



AGENDA

California Avocado Commission Production Research Committee Meeting

Meeting Information

Date: Thursday, October 29, 2026

Time: 8:00 a.m.

Location: Hybrid Meeting

Physical Meeting Location:

Hilton Garden Inn Irvine/Orange County

2381 Morse Ave.

Irvine, CA, 92614

Web Conference URL:

<https://californiaavocado.zoom.us/j/5375836823?pwd=aURBZ3BEL29tcLBRS1ZRY3QrMkhZQT09&omn=83217255671>

Conference Call Number: (507) 473 4847

Meeting ID: 537 583 6823

Passcode: 348652

Meeting materials will be posted online at least 24 hours prior to the meeting at:

<https://www.californiaavocadogrowers.com/commission/industry-calendar>

Committee Member Attendance

As of Wednesday, October 21, 2026, the following individuals have advised the Commission they will participate in this meeting:

Danny Klittich, PRC Chair

Jim Davis

Herman Els

Matthew Fatino

Consuelo Fernandez

Leo McGuire

Daryn Miller

Time	Item
8:00 a.m.	1. Call to Order a. Roll Call/Quorum 2. Opportunity for Public Comment Persons may address the Committee on subjects within the jurisdiction of the Committee. 3. Approval of Production Research Committee Meeting Minutes of July 28, 2026 4. Research Program Consultant's Report 5. Consider request for one-year extension for artificial pollination project 6. Review pre-proposals submitted in response to Fall 2026 request for proposals a. From satellite-derived canopy temperature to irrigation assessment: Decision support for California avocado orchards, PI: Ali Montazar b. What drives consistently high avocado yields in California: A multivariate analysis of grower practices and orchard conditions, PI: Mary Lu Arpaia c. Improving the avocado irrigation calculator, PI: Andre Biscaro d. Development and evaluation of pollen suspensions in avocado: paternity, yield response and Carmen boom management, PI: Monica Castro e. A field-validated AI forecasting tool for avocado lace bug scouting and damage risk, PI: Tibo Duran f. Quantifying mineral availability in marginal irrigation water using lysimeters with mature Hass avocado trees, PI: Ran Erel g. Proactive laurel wilt defense: Rapid digital LAMP diagnostics and integrated regional intake for California avocados, PI: Fatemeh Khodadadi h. Leveraging existing rootstock trials to determine effective leaching fractions for improved salinity management and avocado productivity, PI: Patricia Manosalva i. Building a precision agriculture framework for California avocado: Leaf monitor calibration and statewide variability assessment, PI: Alireza Pourreza j. Investigating conditional host status of GEM and Lamb Hass avocado to fruit fly – Year 2, PI: Liz Dann 7. Reconsider proposals for Carmen/Mendez genetic marker discovery
11:00 a.m.	8. Adjourn Meeting

Disclosures

All meetings of the Commission are open to the public and subject to the Bagley-Keene Open Meeting Act. All agenda items are subject to discussion and possible action.

For information or a request regarding disability-related modification or accommodation for the meeting, please contact April Aymami at 949-341-1955 via email at aymami@avocado.org. Such requests should be made at least 48 hours prior to the meeting.

This meeting schedule notice and agenda is available on the internet at <https://www.californiaavocadogrowers.com/commission/meeting-agendas-minutes> and <http://it.cdfa.ca.gov/igov/postings/detail.aspx?type=Notices>. Contact Tim Spann at tim@spannag.com or 423-609-3451 if you have any questions.

Summary Definition of Conflict of Interest

Committee members are responsible for determining whether they have a conflict of interest.

A member has a conflict of interest in a decision of the Committee if it is reasonably foreseeable that the decision will have a material effect, financial or otherwise, on the member or an immediate family member that is distinguishable from its effect on all persons subject to the Committee's jurisdiction.

No Committee member shall make, or participate in making, any decision in which they know or should know they have a conflict of interest.

No Committee member shall, in any way, use their position to influence any decision in which they know or should know they have a conflict of interest.

**CALIFORNIA AVOCADO COMMISSION
PRODUCTION RESEARCH COMMITTEE
MEETING MINUTES**

July 28, 2026

A meeting of the Production Research Committee (PRC) of the California Avocado Commission (CAC) was held on Tuesday, July 28, 2026, with the following people participating:

MEMBERS PARTICIPATING:

Danny Klittich, Chair
Victor Araiza
Allisen Carmichael
Jim Davis
Herman Els
Consuelo Fernandez (8:10)
Leo McGuire
Daryn Miller
Rachael Laenen (*ex officio*)

CAC STAFF PARTICIPATING:

Ken Melban
April Aymami
Stacia Kierulff

OFFICIALLY PARTICIPATING:

Dr. Tim Spann, Spann Ag Research & Consulting

GUESTS PARTICIPATING:

Ohannes Karaoghlanian
John Berns
Gray Martin
Enrico Ferro
Norm Kachuck
Maria Alejandra Pinto

ITEM #1 CALL TO ORDER

Danny Klittich, Production Research Committee Chair, called the meeting to order at 8:02 a.m. with a quorum present.

ITEM #2 OPPORTUNITY FOR PUBLIC COMMENT

Gray Martin expressed his support for conducting the study to find markers to distinguish the Carmen/Mendez variety from Hass, and specifically recommended funding the proposal from ESB AI Laboratory since they are local to southern California. He further explained his extensive experience with avocados and avocado breeding and asserted that the claim that the Carmen/Mendez variety arose from a mutation or sport is flatly incorrect because he has never personally observed a sport in avocado. He further expressed the belief that the description of Carmen/Mendez as a sport was a “promotional stunt,” and the Committee should consider the appearance of a conflict of interest if some members do not abstain.

Ohannes Karaoghlanian next addressed the Committee stating that today and throughout the coming weeks the PRC will review proposals to determine how to

compare Hass to Carmen. He stated that Carmen flowers at a distinct time and is off cycle from Mexican Hass but ripens at the peak of the California Hass season and currently no one can distinguish the variety in the market, and it directly hurts California growers. He asked the Committee to please read the letters he wrote using Claude AI and then recommended the Committee fund the ESB AI Laboratory proposal for kinship analyses and after that issue a corrected RFP to all interested parties. If Carmen is found to be a sport from the kinship analyses, the new RFP will require detailed analyses. If it is a seedling, then other methods of analysis can be pursued. Committee member Jim Davis asked if Mr. Karaoghlanian saw any value in the other submitted proposals that he did not mention. Mr. Karaoghlanian stated that he used Claude AI to review the proposals and that it recommended the ESB AI Laboratory proposal. Committee member Consuelo Fernandez said that she had spoken to Iñaki Hormaza (who also submitted a proposal) and that he is still looking for markers to distinguish the GEM and Luna varieties, but he says it is so hard to genetically distinguish Carmen because it is a mutation. Member Fernandez also pointed out that Iñaki's proposal also has two stages to identify the varieties, which was pointed to as a strength of the ESB AI Laboratory proposal.

ITEM #3 APPROVAL OF MINUTES OF MAY 28, 2026 PRODUCTION RESEARCH COMMITTEE MEETING

MOTION

To approve the minutes of the May 28, 2026, Production Research Committee meeting.

(Davis/Miller) MSC unanimous

Motion 26-7-28-1

ITEM #4 RESEARCH PROGRAM CONSULTANT'S REPORT

Dr. Spann provided the Committee with a brief update on the GEM fruit fly host status trial work in Australia, with the first round of caged infestation trials underway.

ITEM #5 CONSIDER RESEARCH PROPOSALS TO DEVELOP GENETIC TESTING TO DISTINGUISH 'HASS' FROM 'CARMEN/MENDEZ'

Ken Melban, CAC President, opened the discussion by explaining to the Committee that the issue of being able to distinguish Carmen/Mendez from Hass was brought to the Executive Committee's attention by Mr. Karaoghlanian because of the belief that Mexico is illegally sending Carmen fruit to the U.S. directly competing with California grown Hass fruit. Following the original discussion by the Executive Committee, a zoom meeting was held with Rachael Laenen (CAC Chair), Mr. Melban, Mr. Karaoghlanian, Dr. Tim Spann and Dr. Edwin Solares where the issue was discussed in detail. At the

conclusion of that meeting, it was decided that the path forward was for Dr. Spann to determine who in the world avocado research community would be able to conduct the research necessary to be able to genetically distinguish Carmen from Hass, then request proposals from those individuals.

Dr. Spann informed the Committee that in addition to Dr. Edwin Solares or ESB AI Laboratory, he identified Dr. Alice Hayward, University of Queensland, Australia; Dr. Luis Herrera-Estrella, Texas Tech University; Dr. Noelani van den Berg, University of Pretoria, South Africa; Dr. Iñaki Hormaza, Institute of Subtropical and Mediterranean Horticulture, Spain; and Dr. Shad Ali, USDA ARS; as qualified experts working in the field of avocado genetics/genomics and on June 7, the following was sent via email to each of these individuals:

The California Avocado Commission is interested in determining the genetic markers for the avocado variety known as 'Carmen' or 'Mendez'. In the U.S. plant patent, this variety is described as a "sport discovered by the observation of its early flowering in relation to the 'Hass' avocado." While the flowering pattern of 'Carmen' is distinct from that of 'Hass' the fruit is essentially indistinguishable in taste and shape but tends to be slightly smaller than 'Hass'. Currently, the genetic differences between 'Hass' and 'Carmen' are unknown, thus it is impossible to definitively distinguish the fruit in the marketplace.

The California Avocado Commission is potentially interested in funding a two-phase research project to understand the differences between 'Hass' and 'Carmen'. Phase 1 will entail identifying the genetic markers that distinguish 'Hass' from 'Carmen'. Phase 2 will be to develop commercially viable testing procedures to be used to test and accurately identify 'Hass' and 'Carmen' fruit in the supply chain.

If you are interested in submitting a proposal to the California Avocado Commission for Phase 1 of this research, please do so via email to tim@spannag.com by 15 July 2026. Proposals should be brief, no more than 5 pages and include the following: methodology to be used, detailed budget, project timeline and milestones, summary of yours and your key team members' qualifications.

Thank you for your consideration. If you have any questions, please do not hesitate to contact me.

Dr. Spann then informed the Committee that Drs. Solares, Hormaza, van den Berg and Herrera-Estrella responded to the request by submitting a proposal.

CAC Chair Rachael Laenen then explained to the Committee that they were tasked with determining if this is something that is scientifically possible to do and, if so, are any of the proposals before them worthy of funding.

Discussion ensued and it was pointed out that it was interesting the different approaches that were taken by the different researchers. A question was raised about who would benefit from this research, would there be a benefit to the packinghouses? It was pointed out that the necessity for the grower to be able to distinguish Carmen from Hass was obvious because of the cultural differences between the varieties. Consuelo Fernandez explained that currently there is no genetic test to distinguish Carmen from Hass, the only way to distinguish the varieties from one another is by looking at the tree. It was also pointed out that Carmen is not a popular variety in California because of its winter flowering. The question was then asked, if there is not benefit to the grower then who benefits? Mr. Melban clarified for the Committee that the thinking is to be able to distinguish Carmen from Hass so that it can be established that non-Hass fruit are coming into the U.S. and try to stop it, but it is unknown who or how that would be policed.

Discussion continued with questions being raised about Mexican groves losing their export certifications if it could be proven they are not growing Hass. It was also asked if Mexico could simply get Carmen approved for import to the U.S. to circumvent the entire issue. It was recognized that separating the politics from the science is impossible on an issue like this.

Chairman Klittich asked to bring the discussion back to answering the question of whether it is scientifically possible to be able to distinguish Carmen from Hass? Discussion ensued and there was general agreement that since there are significant difference between the varieties, such as flowering habit, there should be a genetic distinction between them.

MOTION

It is scientifically feasible to find genetic markers to distinguish the Carmen variety from Hass.

(Davis/McGuire) MSC unanimous

Motion 26-7-28-2

Discussion continued, and it was pointed out that the proposal from ESB AI Lab included language about proprietary information and licensing the results of the research as well as paying for patent costs. There was general agreement that CAC should not agree to pay for research and the need to pay to license the product of that research.

Of the remaining three proposals it was believed that any of them can do the work; however, there is a large price difference among them. It was pointed out that Dr. Hormaza has worked on various projects in California in the past and knows the California industry and has the varieties available in Spain to complete the work. Dr. Spann reminded the committee that CAC is currently working with Dr. Hormaza as he is a co-PI on the artificial pollination project that CAC is currently funding. Mr. Karaoghlanian reminded the Committee that whatever project is funded, we need to be sure USDA will accept the results.

Chair Klittich asked each Committee member to express their final opinions on each proposal and indicate which, if any, they supported. Following each member sharing their final thoughts, the following motion was put forward.

MOTION

If the Board desires to fund a project on this topic, the Committee recommends the proposal submitted by Dr. Iñaki Hormaza for \$50,306; however, the support was mixed with four committee members ranking Dr. Hormaza's proposal as their top choice, one ranking Dr. Herrera-Estrella's as their top choice, one ranking Dr. van den Berg's as their top choice, one member was supportive of either of the cheapest proposals (Hormaza or van den Berg) and one member supportive of Dr. Herrera-Estrella's proposal or Dr. Homaza's proposal. Overall, support for the proposals by committee members totaled: Solares, 0; Hormaza, 6; Herrera-Estrella, 2; and van den Berg 2.

(Davis/Fernandez) MSC Unanimous

Motion 26-7-28-3

ITEM #6 RESEARCH PRIORITIES AND 2026 CALL FOR PROPOSALS

Chair Klittinch reminded the Committee that the purpose of having this item on the agenda was to solicit more feedback from the Committee, but none had been received. Discussion ensued and the high cost of water in the southern growing areas was raised.

The question was asked if there would be value in looking at water costs specifically and trying to understand how it affects grower's decision making. It was recommended that item 2 on the research priorities list be revised to specifically call out water costs. It was also recommended to list the currently and recently funded projects at the end of the request for proposals so researchers could see what has been funded and they types of projects that have been successful in receiving funding.

ADJOURN MEETING

Danny Klittich, Production Research Committee Chair, adjourned the meeting at 10:10 a.m.

Respectfully submitted,

Timothy Spann

EXHIBITS ATTACHED TO THE PERMANENT COPY OF THESE MINUTES

- EXHIBIT A July 28, 2026, Production Research Committee AB 2720 Roll Call Vote Tally Summary
- EXHIBIT B Hass avocado variety authentication: A genetic diagnostic for supply chain integrity – Dr. Edwin Solares, ESB AI Lab Corporation
- EXHIBIT C Determining specific genetic markers for 'Carmen Hass/Mendez No1' avocado – Dr. Iñaki Hormaza, La Mayora Institute of Subtropical and Mediterranean Horticulture
- EXHIBIT D Genomic Identification and Molecular Marker Development for the Unique Identification of 'Hass' and 'Carmen' Avocado – Dr. Luis Herrera-Estrella, Texas Tech University
- EXHIBIT E Discovery of Carmen Hass-specific genomic variants through whole-genome resequencing – Dr. Noëlani van den Berg, University of Pretoria

September 29, 2026

Tim Spann, Consultant
California Avocado Commission
Production Research Committee

Re: Request for extension for the project titled “Does artificial pollination improve yield of ‘Hass’ and ‘GEM’ avocado?”

Dear Tim,

On behalf of our research team, I am writing to follow-up on our earlier request for an extension for this project. We are submitting a detailed report and supporting first-cycle results for the Production Research Committee’s internal review. We request an extension to complete a third production cycle and conduct genetic paternity analysis on fruit collected during the first cycle. These activities are needed to determine whether the observed treatment responses are repeatable and whether applied pollen contributed to fertilization. We have also attached our original request for a project extension, sent on May 20, 2026. This document contains the budget request for the extension.

The first cycle established seven orchard sites and generated laboratory, flower-level, fruit retention, and harvest data. The results do not yet support a consistent recommendation. The pollen products showed weak performance in several laboratory and controlled-flower assessments, while field responses varied by orchard and measure. BioPollen was associated with higher yield at the Somis organic Hass site, but lower productivity in other settings; most comparisons showed no significant difference. Fruit drop did not differ significantly among treatments. The enclosed report presents these findings and their limitations in detail.

The second production cycle is underway with expanded flower sampling and the addition of BloomX. A third cycle would allow us to assess treatment performance across changing crop loads and orchard conditions, including alternate bearing, and would provide a second California season for evaluating BloomX. Paternity analysis of the fruit already collected would directly test whether applied pollen contributed to successful fertilization and help interpret the harvest results. The requested work focuses on field outcomes and parentage rather than repeating the mostly complete laboratory program.

We ask the Production Research Committee to review the extension request and the accompanying evidence. Our goal is to provide California growers with a defensible assessment of when, if ever, these technologies improve production. Because the report contains preliminary results and product-specific evaluations, please limit its circulation to authorized personnel involved in reviewing the project.

We would be happy to answer any questions that you or the Production Research Committee may have.

Yours sincerely,



Mary Lu Arpaia
Professor of Extension, Subtropical Horticulture
mlarpaia@ucanr.edu

Project Title: *Does artificial pollination improve yield of ‘Hass’ and ‘GEM’ avocado?*

REQUEST FOR ONE-YEAR EXTENSION OF FUNDING

Researchers: *M.F.S. dos Santos, J.I. Hormaza, M. L. Arpaia*

OVERVIEW OF RESULTS AND SCIENTIFIC JUSTIFICATION FOR EXTENSION

During the completed first production cycle (Year 1) and the ongoing second cycle (Year2), the project established a large, geographically diverse field platform and generated extensive laboratory, field, yield, and fruit samples. The completed first-cycle evaluations revealed an important scientific issue: treatment responses were not consistent across orchards or response variables, while second-cycle evaluations remain in progress. Because avocado exhibits severe alternate bearing and substantial orchard-to-orchard variation was observed, ending the study after only two cycles would create a considerable risk of making a premature recommendation for or against these technologies. A third production cycle (year) is required to distinguish consistent treatment effects from variations across years, orchards, fruit loads, and environmental conditions, in order to capture the effect of alternate bearing on the orchard. This will also provide a second season in California to evaluate BloomX, a technology that has shown promising results elsewhere.

The extension is not intended to seek additional statistical significance or to repeat laboratory evaluations that have already produced consistent results. Its purpose is to resolve the current contradiction between poor pollen performance, isolated field responses, and significant but inconsistent productivity outcomes, thereby providing CAC and California avocado growers with a scientifically defensible final recommendation.

We are requesting additional funds to 1) expand activities in Spring 2027 to repeat the application of all 3 pollen technologies; 2) collect fruit drop data in Fall 2027; 3) collect yield data in Spring 2028; and 4) conduct paternity analysis of fruit samples collected in Spring 2026 (Cycle 1) and Spring 2028 (Cycle 3).

Objective A. Establish Experimental Sites - Completed

Objective B. Standardize Applications by Bloom Development - Completed

Objective C. Pollen Viability, Germination, and Deposition

Laboratory findings (for more details refer to attached appendix)

The laboratory results consistently raise concerns about the biological quality and stability of the pollen-based products under the supplied formulations and recommended storage procedures. Across all three evaluation dates, pollen viability was significantly lower for BioPollen and Dusting than for the locally collected Control (Holm-adjusted $p \leq 0.004$). BioPollen had no viable pollen on the first date, and both products showed many zero-viability observations. In Petri-dish assays, BioPollen germination was significantly lower than the Control on all dates ($p < 0.001$). Dusting also had very low germination and many zeros, although its comparisons with the Control did not reach significance (adjusted $p = 0.091, 0.073$, and 0.062). It is important to note that established avocado pollen germination and viability protocols were developed for fresh, dry pollen rather than BioPollen, which is suspended in a liquid medium. Even after centrifugation and collection of the pollen-containing fraction, these methods appeared unsuitable for BioPollen. Its measured germination and viability may therefore not accurately reflect its actual performance.

Microscopic inspection also showed that Dusting contained abundant non-pollen particles and pollen morphologies inconsistent with avocado, while BioPollen contained less material per unit volume but fewer visible impurities. The controlled fresh-flower sponge assay provided an additional functional test: considering all flowers, BioPollen and Dusting produced significantly less pollen-tube growth than the Control in both applications (adjusted $p \leq 0.020$). Together, these evaluations indicate that the products did not consistently deliver viable, germinating avocado pollen under the tested conditions. Because the laboratory pattern was repeatedly documented, repeating the full viability and germination program in a third cycle is not scientifically necessary; extension resources in the third cycle should instead prioritize field confirmation and paternity analysis.

Field findings and need for a third cycle

Fresh flowers collected after application showed the naturally sparse pattern typical of avocado: most flowers contained no pollen and even fewer showed pollen-tube growth. Across fields, zero counts frequently exceeded 50% and reached 100% in some treatment-by-application combinations. Most comparisons showed no significant increase in pollen deposition or tube growth after BioPollen or Dusting. Isolated differences occurred - for example, greater Dusting deposition at Somis in Application 1 and greater tube growth at Santa Paula West in Application 1 - but they were not reproduced consistently across applications or orchards. In the second cycle, the flower sample was doubled and an additional treatment was included; those evaluations remain in progress. Completing field deposition and tube-growth assessment during a third cycle will determine whether isolated responses are reproducible or reflect highly variable flowering and environmental conditions.

Objective D. Fruit Retention and Tree Productivity - Essential Extension Activity

Current evidence

Fruit drop, collected in July and October, did not differ significantly among treatments in any orchard ($p > 0.05$). Productivity results, however, were heterogeneous in both direction and biological interpretation. At the Somis organic 'Hass' orchard, BioPollen increased total harvested fruit weight by 64%, fruit number by 69%, and yield per canopy volume by 62% relative to the Control ($p < 0.05$). In contrast, at Santa Paula West 'GEM', BioPollen reduced yield per canopy volume by 37% ($p < 0.05$), while changes in total harvested fruit weight and fruit number were not significant. At Fillmore 'Hass', BioPollen significantly reduced total harvested fruit weight and fruit number, but not yield per canopy volume; Dusting significantly increased yield per canopy volume without significantly changing total fruit weight or fruit number. No significant treatment differences were detected for the principal productivity measures at the remaining orchards.

This lack of agreement is the key result at the present stage. A response that appears beneficial in one orchard is absent or negative in another, and significance can disappear when production is normalized by canopy volume. Moreover, the favorable yield observations cannot yet be linked mechanistically to applied pollen because laboratory and stigma-level measurements did not demonstrate a consistent increase in viable pollen deposition or pollen-tube growth. **Any apparent response may be attributable to factors other than the applied pollen, including nutrients, hormones, or other components of the product formulations that were not controlled in this study. Overall, the first-cycle results do not provide consistent evidence of a pollen-related effect across orchards and response variables. Neither the controlled experiments nor the field trials demonstrated a consistent increase in pollen deposition or pollen-tube growth attributable to the applied products. Direct evidence that the applied pollen resulted in successful fertilization will require paternity analysis. Therefore, any positive or negative effects on productivity may reflect unidentified components of BioPollen, SureSet in the Dusting treatment, or climatic conditions that were outside the scope of this study.**

Why two production cycles are insufficient

Avocado is characterized by strong alternate bearing: a high-crop ('on') year is commonly followed by a low-crop ('off') year. Several participating orchards entered an off year during the second cycle. Consequently, two consecutive observations may largely represent opposite positions in the bearing cycle rather than a stable treatment response. With only two cycles, an orchard-specific result can be mistaken for a general effect, and a genuine but context-dependent response can be missed.

A third cycle will provide the minimum temporal replication needed to evaluate whether the direction and magnitude of treatment effects persist across changing crop loads. It will also strengthen treatment-by-environment interpretation across seven distinct orchards and reduce the probability of both false recommendation and false rejection. The extension is therefore not requested simply to increase sample size; it is required to protect the scientific validity and practical usefulness of the recommendation delivered to California avocado growers.

Objective E. Genetic/Paternity Analysis - Essential Activity During Project Extension

The requested extension funds will support paternity analysis of fruit collected during the first production cycle. This is a decisive component because the pollen applied by the technologies originates from cultivars that can be genetically distinguished from the local pollen sources. Paternity testing can therefore determine whether fruit were actually sired by the applied pollen, providing direct evidence of successful fertilization that deposition counts and yield comparisons alone cannot supply.

The value of these samples extends beyond testing the commercial products. Even if no treatment effect is confirmed, linking paternal parentage with data collected across seven environments will provide grower-relevant information on the performance of pollinizer cultivars and their possible role in fruit set and alternate bearing. With assistance from the Brokaw team, fruit weight, length, diameter, seed dimensions, and related traits were recorded for each fruit collected for this analysis. Integrating these measurements with paternity results will allow the project to evaluate whether pollen parent influences fruit and seed characteristics and to develop stronger guidance on pollinizer.

Extension Deliverables, Recommendations and Milestones

1. Complete the expanded second-cycle field evaluations and conduct a third field/production cycle across the established orchard network.
2. Prioritize pollen deposition, pollen-tube growth, fruit retention, yield, and canopy-normalized productivity; do not repeat laboratory assays that have already produced a consistent pattern unless the product formulation or recommended storage conditions change materially.
3. Complete the first-cycle genetic/paternity analysis and integrate parentage with orchard, yield, fruit, seed, and pollinizer information.
4. After the third cycle, treatments will be evaluated jointly across years and orchards, with emphasis on the consistency and direction of treatment effects, treatment-by-environment responses, and evidence of fertilization from paternity analysis. These results will support an unbiased final classification of each technology as beneficial, ineffective, detrimental, or context-dependent.

Milestones for Project Extension				
	June 2027	October 2027	June 2028	September 2028
Already Funded	1) Collection and analysis of samples for Cycle 2 paternity analysis. 2) Tree yield / canopy measurements.			
Extension	1) Apply 4 artificial pollination technologies. 2) Evaluate flowering intensity at trial sites. 3) Collect samples for pollen viability and germination for the three different technologies, quantify pollen germination in vitro and pollen germination and pollen tube-growth in vivo. Initiate examination of samples*.	1) Evaluate fruit set and fruit drop 3 months post treatment. 2) Evaluate fruit set and fruit drop 5 months post treatment. 3) Complete evaluation of microscope work on pollen deposition and pollen tube growth in response to pollination treatments.	1) Harvest trial plots and collect yield and fruit size data. 2) Conduct paternity testing to determine the percentage of origin of the fruits.	1) Compile data and prepare final report.

*To ensure the efficient use of project resources, and given the limitations of pollen viability and germination testing for BioPollen, we will conduct a single assessment of each test and place greater emphasis on pollen deposition and pollen germination on the flower (*in situ*).

Conclusion. The current results show meaningful trends, but not a coherent, repeatable treatment response. A third cycle and paternity analysis for all 3 cycles are necessary to convert these observations into defensible guidance for the industry. Extending the project will ensure that recommendations to CAC and its growers reflect biological performance across alternate-bearing cycles rather than a single orchard, year, or statistical contrast.

APPENDIX 1. CYCLE 1 PRELIMINARY DATA REPORT - OVERVIEW FOR CAC – PRODUCTION RESEARCH COMMITTEE

HIGH CONFIDENTIALITY - THIS REPORT AND THE INFORMATION IT CONTAINS IS PRELIMINARY DATA AND SHOULD NOT BE DISTRIBUTED OR SHARED BEYOND THE PRODUCTION RESEARCH COMMITTEE

Project Title: Does artificial pollination improve yield of ‘Hass’ and ‘GEM’ avocado?

Researchers: M.F.S. dos Santos, J.I. Hormaza, M. L. Arpaia

Objective A. Establish Experimental Sites

Objective A was successfully completed. Seven experimental sites were established across Ventura County, including five ‘Hass’ orchards and two ‘GEM’ orchards representing different tree ages and management systems. During the first production cycle, 50 trees per treatment were marked for Control, BioPollen, and Dusting, totaling 150 trees per site and 1,050 trees across the seven orchards. In 2026, BloomX was added, increasing the experiment to 200 trees per site and 1,400 trees overall. Plot placement and distance from pollinizer cultivars were considered to minimize potential bias.

Objective B. Standardize Applications According to Bloom Development

Objective B was successfully completed. Applications were standardized according to bloom development at each orchard. Treatments were applied at approximately the same time within each site and followed the same application procedures. When operational limitations occurred, the interval among treatments did not exceed two days.

Objective C. Evaluate Pollen Viability, Germination, and Deposition

Laboratory Evaluations

Objective C Laboratory Evaluations was successfully completed. Pollen viability and germination were evaluated at receipt and during the first and second field applications. Dusting, BioPollen, and locally collected pollen were assessed during the first production cycle. Local ‘Zutano’ or ‘GEM’ pollen and BloomX (Year 2) were included during the second cycle. A complementary controlled sponge assay using fresh avocado flowers was also conducted to evaluate pollen germination and pollen-tube growth on fresh stigmas. These protocols were developed in collaboration with J.I. Hormaza based on his research experience.

Field Evaluations

Objective C Field Evaluations (1st cycle completed). Pollen deposition and pollen-tube growth were evaluated in flowers collected approximately 22 hours after product application. More than 3,000 flowers were analyzed during the first production cycle. Second-cycle evaluations remain in progress because the sample size was doubled and an additional treatment was included. Protocols were developed in collaboration with Dr. Hormaza.

Objective D. Evaluate Fruit Retention and Tree Productivity

Objective D Field Evaluations (1st cycle completed). Fruit drop was evaluated at two stages of the production cycle (July and October). Tree height, canopy dimensions, and trunk diameter were also measured to calculate productivity relative to tree size. These measurements were collected after the flowering season. More than 600 trees are currently being analyzed. Canopy dimension data was not collected at 2 sites (Santa Paula East Hass and Moorpark Hass).

Objective E. Conduct Genetic/Paternity Analysis

The original project included paternity analysis for the second production cycle. However, the corresponding samples and fruit information were also collected during the first cycle and are being held for analysis. The requested extension would allow these samples to be analyzed and would strengthen the evaluation of the pollination technologies across production cycles.

A summary of the statistical methods used in data analysis can be found on page 19.

Abbreviations Used in Summary of Results (in order of appearance)

Term	Definition
FDA	Fluorescein diacetate; stain used to assess pollen viability.
p-value (p)	Probability, under the null hypothesis, of obtaining a result at least as extreme as the observed result.
Holm-adjusted p-value	p-value adjusted for multiple comparisons using the Holm procedure.
OR	Odds ratio.
CI	Confidence interval; 95% confidence intervals are reported.
*, **, ***	Statistical significance levels: $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.
ns	Not statistically significant ($p \geq 0.05$).
SD	Standard deviation.
SE	Standard error.
Tukey-adjusted p-value	p-value adjusted for multiple pairwise comparisons using the Tukey method.

Significance symbols refer to adjusted p-values where a multiple-comparison adjustment was applied.

SUMMARY OF RESULTS

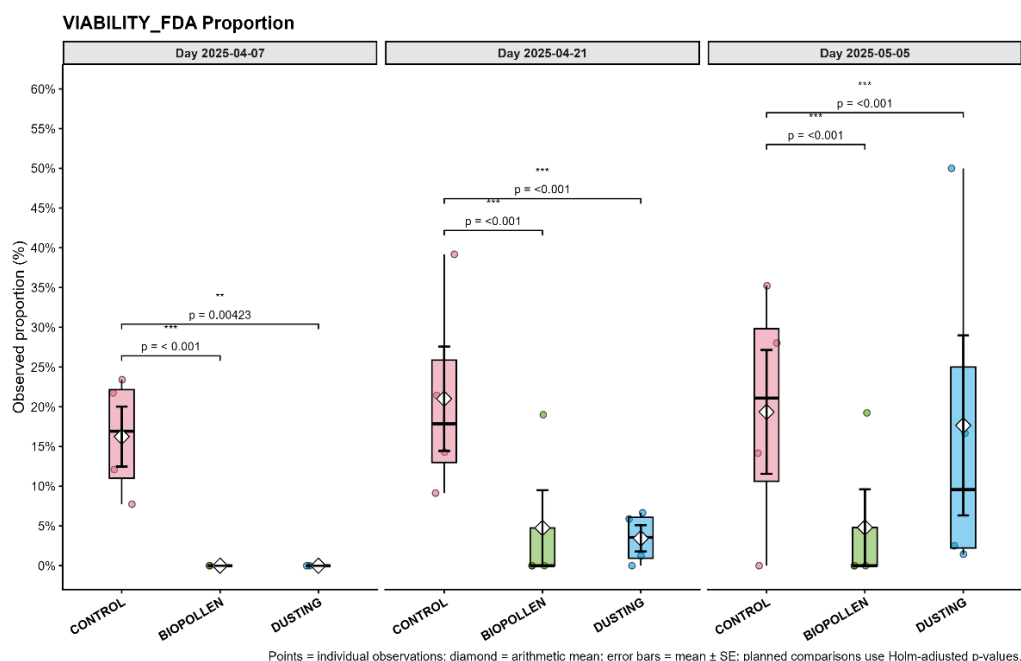
OBJECTIVE C. EVALUATE POLLEN VIABILITY, GERMINATION, AND DEPOSITION

LABORATORY EVALUATIONS

How to read the plots in the following charts regarding pollen: Each dot represents one observation in the sample. The box inside contains the middle 50% of the observations, and the line within it marks the median. The white diamond indicates the mean. The vertical whiskers show the spread of values beyond the box.

POLLEN VIABILITY (FDA)

Pollen viability was significantly lower in the BioPollen and Dusting treatments than in the Control on all three evaluation dates (Holm-adjusted $p \leq 0.004$). BioPollen showed no viable pollen on the first date, while both artificial-pollination treatments had a high frequency of zero-viability observations across the study.



Pollen viability (FDA) by treatment and application date

Treatment	Success/Total	Observed viability (%)	OR (95% CI) vs Control	Holm-adjusted p	Significance
Control	121/708	17.09%	Reference	-	-
BioPollen	0/1,104	0.00%	0.002 (0.0001-0.035)	<0.001	***
Dusting	0/142	0.00%	0.017 (0.001-0.277)	0.004	**
Control	165/809	20.40%	Reference	-	-
BioPollen	76/1,137	6.68%	0.281 (0.210-0.374)	<0.001	***
Dusting	6/228	2.63%	0.114 (0.051-0.253)	<0.001	***
Control	148/804	18.41%	Reference	-	-
BioPollen	50/1,038	4.82%	0.226 (0.162-0.316)	<0.001	***
Dusting	17/235	7.23%	0.354 (0.211-0.595)	<0.001	***

Observed percentage = pooled successes / pooled total. OR < 1 indicates lower odds than Control. P-values are Holm-adjusted within each date; ns = not significant.

POLLEN GERMINATION

Pollen germination was significantly lower for BioPollen than for the Control on all three dates (Holm-adjusted $p < 0.001$). Dusting also showed very low germination and many zero observations, but differences from the Control were not statistically significant ($p = 0.091, 0.073$, and 0.062).

