CALIFORNIA AVOCADO COMMISSION
PRODUCTION RESEARCH COMMITTEE
MEETING MINUTES

May 26, 2023

A web/teleconference meeting of the Production Research Committee (PRC) of the California Avocado Commission (CAC) was held on Friday, May 26, 2023, with the following people participating:

MEMBERS PARTICIPATING VIA TELECONFERENCE:
Leo McGuire, Chair
John Burr (9:07)
Jason Cole
Jim Davis (9:21)
Consuelo Fernandez
Danny Klittich
Daryn Miller (9:15)
Ryan Rochefort

CAC STAFF PARTICIPATING:
April Aymami
Ken Melban
Jeff Oberman

OFFICIALLY PARTICIPATING:
Dr. Tim Spann, Spann Ag Research & Consulting
Raquel Folgado
Fatemeh Khodadadi
Haizhou Liu
Gray Martin
Josette Tin

GUESTS PARTICIPATING:
Ken Weiss
Jamie Shafer

CALL TO ORDER

Leo McGuire, Production Research Committee (PRC) Chairman, called the meeting to order at 9:01 a.m. with a quorum present.

OPPORTUNITY FOR PUBLIC COMMENT

Ken Weiss introduced himself as a small avocado grower in Carpinteria and a retired journalist.

APPROVAL OF MINUTES OF OCTOBER 4, 2022 PRODUCTION RESEARCH COMMITTEE MEETING

MOTION

To approve the minutes of the October 4, 2022 Production Research Committee meeting.

(Cole/Rochefort) MSC Unanimous

Motion 23-5-26-1

RESEARCH PROGRAM DIRECTORS REPORT

Dr. Spann reported that the Avocado Brainstorming meeting was held in Marcoola, Queensland, Australia, March 26-31, 2023. He reported that about 40 people attended the meeting, which was down from the previous two meetings he attended. There was a marked shift in the overall focus of the meeting from the avocado genome, which was heavily discussed at the previous meeting in South Africa, to more focus on adapting to climate change and keeping the world avocado industry productive. Dr. Spann reiterated to the committee that is difficult to say exactly how CAC or the California avocado grower benefits from CAC's participation in the Brainstorming. The Brainstorming meeting wrapped up with the group agreeing to meet in 3-years in Spain.

DISCUSSION ITEMS

A. Consider research suggestions from Gray Martin

Mr. Gray Martin, a former staff researcher at UC Riverside, made a presentation to the Committee about his thoughts on improving yields of avocados in California. He discussed that he believes better understanding of plant "phase," juvenile vs. mature, could help in plant breeding and selection for better rootstock and scion varieties. He concluded by stating that he was requesting, on behalf of the informal group known as AGC (Avocado Growers of California), $15,000 to help AGC build a website and support grower meetings. Leo McGuire informed Mr. Martin that for the Committee to consider a funding request, a formal proposal with a workplan, budget and budget justification would need to be provided to CAC for the Committee to review.

B. Research report, "Development of chloride mitigation strategies for California avocado growers: Technology review and treatment prediction"

Dr. Haizhou Liu, Professor of Chemical Engineering at UC Riverside, presented his preliminary report on his CAC-funded research project to compile information on what technologies exist to mitigate chlorides in irrigation water. He reported that, of the technologies reviewed, three technologies hold the greatest promise of being implemented at the grove level: nanofiltration (NF), electrodialysis reversal (EDR) and chloride-selective ion exchange (IX). Dr. Liu discussed the advantages and disadvantages of each technology, such as whether the technologies are mature and ready for implementation on a large scale or still need further development before commercialization. Dr. Liu concluded that EDR is likely the best technology to explore

for pilot studies in avocado groves because of the low pressure required, low electric energy input, ease of operation, low brine generation and the ability to remove greater than 95% of chloride.

Discussion ensued and some Committee members questioned Dr. Liu's estimates of grove water use, which would directly impact his cost estimates for the various technologies. Questions were raised about where EDR is currently being used and if there is any current agricultural use of the technology. Dr. Liu indicated that the technology has been adopted in industrial settings and the only agricultural use that he is aware of is in large greenhouse operations. The Committee was pleased with the overall result of Dr. Liu's work and made several recommendations for additional details to be included in the final report.

C. Research report, "Micropropagation of avocados in California"

Dr. Raquel Folgado and Ms. Josette Tin from the Huntington Botanical Gardens made a presentation about avocado micropropagation, which was partially funded by CAC, that Ms. Tin presented at the World Avocado Congress in New Zealand. The pair discussed the system they have developed for micropropagating avocados and indicated that the system is working well for several avocado varieties and they are continuing to improve their methodology to expand the number of varieties they can successfully propagate. They indicated that the next phase of their research will involve studies on *in vitro* nutritional needs of the various varieties to further optimize the system.

D. Pine Tree Ranch Lease Renewal

Dr. Spann reminded the Committee that at a prior meeting they had recommended pursuing a renewal of CAC's lease for the Pine Tree Ranch demonstration grove in Santa Paula which is leased from the Cal Poly Pomona Foundation. Dr. Spann informed the Committee that staff had been working with Cal Poly representatives to negotiate the lease renewal and the current offer from Cal Poly was for a 5% rent increase from the current rental rate for the first year of the renewal followed by a 4% increase each year thereafter. He also reminded the Committee that a major issue for CAC was the need for a variable frequency drive (VFD) to be installed on the well so that CAC could irrigate independent of the rest of the ranch. Discussion ensued with the reason for the rent increase being questioned. The group agreed that with current inflation, even with the 5% increase, the rent was reasonable. Further discussion surrounded the VFD with the recommendation that CAC staff be involved in developing the specifications for the VFD to ensure it met CAC's needs, as well as the suggestion that rent be withheld until the VFD was installed.

MOTION
Accept the Cal Poly Pomona Foundation's lease renewal offer with a 5% initial rent increase to be followed by a 4% increase annually thereafter, but with a stipulation that the VFD be installed by December 31, 2023 with no rent payment

until the work is complete and that CAC be involved in developing the specifications for the VFD.

(Davis/Klittich) MSC Unanimous

Motion 23-5-26-2

E. Avocado rootstock trial "Alina Ranch" replacement trees

Dr. Spann reminded the Committee that CAC was currently funding a series of rootstock trials throughout the avocado growing region with Dr. Patricia Manosalva at UC Riverside. One of these trial locations, Alina Ranch, has suffered significant tree death and Dr. Manosalva is asking CAC to cover 50% of the cost for replacement trees at this trial location. Discussion ensued with concern primarily focusing on whether the replacement trees would be of any value for data collection in the trial since they would be significantly different in age from the original trees. In addition, it was questioned whether the trees dying wasn't valid data in and of itself and if replacing them wouldn't simply be a waste of money since the rootstocks didn't seem well suited to that site. There was no support for assisting with the tree replacement costs.

ACTON ITEMS

A. Consider research proposal, "Avocado branch canker disease in California: Pathogen identification, characterization, fungicide efficacy, and interaction between irrigation systems, salinity and disease incidence and severity"

Dr. Fatemeh Khodadadi, plant pathologist at UC Riverside, presented a proposal on avocado branch canker. This proposal was a follow up to work that CAC funded with Dr. Themis Michaelides to understand the causal pathogens of avocado branch canker. The work proposed by Dr. Khodadadi would focus on confirming the causal pathogens, screening fungicides for efficacy in laboratory and field trials, and investigating the effects of salinity on the susceptibility to and severity of avocado branch canker. Following Dr. Khodadadi's presentation discussion ensued. Questions were raised about the efficacy of existing fungicides and whether varieties other than Hass should be looked at in the trials. It was suggested that only major varieties — Hass, Lamb Hass and GEM — be included. Questions were also asked of how the new work would differ from what has already been funded.

MOTION
Recommend funding the proposal with the provision that CAC Staff work with Dr. Khodadadi identify what work has been done to minimize duplication and identify a pathway to registering an effective fungicide and clarify that the salinity parts of the research focus on chloride specifically.

(Burr/Klittich) MSC – Vote Tally: Yea 6, Nay 1

Motion 23-5-26-3

B. Consider research proposal, "Avocado tissue culture"

Dr. Raquel Folgado, Huntington Botanical Gardens, presented a proposal to the Committee on continuing work on avocado tissue culture. The new work proposed by Dr. Folgado had the objectives to: 1) minieral studies of avocado tissue culture to better understand response to the micropropagation conditions, 2) adaptation of the micrografting techniques to regenerate the most susceptible genotypes, and 3) optimization of the cryopreservation protocols for selected cultivars to allow the creation of the first cryobank of avocados in the USA. Following Dr. Folgado's presentation a discussion ensued. Questions were asked about how the work being done by the Huntington relates to work being done in Australia at the University of Queensland. Dr. Folgado explained that there has been some collaboration between the two institutions, such as Chris O'Brien doing some of his PhD research at the Huntington, but there are some intellectual property agreements between the University and their funders that prohibit full disclosure of the University's research results. There was then discussion about the broader applicability of this work and its value to other avocado industries around the world and possibly other crops.

MOTION
Recommend funding 75% of the cost of the proposal if the Huntington Botanical Gardens can secure the remaining 25% from another source.

(Davis/Rochefort) MSF – Vote Tally: Yea 3, Nay 1, Abstain 3 (Cole, Fernandez, Miller)

Motion 23-5-26-4

ADJOURN MEETING

Leo McGuire, Production Research Committee (PRC) Chairman, adjourned the meeting at 1:18 p.m.

Respectfully submitted,

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Timothy Spann

EXHIBITS ATTACHED TO THE PERMANENT COPY OF THESE MINUTES

EXHIBIT A May 26, 2023 Production Research Committee AB 2720 Roll Call Vote Tally Summary

EXHIBIT B Gray Martin Summary for increasing avocado production

EXHIBIT C Development of Chloride Mitigation Strategies for Californian Avocado Groves: Technology Review and Treatment Prediction – Executive Summary of Final Report

EXHIBIT D Research proposal: Characterization and Management of Avocado Branch Canker Disease and Post-Harvest Decay Rots in Avocado Production in California

EXHIBIT E Research Proposal: Avocado Tissue Culture: Mineral Studies, Micrografting, and Cryopreservation

EXHIBIT A

CALIFORNIA AVOCADO COMMISSION
Production Research Committee
AB 2720 Roll Call Vote Tally Summary

To be attached to the Meeting Minutes

Meeting Name:	**Meeting Location:**	**Meeting Date:**
California Avocado Commission Production Research Committee Meeting	*Teleconference*	*May 26, 2023*

Attendees Who Voted	***MOTION 23-5-26-1***	***Motion 23-5-26-2***	***Motion 23-5-26-3***	***Motion 23-5-26-4***
Leo McGuire, Chair	*Did not vote*	*Did not vote*	*Did not vote*	*Did not vote*
John Burr	Yea	Yea	Yea	Yea
Jason Cole	Yea	Yea	Yea	Abstain
Jim Davis	Absent	Yea	Yea	Yea
Consuelo Fernandez	Yea	Yea	Yea	Abstain
Danny Klittich	Yea	Yea	Yea	Nay
Daryn Miller	Absent	Yea	Yea	Abstain
Ryan Rochefort	Yea	Yea	Nay	Yea
Outcome	**Unanimous**	**Unanimous**	**6 Yea, 1 Nay**	**3 Yea, 1 Nay, 3 Abstain**

From: Gray Martin

PRC: Below is a one-page summary for increasing avocado production.

BIO: In partnership with Dr. Bob Bergh, I am the primary inventor of the avocado varieties Lamb/Hass, Sir Prize, GEM, Harvest, and soon-to-be-released BL516. Additionally, I am a co-inventor of rootstocks, Zentmyer, Steddom, and Uzi. I have owned, partnered with, and consulted avocado farms for more than 25 years. I am currently a major California exotic fruit producer, and I am responsible for much of the technology innovation for this new crop. With a degree in Botany, six years on the CAS BOD, three years as Nursery/Variety president, and a regular contributor to CAS Yearbook and Quarterly Magazine, in the 1990s, I come to you with a firm background of experience and insight.

The average yield today is the same as it has been for the past 50 years! This is in spite of declining acreage, advances in irrigation, fertility, horticulture, disease, and pest management, and tens of millions in research dollars! This is not to say progress has not been made, but rather that many advancements have not manifested into our unique California industry farming scheme, which requires greater individual training and networking of mostly non-agrarian land owners. In other words, the industry is following the classic classroom model, where a hierarchy of instructors lead all the dialogue and topics, leaving the student farmers awaiting the next six-month session that may or may not apply to their needs and interest. Some prosper many flounder, and many disconnect. Why? In part, because the instructors are generally not farmers, the instruction is selective, rigid, speculative, and prone to data misinterpretation. Therefore, what is now required, is farmer tutorials led and directed by growers, farm managers, field reps, etc. Utilizing our modern-day communication technology, where we can gain access to ideas, materials, discourse, etc., from a pool of farming experience, on a regular and timely basis.

Enhanced yield resulting from new varieties and reevaluation of historical norms:

When discussing plants, one unique expression that is often underutilized is the plant phase. Plant phase is not linear like plant age, it is changeable, a regenerative function likely adapted from severe environmental stress: Cold, fire, wind, etc. For the purpose of a simplified discussion, I will broadly define the different stages of phase as ***juvenile*** and ***mature***. Juvenility is best recognized as rapidly dividing, elastic cells, expressed as vigorous shoots and branches. And maturity phase is easily observed by the ability of the plant to reproduce, with the expression of flowers. Often, we subcategorize phase in humans with the word "precocious". The child is walking or talking at a young age, he/she is precocious. In plants, we use the same word to define the ability of the plant to reproduce. Therefore, the young flowering plant is precocious. In reality, the young plant tissue has just been signaled as the mature phase. Although, this mature phase could result from several different factors: 1) The result of grafting; 2) Induced from any number of severe stress factors (drought, heat/cold, wounding, etc.; 3) Genetic propensity to flower early in age.

How does understanding the plant phase have anything to do with yield? The answer is EVERYTHING! At least everything from a farmer's perspective. To understand this, we have to look at dominant avocado cultivars; historically Fuerte and Hass. Both are non-precocious varieties. For the last 50+ years, the industry has only known and experimented with this plant phase. So, nurseries have formulated their collective protocols from the expression of non-precocious varieties, and horticulturists have refined their skills only using non-precocious varieties. The result has been, similar average yields year in and year out. Today, there exists new cultivars that are the black sheep in the white wool industry, the new cultivars are precocious! Apparently, the non-farming instructors missed the phase differences as they continue to analyze value comparing varieties strictly from the non-precocious perspective of Hass. In reality, the two cannot be compared in the field, it's like comparing the fox with the hound, they are both canines, but opposites! Fruit quality aside, it appears that the Carmen Hass is precocious compared with standard Hass. Lamb/Hass certainly is precocious; Reed, GEM, Pinkerton, and a host of other varieties are precocious. Precocity can be very troublesome for the nursery, especially if mostly familiar with Hass. The precocious grafted varieties will often flower and therefore stunt vegetatively. First-year planted trees also will encounter the same problem. The solution, besides grafting exclusively to vigorous growing rootstock, is to encourage rapid growth with high fertility and a long first-year growing season to maximize juvenile response. Absent these measures the precocious varieties will flower young, often stunt, set fruit, and stress. The same dynamics occur in the field. Extrapolating the effect of precocity on young trees, it is recognized that ONLY precocious varieties are suitable for high-density planting. Although counter-intuitive, using vigorous rootstocks, high fertility, optimum quality and frequency of water, and building a strong rapid tree base, the juvenile phase aspects will soon result in flowering and fruiting trees. AND THE BEST PRACTICE TO KEEP AVOCADO TREES SMALL IS TO KEEP THEM FRUITFUL. When tree convergence does occur, when pruning becomes part of the management practice, only precocious varieties can be managed and maintained small, as non-precocious varieties often can easily be reset to the juvenile phase, thereby not flowering the following year! No fruit, more vegetative growth, the cycle of tree convergence, and low yields perpetuates! Comparatively precocious varieties are much more forgiving when pruned, and will more often provide a flower set the following Spring. Therefore, ONE solution to increase yield is high-density planting using precocious varieties on vigorous rootstock, including seedling rootstock. Both seedling and clonal trees should be planted and established in the field, and later field grafted to ensure early juvenile vigor of the scion variety. Unlike the nursery practice of 'Heading' the lead shoot to produce a traditional "Standard" tree, field-grafted trees can be encouraged unrestricted and uninterrupted the first year so to aid in early field production. Additionally, this method should reduce the cost of rootstock and prevent long wait times.

Development of Chloride Mitigation Strategies for Californian Avocado Groves: Technology Review and Treatment Prediction

Executive Summary of Final Report

Prepared for the California Avocado Commission Under Project Contract Number 65321-00-000 for the PRC Meeting on May 26, 2023

by

Haizhou Liu, PhD, PE, Xuejun Yu, PhD and Xingyu Tang

Department of Chemical and Environmental Engineering
University of California, Riverside

Executive Summary

The goal of this research project is to conduct a phase-one feasibility study to develop chloride mitigation technologies from irrigation water at Californian avocado groves. The elevated level of chloride in irrigation water is one of the greatest threats to avocado productivity for many growers in California. The development of efficient, cost-effective on-site water desalination technologies to selectively remove chloride from the irrigation water for Californian avocado groves will significantly increase the yield of avocado trees, provide reliably high-quality irrigation water, and consequently increase the profits and competitiveness of Californian avocado groves. The project has **four major tasks**: (1) understand chloride ion activity and chemistry in irrigation water at Californian groves by conducting a comprehensive chemical modeling; (2) screen viable chloride removal technologies uniquely applicable to avocado industry by conducting an extensive literature review; (3) predict the treatment efficiency and economic cost of the most prioritized chloride removal technologies; and (4) recommend the next-phase experimental investigation of candidate technologies.

The first task of the project aimed to predict the chloride ion activity and its chemistry in irrigation water of California avocado industry. A fundamental understanding of chloride chemistry in irrigation water is critical to the design of its removal technologies. Given the fact that irrigation water for avocado groves is typically municipal drinking water or private groundwater wells, two types of irrigation water chemistry that are representative from two avocado production regions were chosen – municipal drinking water in the City of Escondido (San Diego County) and groundwater in the City of Santa Barbara (Santa Barbara County) (**Table E-1**). Chloride concentrations in these two representative water sources are elevated and provides good case examples to evaluate chloride chemistry. Water quality reports from the most recent year in these two regions were obtained and individual chemical compositions were mapped out. Following that, a detailed chemical modeling simulation was conducted using the chemical composition of irrigation water to understand the interactions between chloride ion and other major chemical constituents in irrigation water. All major chloride reactions with water constituents were searched via a comprehensive literature review and constructed into the chemical model.

Chloride chemical modeling results showed that chloride mainly exists in the form of negatively charged free chloride ion Cl^- in the irrigation water (**Table E-2**). In both drinking water and groundwater, the overwhelmingly majority of chloride (>99%) exists in the form of free chloride ion. Although interaction with other metals in the irrigation water were found in the modeling, including with calcium, magnesium and sodium, the interaction itself is minor, and only a small fraction of chloride (<1%) exists as metal-chloride clusters with a different ion charge. For example, the chemical modeling showed that and groundwater interacts with calcium, magnesium and sodium respectively and form $CaCl^+$, $MgCl^+$ and $NaCl_{(aq)}$ clusters that are either positively or neutrally charged or. These metal-cluster chemicals change the surface charge and size of the chloride molecules, but they play a minor role in the chloride composition. The modeling results

clearly show that the negatively charged free chloride ion should be the major target in the design of removal treatment instead of other chloride species.

Furthermore, redox potential calculation showed that the actual redox potential of the chorine/chloride redox couple in a typical irrigation water is higher than that of the oxygen/water redox couple, suggesting that chlorine formation from chloride is unlikely in the irrigation water unless a high energy input (for example, electrolysis) is implemented. Meanwhile, the chloride photochemistry simulation showed that the chloride ions are stable under sunlight irrigation.

Table E-1. Water quality parameters from City of Escondido and City of Santa Barbara.

Chemical Composition	**Unit**	**City of Escondido**	**City of Santa Barbara**
		Municipal drinking Water	Groundwater
Total dissolved solid (TDS)	mg/L	605	1010
pH	-	7.98	6.86
Barium (Ba^{2+})	mg/L	0.1	NA
Fluoride (F^-)	mg/L	0.68	0.27
Chloride (Cl^-)	**mg/L**	**101**	**150**
Sulfate (SO_4^{2-})	mg/L	213	220
Boron	mg/L	0.13	0.14
Manganese	µg/L	2.1	120
Copper (Cu^{2+})	mg/L	0.65	0.53
Total alkalinity as $CaCO_3$	mg/L	120	210
Calcium (Ca^{2+})	mg/L	63	110
Magnesium (Mg^{2+})	mg/L	23	34
Sodium (Na^+)	mg/L	91	77
Potassium (K^+)	mg/L	4.3	1.4
Silica (SiO_2)	mg/L	7.3	NA
Iron (Fe)	µg/L	NA	120
Natural Organic Matter	mg C/L	0.1	2

Table E-2. Modeling of Chloride species distribution in drinking water and groundwater.

Chloride Species	City of Escondido Municipal Drinking Water	City of Santa Barbara Groundwater
	% of total chloride concentration	% of total chloride concentration
Cl^-	99.4%	99.2%
$CaCl^+$	0.2%	0.3%
$MgCl^+$	0.2%	0.3%
$NaCl_{(aq)}$	0.2%	0.2%

The second task of the project aimed to review and evaluate the applicability of both existing and emerging water desalination technologies that can selectively remove free chloride ions for onsite water treatment, and screen viable free chloride ion removal technologies uniquely applicable to avocado industry. The project team conducted an extensive literature review and summarized five categories of technology platforms that can remove chloride for saline water, including pressure-driven physical separation, electricity-driven separation, adsorptive media-based separation, electrode-based separation, and chemical precipitation-based removal (A detailed summary of each technology platform is summarized in **Table E-3**).

The pressure-driven physical separation mainly include reverse osmosis (RO) and nanofiltration (NF), both of which are membrane-based technologies. The electricity-driven separation is based on the principle of an electric field coupled with ion-selective membranes to separate chloride from the irrigation water, with electrodialysis (ED) and electrodialysis reversal (EDR) as the two major platforms. Adsorptive media-based separation includes chloride-selective ion exchange (IX) resins and reactive adsorptive media (ARM) using bimetallic oxides. Electrode-based adsorptive separation is mainly capacitive deionization (CDI) that can remove chloride from water using an electrical potential difference between a pair of electrodes made of porous carbon. Chemical precipitation (CP)-based chloride removal relies on inorganic chemical addition to induce precipitation reactions with chloride and immobilize chloride as a solid after treatment.

Table E-3. A summary of different chloride removal technologies and their working principles.

Technologies	Principle
Reverse Osmosis (RO) & Nanofiltration (NF)	***Pressure-driven physical separation***: A process in which feedwater is demineralized or deionize water by pushed under a hydraulic pressure through a semi-permeable RO or NF membrane. Water will pass through the membrane while chloride and other dissolved chemicals in the feedwater are rejected by membranes to produce a permeate product water, while the rejected stream forms the concentrate (also known as brine) wastewater. RO is effective in the removal of all ions including chloride and other monovalent ions; while NF is very effective in divalent ion removal and partially effective in the removal of chloride and other monovalent ions.

Electrodialysis (ED) & Electrodialysis Reversal (EDR)	***Electricity-driven separation coupled with membrane selectivity***: Electrodialysis (ED) is a membrane process that uses alternating anion-selective membranes and cation-selective membranes, placed between an anode and a cathode. Due to the applied electric field, anions will move towards the anode and cations will move towards the cathode. Anions and cations are stopped by the respective ion-selective membranes, creating a process flow with low ion concentration (desalinated water) and a process flow with high ion concentration (concentrate or brine). During the ED process, suspended solids carrying positive or negative charges will deposit on membrane surface, jeopardize selective ion exchange ability of the membrane. In comparison, electrodialysis reversal (EDR) is a revised ED process during which the membrane surface deposition is eliminated to a large extent by reversing in certain time intervals the polarity of the applied electrical potential, which results in a removal of charged particles that have been precipitated on the membranes.
Ion Exchange (IX)	***Adsorptive media-based separation:*** Ion exchange (IX) is a reversible exchange of one kind of ion present in an insoluble resin with another of like charge present in a feed solution surrounding the resin with the reaction being used especially for softening or making water demineralized and separation of substances. Ion exchange resin can be regenerated. There are ion exchange resins specifically designed for selective chloride removal.
Reactive Media Adsorption (RMA)	***Adsorptive media-based separation:*** Most reactive adsorptive media are magnesium-aluminum oxide-based materials. These materials are typically for one-time use and not regenerated. For example, a mixed phase of magnesium-aluminum oxide ($Mg_{0.8}Al_{0.2}O_{1.1}$) can adsorb chloride and generate a bimetallic chloride solid ($Mg_{0.8}Al_{0.2}(OH)_2Cl_{0.2}$).
Capacitive Deionization (CDI)	***Electrode-based adsorptive separation***: Capacitive Deionization (CDI) is a process that removes charged species from water using an electrical potential difference (electrical driving force on the ions) between a pair of electrodes made often of porous carbon. In CDI systems, ions migrate directionally under the traction of the electric field between the electrodes and then stick to the electrodes (electrosorption process), consequently reducing the salt content of the water. When the electrodes reach a limit to bind extra ions, the power supply and charges on the electrode are reversed, and the ions on the electrodes are released, resulting in a brine stream.

Chemical Precipitation (CP)	***Chemical precipitation-based chloride removal***: There are several chemicals that can induce precipitation reactions with chloride and immobilize chloride as a solid after treatment. Friedel's salt (FS) precipitation: add lime (CaO) and sodium meta-aluminate ($NaAlO_2$), $[Ca_2Al(OH)_6]^+$ is formed in water. This cation has a high adhesion for chloride, resulting in chloride being precipitated. The formular of preipitate is $Ca_2Al(OH)_6Cl{\bullet}2H_2O$. CuCl precipitation: to remove chloride, Cu(0) and Cu(II) are added to react and form Cu(I), which then reacts with Cl^- to generate CuCl precipitation. BiOCl precipitation: Bi_2O_3 is added under acidic conditions to produce Bi^{3+}, which then react with chloride and water to form BiOCl precipitates.

Each technology has its own nuances in applicability to avocado groves. The project team compared each candidate chloride removal technologies, and synthesized the advantages and disadvantages of each option, which is summarized in **Table E-4** below.

Table E-4. Summary of advantages and disadvantages of chloride removal technologies.

Technologies	Advantages	Disadvantages
Reverse Osmosis (RO)	• Effective, removal rate of chloride is >98%. • Mature technology, with highly commercialized equipment and standardized operating procedures.	• Highest energy and chemical cost. • Membrane scaling and cleaning required. • Brine generation. • Antiscalant usually needed.
Nanofiltration (NF)	• Chloride removal rate is 50%-90%. • Lower cost than RO. • Mature technology, with highly commercialized equipment and standardized operating procedures.	• Membrane scaling and cleaning required. • Brine generation. • Antiscalant usually needed.
Electrodialysis (ED)	• Effective on treating feedwater with high	• Brine generation. • uncharged components such as microorganisms or

& Electrodialysis Reversal (EDR)	salinity, removal rate above 90%. • Little feed pretreatment requirement. • No regeneration compared to traditional ion exchange. • Much higher brine concentration can be achieved in electrodialysis than in reverse osmosis since there are no osmotic pressure limitations	organic contaminants will not be removed. • High energy consumption when solutions with high salt concentrations compared to CDI process.
Ion Exchange (IX)	• Easy operation. • Selectively remove chloride using specific commercial resin. • Adsorbents can be regenerated and recycled.	• Requirement of periodic regeneration of IX resin that produces potentially hazardous alkaline brine wastewater. • Possible pH changes of treated water that requires pH adjustment and additional chemical dosages.
Reactive Media Adsorption (RMA)	• High efficiency of removing chloride. • Adsorbents can be regenerated and recycled.	• Difficult regeneration and synthesize of adsorbents. • No commercialized adsorbents available.
Capacitive Deionization (CDI)	• It can replace or outperform RO when feedwater TDS is lower than 1000 ppm. • Moderate energy cost compared to ED process.	• Large surface area of the electrode required. • Lack of commercialization product on the market. • Salt can be efficiently removed at low pressure, low voltage, and room temperature.
Chemical Precipitation (CP)	• Simple operation • Low cost • High selectivity • Suitable for large scale and high-chloride-concentration water treatment.	• Residual chloride level in the treated water can be high. • Waste sludge production • pH and TDS increase in the effluent. • High chemical consumption

In **the third task** of the project, based on the applicability and limitation of each technology, and the unique requirements for avocado groves, the project team developed a list of selection criteria that fits to on-site chloride removal at California Avocado groves. These criteria include:

- A high efficiency in removing chloride from irrigation water;
- Minimize or avoid hazardous byproduct waste generation, for example, brine;
- Easy, automatic and on-and-off operation that requires minimal maintenance;
- Lower capital and operational cost in comparison to RO membrane desalination;
- Easy to scale up and commercialize from academic and lab-scale investigations.

Based on the above selection criteria, **three top candidate technologies** are identified, described below:

- **Nanofiltration (NF).** NF requires a much lower energy input and pressure in comparison to RO, but can still achieve a significant percentage of chloride removal. There are different commercially available NF membranes and modules that are selective towards chloride. Overall chloride removal can reach up to 90%. NF is a manure technology and ready to implement at a large scale.
- **Electrodialysis Reversal (EDR)**. As an electricity-driven technology, EDR can be a good fit to the avocado irrigation requirement. The saline irrigation water will subject to the application of low electric voltage using submerged electrodes. During electrodialysis, chloride ions will migrate towards the anode via ion exchange membranes, thus separating it from the water. EDR process only need low hydraulic pressure pumps and a low electric energy input, thus not energy-intensive in comparison to RO or NF. Another advantage of the EDR process is the easiness to operate and a low volume of brine generated. Overall chloride removal is higher than 95%. This technology is ready for scaling-up and commercialization.
- **Chloride-selective ion exchange resin (IX)**. IX treatment using chloride-selective resin is a low-energy-footprint separation technology, which is its biggest advantages in comparison to other two candidate technologies above. However, IX technology requires periodical cleaning and regeneration of the resin, thus adding to complexity in maintenance and operation. Traditional chloride-selective resins are hydroxide-ion based – also known as strong base resins. Using these traditional resins will produce treated water with a high pH requiring additional treatment afterwards, and the regeneration operation will produce highly alkaline brine wastewater that can become hazardous and difficult to handle onsite. There are emerging chloride-selective IX resins that seems to have a different surface reactivity and are easier to operate, claiming to achieve a chloride removal of more than 95%. Further evaluation of these new resins in field conditions are needed before commercialization.

A comparison of the three top candidate technologies is summarized in **Table E-5** below. A more detailed quantitative comparison results will be discussed during the presentation at the PRC meeting in May 2023.

Table E-5. Comparison of three top candidate technologies for avocado grove-scale irrigation water production.

Technology	Hazardous Brine Generation	Energy consumption	Operation and Maintenance	Overall cost	Readiness to scale up and implement
Nanofiltration (NF)	Moderate	High	Complex: membrane cleaning and brine disposal	Moderate	High
Electrodialysis Reversal (EDR)	Low	Moderate	Moderate: automatic to clean membrane and brine disposal	Moderate/Low	High/medium
Chloride-selective Ion Exchange (IX)	High	Low	Complex: Required-resin regeneration and waste disposal	Unknown	Low

Based on the critical review and analyses on chloride treatment technologies, the project team **recommend the following steps** on selective technologies for a phase-two validation study for future comprehensive development of chloride mitigation strategies:

- Conduct lab-scale electrodialysis reversal (EDR) and nanofiltration (NF) studies using source water collected from avocado groves to generate accurate data on chloride removal efficiency, water production rate, energy consumption rate and capital/maintenance cost.
- Continue to review market progress on emerging chloride-selective ion exchange (IX) resin and its performance in terms of chloride removal, water production, resin regeneration and brine wastewater handling.
- Evaluate onsite brine treatment and freshwater recovery technologies as part of the chloride removal treatment train. The ability to minimize brine volume and convert it to fresh water is a critical bottleneck to the success of all future chloride removal technologies.
- Conduct an industry customer discovery survey to quantify current cost structure using reverse osmosis (RO)-based onsite chloride removal technologies.

Proposal to The CALIFORNIA AVOCADO COMMISSION

Project title: **Characterization and Management of Avocado Branch Canker Disease and Post-Harvest Decay Rots in Avocado Production in California**

Project start date: 1 July 2023
Project end date: 31 July 2026
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Major Collaborator: A Postdoc will be hired in the Microbiology & Plant Pathology Department at UC Riverside to do this work.
Cooperators: Dr. Themis J. Michailides, Dr. Ben Faber, and Dr. Mary Lu Arpaia, PCA's, growers, and homeowners who will kindly provide access to their orchards for sampling and setting up the trials.

1. Background information/justification:

Avocado (*Persea americana* Mill.) is one of the most important crops to California's economy. Two California counties produce 95% of the avocados grown in the United States (Shepherd and Bender, 2013). Avocados are grown on approximately 52,000 acres, primarily in Southern and Central California, typically in coastal climates. Avocados are produced in 15 California counties, but five major Counties including Ventura, San Diego, Santa Barbara, Riverside and San Luis Obispo accounted for 96% of planted acreage in 2021. Ventura County produces nearly 38% of the State's avocado producing acreage, followed by San Diego (29%), Santa Barbara (13%), Riverside (9%) and San Luis Obispo (7%) (California Avocado Commission, 2021). Avocado is susceptible to multiple diseases including avocado branch canker (ABC) disease, caused by several fungal species and genera in the family Botryosphaeriaceae. Members of the Botryosphaeriaceae family are found worldwide and cause cankers, leaf spots, dieback, and fruit rot on a wide variety of woody hosts, which altogether could eventually cause the death in economically important woody perennial trees and ornamental plants (Farr and Rossman, 2011). In the last decade or so, the incidence of Botryosphaeria (Bot) branch canker and consequent post-harvest fruit decays in avocado cultivations in California has risen and developed from a minor problem into a growing threat to avocado production, leading to economic losses (Avenot et al. 2019).

Avocado branch canker could be a stress-related disease. Disease symptoms in avocado are sunken/depressed cankers developing on the trunk, branches, and twigs, causing friable bark, with whitish to brownish exudates. Wilting and dieback of twigs and branches are another typical symptom of ABC is the wilting and dieback of twigs and branches that hold attached dead leaves and fruit which turn totally black and decayed and remain on the trees for a long time. The infection can advance to the xylem and vascular system of a tree and block the water and nutrition flow, weakening the tree, and eventually leading to wilting or death of branches. Pathogens survive as asexual fruiting bodies (pycnidia) on dead leaves, twigs, bark, and canker. Worth noting is that spores can land on freshly cut and damaged woods, so wounds associated with pruning, grafting, girdling, frost injuries, sunburn and mechanical tools are the main sites of infection (Phillips et al. 2005; Eskalen et al. 2013). Avocado branch canker disseminates through contaminated pruning tools, air movement and splash dispersal of spores.

Latent fruit infections of Botryosphaeriaceae species can also occur in orchards affected by branch canker and dieback (Hopkirk et al. 1994; Menge and Ploetz 2003). This infection can remain dormant until the environment is favorable for the pathogen (Aly et al. 2011). Rotting is rarely observed on immature avocado fruit before harvesting, because fruit will ripen only when it is removed from the tree, causing increases in the rate of respiration and ethylene production (Yahia and Woolf 2011). Moreover, as the fruit ripens, there is a decrease in the concentrations of the antifungal component of avocado (Hopkirk et al. 1994; Prusky and Keen 1993), which increases the fruit's susceptibility to infection and reduces the fruit's shelf-life. Branch canker and fruit decay have been frequently reported by growers, extension specialists and nursery managers in California in the past decade. Dr. Mary Lu Arpaia has reported a large occurrence of avocado fruit decay (stem-end rot and anthracnose) post-harvest on avocado fruit specially on 'Hass' avocados once the skin is removed. There is no comprehensive report on specific *Botryosphaeria* species causing fruit rots such as stem-end and anthracnose in our orchards and nurseries. There are some occasions where *Colletotrichum* species have been reported to cause stem-end rot. The first step in successful management of any disease, including ABC in avocados, is identification of causal pathogens and finding ways to detect them so they can be monitored and screened for infectivity, virulence, sensitivity, and resistance to fungicides and other potential control methods. Identifying and characterizing the primary causal agents of this disease will assist in developing the appropriate control measures to reduce yield loss and help monitor and screen those fungi for infectivity, sensitivity, and resistance to fungicides.

Botryosphaeria species are typically opportunistic pathogens, but they can also survive as parasites and endophytes and live latent in trees (Úrbez-Torres, 2011; Slippers and Wingfield, 2007). When pathogens are latent and inside the plant, they become challenging to control. Because species in the Botryosphaeriaceae infect avocados predominantly through wounds, most of the strategies developed to control these pathogens have revolved around protection of pruning wounds using chemicals. A wide spectrum of taxonomically different and sometimes unrelated fungi is associated with ABC disease in avocados so it would be hard for single mode of action fungicides to effectively control them. For instance, most of the fungicides registered for this disease on pistachio, almond and walnut are chemically site specific, and the chance of developing resistance in pathogens is higher after frequent application. Thus, an integrated approach is recommended by using fungicides in combination with cultural practices and the application of proper irrigation system and reduction of plant stresses such as soil salinity. In this project, we will test the efficacy of commercially available chemicals against avocado branch canker pathogens *in vitro* and in the field. Currently, there are no registered chemicals to treat branch canker in avocados. DMI fungicides such as tebuconazole, flusilazole, and cyproconazole, as well as fungicides fluazinam and fludioxonil represent the most promising agents for simultaneous protection of pruning wounds from infection by *Botryosphaeriaceae* spp. in different plant hosts (Pitt et al. 2012). Topsin M (thiophanate-methyl), a benzimidazole fungicide registered in California in 2003, showed a broad-spectrum control between 77 and 82% for four *Botryosphaeriaceae* spp. in vineyards (Rolshausen and Gubler., 2005). The same product has shown very good efficacy against Botryosphaeriaceae in avocado (Avenot et al. 2022). However, since the use of Benlate and Bavistin from the same group was withdrawn by the industry, and since some isolates showed low resistance to Topsin (Twizeyimana et al., 2013; Avenot et al. 2020, unpublished data), more caution around its use should be practiced. All avocado orchards need to be effectively pruned in order to maintain good fruit production and proper tree architecture. Wounds are the main entry for these pathogens and in vineyards, for instance, wounds may remain open and susceptible for up to 4 months (URBEZ-TORRES 2011) but in avocados, depending on the size, wounds can apparently heal faster in about 8 weeks. Although wounds are most susceptible to infection immediately after pruning, more research is also required to evaluate efficacy of fungicides over time in avocado's wounds. Since pruning leads to unnecessary growth near the cuts, repeated farm labor is required to remove the unwanted growth and maintain each tree's energy resources. To avoid such a labor-consuming process, growers use Naphthaleneacetic acid (NAA) (TreHold A-112), a common plant

growth regulator, following tree stumping, tree topping and shoot tip pruning in avocados (Arpaia et al. 2007). However, it is worth assessing how the application of TreHold A-112 on wounds alongside with different fungicides will affect the growth of species in Botryosphaeriaceae and branch canker disease severity. Our research will evaluate the efficacy of fungicides against disease alone and in combination with TreHold A-112 over time for wound application, which distinguishes it from other similar works previously conducted in California (Eskalen et al. 2013; Avenot et al. 2021). There is no study on fungicide efficacy for postharvest rots in Hass (due to the skin color, lesions are not visible on 'Hass' fruits, but such decays affect the shelf life of fruit and damage avocado industry. Hence, we will evaluate the efficacy of fungicides against post-harvest decay rots. Our data from this project along with the previous data on fungicide efficacy in avocado orchards against ABC (Eskalen et al. 2013; Avenot et al. 2021) will pave the way to register the right fungicide for this disease pre- and post-harvest in California.

Mediterranean climate conditions in California predispose avocado production to irrigation, frost, heat, and salinity. Drought stress in California has limited water availability and imposed stressful conditions for avocado producers. Over the past decade, the combination of frequent droughts and deficit irrigations have contributed to higher-than-normal salinity level in the root zone of avocado trees and increased cases of Botryosphaeriaceae infection in avocados in California. Once a pathogen establishes itself in the fruit, then post-harvest rot will become another big challenge to tackle. High salinity (0.6 Sm^{-1}) can negatively impact avocado fruit production resulting in low maximum yields because it limits the water uptake by the roots and exposes the trees to opportunistic pathogens (Wolstenholme & Whiley, 1999; Oster et al., 2007). For productive avocado production, a soil salinity with EC < 1.3 dS/m is needed (Burt, 2013). Once the suitable soil EC level is surpassed, leaves show tip burn, trees become weak and vulnerable, and the yield percentage begins to rapidly decrease. Such stress factors can contribute to either activate disease symptoms/development by latent fungi or to deteriorate the severity of the symptoms caused by *Botryosphaeria* spp. (ÚRBEZ-TORRES, 2011). While researchers know salt is a stress factor, no data is available regarding its impact on avocado branch canker disease. In this proposal we will study the sensitivity of different species of Botryosphaeriaceae to salinity in the lab and determine the impact of salinity on disease incidence and severity in the greenhouse. Such results will be used for further studies on salinity impact in avocado groves in California.

Drip and micro sprinklers are two dominant methods used for avocado production in California (Faber, 2014; Meyer 2014; Smith 2014). A global shift, however, is seen towards drip irrigation using two laterals of driplines per line of trees. Given their lower application rate and wetted pattern well-suited to the avocado tree root zone, micro irrigation systems go well with avocado production as they allow growers to save water and apply water and fertilizers only to the tree root zone. A study was conducted by ITRC in 2003 to compare the long-term impact of drip and micro irrigation on salinity accumulation in orchards, focusing on the salinity concentration pattern across a soil profile. They reported that in orchards irrigated by drip system, a remarkable amount of salt accumulates on the edges of the wetted areas of drippers along tree rows. In orchards with micro sprinkler systems, salt accumulation was mainly focused on the middle of the tree rows, which is on the edges of the wetted patterns (Irrigation Training and Research Center, 2003). The contribution of these two irrigation systems to salinity could depend on several factors such as quality of irrigation water, soil structure, amount of applied water, and leaching procedure. Therefore, knowing the relationship between the irrigation system salinity and ABC disease incidence will help growers to consider new cultural practices and will also develop IPM practices to reduce the use of at-risk fungicides in avocado nurseries and orchards. So, we will evaluate the impact of drip and micro sprinklers, two dominant methods used for avocado production in California, on salinity and incidence of ABC in avocados. Our third objective is contingent upon the results obtained from the salinity assays in the lab and greenhouses in the second objective.

Successful infections of *Botryosphaeria* spp. and *Colletotrichum* sp. causing stem-end rot and anthracnose in avocado fruit are dependent on the host developmental stage. In unripe fruit, these fungi

stay dormant, but in contrast, ripe avocado fruit represent a compatible system for infections and lesions that spread rapidly. During ripening, the physicochemical properties of the fruit tissues change, resulting in compatible conditions for the fruit-pathogen interaction and the reactivation of quiescent pathogens. During ripening, fruit may gradually lose either the ability to activate or the effectiveness of components of the plant immune system, defensive hormone production and signaling, and downstream transcriptional responses. Alternatively, ripening processes such as cell wall breakdown, simple lipid accumulation, changes in pH and secondary metabolite composition, and, in climacteric fruit, increased levels of ethylene may impact the fruit's capability to resist fungal attack (Alkan and Fortes, 2015; Prusky et al., 2013).The widespread nature of this phenomenon in diverse fruit pathosystem suggests that ripening-associated susceptibility is likely to be mediated by combinations of the above factors. However, no information or data are available on the susceptibility of avocado varieties, particularly 'Hass,' to postharvest fruit rots and the interaction between avocado fruit ripening and decay fungi. In this proposal, we investigate the susceptibility of different avocado varieties to stem-end rot and anthracnose and molecular physiological interactions between avocado fruit and pathogens at three developmental stages (unripe, mature, and ripe fruits) along with fungicide efficacy against these pathogens. Postharvest fruit-pathogen interactions are a unique and economically important field of research for combatting food loss. A complete picture of ripening-associated susceptibility of avocado fruit to fruit rot pathogens is highly necessary for avocado breeding program and postharvest control.

For growers to remain competitive in the international market, they must find ways to manage their groves efficiently and significantly increase production. Given the California climatic conditions and reportedly extensive incidence of ABC disease to devastate entire orchards, *Botryosphaeria* species could demolish the California avocado industry if left untreated. Selection of trees free of disease and appropriate controls against pruning wounds are necessary for avocado growers to achieve high yields. Therefore, growers need to stay alert and pro-active and keep their orchards free from inoculum of the pathogens by pruning the diseased parts and following the recommended fungicides programs after pruning. *Botryosphaeria* have been reported to affect other strategic crops in California such as walnut, pistachio, and almond, therefore, to avoid the catastrophic collapse of pistachio industry similar to what happened in Butte County due to the Bot panicle and shoot blight epidemic in the early 1980's, to save avocado industry, there must be collaborative efforts between the nut trees industry and avocado industry to combat this disease. The primary beneficiaries of our research are the approximate 3500 avocado growers represented by the California Avocado Commission, orchard and nursery managers, stakeholders, such as diagnosticians, Pest Control Advisers (PCAs), Farm Advisers, cooperative extension agents, and crop protection manufacturers. All these groups seek updated information about how to quickly diagnose these diseases, which fungicides to use to protect pruning and which alternative management strategies to consider to better control the disease and reduce the risks associated with pathogen resistance to fungicides.

2. Objectives:

Objective 1: Evaluate the *in-vitro* and in-field efficacy of chemical treatments against avocado branch canker disease and post-harvest fruit rots (stem-end rot and anthracnose).
Objective 2. Evaluate the impact of salinity on growth of species in Botryosphaeriaceae *in vitro* and on disease severity and development in greenhouse.
Objective 3: Evaluate the relationship among drip and micro sprinkler irrigation systems, salinity, and avocado branch canker disease incidence in avocado orchards.
Objective 4: Elucidate susceptibility to post-harvest decays of different avocado cultivars including "Hass" and untangling the molecular relationship between fruit physiological changes and decay incidence at various stages of ripening.
Objective 5: Develop and deliver educational materials / programs through extension to stakeholders in the California Avocado Industry.

3. Procedures:

Objective 1: Evaluate the *in-vitro* and in-field efficacy of chemical treatments against avocado branch canker disease in California orchards and nurseries.

We will evaluate the efficacy of a wide range of chemicals in controlling *Botryosphaeria* branch canker in avocado through laboratory and field experiments.

1a. Isolation, identification and characterization. In order to test the efficacy of fungicides in the laboratory and later determine the impact of salinity, we will first isolate, then identify and characterize species in *Botryosphaeriaceae* family using morphological and molecular methods. We will scout all private and commercial avocado orchards and commercial avocado nurseries in Southern and Central California and collect symptomatic leaves, twigs, fruit and branches. We will isolate all potential pathogens/fungi causing ABC and classify them based on their morphological and microscopic characteristics. For isolation, we will first disinfect the symptomatic materials by washing under running tap water, surface sterilizing in 1.5% sodium hypochlorite for 2 min, rinsing twice with sterile distilled water, and plating on potato dextrose agar (PDA). We will incubate cultures at 25°C in darkness until the colonies grow. The isolates will be assessed morphologically and using microscopic methods for colony color, growth rate, and the shape, length, and width of conidia. We will record the colony color after 7 days of incubation on PDA at 25°C in dark. Colony growth rate will be determined by measuring the colony diameter of each isolate grown on PDA daily over the course of 7 days at 25 °C in the dark. Microscopic observations will be made to determine spore shape, size and other reproducing structures. Later, we will extract DNA from the pure culture of isolates using DNeasy Plant Mini Kit (Qiagen, Germantown, MD, US) and amplify the key "fingerprint" fragments of genes such as internal transcribed spacer (*ITS*), glutamine synthetase (*GS*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), calmodulin (*CAL*), and actin (*ACT*) using PCR. We will purify PCR products and sequence them. Finally, we will use the gene sequences to draw phylogenetic trees using respective software. Phylogenetic trees are like a scientific form of a person's family tree that classify the different groups of species or clades, try to diagnose ancestral relationship, origins, or similarity between strains with other references in GenBank. These trees allow us to compare our isolated collection from California with the reference *Botryosphaeria* strains from other researchers worldwide and determine the probability of mixed infections of *Botryosphaeria* species. Identification of the type and dominant species causing avocado branch canker disease in avocado groves in California and access to the sequencing data will pave the way for the next step where we would work to develop a reliable, fast and precise molecular method for pathogen detection from pure cultures and infected tissues.

1b. *In-vitro* chemical efficacy. Our fungal collection identified at the species level in the previous step, will be evaluated for their *in vitro* sensitivity to different fungicides. We will select fungicides from several Fungicide Resistance Action Committee (FRAC) modes of action (FRAC1, Benzimidazoles; FRAC3, Demethylation inhibitors; FRAC7, Carboxamides Succinate Dehydrogenase Inhibitor= SDHI; FRAC11, strobilurins = QoIs; and FRAC12 (phenylpyrroles) and FRAC Group 29 and M1 (Tentative list in Table 1). We will use 6 isolates from each identified species for fungicide sensitivity *in vitro* and the assay will be repeated twice. We will place a 3-mm agar plug taken from the periphery of the seven-day-old fungal cultures in the center of petri dishes containing PDA amended with a range of concentrations of fungicides of interest. Plates will be stored at 25°C for seven days. We will check the efficacy of SDHI fungicides against spore germination of our fungal collection. Sensitivity of our isolates to the SDHI and other fungicides will be determined by the effective concentration which inhibits spore germination/fungal growth by 50% compared to the nonamended control (EC_{50} µg/ml). We will calculate the EC_{50} values of each fungicide for each tested isolate. The most effective chemicals from the *in-vitro* assays will be chosen for field assays.

1c. Chemical field trials. Those fungicides effective against fungal colony growth in the laboratory will be evaluated in the field to protect pruning wounds. We will compare the impact of different fungicides on the most virulent isolate in each species at two time points: applied right after pruning and applied on a two-week schedule (at two weeks interval) over a two-month period. In total, we will set two independent field trials in two different locations. In each trial, we will have four groups of trees; first group: trees which will be treated with fungicides applied immediately after pruning; second group: trees which will be treated with fungicide treatments at two-week interval for two months period; third group: trees which will be treated with combination of respective fungicides and NAA (Tre-Hold A112) right after pruning; and fourth group: trees which will be treated only with NAA (Tre-Hold A112).

We will prune five randomly selected branches per tree (2- to 3-year-old branches, 15 to 20 mm in diameter) to the length of 20 to 25 cm and immediately treat with one of the fungicides in table 1 (one product per tree). The most virulent *Botryosphaeria* isolates from each species identified in the first objective will be used for the inoculation with a 100 µL fungal spore suspension at 10^5 spores/ml, 24 hours and one week after pruning. It's worth noting that before inoculation, we will mist wounds with Sterile Distilled Water (SDW) to wet the entire wound surface and provide humidity and favorable conditions. After inoculation, the pruning wounds will be protected for one week using Parafilm to prevent dehydration and promote spore germination. For NAA treatment, we will repeat the same procedure for evaluating the impact of fungicides in combination with PGRs such as NAA (Tre-Hold A-112) to see their impact on controlling Botryosphaeriaceae species advancement, disease incidence and severity. All details will the same as described above as we will prune selective shoots on the trees to a lateral branch. Following pruning, the trees will be divided into 2 groups. In one group, the pruned shoots will be applied with an application concentration of 1.15% NAA (TreHold A-112 diluted in water and the recommended rates for fungicides used above). In the other group, we will only paint the pruned shoots with NAA (TreHold A-112). Similarly, the same methodology will be applied with the other two trials with this difference that fungicide treatments will be applied at two-week interval for two months. We will include two controls; a wounded negative control with no inoculation and application with fungicides and a positive control which is inoculated and receives no fungicides. We will set our trials in a completely randomized design. The total number of trees in each trial will be dependent on the number of species we identify and the number of effective fungicides. After four months of inoculation, we will cut the branches and evaluate them in the laboratory for disease incidence and severity with presence or absence of necrotic branches and by measuring the length of discoloration, respectively. We will measure canker incidence, canker length, and extent of stem girdling four months after inoculation. To confirm the cause of lesions, we will isolate the pathogens and identify them with morphological and molecular methods described above under the first step of the first objective.

For fungicide efficacy trials for stem-end rot and anthracnose (body rot), we will use four replicate trays of 'Hass' fruit per treatment. Trays are randomly assigned to each of the treatments outlined below (Tentative list). Treatments: 1. Water control 2. Scholar high (500mL/100L, 230 g a.i./L) for 30 sec. 3. Scholar low (250mL/100L, 230 g a.i./L) for 30 sec 4. Sportak standard (0.55 mL/L, 450 g ai /L prochloraz), for 30 s. Efficacy will be tested once fruits ripen and data will be analyzed.

Table 1. Tentative list of fungicides which will be used for *in-vitro* assay, the effective ones will be later used in the field (The list is subject to change).

Trade Name	**Active Ingredients**	**FRAC Group**
Inspire Super	Difenoconazole+Cyprodinil	FRAC group 3
Fontelis	Penthiopyrad	FRAC group 7
Omega	Fluazinam	FRAC group 29
Flint	Trifloxystrobin	FRAC group 11

Merivon	Pyraclostrobin+Fluxapyroxad	FRAC group 11 and 7
Elite	Tebuconazole	FRAC group 3
Scholar	Fludioxonil	FRAC 12
Topsin M	Thiophanate-methyl	FRAC 1
Luna Experience	Fluopyram + Tebuconazole	FRAC group 3 and 7
Boscalid/Pristine	boscalid (25.2% active) and pyraclostrobin (12.8% active)	FRAC 7
BlueShield	Copper Hydroxyde	FRAC group M1
TreHold A-112	NAA (Naphthaleneacetic acid)	

Objective 2. Evaluate the impact of salinity on growth of species in Botryosphaeriaceae *in vitro* and on disease severity in greenhouse.

2a. Salinity effects on *Botryosphaeria* spp. *in vitro*. We will evaluate the impact of salinity on colony growth and spore germination of 10 isolates of each identified species in the laboratory. We will then obtain spore suspension and mycelial plugs from 7-day old colonies grown at 25°C in PDA. We will add the mycelial plugs and a specific amount of spore suspension (concentration are measured using hemocytometer) added to PDA or PDB (Potato dextrose broth) amended with different concentrations of various salts (NaCl, KCl, MgSO4, MgCl2 or CaCl2) and keep them in incubator/shaker incubator at 25°C in dark for a week. Later, petri dishes/glasses will be checked for colony growth (either in the form of surface mycelium or culture turbidity) and spore germination under microscope. Inoculated broth will be examined for visible growth (either in the form of a submerged or surface mycelium or culture turbidity). We will include negative controls (sterile medium without salt) in the experiments. Each treatment will be maintained in triplicates.

2b. Salinity effects on *Botryosphaeria* species in greenhouse. Besides petri dishes, we will also conduct the salinity experiments under controlled conditions to assess the disease severity and fungal growth in the tree tissues. We will use potted Hass trees on either Duke 7 or Toro Canyon and maintain them in a greenhouse before salt treatments are started. At their initiation, we will divide the plants into three groups: one group will continue to receive the irrigation solution with no added salt (at optimum EC for avocado growth), while plants in the other two groups will receive irrigation solution amended with NaCl and CaCl2 (in a 1:1 equivalent ratio). The solution EC in these treatments will be increased in four equal steps over 8 days to a maximum of 7 $dS \cdot m^{-1}$. One day after the maximum salt levels is reached (day 9), we will leach one group of salinized plants and continue irrigating them with non-saline irrigation solution. Plants in the remaining salinized group continued to receive the 7 $dS \cdot m^{-1}$ irrigation solution for the duration of the experiment. So, in total, we will have three salt treatments: no salt (NS), leached salt (LS), and continuous salt (CS). We will regularly monitor the symptom development, disease incidence and fungal growth in our three group of trees.

Objective 3: Evaluate the relationship among irrigation systems, salinity, and avocado branch canker disease incidence in avocado orchards.

Here, we aim to understand the impact of two common irrigation systems used in California (Drip and micro sprinkler) on soil salinity accumulation, soil sodium adsorption ratio (SAR) and the induction of *Botryosphaeria* branch canker disease in avocado orchards. In our experiment, our treatments include

three irrigation treatments; one drip hose per tree row, two drip hoses per tree row, and one micro-sprayer located at the midpoint between two trees in a row; and two soil texture treatments, clay, and sandy loam. In each soil type, we will set up our experimental trials as a randomized complete block design with four blocks, each including one replicate of all irrigation treatments. We will preferably select/use young orchards with known irrigation history and known water quality. Therefore, we will already have good information on the amount of water applied to an orchard and the salt load distributed by the irrigation water. We will gather the data for salinity in our blocks, and the soil and water salinity will be measured before initiating our experimental trials in those orchards. We will consider the wetted soil volumes for one drip hose and two drip hoses treatments and the use of suitable water meters, and valves to control and measure the amount of irrigation water applied to each plot. Trials will receive the same amount of water on a weekly basis but with different frequencies for one and two drip hoses treatments. In each block, we will mark several trees as record trees and use their canopy for sampling soil and soil-water to measure the salinity. We will regularly monitor and measure the electrical conductivity, sodium chloride and chloride calcium of irrigation water, leaves, and soil samples at different depths within two years of trials. Initially, we will monitor the disease incidence daily/weekly by counting the diseased/symptomatic plants in every plot. The data acquired from the proposed experiments will be analyzed using a range of bioinformatic software such as Geneious Prime, Mega 11, Allele ID and other necessary tools and software. For statistical analyses, we will use SAS statistical software (release 9; SAS Institute, Cary, NC) and Graph Pad Prism software v5 (GraphPad Software, San Diego, CA, US).

Objective 4: Elucidate susceptibility to post-harvest decays of different avocado cultivars including "Hass" and untangling the molecular relationship between fruit physiological changes and decay incidence at various stages of ripening.

We will collect avocado fruits from different varieties including 'Hass' at maturity stage (harvest time) and once they are ripening in the storage to monitor and evaluate the incidence of stem-end rot and anthracnose on them. We will examine the impact of different storage conditions in terms of temperature, time and humidity on fruit ripening and postharvest decay development. Later, we will also artificially inoculate fruit of different avocado varieties with fungal pathogens causing stem-end rot and anthracnose in the laboratory and monitor rot development on those fruits to determine the susceptibility level. We leveraged the fact that avocado fruit display an increase in susceptibility to necrotrophic/rot fungal infection as a result of ripening to develop a system for studying compatible and incompatible host-pathogen interactions. The transition from unripe to ripe fruit results in a markedly different physicochemical environment for colonization. We will monitor changes in physiological changes in fruit at three different maturity levels and then we will conduct transcriptomic analysis (RNA-Seq) to find out about the changes in genes expression. A complete picture of ripening-associated susceptibility of avocado fruit to fruit rot pathogens is highly necessary for avocado breeding program and postharvest control.

Objective 5: Develop and deliver educational materials to growers and stakeholders through extension programs and activities.

Results from this project will be disseminated to the avocado growers, avocado stakeholders, packhouses, processors and the general public through local venues like annual national and regional fruit producer meetings, local production meetings, statewide commodity meetings, workshops, field days, grower meetings organized by the California Avocado Commission (CAC), UCCE county-based farm advisors and principal investigator blog posts and extension publications. Newsletters, extension bulletin and factsheet materials related to the project findings will be prepared in cooperation with University Cooperative Extension personnel in relevant counties to educate participating growers and nursery managers on how to diagnose the disease and avoid or reduce the sources of pathogen inoculum and to educate industry personnel (UC Farm Advisors and Pest Control Advisers) on findings of this study and what can be done, cooperatively, to produce plants free of *Botryosphaeriaceae* fungi. Results from this project will be published in peer-reviewed and trade journals for producers and presented at regional,

national, and international professional meetings. Findings attained from this research will be reported to the California Avocado Commission. Results regarding the use of chemical products against avocado canker diseases will be disseminated to the entire community of growers, advisers and consultants involved in avocado production, providing new disease management guidelines to the avocado industry in California. Professional presentations will be made at the American Phytopathological Society's Annual Meeting. We will develop and disseminate educational and outreach materials on the outcomes of the project to stakeholders through several web pages, including University of California, Agricultural and Natural Resources home page (https://ucanr.org/), California Avocado Commission webpage (www.avocado.org) and Hofshi Foundation web site (www.avocadosource.org), presentations at field day and workshop. We will also write extension articles and refereed technical articles. Project outcomes will be shared at grower's field days, in magazines articles, and through the Statewide IPM Program and will be freely available to growers, farm advisors, PCAs, and the academic communities at the University of California webpages. Thanks to the IPM-based solutions that this project will generate, the California fruit industry will greatly benefit, as they will be able to reduce yield losses, thus increasing the productivity and sustainability of this important commodity.

Milestone

The following Milestone Table outlines the activities associated with the project and scheduled completion dates. Milestone Activity Reports are due to the commission by the last day of the scheduled completion month. Variation from this schedule must be communicated in writing to and approved by the commission's Research Project Manager (tspann@avocado.org) no later than the last day of the scheduled completion month for each milestone. Failure to submit a Milestone Activity Report or communicate variations from this schedule according to the specified timeline indicates that the Milestone will not be completed, and the funds associated with that Milestone may be forfeited.

Year 1	**July 2023 - October 2023**		
Milestone	**Activities**	**Scheduled Completion**	**Budget**
1	Interview, recruit, and hire a Postdoc and pay salary/fees for first year. Complete hiring by July 2023	July, 2023	$72,520
		Year 1 Total	**$72,520**
Year 2	**November 2023 – October 2024**	**Scheduled Completion**	**Budget**
1	Sample collection, isolation, DNA extraction and sequencing the genes for identification	November, 2023	$3,000
2	In vitro sensitivity of fungi to the list of fungicides	February, 2024	$3,500
3	In vitro sensitivity of fungi to salts	March, 2024	$2,000
4	Virulence and pathogenicity of species	July, 2024	$1,500
5	Fruit collection for postharvest fungal isolations, identification, and fruit susceptibility	July 2024	$4,000
6	Salary and fringe benefit of the postdoc working on the project		$75,413
7	Fungicide efficacy against stem-end and anthracnose fruit rots	October 2024	$1,500

		Year 2 Total	**$90,913**
Year 3	**November 2024 - October 2025**	**Scheduled Completion**	**Budget**
1	Setting up the trials for the efficacy of fungicides in the field and collecting data	July, 2025	$10,000
2	Studying the physiological changes in fruit-rot pathogen interaction using transcriptomics	July 2025	$5,000
3	Purchasing trees and evaluation of salinity on disease incidence and severity in greenhouse	July, 2025	$5,000
4	50% salary of a postdoc	July 2025	$38,500
		Year 3 Total	**$58,500**
Year 4	**November 2025-October 2026**	**Scheduled Completion**	**Budget**
1	The second-year trials for the efficacy of fungicides in the field and collecting data	June 2026	$12,000
2	Setting up field trials for the interaction between salinity, Branch canker and irrigation systems	July, 2026	$8,000
3	Monitoring the ABC disease in field trials for the interaction between salinity, branch canker and irrigation systems	June 2026	$4000
		Year 4 Total	**$24,000**
		Total Project Budget	**$245,933**

5. Budget Narrative:

Funds are requested to support 80% of the project scientist salary and benefits, and the costs for conducting activities, including the isolation and identification of all fungal isolates from nursery, old, and young orchards with ABC, disease incidence evaluation, *in-vitro* sensitivity to fungicides, determination of fungicide efficacy in field trials, salinity impact on fungal growth and incidence of disease in greenhouse, interaction between the irrigation systems, salinity and branch canker incidence and developments of educational materials. The hired Postdoc will be responsible for designing and completing the work entailed in this project. Supply funds are to purchase materials for 1) fungal isolation and molecular identification, purchase of plant and product materials for pathogenicity studies, to purchase DNA and PCR reagents, sequencing costs, and chemical products; 2) developing educational outreach programs and publications; 3) Travel funds are based on rental of a car from the UC Fleet Services at a daily rate of $40 plus fuel. Overnight lodging and meals at per diem rates, or actual expenses, will be required for trips to the survey locations.

Budget

Year 1	**Budget**
Personnel (includes salary, benefits, fees etc.) (Salary: $60,083+ Benefits: $12,437)	$72,520

Year 1 Total	**$72,520**
Year 2	
Personnel (includes salary, benefits, fees etc.) (Salary: $62,480+ Benefits: $12,933)	$75,413
Materials, equipment and lab supplies	$11,000
Travel (weekly trips to field sites [car rental, gas], meetings etc.) and trees	$4,500
Year 2 Total	**$90,913**
Year 3	
Personnel (includes salary, benefits, fees etc.) (Salary: $62,480+ Benefits: $12,933)	$38,500
Materials and supplies for lab and field work	$18,000
Travel (weekly trips to field sites [car rental, gas], meetings etc.)	$2,000
Year 3 Total	**$58,500**
Year 4	
Materials and supplies	$20,000
Travel (weekly trips to field sites [car rental, gas], meetings etc.)	$2,000
Meetings and publishing costs	$2,000
Year 4 Total	**$24,000**
Total Project Budget	**$245,933**

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THE HUNTINGTON
Library, Art Museum, and Botanical Gardens

AVOCADO TISSUE CULTURE: MINERAL STUDIES, MICROGRAFTING, AND CRYOPRESERVATION

Project proposal to the California Avocado Commission

May, 19 2023

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Background

Like many other fruit crops, the avocado (*Persea americana*) market is dominated by just a few varieties and rootstocks. Threats such as pests, diseases, and climate change are some drivers in the search for propagation improvements and new clonal rootstocks that can better adapt to the biotic and abiotic stress conditions. At The Huntington, we work on tools to improve avocado grower success through mass propagation and ensure the long-term conservation of avocado genetic resources through cryopreserved tissues.

During the last project supported by the California Avocado Commission, we completed studies that led us to learn more about the sensitivity of avocados to tissue culture conditions, including clonal rootstocks, cultivars, and wild relatives. We have developed protocols from the initiation of bud woods to the rooting of microplants. The Huntington hosts the first in vitro repository of avocados in the USA, focused on securing the avocado field collections. Our collection can be used by the California industry, such as breeders and growers. However, more studies are needed to optimize the mass propagation of rootstocks, which will shorten the propagation of avocado plants and positively impact the California avocado industry.

Besides the shoot micropropagation, micrografting is a technique that could allow the regeneration of susceptible avocado genotypes. This technique can help establish tissue cultures and the regrowth of cryopreserved clones. Moreover, the robust tissue culture methods will facilitate the standardization of long-term preservation methods, which will secure the avocado genetic resources.

Objective (s)

The primary objective is to optimize protocols to mass propagate rootstocks and bank avocado germplasm collections.

Other objectives:

- Mineral studies of avocado tissue cultures to better understand the responses to the micropropagation conditions.

- Adaptation of the micrografting techniques to regenerate the most susceptible genotypes.

- Optimization of cryopreservation protocols for selected cultivars will allow the creation of the first cryobank of avocados in the USA.

Research plan

The laboratory research technicians and the interns will perform the micropropagation studies, and Dr. Folgado (Cryopreservation research botanist) will oversee them and perform cryopreservation experiments. Ms. Tin (Conservation Manager) will support the project as a laboratory manager. Other Botanical staff from The Huntington will support this project by helping maintain the trees and collect plant materials.

Partners from nurseries, UC Riverside, botanical gardens, and USDA field stations will continue to provide explants to incorporate into the repositories.

Schedule:

November 2023 to April 2024

Optimization of rooting. Microshoots from the selected varieties will be submitted to various conditions to study the rooting process. We will test plant growth regulators on the previously chosen basal medium.

Mineral studies. Selected cultivars and rootstocks will be submitted to two different media compositions, and samples will be collected. External laboratories will perform mineral analyses, and the collected data will help to adjust the media composition.

Micrografting. The test will be performed using various cultivars. Seeds and in vitro microshoots will be used as rootstocks, and we will collect data.

Optimization of regeneration after cryopreservation. We will use in vitro shoots of Mexican races and Guatemalan-Mexican hybrids to test the first protocol for avocado shoot tips.

May 2024 to October 2024

Initiation in tissue culture. More trees from the field collections will be collected and introduced in tissue culture.

Mineral studies will continue. Obtained results will optimize the media and increase the number of genotypes that can be preserved in tissue culture. Also, the medium for selected rootstocks will be adapted in future experiments.

Test of rooted plants. Some rooted microplants will be acclimated in ex vitro conditions and others will be used in other laboratories as experimental plant materials.

Micrografting will continue to compare different techniques. Results will allow an optimized method based on the feasibility of shoot regeneration.

Results from cryopreservation will be presented at conferences (i.e., the Meeting of the Society for Cryobiology).

November 2024 to April 2025

Publication of results. The obtained results from the micropropagation studies will be published in plant science journals (i.e., Horticultural Science).

Mineral studies. The design of experiments will be based on our previous tests. We will focus on the effect of selected micronutrients combined in adjusted ranges of different media compositions, and samples will be collected. Results will lead to optimizing the mass propagation of the selected rootstocks.

Micrografting will continue to test shoot regeneration after cryopreservation.

The initiation of new cultivars in tissue culture will increase the genetic diversity of the in vitro repository.

May 2025 to October 2025

Initiation of new genotypes in tissue culture.

Rooting experiments. The micropropagated rootstocks will be submitted to rooting trials. The design will be based on prior optimized protocols, and we will study the response of rootstocks to different rooting conditions.

Micrografting tests will continue to test the optimized techniques in other potential targets, such as landraces and wild types.

November 2025 to April 2026

Initiation of new genotypes in tissue culture.

Cryopreservation experiments. Several genotypes representing the three landraces and hybrids will be tested, and we will bank those showing regeneration after cryopreservation. Thus we will start the backup of the in vitro repository, and an avocado cryobank will be created to secure the germplasm. The cryopreserved clonal materials will be available to growers and breeders, like the *in vitro* repository. We will share the results with the avocado industry through their organizations (i.e., report to the California Avocado Commission).

Mineral analyses. Selected genotypes from the Hass lineage will be submitted to micropropagation tests, and samples will be collected to perform mineral analyses.

The optimized mass propagation process will be submitted to be presented at international conferences (i.e., the International Horticultural Congress).

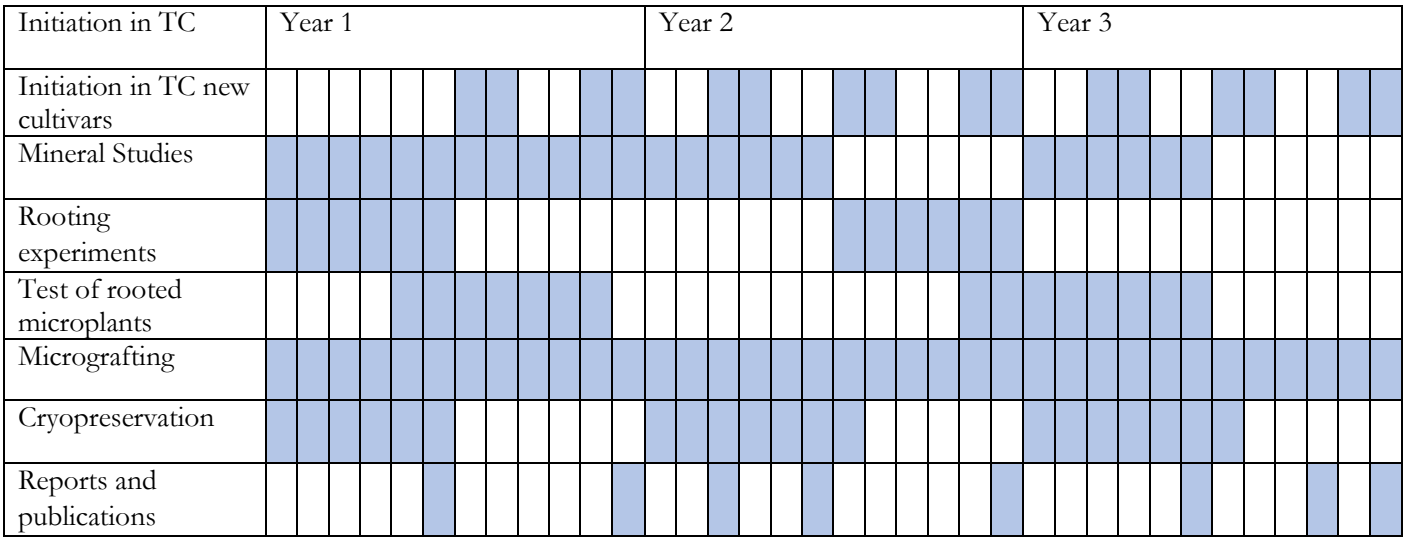
May 2026 to October 2026

Initiation of selected genotypes in tissue culture.

Micrografting will be used to introduce trees that showed sensitivity to the tissue culture.

Results from micropropagation and cryopreservation will be presented at the International Horticulture Science and published in plant science journals (i.e., Acta Horticulturae, Cryoletters).

<u>Chronogram</u>



Detailed budget

The Huntington seeks research support to continue our investigations that could improve avocado regeneration in tissue culture and the mass propagation of rootstocks. The requested support includes two internships, one laboratory technician (30%), scientific research services and supplies, and meeting attendance. The total requested amount is $114,385.

The whole research project includes other expenses which will be covered by The Huntington (i.e., salaries of other staff involved in the research and research equipment), and they are not specified in the budget.

Category	**Amount requested**	**Description**
Summer Internships	$16,800	Interns will support tissue culture and micrografting: Year 2 ($8,400); Year 3 ($8,400)
Staff salary (benefits included)	$50,265	30 % time (approx.) of one full-time laboratory technician: Year 1 ($16,755); Year 2 ($16,755); Year 3 ($16,755)
Travel **Cost of project-related travel**	$2,350	1 Research Staff will attend: -The Avocado Brainstorming 2025, held in Spain. Hotel and trip from Madrid ($350) -The XXXII International Horticultural Congress 2026, held in August 2026, in Kyoto, Japan. Airfare ($1,200) and hotel for eight nights ($800).
Scientific Research Services that are necessary to achieve project objectives.	$42,120	Mineral analyses: Year 1 ($12,960); Year 2 ($14,580); Year 3 ($14,580)
Supplies **(<$5,000/unit) necessary to achieve project objectives.**	$1,550	Supplies include culture vessels and media costs, including consumable lab supplies: Year 1 ($650), Year 2 ($450), Year 3 ($450).
Other **Conference and publication fees**	$1,300	The registration fees for: Avocado Brainstorming 2025 ($300); XXXII International Horticultural Congress 2026 ($1,000)
Total Funds Requested	**$114,385**	