CALIFORNIA AVOCADO COMMISSION
PRODUCTION RESEARCH COMMITTEE
MEETING MINUTES

September 28, 2023

A meeting of the Production Research Committee (PRC) of the California Avocado Commission (CAC) was held on Thursday, September 28, 2023, with the following people participating:

MEMBERS PARTICIPATING:
Leo McGuire, Chair
John Burr (9:18)
Jason Cole (9:04)
Jim Davis (9:02)
Consuelo Fernandez
Darren Haver
Danny Klittich
Daryn Miller (9:03)
Ryan Rochefort
Rob Grether, *ex officio* (9:35)

CAC STAFF PARTICIPATING:
April Aymami
Ken Melban
Jeff Oberman

OFFICIALLY PARTICIPATING:
Dr. Tim Spann, Spann Ag Research & Consulting

GUESTS PARTICIPATING:
John Berns

CALL TO ORDER

Leo McGuire, Production Research Committee (PRC) Chairman, called the meeting to order at 9:00 a.m. with a quorum present.

OPPORTUNITY FOR PUBLIC COMMENT

There were no public comments.

APPROVAL OF MINUTES OF MAY 26, 2023 PRODUCTION RESEARCH COMMITTEE MEETING

MOTION
To approve the minutes of the May 26, 2023 Production Research Committee meeting as amended.

(Klittich/Davis) MSC Unanimous

Motion 23-9-28-1

RESEARCH PROGRAM DIRECTORS REPORT

Dr. Spann reminded committee members that a new PRC will be appointed following the seating of the new Board in November and asked the committee members to think about whether they wish to continue serving. Dr. Spann also informed the committee that the final report on the chloride mitigation project had been received from Dr. Liu and would be sent to the committee for their review. Finally, Dr. Spann updated the committee on the status of the Pine Tree Ranch lease renewal process, stating that CAC had just signed another 3-month lease extension to allow Cal Poly time to complete the installation of the variable frequency drive on the well. It is anticipated the work will be completed and a new lease will be signed starting January 1, 2024.

ACTION ITEMS

A. Consider request for funding for Cal Poly Project #24-044, Avocado Rootstock Trial

Dr. Spann started by reminding the committee that one of the seven rootstock trial sites is on the Cal Poly campus in San Luis Obispo and none of the funding provided to Dr. Manosalva at UC Riverside has been used to support the Cal Poly planting to date. However, the funding that Cal Poly was using to maintain the plot and collect data expired in June 2023 and they are now requesting funds to continue data collection in 2024 and 2025. Discussion ensued and there was general agreement that the Cal Poly site is important to the overall project and maintaining ties with Cal Poly is important as they appear to be the future of applied field research for the avocado industry. Although there was support for the project, there was a strong feeling the cost was too high given the tight budget for the 2023-24 fiscal year. Dr. Spann informed the committee that Dr. Garner had provided him with budget options and the cost to continue basic data collection was $33,463 for the next two years ($16,690 in 2023-24 and $16,773 in 2024-25).

MOTION
To recommend funding the project with Cal Poly for the reduced amount of $33,463 for two years.

(Cole/Davis) MSC Unanimous

Motion 23-9-28-2

B. Reconsider request for funding for project “Characterization and Management of Avocado Branch Canker Disease and Post-Harvest Decay Rots in Avocado Production in California”

Dr. Spann began by reminding the committee that they had reviewed this proposal from Dr. Fatemeh Khodadadi at their previous meeting and recommended it for funding. However, a decision on funding was tabled by the Board and, given the new information about the budget for 2023-24, the committee was being asked to reconsider the proposal. There was general consensus among the committee that supporting Dr.

Khodadadi, a new faculty member, was to the industry's benefit to ensure her support in the future if a critical issue arises. Additionally, there was general agreement that avocado branch canker (ABC) is a serious issue throughout the industry. However, at the present time the industry does not know how much, if any, yield is lost to branch canker and what the appropriate investment should be. There was general agreement that, given current budget constraints, the current proposal's scope and budget are beyond what can be supported. The committee agreed there would be value in meeting in the future with Dr. Khodadadi as well as Dr. Michailides, who worked on ABC prior to Dr. Khodadadi's hiring, to discuss a more paired down study to answer specific questions that the committee feels are important and that placeholder funding should be earmarked in the budget for such a project.

MOTION
To recommend $30,000 of placeholder funding in the 2023-24 budget for ABC research dependent on the committee meeting with Dr. Khodadadi to define the scope of a project and a new proposal being submitted.

(Klittich/Cole) MSC – Vote Tally: Yea7, Nay 1

Motion 23-9-28-3

C. Consider a policy on overhead costs for research projects

Dr. Spann began the discussion by reminding the committee that there has been a longstanding agreement between the commodity boards in California and the UC system that exempts the commodity boards from paying overhead on research grants to the University. However, while Dr. Garner was developing her proposal for rootstock trial funding, it was found that no such agreement exists with the California State University (CSU) system. To avoid paying overhead, the CSU system asked to see CAC's official policy stating that CAC does not pay overhead, but no such policy exists. Discussion ensued and there was general agreement that such a policy should be developed.

MOTION
To recommend CAC staff develop a no overhead payment policy for the Board's consideration.

(Cole/Klittich) MSC Unanimous

Motion 23-9-28-4

ADJOURN MEETING

Leo McGuire, Production Research Committee (PRC) Chairman, adjourned the meeting at 10:56 a.m.

Respectfully submitted,

_____
Timothy Spann

EXHIBITS ATTACHED TO THE PERMANENT COPY OF THESE MINUTES

EXHIBIT A September 28, 2023 Production Research Committee AB 2720 Roll Call Vote Tally Summary

EXHIBIT B Proposal Cal Poly Project #24-044, Avocado Rootstock Trial

EXHIBIT C Proposal Characterization and Management of Avocado Branch Canker Disease and Post-Harvest Decay Rots in Avocado Production in California

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	*Hybrid* *In-person – Irvine* *Online – Zoom*	*September 28, 2023*

Attendees Who Voted	***MOTION 23-9-28-1***	***Motion 23-9-28-2***	***Motion 23-9-28-3***	***Motion 23-9-28-4***
Leo McGuire, Chair	*Did not vote*	*Did not vote*	*Did not vote*	*Did not vote*
John Burr	Absent	Yea	Yea	Yea
Jason Cole	Yea	Yea	Yea	Yea
Jim Davis	Yea	Yea	Yea	Yea
Daren Haver	Yea	Yea	Yea	Yea
Consuelo Fernandez	Yea	Yea	Yea	Yea
Danny Klittich	Yea	Yea	Yea	Yea
Daryn Miller	Yea	Yea	Yea	Yea
Ryan Rochefort	Yea	Yea	Nay	Yea
Outcome	**Unanimous**	**Unanimous**	**7 Yea, 1 Nay**	**Unanimous**

CAL POLY PROJECT #24-044 Lauren Garner

In 2019/2020, a collaboration began between Cal Poly, UCR, and the CAC, resulting in the establishment of a rootstock trial site on Cal Poly's campus in San Luis Obispo. This is the northern-most site in the statewide rootstock trial currently being conducted by the CAC and UCR. With financial and in-kind support from the CAC, members of the avocado industry, and Cal Poly, an avocado orchard was established at a site on campus with a documented and recent history of *Phytophthora* root rot (PRR). Trees of 'Hass' avocado grafted on 'Dusa', 'PP35, 'PP40', or 'PP45' were transplanted at the Cal Poly site on 24 June 2020 using a randomized complete block design with 10 replications of 8-10 trees per rootstock treatment in 3 blocks for a total of 384 trees. Trees were planted on berms at a 15' x 20' tree spacing.

All trees were measured and assessed 2 months after transplanting (August 2020) and during flushing in spring (March/April 2021-23), summer (July 2021-23), and fall (October 2021-2022), with all assessments being overseen by the graduate student, Rashaan Souikane. Harvest data was collected in June 2023. Assessment included tree height (m), above-graft trunk diameter (mm), below-graft trunk diameter (mm), and canopy volume (beginning in 2023), and rating salinity damage, heat damage, vegetative flush and bloom on a scale of 0-5. Also in June 2023, soil and root samples from all 40 replications and sent to Fruit Growers Lab to test for the presence or absence of *Phytophthora cinnamomi*; positive results were found in numerous plots throughout the orchard, which was treated with Orondis this month (August 2023). Our work to date has resulted in several presentations (at grower meetings and scientific conferences), contributions to all intermittent and annual reports required by Patty Manosalva to meet CAC milestones, one Master's thesis, and numerous undergraduate senior projects and class projects.

Since the 2020 establishment of the San Luis Obispo site, no further funding has been requested or received from the CAC for this project and no funding has been received from UCR at any point. Instead, I submitted and was approved for a change of scope to a grant I had from the Agricultural Research Institute (ARI), which added more than $85K in funding to this project. This funding allowed us to support some key aspects of the plot's management and most importantly, all Cal Poly students and faculty working on the project have been solely funded by that (ARI) grant. This grant ended on June 31, 2023, but the work of managing the plot and collecting data for the multi-site project continues.

Objective 1: Continue to collect and analyze data and disseminate results to the avocado grower and research community

To continue to track tree growth, health, and productivity, data will be collected during the spring (2024, 2025), summer (2024, 2025), and fall (2023-2025) flushes, and during harvest (2024, 2025). Leaf samples will be collected from all 40 replications (August 2024, 2025) to assess any treatment effects on leaf nutrient concentrations. Any differences in 'Hass' leaf nutrient concentrations detected between rootstock will help to inform any changes in fertilizer programs or recommendations that might be required. All data collection will be overseen by Rashaan Souikane, who completed his Masters degree based on his work on this project and is currently employed as a part-time lecturer at Cal Poly. He will oversee undergraduate research assistants in data collection and entry and he will work with me and Andrew Schaffner (Professor, Cal Poly

Statistics Department) to analyze the data and to continue to prepare reports for CAC and UCR and to co-author presentations and manuscripts for dissemination to growers and the wider scientific community.

Objective 2: Continue to maintain orchard plot

In addition to employing students as research assistants, having student orchard assistants will allow us to dedicate weekly efforts to regular management and maintenance issues, including tasks such as pruning, weeding, walking irrigation lines, scouting, and harvesting. A mower will be purchased. Having a mower dedicated to this orchard is required to properly maintain the research block, especially with respect to cover crop and weed management, without risking the spread of *Phytophthora cinnamomi* to other orchard blocks. Irrigation supplies and materials will be purchased to complete the installation of a newly donated filtration station, which will help to ensure that the research block has consistent uniform irrigation and chemigation. Two soil and root samples from each of the 3 blocks will be collected (summer 2024, 2025) and sent to Fruit Growers Lab to test for the presence or absence of *Phytophthora cinnamomi* in order to inform PRR control measures.

Sponsor:	CA Avocado Commission
Title:	Avocado Rootstock Trial SLO
Project Term:	10/1/2023-9/30/25
GDO #:	24_044

Personnel	WTUs					CY	AY	SM	Year 1	Year 2	Total
Lauren Garner	0.0	0.00%	Release @	$120,810	/AY		0.00		$0		$0
		15.00	hours overload @	$88.83	/HR	0.09			$1,332		$1,332
		15.00	hours overload @	$92.83	/HR	0.09				$1,392	$1,392
Andrew Schaffner	0.0	0.00%	Release @	$154,794	/AY		0.00		$0		$0
		15.00	hours overload @	$74.42	/HR	0.09			$1,116		$1,116
		15.00	hours overload @	$77.77	/HR	0.09				$1,167	$1,167
CPC Employee 2		120	hrs effort @	$45.00	/HR	0.69			$5,400		$5,400
		120	hrs effort @	$45.00	/HR	0.69				$5,400	$5,400
Undergraduates		488	hours @	18.00	/HR	2.82			$8,784		$8,784
		488	hours @	18.00	/HR	2.82				$8,784	$8,784
								Subtotal Personnel	**$16,632**	**$16,743**	**$33,375**
Fringe Benefits											
Faculty summer & overload		Lauren Garner		7.9%					$105	$110	$215
Faculty summer & overload		Andrew Schaffner		7.9%					$88	$92	$180
Corp intermittent		CPC Employee 2		7.9%	59.8%				$427	$427	$854
Undergraduates		Undergraduates		2.7%					$237	$237	$474
								Subtotal Fringe Benefits	$857	$866	$1,723
								TOTAL Personnel Services	**$17,489**	**$17,609**	**$35,098**
Equipment											
Mower									$13,054	$0	$0
								TOTAL Equipment	**$13,054**	**$0**	**$13,054**
Other Direct Costs											
Materials & Supplies											
Irrigation Supplies									$2,000	$0	$2,000
								SUBTOTAL Materials & Supplies	**$2,000**	**$0**	**$2,000**
Consultant Services											
Fruit Growers Lab									$2,752	$2,752	$5,504
								SUBTOTAL Consultant	**$2,752**	**$2,752**	**$5,504**
								TOTAL Other Direct Costs	**$4,752**	**$2,752**	**$7,504**
								TOTAL DIRECT COSTS	**$35,295**	**$20,361**	**$55,656**
Indirect Costs											
Cal Poly Recovered F&A Base									$22,241	$20,361	$42,602
Cal Poly Recovered F&A		0.0% of Modified Total Direct Costs							$0	$0	$0
								TOTAL SPONSOR COSTS	**$35,295**	**$20,361**	**$55,656**

BUDGET JUSTIFICATION – CAL POLY PROJECT #24-044

PERSONNEL:

- Lauren Garner, Cal Poly- Plant Sciences Professor; PI overseeing project; 15 hours/year
- Andrew Schaffner, Cal Poly- Statistics Professor; Statistical support; 15 hours/year
- Interim employee, Cal Poly- Research technician to oversee data collection and analysis and undergraduate research assistants; 120 hours/year
- Undergraduate employees, Cal Poly- student research assistants 288 hours/year (data collection and entry) and student orchard employees 200 hours/year (assist in orchard management)

SALARIES AND WAGES: The salary and wage rates are based on the California Polytechnic State University (CPSU) and Cal Poly Corporation (CPC), jointly Cal Poly, established salary and wage rates paid during the 2023-2024 Fiscal year (July 1 – June 30). In general, faculty duties at CPSU consist of fifteen units in each of three Academic terms per eight-month Academic contract year, exclusive of academic breaks and summer sessions. Faculty 12-month appointments may include a combination of academic and administrative duties and encompass academic breaks and summers. The salary and wage rates for faculty and non-student staff include a projected 4.5% salary increase per year. he rates shown are for budgetary purposes; the rates in effect at the time the work is performed will be charged to the project.

FRINGE BENEFITS & EMPLOYER PAYROLL TAXES: Benefits for CPSU Faculty summer and overload work include FICA, SUI and Workers Compensation and are calculated at the proposed DHHS pooled rate of 7.9%.

Benefits for CPC intermittent employees include FICA, SUI and Workers Compensation and are calculated at the proposed DHHS pooled rate of 7.9%.

CPC undergraduate student benefits include SUI and Worker's Compensation. The proposed DHHS pooled rate of 2.7% is used for budgetary purposes.

The rates in effect at the time the work is performed will be charged to the sponsor.

EQUIPMENT: (Items $5K and above)

- A dedicated orchard mower is required to properly maintain the research block, especially with respect to cover crop and weed management, without risking the spread of *Phytophthora cinnamomi* to other orchard blocks. This purchase will allow us to dedicate a single mower to the block. A quote is included.

OTHER DIRECT COSTS:

SUPPLIES AND MATERIALS: (Items $5K and under)

- Funds will be used to support the purchase of supplies, including but not limited to, the cost of irrigation supplies and materials required to complete the installation of a newly

donated filtration station, which will help to ensure that the research block has consistent uniform irrigation and chemigation.

CONSULTANT SERVICES:

- Fruit Growers Lab will be used for annual analysis of leaf samples to determine leaf nutrient concentrations for all 40 replications (40 samples @ $61 = $2440 per year) to analyze for possible relationships to rootstock treatment. Fruit Growers Lab will also be used to analyze soil and root samples for the presence of *Phytophthora cinnamomi* in each of 3 blocks (2 samples per block = 6 @ $52 = $312 per year) in order to inform treatment decisions related to PRR.

FACILITIES AND ADMINISTRATIVE (F&A) COSTS:

AVP for Research Administration approved the use of a reduced F&A rate of 0% for this proposal.

EXHIBIT B

SALES QUOTE - PASO ROBLES

CAL-COAST MACHINERY

661 Customer Number

Invoice Number

Name:	Cal Poly c/o John Rosencrans		**Remit To:** P.O. Box 279
Address:	1 Grand Ave Building 15 Accounts Payable		Santa Maria, CA. 93456
City/St./ZIP:	San Luis Obispo, Ca 93407		Phone: (805) 925-0931
Phone:	(805) 756-2548	**Fax:**	FAX: (805) 925-8123

Date:	08/09/23	**Terms:** Net	**Delivery Date:**
Sale Type:	Agricultural	**Delivered By:** CCM	**County:** San Luis Obispo

Brand	Model	Description	Serial Number	Hours	Price
Rears	IFA96K930	Pak Flail Mower - 3pt	New 2023		$11,520.00
		96" cut, 111" overall width			
		1544 lb empty weight			
		Belt housing height - 33.13"			
		Flail housing height - 21.75"			
		FL930 "Y" knives (heavy duty)			
		.25" housing thickness			
		Heavy duty 3pt mast			
		Hard faced skid shoes - replaceable			$41.00
		Chain guard - extended front shelf			$503.00
		6" roller - greasable			
		No rake teeth			
		Rotor shaft 1 15/16” diameter rotor shaft			
		Cut height 0”- 5” adjustable cut height			
		Belt drive 4 groove 5V Powerband®			
		Rears quick-draw tensioner			
		Flail gearbox 1:3 ratio, 85hp rating			
		Input driveline category 4			
		Roller construction 1 3/4” dia. roller			
		shaft in 6 5/8” dia. roller			
		Roller bearing Triple seal ball bearing			
		with eccentric locking collar			
		FL930 - Course mowing and shredding			
		knife - welded channel on drum for			
		blades to bolt to			
			Cal Poly Discount		$893.00
			Freight to P.R.		$1,000.00
			Freight to Farm		$0.00

Additional Notes			
Manufacture time 6-8 weeks	7.250%	Sales Tax	$882.40
Cal Poly Corp Campus Dining Rec Dock/BLD 19 One Grand Ave/Bld 19 SLO		Total	$13,053.40
Cal Poly Corp One Grand Ave BLD 19/Corp CD Rec Dock, SLO		Trade In	$0.00
		Down Payment	$0.00
		Balance	$13,053.40

CAL-COAST MACHINERY RETAINS TITLE TO THE ABOVE GOODS UNTIL BALANCE IS PAID IN FULL

Salesperson: Galen O'Reilly

Customer:

Branch Phone Number: (805) 434-5566

Proposal to The CALIFORNIA AVOCADO COMMISSION

Project title: **Avocado Branch Canker Disease in California: Pathogen Identification, Characterization, Fungicide Efficacy, and Interaction between Irrigation Systems, Salinity, and Disease Incidence and Severity**

Project start date: 1 November 2023
Project end date: 31 October 2026
Project Leader: Fatemeh Khodadadi
Position Title: Plant Pathologist
Address: University of California, Riverside, 900 University Avenue, Riverside, CA 92521
Primary Telephone Contact Number: 951-827-4764 (mobile 845-901-3046)
E-mail Address: fatemehk@ucr.edu
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, Growers, 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. California produces 95% of the avocados grown in the United States within two Counties (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 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 is the leading County, producing 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 have a worldwide distribution 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 in avocado cultivations in California has risen and changed from a minor problem to a growing threat to avocado production, leading to economic losses (Avenot et al. 2019).

Avocado branch canker is 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 the disease which hold attached dead leaves which turn brown 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 lead 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.

Branch canker has been frequently reported by growers, extension specialists, nursery managers in California in the past decade. The first step in successful management of any disease, including ABC in avocado, 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. In avocado-producing countries worldwide, the Botryosphaeriaceae species linked with this disease are *Botryosphaeria obtusa* (Schwein) and *B. rhodina* (Berk. and M.A. Curtis) in Mexico and the USA (Menge and Ploetz et al. 2003); *Lasiodiplodia theobromae*; *Neofusicoccum austral*; *N. luteum,* and *N. mediterraneum* in Spain (Arjona-Girona et al. 2019); and *N. parvum* in other European countries (Zea-Bonilla et al. 2007; Guarnaccia et al. 2016 and 2020). Species causing the ABC in Chile are *N. australe* (Auger et al. 2013), *Diplodia mutila*, *D. pseudoseriata*, *D. seriata, Dothiorella iberica, N. nonquaesitum*, and *N. parvum* (Valencia et al. 2019). In California, species such as *Lasiodiplodia theobromae, Botryosphaeria dothidea, Dothiorella iberica, Lasiodiplodia theobromae; Diplodia mutila* and *Neofusicoccum* spp. (*N. australe, N. luteum, N. parvum, N. ribis, N. vitifusiforme*) have been so far reported from avocado orchards (McDonald et al. 2009; McDonald and Eskalen, 2011; Avenot et al. 2021) with species in genus *Neofusicoccum* seemed to be more common. In California, there has been a study reporting different genera and species associated with this disease (McDonald et al. 2009; McDonald and Eskalen, 2011). However, we know that a wide range of taxonomically different and sometimes unrelated fungi is associated with the ABC disease in avocado, so, it is important to monitor the change in causal agents and determine the primary prevalent and virulent causes across California. 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, it becomes challenging to control them. Because species in the Botryosphaeriaceae infect avocados predominantly through wounds, most of the strategies developed to control these pathogens have been revolved around protection of pruning wounds using chemicals. A wide spectrum of taxonomically different and sometimes unrelated fungi is associated with the ABC disease in avocado 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 best recommended by using fungicides in combination with cultural practices and the application of right irrigation system and reduction of stresses such as soil salinity. In this project, we will test the efficacy of commercially available chemical against avocado branch canker pathogens *in vitro* and in field. Currently, there are no registered chemical for 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, wound may remain open and susceptible for up to 4 months (URBEZ-TORRES 2011) but in avocado 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 keep each tree's energy resources. To avoid such 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 such product 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 make it distinguished from other similar works previously conducted in California (Eskalen et al. 2013; Avenot et al. 2021). However, 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 in California.

Mediterranean climate conditions in California predispose avocado production to irrigation, frost, heat, and salinity. Drought stress in California has made the water availability limited and imposed stress conditions for avocado producers. 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 the past decade in California. 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 exposed 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 decrease rapidly. 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 we know salt is a stress factor, but no data is available on its impact on avocado branch canker disease. So, 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.

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. While 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 on 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 nursery 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 assay in the lab and greenhouse in the objective second.

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. Because these pathogens 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 in Butte County due to the Bot panicle and shoot blight epidemic in the early 1980's, there should be collaborative efforts between the nut trees industry and avocado industry to combat this disease. The primary beneficiaries of our research are an 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 to pathogen resistance to fungicides.

2. Objectives:

Objective 1: Evaluating the *in-vitro* and in-field efficacy of chemical treatments against avocado branch canker disease.
Objective 2. Evaluating the impact of salinity on growth of species in Botryosphaeriaceae *in vitro* and on disease severity and development in greenhouse.
Objective 3: Evaluating the relationship among drip and micro sprinkler irrigation systems, salinity and avocado branch canker disease incidence in avocado orchards.
Objective 4: Develop educational materials / programs through extension to stakeholders in the California Avocado Industry.

3. Procedures:

Objective 1: Evaluating 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 determining the impact of salinity, we will first isolate, 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 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 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 isolates 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 could 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 (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 kept 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 laboratory will be evaluated in field to protect pruning wounds. We will compare the impact of different fungicide 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 applied 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 forth 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. 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 were protected for one week using Parafilm to prevent dehydration and promote spore germination. For NAA treatment, we 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 be similar 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 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 with 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.

Table 1. List of fungicides which will be used for *in-vitro* assay, the effective ones will be later used in the field.

Trade Name	**Active Ingredients**	**FRAC Group**
Inspire Super	Difenoconazole+Cyprodinil	FRAC group 3
Regalia	Extract of Reynoutria sachalinensis (5.0%)	FRAC Code P5
Aprovia	Benzovindiflupyr	FRAC group 7
Fontelis	Penthiopyrad	FRAC group 7
Omega	Fluazinam	FRAC group 29
Flint	Trifloxystrobin	FRAC group 11
Merivon	Pyraclostrobin+Fluxapyroxad	FRAC group 11 and 7
Indar	Fenbuconazole	FRAC group 3
BAS 490F	Kresoxim-methyl	FRAC group 11
Elite	Tebuconazole	FRAC group 3
Scholar	Fludioxonil	FRAC 12
Topsin M	Thiophanate-methyl	FRAC 1
Luna Experience	Fluopyram + Tebuconazole	FRAC group 3 and 7
Luna Priviledge	Fluopyram	FRAC group 7
Approach	Cyproconazole+Picoxystrobin	FRAC group 3 and 11
Boscalid/Pristine	boscalid (25.2% active) and pyraclostrobin (12.8% active)	FRAC 7
Phosphonate	Phosphorous acid	FRAC Code 33
BlueShield	Copper Hydroxyde	FRAC group M1
TreHold A-112	NAA (Naphthaleneacetic acid)	

Objective 2. Evaluating 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 first objective in the laboratory. We will obtain spore suspension and mycelial plugs from 7-day old colonies grown at 25°C in PDA. We will add the mycelial plugs and 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. 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) after 4 weeks. 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 continued to receive the irrigation solution with no added salt (at optimum EC for avocado growth), while plants in the other two groups received 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 symptoms development, disease incidence and fungal growth in our three group of trees.

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

Drip and micro sprinklers are two dominant methods used for avocado production in California. The simultaneous effect of these two irrigation methods on salinity and branch canker disease incidence is unknown. In this objective, we are aiming to seek 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.

Data Analysis:
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: Deliver educational materials to growers and stakeholders through extension programs and activities.
Results and findings 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 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 County to educate participating growers, nursery managers on how to diagnose the disease and avoid or reduce the sources of pathogen inoculum and educate industry personnel (UC Farm Advisors and Pest Control Advisers) on findings of this study and what can be done, cooperatively, to produce plant free of Botryosphaeriaceae fungi. Results and findings from this project will be published in peer-reviewed and trade journals for producers and presented at regional, national, and international professional meetings. The conclusions 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 popular articles and refereed technical articles. Project outcomes will be shared at grower's field days, in magazines articles, and through the Statewide IPM Program 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	**November 2023-October 2024**		
Milestone	**Activities**	**Scheduled Completion**	**Budget**

1	Sample collection, isolation, DNA extraction and sequencing the genes for identification	November, 2023	$5,000
2	Interview, recruit, and hire a Postdoc and pay salary/fees for first year. Complete hiring by December 2023	December, 2023	$77,891
3	In vitro sensitivity of fungi to the list of fungicides	February, 2024	$3,500
4	In vitro sensitivity of fungi to salts	March, 2024	$2,000
5	Virulence and pathogenicity of species	October, 2024	$2,500
		Year 1 Total	**$90,891**
Year 2	**November 2024-October 2025**		
1	Salary and fringe benefit of the postdoc working on the project	November 2024	$83,605
	Continuing the work and data analysis of in-vitro tests for fungicides and salts	November 2024	$500
2	Setting up the trials for the efficacy of fungicides in the field and collecting data	July, 2025	$8,000
	Setting up field trials for the interaction between salinity, Branch canker and irrigation systems	July, 2025	$8,000
3	Purchasing trees and evaluation of salinity on disease incidence and severity in greenhouse	October, 2025	$4,071
		Year 2 Total	**$104,176**
Year 3	**November 2025-October 2026**		
	Salary and fringe benefit of the postdoc working on the project	November 2025	$44,866
1	The second-year trials for the efficacy of fungicides in the field and collecting data	June 2026	$3,000
2	Monitoring the ABC disease in field trials for the interaction between salinity, branch canker and irrigation systems	October 2026	$3,000
		Year 3 Total	**$50,866**
		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) constructing 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	YEAR 2	YEAR 3	CUMULATIVE
SENIOR PERSONNEL	0	0	0	0
OTHER PERSONNEL	64,480	69,209	37,141	170,830
WAGE SUBTOTAL	64,480	69,209	37,141	170,830
BENEFITS	13,411	14,396	7,725	35,532
PERSONNEL TOTAL	77,891	83,605	44,866	206,362
TUITION	0	0	0	0
EQUIPMENT	0	0	0	0
TRAVEL	3,000	2,000	2,000	7,000
PARTICIPANT COSTS	0	0	0	0
				0
SUPPLIES	10,000	18,571	2,000	30,571
PUBLICATION COSTS	0	0	2,000	2,000
COMPUTER	0	0	0	0
SUBAWARDS	0	0	0	0
OTHER	0	0	0	0
TOTAL DIRECT COSTS	90,891	104,176	50,866	245,933
No Indirect Costs Allowed	0	0	0	0
TOTAL DIRECT/INDIRECT	90,891	104,176	50,866	245,933

Year 1 11/1/2023-10/31/2024
Year 2 11/1/2024-10/31/2025
Year 3 11/1/2025-10/31/2026

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