. Efficiency of mechanical harvesting and yield
4. Effect of harvest method on fruit quality

1. Effect of Hand versus Mechanical Pruning + Hand versus Mechanical Harvest on Pull Force

This is a factorial design with 'pruning method' as the main independent variable and 'harvest method' as the independent variable within pruning method. Table 8 below compares fruit removal force before and after mechanical harvest. As can be seen in the T-test results below there were a significant difference in fruit removal force after mechanical harvesting.

Comparison of Fruit Removal Force Before and After Machine Harvest

Sample timing	*N*	*Mean*	*Std. Dev.*	*Std. Err.*	*Minimum*	*Maximum*
Before mechanical harvest	160	**397 A**	169.9	13.4	134	920
After mechanical harvest	67	**348 B**	132.9	16.2	77	712
Diff (1-2)		49	159.9	23.3		

a.

Method	*Variances*	*DF*	*t Value*	*Pr > |t|*
Pooled	Equal	225	2.12	0.04
Satterthwaite	Unequal	156.8	2.34	0.02

b.

Equality of Variances				
Method	*# DF*	*Den DF*	*F Value*	*Pr > F*
Folded F	159	66	1.63	0.02

c.

Table 8. a, b. c. The top table, a, demonstrates there was a significant difference in the 'Manzanillo' fruit removal force before and after mechanical harvesting when analyzed as a T-test results with pf difference significant at 5%.

Effect of Mechanical Harvesting on Fruit Removal Force

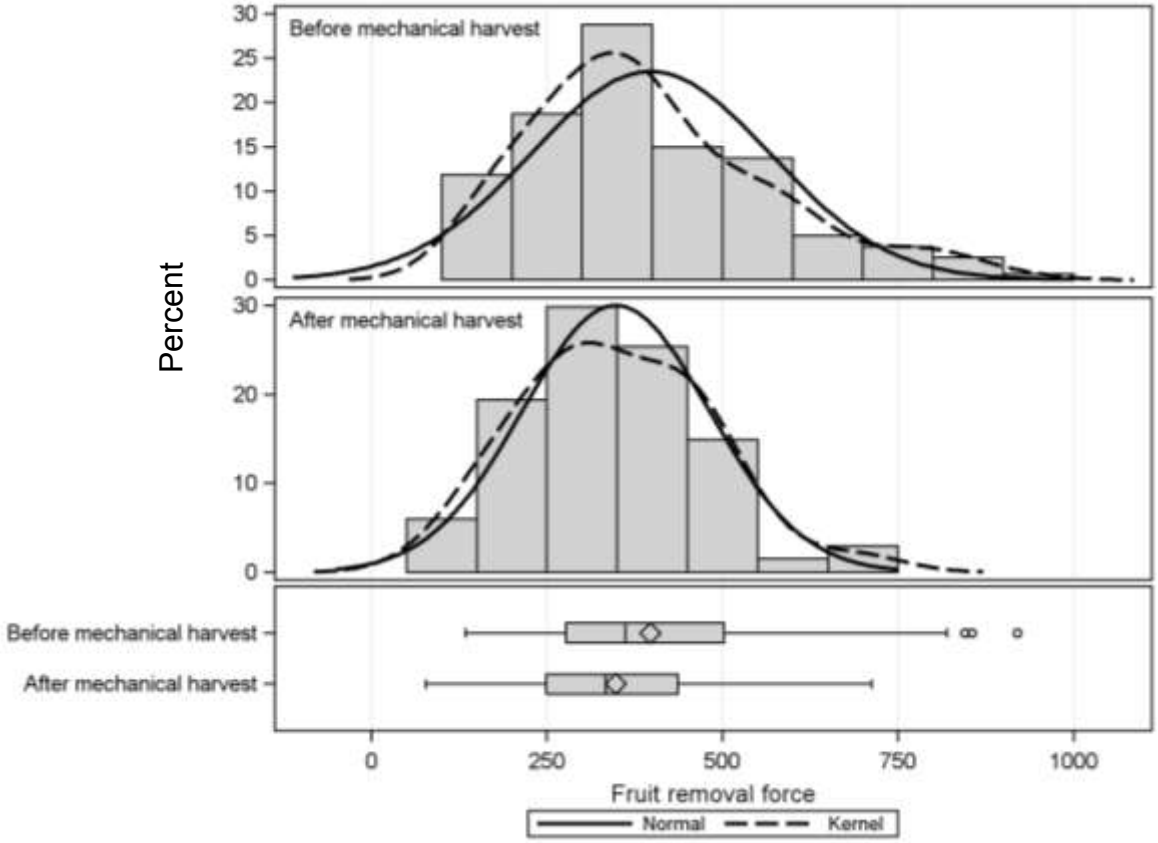


Fig. 4. Percent of olives within each fruit removal force range before and after mechanical harvesting.

Effect of Fruit Mechanical Harvesting on Fruit Removal Force

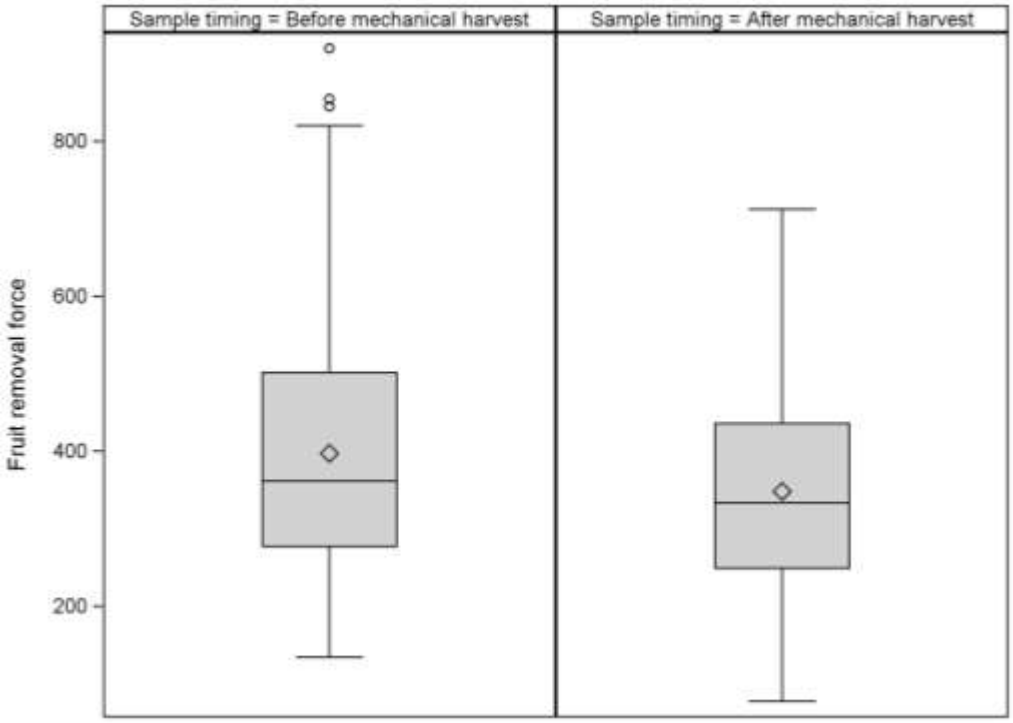


Fig. 5. This is box diagram demonstrating the average fruit removal force before and after mechanical harvesting.

When analyzed as a mixed model linear regression using 'block' (location) as a 'random' factor and 'time of sampling' as a 'fixed' effect to assess the effect of mechanical harvest removal of fruit by 'pull force' found no significant difference in before and after pull force with means separation by Least Squares Means; Table 9 and Fig. 6 below.

Effect of Mechanical Harvesting on Fruit Removal Force

Type 3 Tests of Fixed Effects				
Effect	*# DF*	*Den DF*	*F Value*	*Pr > F*
Time (before and after machine harvest)	1	218	3.72	0.06

Effect=time Method=Bonferroni(P<.05) Set=1

Sample timing	*Mean*	*Standard Error*	*Letter Group*
Before mechanical harvest	397	12.5	A
After mechanical harvest	347	23.1	A

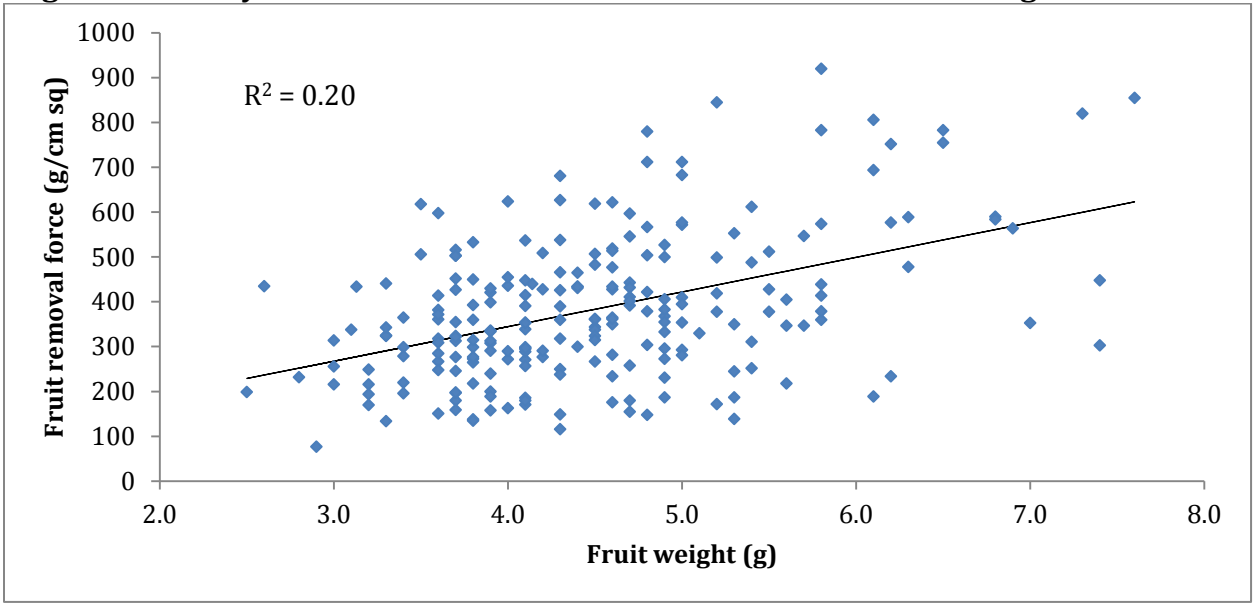
Table 9. This Mixed Model results found no significant difference in the pull force before and after mechanical harvesting.

2. Effect of Hand versus Mechanical Pruning + Hand versus Mechanical Harvest on Fruit Size Before and After Mechanical harvesting

Mechanical pruning, as can be seen in the section 4: **Effect of Pruning Method and Machine versus Hand Harvest on Fruit Quality and Value,** had a significant effect on fruit size distributions and therefor fruit grade and value. Also, while mechanical harvesting did differentially remove larger fruit mechanical harvesting by itself did not affect fruit size.

Figure 6 below demonstrates 20% of the variability associated with fruit weight correlated with fruit removal force. This suggests fruit weight, and not fruit removal force, is more important factor in harvester efficiency.

Regression Analysis of Fruit Removal Force as a Function of Fruit Weight



R^2 = 0.20 for regression of fruit size and removal force (227 fruit)
Pearson Correlation Coefficient = 0.45, N = 227 fruit

Figure 6. Correlation between fruit size and removal force demonstrated only 20% of the pull force was a result of fruit size.

3. Effects of Pruning Method and Machine Versus Hand Harvest on Yield and Harvest Efficiency

While pruning appeared to have a strong effect on yield per tree the replications were so limited that even the 53% and 51% decreases in yield from machine pruning were not statistically significant. ,As expected, the harvest method had no effect on yield per tree as can be seen in Table 10 below

Effect of Harvest Method on Tree Yield

Split-plot analysis of main effects (pruning method), interactions, or subplots (harvest method) within main plots found no significant difference in yield per tree.		
Pruning method	*Harvest method*	*Yield (lb.) per tree*
Hand-pruned	*Hand-harvested*	19.10
	Machine-harvested	10.15
Machine-pruned	*Hand-harvested*	13.95
	Machine-harvested	7.10
Significance	***Pruning method***	***Harvest method***
	NS	**NS**

Table 10. The results within replicates (only 3-4 trees per replicate, 2 replicates per treatment) indicate a wide range in data between replicates, which illustrates the unreliability of such a small data set.

Effect of Pruning Method on Harvester Efficiency

rep	*Pruning method*	*Harvest method*	*Yield (lb.) per tree*	*Efficiency of machine harvest*
1	*Hand-pruned*	*Hand-harvested*	23.5	.
		Machine-harvested	16.9	23.9.
	Machine-pruned	*Hand-harvested*	16.0	
		Machine-harvested	9.7	83.8
2	*Hand-pruned*	*Hand-harvested*	14.7	.
		Machine-harvested	3.4	13.4
	Machine-pruned	*Hand-harvested*	11.9	
		Machine-harvested	4.5	17.8

Table 11. The results within replicates (only 3-4 trees per replicate, 2 replicates per treatment) indicate a wide range in data between replicates, which again illustrates the unreliability of such a small data set.

Interaction Plot of Pruning and Harvest Methods on Yield

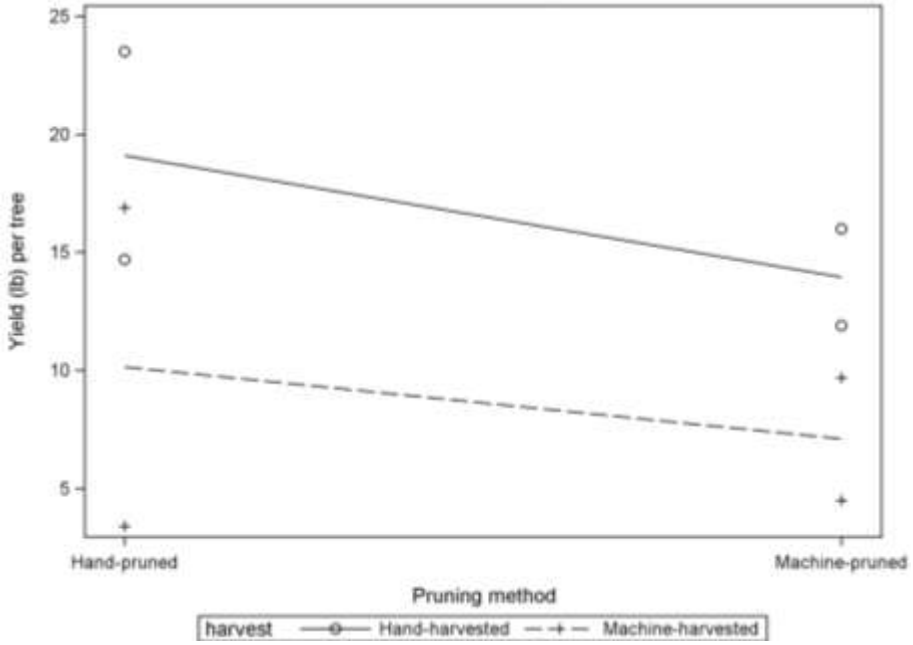


Fig. 7. This Interaction plot shows no interaction between pruning method and harvest method for yield per tree.

Below is a test of simple main (pruning method) effects and simple subplot (harvest method) effects on yield. As with analysis presented earlier, the sharp decreases in yield produced by mechanical pruning were not statistically significant because sample sizes were so limited and variable.

Effects of Pruning and Harvest Method on Yield

Test for simple effects of pruning method on yield, across harvest methods, and of harvest method, across pruning methods		
Simple effects	*Lb./tree*	*H0: LSMean1=LSMean2 Pr > |t|*
Hand-pruned	14.6	0.40
Machine-pruned	10.5	
Hand-harvested	16.5	0.14
Machine-harvested	8.6	

Table 12. Split-plot analysis of main effects (pruning method), interactions, or subplots (harvest method) within main plots found no significant difference in yield. This was probably a result of limited and quite variable sample sizes.

4. Effects of Pruning Method and Machine versus Hand Harvest on Fruit Quality and Value

Tables 13 through 15 below demonstrate the effects of pruning method and harvest method on fruit quality and value from Processor A . Tables 16, 17, and 18, do the same for Processor B.

As expected machine harvesting preferentially removed a higher percentage of larger fruit. Also expected was the lack of interaction between pruning method and harvest method on fruit size suggesting that the pruning method did not influence the efficiency of removal for any particular size of fruit, however, given the small sample sizes, this result should be taken with caution.

Pruning method did increase the proportion of small, petite and sub-petite olives; specifically mechanical pruning significantly decreased the percentages of smaller fruit sizes. In Table 13 with Processor A the three hand pruned samples produced an average of 15.03% small fruits (10.1 + 14.6 + 20.4 = 45.1/3 = 15.1%) versus 6.56% (11.2 + 4.4 + 4.1 = 19.7/3 = 6.56%) for mechanically pruned trees. This is best seen in Table 14 where the grades are combined and evaluated together. Table 15 further supports this point by demonstrating that the hand pruned samples, due to a higher percentage of smaller size fruit, had lower values per ton and %s of cannable fruit. The highest percentages of small fruit were found in the hand pruned and hand gleaned (after machine harvest) samples. The adjusted prices per ton and % of cannable fruit given in Table 10 further support this effect. Tables 16, 17 and 18 with the analyzed results for Processor B give similar results.

Hand pruning resulted in lower percentages of cannable fruit, due to the greater proportion of smaller sizes. Defects in the Processor B samples were higher overall, and included a large percentage of bruised fruit, which was not found in the Processor A samples. The highest percentages of bruised fruit were found in machine-harvested and hand-gleaned trees, indicating an area of concern for the machine harvester development. Deformed fruit were also quite high in the Processor Br samples and were not found in the Processor A samples. Nonetheless, crop values were substantially higher for Processor B than for Processor A. This discrepancy, which needs to be addressed. Much of the difference was due to cullage adjustments in the Processor B samples.

Effect of Pruning and Harvest Methods on Fruit Size Distribution

Effects of pruning and harvest methods on fruit size in a split-plot design (main plot = pruning method, subplot = harvest method) for Manzanillo olive. (The values shown are from Processor A)

Pruning method	*Harvest method*	*Percentage of total sample*								
		Extra large	*Large*	*Medium*	*Small*	*Petite*	*Sub-petite*	*Undersized*	*Cull*	*Trash*
Hand	*Hand*	41.2	21.5	16.6	10.1	4.7	2.1	1.3	1.6	1.0
	Machine	28.5	23.4	20.8	14.6	5.4	2.1	1.4	3.0	1.0
	Hand-gleaned	18.1	16.8	27.8	20.4	7.9	4.1	2.6	1.2	1.0
Machine	*Hand*	36.9	25.6	19.0	11.2	4.2	1.2	0.5	0.7	0.7
	Machine	57.7	19.0	11.0	4.4	1.5	0.7	0.7	3.6	1.5
	Hand-gleaned	5.1	22.4	15.3	4.1	3.1	0.5	0.5	1.0	0.5
*Significance differences between means by LS Means, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).*										
Pruning method		NS	NS	NS	*	*	**	NS	NS	NS
Harvest method		**	NS	NS	NS	NS	NS	NS	*	NS
Pruning x Harvest		NS	NS	NS	NS	NS	NS	NS	NS	NS
Pruning x Replicate		NS	NS	NS	NS	NS	NS	NS	NS	NS
Harvest x Replicate		NS	NS	***	NS	NS	NS	NS	NS	NS

Table 13. This table demonstrates mechanical harvesting preferentially removes larger fruit, and that mechanical pruning significantly decreased the % of fruit in the small through undersize grades.

Effect of Pruning and Harvest Methods on Fruit Size Distribution

Effects of pruning and harvest methods on fruit size in a split-plot design (main plot = pruning method, subplot = harvest method) for Manzanillo olive. The values shown are from Processor A.

Pruning method	Harvest method	Percentage of total sample	
		Small+Petite+Sub-Petite fruit	Undersize+Cull
Hand	*Hand*	16.8	2.8
	Machine	22.0	4.3
	Hand-gleaned	32.4	3.8
Machine	*Hand*	16.6	1.2
	Machine	6.6	4.4
	Hand-gleaned	7.6	1.5
*Significance differences between means by LSMeans, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).*			
Pruning method		*	NS
Harvest method		NS	NS

Table 14. This table demonstrates mechanical harvesting preferentially removes larger fruit, and that mechanical pruning significantly decreased the % of fruit in the small through undersize grades.

Effects of Pruning and Harvest Methods on Crop Value

Effects of pruning and harvest methods on adjusted crop value in a split-plot design (main plot = pruning method, subplot = harvest method) for Manzanillo olive. The grades shown are from Processor A.				
Pruning method	*Harvest method*	*Adjusted total crop value ($)*	*Adjusted price/ton ($)*	*%Cannable fruit*
Hand	Hand	104.49	1082.82	94.30
	Machine	111.97	1032.84	93.70
	Hand-gleaned	100.20	963.46	91.10
Machine	*Hand*	114.31	1112.47	95.15
	Machine	77.56	1132.21	97.10
	Hand-gleaned	114.62	1169.56	96.40
*Significance differences between means by LSMeans, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).*				
Pruning method		NS	*	NS
Harvest method		NS	NS	NS
Pruning x Harvest		NS	*	NS
Pruning x Replicate		NS	NS	NS
Harvest x Replicate		NS	NS	NS

Table 15. This table demonstrates that the hand pruned samples, due to a higher percentage of smaller size fruit, had lower values per ton and %s of cannable fruit. The highest percentages of small fruit were found in the hand pruned and hand gleaned (after machine harvest) samples.

Effects of Pruning and harvest Methods on Fruit Size Distribution

Effects of pruning and harvest methods on fruit size in a split-plot design (main plot = pruning method, subplot = harvest method) for Manzanillo olive. The values shown are from Processor B.

Pruning method	*Harvest method*	*Percentage of total sample*								
		Extra large	*Large*	*Medium*	*Small*	*Petite*	*Sub-petite*	*Undersize*	*Cull*	*Trash*
Hand	*Hand*	30.7	28.7	19.9	11.7	4.5	1.4	0.8	1.7	0.5
	Machine	16.5	22.2	20.3	16.8	9.8	2.8	1.4	8.4	1.7
	Hand-gleaned	29.3	22.0	18.0	11.2	4.3	1.1	0.7	10.6	2.9
Machine	*Hand*	20.8	34.2	22.3	12.7	5.1	1.3	0.5	2.4	0.6
	Machine	43.8	26.2	12.4	5.5	1.8	1.2	0.6	7.5	1.1
	Hand-gleaned	38.3	32.4	15.8	5.5	2.0	0.4	0.4	5.0	0.2
*Significance differences between means by LSMeans, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).*										
Pruning method		NS	NS	NS	***	***	*	NS	NS	NS
Harvest method		NS	NS	NS	***	***	NS	NS	NS	NS
Pruning x Harvest		NS	NS	NS	***	***	NS	NS	NS	NS
Pruning x Replicate		NS	NS	*	NS	NS	NS	NS	NS	NS
Harvest x Replicate		NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 16. These results from Processor B are similar to the results of Processor A demonstrating mechanical pruning significantly improved fruit size grades by decreasing the percentage of small and petite olives.

Effects of Pruning and harvesting Method on Fruit Size

Effects of pruning and harvest methods on fruit size in a split-plot design (main plot = pruning method, subplot = harvest method) for Manzanillo olive. The values shown are from Processor B.			
Pruning method	Harvest method	Percentage of total sample	
		Small + Petite +Sub-Petite fruit	Undersize+ Cull
Hand	*Hand*	17.6	2.6
	Machine	29.4	9.8
	Hand-gleaned	16.5	11.3
Machine	*Hand*	19.1	3.0
	Machine	8.5	8.1
	Hand-gleaned	7.9	5.4
*Significance differences between means by LSMeans, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).*			
Pruning method		***	NS
Harvest method		**	NS

Table 17. As with Processor A in Table 14 mechanical running significantly increased the proportion of larger fruit. The distribution of smaller fruit, combined, was similar to that found in the Processor A grade, with higher significance here for both effects of pruning and harvest methods. Fewer smaller fruit were found with most machine-pruned trees. Undersized and cullage were generally greater in the hand-pruned treatment as well. Both factors contributed to lower crop values; see Table 15.

Effects of pruning and harvest methods on fruit size in a split-plot design (main plot = pruning method, subplot = harvest method) for Manzanillo olive. The grades shown are from Processor B.								
Pruning method	*Harvest method*	*Value ($)*		*Percentage*				
		Adjusted total crop	*Adjusted price/ton*	*Cannable fruit*	*Mutilated*	*Wrinkled*	*Deformed*	*Bruised*
Hand	*Hand*	678.66	1090.71	95.0	1.0	2.5	3.5	0.5
	Machine	440.70	818.67	88.4	1.5	4.0	9.0	64.5
	Hand-gleaned	445.16	842.35	92.8	9.0	6.5	7.5	63.0
Machine	*Hand*	699.10	1074.25	94.2	1.5	1.5	6.0	16.5
	Machine	433.24	999.13	97.2	3.0	3.0	13.0	46.0
	Hand-gleaned	716.68	1126.85	97.7	1.0	7.0	9.0	28.0
*Significance differences between means by LS Means, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).*								
Pruning method		NS	NS	**	NS	NS	NS	NS
Harvest method		NS	NS	NS	NS	NS	NS	NS
Pruning x Harvest		NS	NS	*	NS	NS	NS	NS
Pruning x Replicate		NS	NS	NS	NS	NS	NS	NS
Harvest x Replicate		NS	NS	NS	NS	NS	NS	NS

Table 18. As with Processor A in Table 15 mechanical pruning significantly increased the % of cannable fruit and therefore increased the value per ton.

<u>Summary of Conclusions</u>:
Manzanillo Cultivar spaced @ 12' X 18' (202 trees/acre) hedgerow
Split Plot Experimental Design:

- **Hand pruned versus mechanically pruned: main plot**
- **Canopy contact harvester versus hand harvest: split plot**

Though this data is limited due to sharply decreased yields and therefore replications some conclusions are strongly supported by the data.

The fruit removal force was higher after mechanical harvesting. The mechanical harvester preferentially removed larger fruit. As a poor correlation was found between fruit size and fruit removal force this strongly suggests efficient fruit removal is more of a function of fruit weight and size than of fruit removal force.

The mechanical pruning significantly decreased the percentage of fruit in the smallest size grades; small, petite, sub petite and undersized. This suggests mechanical pruning can increase fruit value.

In these analyses grade and crop values for processors were evaluated separately. Disparities in grading and valuation should be clarified as to how they arise. Cullage, especially as bruising was identified by Processor B but not Processor A and contributed significantly to differences in valuation.

The data was evaluated as a factorial, or split-plot, with Pruning Method as the main plot and Harvest Method as the split-plot. Both factors were analyzed as simple effects and interactive effects. The results, however, should be taken with caution, as the number of trees per replicate (3 or 4), and the number of replicates (2) per treatment combination was quite low, and with crop load as light and variable as it was tree-to-tree, the data are not reliable for 'normal' crop years.

Some significant differences by simple effects and/or interactions were found in each processor's data for some size grades. This type of approach, i.e., looking at individual size grades, may not be an ideal approach as significant differences in some size categories, for some effects, but not conclusively pointing to a trend to smaller or larger size overall, due to specific treatment effects, leads to confusion.

One conclusion that might make sense if it were consistent, would be to see if mostly large fruit predominated in the samples from mechanical harvest, with smaller percentages of the larger sizes in the subsequent hand-gleaning (see Musco grade for Extra Large, for example). Also, if pruning method in a heavy-cropping year were to affect an overall shift toward smaller fruit with heavier crop load, or vice versa, this would also make sense.

However, in a light year such as 2011, crop load is not a limiting factor to size and pruning method did not significantly affect crop load in this very small, machine harvest trial (although it certainly did in the larger, pruning method-only trial). Perhaps the smallest grades that command the lowest prices (undersize, petite and sub-petite) could be lumped together?

The results from crop valuation are ambiguous, as for the size grade data. Perhaps the machine-harvested and hand-gleaned fruit should be combined for valuation in the future , but how to do that and still get the grade separately for each isn't clear.

II. Effect of Mechanical Pruning on Yield, Quality and Value of 'Manzanillo' Olives.

Location I.
Rocky Hill Ranch, Exeter, Tulare County: Manager, Mark Pascoe
Block 17 W
17-year-old 'Manzanillo' orchard with 'Sevillano' pollinators (irregularly placed)
13, 83 tree, rows spaced at 13' X 26' (139 trees per acre)

Pruning Treatments: June 6, 2011 (Laux Pruning)
Split plot with 6 hand-pruned and 6 mechanically pruned rows (replications)
Treatments: (Laux Pruning)

- Mechanically hedged 6' from trunk on the western row side and skirted 4' from ground.
- Hand pruned control; 6 eastern rows. All tree skirted 4' from ground (for harvester catch frame).
- Crop was not thinned with NAA.

Data Collection: October 6 -7, 2011.
6 mechanically pruned rows and 6 hand -pruned rows were harvested by hand
Bins form all 12 rows were:

- Field weighed and a representative 20" sample removed from each for Bell Carter.
- Bins delivered and reweighed at Musco Exeter receiving station.
- Received a COC grade and adjusted value per ton at both Musco and Bell receiving.

Results:

Effects of Mechanical Pruning on Yield: per tree yield in 2011

Pruning method	*Yield (lb./tree)*		
	First 10 trees	*Remainder of row (73 trees)*	*83 tree-row*
Hand-pruned	47.4 b[x]	38.7 b	39.8 b
Machine-pruned	117.8 a**	102.8 a**	104.6 a**
[x]Significant differences between means within a column, 5% level. Significance within columns by LS Means = ***,*,*, NS (0.001, 0.01, 0.05, respectively).			

Table 19. This table demonstrates this fourth year of mechanical pruning significantly increased yield.

Effect of Mechanical Pruning on Yield: 2008 - 2011

Pruning TX	2008 T/A	2009 T/A	2010 T/A	2011 T/A	T/A Cum.	T/A Average
Mechanical	1.34	0.07	6.8	7.3	15.5	3.9
Hand	1.54	0.18	8.5	2.8	13.0	3.2
MP TX	T+HW	T+HE	-	HW		
Sig.	NSD	NSD	P. =0.05	P. = 0.05		

Table 20. This table demonstrates the effect of hedging and topping on yield. Six, 83 tree rows were hedged on one side 6 feet from the trunk and topped at 12 feet in 2008 and 2009, no mechanical pruning was done in 2010 and only hedging on one side in 2011.

Mechanical pruning treatments were begun in 2008. The total crop failures in 2008 and 2009 resulted in no significant differences in yield those years. With the very heavy crop in 2010, and no pruning treatment that year, which in hindsight was a mistake, the mechanically pruned trees produced a significantly 19% less than the hand pruned trees. However, these trees did harvest 8% more efficiently than hand pruned trees. In 2011 the mechanically pruned trees were hedged on one side, and not topped. They produced significantly more crop than the hand pruned trees. And, as can be seen in Figure 8, there was less variability among the trees in yield. The crop on the mechanically pruned trees was also visibly smaller and later maturing. More importantly, the mechanically pruned trees have yielded as well, and slightly better than the hand pruned trees, producing an average 3.9T/A versus 3.2 T/A for the hand-pruned trees over the four years.

This experiment needs to be continued for another 4 years but the results thus far suggest that not only does mechanical pruning better adapt table olive trees for mechanical harvesting, and may partially control alternate bearing but most importantly, does not decrease yields. However, at least 8 years of data is needed, preferably without the total crop failures experienced in the first two years, to demonstrate the latter hypothesis..

Effect of Pruning on Yield Distribution of Trees

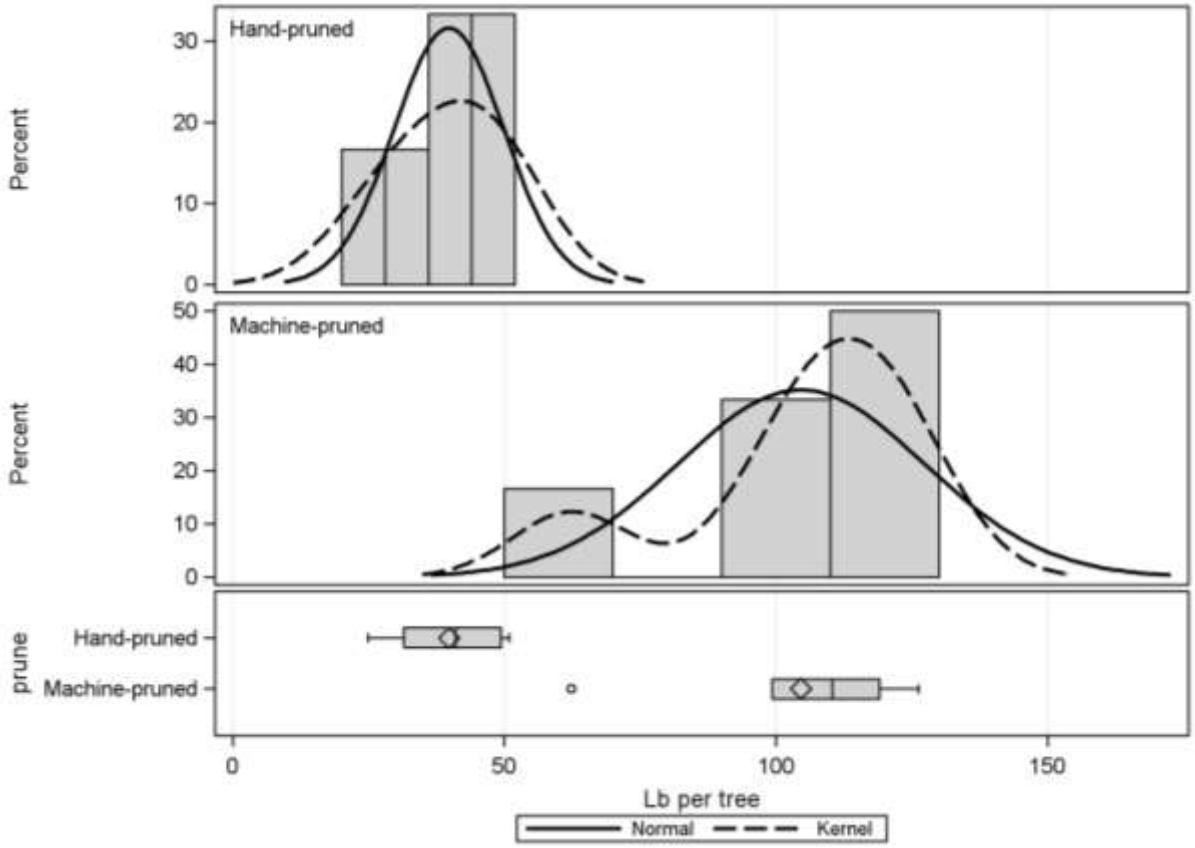


Fig. 8. This graph demonstrates the yield per tree distribution by percentage of trees in each 83tree row. The distribution demonstrates the mechanically pruned trees had much less yield variability than the hand pruned trees.

Effect of Mechanical Pruning on Grade: Processor A

Pruning method	*Percentage of sample*								
	Extra large	*Large*	*Medium*	*Small*	*Petite*	*Sub-petite*	*Undersized*	*Cull*	*Trash*
Hand	59.4 A[x]	27.4 B	6.0 B	2.1 B	1.1 B	0.8 B	0.8	4.0 B	0.9
Machine	31.8 B	41.7 A	19.2 A	8.2 A	4.4 A	2.1 A	1.3	1.9 A	1.5
Significance of pruning method	***	***	***	***	**	*	NS	*	*
Significance of replicate	NS	NS	NS	NS	NS	NS	NS	NS	*

[x]*Significant difference between means by T-test, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).*

Table 21. The data above clearly demonstrates the strong effect of mechanical pruning on crop size distribution in 2011. The higher percentage of Extra Large fruit in the hand-pruned samples is due to much lighter yield, in all likelihood. The larger percentages of fruit in 'Large' and 'Medium' grades for the much heavier cropped machine-pruned samples are probably due to a shift of fruit into the 'Extra large' grade in the hand-pruned samples. Despite the higher percentage of these largest fruit in the hand-pruned trees, the overall adjusted total crop value for the machine-pruned trees warrants this method of pruning in this trial.

Effect of Pruning Method on Crop Value: Processor A

Pruning method	*Adjusted total crop value($)/acre*	*Adjusted price per ton ($)*	*%Cannable fruit*
Hand	2,621.94 B[x]	1139.97	94.4
Machine	7,655.07 A	1048.64	95.8
Significance of pruning method	**	NS	NS
Significance of replicate	NS	NS	NS

[x]*Significant difference between means by LSMeans, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).*

Table 22. The data above clearly demonstrates the mechanical pruning did not significantly decease the adjusted price per ton. However, when the adjusted price per ton is multiplied by the production per acre the mechanically pruned trees generated

$7,655.07 per acre versus the $2,621.94 per acre produced by the hand harvest trees. If reasonable hand harvest costs of $375.00 per ton are deducted the results are still striking with net return of $1,571.93 per acre net return for the hand pruned trees versus $4,918.07 net return for the mechanically pruned tree.

Effect of Pruning on Fruit Grade: Processor B

Pruning method	*Percentage of sample*							
	Extra large	*Large*	*Medium*	*Small*	*Petite*	*Sub-petite*	*Undersized*	*Cull+Trash*
Hand	62.3 A	12.1 B	5.8 B	3.3 B	1.7 B	6.9	6.2 A	7.8
Machine	40.5 B	21.4 A	13.6 A	9.7 A	5.6 A	2.5	1.5 B	6.4
Significance of pruning method	***	***	***	***	**	NS	NS	NS
Significance of replicate	NS	NS	NS	NS	NS	NS	NS	NS
[x]*Significant difference between means by T-test, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).*								

Table 23. This table gives the grade results from Processor B. As with Processor A, the pruning had a significant effect on fruit size.

Effect of Pruning on Crop Value: Processor B

Pruning method	*Adjusted price per ton ($)*	*%Cannable fruit*
Hand	1019.66	82.6
Machine	1025.64	84.2
Significance of pruning method	NS	NS
Significance of replicate	NS	NS
[x]*Significant difference between means by LSMeans, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).*		

Table 24. The adjusted prices per ton were slightly lower than for Processor A, and as with processor A, mechanical pruning had no effect on adjusted price per ton.

Cullage Breakdown: Processor B.

Pruning	*Harvest*	*Mutilated*	*Wrinkled*	*Deformed*	*Bruised*
Hand	*Hand*	10.0	30.0	50.0	10.0
	Machine	8.3	6.2	4.7	80.8
	Hand-gleaned	18.0	14.4	32.1	35.5
Machine	*Hand*	15.0	7.4	37.3	40.2
	Machine	4.6	4.6	20.0	70.8
	Hand-gleaned	2.2	15.6	20.0	62.2
Significance of pruning method	NS	NS	NS	NS	NS
Significance of harvest method	NS	NS	NS	NS	NS
Pruning x harvest	NS	NS	NS	NS	NS
Comparison of harvest method across pruning methods					
Hand		12.5	18.7	43.7	25.1
Machine		7.1	5.7	9.8	77.4
Hand-gleaned		12.7	14.8	28.1	44.4
Significance of harvest method		NS	NS	NS	NS

[x]*Significant difference between means by Tukey or LSMeans, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).*

Table 25. This table clearly demonstrates through lack of significance in any category that cullage rate is not affected by mechanical harvesting. However, lack of significance may be due to very small replication numbers. There appears to be a difference in harvest method across pruning methods, with more or less equal amounts of mutilated, wrinkled and deformed fruit if machine+hand-gleaned are compared to hand-harvested; bruising is far greater in these compared to hand-harvested. As expected there was no effect of pruning method on cullage.

Location II.
Nickels soils Laboratory, Arbuckle, Colusa County, California: Stan Cutter, Manager
'Manzanillo' orchard with center Sevillano pollinator row planted 2002
Olive Hedgerow Block: 13, 31 tree, rows of spaced at 12' X 18' (202 trees per acre)

Experimental Design:
Paired T-Test with pairs being adjacent 2-row blocks of hand or mechanically pruned trees.

Mechanical Pruning Treatments: (July 6th, 2011 with ENE Inc.)

- Mechanically topped 12' from the ground, west side hedged 2' from trunk and skirted 4' from ground for mechanically pruned treatment.
- All branches between 180 and 90* degrees extending directly into the row were removed.
- Hand pruned control simulated mechanical pruning but was done by hand.
- Trees were not thinned with NAA

Data Collection: October 10 - 12th, 2011.

Row replications were harvested:

- Field weighed and a representative 20" sample removed from each for Bell Carter.
- Bins delivered and reweighed at Musco Exeter receiving station.
- Received a COC grade and adjusted value per ton at both Musco and Bell receiving.

Fruit quality and value of the olives was based on:
Yield per tree/or acre
Adjusted price per ton; based on fruit grading (size and quality)
Total canning percentage

Results: Effect of Mechanical Topping and Hedging on Yield*
***The crop was so low these results and analyses are suspect as they do not represent a normal crop.**

The results of the T-test are given below in Table 26. 'N' is number of replicate groups of trees (blocks), which are half-rows. Blocks are a combination of 2 adjacent half-rows. With a T-test at 5% level: mean differences in yield (lb.) per tree are non-significant.

However, when we look at the yields within individual replicates, we find a wide range of yields Table 27 below. The T-test comparison does not allow us to use 'replicate' as a 'random' effect; using a mixed linear model with 'replicate' as a 'random' effect, and 'pruning method' as a 'fixed' effect, we can evaluate the pruning method effect on yield more clearly. When the yields are analyzed by pruning method using individual replicate

means, Table 27, and a mixed linear model with 'rep' as a 'random' and 'pruning method' as a 'fixed' effect the differences pruning method are significant at 5%; Table 16: a,b,c,d,e.

Effect of Mechanical Pruning on Yield

Pruning treatment	*N*	*Mean*	*Std Dev.*	*Std Err*	*Minimum*	*Maximum*
Hand-pruned	5	7.95 A	5.30	2.37	1.72	14.60
Machine-pruned	5	3.98 A	3.62	1.62	1.62	10.10
Diff (1-2)		3.97	4.54	2.87		

Table 26. This table demonstrates the effect of mechanical pruning on yield. With a T-test at 5% level mean differences in yield (lb.) per tree are non-significant. However, this is probably a result of the very limited and variable crop.

Individual Row Replicate Yields

rep	*Pruning method*	*Yield (lb.) per tree*
1	*Hand-pruned*	11.65
	Machine-pruned	4.48
2	*Hand-pruned*	7.77
	Machine-pruned	1.78
3	*Hand-pruned*	1.72
	Machine-pruned	1.91
4	*Hand-pruned*	4.02
	Machine-pruned	1.62
5	*Hand-pruned*	14.60
	Machine-pruned	10.10

Table 27. Individual replication yields demonstrating great variability.

Method	*Variances*	*DF*	*t Value*	*Pr > |t|*
Pooled	Equal	8	1.39	0.20
Satterthwaite	Unequal	7.07	1.39	0.21

a.

Equality of Variances				
Method	*Num DF*	*Den DF*	*F Value*	*Pr > F*
Folded F	4	4	2.14	0.48

b.

Type 3 Tests of Fixed Effects				
Effect	*Num DF*	*Den DF*	*F Value*	*Pr > F*
prune	1	4	9.19	0.04
Rep	4	0	0.00	.

c.

Least Squares Means						
Effect	*Pruning method*	*Estimate*	*Standard Error*	*DF*	*t Value*	*Pr > |t|*
prune	Hand-pruned	7.952	70.125	4	0.11	0.92
prune	Machine-pruned	3.978	70.125	4	0.06	0.96

d.

Effect=prune Method=Bonferroni (P<.05) Set=1

Pruning method	*Mean lb./tree*
Hand-pruned	7.95 A
Machine-pruned	3.98 B

e.

Table 28: a-e: the individual replications are considered and analyzed as a mixed linear model with the replication as 'random' and the 'pruning method' as a 'fixed' effect the differences I pruning method are significant at 5%. With this analysis the mechanical topping and hedging significantly decreased yield within the same growing season.

The distribution of means by pruning treatment, the percentage of each sample population distributed by yield per tree in Figure 8, and as boxplot distribution of mean yields in Figure 9.

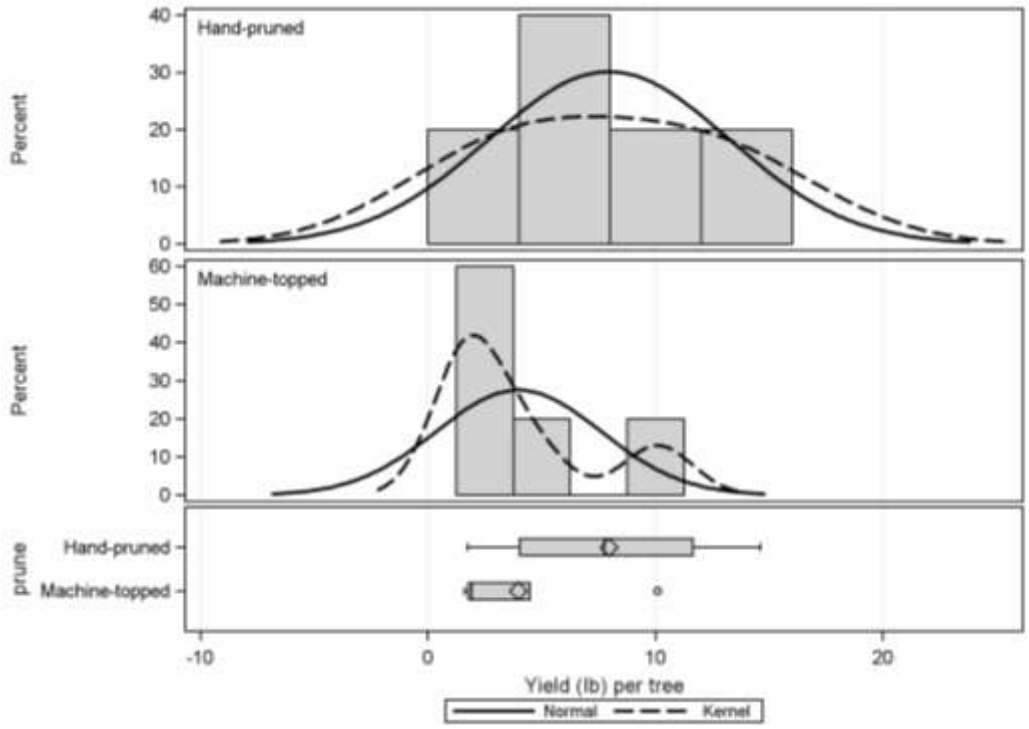


Fig. 9. This graph is the distribution of the pruning treatment means; the percentage of each sample population is distributed by yield per tree.

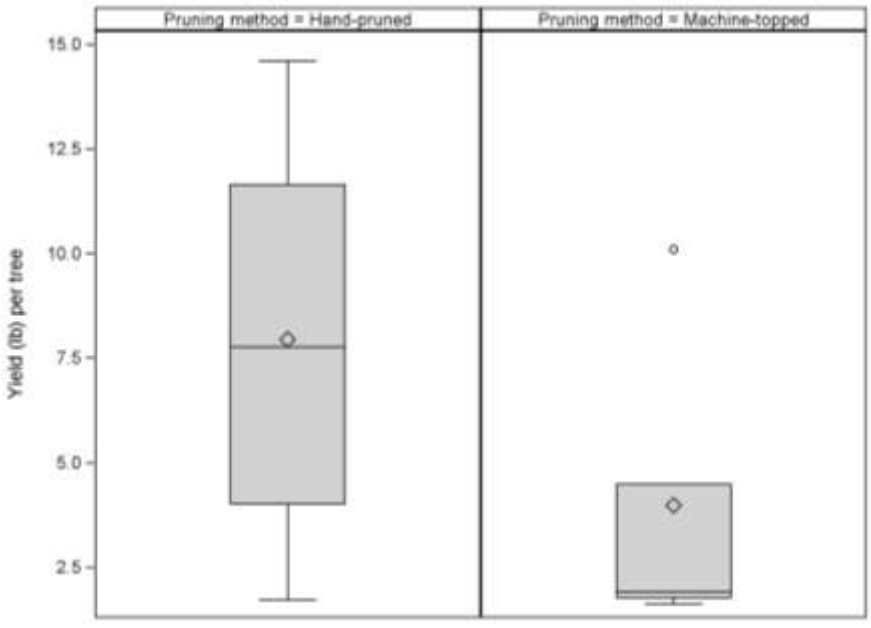


Fig. 10. Distribution of mean yields by pruning method; an extreme outlier is indicated by circle.

Results: Effect of Mechanical Topping and Hedging on Fruit quality and Value*
***The crop was so low these results and analyses are not reliable, as they do not represent a normal crop.**

There were no significant differences for any quality measure or value of the crop between mechanically hedged and topped trees versus the hand pruned control trees when evaluated by t-test or mixed linear model.

However, with the hand-pruned trees, there tended to be a higher variation, a greater spread in the distribution, in the percentages in different size grades compared to the mechanically pruned trees. Figure 10 demonstrates this greater spread an in size distribution for the large size olives in the hand pruning treatment.

Defects were minimal and the percentage of cannable fruit was high in both pruning treatments.

Effect of Pruning Method on Fruit Quality and Value:

Effect of Mechanical Pruning on Fruit Size Distribution: Processor A*

Rep	*Pruning method*	*Percentage in each size grade*								
		Extra large	*Large*	*Medium*	*Small*	*Petite*	*Sub-petite*	*Undersized*	*Cull*	*Trash*
1	*Hand*	75.4	5.9	3.2	1.6	0.5	0.5	0.5	11.8	0.5
	Machine	88.5	3.7	1.4	0.9	0.5	0.5	0.5	3.7	0.5
2	*Hand*	77.3	7.0	3.5	1.7	1.2	0.6	0.6	7.0	1.2
	Machine	87.2	5.9	2.0	1.0	0.5	0.5	0.5	2.0	0.5
3	*Hand*	84.9	4.5	3.6	2.1	0.9	0.3	0.3	2.7	0.6
	Machine	90.1	4.0	2.0	1.0	0.5	0.5	0.5	1.0	0.5
4	*Hand*	94.1	2.4	0.8	0.8	0.4	0.4	0.4	0.4	0.4
	Machine	91.2	3.9	1.6	1.0	0.6	0.3	0.3	0.6	0.3

Processor A only; Processor B received only one rep of 5 as limited crop limited samples and sizes.

Table 29. This table shows the effect of mechanical topping and hedging on fruit quality by replicate.

Effect of Mechanical Pruning on Adjusted Price per Ton and Value: Processor A*

*Processor A**				
Rep	*Pruning method*	*Adjusted total crop value ($)*	*Adjusted price per ton ($)*	*% Cannable fruit*
1	*Hand*	57.28	914.34	98.90
	Machine	95.99	1116.18	94.10
2	*Hand*	196.23	1182.10	103.30
	Machine	154.79	1223.62	101.00
3	*Hand*	127.95	1179.24	97.80
	Machine	122.30	1199.04	98.10
4	*Hand*	121.67	1210.62	98.20
	Machine	187.82	1219.60	99.00

*Processor A only; Processor B received only one rep of 5 as limited crop limited samples and sizes.

Table 30. This table shows the effect of mechanical topping and hedging on value by replicate.

Effect of Mechanical Pruning on Fruit Size Distribution: Processor A*.

Pruning method	*Percentage in each size grade*								
	Extra large	*Large*	*Medium*	*Small*	*Petite*	*Sub-petite*	*Undersized*	*Cull*	*Trash*
Hand	82.93	4.94	2.78	1.56	0.75	0.45	0.45	5.46	0.67
Machine	89.25	4.36	1.74	0.97	0.52	0.44	0.44	1.82	0.44
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS
Significant difference between means by T-test, 5% level. Significance within columns = ***, **,*, NS (0.001, 0.01, 0.05, respectively).									

Processor A only; Processor B received only one rep of 5 as limited crop limited samples and sizes.

Table 31. This table demonstrates the distribution of size grades was unaffected by pruning treatment.

Effect of Mechanical Pruning on Crop Value: Processor A*.

Pruning method	*Adjusted total crop value ($)*	*Adjusted price per ton ($)*	*% Cannable fruit*
Hand	97.14	1121.58	99.55
Machine	140.23	1189.61	98.05
Significance	NS	NS	NS
Significant difference between means by T-test, 5% level. Significance within columns = ***, **,*,NS (0.001, 0.01, 0.05, respectively).			

*Processor A only; Processor B received only one rep of 5 as limited crop limited samples and sizes.

Table 32. This table demonstrates mechanical pruning had no effect on crop value or % cannable fruit.

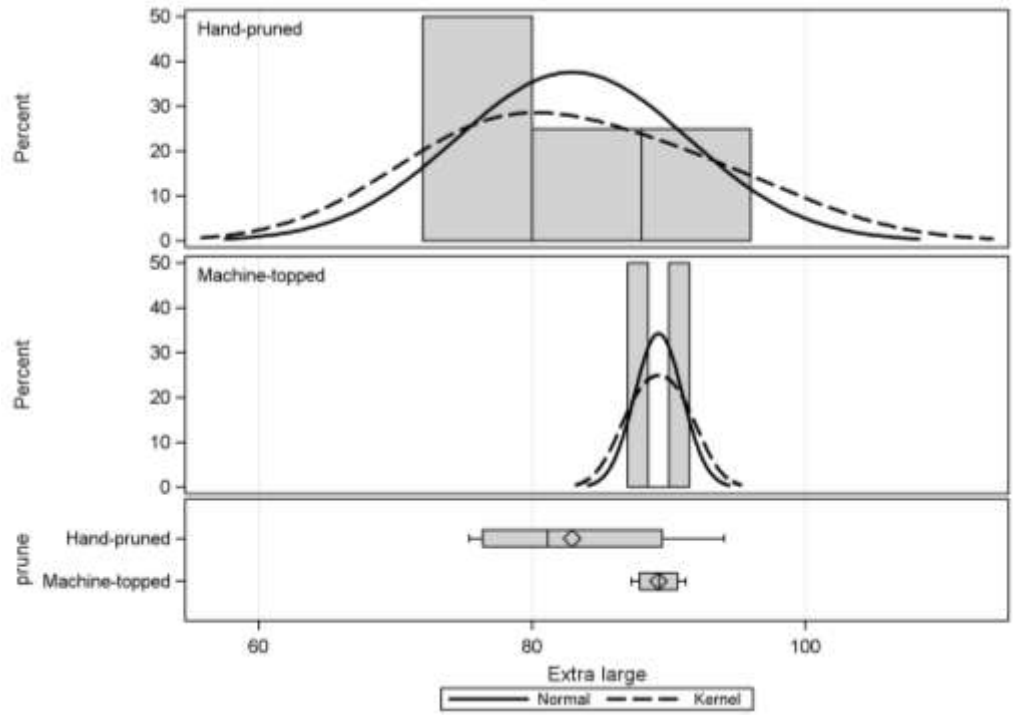


Figure 10.. Using only the large size fruit this graph demonstrates the greater size distribution of fruit sizes in the hand -pruned treatments.

Conclusion:

Effects of Mechanical Pruning versus control Hand Pruning: Location II

Mechanical topping and one-sided hedging strongly decreased yield in the year of treatment. There was no effect on any of the fruit quality or value parameters. However, the yields were so low that this data is not reliable.

Overall Summary and Conclusions: 2006 - 2011

Even with this fourth crop failure in seven years of research: 2006, 2008, 2009 and 2011, progress has been made:

1. Fruit damage with canopy contact harvesters has been eliminated.
2. The operating parameters for fruit removal with a canopy contact head have been have been determined though the head and rod dimensions, and head positioning, need to be changed.
3. 2011 trials strongly demonstrated a smaller, cheaper canopy contact harvester is possible.
4. Trunk damage with shakers has been markedly decreased.
5. Hedgerow training and mechanical pruning is demonstrating the ability to facilitate mechanical harvesting.

However, the major problem remains: final harvest efficiencies at 64-68%. Hopefully, by adapting the harvester modifications based on Dr. Miles analyses made during the 2011 harvest, developing an effective catch frame, and continuing the mechanical pruning trials in the 13 X 26 foot and 12 X 18 foot trials, combined with pruning during harvest, will increase final harvester efficiency.

Also, this may be the time for the COC to again to approach harvesting fabrication companies for canopy contact harvester development. Phil Scott of Agright, Claude Brown of AIM and Oxbo are among those we could approach.

Finally, Louise Ferguson visited the Israeli, Spanish and Italian olive research programs from September through early November 2011. Israel has little to offer us. Our cooperator, Dr. Sergio Castro-Garcia of University of Cordoba in Spain is doing extensive testing of trunk shaking harvesters, (11 total), evaluation of canopy contact heads and development of catch frames for the latter. Cooperating with him offers the best chance of accelerating our engineering improvements. The Italian researchers focus primarily on olive oil trees but the work that is applicable to the California table olive industry is that of Dr. Stephan Tombesi investigating the light levels required to produce fruiting. This research is defining the canopies that are most efficient for fruit production, generally smaller trees and higher density orchards.

Progress Report: Analytical Studies on the Volatile Flavor Constituents of Black-Ripe Olives

Angelina Sansone, Rachelle Woods and Gary Takeoka
Western Regional Research Service, Agricultural Research Service, U. S. Department of Agriculture, 800 Buchanan Street, Albany, CA 94710

Problem and its Significance

Imported black-ripe olives of varying quality have a significant market share in the United States, particularly in the food-service sector. A previous study (Lee, S. M. and Guinard, J. X., "Descriptive Analysis and Consumer Testing of Imported and Domestic Sliced Table Olives," 2009, UC Davis Olive Center) found that many imported olives had sensory defects such as metallic and soapy, and scored lower in consumer testing. Researchers from USDA and UC Davis are collaborating on research to correlate this sensory data with chemical markers.

Objectives

The first objective is to pinpoint and identify impact aroma compounds in black-ripe olives using gas chromatography-olfactometry (GC-O) and gas chromatography-mass spectrometry (GC-MS). The second objective is to identify and quantify volatile constituents responsible for the desirable (i.e., fishy/ocean-like, salty, earthy, and sour) and undesirable (i.e., alcohol, artificial fruity, gassy, rancid and oak barrel) flavor characteristics of black table olives produced in the U.S. (CA), Egypt, Morocco, Portugal and Spain. The third objective is to determine the origin and formation pathways of desirable and undesirable flavors with the goal of optimizing the production of desirable flavors and minimizing the formation of undesirable flavors through selected harvesting and processing parameters. The fourth objective is to develop a reliable automated method to isolate, separate and quantify flavor constituents as a means to rapidly assess black olive quality and desirability.

Materials and methods

Olive samples:

The following thirty-nine samples of black-ripe olives from different cultivars (Manzanilla, Mission, Manzanilla Fina, Picholine, Beldi, Hojiblanca, Caceneras, Picual, Agezi, Gordal, Kalamata, Carrasquena, Ascolano, Barouni, and Sicilian) and different countries of origin (U.S., Egypt, Morocco, Spain, Chile, Greece and Argentina) were studied.

Egypt (2) Festival, (Egypt, Manzanilla, E 576 09:30 SLB) p: 17/10/2007, e: 16/10/2010, can opened 4/21/2011

Morocco (1) Del Destino (Morocco, Beldi, MI 356P DL SLICED 16:53) expiration: 25/12/2010, can opened 4/28/2011

Spain (2) Jackpot (Spain, unknown variety, B13/03/08), no expiration date, can opened 5/4/2011

CA (3) Early California (USA, Manzanillo, T4T0188J), best by JUL 09 2012, can opened 5/11/2011

CA (5) Black Pearls (USA, Manzanillo, T4T0190Q), best by JUL 07 2012, can opened 5/20/2011

CA (4) Mt. Mike's (USA, Mission, 28VSS/175EEE 1341), no expiration date, can opened 5/31/2011

Spain Empress (Spain, Manzanilla, SLICED L-0 26S N 18 09:03), no expiration date, can opened 6/7/2011

Egypt Del Destino (Egypt, Manzanillo, W 10348 SR 13:37:00 S 00284 R.O 1461), no expiration date, can opened 6/14/2011

Morocco Food Meisters (Morocco, Picholine, MI 131 B OL SLICED 08:38), expiration date: 21/05/2013, can opened 6/17/2011

USA Lindsay (USA, Manzanillo, 210SS/70AA 0737), best by 03/01/2015, can opened 6/22/2011

USA Musco Pearls (USA, Manzanillo, T4T0176M), best by Mar 12 2014, can opened 6/27/2011

Spain Roma (Spain, Hojiblanca, S 01-10-2012 26), no expiration date, can opened 6/30/2011

Egypt Bellissimo (Egypt, Manzanillo, W 10330 SR 17:29:14 S 00259 R.O 1370), no expiration date, can opened 7/6/2011

Spain Del Destino (Spain, Hojiblanca, 10333 AG 14:59 SRHO) no expiration date, can opened 7/11/2011

Caceneras (storage spoilage) (USA, Caceneras, 40MFP/102B 1006 (top), 048101532 S756C669C (side), no label, can opened 7/14/2011

Spain Valencia Hojiblanca (Spain, Hojiblanca, 11073AG SRHO11:56), distributed by Bell-Carter, no expiration date, can opened 7/19/2011

Argentina Bell Carter Picual (Argentina, Picual, 17 PLP/193HH 2210), no label, can opened 7/25/2011

Spain Gordal (Spain, Gordal, T2QP084P (bottom) 180102138 N7560669C (side), no label, best by JUL 09 2014, processed in the U.S. by Musco, can opened 7/28/2011

Argentina Manzanilla fina (Argentina, Manzanilla fina, T3FP052L (bottom) 183100551 S756C669C (side), no label, best by JUL 21 2014, processed in the U.S. by Musco, can opened 8/2/2011

Musco Hojiblanca (Spain, Hojiblanca, T3IP1301 (bottom) 334092354 N756C669C (side) 1564D5 (written in marker)), no label, best by FEB 04 2014, processed in the U.S. by Musco, can opened 8/10/2011

Egypt Agezi, (Egypt, Agezi, T2EP056J (bottom) 314090035 N756C669C (side)), no label, best by DEC 10 2013, processed in the U.S. by Musco, can opened 8/15/2011

Greece Kalamata, (Lindsay Greek Kalamata Pitted Olives, Greece, Kalamata, 09089PPS1), best by 9/30/2011, jar opened 8/18/2011

Spain Gordal Bell Carter, (Spain, Gordal, 40WCP/344KK 04:00), no label, no expiration date, processed in the U.S. by Bell-Carter, can opened 8/23/2011

Spain Carrasquena (Spain, Carrasquena, T3RP090G (bottom) 091102310 S756C669C (side), best by APR 08 2014, processed in the U.S. by Musco, can opened 8/29/2011

U.S. Ascolano (U.S., Ascolano, 11SLP/15500 0956), processed in the U.S. by Bell-Carter, can opened 9/1/2011

Spain Manzanilla (Spain, Manzanilla, 41OLP/76AA 06:19), no expiration date, processed in the U.S. by Bell-Carter, can opened 9/28/2011

Bell-Carter Barouni (U.S., Barouni, 110HP/22200 1412), processed in the U.S. by Bell-Carter, can opened 10/4/2011

Lindsay Adventures (U.S., Sicilian, P9P/125D 19:01), processed in the U.S. by De Lallo in Orland, CA, jar opened 10/11/2011

Argentina Manzanilla (Argentina, Manzanilla, 190HP/14DD 14:36), processed in the U.S. by Bell-Carter, can opened 10/26/2011

Spain Manzanilla, (Spain, Manzanilla, T3IP066K). Best by MAR 24 2014, processed in the U.S. by Musco, can opened 10/31/2011

Tuscan Garden (Spain, unknown, (MI01.19CT) L-JCDA/3181 20:16), best by 02/03/2013, Aldi Inc. can opened 11/8/2011

Chile Manzanilla Fina (Chile, Manzanilla Fina, 1BP132L), best by Jun 14 2014, can opened on 10/31/2011

BC Commercial Manzanilla (U.S., Manzanilla, 410LP/730, 1233), can opened on 11/3/2011

Tuscan Garden Classics large olives pitted (Spain, (MI01-19CT) L-JCDA/3181 20:15), best by 2/3/2013, can opened on 11/8/2011

Chile Manzanilla Fina (Chile, Manzanilla Fina, T1BP132L), best by Jun 14 2014, can opened on 11/14/2011

Spain Mario black olives sliced (Spain), best by Nov 02 2013, 23:09, can opened on 11/18/2011

Musco Family Olive Co., Pearls sliced (California, T1T0148R), best by Dec 22 2014, can opened on 12/5/2011

Lindsay sliced olives (California, 210SS/152C 1141), best by 06/01/15, can opened on 12/8/2011

Spain LaRomanella sliced black ripe olives (Spain, L-1 014 E10 07:46), best by 01/2014, can opened on 12/13/2011

Chemicals:

Crystalline sodium chloride (USP/FCC/EP/BP/JP) and anhydrous diethyl ether (certified ACS grade) were purchased from Fisher Scientific. Sodium chloride was heated at 130˚C for 5 hours to remove volatiles. Diethyl ether was freshly distilled through a 60 cm long Pyrex column packed with glass helices and stored in the dark after the addition of 1-2 mg/L of antioxidant 330 (1,3,5-trimethyl-2,4,6-tris[3,5-di-*ter*-butyl-4-hydroxybenzyl]benzene; Ethyl Corp., Richmond, VA). The internal standards 3-hexanone, 2-octanone, and 1-methoxy-4-(prop-1-enyl)benzene (*trans*-anethole) were purchased from Sigma-Aldrich (Milwaukee, WI).

Isolation of volatiles:

Olive samples (30-35 g) were mixed with 75 mL of Milli-Q water and blended for 30 s in a Waring blender. One milliliter of internal standards (50 mg of 3-hexanone, 50 mg of 2-octanone, 12 mg of anethole in 500 mL of 75:25 water:methanol) was added to the mixture which was then blended for an additional 30 s. The mixture was added to a 1 L round-bottom flask containing 54 g of previously heated NaCl. The blender was rinsed with 75 mL of Milli-Q water which was added to the flask. A Tenax trap (10 g of Tenax in a glass column 14 × 2.2 cm) was attached to the flask, and an all-Teflon diaphragm

pump (model UN726 FTP, KNF Neuberger, Inc., Trenton, NJ) was connected (via Teflon tubing) after the trap. The loop was closed by connecting the outlet of the pump to the 1 L flask. The pump circulated air at a flow rate of ~ 6 L/min through the system for 3 h. The olive slurry was continuously stirred during sampling with a magnetic stirrer. The trap was eluted with 70 mL of freshly distilled diethyl ether. The eluate was concentrated to a final volume of about 100 μL using a Vigreux column (15 × 1 cm) and water bath at 40 °C. Extracts were analyzed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS).

Gas chromatography:

A Hewlett-Packard 6890 Series gas chromatograph equipped with a flame ionization detector (FID) (Hewlett-Packard, Avondale, PA) was used. A DB-1 fused-silica capillary column (60 m × 0.25mm i.d., d_f = 0.25 μm; J & W Scientific, Folsom, CA) was employed. The oven temperature was programmed from 30 ˚C (4 min isothermal) to 200 ˚C at 2 ˚C/min (held for 25 min). Helium carrier gas was used at a headpressure of 37 psi. Split injection (1:25) was used. The injector and detector temperatures were 180˚C and 260˚C, respectively.

Gas chromatography-mass spectrometry:

An Agilent Technologies 6890 gas chromatograph coupled to an Agilent Technologies 5973 Network MSD (Agilent Technologies, Palo Alto, CA) was used. A 60 m × 0.25 mm (i.d.) DB-1ms (d_f = 0.25 μm) fused silica capillary column was employed. The oven temperature was programmed from 30 °C (4 min isothermal) to 200 °C at 2 °C/min (final hold = 25 min). Helium carrier gas was employed at a headpressure of 22 psi. MS data were recorded in the electron impact mode with an ionization voltage of 70 eV. The injector, transfer line, ion source and quadrupole temperatures were 180 °C, 200 °C, 170 °C and 130 °C, respectively.

Identification of volatiles:

Identifications of food constituents were made using capillary gas chromatography-mass spectrometry and comparing obtained spectra to that in WRRC's MS library as well as to commercial MS libraries that we have purchased (i.e., Wiley Registry of Mass Spectral Data, 8th Edition, NIST05 Mass Spectral Library, Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Edition [Robert P. Adams], MassFinder 4 [Dr. Hochmuth Scientific Consulting]). Spectral libraries were used for tentative identification but then spectra and retention indices were compared directly to that of authentic standards for more accurate identification.

Quantification of Table Olive Volatiles:

The peak area ratio of constituent to internal standard was used for quantification. Internal standards were used as follows: (i) 3-hexanone for compounds with retention

indices less than 900, (ii) 2-octanone for compounds with retention indices between 900 to 1050, and (iii) *trans*-anethole for compounds with retention indices greater than 1050.

Calculation of Odor Units:

The odor units (odor activity values) were calculated by dividing each volatile's concentration by its odor threshold in water. The water content of canned, black-ripe olives is about 73% (USDA 1977). Odor thresholds were determined by the USDA lab in Albany, CA or were obtained from the literature.

Results and Discussion

The flavor constituents of various samples of domestic and imported black olives were identified and quantified. Volatiles were isolated by dynamic headspace sampling. The isolated volatiles were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Flavor constituents were identified by comparing the unknown's retention index and mass spectrum to that of an authentic reference standard. The constituents were quantified using the following internal standards: 3-hexanone, 2-octanone and *trans*-anethole. Each sample was analyzed in triplicate (i.e., three separate extractions were performed on each sample). A total of 39 samples were studied. GC and GC-MS analyses have been completed on all the olive samples. The large amount of data generated in this study is still being analyzed and interpreted.

Flavor constituents identified in domestic olives included hexanal (green odor), benzaldehyde (almond, cherry odor), 2-pentylfuran (green, vegetable odor), octanal (honey, citrus odor), benzyl alcohol (berry, cherry, grapefruit, citrus, walnut odor), phenylacetaldehyde (honey, nutty odor), octanol (orange, floral odor), 2-methoxyphenol (burnt, smoky odor), methyl benzoate (fruity odor), nonanal (citrus, vegetable odor), linalool (citrus, floral, sweet odor), (*E*)-4,8-dimethyl-1,3,7-nonatriene, ethyl benzoate (floral, plum, vanilla odor), 2-methoxy-4-methylphenol (smoky, chocolate, clove, sweet, vanilla, coffee odor), propyl benzoate (nutty, fruity, sweet odor), theaspirane A and B (green, spicy, woody, vanilla odor), β-damascenone (apple, woody, wine-like, herbaceous, rose odor), α-copaene (woody odor). Domestic olives had a similar profile of flavor constituents while imported olives had a very different composition of flavor compounds. The imported olives contained the previously listed constituents but also contained additional volatiles such as 2-methyl-1-butanol, 3-methyl-1-butanol, styrene, ethyl hexanoate, methyl octanoate, ethyl octanoate, and ethyl decanoate. Another constituent identified in some of the imported samples was ethyl cyclohexanecarboxylate. This compound is an extremely potent odorant with an odor threshold of 0.001 ppb or 1 ppt. We believe that this compound is the major contributor to the artificial fruity off-flavor obseved in some imported olive samples. These compounds provide strong evidence that imported olives (from Spain, Egypt and Morocco) had been fermented by yeasts and/or molds prior to processing. It is believed that the fermentation produced off-flavors in the imported samples. It is not known whether the fermentation was intentional or the result of improper handling and storage prior to processing. There were no signs of

fermentation in the domestic olives. All of the imported olive samples contained moderate to high levels of styrene (up to 797 ppb) while styrene was not detected in the domestic samples. It is important to determine the source of styrene in imported olives since this compound was recently added to a government warning list (12th Report on Carcinogens (RoC) compiled by the National Toxicology Program (NTP)) of known and potential carcinogens. The concentration of styrene found in imported olives was about 100 times higher than had been previously reported in foods such as wheat, oats, peanuts, pecans, coffee beans, tomatoes, peaches, strawberries, beef, chicken and milk. These results are critical for ensuring the safety and acceptability of our food supply.

A list of the constituents identified in black-ripe olives is shown in Table 1. The constituents are listed in increasing elution order and include the experimental and reference Kovats' retention index (from authentic standards or literature) and means of identification.

Table 1. Volatile compounds identified in black-ripe olives

No.	Compound	Kovats' Retention Index (DB-1)		Means of Identification
		Experimental	Reference	
1	3-methylbutanal	636	636	MS, RI
2	2-methylbutanal	646	646	MS, RI
3	(*E*)-2-butenol	658	643	MS, RI
4	pentanal	676	668	MS, RI
5	3-hydroxy-2-butanone	680	674	MS, RI
6	propyl acetate	700	695	MS, RI
7	heptane	700	700	MS, RI
8	(*E*)-2-methyl-2-butenal	720	715	MS, RI
9	3-methylbutanol	725	714	MS, RI
10	2-methylbutanol	729	718	MS, RI
11	toluene	754	748	MS, RI
12	pentanol	759	744	MS, RI
13	hexanal	779	772	MS, RI
14	octane	800	800	MS, RI
15	furfuryl alcohol	837	827	MS, RI
16	ethyl 2-methylbutanoate	841	832	MS, RI
17	ethyl 3-methylbutanoate	844	834	MS, RI
18	(*Z*)-3-hexenol	844	834	MS, RI
19	*p*-xylene	857	834	MS, RI
20	hexanol	860	848	MS, RI

Table 1. Continued

No.	Compound	Kovats' Retention Index (DB-1)		Means of Identification
		Experimental	Reference	
21	cyclohexanone	860	858	MS, RI
22	3-methylbutyl acetate	865	855	MS, RI
23	2-methylbutyl acetate	868	858	MS, RI
24	styrene	873	873	MS, RI
25	heptanal	880	876	MS, RI
26	2-butoxyethanol	889	886	MS, RI
27	benzaldehyde	926	926	MS, RI
28	α-pinene	930	929	MS, RI
29	3-ethylpyridine	930	930	MS, RI
30	(*E*)-2-heptenal	931	931	MS, RI
31	3-ethenylpyridine	937	932[a]	MS, RI
32	camphene	941	941	MS, RI
33	1-octen-3-one	959	953	MS, RI
34	heptanol	960	951	MS, RI
35	2,3-octadione	964	959	MS, RI
36	6-methyl-5-hepten-2-one	967	970	MS, RI
37	β-pinene	968	971	MS, RI
38	phenol	970	971	MS, RI
39	2-pentylfuran	981	977	MS, RI
40	octanal	982	979	MS, RI
41	ethyl hexanoate	985	981	MS, RI
42	(*Z*)-3-hexenyl acetate	990	986	MS, RI
43	hexyl acetate	997	995	MS, RI
44	(*E,E*)-2,4-heptadien-1-ol (tentative)	999	NA	MS
45	benzyl alcohol	1003	1004	MS, RI
46	phenylacetaldehyde	1003	1007	MS, RI
47	α-terpinene	1006	1008	MS, RI
48	*p*-cymene	1009	1010	MS, RI
49	2-ethylhexanol	1016	1013	MS, RI
50	1,8-cineole	1016	1018	MS, RI
51	limonene	1020	1020	MS, RI
52	(*E*)-2-octenal	1033	1032[b]	MS, RI
53	β-(E)-ocimene	1041	1037	MS, RI
54	3-methylbutyl butanoate	1043	1041	MS, RI
55	γ-terpinene	1049	1048	MS, RI
56	*p*-cresol	1057	1059	MS, RI

[a]Tsuneya *et al*. (1993).[b] Takeoka *et al.* (1992).

Table 1. Continued

No.	Compound	Kovats' Retention Index (DB-1)		Means of Identification
		Experimental	Reference	
57	3-ethyl-4-methylpyridine	1057	1059	MS, RI
58	octanol	1059	1062	MS, RI
59	2-methoxyphenol	1059	1063	MS, RI
60	methyl benzoate	1067	1066	MS, RI
61	α–terpinolene	1078	1077	MS, RI
62	propyl hexanoate	1080	1077	MS, RI
63	2-phenylethanol	1081	1081	MS, RI
64	nonanal	1083	1082	MS, RI
65	linalool	1085	1083	MS, RI
66	fenchyl alcohol	1094	1097	MS, RI
67	trigonelline (tentative)	1099	NA	MS
68	(E)-4,8-dimethyl-1,3,7-nonatriene	1106	1105	MS, RI
69	methyl octanoate	1108	1107	MS, RI
70	ethyl cyclohexanecarboxylate	1110	1110	MS, RI
71	camphor	1121	1118	MS, RI
72	benzyl acetate	1133	1131	MS, RI
73	4-ethylphenol	1143	1141	MS, RI
74	ethyl benzoate	1143	1143	MS, RI
75	nonanol	1158	1155	MS, RI
76	terpinen-4-ol	1158	1159	MS, RI
77	2-methoxy-4-methylphenol	1163	1163	MS, RI
78	methyl salicylate	1166	1166	MS, RI
79	α-terpineol	1170	1170	MS, RI
80	2-decanone	1173	1172	MS, RI
81	ethyl octanoate	1182	1180	MS, RI
82	decanal	1184	1184	MS, RI
83	4-vinylphenol	1191	1190	MS, RI
84	2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,7,9-(1)-triene (tentative)	1196	1196[b]	MS[c], RI
85	branched $C_{13}H_{26}$ HC (tentative)	1207	NA	NA
86	2-methoxyphenyl acetone(tentative)	1210	NA	MS
87	2-phenylethyl acetate	1225	1224	MS, RI

[b]Takeoka *et al*. (1992). [c] Buttery *et al.* (1990a).

Table 1. Continued

No.	Compound	Kovats' Retention Index (DB-1)		Means of Identification
		Experimental	Reference	
88	(*E*)-2-decenal	1236	1236	MS, RI
89	2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,7,9-(1)-triene related (tentative)	1239	1236[b]	MS[d], RI
90	propyl benzoate	1242	1242	MS, RI
91	ethyl salicylate	1242	1244	MS, RI
92	4-ethyl-2-methoxyphenol	1250	1250	MS, RI
93	(*E*)-2-decenol	1253	1251	MS, RI
94	vitispirane	1266	1263[b]	MS, RI
95	ethyl nonanoate	1281	1279	MS, RI
96	(*E,E*)-2,4-decadienal	1287	1288	MS, RI
97	theaspirane A	1287	1289	MS, RI
98	nonyl acetate	1290	1293	MS, RI
99	2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,7,9-(1)-triene related (tentative)	1291	1290[d]	MS[d], RI
100	theaspirane B	1300	1304	MS, RI
101	methyl decanoate	1307	1307	MS, RI
102	ethyl 3-phenylpropionate	1316	1316	MS, RI
103	methyl 4-formylbenzoate (tentative)	1316	NA	MS
104	1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene (tentative)	1335	1334[b]	MS[d], RI
105	undec-2-enal	1339	1339	MS, RI
106	β-damascenone	1359	1360	MS, RI
107	cyclosativene	1364	1367	MS, RI
108	α-copaene	1372	1374	MS, RI
109	ethyl decanoate	1380	1379	MS, RI
110	(E)-ethyl cinnamate	1428	1431	MS, RI
111	β-farnesene	1433	1447	MS, RI
112	α-muurolene	1489	1492	MS, RI
113	α-farnesene	1495	1496	MS, RI
114	β-sesquiphellandrene	1511	1513	MS, RI

[b]Takeoka *et al.* (1992). [d]Buttery *et al.* (1990b).

The concentrations and odor unit values have been calculated for the flavor constituents of 11 of the 39 olive samples. We are in the process of completing the calculations for the remaining samples.

Interim report: Correlating sensory data and chemical markers of black-ripe olives for USDA inspectors

Kamolnate Kitsawad, Soh Min Lee, Chirat Sirimuangmoon & Jean-Xavier Guinard

Objective

The purpose of this project is to find chemical markers of sensory quality through the correlation of sensory descriptive and instrumental measurements of the flavor of black-ripe table olives.

Materials and Methods

The sensory component of the study is examining the sensory properties of 10 sliced black ripe table olives from different cultivars (Manzanilla, Manzanilla Fina, Augezi, Gordal, Carrasquena, Barouni, and Ascolano), countries of origins (US, Chile, Spain, Argentina, and Egypt), and processors (Musco Family Olives and Bell-Carter) using descriptive analysis. The labels used for the 10 samples throughout the report are shown in Table 1.

Table 1 – Table olive samples

labels	Country of origin	Brand name	Varietal	Distributor	Store (city)
E1	Egypt	Del Destino	-	Roma	Roma (Livermore)
E2	Egypt	Bellissimo	-	Bellissimo Foods	Cheeseman (Washington)
M1	Morocco	Foodmeisters	Picholine	Cash & Carry	Cash & Carry (Modesto)
S1	Spain	Del Destino	-	Atalanta Corp.	Mike Hudson (Petaluma)
S2	Spain	Roma	Hojiblanca	Roma	Roma (Livermore)
S3	Spain	Empress	Manzanilla	Mitsui Foods	US Foodservice (Livermore)
S4	Spain	La Romanella	-	Smart & Final	Smart & Final (Pleasanton)
S5	Spain	Supremo Italiano	-	Restaurant Depot	Restaurant Depot (San Jose)
U1	USA	Lindsay	Manzanilla	Restaurant Depot	Restaurant Depot (San Jose)
U2	USA	Black Pearl	Manzanilla	Musco Family Olive	N/A

Descriptive analysis

The sensory properties of the olives were measured by descriptive analysis with a trained panel of 7 judges (6 females, 1 male) all of them undergraduate or graduate students at UC Davis.

The panel developed a scorecard with 35 attributes of appearance, flavor (taste and smell), texture, mouthfeel and after-taste (Table 2). After the panel training, all the products were evaluated in triplicate, following a randomized complete block design. Olives without the brines (1 fl. oz.) were presented at room temperature (20 °C), in a spherical glass covered with a plastic lid. The intensity of the attributes was rated on a category line scale labeled with "low" and "high" at the ends of the scale, except for some of the appearance attributes and the lasting flavor which used the labels shown in Table 2 below.

Table 2 – Sensory attributes evaluated in the descriptive analysis

	Attribute	References		Attribute	References
SMELL (AROMA)	Briny/Salty	Olive brine	TASTE/FLAVOR	Overall flavor intensity	-
	Ocean-like	Seaweed*		Saltiness	NaCl solution
	Sugary/Sweet smell	1) Honey* 2) brown sugar*		Sweetness	Sugar solution
	Fermented/Vinegar	1)Sauerkraut* 2) apple cider vinegar*		Bitterness	Caffeine solution
	Sautéed mushroom	Sautéed mushroom*		Umami	MSG + brine
	Earthy/Musty	Potting soil*		Buttery	Melted butter
	Metallic/Canned	Iron tablet solution*		Soapy	Soap*
	Woody	Oak barrel*		Gassy/Propane-like	n/a
	Floral	Chrysanthemum tea*	TEXTURE/MOUTHFEEL	Firmness/Hardness	-
	Fruity	Artificial raspberry extract*		Crunchiness	-
	Green/Grassy	Parsley*		Chewiness	-
	Painty/ Solvent-like	Correction fluid*		Juicy/Moisture release	-
	Rancid/Oxidized	Rancid olive oil*		Astringency/Dryness	Steeped green tea
	Alcohol	Vodka*		Mouthcoating	-
APPEARANCE	Size	Small --- big	AFTERTASTE	Lasting flavor	
	Glossy/Shiny	Matte --- Glossy			
	Skin brownness	(Dark brown color scale)			
	Gradation (Flesh)	Less gradient --- More gradient			
	Brightness-Grey/Green (Flesh)	(Grey/green color scale)			
	Brightness-Grey/Brown (Flesh)	(Light brown color scale)			-

*mixed with olives

Data analysis

The descriptive analysis data was analyzed using a combination of univariate and multivariate statistics. Analysis of variance (ANOVA) was used to examine the effect of each source of variation in the design. Principal component analysis (PCA) was then applied to the matrix of mean intensity ratings across the samples to visually summarize the similarities and differences among the products in the design.

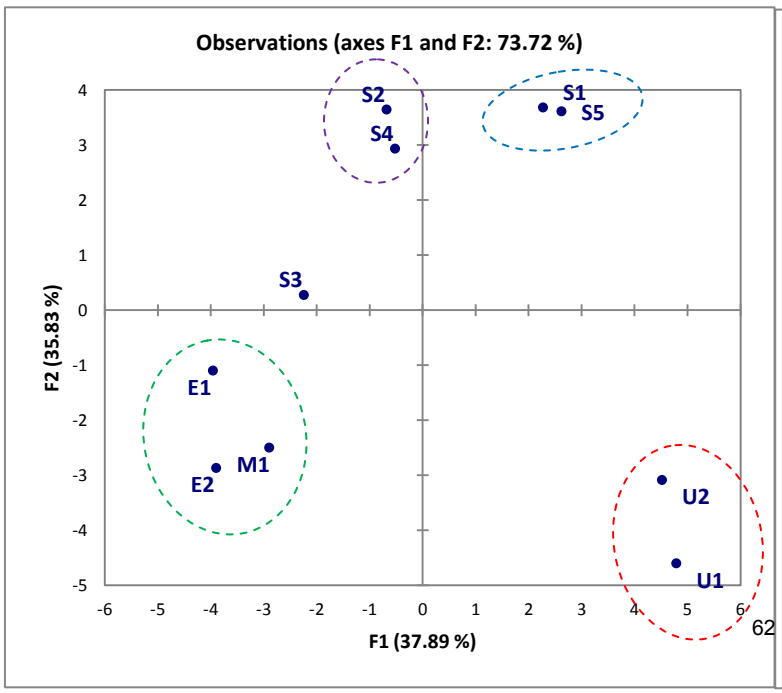
Results and discussion

The ANOVA showed that the 10 sliced olives differed significantly ($P<0.05$) in the intensity of the following sensory attributes:

- Aroma: Ocean-like, Sugary/Sweet smell, Fermented/Vinegar, Sautéed mushroom, Fruity, Painty/Solvent-like, Rancid/Oxidized, and Alcohol
- Appearance: Size, Glossy/Shiny, and Brightness- Grey/Brown (Flesh)
- Taste/Aroma: Overall flavor intensity, Saltiness, Bitterness, Umami, Buttery, and Soapy
- Texture/Mouthfeel: Firmness/Hardness, Crunchiness, Chewiness, Juicy/Moisture release, and Astringency
- Aftertaste: Lasting flavor

The principal component (PC) biplot shows the main sensory features of each table olive product in the design – attributes located close to a given sample tend to be higher for that sample, whereas attributes which are found away from that sample tend to be lower. It also depicts the relationships among the sensory attributes – attributes which are positively correlated tend to form small angles with each other or to be clustered together on the plot, whereas attributes which are negatively correlated are found at opposite ends of the plot.

The biplot of PC1 vs. PC2 is shown in Figure 1 below. PC1 and PC2 explained 73.72% of the variation among products.



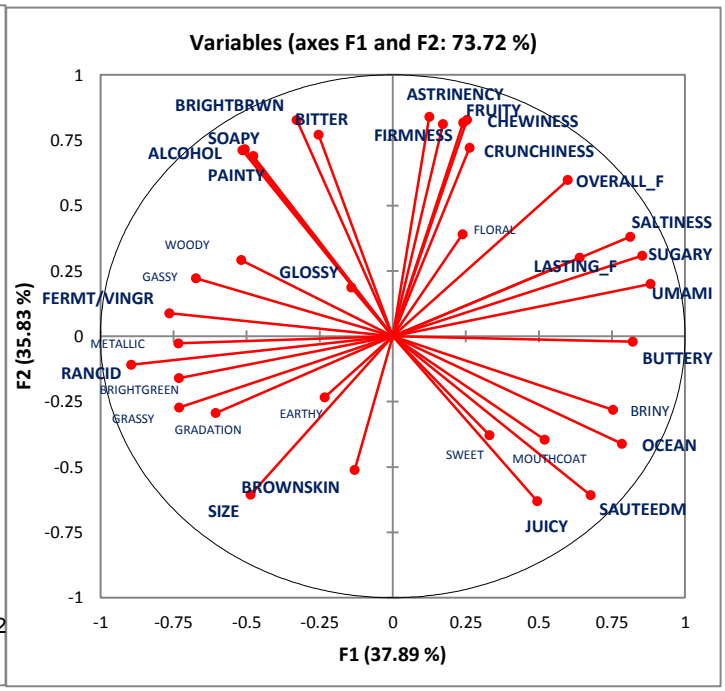


Figure 1 - Principal component analysis of the descriptive analysis data showing the products (left) and sensory attributes (right).

- **There was a clear separation of the table olives based on region of origin, that is, each region produced a distinct set of sensory characteristics. This result only applies to this set of olives and may not be generalized to all olives produced in these countries, however.**
- **The main separation was observed between olives from the US and olives from Epypt and Morocco, along PC1 (the main dimension of variation, 37.9%).** The main attributes in these products can be summarized as follows:

  - USA (U1 and U2):

  Ocean-like, Sautéed mushroom, Sugary/Sweet smell (Aroma);

  Buttery, Umami, Saltiness (Taste/Flavor);

  Juicy/Moisture release (Texutre/Mouthfeel);

  Lasting flavor (Aftertaste)

  - Egypt, Morocco (E1, E2 and M1):

  Rancid, Fermented/vinegar (Aroma);

  Size, Skin brownness (Appearance)

- **The next largest difference was observed between olives from the US and olives from Spain, along PC2 (35.8%).** The attributes of the Spanish olives were:

  - Spanish (S1, S5):

  Sugary, Fruity (Aroma);

  Overall flavor intensity, Saltiness, Umami, Buttery (Taste/Flavor);

  Firmness, Chewiness, Crunchiness, Astringent (Texture/Mouthfeel);

  Lasting flavor (Aftertaste)

  - Spanish (S2, S4):

  Fruity, Painty, Alcohol (Aroma);

  Bitter, Soapy (Taste/Flavor);

  Brightness of brown color (flesh), Glossy (Appearance);

Firmness, Chewiness, Crunchiness, Astringent (Texture/Mouthfeel)

- **S3** was different from the other Spanish olives and had strong fermented/vinegar and rancid attributes.

Preliminary conclusions

The main conclusion for now is that geographical region is the most important factor in differentiating the sensory characteristics for sliced black-ripe olives.

Remaining activities

In the next weeks, we will examine the relationships between the sensory descriptive data presented above and the instrumental analysis data from Dr. Takeoka's laboratory at the USDA, using Partial least square (PLS) regression in order to find chemical markers of desirable and undesirable flavor attributes for black-ripe olives.

PROJECT PLAN/RESEARCH GRANT PROPOSAL PROGRESS REPORT

Project Year: 2010– 2012 **Duration of Project: year 2 of 2**

Project Leader:
Art Hutcheson

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Title: Development and Performance Evaluation of the Hutcheson Mechanical Olive Harvester

Cooperator:
Louise Ferguson PhD
University of California, Davis

2012 Objectives:

I. Redesign of picking arm.

2012 Progress to Date:

I. As stated in interim report 2010 conclusion the two comb style picking heads showed promise but the limitations of the picking arm access to the tree canopy needed to be resolved.

II. The former picking arm was removed and replaced with an expandable scissor-type arm. Due to the construction, the hydraulic system to function the arm proved more complicated than expected and required more engineering. Therefore, the harvester was not ready to test.

III. This year's extremely short crop in the test sight would not allow enough time or olives to evaluate the effectiveness of the new arm.

IV. Currently work is being done to correct the design flaw and make functional for the 2012 season.

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

University of California
Division of Agricultural Sciences

ANNUAL PROJECT/RESEARCH GRANT REPORT
January 2012

Project Year: 2011 – 2012 **Duration of Project: Year 1 of 3**

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

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Overview. Olive knot is a disease that has been disseminated around the world with its olive host and occurs in all known olive production regions including California. Historically, the most susceptible cultivars of olive are: Manzanilla, Sevillano, Ascolano, and Mission, but many of the newer varieties are also susceptible. The disease is caused by the bacterial pathogen *Pseudomonas syringae* pv. *savastanoi* (syn. *P. savastanoi*) and is spread on vegetative host tissue. The pathogen produces tumors, galls, or knots on olive causing defoliation and branch dieback with subsequent reductions in yield, fruit size, and fruit quality. Fruit from diseased trees and the oil produced from these fruit often have off-flavors. Infections generally occur through branch wounds or other injuries including leaf scars. Branch infections that occur in late winter result in water-soaked cavities that contain masses of the bacterial pathogen. As the host begins its seasonal growth, a gall develops around the cavity where hosts cells enlarge (hypertrophy) and multiply (hyperplasia). Occasionally, secondary knots will form from the extension of lysigenous cavities from the original infection court. Due to some host specificity, pathovars have been proposed for the pathogen occurring on olive, oleander, and ash. Despite these differences found among populations of the pathogen, the official name has not been changed. The genetic variability of the pathogen has never been investigated in California and it is not known if a single clone or if several genotypes are present. Thus, this is being studied in one objective of our proposal.

Pathogen biology. The pathogen is a gram-negative, motile, rod-shaped bacterium with one to four polar flagella. Colonies are whitish to cream colored and smooth. The optimum temperature for growth is 23 to 24 C. The organism produces a fluorescent pigment on certain media. Acid production occurs on growth media containing glucose, galactose, and other carbon sources. Starch is also utilized as a carbon source. The organism is capable of producing indole acetic acid (IAA) that is involved in tumor induction on olive and oleander.

Epidemiology. The pathogen survives in the knots where it produces large numbers of cells throughout the year. The bacterium exudes to the gall surface during periods of wetness and is readily water splash-dispersed or may be disseminated by insects or birds. The wetness duration needed for the bacterium to exude to the gall surface is being investigated as part of our studies. The organism can survive on leaves

where it can be found at high populations in the spring. Leaf scars are considered the most common entry points for infection. Any biological (e.g., other diseases) or physical (e.g., mechanical harvesting) causes of defoliation and blossom scars, as well as mechanical injury from wind, pruning, or frost may increase the sites of infection. We initiated studies to determine the duration of susceptibility of injuries to infection and when injury sites are protected by wound healing. This is important because it will define when protective treatments during favorable environmental conditions need to be applied after injuries occur at harvest or from other causes such as hail storms. In California, infection occurs during the rainy season (late fall, winter, and spring) but the knots do not greatly enlarge until new growth starts in the spring. Infections can occur at fairly low temperatures (5-10 C) and thus, wetness is the main limiting factor for the disease.

Disease Control. Management of olive knot is difficult. Sanitation and prevention are the most successful strategies. Pruning and removal of the knots should be done during dry periods (i.e., summer and early fall). This will reduce inoculum and will avoid re-infection at pruning sites. Because the bacteria may be carried on pruning shears, frequent disinfection of equipment is also necessary. Any horticultural practice such as proper irrigation and fertilization programs that promotes growth and minimizes tree stress and results in less leaf drop will reduce infections. Painting galls with Gallex is a successful therapeutic treatment but is very labor intensive. Spray applications of copper-containing bactericides such as Kocide, Kentan, or Badge to protect leaf scars and other injuries have been very effective in minimizing the disease, but they often may need to be repeated to protect new wounds as they appear. A minimum of two applications is usually necessary: one in the fall before the rainy season starts and one in the spring when most leaves have been shed. Recently, new copper formulations (e.g., Kocide 3000, Badge X2, etc.) have been developed to reduce the metallic copper equivalent used in the orchard or grove and to ultimately reduce the amount of copper in the environment. Based on insufficient control of the disease after copper applications, resistance of *P. syringae* pv. *savastanoi* against copper is thought to be common in California, but the extent of resistance is not known.

Based on efforts of our laboratory, recent advances have been made in bacterial disease control with the identification and development of the antibiotic kasugamycin (commercial name Kasumin) for fire blight management on pome fruit and other bacterial diseases of agronomic crops in the United States and elsewhere. The US-EPA registration of Kasumin is pending in 2012. This antibiotic has high activity against *Erwinia* and *Pseudomonas* species and moderate activity against Xanthomonas species and other plant pathogenic bacteria. Our research continues on fire blight and walnut blight and we are currently evaluating the antibiotic against bacterial canker and blast of sweet cherry caused by *Pseudomonas syringae* pv. *syringae* with promising results. As part of this research project we started to evaluate this antibiotic for the management of olive knot and preliminary data are presented in this report.

Additionally, we have also been working on sanitation and biological controls of bacterial diseases of tree crops and we have identified two promising materials that were included in initial greenhouse and field trials. The sanitation treatment kills the pathogen on contact even in wounds where other sanitizers have failed. It has an "exempt status" when applied after harvest and will not affect or leave residues on the harvested crop. Thus, this treatment could be applied to olive trees immediately after harvest to sanitize injuries that were contaminated with the olive knot pathogen at harvest. Biocontrols have several different mechanisms to prevent disease including site exclusion by prolific growth and possibly production of secondary metabolites that function in antibiosis and thus, exclude competitors. Additionally, their persistence could provide a continuous level of protection for extended periods. We evaluated several biocontrols on other tree crops in recent years and demonstrated a reduction of disease in some cases. Thus, these new strategies can be evaluated and developed for an integrated approach with standard copper treatments towards managing olive knot in a highly effective program.

2011 OBJECTIVES:

1) Collection of strains, occurrence of inoculum availability, and period of susceptibility of selected injuries (leaf scars, pruning injuries, etc.) to infection (previous reports indicated 7 to 14 days in one season and shorter periods in other seasons).
    a. Collection of strains to determine genetic variability of populations using molecular approaches.

   b. Monitor galls for production of inoculum over time in relation to conducive environments (e.g., rainfall)
   c. Duration of susceptibility of injuries in selected seasons (e.g., fall, spring, summer)
2) Evaluate populations of the pathogen for laboratory sensitivity to:
   a. Fixed copper compounds
   b. Agricultural chemicals such as kasugamycin (Kasumin), oxytetracycline (Mycoshield), and streptomycin (Agricultural streptomycin) and develop baseline sensitivities of the pathogen to appropriate selected materials.
   c. Biologicals - Blossom Protect, Actinovate, and others
3) Evaluation of protective treatments such as new copper formulations, antibiotics such as Kasumin, Mycoshield, combinations of fungicides and copper or antibiotics, and biologicals to determine optimal usage strategies to obtain high performance and efficacy.
   a. Field trials with and without adjuvants to determine optimal performance (i.e., rates)
   b. Timing trials – spring leaf drop or after harvest treatments. Note: copper or antibiotics will not be used as preharvest treatments.

MATERIALS AND METHODS:

Collection of strains and genetic diversity. Olive knots were collected in collaboration with farm advisors and PCAs. Knots were surface-sterilized with sodium hypochlorite, internal tissue was removed, suspended in sterile water, and the suspension was plated onto KMB medium. Single bacterial colonies were cultured and species identity was verified using primers that target the IAA-lysine synthase gene (Penyalver et al., 2000). The genetic diversity was determined using rep PCR with BOX and REP primers and separation of amplified DNA fragments in agarose gels.

Evaluation of inoculum production and injury susceptibility. Olive knots were sampled in the summer of 2011 and taken to the laboratory. Three knots for each of three replications were soaked in sterile water for up to 24 h. The soaking solutions were periodically sampled, plated onto KMB medium, and colonies of the pathogen were enumerated.

In a wound-healing study in a commercial orchard, terminal twig cuts and lateral wounds (approximately 5 mm in diameter) were made in late October 2011. Wounds were inoculated with *P. syringae* pv. *savastanoi* (10^7 cfu/ml) by hand-spraying immediately after wounding and after 1, 2, or 3 weeks. Inoculated wounds were covered with Parafilm for one week. No knot formation was evident as of mid-January 2012 and thus, wounds will be periodically evaluated throughout the spring of 2012.

Evaluate populations of the pathogen for sensitivity to copper, antibiotics, and sanitizers in the laboratory. The diversity of chemicals evaluated required that assays had to be modified for each chemical type. Thus, three direct exposure assays were done. (1) Sensitivity to copper was evaluated in a continuous exposure assay where the pathogen was grown in wells of microtiter plates. For this, *P. syringae* pv. *savastanoi* was incubated for 24 to 48 h in liquid growth medium without and with the addition of copper at selected concentrations. Measurement of the optical density at 600 nm was used to determine bacterial growth. Growth in the presence of copper was compared to that of the water control. (2) Sensitivity to kasugamycin was determined using another continuous exposure assay, the spiral gradient dilution assay. For this, bacterial suspensions were plated onto agar amended with a continuous gradient of kasugamycin concentrations that was generated using a spiral plater. Reduction and 95% inhibition of bacterial growth was determined after two days. (3) Kasugamycin and the sanitizers Quat 2 Plus, Deccosan 321, Deccosan 315, Vantocil, and chlorhexidine were evaluated in a short-duration exposure assay. For this, bacterial suspensions were incubated in selected concentrations of aqueous dilutions of the test substances. After 1 min, the bacterial-test substance mixture was diluted 1:1000 with sterile water and the resulting suspension was plated out onto nutrient agar using a spiral plater. Bacterial colonies were enumerated after 2-3 days of incubation and compared to those of the water control. Data for chemical toxicity were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.1.

Another laboratory assay was done using inoculated olive twig pieces. Twig sections (5 x 5 x 5 mm) were inoculated with *P. syringae* pv. *savastanoi* (5 x 10^6 cfu/ml) by dipping, treated after 1 or 8 h by dipping for 1 h, and washed in water for 20 min. Sections were then cut up, submersed in water and the aqueous suspension was plated out for viability testing.

Evaluation of protective treatments in greenhouse and field studies. In greenhouse studies, leaf scar wounds were inoculated (10^5 or 10^8 cfu/ml) and then treated with selected bactericides and sanitizers using a hand sprayer. Plants are being periodically evaluated for the presence of knot formation. Field studies on the efficacy and timing of treatments were done in commercial orchards. To evaluate the pre- and post-infection activity, twig end cuts and lateral wounds were inoculated (10^7 cfu/ml) 1 to 3 h before or after hand- or air-blast sprayer applications with Kasumin, Deccosan 231, AgriTitan, PHMB (Vantocil), or Kocide 3000. In a timing study, twigs were inoculated and treated after 0, 1, 2, 3, or 7 days with Kasumin or Kocide 3000. Additionally, in two large-scale field studies trees were harvested and then treated with Kasumin, Vantocil, Deccosan 321, or AgriTitan. A low amount of rainfall occurred between harvest and application at one of the sites. The development of olive knot is currently periodically being monitored. Data for chemical and biological control will be analyzed using analysis of variance and LSD mean separation procedures of SAS 9.1.

RESULTS AND DISCUSSION

Collection of strains and genetic diversity. Eighty strains of *P. savastanoi* were obtained from olive knots in an initial survey from seven olive orchards in Sutter/Yuba, Colusa, and Glenn Co. in northern California and were cultured on nutrient agar and King's medium B. Molecular identification using IAA-lysine synthethase-based primers (Penyalver et al. 2000) confirmed the species identity of the pathogen (Fig. 1). Additional surveys are ongoing and include other growing regions of the state such as San Joaquin Co. and Tulare Co. Our goal was to collect over 100 isolates in the first year of the study to develop baseline sensitivity data for selected antimicrobials. (Note: funding was not available until June 2011 and thus, our goal of obtaining a diverse collection of strains in the first year was achieved.)

In studies on the genotypic diversity of *P. savastanoi* populations using two repetitive primers that generate multiple DNA bands in PCR amplifications, 40 isolates from four orchard locations had identical or near identical banding patterns (Fig. 2). These preliminary results indicate that a clonal population of the pathogen may be present in California olive orchards. More recently obtained isolates from different locations will still need to be tested.

Evaluation of inoculum production and tree injury susceptibility. Some olive knots collected in the field during the summer that were re-hydrated in the laboratory started oozing bacteria after one hour. Nearly all knots tested oozed the pathogen after 18 to 24 h of hydration. Thus, all knots contained viable inoculum that potentially could be spread in the orchard during favorable environmental conditions. Interestingly, the common epiphytic bacterium *Pantoea agglomerans* was also isolated from the bacterial ooze from all hydrated knots evaluated. A recent publication by Marchi et al. (2006) also showed a direct association of this organism with the olive knot pathogen in 70% of the olive knots examined. Both organisms were shown to produce indole-3- acetic acid but cytokinins were only produced by *P. savastanoi*. In future studies, we will evaluate the seasonal availability of inoculum from olive knots during the year.

Evaluate populations of the pathogen for sensitivity to copper, antibiotics, and sanitizers in the laboratory. Strains were tested for their sensitivity to the antibiotic kasugamycin using the spiral gradient dilution method. In these assays, both minimum inhibitory concentrations (MIC) that prevent growth by 95% and lowest inhibitory concentrations (LIC) where a reduction in growth occurs were determined. LIC values ranged from 1.9 to 4.5 µg/ml with a mean value equal to 3.6 µg/ml, whereas MIC values were 3.3 to 6.2 µg/ml with a mean equal to 5.0 µg/ml (Fig. 3). These ranges for LIC and MIC values for the antibiotic are similar to those obtained for other bacterial plant pathogens (Adaskaveg, *unpublished*) and are very encouraging to support continued studies for the development of the antibiotic on olive. In discussions with Arysta LifeScience, this company is willing to support a second tier registration effort poised for 2012 once the first tier registration efforts for the antibiotic are achieved. These registrations on pome fruit, walnut, and tomato/pepper crops are pending in 2012. Thus, efficacy data will need to be generated as described below to support registration plans.

Copper sensitivity was evaluated using a microtiter plate assay. At 12.5 ppm copper (MCE), approximately 50% of the 24 isolates evaluated to date were considered highly sensitive to copper, whereas the remaining isolates were considered less sensitive (Fig. 4). Most isolates were sensitive at 25 ppm copper, but some isolates showed a reduced sensitivity at this level (Fig. 4) and some strains continued to grow even

at 40 ppm. This indicates that other materials besides copper compounds with different modes of action need to be developed to reduce shifts in copper sensitivity.

One strain of *P. savastanoi* was tested against five commercial sanitizers in short-duration direct exposure studies. Deccosan 315, Deccosan 321, and 2-Quat Plus are quaternary ammonia compounds, Vantocil IB is a guanidine, and Nolvasan is a chlorhexidine product. These tests were done because potentially sanitizers can be used to disinfest harvesting equipment as well as olive trees after harvest. Some of these sanitizers are volatile compounds that may not leave any residues. Quaternary ammonia materials are federally registered for use on harvesting and pruning equipment on selected agricultural crops (tomato and citrus). In our studies, all of the tested sanitizers were highly effective in inactivating *P. syringae* pv. *savastanoi* in vitro with reductions of colony forming units of 98% to 100% when using relatively low concentrations (Fig. 5). Kasugamycin was not effective as a bactericidal compound in these assays.

In additional laboratory tests, the efficacy of selected treatments was evaluated on inoculated olive twig segments. Treatments with Kasumin at 100 ppm and Deccosan 321 at 2000 ppm done 1 or 8 h after inoculation inactivated 85 to 100% of the bacteria, similar to Kocide 3000 (Fig. 6). Additional in vivo tests are planned to determine the effect of sanitizer rate, inoculum concentration, and post-inoculation treatment time. Dip treatments were done for 1 h, assuming that during harvest time in the field the treated plant tissues will stay moist for an extended period. Shorter treatment durations, however, will be evaluated in the future.

In summary, these laboratory tests identified treatments that effectively inactivated the olive knot pathogen on agar media, in liquid suspensions, and on woody olive tissues. These tests helped to identify treatments to be subsequently included in field studies.

Evaluation of protective treatments in greenhouse and field studies. In greenhouse studies, the first evidence of knot formation on inoculated potted plants was observed after 1 month. In a preliminary evaluation done after 7 weeks, all treatments effectively prevented knot formation when fresh leaf scars were inoculated with a low concentration of inoculum of a copper-sensitive isolate of the pathogen (Fig. 7). At a higher inoculum concentration, most treatments were less effective. Still, all treatments significantly reduced the disease as compared to the untreated control. Kocide 3000 and AgriTitan reduced the incidence of knot development to zero levels. This experiment is ongoing and plants will be evaluated again in the spring.

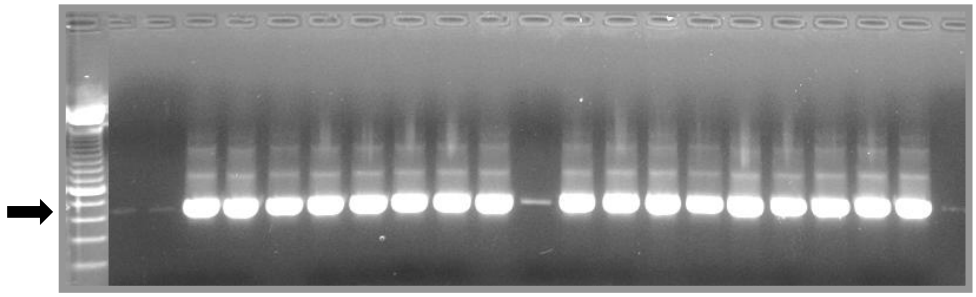
Data for field trials are currently available only from one study where wounded tissues were treated shortly after inoculation with a copper-sensitive isolate of the pathogen and where wounds were covered with Parafilm after treatment. Still, in this study knot formation is at a very early stage and is currently only present at lateral wound sites. Therefore, additional evaluations will be done in the spring. The results of the preliminary evaluation indicate that the sanitizer Deccosan 321 was similarly effective to Kocide 3000 (Fig. 8). Kasugamycin (Kasumin) was also very effective, whereas PHMB and AgriTitan had no or only little effect on knot development.

Our first year's results on the development of new management strategies for olive knot are very promising. In laboratory, greenhouse, and field tests, the sanitizer Deccosan and the antibiotic Kasumin showed consistent very good activity. AgriTitan was effective in the greenhouse but not in the field and this could be explained by the complete covering of the wound sites with several layers of Parafilm in the field during the first 12 weeks of the experiment, whereas in the greenhouse inoculation sites were covered with only a single layer of Parafilm and only for one day. Thus, light activation (i.e., photoactivation) that is required for AgriTitan to be efficacious may not have occurred in the field. Our evaluations of these experiments are ongoing and additional trials are planned for 2012 using strains with reduced sensitivity to copper for inoculation.

Literature Cited

1. Marchi, G. Sisto, A., Cimmino, A., Andolfi, A., Cipriani, M.G., Evidente, A., and Surico, G. 2006. Interaction between *Pseudomonas savastanoi* pv. *savastanoi* and *Pantoea agglomerans* in olive knots. Plant Pathol. 55:614-624.
2. Penyalver, R., Garcia, A., Ferrer, A., Bertonlini, E., and Lopez, M.M. 2000. Detection of *Pseudomonas savastanoi* pv. *savastanoi* in olive plants by enrichment and PCR. Appl. Environ. Microbiol. 66:2673-2677.

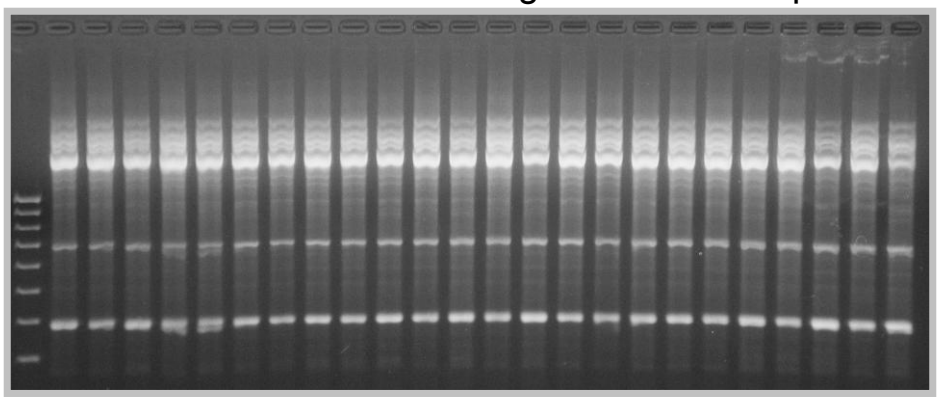
Fig. 1. Specific amplification of *P. syringae* pv. *savastanoi* using primers targeting the IAA-lysine synthethase gene



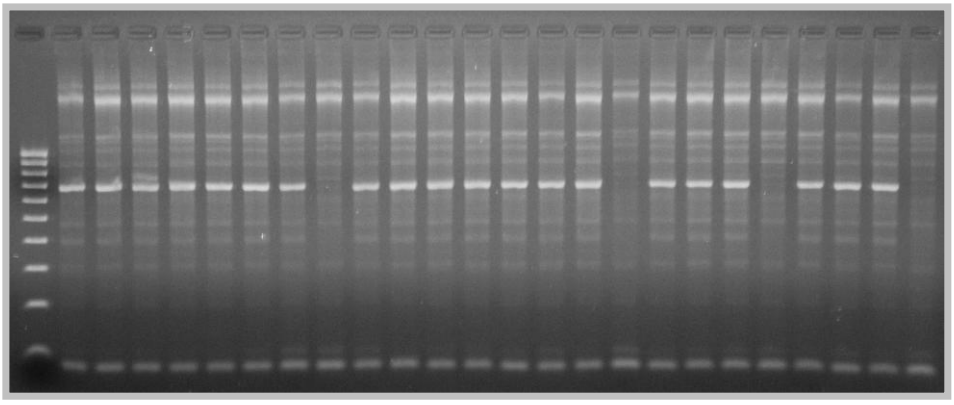
PCR reactions were done using published primers (Penyalver et al. 2000) and amplicons were separated in agarose gels. The intense band at the location of the arrowhead indicates the specific DNA fragment. Based on this method, four isolates in this gel are not *P. syringae* pv. *savastanoi*.

Fig. 2. Genetic diversity among isolates of *P. syringae* pv. *savastanoi* based on PCR using BOX and REP primers

A

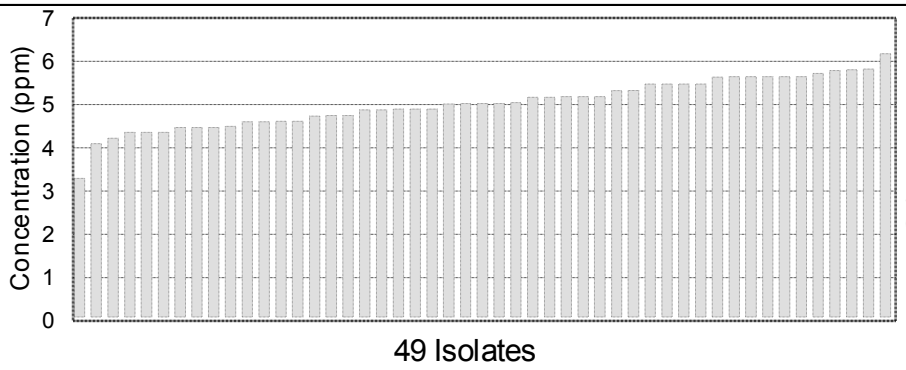


B



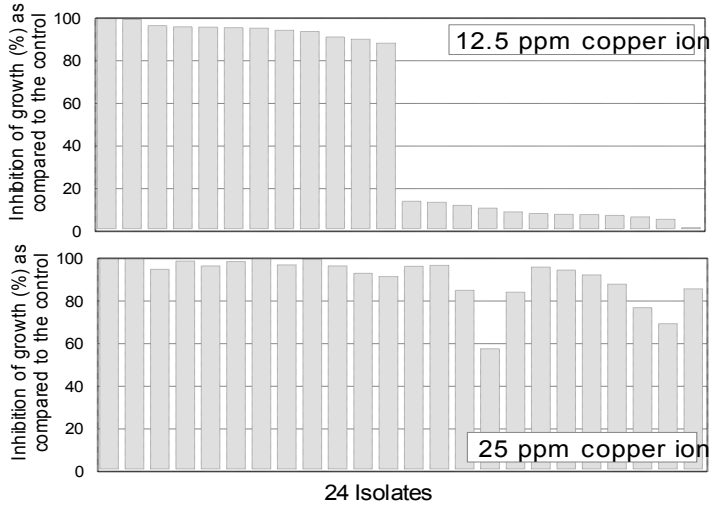
Electrophoretic separation of PCR products from *P. syringae* pv. *savastanoi* using A) BOX and B) REP primers.

Fig. 3. In vitro sensitivity of isolates of *P. syringae* pv. *savastanoi* against kasugamycin in spiral gradient dilution assays



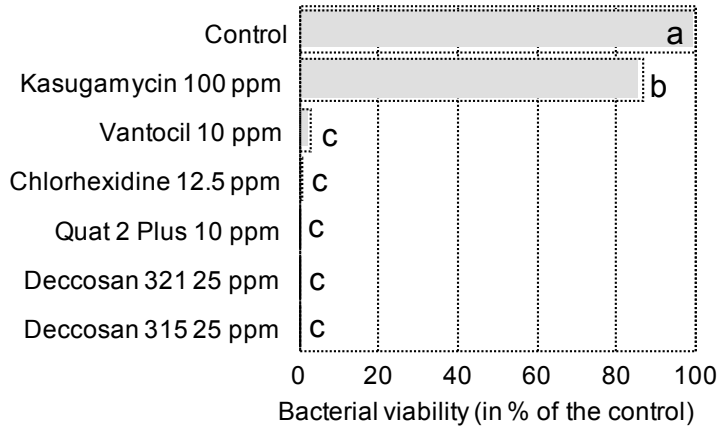
Bacterial suspensions were plated onto agar amended with a continuous gradient of kasugamycin concentrations that was generated using a spiral plater.

Fig. 4. In vitro sensitivity of isolates of *P. syringae* pv. *savastanoi* against copper in microtiter assays



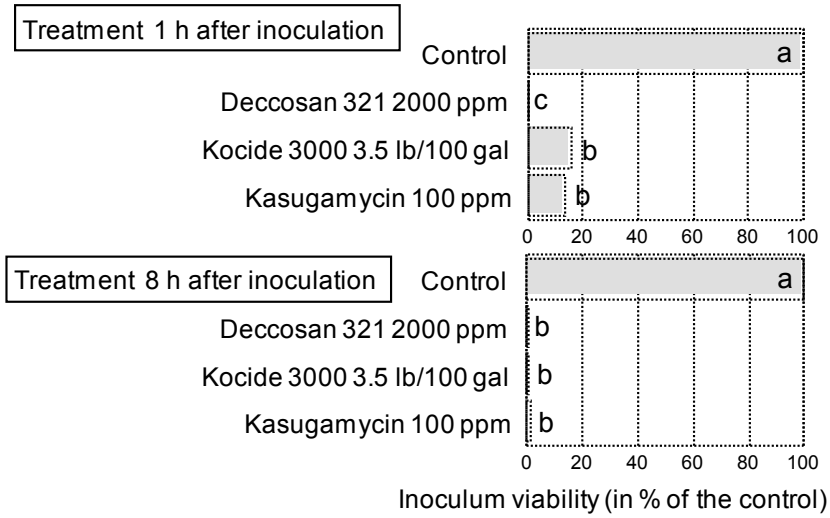
P. syringae pv. *savastanoi* was incubated for 24 to 48 h in liquid growth medium without and with the addition of copper at selected concentrations. Measurement of the optical density at 600 nm was used to determine bacterial growth.

Fig. 5. In vitro toxicity of sanitizers and kasugamycin against *P. syringae* pv. *savastanoi* in short-duration exposure assays in the laboratory



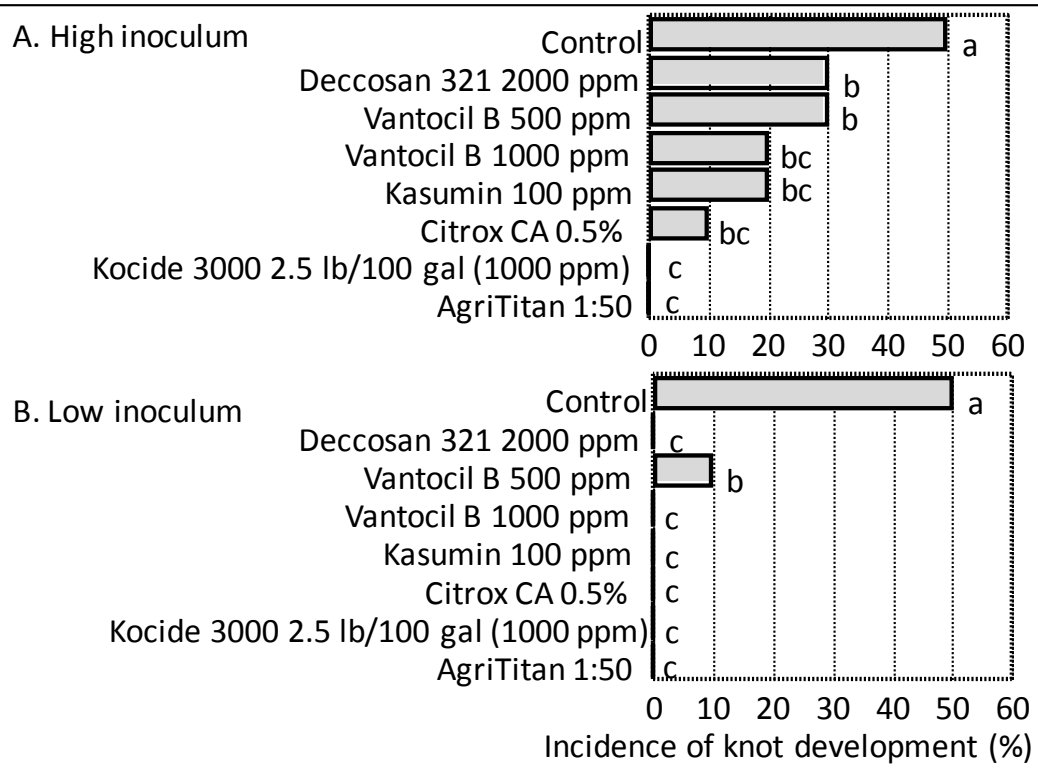
Bacterial suspensions were incubated in aqueous test solutions. After 1 min, the mixture was diluted 1:1000 with sterile water and plated out onto nutrient agar. Bacterial colonies were enumerated after 2-3 days of incubation and compared to those of the water control.

Fig. 6. In vitro toxicity of sanitizers and kasugamycin against *P. syringae* pv. *savastanoi* in short-duration exposure assays in the laboratory



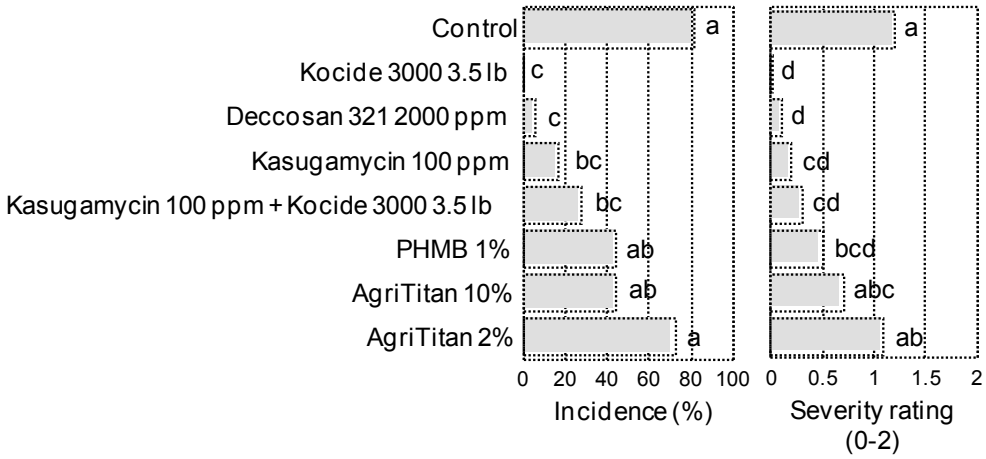
Olive twig sections were inoculated with *P. syringae* pv. *savastanoi* (5 x 10^6 cfu/ml) by dipping, treated after 1 or 8 h by dipping for 1 h, and washed in water for 20 min. Sections were then cut up, submersed in water and the aqueous suspension was plated out for viability testing.

Fig. 7. Evaluation of selected treatments for the control of olive knot in greenhouse studies



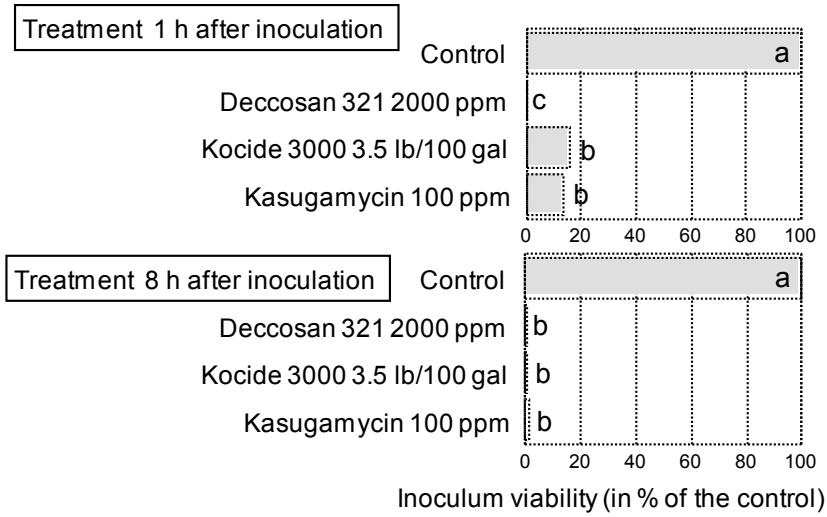
Leaf scar wounds of potted olive trees were inoculated with *P. syringae* pv. *savastanoi* (10^5 or 10^8 cfu/ml) and then treated with selected bactericides and sanitizers using a hand sprayer. A preliminary evaluation was done after 7 weeks.

Fig. 8. Evaluation of selected treatments for the control of olive knot in field studies



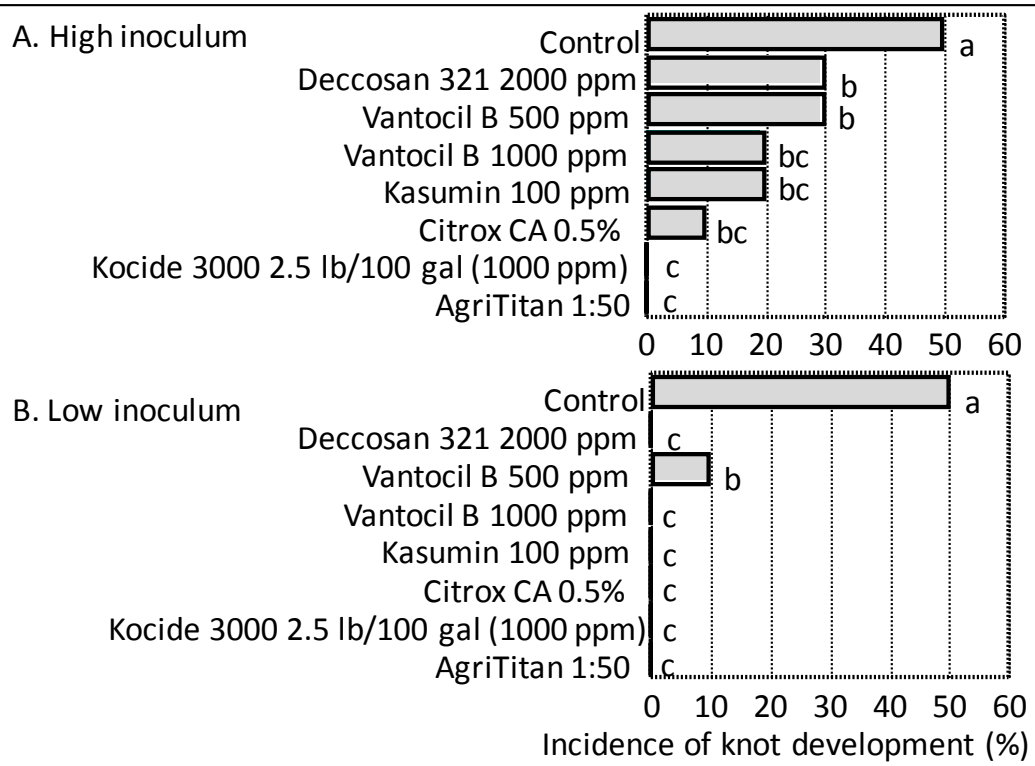
In October 2011, lateral twig wounds of olive trees were inoculated with *P. syringae* pv. *savastanoi* (10^7 cfu/ml), treated with selected bactericides and sanitizers using a hand sprayer, and covered with Parafilm. A preliminary evaluation for was done in early January of 2012.

Fig. 6. In vitro toxicity of sanitizers and kasugamycin against *P. syringae* pv. *savastanoi* in short-duration exposure assays in the laboratory



Olive twig sections were inoculated with *P. syringae* pv. *savastanoi* (5 x 10^6 cfu/ml) by dipping, treated after 1 or 8 h by dipping for 1 h, and washed in water for 20 min. Sections were then cut up, submersed in water and the aqueous suspension was plated out for viability testing.

Fig. 7. Evaluation of selected treatments for the control of olive knot in greenhouse studies



Leaf scar wounds of potted olive trees were inoculated with *P. syringae* pv. *savastanoi* (10^5 or 10^8 cfu/ml) and then treated with selected bactericides and sanitizers using a hand sprayer. A preliminary evaluation was done after 7 weeks.

RESEARCH REPORT TO THE CALIFORNIA OLIVE COMMITTEE

Project Title: Development of Baseline Data for Using Stem Water Potential for Olives

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Key Words: Olives, Stem Water Potential, Regulated Deficit Irrigation
Commodity(s): Olive

Problem and its Significance:

The use of mid-day stem water potential (SWP) for determination of tree water status has become more routine in deciduous tree crops such as almonds. walnuts, and prunes. This information can be used to diagnose the water status of the tree and decide if the tree is being properly irrigated and prevent under or over irrigation. It has been used in almonds and prunes to monitor and regulate deficit irrigation which can result in water savings, decreased disease, more uniform crop maturity with no loss in yield and sometimes improved quality (Stuart, et.al., 2011, Shackel et al., 1997, Shackel et.al., 2000, Teviotdale, et.al. 2001).

To assist with interpreting SWP measurements in olive a baseline must be developed. The baseline is designed to predict what the expected SWP reading would be when soil moisture is near field capacity throughout the root zone and not limiting trees of a given species under specific climatic conditions. To accomplish this SWP and climatic data must be collected on trees when soil moisture is not limiting over a range of weather conditions. This data can then be correlated with air temperature and relative humidity

to develop baseline tables that predict SWP in olive trees across a wide range of weather conditions. Research has shown that the baseline for trees can be quite different for different species. For example SWP reading of - 8 bars for almonds would generally indicate soil moisture is not limited in the root zone while the same reading for walnuts would indicate plant stress severe enough to reduce shoot growth and to begin to affect bud development and kernel quality (Buchner et.al. 2007). Preliminary work that we have done with olives indicates that baseline SWP readings for olive will be significantly more negative than almonds, walnuts or prunes.

Objectives:

1) Understand the influence of variable orchard climatic conditions on SWP in olive trees when soil moisture is not limiting.

2) Develop a baseline table to predict SWP readings for olives grown under different weather conditions when soil moisture is not limiting. This information will be made available to growers for use in interpreting orchard measurement of SWP and managing irrigation of their trees.

Methods:

Preliminary data consisting of olive SWP measurements and corresponding applied water were collected in seasons preceding this 2011 funded project. SWP measurements taken during these preliminary studies where applied water exceeded ETc were pooled with data gathered during 2011 to expand the sample size and include a broader range in climatic conditions. In 2011, SWP and corresponding applied water data was collected from five commercial olive orchards in Glenn and Tehama Counties. The olive variety in each of these orchards was Manzanillo and all of the orchards were at least ten years old with mature canopies and represented common orchard designs and tree densities. These orchards were grown on typical soil series in the olive production areas surrounding Corning and Orland such as the Maywood loam and Arbuckle gravelly loam series. All of the orchards were irrigated with low volume systems (four with drip and one with microsprinkler irrigation).

A specific area was designated for monitoring within each orchard. Small in-line flow meters were installed in the surface line of selected tree rows and the irrigated area below each flow meter was determined. The cumulative gallons of applied water were measured each time SWP measurements were collected. The volume of water applied between SWP measurements was calculated by subtraction using consecutive measurements of total flow. The volume of applied water was converted from gallons to inches and compared to real-time estimates of crop evapotranspiration (ETc) for table olives for corresponding periods of time to describe the water applications as a percentage of ETc. ETc was calculated using crop coefficients for canning olives (Beede et.al. 1994) and grass reference ET (ETo) from CIMIS station #8, Gerber, CA. To the fullest extent possible, we worked with the grower cooperator to apply enough irrigation water to match or surpass ETc and assure that soil moisture was not limiting and influencing SWP measurements.

SWP was monitored on at least five trees per orchard (returning to the same trees each measurement) during the growing season approximately weekly to bi-weekly to determine an average for the orchard. Days for measurement of SWP were selected to provide a wide range in mid afternoon temperature and relative humidity. Two individuals shared the task of collecting SWP measurements in the olive orchards in Glenn County and a third individual collected the SWP measurements in Tehama County. SWP was

measured with a pressure chamber between the hours of 12:00 and 3:00 p.m. Leaves at the end of an olive shoot were inserted into a reflective foil bag for 10 minutes prior to measuring SWP with a pressure chamber to allow the leaves to equilibrate with the larger water conducting xylem tissue in the nearby shoots (Fulton et. al., 2001). Enough leaf pairs (usually 3) were inserted into the bag to allow for adequate stem length to protrude through the lid of the pressure chamber and facilitate the measurement. After a minimum of ten minutes, the leaves were excised from the tree with the leaves remaining in the bag and with the stem extending out of the foil bag about 1/3 inch (1 cm). The bagged leaves were placed inside a pressure chamber by inserting the stem through the lid of the pressure chamber. The lid was placed on the chamber with the bagged olive shoot and leaves inside of the chamber and the cut surface of the stem visible on the top side of the chamber lid. A rubber gasket was tightened around the stem protruding through the lid to seal the chamber and prevent nitrogen used to pressurize the chamber from leaking out. Nitrogen pressure was increased inside the chamber until water exuded from the xylem tissue. The end point of the measurement occurred when water covered the cut surface of the stem but before bubbling was apparent and the pressure (bars) shown on the chamber gauge was recorded to represent the water status of the orchard.

Hourly, mid afternoon air temperature and relative humidity data was acquired from the nearest CIMIS weather station, Gerber station #8. They were used to compute the mid-day vapor pressure deficit for each day and hour bracketing when SWP measurements were taken in each orchard. In-situ temperature and relative humidity sensors and dataloggers were also located in two of the five orchards to provide real-time, on-site measurement of air temperature and relative humidity.

Vapor pressure deficit, or VPD, is the difference (deficit) between the amount of moisture in the air and how much moisture the air can hold when it is saturated. When air becomes saturated water will condense out to form dew on leaves. As the VPD increases or as the air becomes drier the plant water potential becomes greater and the capacity of trees to transpire water increases as long as soil moisture is not limiting. VPD varies with the ambient air temperature and relative humidity. In this study, air temperature and relative humidity data were used to compute the vapor pressure deficit (VPD) for each day and time that SWP was measured in each orchard. To compute VPD for specific weather conditions, saturation pressure was determined by applying a psychrometric equation across the observed ranges in air temperatures, resulting in a range of saturated pressures. Partial pressure was then computed by multiplying the saturated pressure by the observed levels of relative humidity. VPD was then computed as the difference between saturation pressure and partial pressure at various combinations of air temperature and relative humidity. Additional information on the computation of VPD is described by Roseberg et.al. 1983.

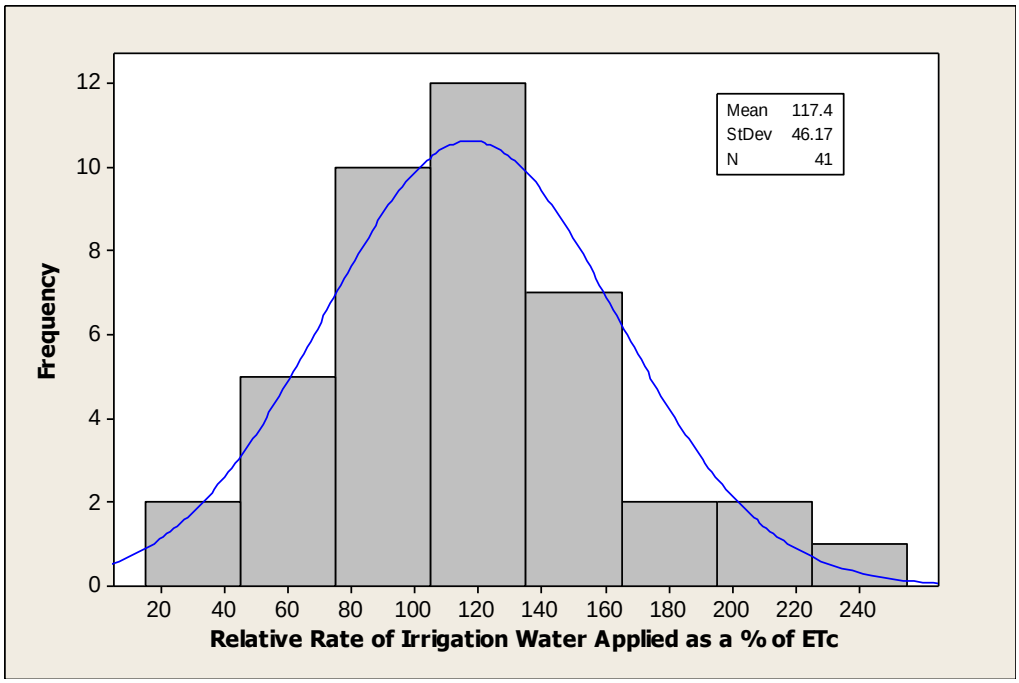
Data from all five orchards were pooled to form a larger data set representing a wider range in orchard climatic conditions which influence SWP and VPD. Minitab Version 16.0 was used to conduct statistical analysis of the field data. A frequency distribution analysis was performed to evaluate the adequacy of irrigation in each of the orchards and to describe the extent that soil moisture may have been limiting the olive orchards, thereby, influencing the mid-day stem water potential measurements. Linear regression was also performed to evaluate the effect of using on-site measurements of orchard air temperature and relative humidity versus off-site, CIMIS weather station data for computing VPD. Finally, linear regression was conducted to evaluate the correlation between mid-day stem water potential and VPD and compute the “best fit” predictive model for this data set. This predictive model was then used to develop a table for estimating the baseline stem water potential levels in olives over a wide range of climatic conditions when soil moisture is not limiting.

Results and Discussion:

Orchard Conditions where Soil Moisture is not Limiting

Figure 1 is a histogram showing the frequency distribution of applied irrigation water when expressed as a relative percentage of real-time ETc. Figure 1 shows a relatively normal distribution in the applied water among the data set. On average, applied irrigation water in this data set was 117 percent of real-time estimated ETc. The illustration shows that 24 of the 41 measurements of SWP (59 percent) occurred when applied water exceeded 100 percent of real-time ETc. Of these 24 measurements, five of the SWP measurements were taken when applied irrigation water greatly exceeded real-time ETc by more than 160 percent. An additional ten measurements of SWP occurred when applied water ranged from 80 to 100 percent of ETc, so 34 of the 41 measurements of SWP (83 percent) were taken when applied water was equal to or greater than 80 percent ETc. Seven (17 percent) of the SWP readings were taken when applied water was less than 80 percent of ETc.

Figure 1. Histogram showing the frequency distribution of the irrigation water applied as a percentage of crop evapotranspiration (ETc) in the experimental orchards.

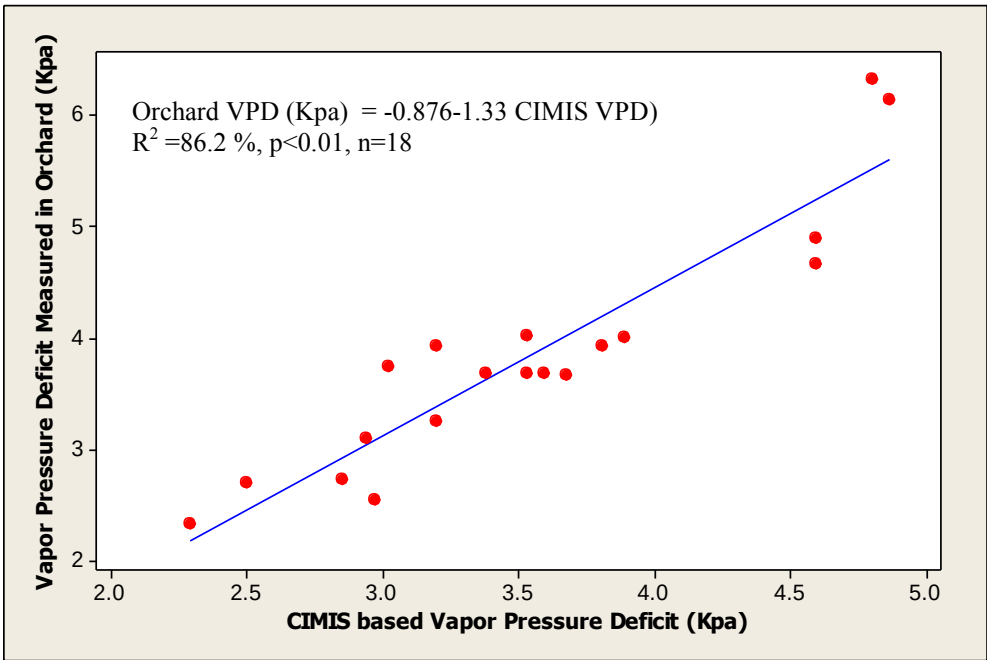


These results suggest that this set of SWP measurements acquired in this study largely represents orchard conditions where soil moisture was not limiting. In those instances where applied water was less than ETc, stored soil moisture in the root zone likely compensated for under irrigation so that a soil moisture deficit should not have influenced the SWP measurements. Statistical tests of the standardized residuals indicated that the data points used in this analysis and that are represented at both ends of the normal distribution curve (Figure 1) did not have an unusually large influence on the computation of the "best fit line" and regression models shown in Figures 3 and 4.

VPD Estimates from CIMIS and Orchard Measurements of Air Temperature and Relative Humidity

Figure 2 shows a linear regression analysis where VPD computed using weather data from the nearest CIMIS station is the independent variable and VPD calculated with weather data within orchards is the dependent variable. A relatively strong linear relationship exists between them. The relationship is positively correlated. As the VPD based upon weather measurements from CIMIS station #8 increased so did the VPD calculated from orchard weather measurements. Over 86 percent of the variation in the VPD within the orchard was explained by the VPD computed with air temperature and relative humidity measurements acquired from the nearest CIMIS station. The slope of the "best fit" line was 1.33 indicating that on average the VPD in the orchard will be approximately 33 percent higher than the VPD based upon the nearest CIMIS weather station.

Figure 2. Relationship between Vapor Pressure Deficit measured in olive orchards and measured at the nearest CIMIS weather station.



Relationships between Mid-day SWP and VPD

Figure 3 shows a negatively correlated, linear relationship between mid-day SWP in canning olives and VPD, where VPD is the independent variable and SWP is the dependent variable. As VPD increases SWP decreases (becomes more negative). The slope of the "best fit" regression line is -2.03 indicating that as the VPD increases 1.0 Kpa due to a combination of higher air temperatures and lower relatively humidity, the SWP is expected to decline -2.0 bars even when soil moisture is not limiting. The y-intercept is -8.26, suggesting that a maximum SWP of about -8.25 bars would be expected when the air temperature is low and the relative humidity is high creating a very low VPD. The correlation is modest with 66.5 percent of the variation explained by VPD. It is lower than correlations reported by McCutchan and Shackel, 1992 where they reported a correlation between VPD and SWP in French prune of 80 percent. However, this correlation between VPD and SWP in table olive is higher than correlations

reported by Goldhamer and Fereres, 2001 where they reported a correlation between VPD and SWP of 42 percent in almonds. Other variables may contribute to the unexplained error in the correlation between VPD and SWP. Variation in observing the end point of SWP measurements is a likely contributor. Goldhamer and Fereres 2001, reported up to -2.0 bars of variation in SWP measurements in almond due to differences in handling SWP samples and observing the end point by different individuals taking the measurements. They also hypothesized that various physiological processes that occur during different stages of tree growth and crop development may contribute to the variation.

Figure 4 shows a similar linear relationship, where VPD is the independent variable and mid-day SWP is the dependent variable. However, in this case, the VPD was either measured in the orchards or was estimated using the linear regression model presented in Figure 2. The correlation between VPD and mid-day SWP was only slightly stronger when VPD based upon measured or predicted orchard weather conditions was used in the regression analysis. Nearly 68 percent of the variation in SWP measurements was explained by VPD. The slope of the "best fit" regression line is -1.56 indicating that as the VPD increases 1.0 Kpa due to a combination of higher air temperatures and lower relatively humidity, the SWP is expected to decline -1.5 bars even when soil moisture is not limiting. The y-intercept is -9.4, suggesting that a maximum SWP of about -9.5 bars would be expected when the air temperature is low and the relative humidity is high creating a lower VPD.

Figure 3. Relationship between mid-day SWP (bars) in canning olives and Vapor Pressure Deficit (Kpa). The VPD was calculated using ambient air temperature and relative humidity measured at the nearest CIMIS weather station on the hour before and after each SWP measurement was taken.

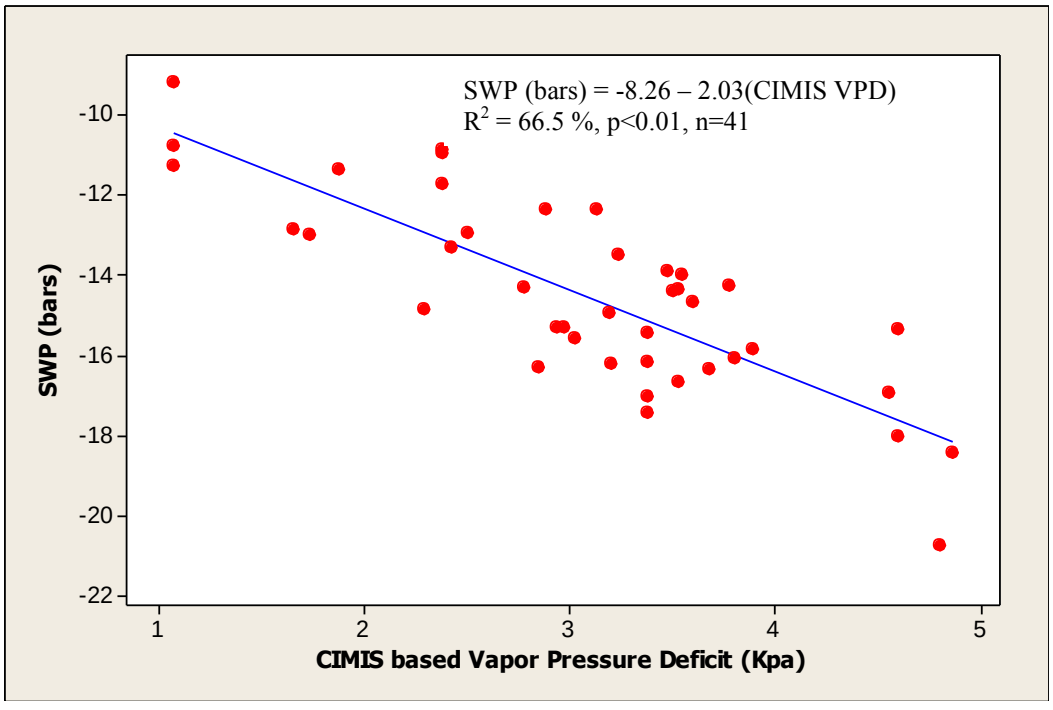
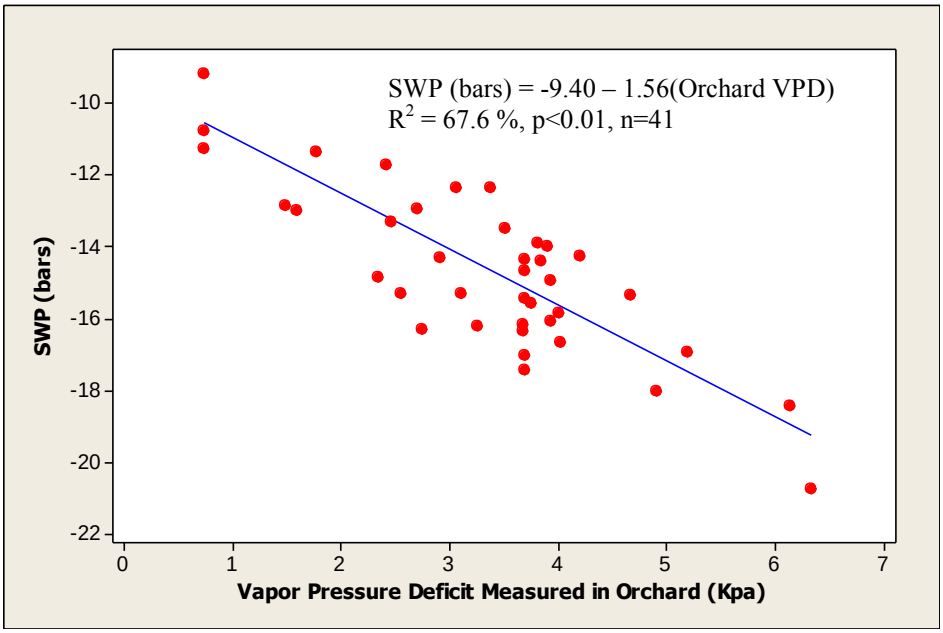


Figure 4. Relationship between mid-day SWP (bars) in canning olive and Vapor Pressure Deficit (Kpa). VPD was calculated using ambient air temperature and relative humidity measured in or estimated for each orchard on the hour before and after each SWP measurement was taken.



Predicted Baseline Stem Water Potential Levels

Table 1 below provides baseline SWP levels that are anticipated for canning olive over a broad range of weather conditions when soil moisture is not limiting orchard water use. This table was derived by applying the "best fit" linear regression model shown and described in Figure 4. Levels in the gray shaded area of the table are the predicted SWP levels that are expected when soil moisture is not limiting and for the more common ranges of temperature and relative humidity that occur in the olive producing regions of California. Even when soil moisture is not limiting, baseline SWP levels are expected to range from about -11.0 to -18.0 bars tension in canning olive when temperature ranges from 86 to 104 °F and 25 to 6 5 percent relative humidity. These baseline SWP levels are more negative than those reported for almond, prune, and walnut. Baseline levels for almond and prune are commonly between -5.9 and -10.7 bars for the same ranges in air temperature and relative humidity and -3.7 to -6.3 bars for walnut (McCutchan et.al., 1992 and Shackel, Personal Communication, 2002).

Application of Predicted Baseline Stem Water Potential

Figure 5 illustrates the application of the predicted baseline SWP for table olives under fully irrigated conditions in comparison to actual orchard measurements of SWP taken in one of the Manzanillo olive orchard involved in this formal study during 2011. Since this orchard was irrigated so that soil moisture should not be limiting the trees, the measured SWP levels were expected to be very similar to the predicted baseline levels. In the figure, the top line with diamond symbols represents the predicted SWP levels when soil moisture was not limited in the root zone of the olive trees. The predicted SWP levels

range from -11.7 bars on July 29 to -21.3 bars on September 9 when the predictive model from Figure 4 was applied. In comparison, actual SWP levels measured in the orchard and denoted by the triangle symbols ranged from -14.3 bars on September 23 to -18.4 bars on September 9. From July 29 through September 2, 2011 the actual measurements of SWP ranged from -0.7 to -4.2 bars below the fully `irrigated baseline, but from September 9 through September 23 the actual SWP measurements ranged from 2.3 bars above to -0.3 bars below the fully irrigated baseline. These results show that the "best fit" line and regression model shown in Figure 4 makes reasonable predictions but has inherent error. This is consistent with the regression coefficient (R^2) of 67.6 percent and the variability among the data points shown in Figure 4 that are not solely correlated with vapor pressure deficit (VPD).

Figure 5. Example application of the "best fit" linear model shown in Figure 4 to predict the fully irrigated baseline stem water potential for one of the Manzanillo table olive orchards used in this formal study.

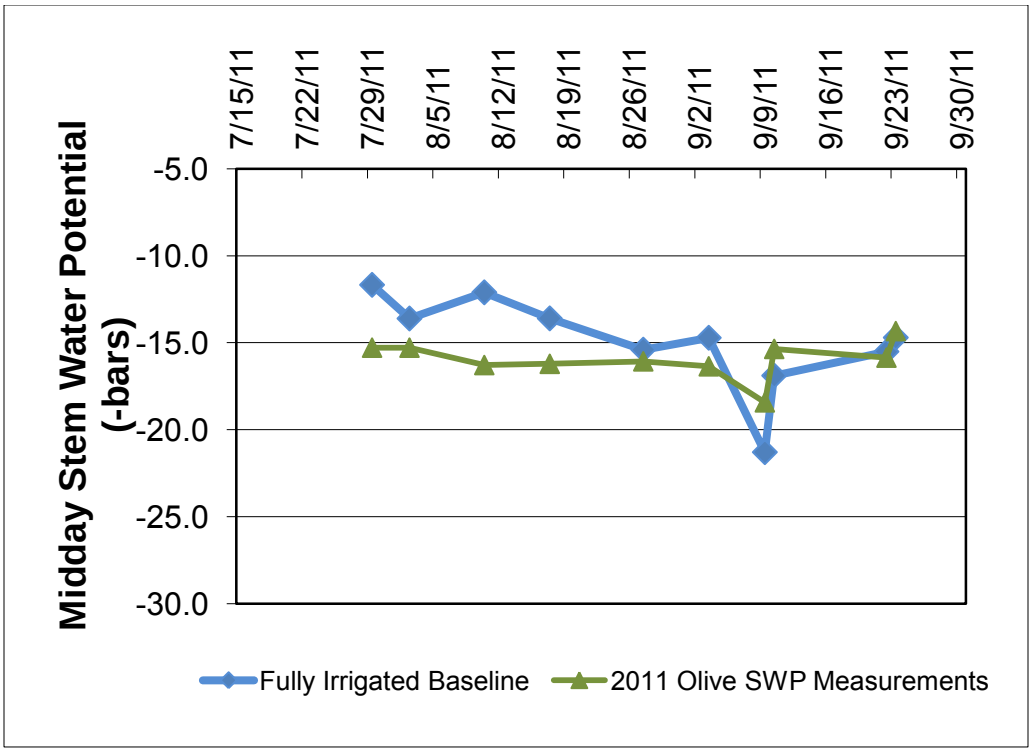
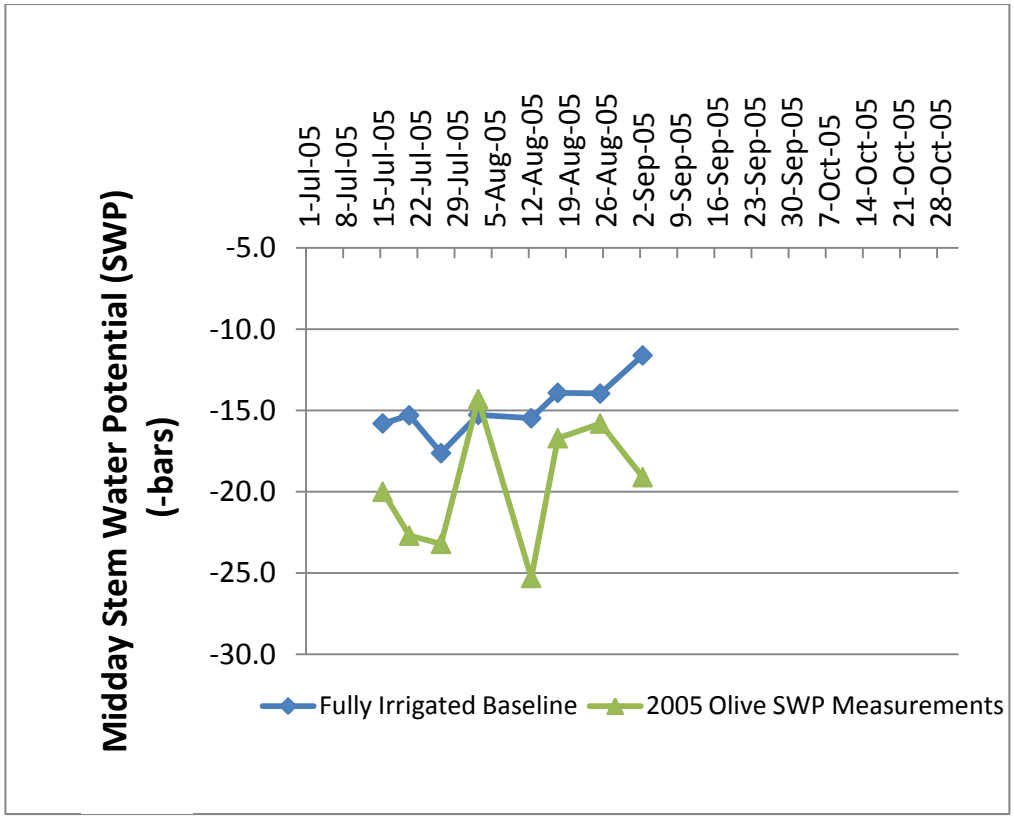


Figure 6 illustrates the application of the predicted baseline SWP for table olives under fully irrigated conditions in comparison to actual orchard measurements of SWP taken in a mature Manzanillo olive orchard west of Corning, CA during the 2005 season. This orchard and SWP data are separate of the orchards and data acquired in this funded study and the preliminary studies that were conducted and provides an independent data set to illustrate application of the predicted baseline SWP. In the figure, the top line with diamond symbols represents the predicted SWP levels that may have been observed on the days that orchard levels of SWP were measured, if soil moisture was not limited in the root zone of the olive trees. SWP levels ranging from -11.6 bars on September 2 to -17.6 bars on July 26 were predicted for fully irrigated conditions when the predictive model from Figure 4 was applied. In comparison, actual SWP levels measured in the orchard and denoted by the triangle symbols ranged from -14.3 bars on August 2 to -25.3 bars on August 12. This indicated that on August 2, 2005 when SWP levels measured -14.3 bars that the orchard water status was at a fully irrigated condition after the orchard had recently

been irrigated. The orchard was not subjected to water stress and the orchard water status was sufficient to promote favorable tree responses and crop development without an immediate need for additional irrigation. However, by August 12, the SWP level averaged -25.3 bars and was -9.8 bars below the fully irrigated baseline indicating a significant change in orchard water status. The other SWP measurements gathered on six other days through July, August, and early September in the 2005 season revealed fluctuating levels of crop stress ranging from -4.2 to -7.1 bars below the fully irrigated baseline, also, indicating some crop stress was evident. While Figures 4 and 5 illustrate that the predictive model resulting from this study has some inherent error it still has useful application. Without its development to estimate the baseline SWP of table olives under fully irrigated conditions, the ability to recognize occurrences of crop stress and interpret the orchard water status would be more difficult.

Figure 6. Example application of the "best fit" linear model shown in Figure 4 to predict the fully irrigated baseline stem water potential for a Manzanillo table olive orchard where midday stem water potential was measured during July, August, and early September of 2005.



Conclusions:

- A strong, positively correlated relationship exists between VPD measured at the nearest CIMIS weather stations and VPD measured in olive orchards. VPD averaged 33 percent higher when measured in olive orchards in comparison to VPD measured at the nearest CIMIS station in irrigated pasture. This reflects differences in climatic conditions and differences in the type and extent of vegetation at the CIMIS weather station and in each orchard. The CIMIS weather station was approximately 15 to 30 miles from the respective orchards where SWP measurements were collected

so some differences in the climatic conditions would be expected. The CIMIS station is also located in a flood irrigated pasture where the grass cover is maintained at about six inches in height and influences the height of the boundary layer where the pasture canopy and atmospheric conditions interface. Plus, the pasture is flood irrigated so that soil surface is completely wetted each irrigation and soil moisture should not be limiting. In contrast, the olive orchards consist of taller trees where the boundary layer between the orchard canopy and the atmospheric conditions interface occurs above the trees. Since the boundary layer is higher in olive trees, greater wind speed, air turbulence, and interception of radiation may be anticipated and contribute to a larger VPD in the orchards. In addition, the olive orchards are irrigated with drip or microspinkler that only partially wet the orchard floor, so heat absorption and release from the orchard floor will be more variable.

- A modest, negatively correlated relationship exists between VPD measured at the nearest CIMIS weather station and SWP. The "best fit" linear regression model for predicting baseline SWP levels in table olives where soil moisture is not limiting was achieved by measuring VPD in olive orchards.

- Prior to this study, it had been suggested that SWP in table olive behaved similar to almonds and French prune, but there was no data to substantiate it and anecdotal evidence to question it. This is important to understand when interpreting SWP in canning olive and gauging how much crop stress an orchard may be under. For example, when SWP measurements in olive orchards were observed to average -16 bars tension and the orchard conditions were 100°F and 35 percent relative humidity, applying the almond and prune baseline prediction model suggested the SWP levels should be about -9.2 bars when soil moisture is not limiting. Further implying that a real-time measurement of SWP averaging -16 bars represented significant crop stress, almost -7 bars more negative than the predicted baseline. Such levels of crop stress may cause concern about negative effects on olive shoot growth and olive fruit sizing and suggest more intensive irrigation management. However, estimates of baseline SWP for canning olive in this study appear more negative than those predicted for almond, prune, and walnut. Based on this study, -16 bars tension when orchard weather conditions are 100°F and 35 percent relatively humidity may not represent significant crop stress.

- Application of the predictive model developed in this study for estimating baseline SWP in table olives was tested against actual orchard measurements of SWP collected in a mature Manzanillo olive orchard in the 2005 growing season. This new predictive model shows the capacity to distinguish instances when an orchard may be fully irrigated and where soil moisture is not limiting from times when an orchard may be in a water deficit and crop stress may be significant.

- The baseline predictions in Table 1 provide a first approximation. Further validation in a more controlled experiment that includes sequential irrigation treatments and corresponding crop loads and direct measurement of tree growth and crop development and yield responses is desirable.

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Table 1. Predicted baseline mid-day stem water potential in olives over a range of weather condition when soil moisture is not limiting.

Air	Air Relative Humidity															
Temp (F)	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85
60	-11.88	-11.74	-11.61	-11.47	-11.33	-11.19	-11.05	-10.92	-10.78	-10.64	-10.50	-10.36	-10.23	-10.09	-9.95	-9.81
62	-12.06	-11.92	-11.77	-11.62	-11.47	-11.32	-11.18	-11.03	-10.88	-10.73	-10.58	-10.44	-10.29	-10.14	-9.99	-9.84
64	-12.26	-12.10	-11.94	-11.78	-11.62	-11.46	-11.31	-11.15	-10.99	-10.83	-10.67	-10.51	-10.35	-10.19	-10.04	-9.88
66	-12.46	-12.29	-12.12	-11.95	-11.78	-11.61	-11.44	-11.27	-11.10	-10.93	-10.76	-10.59	-10.42	-10.25	-10.08	-9.91
68	-12.68	-12.50	-12.32	-12.14	-11.95	-11.77	-11.59	-11.41	-11.22	-11.04	-10.86	-10.68	-10.49	-10.31	-10.13	-9.95
70	-12.92	-12.72	-12.53	-12.33	-12.13	-11.94	-11.74	-11.55	-11.35	-11.16	-10.96	-10.77	-10.57	-10.38	-10.18	-9.99
72	-13.16	-12.95	-12.74	-12.54	-12.33	-12.12	-11.91	-11.70	-11.49	-11.28	-11.07	-10.86	-10.65	-10.45	-10.24	-10.03
74	-13.42	-13.20	-12.98	-12.75	-12.53	-12.31	-12.08	-11.86	-11.64	-11.41	-11.19	-10.97	-10.74	-10.52	-10.29	-10.07
76	-13.70	-13.46	-13.22	-12.99	-12.75	-12.51	-12.27	-12.03	-11.79	-11.55	-11.31	-11.07	-10.83	-10.60	-10.36	-10.12
78	-14.00	-13.74	-13.49	-13.23	-12.98	-12.72	-12.46	-12.21	-11.95	-11.70	-11.44	-11.19	-10.93	-10.68	-10.42	-10.17
80	-14.31	-14.04	-13.76	-13.49	-13.22	-12.95	-12.67	-12.40	-12.13	-11.85	-11.58	-11.31	-11.04	-10.76	-10.49	-10.22
82	-14.64	-14.35	-14.06	-13.77	-13.47	-13.18	-12.89	-12.60	-12.31	-12.02	-11.73	-11.44	-11.15	-10.86	-10.56	-10.27
84	-14.99	-14.68	-14.37	-14.06	-13.75	-13.44	-13.13	-12.81	-12.50	-12.19	-11.88	-11.57	-11.26	-10.95	-10.64	-10.33
86	-15.36	-15.03	-14.70	-14.36	-14.03	-13.70	-13.37	-13.04	-12.71	-12.38	-12.05	-11.72	-11.39	-11.05	-10.72	-10.39
88	-15.75	-15.39	-15.04	-14.69	-14.34	-13.98	-13.63	-13.28	-12.93	-12.57	-12.22	-11.87	-11.52	-11.16	-10.81	-10.46
90	-16.16	-15.78	-15.41	-15.03	-14.66	-14.28	-13.91	-13.53	-13.16	-12.78	-12.40	-12.03	-11.65	-11.28	-10.90	-10.53
92	-16.60	-16.20	-15.80	-15.40	-15.00	-14.60	-14.20	-13.80	-13.40	-13.00	-12.60	-12.20	-11.80	-11.40	-11.00	-10.60
94	-17.06	-16.63	-16.20	-15.78	-15.35	-14.93	-14.50	-14.08	-13.65	-13.23	-12.80	-12.38	-11.95	-11.53	-11.10	-10.68
96	-17.54	-17.09	-16.64	-16.18	-15.73	-15.28	-14.83	-14.37	-13.92	-13.47	-13.02	-12.57	-12.11	-11.66	-11.21	-10.76
98	-18.05	-17.57	-17.09	-16.61	-16.13	-15.65	-15.17	-14.69	-14.21	-13.73	-13.24	-12.76	-12.28	-11.80	-11.32	-10.84
100	-18.59	-18.08	-17.57	-17.06	-16.55	-16.04	-15.53	-15.02	-14.51	-13.99	-13.48	-12.97	-12.46	-11.95	-11.44	-10.93
102	-19.16	-18.62	-18.07	-17.53	-16.99	-16.45	-15.91	-15.36	-14.82	-14.28	-13.74	-13.19	-12.65	-12.11	-11.57	-11.03
104	-19.76	-19.18	-18.60	-18.03	-17.45	-16.88	-16.30	-15.73	-15.15	-14.58	-14.00	-13.43	-12.85	-12.28	-11.70	-11.13
106	-20.38	-19.77	-19.16	-18.55	-17.94	-17.33	-16.72	-16.11	-15.50	-14.89	-14.28	-13.67	-13.06	-12.45	-11.84	-11.23
108	-21.05	-20.40	-19.75	-19.11	-18.46	-17.81	-17.16	-16.52	-15.87	-15.22	-14.58	-13.93	-13.28	-12.64	-11.99	-11.34
110	-21.74	-21.06	-20.37	-19.69	-19.00	-18.31	-17.63	-16.94	-16.26	-15.57	-14.89	-14.20	-13.51	-12.83	-12.14	-11.46
112	-22.47	-21.75	-21.02	-20.30	-19.57	-18.84	-18.12	-17.39	-16.66	-15.94	-15.21	-14.48	-13.76	-13.03	-12.31	-11.58
114	-23.24	-22.47	-21.70	-20.94	-20.17	-19.40	-18.63	-17.86	-17.09	-16.32	-15.55	-14.78	-14.01	-13.25	-12.48	-11.71
116	-24.05	-23.24	-22.42	-21.61	-20.79	-19.98	-19.17	-18.35	-17.54	-16.73	-15.91	-15.10	-14.28	-13.47	-12.66	-11.84
118	-24.90	-24.04	-23.18	-22.32	-21.45	-20.59	-19.73	-18.87	-18.01	-17.15	-16.29	-15.43	-14.57	-13.71	-12.84	-11.98
120	-25.79	-24.88	-23.97	-23.06	-22.15	-21.24	-20.33	-19.41	-18.50	-17.59	-16.68	-15.77	-14.86	-13.95	-13.04	-12.13

Predicted SWP in table olives when soil moisture is not limiting for a common range in weather conditions

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Department of Botany and Plant Sciences
Relevant AES/CE Project No.: 4556

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL PROGRESS REPORT

Project Year: 2011 – 2012 **Duration of Project: Year 1 of 2**
(Year 1 no-cost Extension to 30 June 012)

Project Leaders:

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Title: Use of PGRs to increase bud break and growth of vegetative shoots during the ON-crop year and bud break in Spring the following year to increase return bloom and yield – *proof of concept for olive*

Cooperators:

Byron Fox — Commercial table olive grower, Ivanhoe
Clarence Hill — Commercial table olive grower, Exeter

2011 Objectives:

I. To conduct a proof-of-concept experiment testing Summer (Year 1) and Spring (Year 2) applications of an auxin transport inhibitor, tri-iodobenzoic acid or naringenin, combined with a commercial cytokinin, benzyladenine (BA), or the proprietary cytokinin used in our mandarin research, (i) to increase vegetative shoot growth and, hence, the number of nodes that can produce inflorescences at return bloom, (ii) to increase Spring bud break and (iii) to increase return bloom in olive.

2011 Progress to Date:

I. Per our proposal research plan, Manzanillo olive trees having just produced an ON crop and going into an OFF bloom (OFF crop) were trunk injected with 1g/tree of each of the following compounds prior to vegetative bud break in spring of Year 1 (April 1, 2011): (i) TIBA + 6-BA; (ii) naringenin + 6-BA; (iii) TIBA + the proprietary cytokinin; (iv) naringenin + the proprietary cytokinin; (v) 6-BA; and (vi) the proprietary cytokinin. Treatment (vii) was untreated OFF-crop control trees. Each treatment was applied to seven individual trees in a

randomized complete block design in a commercial table olive orchard in Ivanhoe, CA. At the initiation of the experiment, 10 shoots were tagged per tree. Ribbons were tied just after the 14th node from the apex of the shoot.

On May 25th, we counted the number of racemes (inflorescences) on each of the 10 shoots tagged per tree. Within each raceme, we counted the number of flowers and for open flowers we determined if they were perfect (both male and female floral reproductive structures present) or staminate (male reproductive structures present, female reproductive structure absent). Of the 490 shoots tagged on 49 OFF-bloom trees, only four shoots on four separate trees produced racemes on tagged shoots. Only two additional racemes on untagged shoots could be found on the 49 data trees. Shoots producing racemes were on trees treated as follows: (iii) TIBA + the proprietary cytokinin; (v) 6-BA (two racemes on two shoots); (iv) naringenin + the proprietary cytokinin; (i) TIBA + 6-BA and (vi) the proprietary cytokinin. Note that shoots on untreated OFF-bloom control trees (Treatment vii) produced no racemes. Due to the low floral intensity of the orchard, there were no significant treatment effects on flowering following the ON-crop year. In early winter 2012, we will initiate the trunk injection treatments to determine the optimal application time. We will compare the efficacy of TIBA + 6-BA treatment made in February, March and April.

Due to the fact that no crop was set in orchard 1 in Ivanhoe, we added a second orchard in Exeter, CA, in which to conduct our research in 2011. Per our research proposal plan, this summer and fall, we quantified vegetative shoot growth on a monthly basis in both orchards. Note that the orchard in Exeter had trees that set a good crop. Ribbons were placed at the end of each shoot each time it was measured so that the extension growth that took place each month was bracketed by colored ribbons. In addition, we counted the number of lateral shoots on each tagged shoot and measured their growth. This will enable us to determine when maximum shoot growth occurred. At spring bloom in Year 2, we will determine the contribution that the different monthly units of vegetative shoot growth make to bloom in the two orchards. This information is important for properly timing PGR treatments to stimulate shoot extension growth in order to maximize return bloom and yield.

In orchard 1 (Ivanhoe), which set no fruit, the majority of vegetative shoot extension growth occurred between 15 July and 17 August. Main shoot extension growth averaged only 2 mm during this period. In this orchard, 0.11 new lateral shoots developed per shoot and produced an average of 11.1 mm of new shoot growth between 15 July and 17 August. An additional 0.17 lateral shoots per shoot developed between 18 Aug and 4 October. During this period, the lateral shoots averaged 2.3 mm in new extension growth, whereas the main shoots averaged 1.0 mm in new growth. The values are low due to the fact that a significant portion of the shoots did not grow at all.

In orchard 2 (Exeter), vegetative shoot growth was also greatest between 15 July and 17 August (Table 1). The ON-crop status of the tree significantly reduced the amount of shoot growth that developed compared to that of OFF-crop trees during the period from 15 July to 17 August. Furthermore, for an ON-crop tree, the presence of fruit that set on a shoot (+fruit) significantly reduced shoot extension growth relative to shoots that did not set fruit (-fruit) on ON-crop trees. These data, while preliminary, demonstrate the negative effect of crop load on

vegetative shoot extension growth during the summer. In addition, they provide clear evidence of a whole tree effect of crop load on vegetative shoot growth and the stronger, localized inhibitory effect caused by fruit that set on a shoot. These results are consistent with those obtained in our prior research with 'Pixie' mandarin and 'Hass' avocado. We will continue to measure shoot growth in this orchard and in a new orchard in 2012.

Table 1. Effect of ON- and OFF-crop tree status and the presence (+fruit) or absence (-fruit) of fruit set on a shoot on shoot extension growth. (Orchard 2, Exeter, CA, 2011).

Tree status	No. fruit per shoot	Net shoot growth (mm) and no. of nodes per shoot			
		15 July - 17 Aug		18 Aug - 4 Oct	
ON-crop tree		-- mm --	-- no. --	-- mm --	-- no. --
shoot +fruit	22.8 a[z]	0.0 c	0.1 c	0.0 a	0.1 a
shoot -fruit	0.0 b	9.0 b	0.6 b	1.0 a	0.1 a
OFF-crop tree					
shoot -fruit	0.0 b	24.0 a	1.3 a	1.0 a	0.1 a
P-value	<0.0001	<0.0001	<0.0001	0.4004	0.6024

[z] Values in a vertical column followed by different letters are significantly different at specified *P* levels by Fisher's LSD Test.

NOTE: We received a no-cost extension through 30 June 2012 for this project due to the fact that our original orchard did not flower sufficiently to provide a good test of the capacity of the PGRs that we trunk injected in Spring 2011 on floral shoot development. The no-cost extension enables us to repeat the trunk injections again this spring (2012) and thus, complete Year 1 of this research project.

The treatments to be tested in spring 2012 include: (i) TIBA + 6-BA in January; (ii) TIBA + 6-BA in February; (iii) naringenin + 6-BA in February; (iv) TIBA + the proprietary cytokinin in February; (v) naringenin + the proprietary cytokinin in February; (vi) 6-BA alone in February; (vii) the proprietary cytokinin alone in February; (viii) TIBA alone in February; (ix) naringenin alone in February; (x) TIBA + 6-BA in March; and (xi) TIBA + 6-BA in April. Each treatment will be injected at the rate of 1 g per two syringes per compound into a main branch of each tree (all trees being treated produced fruit last year). The treatments also include control trees that produced ON- and OFF-crops last year. These are the trees for which we monitored shoot growth last summer and fall. The experimental design is a randomized complete block with 7 individual tree replications per 13 treatments. We chose February as the optimal time for trunk injecting the greatest number of treatments because it is sufficiently close but still prior to the final stages of flower development to have a strong effect on bud break and floral bud development. For all trees, except the OFF-crop trees, a minimum of 5 shoots that set fruit last year (with fruit, +fruit) and 5 shoots that did not set fruit last year (without fruit, -fruit) were tagged so we can quantify the effect of the presence and absence of fruit on return bloom on treated and control trees. The number of fruit set last year and/or remaining through January per shoot was quantified. The January treatment will be applied 19 January 2012.

University of California
Division of Agricultural Sciences

Interim Report for 2010-2011

Project Year: 2011-2012 **Duration of Project:** 1/2012-12/2012

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Title: Improving California-Style Black Ripe Olive Quality

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1. Effects of Air Oxidation and Sterilization

During the past review period we have studied the effect of processing on acrylamide formation in California-style black ripe olives (CBRO). Progress to date include the following:

a. The processing steps involved in acrylamide formation in CBRO processing have been identified (**Table 1**). Acrylamide precursors are formed during lye treatment steps when air oxidation is involved and converted into acrylamide during sterilization. Ferrous gluconate (the color fixative) is not involved in acrylamide formation and does not influence acrylamide stability in CBRO.

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

2. Influence of Brine Storage on Acrylamide Formation

During the past review period we have studied the effect of brine storage on acrylamide formation in California-style black ripe olives. Our finding to date include the following:

a. Acrylamide precursors are formed during brine storage, as well as during lye treatments (**Table 2**). The length of brine storage prior to processing influences the level of acrylamide present in the end products. Storage times < 1 month result in relatively lower levels of acrylamide as compared to brine storage times >1 month.

	Acrylamide in olive (ng/g) (wet basis)	
Description	**Modification to CBRO**	**CBRO**
No brine storage	300.3 ± 33.3	668.6 ± 69.3
No ferrous treatment	627.9 ± 49.1	591.2 ± 58.6
Sterilization: No oxygen or ferrous gluconate	461.3 ± 70.8	1078.5 ± 29.7

b. Aeration during brining results in significantly higher levels of acrylamide. This is likely because aeration promotes the hydrolysis of lipids. In general, we believe that the hydrolysis and/or oxidation of lipids during the first months of storage is producing precursors that form acrylamide upon subsequent heat treatment (sterilization) as olives lack the *typical* precursors of acrylamide formation (i.e. high levels of asparagine and glucose).

c. Acrylamide formation is not influenced by lipolytic microbes during brining. The numbers of lipolytic microbes in brined olives stored with and without aeration were counted, and concentrations of acrylamide in the processed olives were measured. The result is shown in **Table 3**. The olives stored in brine with aeration had fewer lipolytic microbes (140 CFU/g olive) than the olives (490 – 650 CFU/g) stored under anaerobic condition. Although the aerated olives contained fewer lipolytic microbes than non-aerated, the level of acrylamide in the final products was higher (693.3 to 749.7 ng/g vs 470.3 to 638.2 ng/g). Therefore, acrylamide formation in CBRO is not influenced by lipolytic microbes present during brine storage. Moreover, aeration does not inhibit acrylamide formation but may promote oxidation of lipids resulting in higher concentration of acrylamide.

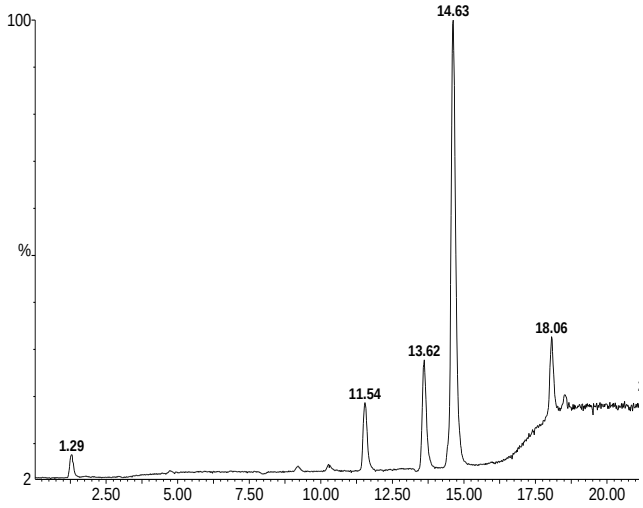
Table 3 Effect of aeration on lipolytic microbes and acrylamide level in olives (Manzanilla var.)

Sample	Olive		Storage		Acrylamide in olives	Lipolytic count in brined olives
	Size	Variety	Aeration	Length	(ng/g olive)	(CFU/g)
1	Small	Manzanilla	No	6 months	638.2(ab) ± 23.5	530
2	Medium	Manzanilla	No	Over 1 year	470.25(c) ± 6.3	490
3	Medium	Manzanilla	No	3 months	548.1(bc) ± 16.9	650
4	Medium	Manzanilla	Yes	3 months	693.3(ab) ± 78.3	140
5	Medium	Manzanilla	Yes	3month	749.7(a) ± 88.5	140

II. Identification of the Mechanism of Acrylamide Formation in California-style Black Ripe Olives

In previous studies Yasuhara[1] et al., 2003 reported the formation of acrylamide from acrylic acid and acrolein, known products of thermal degradation and oxidation of free fatty acids. Based upon our above findings, we tested the hypothesis that lipids are hydrolyzed during brine storage and release free fatty acids. These fatty acids are then subsequently oxidized during lye treatment and are converted into either acrolein or acrylic acid during sterilization.

a. SPE (stationary phase extraction) followed by LC-(ESI-)MS/MS methods, which include separation of free fatty acids from neutral lipids (tri-, di- and monoglycerides) and phospholipids were developed. Six main fatty acids including oleic acid (C18:1), palmitic acid (C16:0), linoleic acid (C18:2), stearic acid (C18:0), palmitoleic acid (C16:1) and linolenic acid (C18:3) have been identified in commercial olives **Figure 1**. The main fatty acids were oleic acid > palmitic acid, > linoleic acid and stearic acid.



b. Sensitive extraction and LC-(ESI)MS/MS methods were developed for the identification of acrolein and acrylic acid in olives. These methods involve the derivatization of acrolein with 2,4-dinitrophenylhydrazine (DNPH) prior to LC-(ESI-)MS/MS analysis.

c. Acrolein is present in sterilized olives whereas acrylic acid is not present in sterilized olives. These findings indicates that acrolein is the acrylamide precursor. Acrolein is formed during sterilization.

III. Continued & Future Work

Progress made over the past few years with the support of the COC has been significant. We have identified key processing steps to target for modification and/or addition of antioxidants to reduce acrylamide levels in CBROs. We have developed the analytical methodology needed to identify the lipids involved in acrylamide formation and to identify the chemical mechanism for acrylamide formation in CBROs. This methodology will allow for evaluating the role of natural and synthetic

[1] Yasuhara A, Tanaka Y, Hengel M, Shibamoto T. 2003. Gas chromatographic investigation of acrylamide formation in browning model systems. J Agric Food Chem , 51, 3999-4003.

antioxidants in reducing acrylamide formation at key processing steps. Future plans include the following:

a. Develop a quantitative method to measure concentration of acrolein in olives.

b. LC-(ESI-)MS/MS methods will be used to evaluate the levels of free FFAs in oil extracted from fresh and brined olives at different storage times.

c. Identify the exact chemical mechanism of acrylamide formation from lipids identified in the formation of acrylamide using isotope-labeled FFAs

d. Determine if antioxidants may reduce formation of acrylamide by reducing the oxidation of hydrolytic products of lipids

Research is expected to continue for another ~9-12 months with funding supported through TA appointments and with monies from the Kinsella Chair Endowment. We will keep the COC updated on research during this time and upon completion of this project.

2011 Southern San Joaquin Valley Olive Fruit Fly Monitoring Project-Final Report

Project Leader:

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Cooperating Personnel:

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Rod Burkett, Leffingwell Ag Sales Co., Lindsay CA
Bob Felts, Leffingwell Ag Sales Co., Ivanhoe
Judy Stewart-Leslie, Consultant, Exeter, CA

Reporting period: 1 April – 26 November 2011

INTRODUCTION

Monitoring of Olive Fruit Fly (OLFF) in Southern San Joaquin commercial olive groves started in 2001 and has continued every year in one form or another for the last 11 years. Over the years, the locations have changed and the number of traps per acre has been reduced. Starting in 2007, trapping was changed to 9 groves in Tulare County and one location in the city of Visalia. Traps are monitored weekly from April through November. OLFF is currently the most serious pest in olives. Black Scale can be a problem in certain years (especially in years with cooler than normal late spring and summer temperatures, as in 2010 & 2011. Olive Scale is currently under excellent biological control. Ivy and Greedy Scales rarely require treatment.

OBJECTIVES

The objective of this project was to continue the monitoring 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 treatments, was the goal of the project. Monitoring gave growers and Pest Control Advisors information on the general OLFF population. This information was specific for only the groves being monitored but was available to aid in making OLFF management decisions in their respective production areas.

PROCEEDURES

Nine sites in commercial olive groves in Tulare County were set up with two traps per site. The locations of the sites were near Ivanhoe, Woodlake, Exeter, South Exeter,

Tonyville, West Lindsay, Strathmore, Porterville and Terra Bella. In addition, an olive tree located at a home situation in Visalia was also monitored. This year, the tree at the Visalia location was pruned heavily and the fruit set was nearly non existent so another nearby tree was trapped in addition to the original location. All traps were in place by the first of April (with the exception of the second Visalia tree) and monitored weekly through November. Reports detailing the number of OLFF male and females per site were e-mailed or faxed to the California Olive Committee office and interested parties. Reports were sent weekly as soon as possible after the traps were checked. An experimental project was also started in the fall using an Ipad and Agrian software to report the data. This part of the project took considerable extra time to implement and was not a time saver for us.

RESULTS AND DISCUSSION

With cooler than normal spring and summer temperatures (except for a very few hot days) high populations of OLFF should have been expected early. Trap results started out in the spring at lower than normal levels. Summer trap results were higher then the 10 year average. The October/November trapping results were well above average and were the highest in all the years of trapping.

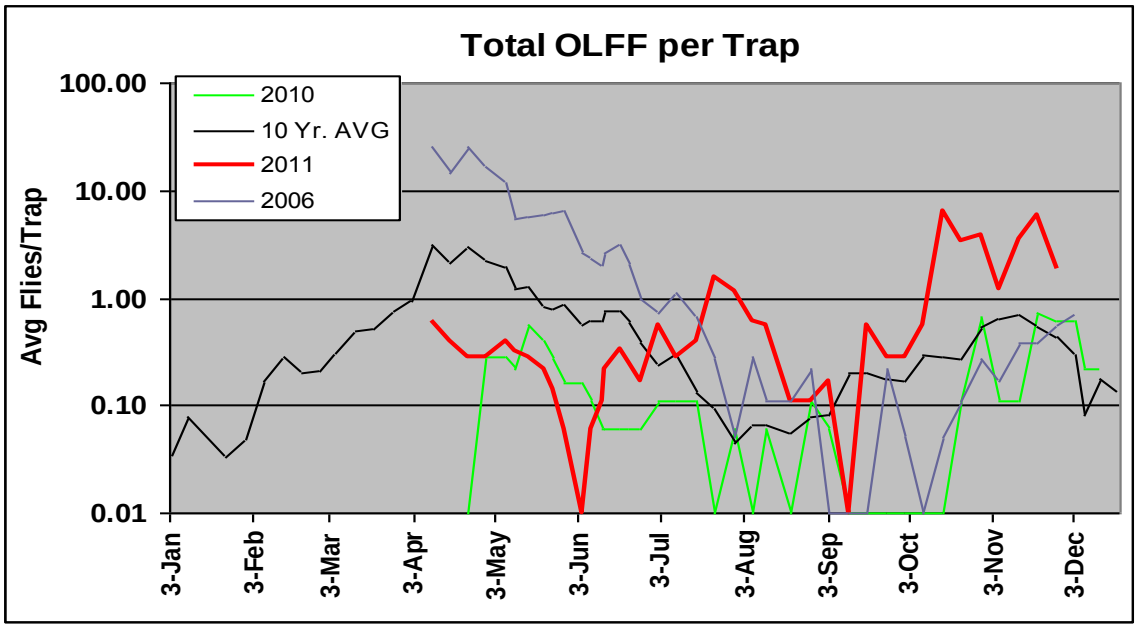

Fig 1. Ten year average of total Olive Fruit Fly (OLFF) caught per trap during the trapping season compared with results trapped in years 2006, 2010 & 2011.

The Ivanhoe site caught the most OLFF followed by the Woodlake Site. The Terra Bell Site normally Traps a significant amount but they were a distant third this tear. Moderate numbers were trapped at Exeter and Tonyville. The lowest numbers trapped were at

South Exeter, West Lindsay, Strathmore and Porterville. The trap at the Visalia location (Thanks to Steve Sibbett and his assistant for taking care of this location) that had fruit on the tree had significant trap catches starting in June and especially in mid October through November. Fruit damage was significant and in late November and was nearly 100%. We did sample fruit at some sites well after harvest (Late November). We sampled 100 fruit at random and examined them for OLFF damage and larvae. The following was the result:

LOCATION	% DAMAGE	LARVAE
WOODLAKE	10	5
IVANHOE	22	10
EXETER	2	1
TONYVILLE	11	3
W. LINDSAY	6	4
TERRA BELLA	6	2

Fig 2. Fruit damage and number of larvae per 100 fruit

The OLFF populations will be interesting to follow this next spring in this area.

FUNDING SOURCES

The following supported this project:

California Olive Committee	$6,000.00
Leffingwell Ag Sales	$6,000.00
Ag IPM Consultants, Inc	$6,000.00
Total	$18,000.00