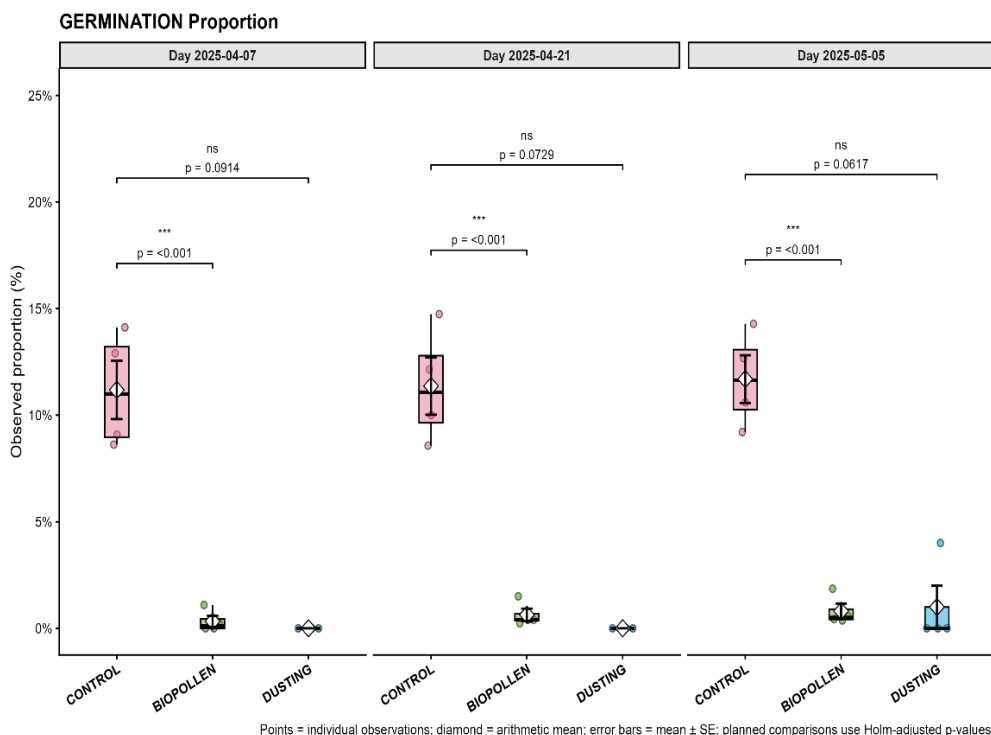


Table 1. Pollen germination by treatment and application date

Treatment	Success/total	Observed germination (%)	OR (95% CI) vs Control	Holm-adjusted p	Significance
Control	48/432	11.11%	Reference	-	-
BioPollen	3/1,237	0.24%	0.022 (0.008-0.067)	<0.001	***
Dusting	0/45	0.00%	0.087 (0.005-1.482)	0.091	ns
Control	55/485	11.34%	Reference	-	-
BioPollen	7/1,365	0.51%	0.043 (0.020-0.093)	<0.001	***
Dusting	0/51	0.00%	0.075 (0.004-1.271)	0.073	ns
Control	62/529	11.72%	Reference	-	-
BioPollen	10/1,465	0.68%	0.054 (0.028-0.105)	<0.001	***
Dusting	1/55	1.82%	0.206 (0.039-1.081)	0.062	ns

Observed percentage = pooled successes / pooled total. OR < 1 indicates lower odds than Control. P-values are Holm-adjusted within each date; ns = not significant.

SPONGE ASSAY USING FRESH AVOCADO FLOWERS AFTER APPLICATION

POLLEN-TUBE GROWTH

Considering all flowers, BioPollen and Dusting produced significantly less pollen-tube growth than the Control in both applications (Holm-adjusted $p \leq 0.020$). Many flowers had no deposited pollen and were therefore recorded as 0%. Among flowers where pollen was deposited, both treatments were significantly lower than the Control in Application 1. In Application 2, Dusting remained significantly lower ($p < 0.001$), whereas BioPollen was not significantly different ($p = 0.917$).

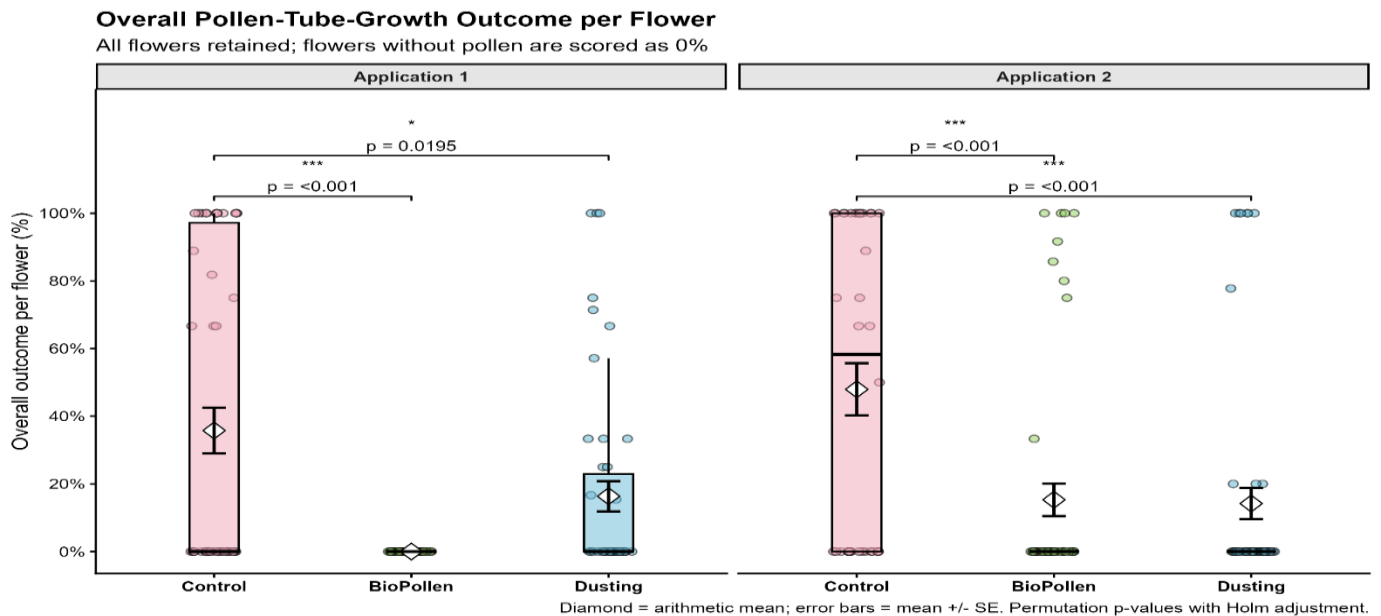


Table 3. Overall pollen-tube-growth outcome per flower (All Sites)

Application	Treatment	Flowers (n)	Without pollen (n)	Mean overall outcome (%)	Difference vs Control (percentage points)	Holm-adjusted p	Significance
Application 1	Control	46	26	35.78%	Reference	-	-
Application 1	BioPollen	43	28	0.00%	-35.78	<0.001	***
Application 1	Dusting	46	11	16.35%	-19.42	0.020	*
Application 2	Control	38	14	47.95%	Reference	-	-
Application 2	BioPollen	50	41	15.31%	-32.64	<0.001	***
Application 2	Dusting	52	18	14.19%	-33.77	<0.001	***

All flowers were retained. Flowers with no pollen were assigned an overall outcome of 0%. Differences are treatment minus Control; p-values are permutation tests adjusted by Holm within each application.

Table 4. Pollen tube-growth proportion among deposited pollen (All Sites)

Application	Treatment	Tube growth/ total pollen	Observed tube growth (%)	OR (95% CI) vs Control	Holm-adjusted p	Significance
Application 1	Control	41/50	82.00%	Reference	-	-
Application 1	BioPollen	0/209	0.00%	0.0005 (0.0003-0.010)	<0.001	***
Application 1	Dusting	29/160	18.13%	0.051 (0.023-0.116)	<0.001	***
Application 2	Control	46/56	82.14%	Reference	-	-
Application 2	BioPollen	30/36	83.33%	1.060 (0.355-3.159)	0.917	ns
Application 2	Dusting	28/154	18.18%	0.051 (0.023-0.112)	<0.001	***

This secondary table includes only flowers with deposited pollen. OR < 1 indicates lower odds than Control. Comparisons use separation-safe bias-reduced logistic regression with Holm adjustment.

FIELD EVALUATIONS (FLOWERS COLLECTED 22 HOURS AFTER APPLICATION)

FILLMORE HASS

No significant differences among treatments were detected for total pollen or pollen-tube growth in either application ($p > 0.05$). All treatments had a high frequency of flowers with zero counts (54.8-90.6%).

Table 1. Total pollen - Fillmore Hass, Applications 1 and 2

APP	Treatment	Zero flowers, n/N (%)	Observed mean	Observed SD	Estimated mean	Model SE	95% CI lower	95% CI upper
1	Control	35/54 (64.8%)	1.63	4.04	1.63	0.65	0.75	3.55
1	BioPollen	44/53 (83.0%)	0.55	1.84	0.55	0.23	0.24	1.27
1	Dusting	46/54 (85.2%)	0.57	1.78	0.57	0.24	0.25	1.31
2	Control	47/54 (87.0%)	0.48	1.58	2.38	1.51	0.69	8.25
2	BioPollen	40/53 (75.5%)	0.47	1.05	0.78	0.51	0.22	2.80
2	Dusting	45/54 (83.3%)	0.76	2.70	3.15	1.78	1.04	9.52

Tukey-adjusted pairwise comparisons

APP	Comparison	Adjusted p-value	Significance
1	Control - BioPollen	0.148	ns
1	Control - Dusting	0.170	ns
1	BioPollen - Dusting	0.997	ns
2	Control - BioPollen	0.211	ns
2	Control - Dusting	0.904	ns
2	BioPollen - Dusting	0.062	ns

Table 2. Total pollen tube growth - Fillmore Hass, Applications 1 and 2

APP	Treatment	Zero flowers, n/N (%)	Observed mean	Observed SD	Estimated mean	Model SE	95% CI lower	95% CI upper
1	Control	36/54 (66.7%)	1.48	3.62	1.13	2.41	0.02	74.52
1	BioPollen	48/53 (90.6%)	0.43	1.81	1.19	2.66	0.01	96.41
1	Dusting	48/54 (88.9%)	0.50	1.69	1.15	2.55	0.01	89.36
2	Control	48/54 (88.9%)	0.44	1.55	2.96	1.61	1.02	8.60
2	BioPollen	41/53 (77.4%)	0.43	0.97	0.90	0.50	0.31	2.65
2	Dusting	45/54 (83.3%)	0.74	2.58	3.40	1.55	1.39	8.33

Tukey-adjusted pairwise comparisons

APP	Comparison	Adjusted p-value	Significance
1	Control - BioPollen	0.998	ns
1	Control - Dusting	1.000	ns
1	BioPollen - Dusting	1.000	ns
2	Control - BioPollen	0.154	ns
2	Control - Dusting	0.972	ns
2	BioPollen - Dusting	0.057	ns

Observed mean and SD are arithmetic summaries of all flowers, including zeros. Estimated mean, model SE and 95% CI come from the selected count model. ns = not significant (Tukey-adjusted $p \geq 0.05$).

SANTA PAULA NORTH GEM

No significant differences among treatments were detected for total pollen or pollen-tube growth in either application ($p > 0.05$). A high frequency of flowers with zero counts was observed, particularly for pollen-tube growth (84.4-90.6%).

Table 1. Total pollen - Santa Paula North GEM, Applications 1 and 2

APP	Treatment	Zero flowers, n/N (%)	Observed mean	Observed SD	Estimated mean	Model SE	95% CI lower	95% CI upper
1	Control	21/39 (53.8%)	2.10	3.34	2.10	0.66	1.14	3.88
1	BioPollen	28/54 (51.9%)	2.94	5.06	2.94	0.77	1.77	4.91
1	Dusting	20/44 (45.5%)	3.52	8.75	3.52	1.01	2.01	6.18
2	Control	49/54 (90.7%)	0.20	0.88	0.20	0.10	0.08	0.51
2	BioPollen	45/54 (83.3%)	0.41	1.21	0.41	0.17	0.18	0.93
2	Dusting	44/53 (83.0%)	0.26	0.62	0.26	0.12	0.11	0.64

Tukey-adjusted pairwise comparisons

APP	Comparison	Adjusted p-value	Significance
1	Control - BioPollen	0.686	ns
1	Control - Dusting	0.443	ns
1	BioPollen - Dusting	0.889	ns
2	Control - BioPollen	0.518	ns
2	Control - Dusting	0.917	ns
2	BioPollen - Dusting	0.764	ns

Table 2. Total pollen tube growth - Santa Paula North GEM, Applications 1 and 2

APP	Treatment	Zero flowers, n/N (%)	Observed mean	Observed SD	Estimated mean	Model SE	95% CI lower	95% CI upper
1	Control	34/39 (87.2%)	0.18	0.51	0.18	0.12	0.05	0.69
1	BioPollen	47/54 (87.0%)	0.24	0.73	0.24	0.14	0.08	0.73
1	Dusting	38/44 (86.4%)	1.27	6.51	1.27	0.71	0.43	3.81
2	Control	51/54 (94.4%)	0.15	0.83	0.15	0.08	0.05	0.43
2	BioPollen	48/54 (88.9%)	0.17	0.54	0.17	0.09	0.06	0.47
2	Dusting	48/53 (90.6%)	0.11	0.38	0.11	0.07	0.04	0.36

Tukey-adjusted pairwise comparisons

APP	Comparison	Adjusted p-value	Significance
1	Control - BioPollen	0.942	ns
1	Control - Dusting	0.070	ns
1	BioPollen - Dusting	0.090	ns
2	Control - BioPollen	0.987	ns
2	Control - Dusting	0.939	ns
2	BioPollen - Dusting	0.876	ns

Observed mean and SD are arithmetic summaries of all flowers, including zeros. Estimated mean, model SE and 95% CI come from the selected count model. ns = not significant (Tukey-adjusted $p \geq 0.05$).

SANTA PAULA NORTH HASS

No significant differences among treatments were detected for total pollen or pollen-tube growth in either application ($p > 0.05$). Zero counts were extremely frequent, especially for pollen-tube growth (90.7-100%).

Table 1. Total pollen - Santa Paula North Hass, Applications 1 and 2

APP	Treatment	Zero flowers, n/N (%)	Observed mean	Observed SD	Estimated mean	Model SE	95% CI lower	95% CI upper
1	Control	44/57 (77.2%)	0.81	1.78	0.81	0.27	0.42	1.56
1	BioPollen	54/54 (100.0%)	0.00	0.00	0.00	0.00	0.00	NA
1	Dusting	39/54 (72.2%)	0.67	1.29	0.67	0.23	0.33	1.33
2	Control	54/57 (94.7%)	0.05	0.23	0.05	0.03	0.02	0.16
2	BioPollen	51/54 (94.4%)	0.07	0.33	0.07	0.04	0.03	0.20
2	Dusting	48/54 (88.9%)	0.13	0.39	0.13	0.05	0.06	0.27

Tukey-adjusted pairwise comparisons

AP P	Comparison	Adjusted p-value	Significance
1	Control - BioPollen	1.000	ns
1	Control - Dusting	0.918	ns
1	BioPollen - Dusting	1.000	ns
2	Control - BioPollen	0.896	ns
2	Control - Dusting	0.392	ns
2	BioPollen - Dusting	0.645	ns

Table 2. Total pollen tube growth - Santa Paula North Hass, Applications 1 and 2

AP P	Treatment	Zero flowers, n/N (%)	Observed mean	Observed SD	Estimated mean	Model SE	95% CI lower	95% CI upper
1	Control	54/57 (94.7%)	0.09	0.43	0.09	0.06	0.03	0.30
1	BioPollen	54/54 (100.0%)	0.00	0.00	0.00	0.00	0.00	NA
1	Dusting	52/54 (96.3%)	0.04	0.19	0.04	0.03	0.01	0.19
2	Control	56/57 (98.2%)	0.02	0.13	0.02	0.02	0.00	0.12
2	BioPollen	51/54 (94.4%)	0.06	0.23	0.06	0.03	0.02	0.17
2	Dusting	49/54 (90.7%)	0.09	0.29	0.09	0.04	0.04	0.22

Tukey-adjusted pairwise comparisons

APP	Comparison	Adjusted p-value	Significance
1	Control - BioPollen	1.000	ns
1	Control - Dusting	0.693	ns
1	BioPollen - Dusting	1.000	ns
2	Control - BioPollen	0.578	ns
2	Control - Dusting	0.282	ns
2	BioPollen - Dusting	0.764	ns

Observed mean and SD are arithmetic summaries of all flowers, including zeros. Estimated mean, model SE and 95% CI come from the selected count model. ns = not significant (Tukey-adjusted $p \geq 0.05$).

SANTA PAULA WEST GEM

No significant differences were detected for total pollen. Dusting had greater pollen-tube growth than the Control in Application 1 ($p = 0.044$), despite the very high frequency of zero counts (83.3-98.1%).

Table 1. Total pollen - Santa Paula West GEM, Applications 1 and 2

APP	Treatment	Zero flowers, n/N (%)	Observed mean	Observed SD	Estimated mean	Model SE	95% CI lower	95% CI upper
1	Control	52/54 (96.3%)	0.07	0.38	0.07	0.07	0.01	0.41
1	BioPollen	50/54 (92.6%)	0.56	3.41	0.56	0.41	0.13	2.39
1	Dusting	47/54 (87.0%)	0.83	3.02	0.83	0.61	0.20	3.54
2	Control	45/54 (83.3%)	0.33	0.89	0.33	0.13	0.16	0.72
2	BioPollen	37/53 (69.8%)	0.57	1.20	0.57	0.21	0.28	1.15
2	Dusting	41/54 (75.9%)	0.93	2.96	0.93	0.32	0.47	1.81

Tukey-adjusted pairwise comparisons

APP	Comparison	Adjusted p-value	Significance
1	Control - BioPollen	0.187	ns
1	Control - Dusting	0.088	ns
1	BioPollen - Dusting	0.921	ns
2	Control - BioPollen	0.580	ns
2	Control - Dusting	0.119	ns
2	BioPollen - Dusting	0.584	ns

Table 2. Total pollen tube growth - Santa Paula West GEM, Applications 1 and 2

APP	Treatment	Zero flowers, n/N (%)	Observed mean	Observed SD	Estimated mean	Model SE	95% CI lower	95% CI upper
1	Control	53/54 (98.1%)	0.04	0.27	0.04	0.04	0.01	0.24
1	BioPollen	50/54 (92.6%)	0.28	1.52	0.28	0.19	0.07	1.09
1	Dusting	47/54 (87.0%)	0.61	2.24	0.61	0.41	0.16	2.28
2	Control	51/54 (94.4%)	0.09	0.45	0.09	0.05	0.03	0.28
2	BioPollen	41/53 (77.4%)	0.42	0.97	0.42	0.17	0.19	0.91
2	Dusting	46/54 (85.2%)	0.35	1.14	0.35	0.14	0.16	0.78

Tukey-adjusted pairwise comparisons

APP	Comparison	Adjusted p-value	Significance
1	Control - BioPollen	0.205	ns
1	Control - Dusting	0.044	*
1	BioPollen - Dusting	0.694	ns
2	Control - BioPollen	0.073	ns
2	Control - Dusting	0.129	ns
2	BioPollen - Dusting	0.954	ns

Observed mean and SD are arithmetic summaries of all flowers, including zeros. Estimated mean, model SE and 95% CI come from the selected count model. ns = not significant (Tukey-adjusted $p \geq 0.05$).

SOMIS HASS

Dusting had greater total pollen than Control and BioPollen in Application 1 ($p \leq 0.010$); no differences occurred in Application 2. For pollen-tube growth, only Dusting versus BioPollen differed in Application 1 ($p < 0.001$), with zero counts ranging from 56% to 96%.

Table 1. Total pollen - Somis Hass, Applications 1 and 2

APP	Treatment	Zero flowers, n/N (%)	Observed mean	Observed SD	Estimated mean	Model SE	95% CI lower	95% CI upper
1	Control	40/50 (80.0%)	0.58	1.81	0.58	0.22	0.27	1.24
1	BioPollen	39/50 (78.0%)	0.44	1.09	0.44	0.18	0.20	0.96
1	Dusting	31/50 (62.0%)	2.64	6.87	2.64	0.92	1.33	5.24
2	Control	42/50 (84.0%)	0.80	2.07	4.97	0.80	3.62	6.81
2	BioPollen	46/50 (92.0%)	0.22	0.84	2.53	0.86	1.30	4.94
2	Dusting	39/50 (78.0%)	0.76	1.60	3.33	0.58	2.37	4.68

Tukey-adjusted pairwise comparisons

APP	Comparison	Adjusted p-value	Significance
1	Control - BioPollen	0.873	ns
1	Control - Dusting	0.010	*
1	BioPollen - Dusting	0.002	**
2	Control - BioPollen	0.174	ns
2	Control - Dusting	0.210	ns
2	BioPollen - Dusting	0.753	ns

Table 2. Total pollen tube growth - Somis Hass, Applications 1 and 2

APP	Treatment	Zero flowers, n/N (%)	Observed mean	Observed SD	Estimated mean	Model SE	95% CI lower	95% CI upper
1	Control	41/50 (82.0%)	0.44	1.54	0.44	0.18	0.20	0.98
1	BioPollen	46/50 (92.0%)	0.12	0.48	0.12	0.06	0.04	0.34
1	Dusting	33/50 (66.0%)	1.52	3.80	1.52	0.56	0.74	3.13
2	Control	46/50 (92.0%)	0.08	0.27	0.08	0.04	0.03	0.21
2	BioPollen	49/50 (98.0%)	0.02	0.14	0.02	0.02	0.00	0.14
2	Dusting	48/50 (96.0%)	0.04	0.20	0.04	0.03	0.01	0.16

Tukey-adjusted pairwise comparisons

APP	Comparison	Adjusted p-value	Significance
1	Control - BioPollen	0.133	ns
1	Control - Dusting	0.063	ns
1	BioPollen - Dusting	0.000	***
2	Control - BioPollen	0.430	ns
2	Control - Dusting	0.703	ns
2	BioPollen - Dusting	0.838	ns

Observed mean and SD are arithmetic summaries of all flowers, including zeros. Estimated mean, model SE and 95% CI come from the selected count model. ns = not significant (Tukey-adjusted $p \geq 0.05$).

OBJECTIVE D. EVALUATE FRUIT RETENTION AND TREE PRODUCTIVITY

FRUIT DROP/RETENTION

No significant differences in fruit drop were detected among treatments in any field ($p > 0.05$). Fruit drop varied among fields, but this variation was unrelated to treatment.

Fruit drop 2025 - summary of all fields

Field	Cultivar	Control mean $\pm$ SE	BioPollen mean $\pm$ SE	Dusting mean $\pm$ SE	BioPollen vs Control adjusted p	BioPollen vs Dusting adjusted p	Control vs Dusting adjusted p	Result
Santa Paula West	GEM	14.90 $\pm$ 2.86	11.00 $\pm$ 2.18	10.00 $\pm$ 2.00	0.513	0.939	0.321	ns
Santa Paula North	Hass	11.80 $\pm$ 2.34	9.40 $\pm$ 1.91	10.90 $\pm$ 2.18	0.702	0.862	0.957	ns
Santa Paula North	GEM	21.50 $\pm$ 4.51	24.50 $\pm$ 5.11	12.50 $\pm$ 2.72	0.898	0.066	0.172	ns
Fillmore	Hass	6.50 $\pm$ 1.45	11.80 $\pm$ 2.45	6.30 $\pm$ 1.42	0.124	0.101	0.995	ns
Somis	Hass	11.10 $\pm$ 2.26	13.60 $\pm$ 2.72	13.10 $\pm$ 2.63	0.757	0.990	0.831	ns

Values are negative-binomial estimated means $\pm$ model SE ($n = 10$ per treatment). P-values are Tukey-adjusted. ns = not significant at $\alpha = 0.05$. No treatment comparison was significant in any field.

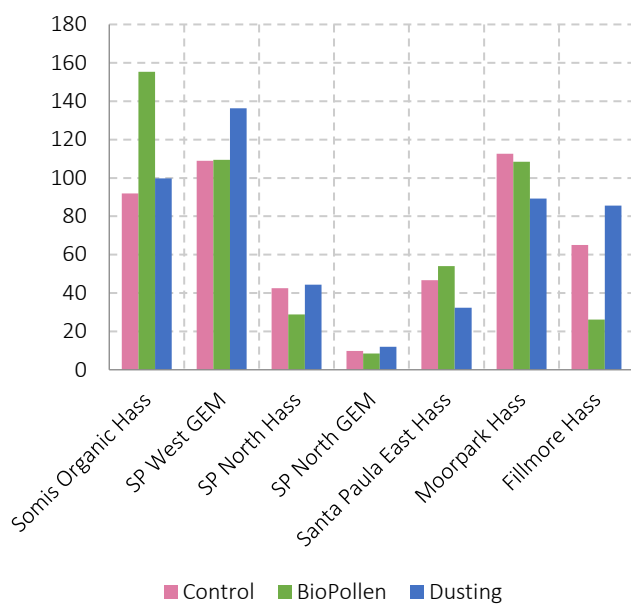
TREE PRODUCTIVITY

SUMMARY

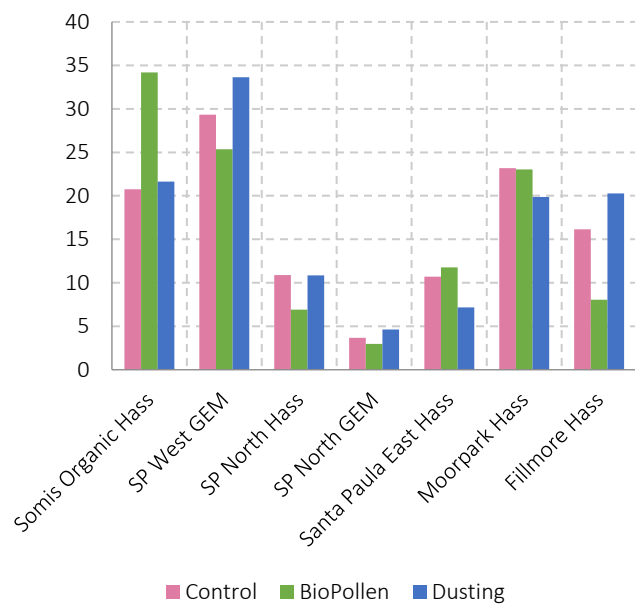
Harvest 2026 - consolidated productive indices

Field	Cultivar	Treat.	Fruits/tree	Weight per tree (kg)	Mean fruit weight (g)	Fruits/m ³	kg/m ³	Fruits/cm ² TCSA	kg/cm ² TCSA
Somis Organic	Hass	Control	91.83	20.76	226.05	12.88	2.92	1.40	0.32
Somis Organic	Hass	BioPollen	155.37	34.21	220.16	21.56	4.74	2.39	0.53
Somis Organic	Hass	Dusting	99.83	21.64	216.73	13.64	2.94	1.26	0.27
S. Paula West	GEM	Control	109.00	29.32	268.97	20.10	5.37	1.11	0.30
S. Paula West	GEM	BioPollen	109.41	25.35	231.73	14.53	3.37	1.14	0.26
S. Paula West	GEM	Dusting	136.38	33.65	246.72	19.89	4.98	1.28	0.32
S. Paula North	Hass	Control	42.60	10.88	255.32	7.33	1.86	0.76	0.19
S. Paula North	Hass	BioPollen	28.77	6.92	240.41	5.43	1.31	0.61	0.15
S. Paula North	Hass	Dusting	44.40	10.85	244.30	6.93	1.71	0.76	0.19
S. Paula North	GEM	Control	9.87	3.68	372.64	18.34	6.94	0.71	0.27
S. Paula North	GEM	BioPollen	8.40	2.96	352.82	11.51	4.08	0.62	0.22
S. Paula North	GEM	Dusting	11.93	4.63	388.16	14.21	5.60	0.72	0.28
Fillmore	Hass	Control	64.97	16.17	248.86	12.32	3.07	1.05	0.26
Fillmore	Hass	BioPollen	26.13	8.04	307.73	7.21	2.23	0.65	0.20
Fillmore	Hass	Dusting	85.53	20.29	237.17	40.46	9.56	2.23	0.53
S. Paula East	Hass	Control	46.67	10.69	229.09	EXTRA FIELD			
S. Paula East	Hass	BioPollen	54.00	11.76	217.78				
S. Paula East	Hass	Dusting	32.37	7.18	221.73				
Moorpark	Hass	Control	112.68	23.19	205.78	EXTRA FIELD			
Moorpark	Hass	BioPollen	108.50	23.03	212.22				
Moorpark	Hass	Dusting	89.32	19.89	222.65				

Fruit number per tree



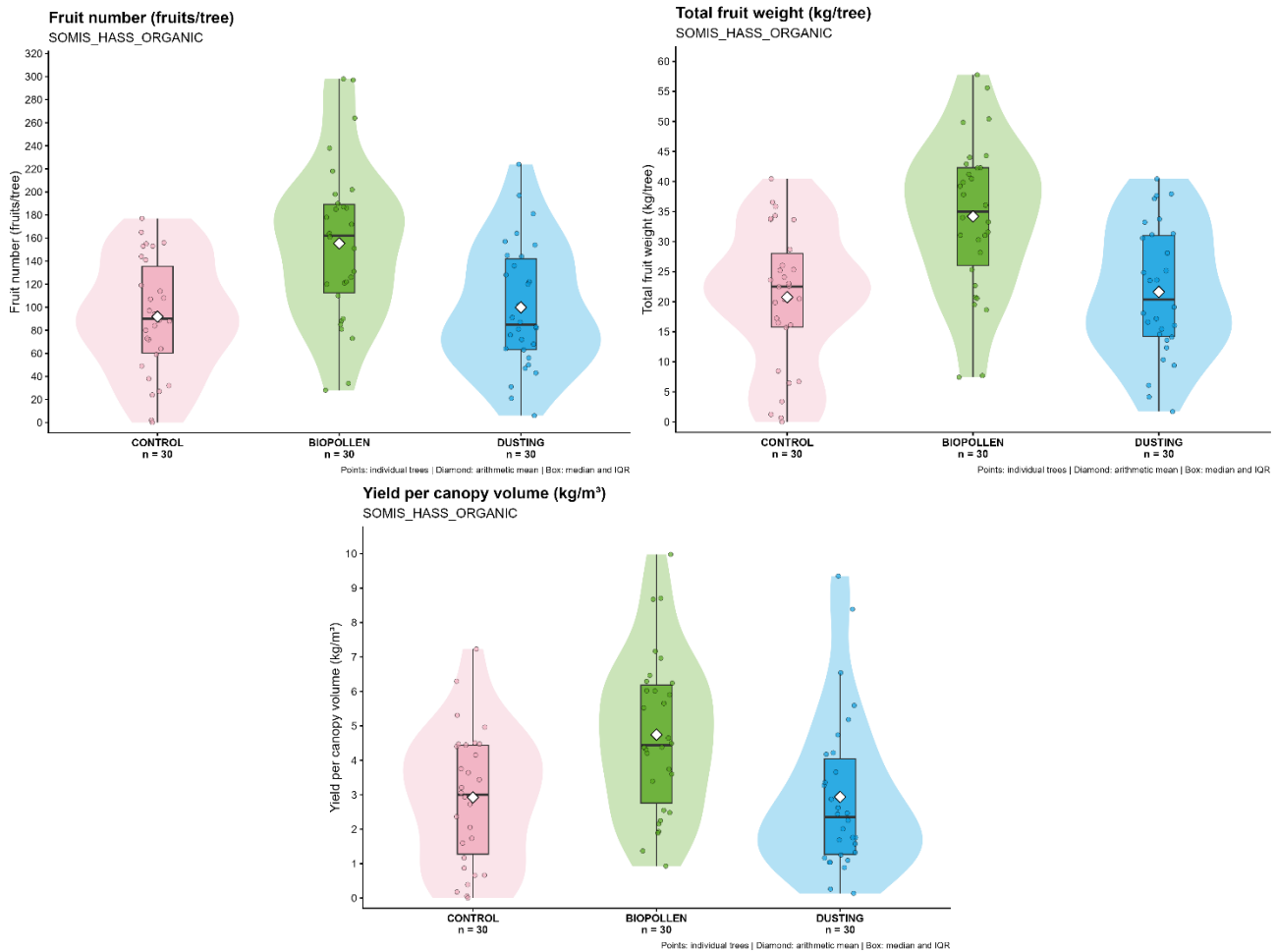
Total harvested weight per tree (kg)



How to read the plots in the following charts: These are a combination of a violin and box plot. Each dot represents one observation in the sample. The outer violin shows the shape of the data distribution: wider sections indicate more observations concentrated in that range, while narrower sections indicate fewer. The box inside contains the middle 50% of the observations, and the line within it marks the median. The white diamond indicates the mean. The vertical whiskers show the spread of values beyond the box.

SOMIS HASS ORGANIC

Compared with the Control, BioPollen increased total harvest fruit weight (kg/tree) by 64.4%, fruit number by 69.3%, and yield per canopy volume by 62.1% ($p < 0.05$). Dusting increased the kg/tree by 3.8% and fruit number by 8.7%, with no change in yield; none were significant.



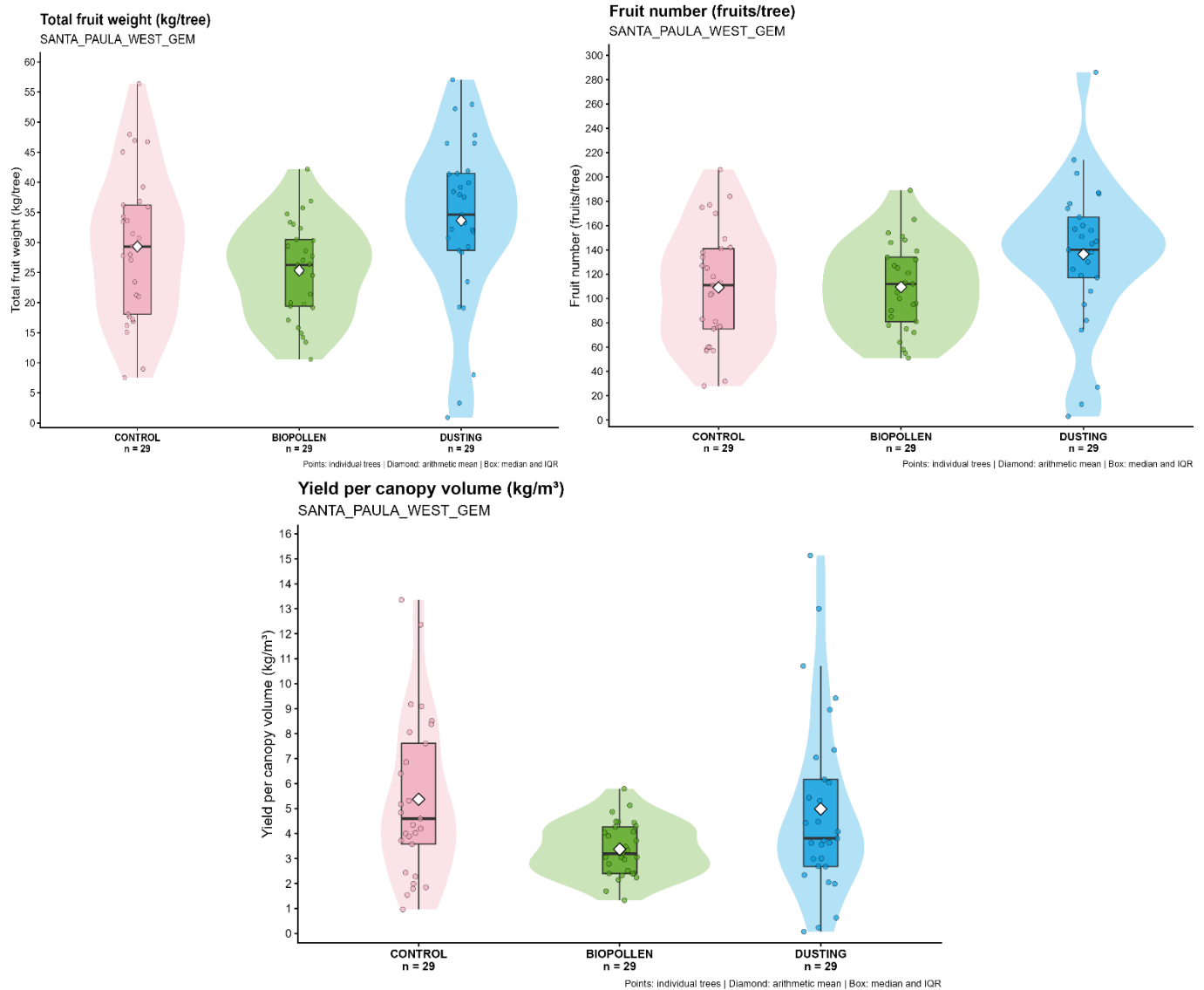
SOMIS HASS ORGANIC

Indicator	Control	BioPollen	Dusting
Total fruit weight (kg/tree)	20.8	34.2*	21.6 ns
Fruit number (fruits/tree)	91.8	155.4*	99.8 ns
Yield per canopy volume (kg/m³)	2.9	4.7*	2.9 ns

Values are treatment means. * Significant vs Control ($p < 0.05$); ns = not significant vs Control.

SANTA PAULA WEST GEM

Compared with the Control, BioPollen reduced total harvested total fruit weight (kg/tree) by 13.3% and yield per canopy volume by 37.0%, while fruit number increased by 0.4%; only the yield reduction was significant. Dusting increased the kg/tree by 14.7% and fruit number by 25.1%, while yield decreased by 7.4%; none were significant.



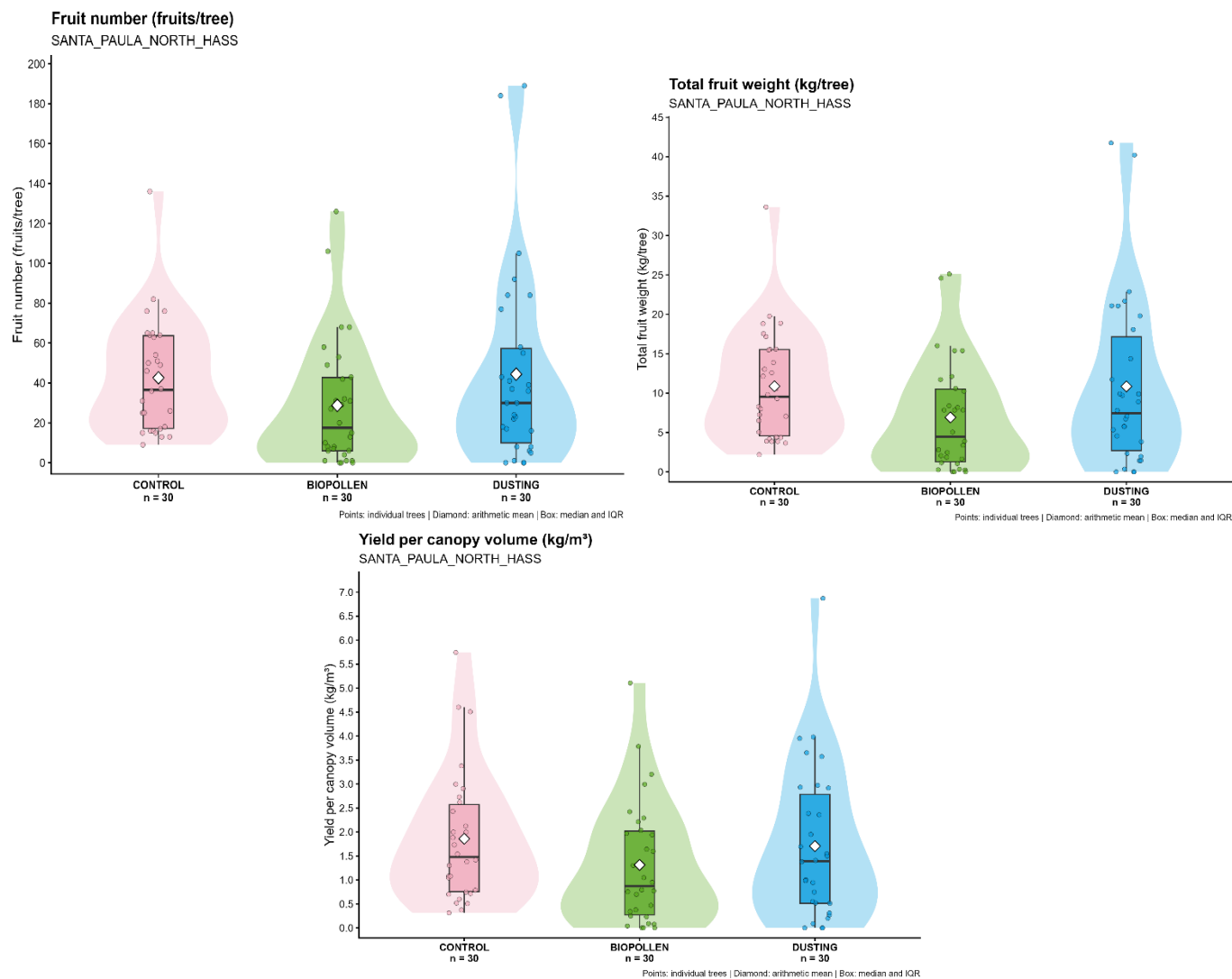
SANTA PAULA WEST GEM

Indicator	Control	BioPollen	Dusting
Total fruit weight (kg/tree)	29.3	25.4 ns	33.6 ns
Fruit number (fruits/tree)	109.0	109.4 ns	136.4 ns
Yield per canopy volume (kg/m³)	5.4	3.4*	5.0 ns

Values are treatment means. * Significant vs Control ($p < 0.05$); ns = not significant vs Control.

SANTA PAULA NORTH HASS

No significant differences were detected among treatments for total harvested fruit weight (kg/tree), fruit number, or yield per canopy volume ($p > 0.05$).



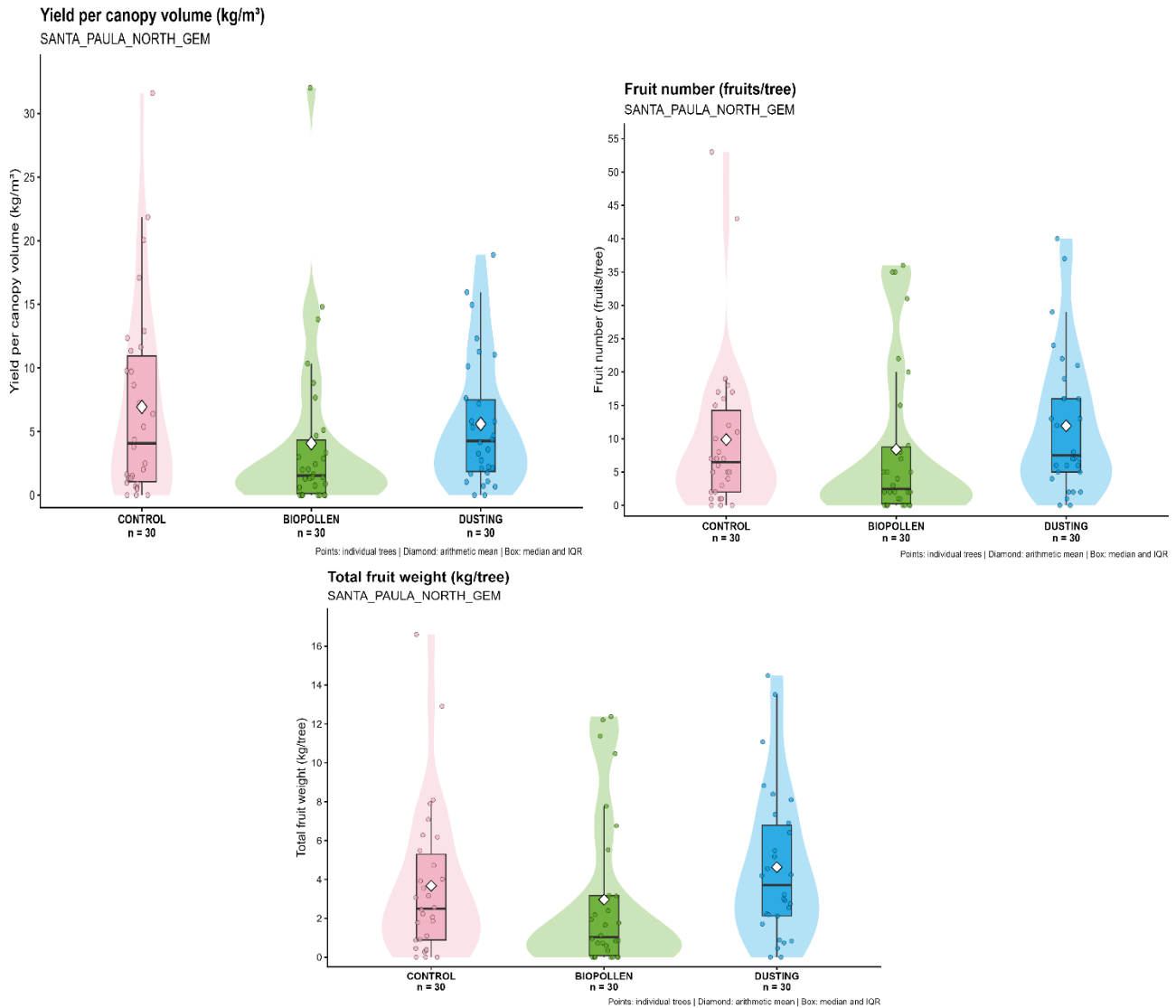
SANTA PAULA NORTH HASS

Indicator	Control	BioPollen	Dusting
Total fruit weight (kg/tree)	10.9	6.9 ns	10.8 ns
Fruit number (fruits/tree)	42.6	28.8 ns	44.4 ns
Yield per canopy volume (kg/m³)	1.9	1.3 ns	1.7 ns

Values are treatment means. * Significant vs Control ($p < 0.05$); ns = not significant vs Control.

SANTA PAULA NORTH GEM

No significant differences were detected among treatments for total harvested fruit weight (kg/tree), fruit number, or yield per canopy volume ($p > 0.05$).



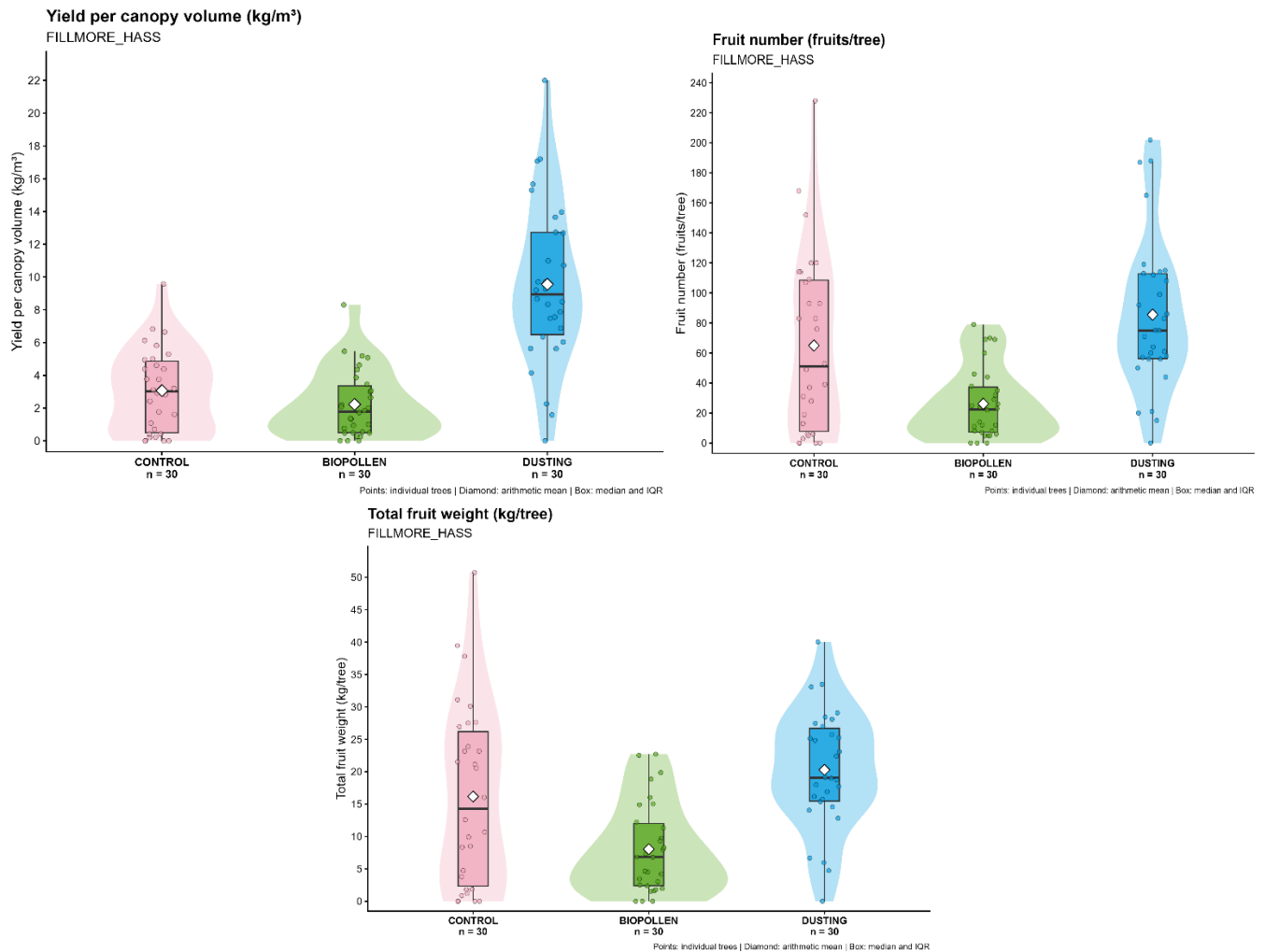
SANTA PAULA NORTH GEM

Indicator	Control	BioPollen	Dusting
Total fruit weight (kg/tree)	3.7	3.0 ns	4.6 ns
Fruit number (fruits/tree)	9.9	8.4 ns	11.9 ns
Yield per canopy volume (kg/m³)	6.9	4.1 ns	5.6 ns

Values are treatment means. * Significant vs Control ($p < 0.05$); ns = not significant vs Control.

FILLMORE

Compared with the Control, BioPollen significantly reduced total harvested fruit weight (kg/tree) and fruit number, whereas its effect on yield per canopy volume was not significant. Dusting significantly increased yield per canopy volume but did not significantly affect total fruit weight or fruit number.



FILLMORE HASS

Indicator	Control	BioPollen	Dusting
Total fruit weight (kg/tree)	16.2	8.0*	20.3 ns
Fruit number (fruits/tree)	65.0	26.1*	85.5 ns
Yield per canopy volume (kg/m³)	3.1	2.2 ns	9.6*

Values are treatment means. * Significant vs Control ($p < 0.05$); ns = not significant vs Control.

SANTA PAULA EAST HASS

No significant differences were detected among treatments for total harvested fruit weight (kg/tree) and fruit number ($p > 0.05$).



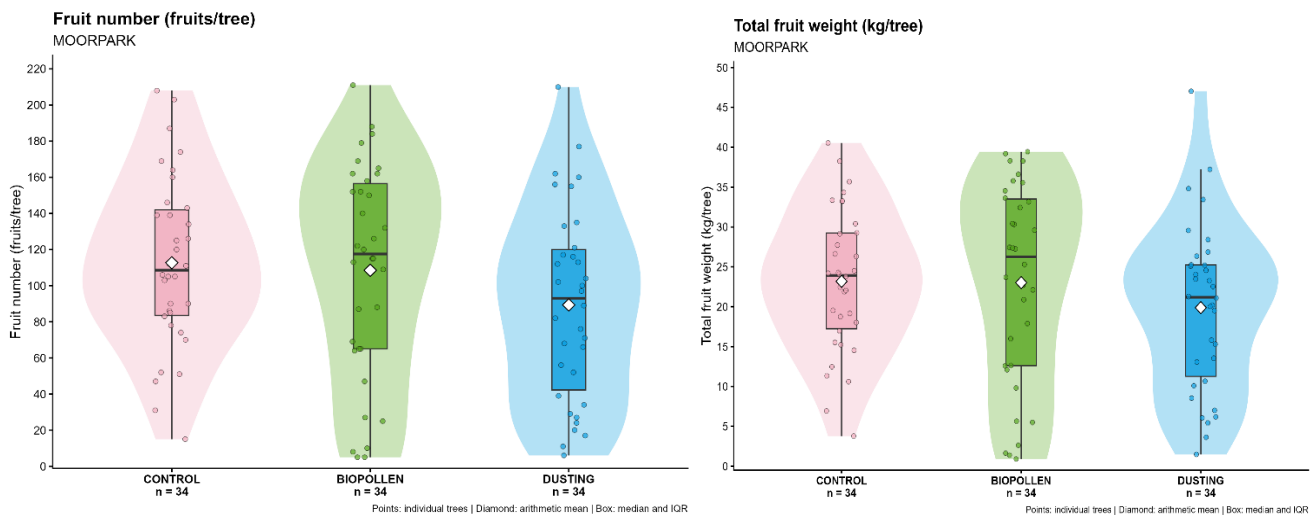
SANTA PAULA EAST HASS

Indicator	Control	BioPollen	Dusting
Total fruit weight (kg/tree)	10.7	11.8 ns	7.2 ns
Fruit number (fruits/tree)	46.7	54.0 ns	32.4 ns

Values are treatment means. ns = not significant vs Control.

MOORPARK HASS

No significant differences were detected among treatments for total harvested fruit weight (kg/tree) and fruit number ($p > 0.05$).



MOORPARK HASS

Indicator	Control	BioPollen	Dusting
Total fruit weight (kg/tree)	23.2	23.0 ns	19.9 ns
Fruit number (fruits/tree)	112.7	108.5 ns	89.3 ns

Values are treatment means. ns = not significant vs Control.

STATISTICAL APPROACH

1. Pollen viability assessed by FDA staining

Pollen viability was analyzed separately for each evaluation day. The number of viable pollen grains was modeled relative to the total number of pollen grains evaluated. Overall treatment effects within each day were assessed using beta-binomial regression and likelihood-ratio tests. The beta-binomial distribution was used to account for proportional count data and additional variability among observations. Planned comparisons between each experimental treatment and the control available on the same day were performed using bias-reduced binomial logistic regression, which provided finite estimates when treatment groups contained no viable pollen. Treatment effects were expressed as odds ratios with 95% confidence intervals. P-values were adjusted across the planned comparisons within each day using the Holm procedure. Observed zeros were retained without modification, and statistical significance was assessed at $\alpha = 0.05$.

2. Pollen germination in Petri-dish assays

Pollen germination was analyzed separately for each evaluation day. The number of germinated pollen grains, defined by pollen-tube growth, was modeled relative to the total number of pollen grains evaluated. Overall treatment effects within each day were assessed using beta-binomial regression and likelihood-ratio tests. The beta-binomial distribution was used to account for proportional count data and additional variability among observations. Planned comparisons between each experimental treatment and the control available on the same day were performed using bias-reduced binomial logistic regression, which provided finite estimates when treatment groups contained zero germinated pollen grains. Treatment effects were expressed as odds ratios with 95% confidence intervals. P-values were adjusted across the planned comparisons within each day using the Holm procedure. Observed zeros were retained without modification, and statistical significance was assessed at $\alpha = 0.05$.

3. Pollen-tube growth in the controlled sponge assay - overall flower-level outcome

The overall pollen-tube-growth outcome was analyzed separately for each application at the flower level. All flowers were retained, and flowers without deposited pollen were assigned an overall outcome of 0%. For flowers with pollen, the outcome was calculated as the number of pollen grains producing tubes divided by the total number of deposited pollen grains. Because the resulting proportions contained many zeros and showed a strongly non-normal distribution, treatment effects were evaluated using permutation tests with 99,999 permutations. A global permutation test was followed by planned comparisons of BioPollen and Dusting against the Control. P-values from these comparisons were adjusted within each application using the Holm procedure. No pseudocounts were added, and statistical significance was assessed at $\alpha = 0.05$.

4. Sponge assay - conditional tube-growth proportion

The conditional pollen-tube-growth analysis included only flowers on which at least one pollen grain was detected. The number of pollen grains producing tubes was modeled relative to the total number of deposited pollen grains. Analyses were conducted separately for each application. Overall treatment effects were evaluated using beta-binomial regression and likelihood-ratio tests to account for additional flower-to-flower variability beyond that expected under a standard binomial distribution. Planned comparisons of BioPollen and Dusting against the Control were performed using bias-reduced binomial logistic regression to obtain finite estimates in the presence of zero-success treatment combinations. Treatment effects were expressed as odds ratios with 95% confidence intervals. P-values were adjusted within each application using the Holm procedure, and statistical significance was assessed at $\alpha = 0.05$. This conditional analysis evaluated pollen performance after deposition and was interpreted as complementary to the overall flower-level analysis.

5. Field pollen deposition and pollen-tube growth in situ

Total pollen deposition and total pollen-tube growth per flower were analyzed as count responses. Each flower was considered an independent experimental unit, and analyses were conducted separately for each orchard, application, and response variable. Poisson, negative binomial, zero-inflated negative binomial, and hurdle negative binomial models were initially fitted. Poisson dispersion was evaluated using Pearson residuals. Because the datasets exhibited overdispersion and high frequencies of zero observations, final model selection was conducted among the negative binomial, zero-inflated negative binomial, and hurdle negative binomial models using the lowest Akaike Information Criterion. All flowers, including zero-count observations, were retained. Model-estimated treatment means and standard errors were obtained on the response scale. Pairwise comparisons among Control,

BioPollen, and Dusting were adjusted using the Tukey method. Simulation-based residual diagnostics were examined for models fitted using glmmTMB. Statistical significance was assessed at $\alpha = 0.05$.

6. Fruit drop

Fruit drop was analyzed separately for each orchard and cultivar as count data. Poisson and negative binomial generalized linear models with logarithmic link functions were initially fitted. Poisson dispersion was evaluated using the ratio of the sum of squared Pearson residuals to the residual degrees of freedom, and model fit was compared using the Akaike Information Criterion. Because the data exhibited overdispersion and the negative binomial model provided the more appropriate representation of the observed variability, final inference was based on the negative binomial model. Treatment was included as a fixed categorical effect. Model-estimated treatment means and standard errors were obtained on the original count scale, and all pairwise treatment comparisons were adjusted using the Tukey method. Statistical significance was assessed at $\alpha = 0.05$.

7. Harvest, tree characteristics, and productivity indices

Harvest and vegetative variables were analyzed separately for each orchard. Individual trees were considered independent experimental units, and treatment was included as a fixed categorical effect. Replicate numbers identified individual trees and were not included as blocks. Fruit number per tree was analyzed using a negative binomial generalized linear model with a logarithmic link because it is count data with overdispersion. All other variables-tree dimensions, trunk diameter, total fruit weight, canopy volume, fruit and yield efficiency per canopy volume, trunk cross-sectional area, and production efficiency per trunk cross-sectional area-were analyzed using linear models with HC3 heteroscedasticity-robust covariance estimates. Planned comparisons of each treatment against the Control used Dunnett-type multivariate-t adjustment, while comparisons among all treatment pairs used Tukey adjustment. Global treatment-effect p-values were additionally adjusted across response variables using the Benjamini-Hochberg procedure. Zeros were retained, missing values were excluded separately for each response, and no transformations, pseudocounts, or automatic outlier removal were applied. Statistical significance was assessed at $\alpha = 0.05$.

Marllon Fernando Soares dos Santos

Postdoctoral Researcher

University of California, Riverside

Riverside, California

September 28, 2026

Request for One-Year Project Extension

Project contract number: 65325-00-000

Project title: *Does artificial pollination improve yield of 'Hass' and 'GEM' avocado?*

Project start date: January 2025

Revised Project end date: September 2028

Project Leader: Mary Lu Arpaia

Position Title: Professor of Extension

Research Institution: University of California, Riverside, Department of Botany and Plant Sciences, 900 University Ave, Riverside, CA 92521

Primary Telephone Contact Number: 559-288-8507 (cell)

E-mail Address: mlarpaia@ucanr.edu

Collaborators: Inaki Hormaza, Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM La Mayora- CSIC-UMA, Avda Dr. Wienberg s/n. 29750 Algarrobo, Málaga – Spain

Marllon Fernando Soares dos Santos, University of California, Riverside, Department of Botany and Plant Sciences, 900 University Ave, Riverside, CA 92521

Justification for 1-Year Extension (07/01/2027 – 06/30/2028)

The current funded project has generated the most extensive datasets currently available under commercial California avocado production systems involving artificial pollination technologies, flowering dynamics, fruit set, and yield responses under highly heterogeneous orchard conditions. Across the 2025 and 2026 seasons, the project expanded from the originally proposed four orchards to seven commercial orchards distributed across Ventura County to better capture the substantial environmental and biological variability naturally present in avocado production systems.

This expansion was necessary because avocado production is strongly influenced by multiple interacting factors that cannot be fully controlled experimentally under commercial conditions. These include orchard age, rootstock-scion combinations, local management practices, climatic variability, and flowering behavior. The orchards included in the project range from approximately 3- to 6-year-old trees and involve multiple rootstocks, including Dusa, Duke 7, and Toro Canyon. Furthermore, the project includes both 'Hass' and 'GEM' varieties, allowing broader evaluation across commercially important cultivars, although responses may still differ in additional avocado varieties not evaluated in the current study.

Ventura County itself presents substantial microclimatic variation. Temperature differences exceeding 18°F may occur between orchards located a few miles apart, directly affecting flowering synchronization, pollen viability, pollinator activity, fruit set, and final yield responses. In addition, each orchard presents unique local management practices involving fertilization

programs, irrigation management, pruning history, pest control programs, and other cultural practices that may substantially influence productivity and treatment responses.

One of the major scientific challenges associated with avocado research is the naturally high alternate bearing behavior of the crop together with its strong interaction with climatic events such as El Niño and La Niña. These climatic conditions may shift flowering timing by up to one month between years as well as affecting the length of the flowering season substantially affecting overlap between male and female flower stages, fruit set dynamics, and productivity. Because of this, avocado experiments generally require multiple production cycles before robust conclusions can be made. Scientifically, evaluation across at least four harvest seasons (two cycles of on/off years) would be ideal to properly capture the natural biological variation associated with the crop.

The incorporation of the BloomX electrostatic pollination system during the 2026 season further reinforces the need for an extension of the project. Due to logistical delays associated with the current geopolitical situation involving Israel, where the company is headquartered, the BloomX equipment arrived later than originally planned. As a result, the first applications in several orchards were delayed relative to the remaining three treatments. Although the applications were ultimately standardized operationally, the delayed incorporation of the technology may influence the interpretation of the first-year BloomX results. Under these conditions, drawing definitive conclusions based on a single production season could generate scientifically misleading interpretations and potentially create unintended impacts for current commercial operations and technology adoption decisions.

The variability observed in the current dataset strongly supports the need for an additional season of evaluation which would provide the California industry with 3 years of data. Preliminary analyses from Year 1 (2025 applications) examined 639 trees and more than 11 metric tons of harvested fruit. We observed extremely high biological variability among orchards and treatments. Coefficients of variation (CV) ranged from 32.59% to 167.85% for fruit number per tree and from 31.41% to 146.01% for total fruit weight. Similarly, standard errors ranged from 4.39 to 65.72 fruits per tree and from 0.79 to 15.84 kg for total fruit weight. In practical terms, the CV reflects how variable individual trees were within each treatment at an individual location, whereas the standard error reflects the uncertainty associated with estimating the treatment average. The higher the value for both CV and SE, the greater the uncertainty in the data. Additionally, the dataset contains a substantial excess of zero values, with 5% of the trees producing no fruit during the 2026 harvest; and an additional 13% of the trees yielding less than 5 fruit per tree for a total of 18% of the 639 data trees with extremely low yield. These characteristics indicate a high heterogeneous biological response typical of avocado production systems under commercial field conditions.

Under this level of variability, interpretation based solely on arithmetic means may lead to incorrect conclusions regarding treatment effectiveness. Even with a relatively large experimental scale involving seven orchards, 639 evaluated trees, and approximately 11 tons of harvested fruit, substantial uncertainty remains due to the natural variability of the avocado production systems. Therefore, long-term sampling combined with robust statistical analyses is

essential to correctly interpret treatment responses while minimizing the risk of inaccurate recommendations.

Additionally, future paternity analyses represent a critical component of the project and will provide essential information regarding pollen donor origin and the effectiveness of each pollination technology under commercial orchard conditions. Ideally, paternity testing should also be conducted across multiple years to ensure consistency and biological reproducibility of the observed results. We collected embryo samples from all treatments for each site in 2026. Continuation for an additional year will give us 3 years of paternity results and result in a robust data set, allowing us to draw meaningful conclusions from the data.

Based on the scientific, biological, operational, and statistical considerations described above, we respectfully request a one-year extension of the project through September 2028. Data collection (yield data) should be completed by June of 2028 and we are requesting an additional 3 months to complete paternity analysis, data analysis and preparation of the final report. This will provide the California avocado industry with 3 years data regarding the BioPollen and Dusting treatments and 2 years data regarding the BloomX treatment. This additional year is essential to ensure that any future recommendations provided to growers and industry stakeholders are supported by sufficiently replicated and biologically reliable data.

Objectives of the Research:

- Evaluate the effectiveness of three artificial pollination systems in improving fruit set, increasing productivity and fruit quality.
- Measure the costs associated with using technologies.

Deliverables:

- The data from this project will assist producers in decision-making regarding the selection of the best artificial pollination technology, either as a complement to or replacement for the use of honey bees, potentially reducing the costs associated with pollination processes. Additionally, the most effective technology could serve as a valuable tool in mitigating the impacts of adverse climate conditions during California's flowering season.

REVISED Milestone Table:

The following Milestone Table outlines the activities associated with the Project and scheduled completion dates from the onset of the project. **Additional milestones are highlighted in RED and are listed chronologically in terms of completion dates.**

Milestone	Activities	Scheduled Completion	Budget
1	• Select cooperating orchards and develop the experimental design. • Apply three artificial pollination technologies • Evaluate flowering intensity at trial sites	May 2025	\$31,360

	Evaluate pollen viability and germination for the three different technologies, quantify pollen germination in vitro and pollen germination and pollen tube-growth in vivo.		
2	- Evaluate fruit set and fruit drop 3 months post treatment. - Evaluate fruit set and fruit drop 5 months post treatment	October 2025	\$31,359
Year 1 Total:			\$62,719
Year 2			
3	- Apply three artificial pollination technologies - Evaluate flowering intensity at trial sites - Evaluate pollen viability and germination for the three different technologies, quantify pollen germination in vitro and pollen germination and pollen tube-growth in vivo.	May 2026	\$31,058
4	- Evaluate fruit set and fruit drop 3 months post treatment. - Evaluate fruit set and fruit drop 5 months post treatment - Harvest trial plots and collect yield and fruit size data	October 2026	\$31,058
Year 2 Total:			\$62,116
Year 3			
5 THIS IS A NEW MILESTONE WITHIN THE CURRENT FUNDED TIMELINE	- Apply four artificial pollination technologies - Evaluate flowering intensity at trial sites - Collect samples for pollen viability and germination for the three different technologies, quantify pollen germination in vitro and pollen germination and pollen tube-growth in vivo. Initiate examination of samples.	May 2027	\$16,398
6	- Harvest trial plots and collect yield and fruit size data - Conduct paternity testing to determine the percentage of origin of the fruits.	June 2027	\$47,990
Year 3 Total			\$64,388-

Year 4 (ONE YEAR EXTENSION)			
7	- Evaluate fruit set and fruit drop 3 months post treatment. - Evaluate fruit set and fruit drop 5 months post treatment - Complete evaluation of microscope work on pollen deposition and pollen tube growth in response to pollination treatments	October 2027	\$46,543
8	- Harvest trial plots and collect yield and fruit size data - Conduct paternity testing to determine the percentage of origin of the fruits	June 2028	\$31,543
9	Compile data and prepare final report	September 2028	\$15,000
Year 4 Total			\$93,087
REVISED Total Project Budget:			\$282,310

**Project Proposal Budget
FY 2025 - 2028**

	ORIGINAL PROPOSAL			ONE YEAR EXTENSION
	Year 1 01/01/2025 – 12/31/2025	Year 2 01/01/2026 – 12/31/2026	Year 3 01/01/2027 – 06/30/2027	Year 4 07/01/2027– 09/30/2028
Salaries and Benefits				
Postdocs/Research Associates	34,605	35,746	18,463	45,452
SRAs	0	0	0	0
Lab/Field Assistance	0	0	0	0
Benefits	7,717	7,971	4,117	21,635
Supplies and Expenses	2,000	2,000		2,000
Equipment	0	0	0	0
Services (Paternity Analysis)	0	0	24,000	24,000
Travel to research sites	16,398	16,398	1,409	16,398*
Other Travel	0	2,000	0	
Annual Total Amount Requested	\$60,719	\$64,116	\$47,989	\$109,485
REVISED TOTAL AMOUNT REQUESTED	\$172,825 ORIGINAL TOTAL AMOUNT ALLOCATED			\$282,310

* See budget justification (Travel) for explanation.

Budget Justification for 1-Yr Extension covering Milestones 5,7, 8.

The requested budget for the 1-Yr Extension covers new expenses that will be incurred during the current project timetable (Milestone 5) which are necessary to complete Milestones 7 and 8. The budget for Milestone 5 (Year 3) is \$16,398. The budget for Milestones 7 and 8 is \$93,087.

Salary: Funds are requested to cover 0.50 FTE time for Assistant Project Scientist, Marllon Fernando Soares dos Santos. Dr. Soares dos Santos will continue to be responsible for the day-to-day oversight of the harvest activities across the proposed research sites during the 2028 production season, including coordination of fruit harvest, individual tree yield evaluations, fruit counting, organization of field datasets, and statistical analyses associated with the project. He will also coordinate with Dr. Hormaza on the paternity analysis workflow and integration of the production and molecular datasets. Salary projections are based on recommendations by our campus administrative officials for merit and range adjustments.

Benefits: Fringe benefits are calculated according to current University of California benefit rates and institutional recommendations associated with Assistant Project Scientist appointments.

Travel: Travel funds are requested to support field activities associated with the 2027 pollination season (Milestone 5), final experimental evaluations across seven commercial avocado orchards located throughout Ventura County, California, and harvest of the research sites (Milestones 7, 8). The funds originally allocated for travel in Year 3 (\$1,409) will be applied to the travel expenses occurred in the completion of Milestones 5, 7 and 8. Additional funds (similar to Years 1 and 2) are also requested to reflect a general increase in cost in fuel, lodging and food. We estimate that the total cost of travel for Year 3 (with extension) and Year 4 will be \$17,807.

Due to the geographic distribution of the orchards and the operational requirements associated with individual tree harvest assessments, multiple overnight trips will be required. Based on previous harvest evaluations, each orchard is expected to require approximately three field days, resulting in an estimated total of 21 field days distributed throughout the harvest season. Travel activities will include repeated round trips between Riverside and Ventura County, in addition to daily transportation between orchards and lodging locations. Lodging expenses are estimated at approximately \$1,300 to \$1,500 (room rental) per month during the harvest evaluation period, depending on seasonal availability. Per diem expenses are estimated at approximately \$65 per day to cover meals and field-related operational costs during travel periods. Additional travel expenses include UC vehicle leasing costs estimated at approximately \$109 per day, fuel expenses, and transportation between orchards. Travel is essential due to the extensive geographic distribution of the orchards, the large volume of harvested fruit and individual tree evaluations, and the time-sensitive nature of avocado harvest operations, where harvest notifications are often provided with limited advance notice.

From Satellite-Derived Canopy Temperature to Irrigation Assessment: Decision Support for California Avocado Orchards

Research Priority Topics Addressed: 15. Irrigation Planning/Management tool; 12. Precision Farming.

Principal Investigator: Ali Montazar, Irrigation and Water Management Advisor, UCCE San Diego, Riverside, and Imperial Counties; email: amontazar@ucanr.edu.

Collaborators: (1) Ben Faber, Subtropical Crops Advisor, UCCE Ventura and Santa Barbara Counties, (2) Andre Daccache, Associate Professor, Dept. of Biological and Agricultural Engineering, University of California Davis.

Introduction and Rationale

Efficient irrigation management in California avocado orchards is challenged by within-orchard variability in topography, irrigation performance, soils, salinity, root-zone water availability, and canopy condition. Irrigation decisions are commonly made at the block or orchard scale, while ET-based crop water demand, soil-moisture sensors, and plant measurements at limited locations may overlook localized differences in water status or irrigation performance.

With support from the California Avocado Commission (CAC), our current research developed and evaluated a framework for estimating actual avocado canopy temperature from Landsat thermal observations. Using satellite observations and continuous field measurements from six commercial Southern California avocado orchards, the framework achieved a cross-validated RMSE of 1.58 °C and predictive R^2 of 0.919, with strong performance across independent orchards. The associated manuscript, “*Satellite-derived canopy temperature for irrigation assessment in avocado orchards: A transferable framework with physiological validation*,” is under review in *Smart Agricultural Technology*. This work provides the foundation for extending satellite canopy-temperature estimation into field-validated irrigation assessment and decision support.

A critical gap remains. Satellite canopy temperature can identify where orchard conditions differ, but not why. Warm canopy areas may reflect inadequate or nonuniform irrigation, root-zone water limitations, excessive wetness, salinity/chloride, soil or root constraints, or plant/canopy conditions. Thermal differences may also be seasonal or transient. Interpreting warm canopies simply as water stress could lead to inappropriate irrigation decisions.

This project will analyze three years of historical Landsat observations to identify recurrent and seasonal thermal anomalies in avocado orchards. Targeted field measurements and high-resolution UAV imagery will investigate contributing factors and management relevance. Where corrective actions are implemented, follow-up measurements will assess responses. The resulting irrigation-assessment framework will help growers answer three practical questions: *Where should I look? Why is this area different? What should I evaluate or address next?*

Objectives and Deliverables

Objective 1. Identify and characterize recurrent and seasonally expressed canopy thermal anomalies across commercial avocado orchards.

Objective 2. Determine factors associated with satellite-detected anomalies by integrating irrigation performance, root-zone water availability, salinity/chloride, and plant/canopy evidence, distinguishing conditions warranting management action from those requiring further evaluation or no intervention.

Objective 3. Translate satellite detection and field diagnosis into practical irrigation-assessment and management guidance, including response evaluation where feasible.

Key deliverables. (1) Multi-year orchard thermal histories characterizing recurrent, seasonal, intermittent, and transient patterns; (2) field-validated relationships between thermal behavior and irrigation, root-zone soil water, salinity/chloride, and plant conditions; (3) orchard diagnostic cases documenting thermal patterns, likely contributing factors, diagnostic confidence, management relevance, and responses where feasible; and (4) a practical satellite-guided irrigation-assessment protocol describing where to investigate, which field measurements to prioritize, and when management attention is warranted.

Methods/Approach

Satellite thermal assessment. Ten commercial avocado orchards representing contrasting production conditions will be selected with cooperating growers to capture variability in irrigation systems, topography, soils, and management. Orchard boundaries, irrigation configuration, soil/water information, and management history will be documented. Three years of historical Landsat 8/9 and weather data will be analyzed using the previously developed and physiologically validated avocado canopy-temperature framework. Multi-temporal analysis will characterize anomaly magnitude, spatial extent, frequency, seasonal recurrence, and interannual persistence, and identify anomaly and reference zones. Thermal anomalies will be treated as screening signals requiring field diagnosis rather than direct evidence of water stress or inadequate irrigation.

Targeted field diagnosis. Satellite-identified anomaly and representative reference locations will be established within each orchard. Root-zone soil water will be monitored at multiple depths using a combination of project-funded instrumentation and existing PI laboratory equipment, with monitoring intensity tailored to orchard conditions and diagnostic needs. Orchard-specific atmospheric data will support ET-based crop-water-demand estimates. Irrigation timing, applied water, and system performance will be evaluated to identify inadequate, excessive, or nonuniform delivery. Soil and irrigation-water samples will be analyzed for salinity, chloride, and other targeted parameters, together with relevant plant/canopy assessments. Thermal patterns will be interpreted jointly with these measurements to identify plausible contributing factors, evaluate consistency among indicators, and assign diagnostic confidence. Cases with insufficient or conflicting evidence will be identified as requiring further evaluation rather than assigned a definitive cause.

UAV spatial assessment. High-resolution UAV thermal and visible/multispectral imagery will be collected during a single summer campaign in Year 1. Imagery will resolve fine-scale variability within satellite-detected anomaly and reference zones, evaluate within-pixel heterogeneity, and strengthen satellite-to-UAV-to-ground comparisons. Coordinated field measurements will support interpretation of thermal differences across spatial scales.

Integrated diagnosis and management guidance. Thermal behavior will be evaluated jointly with crop water demand, irrigation performance, root-zone soil water, salinity/chloride, plant evidence, and UAV observations. Each orchard will provide a diagnostic case linking detected patterns with field evidence, likely contributing factors, diagnostic confidence, and management relevance. Findings will be reviewed with growers to identify appropriate management options or additional measurements. Where corrective actions are implemented early enough, follow-up satellite and field measurements will assess responses. The resulting framework will provide a practical sequence for anomaly detection, targeted diagnosis, and management interpretation without assuming that every warm canopy requires additional irrigation.

Timeline/Milestones

Project Period	Major Activities and Milestones
Year 1	Select 10 orchards; analyze three years of historical Landsat data; identify recurrent anomalies and reference zones; establish field monitoring; conduct UAV imaging and initial diagnosis.
Year 2	Complete field diagnosis; integrate satellite, UAV, and field evidence; develop orchard diagnostic cases and management guidance; assess responses where feasible; finalize the irrigation-assessment protocol and grower guidance.

Estimated Total Budget**Project Duration:** 2 years**Total Funds Requested:** \$150,000

Budget Category	Year 1	Year 2	Total
Personnel	\$45,584	\$47,407	\$92,991
Travel	\$2,500	\$2,500	\$5,000
Materials and supplies	\$34,929	\$2,080	\$37,009
Equipment >\$5,000/item	\$0	\$0	\$0
Other costs	\$12,500	\$2,500	\$15,000
Total Direct Costs	\$95,513	\$54,487	\$150,000

Budget Justification

Personnel (\$92,991). Support is requested for one Staff Research Associate at 40% FTE for 24 months, including UC fringe benefits and salary escalation. The associate will conduct satellite data processing, establish and maintain field monitoring, collect and quality-control data, coordinate UAV imaging, integrate datasets, and develop orchard diagnostic cases. The PI will provide scientific leadership, supervision, grower coordination, interpretation, and reporting as in-kind support.

Travel (\$5,000). Funds will support visits to 10 cooperating avocado orchards for site characterization, instrument installation and maintenance, field sampling, irrigation assessments, UAV coordination, grower meetings, and follow-up evaluations.

Materials and supplies (\$37,009). Funds will support four ATMOS 41 weather stations (\$10,000); 16 IRRoCloud IC-10 monitoring systems, including first-year service (\$16,000); second-year subscriptions for 16 systems (\$2,080, Year 2); and soil-water and irrigation monitoring supplies (\$8,929). Instruments will be distributed across orchards to monitor contrasting thermal anomaly and reference locations and multiple root-zone depths. Available weather data will supplement the four weather stations where needed. The PI's laboratory will provide supplementary monitoring supplies and field equipment from existing inventory at no cost to CAC.

Equipment (\$0). No individual equipment item exceeding \$5,000 is requested.

Other costs (\$15,000). Commercial UAV thermal and visible/multispectral imaging (\$10,000, Year 1 only) will be conducted during one summer campaign to characterize fine-scale canopy-temperature variability and support satellite-to-UAV-to-ground comparisons. Targeted external laboratory analyses (\$5,000; \$2,500 per year) of soil, irrigation-water, and plant/tissue samples will evaluate salinity, chloride, and related diagnostic factors during both project years.

Total direct costs: \$150,000. No indirect or overhead costs are requested.

Principal Investigator and Collaborator Background and Avocado Research Experience

Ali Montazar — Principal Investigator University of California Cooperative Extension

Dr. Ali Montazar leads an integrated research and extension program focused on improving water management, resource-use efficiency, and sustainability of California avocado production. His research is conducted primarily in commercial orchards across contrasting Southern California production environments and integrates crop evapotranspiration and crop coefficients, irrigation management and system performance, root-zone soil-water monitoring, salinity assessment, plant-based sensing, canopy characterization, UAV imaging, and satellite remote sensing. This integration of field-scale irrigation science, plant and soil measurements, and spatial technologies provides a foundation for developing and validating practical irrigation-management tools under commercial orchard conditions.

A major component of Dr. Montazar's research has been the development of updated information on actual avocado water use and crop coefficients. Multi-year research across several commercial avocado production environments quantified actual evapotranspiration (ET) and evaluated how avocado water use and crop coefficients vary with climate, canopy characteristics, topography, salinity, soil conditions, and management. This work was published in *Agricultural Water Management* in 2025 as "Quantifying evapotranspiration and crop coefficients of California 'Hass' avocado affected by various environmental and plant factors." His program subsequently expanded from field-scale crop water-use measurements to satellite-based irrigation assessment. Multi-year OpenET estimates were evaluated against field-measured evapotranspiration across commercial avocado orchards, demonstrating both the potential and limitations of satellite ET for operational irrigation assessment and the importance of field validation in heterogeneous orchard systems. This research was published in *Agricultural Water Management* in 2026 as "Persistent daily OpenET bias drives seasonal water-use divergence in California avocado orchards."

Most recently, Dr. Montazar's program has advanced satellite-derived canopy-temperature estimation and physiological validation. A Landsat-based framework was developed and evaluated using satellite observations and continuous field measurements from six commercial Southern California avocado orchards. This research established the capability to characterize spatial canopy-temperature differences while highlighting the need for additional field evidence to interpret their underlying causes. The associated manuscript, "Satellite-derived canopy temperature for irrigation assessment in avocado orchards: A transferable framework with physiological validation," is currently under review in *Smart Agricultural Technology*.

Together, these efforts demonstrate a progression from avocado water-use and crop-coefficient research to field-based irrigation and root-zone assessment, satellite ET evaluation, and satellite canopy-temperature estimation supported by physiological validation. The proposed project represents the next stage of this program: moving from thermal detection to targeted field diagnosis and grower decision support. Dr. Montazar's experience integrating crop water demand, irrigation applied and system performance, root-zone soil water, salinity, plant response, UAV imaging, and satellite observations provides the multidisciplinary foundation needed to accomplish this work.

A distinguishing strength of Dr. Montazar's avocado program is its direct integration of research and grower extension. He established and leads a continuing avocado irrigation workshop and education program that has delivered research-based irrigation information to California avocado growers and industry professionals for more than six years. Research findings and practical irrigation information developed through his program have also been disseminated through numerous Cooperative Extension and industry publications, grower articles, webinars, field demonstrations, and presentations at grower and professional meetings. This sustained research-to-extension program demonstrates an established ability to develop, validate, and translate irrigation research into practical information delivered directly to California avocado growers.

Dr. Montazar has received CAC support for two projects: “Developing tools and information on crop water use and effective irrigation management for more profitable and sustainable avocado production” (2022–2025) and “Assessing irrigation management tools and strategies on avocado fruit quality and yield impacts” (2025–2028). He also currently leads the CDFA-funded project “Developing tools and information to enhance water-nitrogen use efficiency and sustainability of avocado production systems.” These projects provide commercial-orchard infrastructure, long-term datasets, monitoring equipment, and grower partnerships that will support the proposed research.

Ben Faber — Collaborator
University of California Cooperative Extension

Dr. Ben Faber is a long-standing leader in California avocado research and extension, bringing decades of experience working directly with avocado growers and commercial orchards. His expertise spans avocado production, irrigation and water management, soil and salinity management, root-zone conditions, tree health, and orchard responses to environmental and management factors. His extensive research and extension experience provides critical production expertise for interpreting whether satellite-detected thermal patterns are associated with irrigation management, soil/root-zone conditions, salinity, tree condition, or other orchard factors.

Dr. Faber has been a key collaborator in Dr. Montazar’s avocado irrigation research program and is a coauthor of the recent *Agricultural Water Management* studies on California avocado evapotranspiration and crop coefficients and satellite-based OpenET performance. His established relationships with the California avocado industry and extensive grower-extension experience strengthen the project’s connection to commercial production. In the proposed project, Dr. Faber will contribute to orchard selection, interpretation of thermal and field conditions, diagnosis of irrigation, salinity, root-zone, and plant-related constraints, development of practical management guidance, and communication of findings to growers.

Andre Daccache — Collaborator
University of California, Davis

Dr. Andre Daccache brings complementary expertise in remote sensing, precision agriculture, agricultural water management, and spatial data analysis. His expertise in remote sensing and spatial analysis complements the avocado irrigation and production expertise of Drs. Montazar and Faber. Dr. Daccache has collaborated with the team on the evaluation of satellite-based water-use information in California avocado orchards and is a coauthor of the 2026 *Agricultural Water Management* paper “Persistent daily OpenET bias drives seasonal water-use divergence in California avocado orchards.”

In the proposed project, Dr. Daccache will contribute to satellite data analysis and interpretation, spatial characterization of thermal patterns, remote-sensing methodology, and integration of Landsat, UAV, and ground observations. His precision-agriculture and remote-sensing expertise will strengthen the methodological robustness and transferability of the satellite-guided assessment framework across contrasting commercial orchard conditions.

Proposal Title: What Drives Consistently High Avocado Yields in California: A Multivariate Analysis of Grower Practices and Orchard Conditions

Research priority topic addressed: Cultural Methods 2. Above Average Grower Survey

Lead Principal Investigator: *Mary Lu Arpaia*, Dept. of Botany & Plant Sciences. E-mail: mlarpaia@ucanr.edu

Co-Principal Investigators: *Caroline Tardivo and Marllon F. S. dos Santos*, Dept. of Botany & Plant Sciences, *Evangelos E. Papalexakis*, Dept. of Computer Science & Engineering; *Patricia Manosalva*, Dept. of Microbiology & Plant Pathology. All are affiliated with the University of California, Riverside.

Introduction and Rationale

California avocado yields vary substantially among orchards and years, yet the management practices and environmental conditions associated with consistently high yields remain poorly quantified. Previous work by Campisi-Pinto, Crowley, and Lovatt examined nutrient balance in relation to total yield but did not include environmental factors. We will assemble standardized historical records from at least 50 commercial orchards distributed across California's main avocado-producing regions, targeting approximately 500 orchard-year observations. Records will cover yield, management, soil, water, climate, pollination, cultivar, rootstock, and orchard characteristics. Compatible observations from an existing UCR longitudinal database of California research plots will provide complementary data; source, location, orchard, tree, cultivar, year, and sampling protocol will remain identifiable after harmonization. Mixed-effects, multivariate, and machine-learning analyses will identify factors associated with yield, yield stability, and alternate bearing while accounting for repeated observations. Results will inform a prototype interactive tool that lets growers compare their orchards with similar high-performing orchards and identify potential management priorities to improve yield and stability. All results will be made available to California avocado industry stakeholders.

Objectives and Deliverables

There are 3 objectives:

- A) Collect, verify, harmonize, and organize historical yield, orchard-management, soil, water-quality, climate, pollination, cultivar, rootstock, tree-age, planting-density, and nursery-origin data from at least 50 commercial avocado orchards statewide, representing all production areas.
- B) Characterize orchard productivity using long-term average yield, year-to-year variability, yield stability, and alternate-bearing intensity using multivariate statistical, mixed-effects, and machine-learning approaches to identify management, environmental, soil, water, pollination, cultivar, rootstock, and orchard characteristics associated with consistently higher and more stable production.
- C) Develop and evaluate a prototype interactive benchmarking tool that enables growers to compare their orchards with similar production profiles and identify factors associated with yield level and stability.

The project will deliver: (1) a standardized database integrating historical yield, orchard characteristics, management practices, and environmental conditions; (2) productivity profiles describing consistently higher-, average-, lower-, and more variable-yielding orchards; (3) identification of the management and environmental factors most consistently associated with yield level, stability, and alternate bearing; and (4) a prototype interactive benchmarking tool that allows growers to compare their orchards with similar production profiles and identify factors associated with higher, lower, or less stable performance. The project will provide California avocado growers with quantitative benchmarks for evaluating orchard performance and identifying management practices associated with higher and more stable yields. By separating potentially modifiable management factors from characteristics related to climate, soil, water, orchard age, and location, the results will help growers prioritize practices and investments that are better suited to their production conditions.

Methods/Approach (*References available upon request*)

Objective A. Data collection and database development. At least 50 commercial avocado orchards will be selected (25, Ventura County/Santa Barbara Counties; 15, Riverside/San Diego Counties; 5, San Luis Obispo County; 5, Tulare/Sacramento Counties). The research team will work with CAC, UCANR and other entities to

identify orchards. Selection will be based on a range of historical productivity, environmental conditions, and management systems and will prioritize the completeness, duration, and reliability of available records. Structured interviews with growers/farm managers will document orchard characteristics, historical yield, and overall cultural, irrigation, and fertilization practices. Thirteen orchards will receive in-person visits for detailed interviews, record verification, site documentation, and direct sampling. At the remaining orchards, soil and irrigation-water samples will be collected by the collaborators. One composite soil sample and 1 water sample will be obtained from each orchard to characterize current site conditions. Historical soil and water analyses will also be collected when available. Variables known to change over time will be interpreted as current orchard descriptors and will not be assumed to represent conditions throughout the entire historical yield period. Soil and water samples will be collected at all sites using a standardized protocol. Soil samples will be analyzed for texture, pH, EC, organic matter, and available nutrients. Water samples will be analyzed for NO₃, EC, pH, Cl, B, and related water-quality indicators. Orchard records will be integrated with compatible multi-year yield, cultivar, management, and environmental records from the existing UCR longitudinal avocado database, which includes repeated observations from numerous trees, cultivars, and locations across California from various research plots. A harmonization protocol will standardize variable definitions, measurement units, temporal resolution, and missing-data codes. The resulting database will target approximately 500 commercial orchard-year records, supplemented by the compatible UCR historical records.

Objective B. Statistical and multivariate analysis

Long-term average yield, year-to-year variability, yield stability, and alternate-bearing intensity will be calculated for each orchard. Alternate bearing is a major source of temporal variation in avocado productivity and can be influenced by interactions among crop load, vegetative growth, and orchard management (Lovatt, 2014). Management, soil, water, climate, pollination, cultivar, rootstock, tree age, and planting density will then be analyzed to identify the factors most consistently associated with higher and more stable production. These variables reflect major constraints and management decisions affecting commercial avocado production in California, including water scarcity, salinity, orchard design, pruning, and cultural practices (Reints et al., 2020; Bender, 2019). Multivariate statistical methods will be used to group orchards with similar production conditions and management practices. Mixed-effects and machine-learning models will evaluate relationships between orchard characteristics and yield while accounting for repeated observations across orchards and years. Model development and validation will be conducted in collaboration with Dr. Papalexakis and his postdoctoral researcher, keeping all records from the same orchard together during model evaluation. The commercial-orchard database will serve as the primary dataset. Model complexity will be limited according to the number of participating orchards, with emphasis on parsimonious and regularized models to reduce overfitting. Compatible records from the existing UCR longitudinal database will be included in complementary analyses to determine whether the identified relationships remain consistent across cultivars, locations, years, and production conditions.

Objective C. Decision-support tool development and validation

Validated statistical and machine-learning models will be integrated into an interactive decision-support tool. Growers will enter orchard characteristics and available management, soil, water, pollination, climate, and yield information. The tool will compare the orchard with similar production profiles and provide an expected yield range, an estimate of yield stability, the principal factors associated with observed performance, and management factors that may warrant further evaluation. Outputs will reflect model uncertainty and will not present statistical associations as evidence of causation. Model validation will be conducted at the orchard level.

Timeline/Milestones

January/2027: Finalize the survey, data protocols, orchard recruitment plan, and team responsibilities.

February-July/2027: Recruit orchards; collect and verify historical records; complete planned sampling.

August/27-January/2028: Harmonize and refine the database; calculate yield and stability measures and develop model.

February-July/2028: Work with the computer science team to evaluate models and develop and test the benchmarking prototype.

August/2028: Finalize the tool, project report, and grower-facing results.

Estimated Total Budget
20 months (1/1/2027 – 8/31/2028)

	Year 1 1/1/2027 – 12/31/2027	Year 2 1/1/2028 – 8/31/2028
Personnel Costs		
Postdocs/Research Associates	\$27,790	\$31,493
Asst. Specialist Research (ASR)	\$12,425	\$9,308
Graduate Student Researcher (GSR)	\$10,611	\$0
Fringe Benefits	\$19,404	\$15,901
Supplies and Expenses	\$1,372	\$0
Other: Services (Samples Analysis)	\$9,100	\$0
Travel	\$9,009	\$5,148
Annual Total Amount Requested	\$89,711	\$61,850
TOTAL AMOUNT REQUESTED	\$151,561	

Budget Justification for 20-month project

Personnel Costs/Benefits: \$126,932 is requested for salaries/benefits. Funds will support Dr. Marllon Santos at 35% FTE in both years (\$69,267). He will serve as the project manager, coordinate site selection, grower interviews, field activities, soil/irrigation-water sampling, integration of commercial and UCR historical yield databases, statistical analyses, interpretation, and reporting. An ASR in Dr. Manosalva's laboratory (20% FTE) is requested for both years (\$32,079). They will assist with grower interviews, collection and organization of historical data, soil and irrigation-water sampling, sample tracking, and integration of project data into the analytical database. A Postdoctoral Scholar (Dr. Papalexakis) is requested at 30% FTE for 6 months in Year 2 (\$14,714). They will support machine-learning analyses, out-of-sample model evaluation, assessment of model performance, and development of the prototype benchmarking tool. A GSR (Dr. Tardivo) is requested at 20% FTE for 6 months in Year 1 (\$10,871). The GSR will assist with grower-data collection, record verification, sample tracking, database organization, and preliminary data processing.

Supplies: A total of \$1,372 is requested in Year 1 and includes 2 soil augers/probes, 2 sampling buckets, 75 sample bags, 12 permanent markers, 4 mixing trays or containers, distilled water, cleaning solution, and 2 sets of coolers and ice packs. These materials will support sample collection, processing, labeling, equipment sanitation, temporary storage, and transportation. At orchards not visited directly, the cooperators will collect samples using local equipment. The project will provide sample bags, labels, and collection instructions/protocols.

Services: A total of \$9,100 is requested (Year 1). This includes \$3,550 for 50 soil samples (texture, pH, EC, organic matter, and available nutrients), and \$5,050 for 50 irrigation-water samples (NO₃, EC, pH, Cl, B, and related indicators) conducted at Fruit Growers' Laboratory in Santa Paula, CA. An additional \$500 for 18 months of secure database and cloud-storage services is requested at UCR. Database services will support the organization, backup, and preservation of commercial-orchard records, UCR historical data, laboratory results, management information, and environmental data.

Travel: A total of \$14,157 is requested; \$9,009 (Year 1) and \$5,148 (Year 2). During the initial data-collection phase (Year 1), Dr. Santos and the GSR will complete 7 2-day trips to ~13 orchards in Ventura, Santa Barbara, and San Diego counties for grower interviews, record verification, site documentation, and soil and irrigation-water sampling. Year 1 includes \$1,596 for mileage (7 trips x 300 miles x \$0.76 per mile), \$4,662 for lodging (7 trips x 2 travelers x 1 night x \$333), \$2,576 for meals and incidental expenses (7 trips x 2 travelers x 2 days x \$92), and \$175 for parking/tolls (7 trips x \$25), totaling \$9,009. In Year 2, the team will complete 4 2-day trips to conduct grower outreach and present project results in the principal California avocado-producing regions. Year 2 travel includes \$912 for mileage (4 trips x 300 miles x \$0.76 per mile), \$2,664 for lodging (4 trips x 2 travelers x one night x \$333), \$1,472 for meals and incidental expenses (4 trips x 2 travelers x 2 days x \$92), and \$100 for parking/tolls (4 trips x \$25), totaling \$5,148. All travel will use one vehicle per trip.

Summary of Principal Investigator and Partner(s)/Collaborator(s) background and previous avocado research experience.

Dr. Mary Lu Arpaia, the Lead Principal Investigator, is a UC Cooperative Extension specialist in subtropical horticulture with extensive experience in avocado production, cultivar evaluation, orchard management, and grower outreach in California. She guides UCR's avocado scion breeding program and has worked with long-term field evaluations that document tree performance under different growing conditions. Her knowledge of avocado physiology and production practices will help the team distinguish meaningful productivity patterns from differences attributable to cultivar, tree age, site conditions, and management history. Her longstanding relationships with growers, industry partners, and research organizations will be essential for identifying participating orchards and seeking access to historical yield and management records. She will also help identify relevant datasets from previous experiments and collaborations, assess whether their measurements and protocols are suitable for integration, and interpret the resulting orchard comparisons. As Lead Principal Investigator, she will oversee the study, guide its horticultural priorities, and help translate the findings into practical information for California growers.

Dr. Caroline Tardivo, Assistant Professor of Horticulture at UCR, brings expertise in tree-crop physiology, rootstock evaluation, field experimentation, and statistical analysis of orchard data. Her research has followed citrus rootstocks from nursery production through field performance, examining how rootstock selection and management treatments relate to tree health, yield, and fruit quality. She has contributed to study design, data analysis, and the communication of field-trial findings to growers. In this project, she will help design the survey and field protocols, select and interpret horticultural variables, support grower recruitment and data collection, and evaluate how cultivar, rootstock, tree age, and management relate to avocado yield and stability. Her experience connecting field measurements, quantitative analysis, and grower needs will strengthen the practical relevance of the project's benchmarks.

Dr. Marllon F. S. dos Santos combines research in avocado production and reproductive physiology with expertise in applied statistics and data analytics. He holds an MBA in Data Science and Analytics, and his doctoral thesis focused on integrating statistical methods into research on 'Hass' avocado trees. His work applies quantitative approaches to practical questions in avocado production, including flowering and pollination, plant performance, and the analysis of repeated measurements and other complex agricultural data. He has used mixed-effects models, multivariate methods, and machine-learning approaches to evaluate biological variation and identify factors associated with outcomes of interest. At UCR, he has also contributed to research on avocado flowering patterns. In this project, he will coordinate implementation, harmonize historical records from different sources, and lead analyses of yield level, year-to-year variability, stability, and alternate bearing.

Dr. Patricia Manosalva, Associate Professor at the UCR Microbiology and Plant Pathology Department. Since 2015, she has overseen the UCR avocado rootstock breeding program, which aims to develop rootstocks exhibiting resistance to major avocado pathogens and abiotic stressors including salinity. This is a long-term collaboration with the avocado industry and growers in California and currently hosts 8 on-farm rootstock trials evaluating several advanced UCR rootstocks. Dr. Manosalva brings expertise in how rootstock genetics, plant health, soil conditions, and irrigation-water quality can interact to affect tree performance. In this project, she will help identify participating growers, define questions on rootstock and orchard health for the survey, and guide the interpretation of soil and water measurements alongside historical yield records. Her contribution will be particularly important when comparing orchards with different underlying production constraints and assessing which observed differences may be associated with management practices.

Dr. Evangelos E. Papalexakis is the Ross Family Chair and Professor of Computer Science and Engineering at UCR. His research develops machine-learning and data-mining methods for extracting interpretable insights from complex data with multiple sources and dimensions. He is particularly recognized for his work on tensor methods and scalable algorithms, and has received an NSF CAREER Award and early-career awards in data mining. This expertise is well suited to a project that integrates repeated yield observations with management, soil, water, climate, and orchard characteristics. Dr. Papalexakis will guide the selection and evaluation of machine-learning methods, assess the reliability and interpretability of model outputs, and supervise a postdoctoral researcher developing the interactive benchmarking prototype. His involvement will help turn the project's analyses into

rigorous, understandable comparisons that growers can use to evaluate orchards with similar production conditions.

Together, the team brings complementary experience in California avocado production, tree-crop field research, rootstock and plant health, longitudinal data analysis, and computational methods. These strengths support each stage of the project, from assembling reliable orchard records to producing benchmarks that growers can use to assess their own production conditions.

Roles and Responsibilities of each research team member for the proposed project.

The work will be conducted at the University of California, Riverside. Data will be collected in collaboration with California avocado growers, grower associations, industry organizations, and research and extension institutions, and supplemented with relevant publicly available soil, water, climate, and production datasets.

Project Planning: Dr. Arpaia, Dr. Tardivo, Dr. Manosalva, Dr. Santos, and Dr. Papalexakis will jointly develop the survey design, data-collection procedures, and analytical framework.

Grower Recruitment and Data Collection: Dr. Arpaia, Dr. Manosalva, and Dr. Tardivo will lead grower recruitment and provide horticultural guidance. Dr. Santos will coordinate survey administration, data organization, and quality control. The GSR and ASR supervised by Dr. Tardivo and Dr. Manosalva will assist with grower-data collection, record verification, sample tracking, and database organization during the initial data-collection phase of Year 1.

Statistical Analysis and Tool Development: Dr. Santos will lead data harmonization, integration of the commercial-orchard and historical databases, and the statistical and multivariate analyses. Dr. Papalexakis and the postdoctoral researcher from his group will support machine-learning analysis, model evaluation, and development of the interactive benchmarking tool.

Reporting and Outreach: All team members will contribute to interpreting results and preparing reports. Dr. Arpaia, Dr. Manosalva, and Dr. Tardivo will lead outreach to California avocado growers, with contributions from the full project team.

Current and Previous California Avocado Commission Funding

Dr. Arpaia and Dr. Santos are currently involved in a California Avocado Commission-funded project evaluating supplemental pollen applications in commercial ‘Hass’ and ‘GEM’ avocado orchards in Ventura County. The project, funded at \$172,825, began in January 2025 and includes multi-orchard field trials, flower sampling, pollen deposition and pollen-tube analyses, productivity assessment, and grower outreach. Dr. Arpaia serves as Principal Investigator, and Dr. Santos contributes to project coordination, field implementation, data analysis, and interpretation. This ongoing work has established relationships with California avocado growers and provides direct experience coordinating research across commercial orchards.

Patricia Manosalva has been funded by the California Avocado Commission (CAC) for her UCR rootstock breeding program through several funding cycles since 2015. CAC funding cycles from 2019 to 2025 aimed to establish large-scale trials in California with the 5 most advanced UCR rootstocks to gather comprehensive data for commercial release. The establishment and monitoring of the rootstock trials established by the UCR rootstock breeding program was funded by CAC and USDA and will provide historical data to this project. Recently, Drs. Manosalva and Adaskaveg received funding from CAC (2025-2028) to assess the risk for *Phytophthora cinnamomi* resistance to new oomycete fungicides under laboratory, greenhouse, and field conditions.

Improving the Avocado Irrigation Calculator

CAC research pre-proposal

Research priority topic addressed: No. 15, Irrigation Planning/Management tool including water budgeting tool.

Principal investigator: Andre Biscaro, UC Agriculture and Natural Resources, Irrigation and Water Resources Advisor, Ventura County; asbiscaro@ucanr.edu

Partners and collaborators: Andy Lyons, UC Agriculture and Natural Resources Informatics and GIS Statewide Program; Ben Faber, UC Agriculture and Natural Resources Cooperative Extension, avocado and subtropical crops advisor.

Introduction and Rationale

The California Avocado Commission (CAC) funded the development of an online avocado irrigation calculator (<https://ucanr-igis.shinyapps.io/irrigation-calc-avo/>) to turn local weather data and grove conditions into practical irrigation guidance. CAC has also supported expansion of the weather-station network serving avocado production areas. The next step is to connect these investments: incorporate four new stations, refine spatial assignment of reference evapotranspiration (ET_o), improve the estimation of effective rainfall on sloping groves, and make the application more dependable for everyday grower use. In addition, certain growers may need the flexibility to correct local rainfall and, where they have measured values, adjust distribution uniformity (DU) and leaching fraction. These changes will strengthen an existing tool rather than duplicate its original development.

Objectives and Deliverables

- Expand weather coverage: connect four new stations, define and document new ET_o zones.
- Improve data delivery: replace the Synoptic dependency with the CIMIS API where the required station data are available; verify completeness, latency, units and continuity before cutover.
- Improve effective precipitation estimates for slope and heavy rain
- Allow users to replace station rainfall with measured grove rainfall, with the source and edits recorded in the calculation.
- Add optional advanced inputs for DU and leaching fraction; provide a downloadable Excel sheet of the calculation records
- Improve the stability and performance of the calculator by migrating the application to a newer hosting platform (Posit Connect Cloud), reducing the launch time, automating checks on configuration settings and calculations, and alerts for missing data.
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Methods and Approach

1. Weather data and coverage. Inventory station locations, data access and quality flags. Integrate the four new stations and compare overlapping ETo records with existing sources. Define zone boundaries using geography, elevation, coastal influence, station coverage, and observed ETo differences. Document rules for zone assignment and fallback when a station is offline. Replace the Synoptic ingestion path with the CIMIS API after confirming that CIMIS exposes the required data at an acceptable refresh interval; retain a documented contingency if any station is unavailable through that API.
2. Water-balance algorithm. Review the current effective-precipitation logic and use a bounded approach that accounts for grove slope and storm intensity. Compare outputs for representative flat and sloping orchards, light and intense rainfall, and dry periods. Permit growers to enter local precipitation values and distinguish observed, imported, and edited values in the output. Make DU and leaching-fraction fields optional, with documented defaults and sensible input limits.
3. Application reliability and validation. Implement Excel export with dates, zone, station source, input values, assumptions, calculation steps and recommendation. Profile launch time and reduce avoidable startup processing. Migrate to Posit Connect Cloud, establish environment-specific configuration, and run regression tests against known calculator examples. Have collaborators and a small group of avocado growers review usability and representative recommendations before release.

Timeline and Milestones

Period	Milestone
Months 1–4	Weather station zone design, baseline test cases. Posit Connect Cloud migration, startup optimization, configuration and testing.
Months 5–8	Three-station integration, ETo zones, CIMIS transition, effective-rainfall prototype.
Months 9-15	User rainfall edits, advanced settings, Excel export.
Months 16-24	Grower review, revisions, documentation, deployment, and final report.

Success measures: three additional stations available in the calculator; documented zone assignment and data-source fallback; tested recommendations across defined scenarios; functioning Excel exports and advanced inputs; production deployment; and measured improvement in launch time and data latency relative to baseline.

Estimated Total Budget

Twelve-month planning estimate in US dollars. Figures below are placeholders for confirmation with UC ANR rates, collaborator effort, vendor charges, and the final scope. Only direct project costs are requested; no indirect or overhead costs are included.

Category	Basis	Estimate
Personnel	IGIS programing time, 150 hours × \$99.62 direct rate	\$14,943
Travel	NA	\$0
Materials and supplies	NA	\$0
Equipment over \$5,000	None	\$0
Other costs	NA	\$0
TOTAL DIRECT COSTS	Planning estimate	\$14,943

Budget assumptions: The four weather stations themselves are funded separately and are not charged here. Hosting is included only for the proposed project period; ongoing operating costs after the project require a separate commitment. Personnel rates and eligibility for PI effort must be confirmed before submission. There are no items over \$5,000 classified as equipment.

Cost controls: Confirm CIMIS access and data availability in Month 1 before committing to the API migration. Scope any station-specific integration outside CIMIS separately if required.

Reuse the existing calculator code and weather infrastructure where practical.

Investigator and Collaborator Background

Andre Biscaro — Principal investigator. Biscaro is UC ANR Irrigation and Water Resources Advisor based in Ventura County. His research and extension program evaluates irrigation management, weather and sensor data, and decision-support tools for regional crops. He led the CAC-funded project “Adapting a user-friendly online irrigation calculator to avocados” (2024–2025) and the CAC-funded “Creating a weather station network to guide irrigation decision of avocados” (2025–2027). The avocado calculator incorporates grove characteristics, including a south-facing slope adjustment, in irrigation guidance. His other calculator and weather-network projects provide experience translating field measurements into practical recommendations and grower education.

Andy Lyons — CoPI. Lyons is affiliated with UC ANR’s Informatics and GIS Statewide Program. He has collaborated on adaptation of the online irrigation calculator for avocado production and brings experience in spatial data, application development, and geospatial workflows. His proposed role is to guide ETo-zone integration, data pipelines, application features, testing, and deployment.

Ben Faber — Collaborator. Faber is a UC ANR advisor with extensive avocado and subtropical crop experience. He has collaborated on developing the avocado version of the calculator and on weather-station planning and grower-facing irrigation work. His role is to review agronomic assumptions, effective-rainfall and leaching settings, field scenarios, and grower usability.

Development and evaluation of pollen suspensions in avocado: paternity, yield response and Carmen® bloom management

Principal investigator: Prof. Mónica Castro, Pontificia Universidad Católica de Valparaíso (PUCV), Chile; e-mail: monica.castro@pucv.cl. Project lead and co-investigator: Maite Arancibia, PUCV; e-mail: maite.arancibia@pucv.cl. Co-investigator: Prof. Ricardo Cautin, PUCV; e-mail: ricardo.cautin@pucv.cl. Proposed genetics collaborator: Dr. Patricio Hinrichsen, INIA La Platina, Chile; e-mail: phinrichsen@inia.cl. Genetic paternity verification through DNA analysis of seedlings grown from fruit seeds.

Introduction and rationale

Successful avocado pollination requires female-phase flowers, viable pollen and active insect pollinators to coincide. Temperature fluctuations can alter floral behavior and reduce bee visitation, as documented in central Chile (Monzón et al., 2020). Against the backdrop of climate change, this project will examine the relationships between temperature, relative humidity, pollination and fruit set, and assess whether pollen sprays can supplement natural pollination.

The project will develop pollen suspensions from different donors and evaluate their genetic contribution and effects on fruit set, fruit retention, yield and fruit size distribution. Weather conditions and insect visitation will also be recorded. The work will complement artificial pollination research by developing a reproducible protocol, comparing pollen donors and evaluating fall and spring flowering in Carmen®. The proposal does not correspond directly to a numbered priority in the call; findings from Chile would require further validation under California conditions.

The orchard available for the trial is located at La Palma Experimental Station, School of Agronomy, Pontificia Universidad Católica de Valparaíso (PUCV), Quillota, Chile (32°49' S, 71°16' W). It was established through the CORFO project “Paquete tecnológico para el desarrollo de nuevas variedades de palto”, within the program “Plataforma de Innovación Colaborativa en el Desarrollo de Paquetes Tecnológicos Frutícolas para la Zona Centro-Norte” (16PTECFN-66646). The project ended in 2024; the team has continued studying the trees, with tree-level yield and fruit size records since 2022.

Planted in December 2019 at 5 × 3 m spacing, the orchard contains 272 experimental trees and 24 Edranol pollenizers (296 trees). We will use 240 Carmen®, Palta Gem® (UC 3295), Maluma® and Hass trees on Dusa® and Zutano, with 30 replicates per combination. The other 32 trees are Isabel, with 16 per rootstock, and will be included as candidate pollen donors. Results from the CORFO-funded project were published in “Evaluación de variedades y portainjertos de palto en la zona centro-norte de Chile” (2025). This platform provides production history and allows study of both Carmen® bloom seasons.

General objective

Develop and evaluate a supplementary pollination strategy using pollen suspensions from different avocado donors, determine their genetic contribution and effects on fruit set, fruit retention, yield and fruit size distribution, and assess fall and spring flowering and bloom management in Carmen®.

Objectives, methods and deliverables

The pollen protocol will be developed and validated before field evaluation. Individual trees will serve as experimental units, with flowers and fruit treated as subsamples. Carmen® bloom management will be evaluated in a separate cycle. Treatments will be assigned at random, taking tree location and yield history into account.

Specific objective	Methods and approach	Deliverable
1. Develop and validate a pollen collection and application protocol.	Compare pollen collection, storage and suspension formulations for Hass, Maluma®, Carmen®, Palta Gem® (UC 3295) and Edranol. Assess pollen concentration, germination, stability and viability after passage through the spray nozzle. Conduct a pilot trial on inflorescences to establish application rates and conditions.	Documented technical protocol with quality criteria, application conditions and validation results, including storage and use limitations.
2. Determine paternity and the effects of supplementary pollination on yield.	Randomly assign seven treatments within each cultivar–rootstock combination, using four trees per treatment: open pollination without spraying, a pollen-free carrier, and pollen from each of five donors. All trees will remain exposed to ambient pollination. Apply sprays during the female floral phase at equivalent viable pollen doses, with measures to limit drift. Measure fruit set, retention, yield and fruit size by tree; record weather and insect visitation; and analyze seedling DNA. Validate the ability to distinguish donors, particularly Hass and Carmen®, and report unresolved paternity assignments.	Paternity assignment procedure, experimental database, and a report on each donor's contribution and effects on fruit set, retention, yield and fruit size distribution, including estimates of uncertainty.
3. Evaluate supplementary pollination during Carmen® fall and spring blooms.	Identify fall and spring flowering cohorts and apply treatments when viable pollen is available. Assess paternity and yield response for each flowering season, comparing treatments used in both seasons.	Comparative report on the genetic contribution and yield effects of supplementary pollination during fall and spring flowering.
4. Evaluate effects of Carmen® bloom management on yield and fruit size.	Compare three treatments: retain fall flowers only, spring flowers only, or both. Use ten trees per treatment and rootstock, in a cycle separate from the pollen spray trial.	Report on yield under each bloom management treatment, with recommendations based on yield and fruit size distribution.

Economic assessment: pollen collection, storage, formulation and application costs will be recorded throughout the project. An economic report will estimate returns based on the observed changes in yield and fruit size distribution.

Timeline and milestones

The proposed project duration is 36 months. Field activities will follow the flowering and harvest calendar of each cultivar.

Period	Main activities	Milestones
Months 1–12	Organize data and trees; characterize blooms; develop and assess collection, storage and suspensions; validate markers and spray pilot. Initiate separate Carmen® bloom management according to phenology.	Preliminary protocol and genetic panel assessed; advancement conditions defined; bloom management established.
Months 13–24	Apply treatments in spring and, for Carmen®, in fall if viable pollen is available. Record weather, insects, set and retention; sample for paternity and harvest according to schedule.	Field trial implemented; preliminary genetic and production results by bloom season.
Months 25–36	Complete harvests, genotyping and costs; integrate results; conduct statistical and economic analyses; finalize protocol and reports.	Final database; technical protocol and reports on paternity, yield, bloom management and economic returns.

The Carmen® bloom management trial will be scheduled to avoid interference with the pollen spray trial. Fall applications will proceed only if pollen with validated viability is available.

Estimated budget for direct costs

Category	Estimated amount (USD)	Description
Personnel costs	60,000	Coordination, pollen collection and preparation, laboratory assessments, applications, flowering, set and retention monitoring, harvests and data analysis.
Travel	9,000	Travel associated with experimental work, coordination with the genetics laboratory and dissemination of results.
Materials and supplies	13,000	Materials for pollen collection and storage, formulations, germination assays, sample identification, spraying and field assessments.
Equipment costing over USD 5,000 per item	0	No equipment purchase in this category is proposed; available infrastructure at participating institutions will be used.
Other costs	38,000	Genotyping and paternity assignment services, marker validation and specialized laboratory analyses.
Total requested	120,000	Direct costs for 36 months.

The budget is an estimate for the preproposal and will be refined using quotations and a detailed work plan. It excludes institutional overhead and other indirect costs.

Team background and avocado research experience

Mónica Castro — Principal investigator

Professor at the School of Agronomy of Pontificia Universidad Católica de Valparaíso (PUCV), with extensive experience in plant propagation and evaluation of avocado rootstocks and cultivars. Her recent work includes “Optimizing In Vitro Establishment Protocols for ‘Merensky 2’ Avocado Rootstock” (*Horticulturae*, 2025, 11, 900). She is an author and editor of “Evaluación de variedades y portainjertos de palto en la zona centro-norte de Chile” (2025). She led the project “Evaluación de variedades y portainjertos de palto en la zona centro-norte de Chile” for eight years (2016–2024). Her experience in protocol development and plant material evaluation will support scientific leadership and supervision of laboratory and field activities.

Maite Arancibia — Project lead and co-investigator

Agronomist and MSc in Environmental Sciences, with approximately 20 years of agricultural research and development experience. At PUCV’s La Palma Experimental Station, she participates in avocado cultivar and rootstock trials, monitoring tree-level yield, fruit size distribution, fruit growth, dry matter and quality. She is a coauthor of “Evaluación de variedades y portainjertos de palto en la zona centro-norte de Chile” (2025). She will coordinate experimental implementation, field assessments, data management and interpretation of production outcomes.

Ricardo Cautín — Co-investigator

Professor at PUCV’s School of Agronomy, with extensive experience in research and management of subtropical fruit crops, especially avocado. He is a coauthor of the study on in vitro establishment of ‘Merensky 2’ rootstock (*Horticulturae*, 2025, 11, 900) and of “Evaluación de variedades y portainjertos de palto en la zona centro-norte de Chile” (2025). He served as Alternate director of the project “Evaluación de variedades y portainjertos de palto en la zona centro-norte de Chile” for eight years (2016–2024). His extension work includes “Manejos agronómicos: Rol de la poda en verde en paltos” (*MundoAgro*, 2026, 190, pp. 38–42). He will contribute to the design and interpretation of field assessments and Carmen® bloom management.

Patricio Hinrichsen — Proposed genetics collaborator, INIA La Platina

Researcher at INIA La Platina with experience in genetic characterization and molecular markers in fruit crops. His publications include identification of SNPs and InDels associated with table grape berry size (*BMC Plant Biology*, 2020, 20:365), analysis of loci and candidate genes for table grape sweetness and acidity (*Australian Journal of Grape and Wine Research*, 2021, 27:308–324), and sweet cherry cultivar characterization using microsatellites (*Chilean Journal of Agricultural Research*, 2021, 81:326–337). His background includes a FONDECYT project initiated in 2022 on genetic determinants of table grape rachis architecture. His proposed role includes marker selection and validation, characterization of maternal trees and pollen donors, and paternity assignment using DNA from seedlings grown from seeds.

CAC funding disclosure

Team members do not currently receive and have not previously received funding from the California Avocado Commission.

Title: A Field-Validated AI Forecasting Tool for Avocado Lace Bug Scouting and Damage Risk

Research Priority Topic Addressed: Priorities 18a-b, Avocado Lace Bug Scouting Methods and the Empirical Foundation for Treatment Thresholds

Principal Investigator

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Assistant Professor, Department of Microbiology and Plant Pathology

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Co-Principal Investigators/Collaborators

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University of California, Riverside

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Introduction and Rationale

Avocado lace bug (ALB), *Pseudacysta perseae*, is an invasive foliar pest of increasing concern to California avocado production. In 2024, California growers produced 182,420 tons of avocados valued at \$523.5 million across approximately 48,000 harvested acres.¹ ALB adults and nymphs feed on mature leaves, causing chlorotic and necrotic lesions, reduced photosynthetic area, and premature defoliation. Severe infestations may weaken trees and increase fruit-sunburn risk. ALB has expanded into commercial ‘Hass’ avocado-producing areas, including Ventura and Santa Barbara Counties, yet growers lack validated methods for interpreting scouting counts and identifying increasing population pressure.

A strong biological foundation for predictive management is available. Recent research led by Dr. Hoddle quantified ALB development and reproduction under six field-informed fluctuating-temperature profiles representative of California avocado-producing regions.² The study estimated a thermal requirement of approximately 476 degree-days and established developmental thresholds suitable for phenology modeling. The resulting dataset provides the biological basis for constructing degree-day, thermal-suitability, and expected life-stage features for machine learning. This project will therefore use the existing ALB thermal-development data and will not require new labor-intensive temperature-cabinet experiments.

The **critical remaining gap** is the relationship among thermal development, ALB abundance, cumulative leaf injury, and yield. Scouting lacks validated guidance on where, when, and how intensively to monitor, and the leaf-damage level associated with canopy decline, photosynthetic stress, fruit sunburn, and yield loss remains unknown. Because phenology, natural enemies, orchard conditions, and management may modify these relationships, growers cannot yet determine when treatment may be avoided or is needed to prevent yield-impacting damage.

We **propose** a three-year project integrating Dr. Hoddle’s existing ALB thermal-development dataset with two seasons of standardized field surveillance and AI/machine learning models. A biology-informed XGBoost framework will integrate thermal features, public weather data, ALB abundance, avocado phenology, natural enemies, management history, and injury progression to forecast population, canopy-damage, and yield-impact risk. The project will establish a practical scouting protocol and provisional yield-impact levels as the empirical foundation for future treatment thresholds. The collaboration combines Dr. Tibo Duran’s expertise in AI/ML and predictive modeling, Dr. Mark S. Hoddle’s expertise in ALB biology and thermal modeling, and Dr. Hamutahl Cohen’s expertise in field IPM and grower engagement. The resulting web-based tool will support earlier detection, better-timed intervention, and more sustainable ALB management.

Objectives and Deliverables

Objective 1. Establish a practical, multi-season ALB scouting protocol. Deliverables: a standardized sequential sampling plan and quality-controlled dataset spanning two field seasons. **Objective 2.** Develop an explainable AI/ML model for ALB population, canopy-damage, and yield-impact risk. Deliverables: 7–14-day population and yield-impact forecasts, model explanations, and provisional risk levels supporting future thresholds. **Objective 3.** Prospectively validate and translate the AI/ML framework. Deliverables: independent third-season validation, grower scouting guidance, and a documented web-based decision-support prototype.

Methods/Approach

Aim 1. Establish Multi-Season ALB Population–Injury–Yield Relationships and an Efficient Scouting Protocol. During Years 1–2, six confirmed-infested Ventura County orchards will be monitored weekly for 18 weeks using 15 sentinel trees per orchard (216 orchard-weeks). We will record ALB stages, natural enemies, phenology, management, and ALB-attributed injury. Marked trees will be followed through harvest for defoliation, fruit retention, sunburn, fruit number and weight, and marketable quality. Fixed-count, presence–absence, and sequential designs will identify a precise, labor-efficient scouting protocol.

Aim 2. Develop an Explainable, Biologically Informed AI Model for ALB Population and Yield-Impact Risk Forecasting. XGBoost will integrate thermal features, public weather, ALB counts, phenology, natural enemies, management, initial crop load, cumulative leaf injury, and yield outcomes to generate a 7-day forecast and 8–14-day outlook for population pressure and the probability of reaching a yield-impacting damage level. Orchard-season-grouped validation and regularization will reduce overfitting.

Aim 3. Prospectively Validate the AI Forecasting Framework and Develop a Grower-Oriented Web Tool. During Year 3, locked models and the reduced protocol will be tested for 18 weeks in three independent orchards. Forecasts will be compared with ALB abundance, cumulative leaf injury, fruit sunburn, yield, and marketable fruit quality. A web-based AI tool will report population risk, provisional yield-impact risk, confidence, drivers, and scouting intensity. Results will be shared through CAC, California Avocado Society, From the Grove, and UC ANR extension outlets.

Timeline and Milestones

Period	Key Activities	Milestones
Year 1 Months 1-3	Finalize protocols; recruit 6 confirmed-infested orchards; select 15 sentinel trees per orchard; establish public-weather, sensor, injury-diagnosis, yield, and data workflows.	Field sites, sampling protocol, database, and thermal-feature workflow established.
Year 1 Months 4-12	Complete first 18-week field season; quantify ALB life stages, natural enemies, phenology, management, ALB-attributed injury, and initial crop condition; develop preliminary AI/ML model.	108 orchard-week records; preliminary scouting protocol and AI/ML model developed.
Year 2 Months 13-20	Complete second 18-week season in 6 orchards using retained and new sites; test the reduced scouting protocol; continue delayed injury, fruit-sunburn, yield, and quality assessments.	216 cumulative orchard-week records; multi-season dataset and field-tested scouting protocol completed.
Year 2 Months 21-24	Integrate thermal, public-weather, population, injury, and yield features; train and validate explainable AI/ML models; quantify uncertainty and lock the forecasting framework.	Locked 7–14-day population and damage-risk forecasts and provisional yield-impact risk levels developed.
Year 3 Months 25-30	Prospectively evaluate the locked models and reduced scouting protocol in 3–5 independent Ventura County orchards monitored weekly for approximately 18 weeks.	54–90 independent orchard-week records; prospective validation completed.
Year 3 Months 31-36	Complete leaf-injury, fruit-sunburn, yield, and marketable-quality assessments; finalize and deploy the web tool; prepare grower guidance, outreach materials, and final report.	Validated forecasting tool, scouting guidance, technical report, and grower outreach completed

Estimated Total Budget:

Title: A Field-Validated, AI-Enabled Forecasting Tool for Avocado Thrips Phenology and Fruit-Damage Risk				
Budget category	Year 1	Year 2	Year 3	Three-year total
GSR support	\$34,123	\$34,847	\$35,592	\$104,562
Ventura field study travel	\$9,050	\$9,050	\$9,050	\$27,150
Field study personnel	\$12,000	\$12,000	\$12,000	\$36,000
Materials and supplies: gloves, foreceps, temperature sensors	\$2,000	-	-	\$2,000
Total	\$57,173	\$55,897	\$56,642	\$169,712

Principal Investigator:

Dr. Tibo Duran is an Assistant Professor in the Department of Microbiology and Plant Pathology at the UC, Riverside. His research integrates artificial intelligence, machine learning, mechanistic modeling, and environmental data analysis to develop biologically informed forecasting and decision-support tools. His expertise includes longitudinal data integration, interpretable predictive modeling, model validation, and web-based research tools.

Dr. Duran has not previously received and does not currently hold funding from the California Avocado Commission.

Co-Principal Investigator:

Dr. Hamutahl Cohen is an Entomology Advisor with UC Cooperative Extension in Ventura County. She received her Ph.D. from UC Santa Cruz in Environmental Studies and has been working in the field of agricultural entomology since with growers. Her fields of expertise include cultural control, biological control, and chemical control of tree and vegetable pests, as well as pesticide resistance monitoring and pollinator conservation.

Dr. Cohen currently has two grants supported by the California Avocado Commission.

- The first is entitled “Enhancing integrated pest management for avocado lace bug in organic production” (July 2026-October 2027)
- The second is entitled “A pesticide resistance monitoring program for avocado thrips” (July 2025-October 2030).

Co-Principal Investigator:

Dr. Mark Hoddle is an extension specialist in biological control in the Department of Entomology, UC Riverside. Hoddle has worked on numerous invasive avocado pests, including avocado thrips, persea mite, and avocado lace bug. Proactive work on avocado pests overseas includes projects on avocado seed moth in Guatemala and Peru, and avocado seed weevil in Mexico.

Dr. Hoddle currently has two grants supported by the California Avocado Commission.

- The first is entitled “Chemical Synthesis and Field Evaluation of an Enantiopure (+)-Grandisol, the Putative Avocado Seed Weevil (*Heilipus lauri*) Aggregation Pheromone” (April 2025–October 2027; Award No. 65133-000-00; \$349,212).
- The second is entitled “Surveys for Avocado Fruit-Feeding Insect Pests in Guatemala” (April 2025–October 2027; Award No. 65132-000-00; \$510,202).

Quantifying Mineral Availability in Marginal Irrigation Water Using Lysimeters with Mature Hass Avocado Trees

Research priority topics:

3a. Leaching Fractions with Modern Rootstocks and Water Sources

4a. Nitrogen planning and nitrate credits

Principal investigator

Ran Erel, Institute of Soil, Water and Environmental Sciences, Agricultural Research Organization (ARO)–Volcani Institute, Gilat Research Center, Israel; ranerel@volcani.agri.gov.il

Partners and collaborators

Noam Alkan, Department of Postharvest Science, Agricultural Research Organization–Volcani Institute.

Introduction and rationale

As reclaimed water becomes more common in avocado production, growers need to know whether minerals carried in the irrigation water can replace part of the fertilizer supply and whether the same water changes tree water uptake. Reclaimed water differs from high-quality water in salinity, chloride, sodium, and nutrient content, mainly N (as nitrate) and potassium. Input concentrations alone cannot establish plant availability because some minerals leave the root zone in drainage.

At Gilat Research Center, we established a large lysimeters system with mature ‘Hass’ avocado trees grown in 1,000-L perlite containers with independent fertigation lines and continuous records of irrigation and drainage. The inert medium and a long-running experimental platform established in 2019 permit a direct input–output assessment without soil interactions. This project will compare local high-quality water-source with tertiary-treated wastewater under the same added fertilizer regime.

Research questions: How much of the mineral load delivered in marginal irrigation water is retained or taken up by mature Hass avocado trees? To what extent does water quality change tree water uptake? and how the salts affect avocados’ productivity?

Objectives and deliverables

1. Quantify the water and mineral input–drainage balances for both water sources over two production years.
2. Test differences in tree water uptake, leaf and fruit mineral composition, yield, fruit size, and postharvest quality.
3. Translate the measured waterborne mineral contribution and water-use response into practical guidance on fertilizer credits and drainage management.

Methods and approach

Site and treatments. Twelve mature Hass avocado trees grafted on modern, vigorous, vegetative clone rootstock (VC-66) will be assigned to two irrigation-water treatments (six trees per source) in existing 1,000-L perlite lysimeters at Gilat. Local high-quality line water supplied from the Ashkelon desalination system has an approximate electrical conductivity (EC) of 0.36 dS m⁻¹. Tertiary-treated effluent from the Be’er Sheva wastewater treatment plant has an approximate EC of 1.1 dS m⁻¹ with considerable concentration of nutrients (Table 1).

Table 1: chemical composition of the two sources

	High quality FW	Recycled water
EC (dS/m)	0.36	1.08
pH	7.82	7.74
Cl (mg/L)	35	154
Na (mg/L)	26	99
N-NO ₃ (mg/L)	0.7	1.86
N-NH ₄ (mg/L)	0	10.0
Total P (mg/L)	0.08	0.96
K (mg/L)	1.1	22.6
Mg (mg/L)	2.3	17
Ca (mg/L)	38	79

Fertigation and water balance. Both groups will receive the same added fertilizer concentrations: 30 mg L⁻¹ N, 5 mg L⁻¹ P, 25 mg L⁻¹ K, and micronutrients including 0.6 mg L⁻¹ chelated Fe (Bar-Koret, ICL). Mineral inputs from the water sources will be quantified separately every month. Irrigation will occur daily, with applications adjusted to target drainage of about 30% of applied water. Dedicated drainage collection tanks are weighed and logged daily. Tree water uptake will be estimated from irrigation minus drainage, accounting for any material change in lysimeter water storage (Sperling et al., 2023).

Mineral balance. Once each month, drainage will be sampled manually from every study tree while the corresponding irrigation water is sampled. Measured concentrations will be combined with logged irrigation and drainage volumes to estimate mineral inputs, losses, and net retention for each tree. Monthly chemistry provides 12 sampling periods per year; daily mineral uptake estimates will require concentration interpolation and will be identified as estimates rather than direct daily measurements.

Tree and fruit responses. Leaves will be sampled in May–June after the spring flash and again in October. Flowering intensity, fruit-set percentage, and determinate versus indeterminate inflorescences will be assessed each year. Leaf area index will be measured five times annually using a LI-COR instrument. At harvest, anticipated around January when fruit dry matter reaches approximately 25%, all fruit will be collected to determine yield per tree, fruit size, and fruit mineral composition. Twenty fruit per tree will be sent to Dr. Noam Alkan's laboratory for quality assessment after 7–8 days of simulated shelf life.

Analysis and related work. The tree will be the experimental unit. Water-source effects will be evaluated across repeated sampling dates and two seasons, with the assignment structure included in the model. The project will run alongside a separate experiment on nitrogen application timing and avocado yield and postharvest quality.

Timeline and milestones

The expected experiment duration is January 2027–January 2029

January - March 2027 – Establishment: We will establish water-source treatments and baseline composition.

March 2027 Treatments initiation: log daily water balances and sample monthly mineral balances throughout; measure flowering, fruit set, leaf chemistry, and LAI seasonally; harvest and test fruit around January 2028 and January 2029; complete integrated analysis and grower recommendations by project end.

January 2028 First harvest and report. First report on nutrients uptake dynamics, yield, quality, and plants' minerals composition

January 2029 Second harvest and final report. Final report on nutrients uptake dynamics, yield, quality, and plants' minerals composition



The large lysimeter platform at Gilat Research Center. April 2026

Estimated total budget

Project period: January 2027–January 2029 (24 months). Amounts in US dollars.

Direct-cost category	Year 1	Year 2	Total
Personnel: Technician for platform maintenance, sampling, recording, and field operation	40,000	40,000	80,000
Personal: Technician for laboratory plant and solution analysis	20,000	20,000	40,000
Travel	0	0	0
Materials and supplies: water delivery, sampling, analytical consumables (per year: 144 water analysis and 36 plant tissue analysis)	14,000	14,000	28,000
Equipment: list each item above \$5,000 separately, if any	0	0	0
Other direct costs: external assays and postharvest evaluation	5,000	5,000	10,000
Total direct costs	79,000	79,000	158,000

Principal investigator background

Dr. Ran Erel is a plant nutrition researcher in the Department of Soil Chemistry, Plant Nutrition and Microbiology, Institute of Soil, Water and Environmental Sciences, Agricultural Research Organization–Volcani Institute, at Gilat Research Center. His research focuses on nutrient acquisition, fertigation, and the effects of mineral nutrition on tree productivity and fruit quality. His previous work on long-term irrigation with reclaimed wastewater demonstrated the importance of accounting for nutrients supplied in irrigation water when managing orchard fertilization (Erel et al., 2019).

Before establishing the avocado platform, Dr. Erel used large drainage lysimeters to study water use and mineral nutrition in olive trees. This work, together with long-term field trials, generated insights that helped revise fertilization practices for intensive olive oil production, including lower nitrogen recommendations and explicit accounting for nutrients supplied in irrigation water. His olive research has produced 21 peer-reviewed journal articles, including two studies directly addressing irrigation with reclaimed wastewater (Segal et al., 2011; Erel et al., 2019). The experience in measuring tree water and mineral balances and translating the results into orchard management underpins the proposed avocado study.

At Gilat, Dr. Erel leads a controlled experiment established in 2019 with Hass avocado trees grown in large perlite lysimeters. The system records irrigation and drainage and has already been used to characterize seasonal nutrient uptake from mineral input–output balances in avocado (Sperling et al., 2023). In the 2023–2025 avocado project, daily water balances indicated annual tree water uptake of approximately 1,000–1,200 mm. Mature trees under moderate fertilization produced approximately 20–30 kg of fruit per tree in 2024. The project also linked fertilizer supply to flowering, fruit set, fruit mineral composition, and postharvest disorders: higher N, P, and K supply was associated with more internal grey pulp and decay after storage. These results, together with the established lysimeter and analytical infrastructure, provide a strong basis for testing how minerals supplied by reclaimed irrigation water affect nutrient and water uptake and Hass fruit quality.

Relevant experimental platform

The Gilat lysimeter orchard was established in 2019. Hass trees in 1,000-L perlite containers are planted at 4 × 5.5 m spacing, with Ettinger pollinizers in the orchard. A computerized SAS Control fertigation system operates separate irrigation lines and ProMinent gamma/X fertilizer pumps. Each study tree drains into a dedicated weighing vessel whose daily records are stored by the control system. The system has supported long-term measurement of nutrient and water dynamics through 2026 and will be available starting November 2026.

California Avocado Commission funding history

Dr. Erel did not received any CAC funding

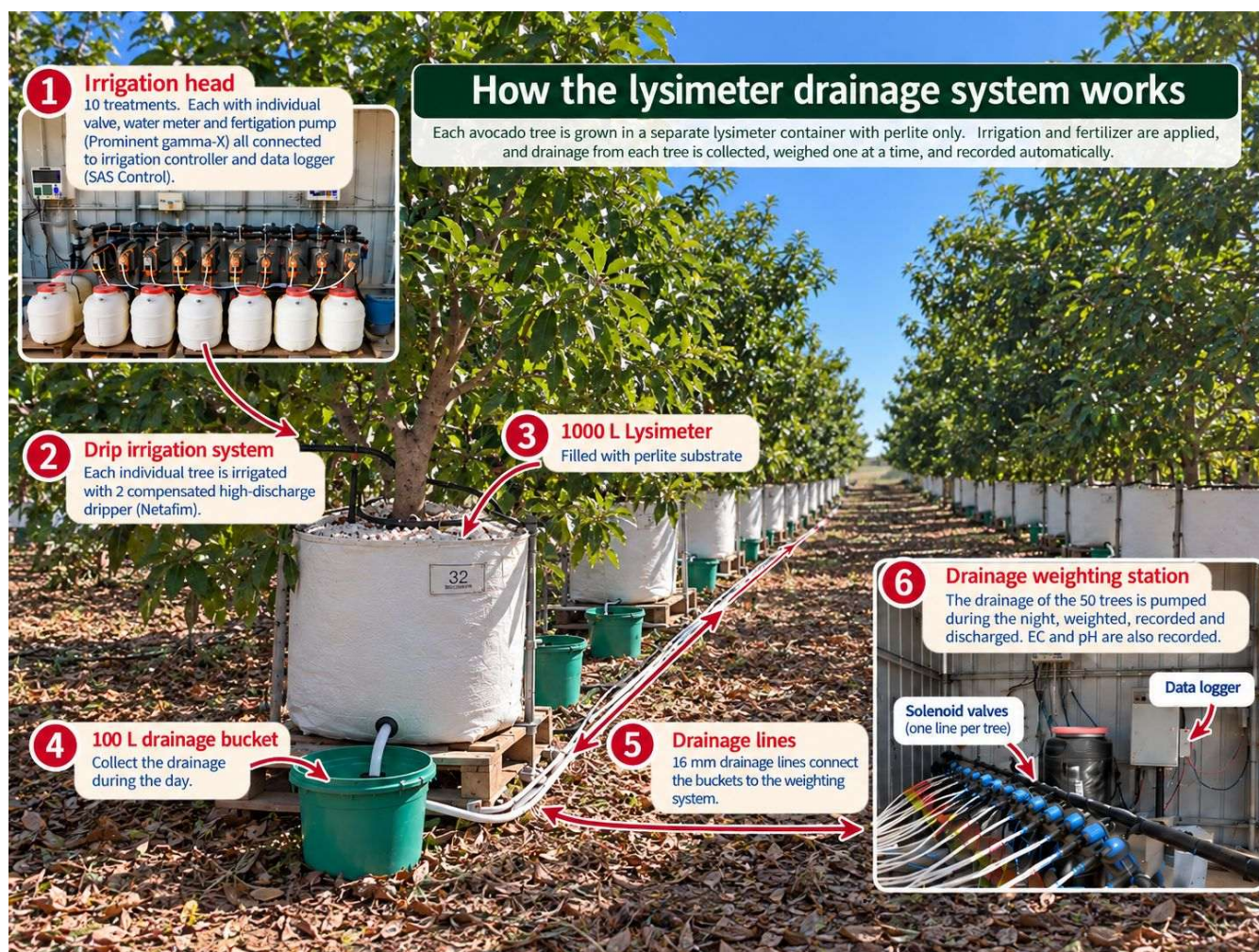


Figure 1: Schematic diagram of the lysimeters platform at Gilat Research Center (summer 2026) with description of the water mass-balance procedure.

Partner and collaborator backgrounds

Dr. Noam Alkan, Ph.D., is a Research Scientist in the Department of Postharvest Science, at the Agricultural Research Organization–Volcani Institute. He has directed the department since 2022 and leads its postharvest fruit pathology laboratory. His avocado research includes assessment of chilling injury, decay, and firmness in ‘Hass’ fruit after cold storage and shelf life; assessment of stem-end rot and side decay after storage (Shimshoni et al., 2020); and a recent study with Dr. Erel linking nitrogen management to avocado yield and postharvest quality (Grozdoz et al., 2026). In the team’s previous avocado nutrition study, fruit from individual trees underwent three weeks of cold storage followed by one week of simulated shelf life, after which internal gray pulp and fungal decay were scored. In the proposed study, Dr. Alkan’s laboratory will assess 20 fruit per tree after 7–8 days of simulated shelf life and relate fruit defects to mineral composition and irrigation-water treatment.

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Project Title: Proactive Laurel Wilt Defense: Rapid Digital LAMP Diagnostics and Integrated Regional Intake for California Avocados

Research priority topic(s) addressed (indicate by using the number shown in the research priorities document): Pest Research (High Priority) / Biosecurity and Invasive Species.

Laurel Wilt isn't in California yet, the fungus and its beetle vectors are moving west, placing our \$1.3 billion industry at risk. We currently lead a 3-year USDA extension grant to conduct orchard audits, trapping, and grower education across California. However, **our USDA grant lacks a rapid diagnostic lab component**. This proposal provides the missing piece of the puzzle: setting up a dedicated California referral lab equipped with a fast digital test that detects Laurel Wilt in under 60 minutes. By funding this proposal, CAC completes our state's defense system, allowing us to spot, test, and stop suspect samples flagged during our USDA orchard audits before an outbreak can take hold in your groves.

Project start date: 1 November 2027

Project end date: 31 October 2028

Project Leader: Fatemeh Khodadadi

Position Title: Assistant Professor of Extension and Assistant Plant Pathologist

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Present Title: Associate Professor

Introduction and Rationale

The devastating impact of Laurel Wilt (LW) on the Florida avocado industry provides a stark \$54 million cautionary tale for California. Since its introduction in 2012, LW has resulted in the loss of more than 300,000 trees, a 60% decline in production, and a 45% reduction in total acreage^{1,2}. This lethal vascular disease is caused by the fungal pathogen *Harringtonia lauricola* (formerly *Raffaelea lauricola*), which is vectored by the invasive Redbay Ambrosia Beetle (*Xyleborus glabratus*)³. The disease results from a tightly coupled vector-pathogen interaction: the beetle introduces the fungus into the sapwood, triggering a host defense response that rapidly disrupts vascular function, leading to canopy wilt and tree death within weeks. Once established, infections spread quickly through root-to-root transmission, often resulting in widespread orchard decline and abandonment⁴. Unlike Florida's "West Indian" varieties, California's industry is dominated by the Hass variety, which is highly susceptible to LW^{5,6}. Although the disease is not yet established in California, the state's microclimates and widespread presence of susceptible Lauraceae species,

including common ornamental trees like Camphor (*Cinnamomum camphora*) and California Bay Laurel (*Umbellularia californica*) intermixed near commercial production regions, create a high-risk environment for rapid establishment and spread if introduced^{7,8}. With the pathogen already detected as far west as Texas, California faces an increasingly imminent risk of introduction. To complement our newly awarded three-year USDA Extension grant, which focuses on grower outreach, orchard audits, and a LW web portal, establishing an official diagnostic lab point of contact is the vital next step to ensure any suspicious cases reported statewide can be immediately evaluated. To make this defense work, California needs an established diagnostic contact point equipped with rapid detection. Traditional culturing and PCR take days or weeks, a delay we cannot afford during an outbreak. This project fills that gap by validating a digital LAMP assay in our lab. As a dedicated referral hub, we will confirm suspect samples from Hass trees, ornamental hosts, or beetle vectors within hours, protecting California's \$1.3 billion industry before an outbreak takes root.

Objectives and Deliverables

1. **Develop Rapid Isothermal Molecular Diagnostics:** Design and validate a high-sensitivity digital LAMP assay capable of detecting *H. lauricola* DNA in under 60 minutes from Hass avocado wood, ornamental host tissues, and ambrosia beetle vectors.
2. **Establish an Operational Regional Diagnostic Hub & Outreach:** Create a streamlined sample intake pipeline for growers, PCAs, and industry via our UC ANR portal, while distributing protocols and findings across our ongoing USDA grant focused on extension events, articles, and web portals.

Methods:

Objective 1: Diagnostic Assay Optimization & Validation: Following protocols established by Dashtarzhaneh et al. (2025), sequence alignments of *H. lauricola* and related fungi will be analyzed to design six target LAMP primers, rigorously screened to prevent false positives from common wood-decay fungi or non-pathogenic endophytes. Reference *H. lauricola* DNA will be obtained from the University of Florida under our active USDA permit. We will optimize testing parameters on digital LAMP microfluidic platforms to establish exact detection limits, benchmarking accuracy against quantitative PCR (qPCR). Assay performance will be validated across real-world plant matrices, including DNA-spiked Hass wood, bark, and leaves, ornamental Lauraceae, and ambrosia beetles (*Xyleborinus saxesenii* and *Euwallacea* spp.), using specialized DNA extraction steps to eliminate chemical inhibitors. Secure chain-of-custody protocols compliant with state regulatory standards will be integrated into our intake pipeline to ensure rapid sample processing.

Objective 2: Sample Intake & Result Dissemination: We will establish an efficient sample intake workflow to process suspect plant tissues and beetle vectors sent in by growers, PCAs, or field inspectors via our UC ANR web portal. Samples arriving at our lab will be logged immediately and processed using our optimized digital LAMP test, delivering confirmed answers

to growers within 24 hours (with qPCR used for secondary confirmation on ambiguous samples). To empower early field detection, we will author step-by-step sampling guides and extension articles. Furthermore, diagnostic findings and project updates will be actively cross-promoted and distributed during every grower meeting, field day, and workshop conducted under our current USDA extension grant, as well as published directly on our dedicated Laurel Wilt web portal.

Timeline/Milestones

Quarter	Key Milestones & Project Activities
Months 1–4	Complete primer design and <i>in silico</i> specificity screening; acquire control DNA/reference targets; optimize core LAMP reaction parameters.
Months 5–9	Transition assay to the digital LAMP platform; determine analytical limit of detection and compare against qPCR; begin plant matrix testing (avocado and ornamentals).
Months 10–12	Optimize DNA extraction workflows for California ambrosia beetle vectors; complete cross-reactivity testing against common avocado pathogens.
Months 1–12	Finalize diagnostic SOPs; fully operationalize the diagnostic point of contact for CAC/industry; integrate lab submission workflows into ongoing USDA orchard audits and extension workshops.

Budget:

Year 1	Budget
GSR Personne (includes salary, benefits, fees etc.) (Salary: \$28,600+ Benefits and fees: \$22000)	\$50,600
Materials, equipment and lab supplies	\$15,000
Travel (weekly trips to field sites [car rental, gas], meetings etc.) and trees	\$4,400
Total Project Budget	\$70,000

Principal Investigator & Collaborator Background

Dr. Fatemeh Khodadadi (UC Riverside) leads an applied plant pathology and diagnostics laboratory dedicated to safeguarding California avocado production against high-consequence

fungal pathogens. Her research focuses on high-sensitivity molecular diagnostic platforms (digital LAMP and ddPCR) and vector-borne pathogen surveillance such as avocado sunblotch viroid and integrated disease management for avocado diseases. Supported by experienced UC Cooperative Extension specialists and regional diagnosticians, the team brings extensive expertise in avocado disease epidemiology and statewide extension outreach. The PI currently lead a USDA-funded project on Laurel Wilt preparedness and orchard auditing in California.

CAC Funding History: The PI previously received funding from the California Avocado Commission for the project titled "*Integrating Chemical and Cultural Practices for Bot Canker Control in Avocado*" (initiated November 1, 2025).

Dr. Romina Gazis-Seregina is an Associate Professor in the Department of Plant Pathology at the University of Florida (UF-IFAS) Tropical Research and Education Center (TREC) in Homestead, holding a split appointment of 40% research and 60% extension. Specializing in tropical plant pathology, she leads the plant pathology program focused on major diseases impacting avocado production in Florida. In this project, Dr. Gazis-Seregina will provide us with potential samples needed for testing.

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Leveraging Existing Rootstock Trials to Determine Effective Leaching Fractions for Improved Salinity Management and Avocado Productivity

Cultural Methods, Research Priority 3: Leaching Fractions with Modern Rootstocks and Water Sources

Principal Investigator: Dr. Patricia Manosalva, Associate Professor, Microbiology and Plant Pathology Department, The Regents of The University of California, patricia.manosalva@ucr.edu.

Co-Principal Investigator: Dr. Aliasghar Montazar, Irrigation and Water Management Advisor, University of California Division of Agriculture and Natural Resources (UC ANR), amontazar@ucanr.edu

INTRODUCTION AND RATIONALE

Salinity and chloride accumulation are persistent challenges for California avocado production, particularly when orchards are irrigated with water containing elevated dissolved salts. Avocado is highly susceptible to salinity, with yield reductions reported when irrigation-water electrical conductivity (EC) exceeds 0.75 dS/m and chloride concentrations exceed 100 ppm. As growers increasingly use alternative water sources, irrigation and leaching practices need to be adjusted to account for differences in water quality. However, determining appropriate leaching requirements remains challenging because they depend on water quality, soil properties, irrigation practices, rootstock salt tolerance, and tree health. Modern rootstocks may differ in salinity tolerance through mechanisms such as ion exclusion, accumulation, or sequestration, potentially affecting root-zone salinity thresholds and leaching needs. Greater rootstock salt tolerance, however, does not necessarily translate to lower leaching requirements.

Table 2. Characteristic of the UCR advanced rootstocks that will be used in this study.

Trait	PP35	PP40	PP80	PP42	PP45
Race	M x G	M x G	M	M	M
Tree size compared to Dusa®	Small or Same	Same	Same	Same	Same
Salinity tolerance	EC = 3.7dS/m Chloride 100-300 ppm	EC = 3.7dS/m Chloride 100-600 ppm	EC = 3.7dS/m Chloride 100-300 ppm	Low	Low
Heat tolerance	Moderate	Moderate	High	Low	High
Tolerance to high pH	pH= 7 – 8.2	pH= 7 – 8.2	pH= 7 – 8.2	pH= 7 – 8.2	pH= 7 – 8.2
Tolerance to CaCO₃	> 200 ppm	> 200 ppm	> 200 ppm	> 200 ppm	> 200 ppm
Resistencia a Phytophthora root rot	High	High	High	Highly resistant better than Duke 7, Dusa®, and other commercial rootstocks	Highly resistant better than Duke 7, Dusa®, and other commercial rootstocks
Tolerance to other avocado diseases	White root rot & laurel wilt	Avocado branch canker	Laurel wilt		
Soil types	Sandy – Loam – Rocky – Clay soils	Sandy – Loam – Rocky – Clay soils	Sandy – Loam – Rocky – Clay soils	Sandy – Loam – Rocky – Clay soils	Sandy – Loam – Rocky – Clay soils
Compatible with other varieties: 'Hass', 'GEM', 'Lamb Hass', 'Reed', 'Carmen', 'Donnie', and 'Sharwil'.					

Table 1. Description of the existing rootstock trials in California that will be used to select the trials for this study based on new water and soil analyses.

County	Rootstock	Field conditions
Temecula/Riverside (2 fields)	Scion: Hass; Rootstock: Field 1 (2019): PP35 (T), PP40 (T); Design: planted by rows/rootstock Field 2 (2021): Dusa (T), PP42 (MT), and PP80(U); Design: planted by blocks/rootstock	2025: Soil salinity EC 1.54 dS/m, Cl ⁻ = 146 ppm; water pH= 8.2, water salinity EC= 0.82 dS/m, Cl ⁻ = 94 ppm, CaCO ₃ = 110 (moderate). High PRR incidence (Orondis). Loamy sand to sandy loam.
Temecula/Riverside (2020)	Scion: Hass; Rootstock: Dusa (T), PP35 (T), PP40 (T), PP45 (S); Design: planted by blocks/rootstock	2025: Soil salinity EC 1.11 dS/m, Cl ⁻ = 119 ppm; Water pH= 8.2, salinity EC= 0.86 dS/m, Cl ⁻ = 95 ppm, CaCO ₃ = 110 ppm (moderate). High PRR incidence (Orondis). High clay composition.
Pala/San Diego (2022)	Scion: Hass, Gem, Lamb-Hass, and Reed; Rootstock: Dusa (T), PP35 (T) PP40 (T), Toro Canyon (T), Steddom (T), PP80 (U); Design: planted by blocks of scions where rootstocks are completely randomized.	2025: Soil salinity EC 1.0 dS/m, Cl ⁻ = 135 ppm, pH 6.8; water EC = 0.34 dS/m, Cl ⁻ = 26 ppm, pH= 7.3. Loamy sand to Loam. Incidence of PRR. No fungicide.
Camarillo/Ventura (2 fields)	Scion: Hass; Rootstock: Field 1 (2019): PP35 (T), PP40 (T); Design: planted by rows/rootstock Field 2 (2021): Dusa (T), PP42 (MT), and PP80(U); Design: planted by blocks/rootstock	2025: Soil salinity EC 3.88 dS/m, Cl ⁻ = 171 ppm; Water pH= 7.4, water salinity EC= 2.23 dS/m, Cl ⁻ = 97 ppm, water CaCO ₃ = 320 ppm (severe problem), Boron = 0.8 ppm possible problem. No PRR incidence. Loamy sand to silt loam. High limestone. No fungicides
Ventura (2020)	Scion: Hass; Rootstock: Dusa (T), PP35 (T), PP40 (T), PP45 (S); Design: planted by blocks/rootstock	2025: Soil salinity EC 1.09 dS/m, Cl ⁻ = 16 ppm; Water pH= 7.5, water salinity EC= 1.01dS/m, Cl ⁻ = 69 ppm, water CaCO ₃ = 380 ppm (severe problem).
Ventura (2020)	Scion: Hass; Rootstocks: Dusa, Leola, Zerala, Steddom, Tami, Uzi, PP35, PP40, PP45, PP42, PP80. Design: planted by blocks/rootstock and by rows/rootstocks.	2025: Soil salinity EC 1.61 dS/m, Cl ⁻ = 64 ppm, Water pH= 7.5, water salinity EC= 1.01dS/m, Cl ⁻ = 69 ppm, water CaCO ₃ = 380 ppm (severe problem).

T= Salinity Tolerant, MT = Salinity Moderate Tolerant, S = Salinity Susceptible, U = unknown, PRR = Phytophthora root rot.

We will leverage existing UCR rootstock trials to select field sites under salinity stress based on current water and soil analyses (Table 1). At each site, we will evaluate 'Hass' grafted onto UCR phytophthora root rot (PRR) resistant rootstocks (Table 2), along with Dusa® or other commercial rootstocks as appropriate. By integrating water and soil characterization, irrigation management, root-zone salinity monitoring, tree physiological and phenotypic assessments, and yield data, this project will determine how rootstocks interact with soil and water properties to influence leaching requirements and tree performance. The goal is to develop practical, science-based leaching recommendations that maintain productivity while improving irrigation-water-use efficiency.

PROJECT OBJECTIVES AND DELIVERABLES. We will determine how rootstock, soil properties, irrigation-water quality, and leaching management affect root-zone salinity, tree performance, and productivity.

01. Characterize seasonal soil and irrigation-water quality changes in root-zone salinity and chloride accumulation across different rootstocks in two different regions.

Deliverables.

- *Comprehensive soil and water characteristics influenced by seasonal changes.*
- *Comparison of the root-zone salinity and chloride accumulation of Dusa® and other commercial rootstocks with the UCR advanced rootstocks.*

O2. Evaluate how contrasting leaching treatments affect soil salinity, chloride accumulation, tree performance, and productivity, and determine whether soon-to-be-released UCR rootstocks differ from Dusa® and other commercial rootstocks in their leaching requirements.

Deliverables

- *Leaching requirements for Dusa® and advanced UCR rootstocks under salinity stress.*
- *Provide practical, rootstock-specific leaching management recommendations to maintain acceptable root-zone salinity and tree performance while minimizing unnecessary irrigation-water applications.*
- *Reduction on production inputs and increase grower profitability.*

METHODS/APPROACH

O1. Soil and irrigation-water samples will be collected at each rootstock trial (**Table 1**) to measure EC, pH, CaCO₃, chloride, sodium, and other ion levels using Fruit Growers Lab (FGL) services. Soil moisture and salinity will be monitored at multiple depths within the active root zone using sensors and periodic soil sampling. Changes in salinity and ion accumulation will be assessed across rootstocks and irrigation treatments to evaluate their effectiveness in managing soil salinity over time.

O2. Three irrigation treatments corresponding to 100%, 115%, and 130% of estimated crop evapotranspiration (ETc) will be implemented across rootstocks grafted to ‘Hass’ at two experimental sites selected based on **O1**. These treatments are designed to establish contrasting irrigation regimes and evaluate the extent to which additional water applications influence root-zone salinity, chloride accumulation, tree performance, and productivity. The 100% ETc treatment will serve as reference, while the 115% and 130% ETc treatments will represent moderate and elevated irrigation applications to promote salt leaching. Treatment levels will be verified against site-specific irrigation efficiency, soil conditions, and crop water requirements to ensure adequate water supply while minimizing the risk of prolonged root-zone saturation. Applied irrigation volumes will be quantified using flow meters. Soil moisture and salinity levels will be monitored at multiple depths to characterize temporal and vertical variations in root-zone conditions. Periodic soil sampling will provide quantitative assessments of salinity and chloride accumulation. Because drainage will not be directly measured, the irrigation treatments will be expressed as percentages of estimated ETc rather than actual leaching fractions. Treatment effectiveness will be assessed based on changes in root-zone salinity, chloride accumulation, tree performance, productivity, and root & leaf sodium and chloride accumulation (**Table 3**) rather than direct drainage quantification or salt export. Linear mixed-effects models (LMMs) will assess the effect of contrasting leaching treatments on soil salinity, chloride accumulation, and rootstock performance.

Table 3. Field site data to be collected	
<i>Data to collect</i>	Weather data; soil and water analyses; tree measurements; phenotypic measurements using the UCR rootstock breeding program scoring system (overall tree health and stressor damage [salinity, heat, cold]); blooming and fruit set scores; physiological measurements.
<i>Once per year:</i>	Total yield and fruit number; yield and fruit number/tree; fruit size; dry weight (48 fruits); yield efficiency (yield/unit of canopy; yield/canopy volume to compare yield across trees with different sizes; cumulative yield to determine alternate bearing; packing data; and other characteristics including fruit size and quality.

TIMELINE/MILESTONES

Obj.	Objective (Lead)/Sub-task Description (performers)	Year 1				Year 2			
1	Characterize irrigation-water quality and quantify seasonal changes in root-zone salinity and chloride accumulation across different rootstocks in two different regions.								
2	Evaluate the effects of contrasting leaching treatments on soil salinity, chloride accumulation, tree phenotypic & physiological responses, and productivity to determine whether leaching requirements differ among rootstocks								
2.1	Leaching treatments								
2.2	Determine if there is specific leaching requirement per rootstock based on changes on root-zone salinity and chloride and other ions accumulation								
2.3	Tree phenotypic and physiological data								
2.4	Harvest data								
2.5	Leaf and root ionomic analyses								

TOTAL PROJECT BUDGET: \$216,402 for two-year project.

	Year 1	Year 2	Total
Estimated Budget for Dr. Manosalva			
Personnel	\$35,173	\$36,229	\$71,402
Travel	\$2,500	\$2,500	\$5,000
Materials and supplies	\$2,000	\$2,000	\$4,000
Equipment (>\$5,000 per item)	\$0	\$0	\$0
Other costs: Ionomic analyses (root and leaf)	\$18,000	\$18,000	\$36,000
Sub Total	\$32,400	\$32,400	\$116,402
Estimated Budget for Dr. Montazar			
Budget Category			
Personnel	\$22,792	\$23,704	\$46,496
Travel	\$2,500	\$2,500	\$5,000
Materials and supplies	\$30,000	\$8,544	\$38,544
Equipment (>\$5,000 per item)	\$0	\$0	\$0
Other costs: Soil and water laboratory analyses	\$4,980	\$4,980	\$9,960
SubTotal	\$60,272	\$39,728	\$100,000
		TOTAL	\$216,402

BUDGET JUSTIFICATION

Patricia Manosalva and Aliasghar Montazar. No salary or benefits are requested by Manosalva and Montazar. They will provide scientific leadership, personnel supervision, grower and team coordination, experimental oversight, data interpretation, and will be responsible for the project reporting activities.

Personnel Costs/Benefits (\$117,898). \$71,402 is requested to cover Manosalva's Assistant Specialist, Amber Newsome, at 35% FTE for the duration of the project (Year 1: [\$23,721 salary & \$11,452 benefits] and Year 2: [\$24,433 salary & \$11,796 benefits]). Amber Newsome oversees the data collection at the UCR rootstock program trials in California. Amber Newsome will continue to monitor and conduct the data collection for the experimental sites for this project as indicated in **Table 2**. She will be working closely with Dr. Montazar and his Staff Research Associate (SRA) which will conduct the irrigation treatments.

A total of \$46,496 will be used to cover salary and benefits of one SRA that will be working under the supervision of Dr. Montazar at 20% FTE for the duration of the project. This SRA will coordinate irrigation treatment implementation at the two experimental sites selected, install, inspect, and maintain irrigation and monitoring systems. This SRA will work in the activities from O2 for the irrigation treatments. The SRA will interface with Amber Newsome regarding the data collection and sample collection and characterization.

Travel (\$10,000). A total of \$2,500 per Year 1 and Year 2 is being requested by the Manosalva and Montazar labs to conduct travel to the experimental sites to conduct the research activities described in this project. These funds will cover gas, mileage, and meals & overnight trips when need it.

Supplies (\$42,544). Manosalva is requesting \$2,000 for supplies in each year of the project. These funds will cover supplies required for the measurements of stem water potential (SWP) and supplies associated with sample collections, water and soil analyses, yield data (harvest bins), and laboratory supplies and consumables to identify pathogens natural occurring in the field at the time of data collection.

Montazar is requesting a total of \$30,000 in Year 1 and \$8,544 in Year 2. These funds will cover the installation, modification, and maintenance of the irrigation systems required to apply three irrigation treatments at each site as described in **O2**. Funds will be used to purchase flow meters to quantify applied irrigation and valves, tubing, regulators, and fittings will enable treatment implementation. The requested amount will also support the purchase of soil moisture and EC sensors, data acquisition system, and associated supplies to monitor the experimental site treatments.

Services (\$45,960). Manosalva is requesting a total of \$18,000 in years 1 and 2 to conduct leaf and root ionomic analyses of the rootstocks at each of the experimental sites to determine sodium and chloride accumulation under the different irrigation treatments. These analyses will be conducted at Fruit Growers' Laboratories (Santa Paula, CA).

A total of \$9,960 (\$4,980/year) is requested by the Montazar lab to cover the cost for all water and soil analyses that will be done periodically at these experimental sites.

Dr. Patricia Manosalva. Dr. Manosalva is an Associate Professor in the Department of Microbiology and Plant Pathology at the University of California, Riverside (UCR). Since 2015, Dr. Manosalva has led the UCR avocado rootstock breeding program, which aims to develop rootstocks with resistance to major avocado pathogens and abiotic stressors, including salinity. Her avocado research focuses on developing short-, mid-, and long-term solutions to address major challenges affecting avocado production.

As part of her short- and mid-term research efforts, Dr. Manosalva has monitored and characterized the genetic and phenotypic diversity of *Phytophthora cinnamomi* populations, the causal agent of avocado Phytophthora root rot (PRR). This work has identified isolates with resistance to commonly used fungicides. In addition, her greenhouse fungicide efficacy trials with several oomycete fungicides have generated data supporting new fungicide registrations. This research is conducted in collaboration with Dr. Jim Adaskaveg, who is currently conducting fungicide efficacy field trials funded initially by the USDA and, more recently, by the California Avocado Commission (CAC), with Dr. Manosalva serving as Principal Investigator.

With funding from the USDA Organic Research and Extension Initiative (OREI), Dr. Manosalva's laboratory has also evaluated essential oils as potential tools for managing pre- and post-harvest avocado diseases in certified organic orchards. In collaboration with scientists from other U.S. and international institutions, her laboratory is evaluating UCR rootstocks for resistance to laurel wilt and white rot diseases.

Dr. Manosalva has maintained a long-standing collaboration with the California avocado industry and growers, who are currently hosting eight on-farm rootstock trials evaluating several advanced UCR rootstocks. Her expertise encompasses the interactions among rootstock genetics, plant health, soil conditions, and irrigation-water quality and how these factors influence tree performance and productivity. This expertise directly supports the proposed research on rootstock responses to salinity and the interactions among rootstock genetics, soil conditions, irrigation-water quality, and tree performance.

The California Avocado Commission (CAC) has historically supported the UCR rootstock breeding program and Manosalva's research through multiple funding cycles since 2015. CAC funding cycles from 2019 to 2025 supported the establishment of large-scale field trials in California using the five most advanced UCR rootstocks (**Table 2**) to generate comprehensive performance data needed for commercial release. The establishment and monitoring of these rootstock trials were supported by CAC and USDA funding, and the proposed research will leverage these established field trials. More recently, Dr. Manosalva and Dr. Adaskaveg received CAC funding for 2025–2028 to assess the risk of *P. cinnamomi* developing resistance to new oomycete fungicides under laboratory, greenhouse, and field conditions. In addition, Dr. Manosalva leads an ongoing California Department of Food and Agriculture (CDFA) Specialty Crop Block Grant Program project (2025–2028) focused on determining the mechanisms underlying salinity tolerance in avocado under both greenhouse and field conditions.

Dr. Ali Montazar. Leads an integrated research and extension program focused on improving water management, resource-use efficiency, and sustainability in California avocado production. His research is conducted primarily in commercial orchards across contrasting Southern California production environments and integrates crop evapotranspiration and crop coefficients, irrigation management and system performance, root-zone soil-water monitoring, salinity assessment, plant-based sensing, UAV imaging, and satellite remote sensing. This multidisciplinary program provides a strong foundation for developing and validating practical irrigation-management tools under commercial orchard conditions. A major component of Dr. Montazar's research has been quantifying actual avocado water use and crop coefficients and determining how they vary with environmental, orchard, and management conditions. This work was published in *Agricultural Water Management* in 2025. His program subsequently evaluated multi-year OpenET estimates against field-measured evapotranspiration in commercial avocado orchards, with results published in *Agricultural Water Management* in 2026. Most recently, his research has advanced satellite-derived canopy-temperature estimation and physiological validation across commercial Southern California avocado orchards, with the associated manuscript currently under review in *Smart Agricultural Technology*. These efforts demonstrate a

progression from crop water-use quantification and field-based irrigation assessment to satellite-based detection, field diagnosis, and grower decision support.

Dr. Montazar has an established history of California Avocado Commission (CAC)-supported research, including a completed 2022–2025 project on avocado crop water use and irrigation management and a current 2025–2028 project examining irrigation-management tools and their effects on avocado fruit quality and yield. He also leads an ongoing CDFA-funded project addressing water and nitrogen use efficiency in avocado production. In parallel, he has led a continuing avocado irrigation workshop and education program for more than six years, translating research findings into practical information for growers and industry professionals. These ongoing research programs provide established commercial-orchard infrastructure, long-term datasets, monitoring capabilities, and grower partnerships.

Building a Precision Agriculture Framework for California Avocado: Leaf Monitor Calibration and Statewide Variability Assessment

Research Priority Topic Addressed	Cultural Methods Priority 12 - Precision Farming: Introduce the concepts of precision farming into avocado production in California
Principal Investigator	Alireza Pourreza, Associate Professor of Digital Agriculture, Department of Biological and Agricultural Engineering, University of California, Davis; apourreza@ucdavis.edu
Partner/Collaborator	Mohsen B. Mesgaran, UC Davis; Aliasghar (Ali) Montazar (UCCE Irrigation and Water Management Advisor)

Introduction and Rationale

Precision farming requires three practical steps: identify where variability exists, diagnose what is causing it, and apply the right management response. This multi-year project will build an avocado framework that connects spatial monitoring, ground diagnosis, and science-based recommendations.

The framework follows four connected steps: remote sensing identifies spatial and seasonal variability; targeted field observations diagnose likely causes; scientific recommendations address the problem; and repeated monitoring evaluates the response. Satellite and aerial imagery show where trees differ, while tools such as the UC Davis Leaf Monitor provide ground-truth information for diagnosis.

1. Detect	2. Diagnose	3. Address	4. Monitor
Satellite / aerial imagery Locate persistent variability	Leaf Monitor + field observations Identify likely causes	Science-based recommendations Target management by zone	Repeat sensing Evaluate response over time

Precision agriculture framework. Year 1 establishes the statewide variability screening and avocado-specific Leaf Monitor calibration needed for the first two steps; later years will connect these tools to targeted recommendations and management-zone validation.

Year 1 will establish two core components. First, we will calibrate and validate [Leaf Monitor](#) for avocado. The existing [UC Davis system](#) uses a portable full-range spectrometer, a smartphone application, cloud-based AI models to estimate leaf nutrients, and a web application. Preliminary cross-crop results show that models trained on one crop may not perform optimally on another, while performance improves when target-crop data are added. Avocado-specific spectral and laboratory measurements are therefore needed.

Second, we will use multi-date satellite imagery to quantify within-grove variability across California avocado production, determine whether patterns persist through time, and identify groves with the strongest potential for precision management and zoning.

Year 1 will also estimate the production and economic opportunity associated with persistent variability. Where yield records are available, satellite patterns will be compared with yield differences. At the broader scale, scenario analysis using industry yield and crop-value information will estimate the potential yield and gross-income opportunity if consistently underperforming areas can be moved toward typical grove performance.

Objectives and Deliverables

Objective 1. Calibrate and validate Leaf Monitor for avocado.

Develop paired avocado leaf spectra and laboratory nutrient measurements, evaluate transfer of the existing model, and train or fine-tune avocado-specific models where needed.

Objective 2. Quantify statewide within-grove variability from satellite imagery.

Use multi-date satellite data to measure the magnitude and persistence of within-grove variability and identify where precision management has the greatest potential.

Objective 3. Establish the Year 1 precision-agriculture opportunity baseline.

Develop a framework that integrates Leaf Monitor observations with remote sensing to define economically justified management zones and guide field monitoring and precision management.

Year 1 Deliverables

- Avocado-specific paired spectral and laboratory nutrient dataset and validated nutrient models for supported outputs.
- Smartphone application: integration of the validated avocado model so users can collect spectra in the grove and receive rapid nutrient estimates in real time.
- Web application: integration of avocado models so users can upload, review, organize, and analyze spectral measurements and nutrient estimates online.
- Statewide satellite assessment of within-grove variability, persistence, and potential management-zone structure.
- Precision-agriculture framework for later years linking satellite/aerial monitoring, directed scouting, Leaf Monitor diagnosis, recommendations, and response monitoring.

Methods/Approach

1. Avocado Leaf Monitor calibration

Leaf samples will be collected from cooperating commercial avocado grove(s) across the growing season to capture natural variation in tree vigor, nutrient status, soil, irrigation, and management. Representative leaves will be scanned with the existing full-range spectrometer and then submitted for laboratory analysis of N, P, K, Ca, Mg, B, Zn, Mn, Fe, and Cu. Mean spectra will be paired with the corresponding laboratory results for model development.

The existing Leaf Monitor model will first be tested without avocado adjustment. Avocado data will then be used for avocado-only training and fine-tuning of the existing multi-trait model. Spectra will follow the established Leaf Monitor quality-control and preprocessing workflow. A portion of the data will be reserved for validation, with performance reported by nutrient. Supported models will be integrated into the smartphone and web applications.

2. Statewide satellite assessment of avocado variability

Available avocado grove boundaries will be assembled. Time-series Sentinel-2 imagery will characterize canopy reflectance and vegetation-index patterns. For each grove, we will quantify spatial variability and determine whether the same low- and high-performing areas persist over time, separating short-term variation from recurring patterns useful for management zoning. Groves will be summarized using comparable variability indicators. Where supporting information such as grove age, variety, or location is available, comparisons will be made among similar groves. The result will be a statewide baseline showing where precision monitoring and zoning are most likely to provide value.

3. Precision-farming opportunity and multi-year development

The satellite analysis will identify where variability occurs, and Leaf Monitor will provide one of the tools needed to diagnose it on the ground. In later years, satellite screening will be combined with higher-resolution aerial imagery and directed scouting to determine whether zones are associated with nutrition, irrigation, soil, pest pressure, or other factors. Recommendations can then be developed and tested by zone, followed by repeated monitoring.

Year 1 will also develop a preliminary economic-opportunity analysis. Where reliable yield records are available, persistent satellite zones will be compared with yield. Statewide, industry yield and value benchmarks will be used in scenarios that estimate the potential yield and gross-income opportunity if underperforming zones improve toward typical grove performance.

Timeline/Milestones

Period	Planned work
Early project period	Confirm cooperating grove(s), laboratory arrangements, grove-boundary sources, and field protocol; assemble satellite archive.
Growing season	Collect avocado leaf spectra and laboratory samples; continue satellite time-series processing and statewide variability screening.
Mid-project	Evaluate Leaf Monitor transferability; quantify grove-level variability and persistence; identify candidate management-zone patterns.
Later project period	Train/fine-tune and validate avocado models; complete statewide variability summaries and preliminary yield/income opportunity scenarios.
Final phase	Integrate supported avocado models into smartphone/web applications; report Year 1 findings and define targeted aerial/ground validation for the next project phase.

Estimated Total Budget

Estimated total request: \$69,000

Category	Estimated amount
Personnel costs	\$39,000
Travel	\$3,000
Materials and supplies	\$8,000
Equipment	\$0
Other costs - GSR tuition and fees	\$19,000
Total	\$69,000

Budget Justification

Personnel costs: Graduate Student Researcher (50% FTE) salary and associated fringe benefits. Responsibilities will include field data collection, spectral processing, satellite data analysis, model development and validation, statewide variability analysis, reporting, and presentations.

Travel: Travel to cooperating California avocado grove(s) for seasonal sampling, sample transport, and project-related grower or industry meetings.

Materials and supplies: Laboratory leaf nutrient analyses, field and sampling consumables, sample handling and shipping, instrument calibration/cleaning supplies, and cloud computing/storage for satellite processing and model training.

Other costs: Graduate Student Researcher tuition and fee remission.

Equipment: No equipment is requested. The UC Davis Digital Agriculture Laboratory will provide the existing full-range spectrometer, Leaf Monitor smartphone and web applications, modeling infrastructure, and computing resources. Higher-resolution aerial data collection is planned for later project phases and is not requested in this Year 1 budget.

No indirect or overhead costs are included. Only direct research costs are requested.

Principal Investigator and Collaborator Background

Alireza Pourreza - Principal Investigator

Alireza Pourreza is an Associate Professor and Extension Specialist of Digital Agriculture in the Department of Biological and Agricultural Engineering at the University of California, Davis, and Director of the UC Davis Digital Agriculture Laboratory. His research focuses on precision agriculture, proximal and remote sensing, spectroscopy, artificial intelligence, spatial crop monitoring, and development of practical decision-support technologies for specialty-crop production.

Pourreza's research program combines field sensing with satellite, aerial, and proximal measurements to characterize crop variability and convert sensing information into management decisions. His group has developed methods for mapping within-field variability, monitoring crop nutrition, detecting stress and disease, estimating yield, and evaluating spatial management zones in California specialty crops. This experience directly supports the proposed avocado precision-agriculture framework, which requires both large-area remote sensing and targeted ground diagnostics.

Pourreza's group developed the Leaf Monitor platform and its smartphone, web, cloud-computing, and spectral-modeling components. The underlying research includes hyperspectral methods for estimating leaf nutrients and biochemical traits in grape, almond, pistachio, walnut, and other crops. A recent grapevine study used approximately 1,000 samples collected over three growing seasons and developed a multi-trait model for simultaneous estimation of multiple leaf traits. Related almond research combined hyperspectral radiative-transfer modeling and machine learning for leaf nitrogen assessment.

The proposed avocado project builds on these existing capabilities rather than developing a sensing system from the beginning. Year 1 will adapt Leaf Monitor to avocado and use satellite data to establish a statewide picture of within-grove variability. Subsequent phases will integrate satellite and aerial monitoring with directed ground scouting, diagnosis, management-zone recommendations, and response monitoring.

California Avocado Commission funding history: N/A

Mohsen B. Mesgaran - Collaborator

Mohsen B. Mesgaran is an Associate Professor in the UC Davis Department of Plant Sciences. His research includes plant physiology, crop production, field experimentation, plant-environment interactions, and quantitative analysis of crop responses to stress. His role in this project will include input on field sampling, interpretation of plant responses, and development of scientifically grounded hypotheses for follow-up diagnosis of spatial variability.

California Avocado Commission funding history: N/A

Additional avocado expertise and industry participation

Aliasghar (Ali) Montazar (UCCE Irrigation and Water Management Advisor) will serve as a collaborator on the project. His expertise in avocado irrigation, water and nutrient management, sensor-based monitoring, and remote sensing will support grove selection, interpretation of spatial variability, and development of practical management recommendations. He is currently leading several avocado research projects related to irrigation management, water and nitrogen use efficiency, crop water use, and impacts on yield and fruit quality. UC Agriculture and Natural Resources, CAC, and participating growers will also be important partners in identifying representative groves and, where available, providing management and yield information to support the statewide variability and economic-opportunity analyses.

Relevant project capabilities

- Leaf-level hyperspectral nutrient sensing and multi-trait machine learning.
- Satellite and aerial remote sensing for specialty crop monitoring and within-field variability analysis.
- Development of smartphone, web, and cloud-based agricultural decision-support tools.
- Field validation of sensing models across California specialty-crop production systems.
- Integration of remote sensing with directed ground scouting and spatial management-zone concepts.



Project pre-proposal

21 October 2026

Investigating host status of GEM® and Lamb Hass avocados to fruit fly

Research Project Title: Investigating host status of GEM and Lamb Hass avocados to fruit fly

Research Priority Topic: Pest research high priority topic #19 Fruit flies

California has seen an unusually high number of fruit fly invasions in recent years. Hass avocados are recognized by the USDA as a non-host of fruit flies in their mature, hard green state on the tree, but this status cannot be extended to other varieties without data to support each variety's non-host status. Data are needed for GEM, Lamb Hass and other minor varieties to establish their non-hosts status. Ideally, these data will be collected in areas where fruit fly species of importance (Oriental fruit fly, Mediterranean fruit fly, Mexican fruit fly, Queensland fruit fly) are naturally occurring, in cooperation with local cooperators to avoid having to establish colonies in quarantine facilities in California.

Primary Researcher(s): Dr Elizabeth Dann, liz@horthealth.au

Research Institution: Hort Health Pty Ltd, PO Box 5182, Kenmore East, QLD 4069, Australia

Collaborators: Dr Brendan Missenden, Dr Pauline Wyatt and staff (Market Access team, Queensland Department of Primary Industries, Brisbane), <https://www.dpi.qld.gov.au/business-priorities/agriculture/research/teams-facilities/market-access>. This team has extensive experience with host status trials and disinfestation studies to support domestic and international trade of horticultural produce.

Dr Peter Follett (USDA-ARS, Hilo, Hawaii), has expertise in host status studies and approaches for minimising threats from quarantine pests.

Avocado growers.

Introduction and Rationale:

Californian GEM avocado orchards are subjected to quarantine treatment protocols whenever there is an incursion of fruit fly (including Oriental fruit fly, Mediterranean fruit fly, Tau fruit fly, Mexican fruit fly and Queensland fruit fly) detected in the state. This is because there are no data to demonstrate the susceptibility or resistance of hard green fruit on trees to various fruit fly species. This situation contrasts strongly to Hass, which is recognized by USDA and California Dept Food and Agriculture as a non-host (in hard green unblemished condition) for fruit flies, and as such, there are no quarantine restrictions (pre-movement treatment requirement) for green Hass fruit.

Queensland fruit fly (Q-fly, *Bactrocera tryoni*) is endemic to eastern Australia, originating in the rainforests of Queensland and northern NSW. It is one of Australia's most damaging horticultural pests, affecting over 100 fruit and vegetable species. The Market Access team within the Queensland Department of Primary Industries (QDPI), has worked extensively with the

horticultural industries to investigate host status and disinfestation strategies of different horticultural fruit crops. Mature hard-green Hass is known to have non-host status, however, the studies have not been undertaken for GEM or Lamb Hass. The Market Access team maintain colonies of fruit flies and have dedicated equipment, laboratories and staff in Brisbane, QLD to support research on host status testing.

Preliminary no-choice laboratory cage trials with harvested mature GEM fruit were undertaken July-September 2026, demonstrating that Q-fly could not complete its lifecycle when gravid females were placed in cages with intact GEM fruit (hard green, unripe Day 0 and Day 2 after harvest or ripe, coloured fruit at Day 10 after harvest). However, the laboratory cage trial may not reflect what happens in the field.

Objectives and Deliverables:

To determine the host status of GEM and Lamb Hass avocados under field conditions

Methods/Approach:

Methodology will follow guidelines for host status determination as per the FAO's ISPM 37 and RSPM No. 4, as well as other key publications.

1. Preliminary oviposition and fecundity tests

A preliminary field infestation with only preferred host (e.g. organically-grown red peppers or Valencia oranges) and/or artificial eggging cups, will be undertaken in early July to determine effects of cool to cold conditions on fruit fly oviposition. This will determine approximate dates for avocado field infestation trials, and possibly numbers of gravid females required per cage.

Immediately prior to each field trial infestation, potential oviposition (i.e. egg load) will be determined by transferring 10 gravid female flies to a cage and exposing a prepared eggging cup for 24 h, replaced with a fresh cup for another 24 h. Eggs will be washed from the cups and numbers of eggs/ml estimated. Aliquots containing about 200 eggs will be transferred to moist filter paper over carrot media, and examined over 3 days to estimate the mean number of viable eggs per female. There will be 5 replicates.

2. Semi-natural field cage trials with intact fruit

Collaborators with orchards at Glass House Mountains (about 80 km north of Brisbane) and Ravensbourne (about 130 km north west of Brisbane), growing GEM and Lamb Hass varieties have been identified. About 10 trees of each variety will be tagged and excluded from grower pesticide spray programs. Individual GEM or Lamb Hass fruit will be bagged 6 weeks prior to field tests to exclude insects and risk of mechanical damage.

Batches of *Bactrocera tryoni* will be provided by project collaborators from the Market Access Team, Queensland DPI. Fruit fly colonies are typically maintained for 2 years, then replaced. It is unlikely that F3-F5 generation flies will be available, due to the lengthy process involved with culturing wild flies to maintain a population stable enough to conduct host status tests.

For infestation, individual intact (blemish-free) hard green fruit will be enclosed in cylindrical sleeve cages and sugar and water will be provided in each cage. Females will be introduced to cages on trees to provide an oviposition pressure of at least 1,500 viable eggs per cage, (RPSM No. 4, approx. 25-50 gravid females). An infestation period of 48 h will allow for lab-reared flies to acclimatise in the field (as per APPPC RPSM No. 4). Cages will be checked at 24 h for numbers of dead flies, which will be replaced with females from the same cohort to maintain the same infestation pressure (ISPM 37). Maximum and minimum temperatures will be recorded for each

day during the trial. High adult mortality likely indicates unfavourable conditions for fruit fly survival.

For each variety, there will be 20 (?) fruit* infested for each of two collection times. It is important that blemish-free fruit are chosen for infestation. GEM will be infested in July and again in August, and Lamb Hass in September and October. Extra fruit will be collected and processed to determine dry matter content.

For each variety and collection time, there will be 5 replicates of organically-grown preferred host fruit (e.g. red peppers or Valencia oranges), suspended from a branch in netting produce bags, enclosed in cylindrical sleeve cages and infested with flies as described for avocados. These fruit will be punctured with an entomological pin (as in RSPM No. 4 and ISPM 37).

After the infestation period, fruit will be removed and taken to the laboratory. Each fruit will be weighed, firmness recorded using a penetrometer, and transferred to individual ventilated boxes containing a layer of moist vermiculite for larval development. Boxes will be maintained at approximately 25-26°C and 70% relative humidity.

At 2 and 3 weeks after incubation, vermiculite will be sieved to separate and count pupae. Fruit will be maintained under these conditions until no more pupae are recovered. Recovered pupae will be transferred to ventilated, meshed cups containing moist vermiculite for adult emergence. Finally, fruit will be dissected and examined for presence of larvae before being discarded.

Data will be appropriately analysed and final report prepared.

References:

FAO APPPC. (2005). Guidelines for the confirmation of non-host status of fruit and vegetables to Tephritid fruit flies. *Regional Standards for Phytosanitary Measures, No. 4.* (RSPM No. 4).

FAO IPPC. (2016). Determination of host status for fruit flies (Tephritidae). *International Standard for Phytosanitary Measures, 37.* (ISPM 37).

Follett, P. A., Asmus, G., Hamilton, L. and Stockton, D. (2022) Susceptibility of 'Malama' avocados to *Bactrocera dorsalis* (Diptera: Tephritidae) in Hawai'i and a proposed systems approach for market access, *Journal of Economic Entomology* 115:1894-1900.

Notes/questions

- *B. tryoni* colony may not be F5 or younger generation. F32 and F33 were used for cage trials in 2026, and there was good infestation of preferred host. The Market Access team replenish their colonies periodically.
- A complication here is Fruit Spotting and Banana Spotting Bugs (FSB, BSB), which are very severe pests on the east coast, and growers need to spray insecticides to manage the damage. For fruit fly work, fruit on trees need to be unsprayed for about 6 weeks prior to collection, and probably bagged as well to protect from stings and/or other damage. QFly can use the wounds created by these bugs to lay eggs, and in such cases can complete their lifecycle. Samples of fruit can be sent for analysis of insecticide residues if necessary (to show low or zero residue)
- Fruit fly trapping and collection of fruit to assess natural host status has not been included in the proposal at this stage, primarily due to the limited value given the widespread use of pesticides for FSB (discussed above)
- Timing of field infestations may need to be altered to suit fruit fly activity (although this means that DM will be higher, and maybe outside of commercial practice). Flies will be lab reared at 26°C, and subjected to cool-cold temperatures in the field. In Queensland, GEM

are usually harvested July-August, and Lamb Hass in August-September. See average min and max for 1996/99-2026 below. This timing is a bit different to when these cultivars would be picked in California, i.e. GEM March through August/September (peak April-July) and Lamb Hass late May through Nov/December, peak July-September. Temperatures during harvest in California are generally a bit warmer than south east Queensland.

- GEM Glass House Mountains (Beerburum Forest) June 10.8-21.7°C; July 9.5-21.7°C; August 9.6-23.4°C
- Lamb Hass Ravensbourne (Toowoomba airport) July 6.7-16.7°C; August 7.7-18.9°C; September 10.6-22.4°C.
- San Joaquin March 8-21°C; April 9-25°C; May 12-30; June 15-34°C; July 18-36°C; August 17-36°C; September 15-33°C.
- Ventura March 9-18°C; April 10-19°C; May 12-21; June 14-22°C; July 16-24°C; August 16-24°C; September 15-24°C

DRAFT

Milestones:

Year 1 (May 2027 to October 2027)			
Milestone	Activities	Scheduled Completion	Budget (USD)^a
1	Finalise trial protocol, secure access to orchards, facilities, equipment, flies	May 2027	\$6,300
2	- Preliminary field trial with small number of preferred host fruit (organic red pepper, Valencia oranges) - Bag GEM fruit to exclude insects - Commence first GEM field infestation trial	July 2027	\$14,000
3	- Bag Lamb Hass fruit to exclude insects - Complete first GEM trial, report results - Commence second GEM field infestation trial	August 2027	\$14,000
4	- Complete second GEM trial, report results - Commence first Lamb Hass field infestation trial	September 2027	\$14,000
5	- Complete first Lamb Hass trial, report results - Commence second Lamb Hass field infestation trial	October 2027	\$14,000
Year 1 Total:			\$62,300
Year 2 (November 2027 to January 2028)			
6	Complete second Lamb Hass field infestation trial, report results	November 2027	\$11,700
7	Complete final report	January 2028	\$10,000
Year 2 Total:			\$21,700
Total Project Budget:			\$84,000

Budget:

Year 1 (Nov 2026 to Oct 2027)	Amount (USD)^a
Personnel time	\$46,400
Travel	\$3,600
Facilities, equipment and supplies	\$5,800
Fruit compensation (GEM)*	\$6,500
Total Year 1	\$62,300
Year 2 (Nov 2027 to Oct 2028)	
Personnel time	\$15,200
Fruit compensation (Lamb Hass)*	\$6,500
Total Year 2	\$21,700
Project Total	\$84,000

* maximum estimated based on compensating for all fruit from 20 trees if infestation trials extend beyond the regular commercial harvest period.

^a Calculated based on exchange rate of USD 1 = AUS 0.725 (the ANZ bank conversion rate is higher than the rates reported online, e.g. Google finance).



Prepared by Elizabeth Dann, Hort Health Pty Ltd.

Determining specific genetic markers for ‘Carmen Hass/Mendez No1’ avocado

The availability of molecular tools to identify genetic differences among genotypes is essential for breeding programs and the management of genetic resources (Kämper et al., 2021), as these tools support both crop improvement and the conservation of genetic diversity. In avocado, after more than a century breeding programs (Lahav et al., 2024), a wide range of molecular markers have been used to identify accessions and clarify genetic relationships, including minisatellites, VNTRs, RAPDs, RFLPs, SSRs (Alcaraz & Hormaza, 2007), and SNPs (Kuhn et al., 2019; Talavera et al., 2019; Rubinstein et al., 2019; Rendón-Anaya et al., 2019; Solares et al. 2022; Berdugo-Cely et al., 2023; Cañas-Gutiérrez et al., 2025).

Despite these advances, global production remains highly dependent on a limited number of cultivars, with a single cultivar, ‘Hass’ accounting for approximately 90% of the global avocado market (Pérez et al., 2025; Talavera et al. 2019). In addition to most cultivars developed by selection from seedlings, occasional spontaneous somatic mutations (bud sports) have been reported in avocado (Lahav et al., 2024). In the case of ‘Hass’ examples include ‘Carmen Hass’/‘Mendez No 1’, ‘Jimenez-1’, ‘Jimenez-2’ and ‘Flor de Maria’ in Mexico, ‘Andes 3’ and ‘Andes 4’ in Chile or ‘Turner Hass’ in Australia. The most economically important of those mutants is ‘Carmen Hass’, which is grown in several countries, due to its ability to produce fruit outside the typical ‘Hass’ season, allowing growers to access premium market prices. This cultivar originated from a spontaneous sport identified by the observation of its early flowering compared to ‘Hass’ in Uruapan, Michoacan, Mexico. Because these bud sports generally result from single or very few mutations, distinguishing them at the molecular level remains challenging. Consequently, currently available fingerprinting methods based on molecular markers have not been able to unequivocally distinguish ‘Carmen Hass’ from ‘Hass’.

To address this challenge, we propose the use of whole-genome resequencing at 30x coverage to identify Single Nucleotide Polymorphisms (SNPs) specific to ‘Carmen Hass’ and validate them using complementary molecular approaches to determine the most suitable method for routine application. This approach should provide higher resolution and accuracy, particularly given the availability of recently published reference genomes (Nath et al. 2022; Solares et al, 2023), making the detection of these markers more feasible. This strategy is expected to enable the identification of validated molecular markers capable of reliably distinguishing ‘Carmen Hass’ from ‘Hass’ fruit throughout the supply chain.

● Materials and Methods.

Leaf tissue will be collected from a total of 10 avocado trees each of the ‘Carmen Hass’ and ‘Hass’ cultivars provided by The California Avocado Commission. Samples will be obtained from trees showing clear phenotypic differences in the field. Genomic DNA will be extracted from dried young leaves using the Qiagen DNeasy plant Mini kit. DNA quality will be determined using a NanoDrop spectrophotometer (ND-1000; Thermo Scientific, New York, USA), while DNA integrity and fragment size will be evaluated by 1% agarose gel electrophoresis.

Paired-end sequencing libraries will be prepared following the NovaSeq PE150 library preparation protocol and sequenced on the Illumina platform. Raw sequencing reads will be processed using Trimmomatic (0.39) (Bolger et al. 2014) and mapped to the reference genome (Nath et al. 2022) using BWA-mem (2.2.1) (Li, 2013). PCR duplicates will be identified and removed using GATK MarkDuplicates (Poplin et al. 2018). Variant discovery will be conducted using GATK Best Practices (Van der Auwera & O'Connor, 2020) using HaplotypeCaller. Variant files from all chromosomes will be concatenated and filtered to retain high-quality biallelic SNPs using GATK SelectVariants and VariantFiltration with hard-filtering criteria. VCFtools (0.1.14) (Danecek et al. 2011) will be used to remove missing data. Only variants passing all filtering criteria will be retained.

Genetic relationships between the samples will be analysed using Principal Components Analysis (PCA) and phylogenetic approaches using R. Putative private SNPs (specific to 'Carmen Hass') will be identified and validated using DNA extracted from fruit skin of 'Carmen Hass' using the DNA extraction kit indicated above. Different validation approaches, such as Kompetitive Allele-Specific PCR (KASP) (He et al. 2014), High-Resolution Melting (HRM) (Erali & Wittwer 2010) or Sanger sequencing (Hyten et al. 2010) will be evaluated to identify the most reliable and cost-effective method. For this purpose, specific primers will be designed, and the protocols will be optimized for each validation method.

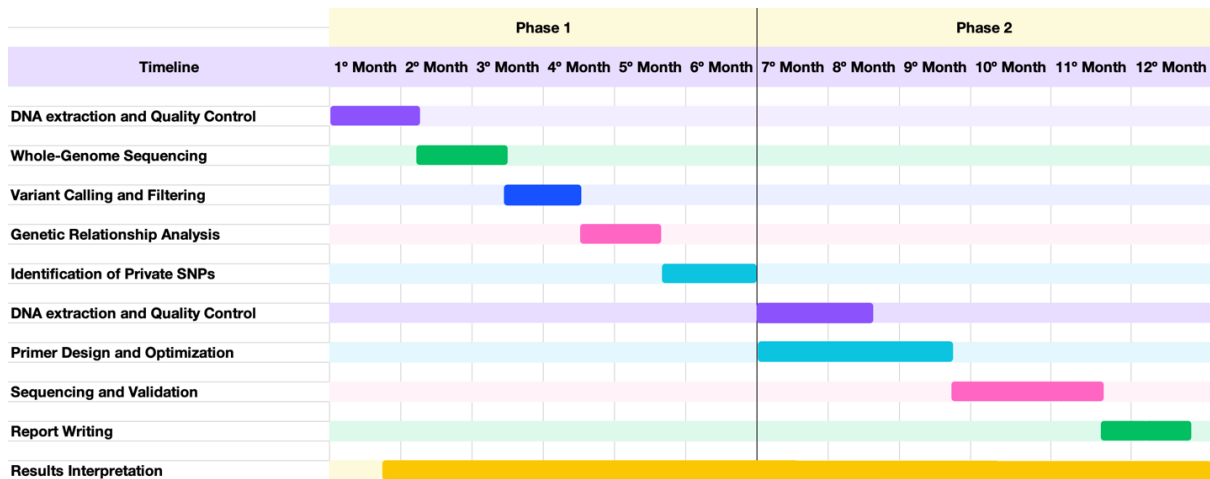
- **Contingency plan.**

While most bud sports in fruit trees result from DNA sequence mutations, some are driven by stable epigenetic modifications, including changes in DNA methylation or histone modifications, that alter gene expression without changing the underlying DNA sequence of the plant (Zhou et al., 2023). Therefore, it is possible that the phenotypic differences between 'Carmen Hass' and 'Hass' are epigenetic rather than genetic. If whole-genome resequencing does not identify robust cultivar-specific genetic markers, we propose to extend this work to investigate epigenetic differences between the two cultivars using appropriate epigenomic approaches.

- **Budget.**

	Price \$
Phase 1	19,500
Phase 2	21,250
Indirect cost	9,556
Total	50,306

- **Timeline and milestones.**



- **Research Team and scientific contributions.**

This project will be executed by a group of three scientists from La Mayora Institute of Subtropical and Mediterranean Horticulture (IHSM La Mayora) with extensive experience in genetics, genomics and bioinformatic analyses in avocado and other subtropical fruit crops: Alicia Talavera (orcid: [0000-0002-1285-7891](https://orcid.org/0000-0002-1285-7891)), Noé Fernández-Pozo (orcid: [0000-0002-6489-5566](https://orcid.org/0000-0002-6489-5566)) and Iñaki Hormaza (orcid: [0000-0001-5449-7444](https://orcid.org/0000-0001-5449-7444)). The group has considerable expertise in the identification and characterizing of avocado cultivars from different collections worldwide and has been involved in numerous avocado projects, as well as the development and maintenance of Avobase, a web portal dedicated to avocado genomics (https://www.avocado.uma.es/easy_gdb/index.php). Below we attach some of the publications of our group since 2019 on avocado research.

- Alcaraz ML, Hormaza JI (2021). Fruit set in avocado: Pollen limitation, pollen load size, and selective fruit abortion. *Agronomy*, 11(8), 1603.
- Alcaraz ML, Hormaza JI (2024). Inadequate pollination is a key factor determining low fruit-to-flower ratios in avocado. *Horticulturae*, 10(2), 140.
- Beiro-Valenzuela MG, Serrano-Garcia I, Monasterio RP, Moreno-Tovar MV, Hurtado-Fernandez E, Gonzalez-Fernandez JJ, Hormaza JI, Pedreschi R, Olmo-García L, Carrasco-Pancorbo A (2023). Characterization of the Polar Profile of Bacon and Fuerte Avocado Fruits by Hydrophilic Interaction Liquid Chromatography–Mass Spectrometry: Distribution of Non-structural Carbohydrates, Quinic Acid, and Chlorogenic Acid between Seed, Mesocarp, and Exocarp at Different Ripening Stages. *Journal of agricultural and food chemistry*, 71(14), 5674-5685.
- Cañas-Gutiérrez GP, Alcaraz L, Hormaza JI, Isaza REA, Saldamando-Benjumea CI (2019). Diversity of avocado (*Persea americana* Mill.) cultivars from Antioquia (Northeast Colombia) and comparison with a worldwide germplasm collection. *Turkish Journal of Agriculture and Forestry*, 43(4), 437-449.
- Hormaza JI, Garner LC, Lovatt CJ (2024) Reproductive Biology. In: The Avocado: Botany, Production and Uses. 3rd edition. D. Carrillo, B. Schaffer, A. W. Whiley, B.N. Wolstenholme. CABI, Wallingford, UK. DOI: 10.1079/9781800621824.0000

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- Pérez V, Larranaga N, Alcaraz ML, Hormaza JI (2025) Genotyping and diversity analysis of local avocado landraces in La Palma, Canary Islands. *Frontiers in Plant Science* 16: 1572870.
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- Pliego-Alfaro F, Hormaza JI, Pliego C, Folgado R, Barceló-Muñoz A, López-Gómez R, Mercado JA, Narváez I, Palomo-Ríos E, Litz RE (2024) Biotechnology. In: The Avocado: Botany, Production and Uses. 3rd edition. D. Carrillo, B. Schaffer, A. W. Whitley, B.N. Wolstenholme. CABI, Wallingford, UK. DOI: 10.1079/9781800621824.0000
- Rodríguez L, Hormaza JI, Laich F, Pérez V, Medina-Alonso MG, Ríos D (2026). Determination of the Genetic Diversity of Avocado (*Persea americana* Mill.) Germplasm in the Canary Islands (Spain) Using Morphological and ISSR Molecular Markers. *Horticulturae*, 12(2), 182.
- Sagili RR, Chakrabarti P, Melathopoulos A, Delaplane KS, Dag A, Danka, RG, Freitas BM, Garibaldi, LA, Hormaza JI, Steinhauer N. (2024) Standard methods for pollination research with *Apis mellifera* 2.0. *Journal of Apicultural Research* 64: 612-646.
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PHASE 1 RESEARCH PROPOSAL
Genomic Identification and Molecular Marker Development
for the Unique Identification of ‘Hass’ and ‘Carmen’ Avocado

Submitted to: California Avocado Commission

Principal Investigator: Luis Herrera-Estrella

Co-Investigators: Dr. Gerardo Alejo Jacuinde

Project Duration: 18 months | Total Budget: \$331,166

Executive Summary

The ‘Hass’ and ‘Carmen’ avocado varieties are morphologically indistinguishable by vegetative and fruit characteristics, differing principally in flowering time. This phenotypic similarity creates critical vulnerabilities across the supply chain, from nursery certification and orchard management to post-harvest variety verification and intellectual property protection, with direct economic consequences for California growers, packers, and retailers. Phase 1 of this two-phase research program will deliver a definitive set of validated genetic markers that unambiguously distinguish ‘Hass’ from ‘Carmen’ at the DNA level, grounded in haplotype-resolved, chromosome-level genome assemblies of both varieties. Our preliminary genomic analysis has already confirmed that ‘Carmen’ harbors distinct structural architectures and allelic variations that are entirely absent from the existing public ‘Hass’ reference genome, underscoring the absolute necessity of de novo assembly for both varieties before robust markers can be identified. By the end of Phase 1, we will deliver a validated, commercially deployable panel of 3–5 diagnostic molecular markers, establishing the scientific foundation for the field-ready testing procedures to be developed in Phase 2.

Problem Statement and Industry Relevance

California produces most of the avocados grown in the United States, with ‘Hass’ accounting for most commercial production. ‘Carmen’ (also known as ‘Mendez’) has emerged as a variety of growing commercial interest, valued particularly for its early-flowering phenology, which offers advantages for orchard management and market scheduling. However, the near-identical leaf morphology, tree architecture, and fruit characteristics of the two varieties mean that visual inspection cannot reliably distinguish them at any point in the supply chain.

This creates three categories of industry risk that a validated DNA-based marker system would directly address:

- **Nursery and orchard integrity:** Mislabeled planting material leads to unintended variety mixing, with consequences for orchard management, pollination strategy, and harvest scheduling that may not become apparent for years.
- **Post-harvest and supply chain verification:** Without a rapid, reliable testing method, packing houses and retailers cannot confirm the variety identity of incoming fruit, creating exposure to mislabeling claims and brand integrity issues.
- **Plant variety protection and grower investment:** Growers and breeders investing in ‘Carmen’ production require legally defensible tools for variety verification to protect their intellectual property and commercial interests.

Scientific Rationale and Intellectual Merit

The phenotypic difference between ‘Hass’ and ‘Carmen’, early flowering, is most likely driven by a low-frequency somatic mutation, a small structural variant (SV), or a transposable element (TE) insertion near a gene that modulates flowering time. Because the *Persea americana* genome is highly heterozygous (1.96–2.08% for ‘Hass’; 1.61–1.92% for ‘Carmen’ based on our *k*-mer analyses), this early-flowering phenotype

may be driven by a mutation occurring on only one of the parental haplotypes, making it invisible to standard collapsed (haploid) assemblies.

Our preliminary *k*-mer spectral analysis using Merqury (**Figure 1**) against the publicly available ‘Hass’ reference genome (Nath et al., 2022) reveals a critical limitation: the current reference fails to capture a substantial pool of heterozygous *k*-mers shared by both lines. Crucially, the ‘Carmen’ (‘Mendez’) profile shows a broader, enriched read-only signature relative to ‘Hass’, demonstrating that ‘Carmen’ harbors distinct structural architectures and allelic variations entirely absent from the standard reference. Attempting to identify the mutations responsible for the early-flowering phenotype via standard reference mapping would fundamentally risk false negatives by ignoring this unrepresented allelic space. Although not strictly necessary for the main objective of this proposal, it would be highly desirable to have strong candidates for the causal mutation underlying the ‘Carmen’ flowering phenotype to enable more precise identification and future gene-editing-based breeding programs.

This empirical bottleneck underscores the necessity of our proposed haplotype-resolved, de novo assembly strategy for both varieties, which will capture the true genetic markers that distinguish ‘Carmen’ from ‘Hass’ with the fidelity required for commercial deployment.

Specific Objectives

1. Generate chromosome-level, haplotype-resolved de novo genome assemblies for both ‘Hass’ and ‘Carmen’.
2. Identify genome-wide sequence polymorphisms, SNPs, indels, structural variants, and TE insertions that consistently and exclusively distinguish ‘Carmen’ from ‘Hass’.
3. Validate a minimal panel of 3–5 diagnostic molecular markers across geographically diverse populations of both varieties.
4. Deposit all genomic assemblies and marker sequences in publicly accessible repositories as a permanent resource for the California avocado industry.

Methodology

Task 1: Sample Collection and DNA Extraction (Months 1–3)

Verified ‘Hass’ and ‘Carmen’ mother trees will be sourced from certified populations in California’s principal avocado-producing counties (Ventura, San Diego, and Santa Barbara). For de novo genome assembly, a single high-quality representative individual per variety will be selected. For population-scale validation, leaf material will be collected from 20 verified ‘Hass’ and 20 verified ‘Carmen’ individuals from geographically distinct orchards.

High-molecular-weight (HMW) DNA will be extracted from young leaf tissue using an optimized CTAB and magnetic bead-based purification protocol validated for the high-lipid, high-polyphenol content of avocado leaf tissue, targeting fragment sizes in the 50–300+ kb range required for PacBio HiFi library preparation. Hi-C crosslinked nuclei will be prepared from fresh leaf tissue following the Arima-HiC 2.0 protocol.

Task 2: De Novo Genome Sequencing and Assembly (Months 2–6)

Each genome will be sequenced using a three-technology strategy designed to maximize both base-level accuracy and chromosomal contiguity, targeting the known ~920 Mb avocado genome:

- **PacBio HiFi sequencing** (50× coverage): Long, highly accurate reads essential for phasing heterozygous haplotypes and spanning the repeat-rich regions that dominate the avocado genome.
- **Hi-C chromatin conformation capture** (184 M PE reads; approximately 55 Gb): Proximity ligation data to scaffold assembled contigs into chromosome-level pseudomolecules.

- **Illumina paired-end sequencing** (PE-150 bp, 50× coverage): High-accuracy short reads for base-level polishing and SNV validation.

Phased assembly will be performed using Hifiasm, with Hi-C scaffolding into pseudochromosomes using HapHiC. Assembly completeness, continuity, and phasing precision will be rigorously evaluated using BUSCO completeness benchmarks and Merquy spectral analyses. Repetitive sequences will be characterized using RepeatModeler (de novo) combined with homology-based approaches using RepBase and PlantRep, followed by RepeatMasker annotation. Structural gene annotation will be performed using BRAKER3, integrating public *Persea americana* protein databases and available transcriptomic evidence.

Both assemblies will be generated using identical sequencing technologies and bioinformatics pipelines to eliminate technical artifacts arising from platform or methodological differences, ensuring that observed genomic differences between varieties reflect true biological variation.

Task 3: Comparative Genomics and Variant Discovery (Months 6–10)

A parallel bioinformatic discovery pipeline will be applied to separate true somatic mutations and variety-specific variants from technical assembly artifacts:

Genome-to-genome alignment

Direct whole-genome alignments between the finalized phased 'Hass' and 'Carmen' assemblies will be performed with minimap2, and large-scale insertions, deletions, inversions, and translocation breakpoints will be systematically extracted using paftools.js.

Read-based cross-validation

Short and long reads from both varieties will be reciprocally cross-mapped to both assemblies to establish a high-confidence baseline for variant calling. Short-read alignments (Bowtie2) will be used to run VarScan2 for SNV and micro-indel calling; long-read alignments (minimap2) will be used to run PBSV for structural variant detection. Only variants identified independently by both genome-alignment and read-mapping approaches will advance as high-confidence candidates.

Population-scale filtering

Candidate variants will be screened against an expanded cohort of 40 commercial populations (20 'Hass', 20 'Carmen') sequenced at 30× depth (Illumina). Short-read data from the 40-population cohort will be processed using the GATK HaplotypeCaller pipeline. Subsequently, custom Bcftools filters and PLINK association analyses will be applied to exclude intra-variety polymorphisms and isolate stable, high-

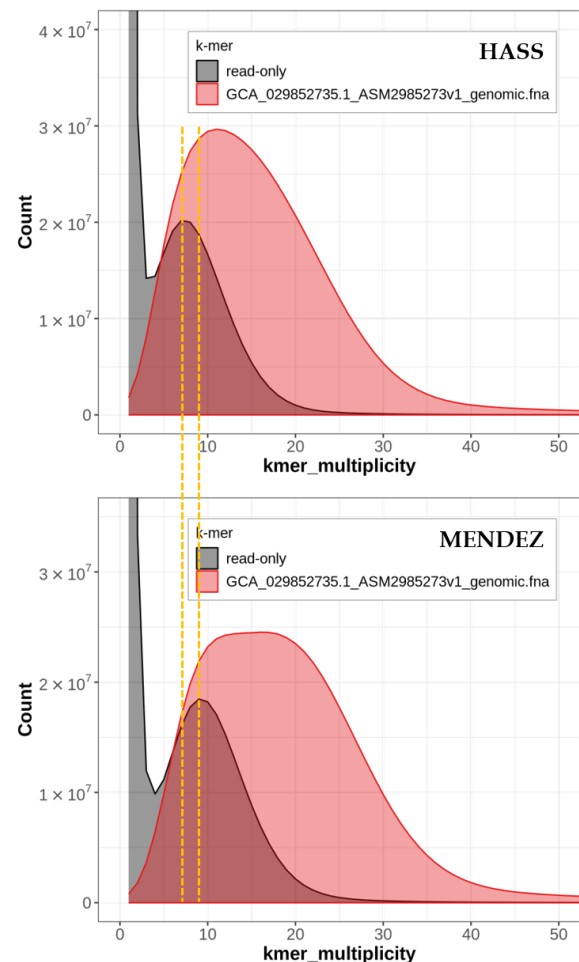


Figure 1. *k*-mer spectrum analysis (Merquy) of 'Hass' and 'Mendez' ('Carmen') genomes against the public haploid reference. Whole-genome Illumina reads from 'Hass' (top) and 'Mendez' (bottom) were tracked against the standard collapsed 'Hass' reference assembly. The prominent gray/brown peaks spanning the heterozygous multiplicity space (marked by yellow dashed lines) represent high-confidence read *k*-mers completely absent from the reference genome. The expanded read-only signature and altered assembly coverage profiles in 'Mendez' highlight significant, unrepresented genomic divergence between the two varieties.

confidence variants that segregate perfectly with the 'Carmen' early-flowering phenotype. The software Paragraph will be used to assess candidate structural variants, while transposable element (TE) insertions will be profiled across the cohort using tools such as PopoolationTE2. Priority will be given to stable structural variants and TE insertions, which offer the most reliable and unambiguous diagnostic signatures.

Task 4: Molecular Marker Development and Validation (Months 10–18)

From the validated candidate locus pool, PCR primers will be designed flanking variety-diagnostic polymorphisms. Three marker formats will be developed in parallel to accommodate different deployment contexts and equipment levels:

- **Indel markers:** Size-polymorphic amplicons distinguished by agarose gel electrophoresis — lowest cost, no specialized equipment required, suitable for nurseries and field laboratories.
- **CAPS markers (Cleaved Amplified Polymorphic Sequences):** SNPs creating or abolishing restriction enzyme sites, distinguished by restriction digest of a PCR product — no specialized equipment required beyond standard PCR and gel electrophoresis.
- **KASP assays (Kompetitive Allele-Specific PCR):** High-throughput fluorescence-based SNP genotyping for large-scale certification programs and high-volume supply chain screening.

An initial panel of 20 candidate markers will be developed to identify a minimally validated set of 3–5 markers that provide complete, unambiguous discrimination of variety. Final validation will be performed by blind genotyping of 50 'Hass' and 50 'Carmen' individuals from independent orchards not used in variant discovery, plus 20 individuals of other commercially relevant California varieties (Lamb Hass, GEM, Maluma) to confirm specificity.

Phase 1 Deliverables

- **Two chromosome-level, haplotype-resolved genome assemblies** for 'Hass' and 'Carmen', fully annotated and deposited in NCBI, represent the first variety-resolution genomic resource for the California avocado industry and a permanent platform for future research.
- **A high-confidence catalog of variety-specific genetic variants** distinguishing 'Carmen' from 'Hass', validated across 40 geographically diverse populations.
- **A validated molecular marker panel** of 3–5 diagnostic loci capable of unambiguously distinguishing 'Hass' from 'Carmen' from any tissue — leaf, seed, or fruit — at any developmental stage, in three marker formats (indel, CAPS, KASP).
- **Peer-reviewed publications** reporting the genome assemblies and marker development, ensuring scientific credibility and long-term visibility for the Commission's investment.
- **A genomic and bioinformatic framework** ready to support Phase 2 commercial testing protocol development without additional sequencing.

Significance and Connection to Phase 2

Phase 1 will resolve the fundamental scientific question of what makes 'Carmen' genetically distinct from 'Hass' and deliver validated genetic markers on which all subsequent commercial testing depends. The haplotype-resolved genome assemblies generated in Phase 1 will serve as permanent, publicly available resources that benefit the California avocado industry far beyond this project, providing a foundation for future research into avocado breeding, disease resistance, and climate adaptation.

The marker panel and genomic data from Phase 1 will directly and efficiently enable Phase 2, where the focus shifts from marker discovery to the development of commercially viable testing procedures deployable across the supply chain, from nursery certification to packing house verification, at the speed, cost, and simplicity required for routine industry adoption.

Research Team

The proposed project will be led by Dr. Luis Herrera-Estrella, a member of the United States National Academy of Sciences and the Royal Society of London, one of Latin America's most distinguished plant scientists with over 30 years of experience in plant biotechnology and genomics. Dr. Herrera-Estrella has direct and relevant expertise in large-scale plant genome sequencing, having led sequencing and assembly projects for avocado, chili pepper, and chia, among others, which makes him uniquely positioned to lead the de novo assembly of the 'Hass' and 'Carmen' genomes. He will be joined by Dr. Gerardo Alejo-Jacuinde, an expert in plant genomics with extensive experience in the sequencing and assembly of complex, highly heterozygous plant genomes, including multiple *Selaginella* species, chia, and most recently, the genome sequencing of five species of resurrection plants, organisms renowned for their genomic complexity and repeat content. Both investigators are faculty members of the Institute of Genomics for Crop Abiotic Stress Tolerance (IGCAST) within the Department of Plant and Soil Science at Texas Tech University, an institute purpose-built for the kind of large-scale, integrative genomics research proposed here. We are requesting funds for a MSc student, who will be trained to participate in the sample collection, data processing and analysis. Together, the team brings directly applicable expertise in every technical component of the proposed Phase 1 strategy, from high-molecular-weight DNA extraction and long-read sequencing to haplotype-resolved assembly, comparative genomics, and molecular marker development.

Project Timeline

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Sample Collection and DNA Extraction																		
De Novo Genome Sequencing and Assembly																		
Comparative Genomics and Variant Discovery																		
Molecular Marker Development and Validation																		

Budget Summary

Category	Amount
Personnel	\$179,824
Graduate tuition and fees	\$13,696
Travel - Sample collection	\$22,452
Sequencing and Genomics	\$30,000
Bioinformatics and Computing	\$4,000
Supplies and Other Direct Costs	\$26,000
Subtotal (Direct Costs)	\$275,972
Indirect Costs (indirect rate applied to direct costs)	\$55,194
TOTAL PHASE 1 REQUEST	\$331,166*

*Budget reflects estimated direct and indirect costs; final indirect cost rate to be confirmed by administering institution. The personnel costs above reflect estimated totals, including benefits.

Discovery of Carmen Hass-specific genomic variants through whole-genome resequencing

Robert Backer and Noëlani van den Berg

Hans Merensky Chair in Avocado Research

Forestry and Agricultural Biotechnology Institute

University of Pretoria

Key team members

Dr Robert Backer

Bioinformatics Researcher

Dr Backer has expertise in whole-genome resequencing, reference-based read alignment, variant discovery, comparative genomics and the development of reproducible bioinformatics workflows. He will lead the analysis of SNPs, indels, optional structural variant candidates, candidate-marker prioritisation and evaluation of marker specificity across avocado resequencing datasets.

Prof. Noelani van den Berg

Chair of the Hans Merensky Chair in Avocado Research

Prof. van den Berg has extensive experience in avocado research, plant pathology and the coordination of multidisciplinary research programmes. She will provide project leadership, ensure alignment with avocado-industry priorities and support access to authenticated plant material and relevant industry networks.

Dr Nicky Olivier

Senior Lecturer

Co-manager of the University of Pretoria Genomics Laboratory (UPGL)

Dr Olivier has expertise in genomics, molecular biology and high-throughput sequencing. As co-manager of the UPGL, he will provide oversight of DNA quality control, sequencing-library preparation and generation of high-quality whole-genome resequencing data.

Prof. Fourie Joubert

Director of the Bioinformatics and Computational Biology Facility, African Centre for Gene Technologies

Prof. Joubert has extensive expertise in bioinformatics, computational genomics and research infrastructure. He will provide senior bioinformatics oversight, support workflow optimisation and assist with computational resource planning, data management and quality assurance.

Executive summary

Hass is the dominant commercial cultivar of *Persea americana*, accounting for approximately 80% of global avocado consumption (Nath et al., 2022). Carmen Hass, originally described as Mendez No. 1, is widely accepted as a somatic sport of Hass identified through earlier flowering and fruit production, while retaining fruit highly similar to Hass in appearance and quality (Mendez Vega,

2000). This extends Hass-type fruit availability, but their close similarity creates a barrier to cultivar authentication and enforcement of plant breeders' rights.

Previous avocado genotyping studies have been unable to distinguish Hass and Carmen Hass confidently. A 384-SNP study placed Hass and Carmen accessions in the same near-identical genotype group, with only 15 differences among 8,671 genotype comparisons and no opposing homozygous calls (Kuhn et al., 2019). A ddRAD-seq study likewise identified 52 homozygous SNPs distinguishing nine other cultivars, but no private homozygous SNPs separating Hass from Carmen Hass (Kämper et al., 2021). These studies demonstrate their close relationship, but were restricted to reduced genomic fractions and focused mainly on homozygous variation, making them poorly suited to detecting rare heterozygous somatic mutations.

Recent low-coverage whole-genome resequencing (WGR) of 205 avocado trees identified more than 64 million SNPs at approximately 4.69x mean depth, but was designed for population structure rather than rare variant discovery between clonally related cultivars (Cañas-Gutiérrez et al., 2025). These data are useful as a specificity panel, but low coverage limits confidence in the absence of individual heterozygous variants. In contrast, preliminary GATK Mutect2 analysis by the Hans Merensky Chair in Avocado Research identified candidate heterozygous SNPs present in Carmen Hass but absent from Hass. Recovery against both the Nath et al. Hass genome and an independently assembled West-Indian genome reduces the likelihood of mapping artefacts and supports targeted whole-genome investigation, consistent with clonal-crop precedents (Calderón et al., 2021; Wang et al., 2024).

This Phase 1 project will resequence approximately four confirmed source trees of each cultivar using PCR-free, 150-bp paired-end Illumina sequencing at approximately 30x usable genome coverage. Closely related cultivars will support stringent filtration. Reads will be mapped to the chromosome-level Hass reference genome, and complementary variant-calling approaches will identify candidate variants distinguishing Carmen Hass from Hass. Variants will be retained only if reproducible across Carmen Hass samples, absent from Hass, close relatives and public datasets, supported by high-quality evidence and suitable for assay development.

The anticipated outcome is three to six independent, high-confidence Carmen Hass-specific genomic variants suitable for KASP, TaqMan or targeted amplicon assay development for cultivar authentication across the avocado supply chain.

Methodology

Discovery material and sampling

To identify diagnostic markers, WGR will be performed on a curated set of authenticated samples:

- **Target accessions:** four authenticated Carmen Hass trees (original/early-generation source material) and four authenticated Hass trees from verified clonal lines.
- **Comparative controls:** Representative sequencing (n = 2 each) of close relatives to ensure marker specificity, including Gwen, GEM, Lamb Hass, and Thille.
- **Background specificity panel:** A curated set of public avocado resequencing datasets with $\geq 10x$ mean genome coverage will be reprocessed against the same reference and used downstream to filter out common or non-Carmen variants.

DNA extraction and sample requirements

As Carmen Hass is a clonally propagated sport, distinguishing mutations may vary in allele frequency across tissues. Consequently, DNA will be obtained from both **young, recently expanded flush material** (for high-quality initial discovery) and **unripe fruit peel** (for supply-chain relevance).

Three options for sample provision are available:

1. **Provision of extracted DNA:** The California Avocado Commission may provide extracted DNA meeting the following specifications: $A_{260/280}$ ratio of 1.8-2.0; $A_{260/230}$ ratio $\approx$ 2.0 (minimum 1.6); fluorometric concentration (Qubit) > 20 ng/ μ l; and a minimum of 1 μ g total DNA per sample (2 μ g preferred).
2. **Fresh tissue provision:** Alternatively, young flush and unripe fruit peel may be sent to the Hans Merensky Chair in Avocado Research for extraction using an optimised in-house CTAB-based protocol.
3. **Fresh tissue acquisition:** Finally, fresh tissue may be acquired in South Africa, through local industry partners. This would, however, lead to an increased risk of IP complications. It is a viable route, nonetheless.

Strategic application: Initial variant discovery will leverage high-quality leaf DNA. However, all candidate variants must be confirmed in matched fruit peel DNA before assay development. Shortlisted variants will undergo targeted PCR-based validation and Sanger sequencing in peel samples; the final assay format (e.g. KASP/TaqMan for clear heterozygosity, or amplicon deep sequencing/ddPCR for variable allele frequencies) will be determined by the observed tissue-specific representation.

Sequencing strategy

A high-fidelity sequencing approach will be employed to maximise variant sensitivity:

- **Platform:** Illumina 150-bp paired-end, PCR-free libraries.
- **Coverage:** Target 30x usable mapped coverage (generating ~ 35 -40x raw coverage) to account for trimming and duplication removal.
- **Consistency:** Identical library preparation and sequencing platforms will be used for Hass, Carmen Hass and close relatives to minimise technical bias.

Bioinformatics pipeline

Processing and alignment

Reads will be processed via **fastp** (QC/trimming) and **MultiQC**. Alignment will be performed against the *Nath et al.* Hass reference genome using **BWA-MEM2**. BAM files will be processed with **samtools/GATK MarkDuplicates**, and coverage verified using **mosdepth**. Samples failing quality thresholds (mean depth < 20 x, excessive duplication, or evidence of contamination) will be excluded.

Variant discovery

A dual-analysis approach will be used, with joint cohort variant calling providing the primary genotype matrix and somatic-style calling providing an independent Carmen-versus-Hass candidate-locus set:

- **Joint cohort variant calling:** **GATK HaplotypeCaller** will be used in GVCF mode, followed by GenomicsDBImport and GenotypeGVCFs, to identify variants across the newly generated high-coverage Carmen Hass, Hass and close-relative samples.
- **Somatic-style calling:** **GATK Mutect2** will be used for Carmen-versus-Hass comparisons, treating Carmen Hass as the case genotype and authenticated Hass accessions as pseudo-normal controls, including a pooled Hass “panel-of-normals” with subsequent FilterMutectCalls refinement.
- **Structural variant screening:** SVs may be considered as a secondary candidate class, but the primary focus will be SNPs and small indels suitable for targeted assay development.

Candidate loci from the **joint cohort** and **somatic-style** analyses will be normalised and reconciled at the locus level. The joint cohort VCF will provide the primary genotype matrix across Carmen Hass, Hass and close-relative samples. Initial Carmen-private candidates will be defined as loci where the alternate allele is reproducibly present in Carmen Hass and absent from Hass and close relatives. To generate the highest-confidence candidate set, these loci will be intersected with the independent Carmen-versus-Hass Mutect2 candidate-locus set. If this strict intersection yields too few candidates for validation, secondary candidates may be considered where they show a clean Carmen-private pattern in the joint cohort VCF and strong read-level support, but these will be ranked below candidates supported by both analytical approaches. Candidates will then be screened against the curated public WGS background panel, which will be used as a downstream exclusion panel. Any candidate with alternate-allele support in non-Carmen public data will be removed.

Candidate marker selection and specificity

To ensure markers are robust for diagnostic use, candidates must meet the following criteria:

- **Distribution:** Primary candidates must be present in 100% of authenticated Carmen samples, absent from all Hass and close relatives, independently supported by Carmen-versus-Hass Mutect2 analysis, and absent from the curated public non-Carmen WGS background panel where locus-level coverage is informative.
- **Quality metrics:** Mapping quality ≥ 50 ; coverage ≥ 20 with at least 8-10 alternate reads; allele frequency ≥ 0.4 and ≤ 0.6 .
- **Genomic context:** Located outside repetitive or low-mappability regions, surrounded by clean, assayable flanking sequences for downstream primer design.
- **Validation against public data:** Candidate markers must be absent from the curated $\geq 10\times$ public WGS background panel. Any candidate with alternate-allele support in a non-Carmen public accession will be excluded, provided the locus has sufficient coverage and read quality for interpretation.

Targeted validation in fruit peel DNA

Shortlisted candidate loci will be validated by PCR amplification and bidirectional Sanger sequencing in matched fruit peel DNA from authenticated Carmen Hass and Hass samples. This

step will confirm that candidate markers discovered from leaf-derived WGS data are reproducibly detectable in fruit peel, the tissue most relevant for supply-chain authentication. At minimum, validation will include fruit peel DNA from the four Carmen Hass and four Hass accessions used in the discovery panel. Candidate loci showing clear Carmen-specific heterozygous signal in fruit peel and absence from Hass fruit peel will be prioritised for downstream KASP, TaqMan or targeted amplicon assay development. Where Sanger chromatograms indicate reduced or variable allele representation, candidates may be redirected to amplicon deep sequencing or ddPCR-based validation.

Budget

Cost category	Description	Quantity basis	Estimated cost
<i>DNA extraction reagents and consumables</i>	Extraction and QC of leaf and/or fruit peel DNA from authenticated Carmen Hass, Hass and control accessions.	16 leaf samples + 8 fruit peel samples = 24 samples; 32 samples including contingency	\$470.59** (R8,000.00)
<i>WGS Sample QC, library preparation, sequencing</i>	TruSeq PCR-free libraries, NovaSeqX, 150 bp paired-end sequencing to achieve ~30x usable mapped coverage.	16 libraries	\$ 4,281.39** (R 72,783.59)
<i>Data delivery</i>	Cloud	1 unit	\$ 68.29 (R1161.00)
<i>Candidate primer sets</i>	Synthesis of primers for 20 shortlisted candidate loci.	20 primer sets	\$ 705.88** (R12,000.00)
<i>PCR reagents and consumables</i>	PCR validation of candidate loci across Carmen Hass and Hass fruit peel DNA.	20 loci x 8 fruit peel DNA samples plus controls/repeats = 200 PCRs	\$ 964.71** (R16,400.00)
<i>Sanger sequencing</i>	Bidirectional sequencing of amplified candidate loci.	Up to 160 amplicons x 2 reads = 320 Sanger reads	\$ 2,020.35** (R34,346.00)
<i>Student Bursary</i>	One Masters student will be trained to assist in data analysis (alongside Dr R Backer) and academic article writeup.	2 years	\$ 21,176.47 (R360,000.00)
<i>UP overhead</i>	Overhead applied to all funds entering University cost-centers.	15%	\$ 4,453.15** (R75,703.56)
Total project cost			\$ 34,140.83 (R580,394.15)

This budget reflects only the proposed sponsor/funder contribution and excludes the full economic cost of research, including facilities, staff time, student support, supervision, additional overheads, and indirect costs.

* Based on ZAR (R) to U.S. Dollar (\$) exchange rate of \$1.00 = R17.00

** If required, cost may be reduced by lowering the number of samples in the resequencing phase and/or reducing the number of candidate loci screened in fruit peels. However, the experimental design is, in our opinion, balanced in terms of cost-benefit ratio.

Timeline and milestones

Activity	Milestone	Time
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<i>Sample confirmation and DNA preparation</i>	Authenticated leaf and fruit peel DNA panel prepared and QC-verified.	2 months (DNA extraction)
<i>Whole-genome resequencing</i>	PCR-free WGS data generated for Carmen Hass, Hass and close relatives.	3 months
<i>Bioinformatics processing</i>	Pipeline development and QC-passed, reference-aligned BAM files generated.	2 weeks
<i>Variant discovery</i>	Preliminary Carmen Hass-associated SNP/indel candidate list produced.	1 month
<i>Candidate filtering and specificity assessment</i>	High-confidence candidate markers shortlisted after filtering against Hass, close relatives and public WGS data. Manual curation and verification.	2 months
<i>Fruit peel validation</i>	Shortlisted loci PCR/Sanger-validated in Carmen Hass and Hass fruit peel DNA.	4 months
<i>Final marker recommendation</i>	Ranked diagnostic marker list and assay-development recommendation delivered.	1 month

**Estimated time is based on prior experience along with safety margins. Overall, this project is not expected to take more than 18 months to complete.*

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UNIVERSITY OF CALIFORNIA, RIVERSIDE
PHASE 1 RESEARCH PROPOSAL

Hass Avocado Variety Authentication
Marker Discovery and Validation

Submission for the California Avocado Commission, Production Research Program

Project title	Hass avocado variety authentication: Phase 1 marker discovery and validation
Submission date	October 21, 2026
Lead Principal Investigator	Mary Lu Arpaia, PhD, Department of Botany and Plant Sciences, University of California, Riverside
Co-Principal Investigators	Edwin A. Solares, PhD, ESB AI Lab Peggy A. Mauk, PhD, Department of Botany and Plant Sciences, University of California, Riverside
Collaborator	Melanie L. Oakes, PhD, Genomics Research and Technology Hub, University of California, Irvine
Submitting institution	University of California, Riverside
Proposed duration	8 to 12 weeks from execution of agreement
Total amount requested	\$58,232

1. Executive Summary

Project at a Glance:

- ‘Carmen’ and ‘Hass’ look and taste nearly the same, and no existing test can tell them apart.
- This project uses DNA to find out where ‘Carmen’ came from.
- It looks for small genetic differences that past studies missed.
- Any difference found becomes a simple lab test, checked on trees whose identity is hidden from the testers.
- If no test is possible, the project will show that clearly.
- This project builds on data the team already discovered.

In 2000, Vega patented a new avocado variety, ‘Mendez No. 1’, which is also commonly known as ‘Carmen Hass’, ‘Carmen’, or ‘Carmen Mendez’ (thereafter in this proposal called ‘Carmen’). Vega noted that main distinction between the 2 cultivars is the profuse “off bloom” in ‘Carmen’ which occurs approximately 6 to 7 months prior to the primary spring bloom of both cultivars. For this reason, ‘Carmen’ garnered the interest of Mexican growers since the “off-bloom” corresponds to the “flor loca” bloom in Mexico. The fruits of the two cultivars are nearly indistinguishable.

Phase 1 will determine the genetic origin of ‘Carmen’ relative to ‘Hass’ and identify and validate candidate markers distinguishing them. The work is scoped to 8 to 12 working weeks and \$58,232 due to the reference assemblies, resequencing panels, unreleased long-read data and analysis framework already being in place.

Published marker work has not been able to separate the two cultivars. Both Rendón-Anaya et al. (2019) and Solares et al. (2023) reported ‘Hass’ and ‘Mendez’ (‘Carmen’) tightly clustered on principal component analysis (PCA) of genome-wide SNPs using different reference genome assemblies. Additionally, Kämper et al. (2021) found no private allele separating the pair. Previous marker-panel studies filtered toward homozygous variation across a reduced fraction of the genome, and the genome-wide analyses resolved population structure rather than near-identical accessions, so none could detect the variant classes a clonally propagated lineage carries: heterozygous somatic variants, loss of heterozygosity (LOH), and structural variants (SVs).

Current tools collapse indels and structural variants that differ by only a few base pairs into a single event, and their resolution is insufficient for comparing clonal accessions. This project applies unpublished high-resolution genomic

scans and AI/ML approaches developed by Solares (EAS) to identify differences between 'Carmen' and 'Hass', and to determine whether 'Carmen' is a clone or a self-fertilized seedling. The framework has been applied to public data on chromosomes 7 and 10 (Section 3.4), and the project draws on prior research and unreleased PacBio HiFi data from EAS and Arpaia (MLA) on 'Hass' clonal selections. Phase 1 extends the analysis to all twelve chromosome-scale scaffolds, sequences six independent 'Carmen' and 'Hass' trees, screens multiple primer pairs, and validates a panel of a subset of primers on a blind sampling at UCI.

Phase 1 succeeds if it delivers a defensible origin determination with stated confidence, a ranked catalog of candidates exceeding the measured null, and PCR confirmation on authenticated leaf material. A rigorous demonstration that no such marker exists at this resolution, with power analysis, is also a success.

2. Justification and Benefit to the California Avocado Industry

While the U.S. fresh market for avocados has grown significantly in the last 2 decades, that growth has been due to the increase in fruit imports into the U.S. California acreage and overall production has stayed relatively constant during this time resulting in the California avocado industry losing market share. United States production, of which California supplied over 85 percent, contributed more than 80 percent of domestic sales in the 1990's but has since declined to under 20 percent (USDA ERS 2024). Imported fruit may include Hass-like varieties that cannot be distinguished from 'Hass' by visual inspection. 'Carmen', the most prominent variety, is identified in the orchard by multiple blooms per year and off-season fruit (Illsley-Granich et al. 2011) but is essentially indistinguishable from 'Hass' in taste and shape, differing mainly by a modest reduction in size.

3. Related, Current, and Previous Work

3.1 Prior attempts at cultivar discrimination

Rendón-Anaya et al. (2019) reported 'Hass' and 'Carmen' tightly clustered on genome-wide SNPs, and Solares et al. (2023) noted the same result across a 34-accession panel: the two are adjacent on principal component analysis, carry indistinguishable ancestry proportions at $K = 2, 3$ and 4, and sit on adjacent tips of the TreeMix graph (Figure 3). Kuhn et al. (2019) genotyped 1,524 individuals with 384 SNPs, a density that cannot separate accessions differing at a handful of sites. Kämper et al. (2021) applied ddRADseq across 10 cultivars and identified 52 homozygous SNPs distinguishing nine; 'Carmen' and 'Hass' produced no private alleles and were grouped as likely isogenic. These results are not decisive. Kämper and Kuhn sampled a small non-random fraction of a 912.9 Mb genome and filter toward homozygous variation, while somatic mutations accumulate almost exclusively as heterozygous variants and are readily discarded under germline filtering (Calderón et al. 2021). The genome-wide analyses resolve population structure, at which scale two near-identical accessions co-locate whether or not fixed differences exist between them.

3.2 Evidence from other clonally propagated fruit crops

Calderón et al. (2021) resequenced four 'Malbec' wine grape accessions at 30x and recovered 941 clone-distinguishing variants against 2.12 million cultivar-level variants, genotyping 214 accessions with 41 of them. Scaglione et al. (2025) targets traceability directly: SNPs, SVs and large hemizygous deletions specific to individual clones were cataloged from 20 deeply resequenced sweet orange accessions, then deployed across 221, and Wang et al. (2024) recovered twice as many somatic mutations from a haplotype-resolved sweet orange genome as a collapsed reference exposes. In grapevine, one in seven Chardonnay genes is hemizygous on the basis of SVs, which accrue as recessive heterozygotes specifically in clonal lineages (Zhou et al. 2019).

Where the causal variant underlying a bud sport has been resolved it has been structural rather than epigenetic: a long LOH tract on Pp06 that may be responsible for flat peach fruit converting to round (Tan et al. 2019), retrotransposon insertion in the VvmybA1 promoter producing white grape skin (Kobayashi et al. 2004), rearrangement deleting 313 genes in Tempranillo Blanco (Carbonell-Bejerano et al. 2017), and a spur-type-specific 167 bp deletion in the MdTCP11 promoter among 74 'Fuji' varieties (Cai et al. 2024); outside bud sports the tb1 insertion in maize acts as a regulatory enhancer and is causative (Studer et al. 2011). We therefore take structural variation as the working hypothesis for any difference between 'Carmen' and 'Hass' if 'Carmen' is a clonal derivative: better supported across this literature, and directly assayable by PCR. LOH and SV detection is the appropriate primary strategy whatever the outcome, since selfing itself produces biological loss of heterozygosity, provided the calling is somatic-aware and the null measured rather than assumed.

3.3 Relationship to existing methods

LOH is mitotic recombination converting heterozygous loci to homozygous loci, widespread in asexually reproducing organisms, exceeding the point mutation rate and acting on contiguous tracts rather than scattered sites (Smukowski Heil 2023; Dutta et al. 2021, 2025); bud sports are stable somatic mutations preserved through

vegetative propagation (Foster et al. 2018). Both signals used here exist in published software: JLOH locates LOH blocks from SNP density and coverage and separates copy-neutral from hemizygous blocks by block-against-flanking depth (Schiavinato et al. 2023), and clonal identity in fruit crops is normally established from identity-by-state and kinship (Liu et al. 2022). It's important to note that Liu (2022) state epigenetics as likely but fall short of claiming it as the cause, the corresponding authors then follow up with a new study in Fuji apples (Cai et al. 2024) where they do find structural variation for the cause in differences and the cause for epigenetic change. Neither completes the inference here, since identity-by-state cannot separate a self-fertilized seedling from a clone and existing LOH tools locate blocks once origin is assumed.

Standard structural variant workflows are poorly suited to comparing two near-identical genomes. Multi-sample analysis requires deciding when two calls represent the same event, and common comparison approaches were developed for technologies that produce ill-defined breakpoints, so calls falling within a positional tolerance are merged into a single event. Over-merging of this kind inflates allele frequency by up to 2.2-fold relative to sequence-aware comparison (English et al. 2022), and identifying de novo SVs and indels between closely related individuals remains difficult due to shortcomings in both inference and comparison (Kirsche et al. 2023). For a clonal lineage the consequence is direct: indels and SVs that differ by only a few base pairs between 'Carmen' and 'Hass' can be collapsed into one event and reported as shared, removing the class of difference this project seeks. EAS will therefore apply unpublished high-resolution genomic scan methods developed while at the University of California, which retain variant alleles at base-pair resolution rather than collapsing them within a positional window, together with AI/ML approaches to identify and prioritize candidate differences. The same data address whether 'Carmen' is a clone of 'Hass' or a self-fertilized seedling, using the joint conversion-ratio and opposite-homozygote framework described in Task 3.

3.4 Preliminary results and prior research

The framework was implemented and applied to public data prior to this submission, against the University of Queensland 'Hass' genome (Nath et al. 2022; GenBank GCA_029852735.1), using three Illumina accessions from Rendón-Anaya et al. (2019): two independently sampled 'Hass' trees and 'Carmen', with alignment rates of 99.54, 99.78 and 99.76 percent. Genotype concordance, the windowed LOH conversion ratio, depth-ratio analysis at 50, 25 and 10 kb, and the index-of-dispersion scan (Task 3) were run using public Illumina data on chromosomes 7 and 10, which carry the flowering-associated loci. The two 'Hass' accessions, propagated clones of one cultivar, form an internal null rather than a positive control: any signal distinguishing 'Carmen' must exceed that separating two 'Hass' clones.

On chromosomes 7 and 10, no large copy-number difference is resolved in 50, 25 or 10 kb genomic windows. Of the 13,620 test windows at 10 kb, 13,618 have a 'Carmen'-to-'Hass' depth ratio within the range expected for equal copy number (0.7 to 1.3), short of a heterozygous deletion (0.5) or duplication (1.5), and none reaches a doubling of copy number (2.0) (Figure 4). Even so, the depth ratio between 'Carmen' and 'Hass' scatters 12 to 15 percent more widely than the ratio between the two 'Hass' trees at all three window sizes, consistent with small differences that are too short to shift a whole window. At finer 5 kb resolution across the 1.5 Mb surrounding SDMYB, SPA1 and EOBI, individual windows rise near 1.2 or fall below 0.5, the level of a heterozygous deletion (Figure 2), consistent with small local gains or losses of sequence that window averaging cannot resolve and that the dispersion scan is designed to detect. Ten of the twelve chromosome-scale scaffolds remain unexamined using publicly available Illumina data, no independent trees have yet been compared, and no candidate has been tested in the laboratory, and this analysis has not been done using our Carmen HiFi data, so the origin of 'Carmen' is not asserted here; Phase 1 addresses all three.

This work also builds on prior research by EAS and MLA on 'Hass' clonal selections. PacBio HiFi resequencing of the closest clone to the original 'Hass' tree, 'Carmen', and the 'Hass' clonal selections 'Eugenin', 'Flavia' and an unnamed clone was completed from a separate study and is contributed ahead of publication, together with the 'Gwen' reference and 34-accession resequencing panel of Solares et al. (2023). EAS annotated EOBI and SPA1 in avocado, with coverage analysis showing an SV differing between the A and B flowering types (Solares et al. 2023), and co-authored the identification of SDMYB, likewise subject to a deletion and a transposable element insertion (Groh et al. 2026).

4. Objectives

1. Determine whether 'Carmen' is a clonal derivative of 'Hass', a chimera, an outcrossed seedling, a self-fertilized seedling, a selection of separate parentage, or a mixture, by applying the joint conversion-ratio and opposite-homozygote framework genome-wide, and state the determination with its confidence.

2. Assemble an authenticated comparison set of 'Carmen' and 'Hass': six newly sequenced trees from independent UC field sites, unreleased HiFi data on the original 'Hass' tree, 'Carmen' and three 'Hass' clonal selections, and the 'Hass' HiFi data of Nath et al. (2022). Existing public data are used only to confirm marker specificity.
3. Identify candidate distinguishing variants genome-wide, including SNPs, indels and SVs, drawing first on the LOH tracts and copy-number transitions recovered under Objective 1, and resolve their boundaries with the long-read data.
4. Screen numerous primer pairs in vitro, confirm discriminating amplicons by Sanger sequencing, and validate a panel of a subset of primer pairs blind at UCI against six independent trees, reporting per-marker performance.
5. Deliver a final report including a plan for transition to a Phase 2 application.

5. Workplan and Methods

Computational analysis is performed by EAS; primer screening is performed in-house at UC Riverside under Mauk (PAM); DNA extraction, Illumina sequencing are performed under PAM, and blind PCR validation are performed at the UCI Genomics Research and Technology Hub (GRT Hub) under Dr. Oakes. Analyses use established open-source software, including BWA-MEM, SAMtools, bcftools, Manta, Delly, Sniffles2, hifiasm, minimap2 and Primer3, together with unreleased research tools developed by EAS while at the University of California. EAS will perform all computation on his existing compute cluster, on NSF ACCESS allocation MCB180035, and on National Artificial Intelligence Research Resource (NAIRR) Pilot resources.

Task 1. Sampling strategy and chain of custody (Weeks 1 to 4)

MLA designs the sampling strategy and selects accessions. Material is drawn from the UCR germplasm collection and UC field sites; UCR accessions are cataloged and budwood is distributed worldwide, so any marker reported here can be re-tested by a third party against the same verifiable material. Two 'Hass' and two 'Carmen' trees have already been sampled and are in hand, so procurement is off the critical path and sequencing begins in Week 2. Six trees enter new sequencing, three 'Carmen' and three 'Hass', each an independent individual sampled from a separate UC field site. Each sample is barcoded at collection against source tree, accession, site, rootstock, date and collector, with identity certified by MLA independently of the analysis.

Task 2. DNA extraction and sequencing (Weeks 2 to 6)

DNA is extracted from young leaves under PAM using a modified CTAB protocol with polyvinylpyrrolidone and a chloroform partition, given the polyphenol and lipid content of avocado tissue. Libraries are sequenced as 2 x 150 bp paired-end reads, avoiding PCR amplification where possible, since amplification introduces substitution errors indistinguishable from low-frequency heterozygous somatic variants.

Long-read data are already in hand: the unreleased PacBio HiFi data on a second generation clone from the original 'Hass' tree (Mother Hass), 'Carmen' and three 'Hass' clonal selections, and the 'Hass' HiFi data of Nath et al. (2022), deposited as three SMRT cells at 47.9x under SRA BioProjects PRJNA694184 and PRJNA818813. No new long-read sequencing is required.

Task 3. Genome-wide analysis (Weeks 1 to 8)

3.1 Alignment, variant calling, and thresholds

The analysis is applied to all twelve chromosome-scale scaffolds and to the new accessions alongside the existing data. Reads are trimmed with Trimmomatic (Bolger et al. 2014), aligned with BWA-MEM v0.7.17 (Li and Durbin 2009) and indexed with SAMtools v1.23.1 (Danecek et al. 2021). Variants are called with bcftools mpileup (-q 20 -Q 20 -d 500 --no-BAQ) and bcftools call -mv, filtered to biallelic SNVs at QUAL>30 and DP>10. Structural variants are called separately from split-read and discordant-pair evidence with Manta (Chen et al. 2016) and Delly (Rausch et al. 2012), and from the long reads with Sniffles2 (Smolka et al. 2024). Short-read call sets are compared at base-pair resolution rather than merged within a breakpoint window, so nearby events of different length are retained as distinct alleles, and agreement between callers is used as supporting evidence rather than as a merging criterion. Thresholds are set high due to much of the apparent difference in short-read data arising from misalignment: against assemblies of the maize NAM founder lines, calls falling in assembly-resolved deletions are erroneous at 8 to 17 percent, mode near 10 percent (Ross-Ibarra et al. 2022; Figure 5).

3.2 Genotype concordance and the empirical null

Concordance is computed with bcftools gtcheck, discordant sites partitioned into opposite homozygotes, novel heterozygotes at sites homozygous in 'Hass', and heterozygous-to-homozygous mismatches, the latter recomputed under MIN(SUM(AD))>20. Discordant sites are interpreted by class. At sites homozygous in 'Hass', a heterozygous

'Carmen' genotype carries an allele absent from 'Hass', which only an outcrossed pollen parent can contribute, so the count of such novel heterozygotes separates outcrossing from selfing and clonality. Opposite homozygotes cannot arise from any seedling of 'Hass', since every 'Hass' gamete carries the 'Hass' allele, so their count beyond the genotyping error rate measured between the two 'Hass' accessions tests whether 'Hass' is a parent at all. Identity-by-state and kinship are also computed.

3.3 Windowed loss of heterozygosity and coverage

LOH is quantified in 100 kb windows as the proportion of sites heterozygous in both 'Hass' accessions that are homozygous in 'Carmen', excluding windows with fewer than 20 qualifying sites. Both sexual hypotheses predict this ratio at 0.50 uniformly along every chromosome, since meiosis converts half of maternal heterozygous sites and segregates independently across loci; vegetative propagation predicts near zero, with elevation confined to discrete tracts. The histogram is reported, unimodal at 0.50 indicating meiosis and bimodal indicating discrete somatic events.

Three controls guard against conversion of technical origin: a site is scored only where both 'Hass' accessions are independently heterozygous, which also sets the internal null; only where the alternate allele fraction lies between 0.3 and 0.7 in both with depth at least 10 in all three, a skewed fraction being more consistent with a collapsed paralog; and any window above 0.85 is depth-checked first, with thresholds derived from the control pair rather than fixed a priori. Per-base depth, retaining zero-depth positions, is summarized as the median across 50, 25, 10, 2 and 1 kb windows and normalized per accession. Coverage also fixes assay format: gene conversion and mitotic recombination preserve copy number, giving a haplotype difference for an allele-specific assay; hemizygous deletion halves depth, giving an absence for a junction-spanning assay.

3.4 Index-of-dispersion scan for sub-window structural variants

Windowed depth statistics miss variants much smaller than the window and so do not reach the 200 to 2,000 bp range in which somatic SVs are concentrated, as Figures 2 and 4 illustrate. A uniformly diploid or hemizygous window is Poisson with an index of dispersion, variance over mean, of unity; a part-diploid, part-hemizygous window is a two-Poisson mixture and is over-dispersed. For a deletion spanning fraction f at diploid depth λ the expectation is $1 + f(1 - f)\lambda / (4(1 - f/2))$, with standard deviation from n covered bases of the square root of $2/n$. Simulation at $\lambda = 22$ and $W = 10,000$ reproduced this to three decimal places, giving five-sigma limits of 289 bp in 50 kb windows, 130 bp in 10 kb, 58 bp in 2 kb and 41 bp in 1 kb. Technical overdispersion is a property of reference position and cancels between accessions, so two contrasts are formed per window, a control between the two 'Hass' accessions and a test against 'Carmen'. A window is called at five control standard deviations above the control mean, with control windows crossing that threshold reported as an empirical false positive rate.

3.5 Long-read boundary resolution and targeted loci

HiFi reads from 'Carmen' and the original 'Hass' tree are assembled with hifiasm (Cheng et al. 2021) and aligned with minimap2 (Li 2018), with the three 'Hass' clonal selections as a long-read within-cultivar null and the Nath et al. (2022) data as an external reference. Structural differences are compared against the short-read tracts, transitions, SV calls and dispersion intervals, and a candidate promotes only where window evidence and long-read boundary agree, since reads spanning the boundary give the junction sequence the assay requires. On chromosomes 7 and 10, no large structural variant is resolved at 50, 25 or 10 kb, and at 5 kb across the regions flanking the flowering-associated loci, individual windows reaching 1.2 and falling below 0.5 are consistent with structural variants below roughly 1 kb. Three flowering-associated loci localized on this assembly are examined at 5 kb resolution across 1.5 Mb (Figure 2): SDMYB (chromosome 10, 41,397,672 to 41,405,576; MRB53_031466), mapped to the synchronized dichogamy locus (Groh et al. 2026), and tblastn positions (Camacho et al. 2009) for SPA1 and EOBI, both multi-copy families and therefore candidate orthologs.

3.6 Marker specificity

Candidate markers are checked for specificity against existing public resequencing data (Rendón-Anaya et al. 2019; Solares et al. 2023), and against unreleased data from additional 'Hass' clonal selections and the 'Hass' lineage, so that no marker is shared with other cultivars or varies within 'Hass', reducing the risk of misclassification. No varieties other than 'Carmen' and 'Hass' are sequenced.

3.7 Layer-restricted variants and chimerism

The shoot apical meristem comprises three cell layers, L1, L2 and L3, and a somatic variant arising in one layer can persist through vegetative propagation as a periclinal chimera. Leaf tissue draws on all three layers in unequal proportion, so a variant present in every layer appears at an allele fraction near 0.5, while a variant restricted to one or two layers appears at a reduced fraction proportional to those layers' contribution to the sampled tissue. Each

candidate is therefore classified by allele fraction and read depth as present in all layers or restricted to a subset, with the probability of each class estimated from the depth-adjusted allele fraction. Long reads support this directly, and Sniffles2 includes a mode for low allele-fraction structural variants (Smolka et al. 2024). Layer-restricted variants are noted for a future Phase 2 application. Chimerism also bears on the origin determination, since layer-restricted alleles can produce allele patterns at a locus that differ from those expected in a uniform genotype (Franks et al. 2002).

Task 4. Primer design, screening, and blind validation (Weeks 7 to 12)

Candidate markers are screened in silico by EAS, who designs and ranks primer pairs with Primer3 (Untergasser et al. 2012) by margin above the null, boundary precision, distance from repeats and designability, for 150 to 400 bp amplicons, junction-spanning for LOH and copy-number boundaries and allele-specific for nucleotide candidates. The candidate set comprises SNP-targeted assays where candidate SNPs are identified, indel-spanning assays, and a set of unreleased primers developed in prior work.

Primer pairs are ordered from commercial vendors, and PAM's laboratory performs in vitro screening and initial testing in house by PCR on authenticated young leaf DNA, with amplicons from discriminating pairs sent for Sanger sequencing (Calderón et al. 2021). Structural variants can be shared among clonal lineages, so a marker specific to 'Carmen' must be absent from every 'Hass' selection rather than from a single 'Hass' tree; initial testing therefore uses DNA from the original 'Hass' tree, 'Carmen' and the 'Hass' clonal selections 'Eugenin', 'Flavia' and an unnamed 'Hass' clone.

The resulting panel of a subset of internally verified primer pairs, together with the PCR protocol developed by PAM and EAS, is transferred to the GRT Hub, where Dr. Oakes performs blind validation of the panel and the protocol on a new independent set of trees that include two controls. The six newly sequenced trees are submitted with identities masked, MLA holds the key, and the panel is scored before identities are revealed. Reported performance states the number of trees tested and does not extrapolate beyond it.

Task 5. