

CALIFORNIA AVOCADO COMMISSION
PROJECT PLAN – RESEARCH GRANT PROPOSAL

Proposal Budget Requested: \$ 82,892

CAC Fiscal Year 2008: November 1, 2007 – October 31, 2008

Anticipated Duration of Project: 4 Years

This project is: New

Project Title: **Salinity-Chloride Interactions: Their Influence on Yields**

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Research Priority Areas:

Irrigation and salinity management in avocado, including water management strategies designed for optimal yield, with attention being given to varying: 1) rootstocks; 2) soil types; 3) water quality; 4) environmental conditions, and 5) development of thresholds for leaching and crop coefficients under saline environments.

Relevant Published Research (Adobe pdf files available at avocadosource.com)

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Literature Review

Avocado is one of the most salinity sensitive horticultural crops, but is commonly grown in areas of California having saline irrigation water (an EC greater than 0.75 dS/m and chloride >100 ppm). Resulting problems associated with high soil salinity and chloride toxicity include reductions in fruit yield and tree size, lowered leaf chlorophyll content, decreased photosynthesis, poor root growth, and leaf scorching. In California, this problem is common as the cost for irrigation water has increased and growers have come to rely on groundwater that may be saline. A confounding problem is that certain district waters, eg. Lake Skinner that serves most of San Diego water districts are also marginal quality from a salinity perspective. Since the 1950s there has been extensive research on irrigation management, and it has been well established that the impacts of salinity are caused both by high salt, measured as EC. It has been difficult in both controlled studies as well as field studies to separate the negative influence of specific ion toxicities, namely chloride and sodium, from those of salinity. Thus, one of the major questions today is whether to manage irrigation with respect to total salinity (EC) or chloride, or perhaps both. The principle objective of this research is to clarify this question and examine the reduction in root growth and tree yields that are associated with chloride and salinity or a complex of both over the broad range of conditions that are represented in the avocado production region of southern California.

Management of salinity in avocado orchards requires a multifaceted approach that considers not only the tolerance of different rootstocks to salinity and chloride uptake, but also management of irrigation practices that influence water availability and stress in avocado trees. In Israel, which uses primarily West Indian (WI) or WI hybrid seedling rootstocks, avocado trees are grown using high chloride containing water (over 220 ppm) that has a low EC and trees are purported to show relatively minor leaf burn symptoms that are associated with chloride damage. This suggest that water availability is equally critical to managing salinity in avocado groves, and that if the irrigation water is properly managed, chloride toxicity symptoms will not develop. On the other hand, recent studies show that root growth is much more sensitive than shoot growth to specific toxicities caused by NaCl (Bernstein et al., 2004). In that study, the threshold NaCl concentration that causes root and shoot growth reduction occurred between 5 and 15 mM. A concentration of 15 mM NaCl decreased leaf biomass production by 10%, but induced a 43% reduction in the rate of root elongation and decreased the root volumetric growth rate by 33%.

Accumulation of chloride in the leaf tissues by itself is not always associated with leaf scorching symptoms, but when combined with water stress may result in the familiar foliar symptoms of leaf burn on the tips and margins of the older leaves. Results of our field studies also show that the EC of water below avocado trees seldom exceeds 4 dS/m, which suggest that avocado trees do not efficiently extract water from solutions having this level of total salinity. This is also supported by the production function model developed by Oster and Arpaia which is based on results from a multiyear study at Covey Lane (Oster et al., 2007). In this case, Oster predicts a linear decrease in yield with increasing salinity (total salts), and suggests that there is no threshold, such that yields are already reduced below their maximum potential even with what most growers would consider to be acceptable quality irrigation water. On the other hand, researchers in Israel contend that chloride is the main limiting factor. (Lahav, 2003). In their studies, the rule of thumb given by Lahav was that “there is 12% loss of production for every milliequivalent of chloride in the irrigation water (1 meq/L Cl = 35.5 ppm)” More recently, Mickelbart et al (2007b) demonstrated under sand tank conditions the relationship between leaf Cl levels and necrosis for 3 rootstocks, Thomas, Duke 7 and Toro Canyon. Leaf necrosis occurred particularly in older leaves and at similar concentrations of Cl (~ 4000 ppm).

Although there is still controversy about which is most important, it is likely that both total salinity and concentrations of NaCl in the irrigation water and levels in the soil will affect avocado growth and yields. Previously, most of the concern over chloride has been driven by the appearance of leaf scorch symptoms that are associated with chloride toxicity and that typically manifest in late summer and early fall. Nonetheless, if Lahav and Oster are correct, there is a hidden cost to salinity and chloride that is not fully appreciated by most growers. Among the likely reasons for reduced yields are reduced water uptake and stress during flowering and fruit set. This would be explained as a combination of lower root volume and high water osmolality, the latter contributing to diminished ability to extract water from the soil during key periods of growth. It has also been suggested that water deficits cause changes in the nitrogen metabolism of plants, which leads to ammonia accumulation and leaf burn symptoms (Lovatt et al. 1987).

Local water management districts have set 100 ppm of Cl as a target for maximum chloride levels for irrigation water. However, this value is controversial since the data on yield coefficients at different salinity and chloride levels are not yet available. In conjunction with chloride, soil factors are also important in causing salinity problems in avocado trees and other crops. Generally, high clay soils that have poor drainage are particularly problematic in that salts are not easily leached, and irrigation water may be perched in the soil above hardpan layers where salts accumulate. For example, studies with grape vines suggest that soil drainage and waterlogging following irrigation greatly affect salt uptake by plants (Stevens and Harvey, 1995). Under saline conditions, the use of chloride excluding grape rootstocks reduced leaf chloride concentration by 60% in vines with free-draining root zones but by only 18% in vines with waterlogged root zones. This suggests that over watering may also cause problems. Poor tree performance especially on heavy soils is also influenced by low oxygen and asphyxia (Schaffer, 2006). Altogether, it is becoming increasingly clear that along with rootstock selection, irrigation management and determination of the proper leaching fraction are critical aspects of an integrated strategy for salinity and chloride management.

Over the past several years of our research in a prior salinity study, and in earlier published research (Micklebart and Arpaia, 2002) and by researchers in Israel, a large number of rootstocks have been evaluated for salinity tolerance. Early data has suggested that salt tolerance is greatest in West Indian rootstocks and poorest in the Mexican rootstocks (Embleton, et al., 1955; Ben-Ya'acov, 1970; Gustafson et al., 1970). In southern California, West Indian rootstocks have not been used in breeding programs or as commercial rootstocks because of their putative poor cold tolerance. However, several West Indian varieties have been identified by Israeli researchers as having excellent salinity tolerance. In all of this previous work, one of the critical shortcomings has been the lack of information on the true heritage of the rootstock materials. New molecular methods that are being used to classify rootstocks will help to determine whether such a link between salt tolerance and race origin exists. In spite of this, we now know that within the Mexican race rootstocks used in California, there are differences in salinity tolerance (Oster and Arpaia, 1992; Micklebart and Arpaia, 2002; and Micklebart et al, 2007b). In these studies, two commonly used clonal rootstocks, Toro Canyon and Duke 7, have consistently demonstrated higher tolerance as compared to a third rootstock, Thomas. This is good news in the sense that it demonstrates considerable variability in salt tolerance likely exists within avocado races.

Other relevant research indicating that certain rootstocks may confer resistance to stress caused by salinity has been conducted with citrus, which is similarly damaged by high chloride concentrations in the foliage. In a 6 year study with three rootstocks, fruit yield and growth of

lemon trees were differentially affected depending on which rootstocks were used (Cerda et al., 1990). The threshold values for salinity stress were 1.5, 2.1, and 1.0 dS m⁻¹ for sour orange, Cleopatra mandarin, and macrophylla, respectively, after which yield reductions were 10-15% for each unit increase in salinity. Physiological mechanisms of salt tolerance include a number of plant responses that have been characterized in various model plant species, but not in avocado. As a general principle, high sodium is thought to displace calcium from the root cell walls, which causes leakage of potassium and other plant metabolites from the root (Picchioni et al., 1991). As reviewed by Kafkafi and Bernstein (1997), maintenance of adequate potassium concentrations and the proper potassium/sodium ratios in plant tissues is necessary for cellular function under saline conditions. Interestingly, in citrus chloride accumulation in the leaf tissue is also scion dependent when different scions are grown on the same rootstock (Garcia-Legaz et al., 1993). Once chloride is taken up and transported to the scion, the physiological affects of high chloride are manifested by reduced photosynthesis, and decreased stomatal conductance, transpiration, and decreased gas exchange (Garcia-Legaz et al., 1993).

In citrus, highly saline water has been shown to reduce potassium, calcium, and magnesium uptake and rootstocks that accumulate calcium appeared to have reduced salinity stress (Banuls et al., 1990; Alva and Syvertsen, 1991). In lime trees, resistance to salinity is associated with chloride exclusion and high selectivity of the roots for potassium as opposed to sodium (Storey and Walker, 1987). Nutrient interactions that influence uptake of chloride by avocado are not yet understood. Nitrate has been shown to ameliorate the uptake of chloride, provided that nitrate is supplied continuously at a molar concentration equivalent to half that of chloride (Bar et al. 1997). Selectivity in the transport of Cl and Na to the scion is also affected by the rootstock (Banuls et al., 1990). Altogether, these studies suggest that fertilization and plant nutrient interactions may play an important role in salinity tolerance, along with other management practices (Lahav 1987). Mickelbart et al (2007a) demonstrated that nutrient levels in 'Hass' can be influenced by both clonal rootstock and yield. Among the 3 rootstocks, Toro Canyon, Duke 7 and Thomas, there were consistent differences in leaf nutrient levels through the course of the study. The authors also found that P, Ca and S were highest in leaf tissue in "on-years: while Fe was highest in "off-years". Mickelbart et al (2007b) showed that among these 3 rootstocks under sand tank conditions, similar trends for 'Hass' leaf levels although salinity moderated this effect. In this study, unlike salinity's impact on Ca levels, the authors observed that salinity had no effect on Ca concentration. The results of this work suggest that Cl and Na:K ratio in older leaves may be a useful marker in studying salinity tolerance in 'Hass' on different avocado rootstocks.

Breeding for salinity tolerance to date has relied on traditional approaches in which large numbers of seedlings are planted in highly saline soils and are then selected based on observations of their field performance when grafted to commercial cultivars. Another more technologically sophisticated approach with citrus has been to genetically engineer trees with a gene for salt tolerance that has been taken from yeast (Cervera et al., 2000). To date, the gene has been successfully transferred, but the trees have not yet been actually tested. One of the long term goals of earlier research conducted by us has been to develop new avocado lines through traditional breeding blocks. Another approach that holds promise for evaluation of new materials is the use of microsatellite markers which can be used to draw linkage maps for important traits.

Soil conditioners have been marketed for increasing salinity tolerance in citrus and other tree crops. Presumably, these materials help to increase water availability in soils and offset the effects of drought on salt burn caused by a combination of high salt concentrations in the leaf tissue and low leaf tissue water content. However, a recent study comparing three different commercial

products showed that none of the soil conditioners had any effect on salinity tolerance in citrus (Boman, 1998), and thus are unlikely to be of benefit to other tree crops.

Listed Research Priority Area to which this Project is Related

Irrigation and salinity management in avocado, including water management strategies designed for optimal yield, with attention being given to varying: 1) rootstocks; 2) soil types; 3) water quality; 4) environmental conditions; and 5) chloride and 6) development of thresholds for leaching and crop coefficients under saline environments.

Research Summary

One of the continuing questions by avocado producers is the cost benefit ratio when using different irrigation water supplies to produce avocados. Should the focus be on total salinity, or on chloride or both? How much yield reduction can be anticipated if intermediate grade water supplies, eg., salinities of EC 0.25-0.75 dS/m (USDA Handbook 60), chloride 50 – 100 ppm, are used? Is it more important to control salinity in the root zone during critical periods such as fruit set? What degree of benefit is there in switching to salt tolerant root stocks when dealing with intermediate levels of salinity? If higher quality, more expensive water is available for irrigation, how much increase in yields might be expected, and can this justify the increased water costs? To answer these questions, the proposed research will examine the relationship between soil water availability, chloride, and salinity across a range of orchards in California using different water supplies that vary in total salts and chloride contents. The research will take advantage of preexisting plantings of rootstocks that are currently being investigated for *Phytophthora* resistance and salinity tolerance.

Project Objectives:

To compare the salinity tolerance of currently used and newly developed *Phytophthora* root rot resistant rootstocks in field experiments which will allow us to ascertain the physiological basis of salinity tolerance, and make recommendations for specific rootstocks which can be used by avocado growers in California. We will be able to test the hypothesis of Mickelbart et al (2007b) that leaf Cl concentrations as well as the Na:K ratio of older leaves may be a useful marker in screening for salinity tolerance.

We hypothesize that water quality is a primary determinant of differences in tree root growth and yield that will be apparent in a broad based survey of orchards. In our study, we will select trees that are similar in age, and under similar levels of *Phytophthora* pressure, and will monitor root growth, annual relative canopy volume increases, fruit yields, leaf chloride and sodium, soil water status, and soil water salinity. Relevant cultural information such as fertilizer applications and timing will be obtained from the growers for use in the data analysis and interpretation. If salinity and chloride are associated with measurable differences in yield reduction as has been indicated in the literature, a broad based survey of orchards should still reveal this phenomenon when examined across the full range of environmental and management variables. Where possible, we will also work with growers at individual locations to use different irrigation water supplies on different parts of their orchard to examine site specific salinity effects. The trees available for this research are described in Table 1, and are located across a

range of soil types in the northern and southern avocado regions in California. The trees range in age from 3 to 5 years old, and are all now of fruit bearing age. The same trees are currently being monitored by Dr. Greg Douhan, who is carrying out the Phytophthora root rot surveys initiated by Menge, and who will provide us with data on the status of the Phytophthora root rot infection pressure in the different orchards we have selected. Additional orchards may be selected after final consultations with the project area subcommittee and UCCE farm advisors.

Table 1. Rootstock selections and locations of candidate trees for the proposed study. Orchards listed below are those that are being actively monitored in Douhan’s rootstock field trials. Disease pressure indices provided below each rootstock are from 2006 survey.

Southern CA										Northern CA																		
Rootstocks	Beck (2002)	Beckstead 1(2003)	Beckstead 2(2005)	Cornell(2005)	Krusser(2006)	Mellano 3(2005)	Malone(2000)	Marke(2003)	Pettygrill(2002)	Perkins 31(2002)	Richards(2004)	Stahley(2003)	Rootstocks	Bishop clay(2004)	Bishop sand(2004)	Baker(2004)	Broome(2005)	Cavalleto(2003)	Cal Poly(2003)	Gerry(2003)	Gunderson(2006)	Moro creek(2003)	Smith 2(2001)	Smith#3(2006)	Tobias(2002)	Varan #1(2005)	Varan #2(2005)	
Thomas	X	X				X	X	X	X	X	X		Thomas	X	X						X	X	X	X	X	X		
Merensky II (Dusa)			X	X	X		X						Merensky II (Dusa)								X	X	X	X	X	X	X	X
Merensky I (Latas)		X					X						Merensky I (Latas)			X	X											
Toro Canyon		X						X					Toro Canyon															
VC801	X		X	X		X				X		X	VC801								X	X	X	X	X			
PP14 Uzi		X		X			X	X		X	X		PP14 Uzi					X	X	X	X	X	X	X	X			X
PP24 Steddum		X					X	X			X	X	PP24 Steddum					X	X			X	X					
PP41 Witney			X	X		X			X				PP41 Witney	X	X	X					X	X				X	X	
Number of trees	200	200	180	260	100	120	300	200	200	100	200	200		200	200	180	180	180	140	200	300	160	180	280	200	160	80	
Disease Pressure	1	2	2	1	?	2	3	1	1	2	3	2		2	2	1	1	2	1	2	3	1	1	3	1	3	3	

- 1 = low
- 2 = medium
- 3 = high

The proposed study will focus on tree growth responses and yields in relation to chloride and total salinity. An inherent difficulty of this research is the large variability in soil types and variation in local cultural and climatic factors that influence avocado yields at different locations. Individual orchards will vary in their tree spacing, topography, pruning methods, irrigation system layout (differences in wetted soil volume), water quality, fertilization practices, and soil chemical and physical properties (pH, CEC, soil compaction). Thus there are many interacting variables that will potentially influence yields other than salinity and chloride that will be the focus of this study. This is both a strength and weakness. If salinity and chloride are primary determinants of yield, this should be evident from the data even when examined across a wide range of locations. Irrigation frequency and fertilization practices are probably less of a concern that might first appear, as good orchard managers seek to optimize the nutritional and irrigation requirements of their trees, leaving differences in water quality, leaching practices, and disease pressure as the main variables that determine differences in yield. The impact of irrigation variables can be ascertained by soil water monitoring and will be related to dependent variables for each tree to include fruit yield, root growth, and leaf tissue contents of toxic chloride. A potential pitfall is that large variation associated with all of the myriad management practices may increase the difficulty to detect statistically significant differences associated with chloride and salinity. In any event, based on prior studies examining yield, it is likely that three to four years will be required to determine yield differences. Measurements of root growth on the

other hand are predicted to be a more robust variable and may provide insight into tree responses for different rootstocks across a range of soils and irrigation water chemistries.

To establish the experiment, the research will be initiated by a series of visits to each of the orchards selected from Table 1, at which time we will examine the status of the orchard, the location of individual trees, and examination of their suitability for inclusion in the experiment. Initial canopy volumes will be measured, and selected trees will be tagged and mapped for inclusion in the experiment. Grower records will be consulted to obtain information on the cultural practices, including irrigation system details, water source, historical water analysis records, fertilization practices, and Phytophthora and insect pest histories in the orchard. Water and soil samples will be collected for analysis to determine total salinity (EC), pH, potassium, calcium, chloride, and sodium, in the irrigation water, and soil texture and bulk density under the trees. The latter data will be used to estimate soil water holding capacity. After the final selection of trees and locations for the study, the sites will be revisited to install irrometer water mark soil water monitoring devices at the canopy line of each tree included in the study. Each tree will be further monitored with the installation of a porous ceramic cup suction lysimeters at 12 inches in the middle of the wetted soil zone occupied by the roots. Intact soil cores will be taken in the period following the fall root flush in November to ascertain root densities. The soil cores will be taken from beneath each tree in the middle of the wetted zone, and will be taken to a one foot depth. We will also monitor temperature, wind, and rainfall at each experimental site.

During the year, the soil water data will be collected from each tree at 1 month intervals. Soil water samples will be extracted from the lysimeters at the base of each tree for analysis of chloride and total salinity. The extraction tubes will be located in the “average zone of wetting” within the sprinkler distribution pattern as described by Oster et al. 2007. The soil salinity levels will be normalized with respect to total soil water at field capacity using data readings from the soil water monitoring equipment. Leaf samples will be collected in late September for analysis of chloride and sodium concentrations for each tree.

Given all of the factors that affect yield, detection of statistical differences will require use of multivariate analysis methods to take into account variation due to different cultural factors. One of the most powerful statistical tools for examining multiparametric data in which there may be nonlinear interactions between variables is the use of a type of artificial neural network called a Kohonen self organizing map. This analytical method provides a visual output that reveals patterns within complex data sets and possible correlations between different variables. In this manner, the data set can be explored to examine for covariation, for example interactions of soil texture and salinity, which can then be examined using more traditional multivariate analysis such as multivariate analysis of variance and regression modeling. If particular variables are consistently linked and are of importance for further understanding the problem, specific experiments can be designed to tests hypotheses with greater precision than in the trans-industry survey. Crowley has recently explored the utility of these methods for sorting soil quality variables in Australia. The statistical tools developed here will eventually lead to a computer based decision support tool to assist avocado growers with salinity management.

Summary of Contribution to New Knowledge

The proposed research will provide a scientifically based evaluation of the relative impacts of salinity and chloride on root growth, tree growth, and fruit yield for avocado produced on different rootstocks. Our previous data has shown remarkable differences between rootstocks with some providing 40% lower chloride levels in the leaf tissue as compared to the least tolerant

rootstocks. General recommendations for irrigation water now recommend 100 ppm as the maximum chloride levels in water. In our prior research, several of the rootstocks we examined performed well with good growth and moderate leaf salt burn using irrigation water containing 250 ppm chloride. There were also differences in growth, which suggest that some rootstocks are better able at extracting water from the soil at high salinity. If successful, this project will help to determine the upper limit for chloride tolerance that can be conferred using different rootstocks, and will allow us to identify those rootstocks that have the greatest water use efficiency under saline conditions. The research team will also work to develop irrigation management strategies that consider all of the different management parameters including irrigation frequency, amount, EC, and chloride levels. These strategies will be developed into formal recommendations that will assist avocado growers with an integrated plan for salinity management.

Project's Benefit to the Industry:

This project will provide avocado growers with information on the hidden cost of salinity and chloride in causing yield reductions in avocado. With this information, growers may obtain data to support their decisions with respect to the potential cost and economic benefits of using various water sources when available. The research will also help to show whether there is a cost benefit in switching to some of the new salinity tolerant rootstocks (eg., Latas, VC801, VC 207, DUSA) even in situations where salt burn is minimal or nonexistent, but where salinity or chloride may still be causing measurable decreases in yield. Water costs are increasing every year, with frequent droughts and soil salinization becoming increasingly severe problems in California. Research on the interrelationship of tree responses to salinity and chloride for different rootstocks and soils is expected to provide fundamental information that will lead to development of integrated management practices that are critical to long term viability of the avocado industry in California.

Schedule of Expected Accomplishments

The orchards to be included in the study will be surveyed in November and December 2007. Soil water monitoring equipment will be installed in January and February. Soil water monitoring will commence in March and will continue through the project with monthly visits to each tree. Soil cores, root densities, and soil analysis will be conducted in November. Leaf samples will be collected in late September 2008 and at a similar time annually thereafter. Tree canopy volumes are measured in the spring, along with fruit yield data. Data will be analyzed and summarized for the semiannual and annual reports as soon as possible to provide a continuing update on the experiment and water use efficiency for the rootstocks over the growing season in relation to changes in soil and irrigation water salinity.

Dates on which semiannual reports will be filed: April 15, 2008.

Expected Duration of this Project: This project is suggested for a total of 4 years.

PROPOSED PROJECT BUDGET

Budget Year 2007-08 (Nov 1, 2007 – Oct 31, 2008)

Salaries & Benefits:

Primary Researcher/Project Leader:	_____
Benefits:	<u>\$18,862</u>
Postdocs/Research Assistants:	_____
SRAs:	<u>\$38,030</u>
Subtotal:	<u>\$56,892</u>

Supplies /Leaf Analysis \$6,000

Equipment: \$12,000

Operating Expenses: _____

Travel: \$8,000

Other: _____

Total: \$82,892

*Supplies/Leaf tissue analysis funds are for chloride and sodium analysis on 100 trees in September and for analysis of water samples (~1000 samples from soil lysimeters).

Equipment funds are requested to purchase soil lysimeters and soil water monitoring equipment that will be installed at each location. Our plans are to include 100 trees in this study. Suction lysimeters are priced at \$68 dollars per unit, water mark water sensors are \$32 each. Additional water mark sensors will be installed with a data logger at individual orchards to obtain records of watering frequency for use in irrigation calculations. Costs are based on \$100 per tree included in the study, and an additional \$2,000 for purchase of data loggers and accessory equipment for reading and servicing the water monitoring equipment.

Travel costs are based on rental of a sedan from fleet services at a daily rate of \$16.62 and 24 cents per mile. Overnight lodging and meals at per diem rates, or actual expenses, whichever is lower) will be required for trips to the northern locations.

Salary funds are requested to support a Staff Research Associate at 100% time. The designated SRA Steven Qi will be responsible for maintaining the lysimeters, collection and analysis of soil water samples, data entry, soil and plant tissue analyses, and for providing routine oversight of the field experiment.

Indirect cost (overhead) is not allowed by the California Avocado Commission.

Is satisfactory execution/completion of this research project contingent upon receiving support funding **in addition to** CAC funding?

Yes

No

Indicate approximate total support funds for this project expected from other sources; indicate source of all funds, in-kind or otherwise).

None anticipated

If multiple years are anticipated for this 2007-08 project, what is your projected budget for each year? FY 2008-09 \$ 65,000 FY 2009-10 \$ 65,000

Note: Each year of a project must be approved prior to the fiscal year in which it will occur. There is no guarantee of funding.

Payment/Disbursement

CAC payment/disbursement is quarterly. A minimum of 20% of approved funding will be **withheld** pending receipt and approval of final report.

All approved researchers will be required to sign a CAC Research Agreement for each year CAC funding is provided.

Signature (Project Leader): _____

Date: May 30, 2006

Approved By:

(Organization's Authorized Representative): _____

Date: _____

CALIFORNIA AVOCADO COMMISSION Approval:

Tom Bellamore, Corporate Counsel and Senior Vice President Date

Date: _____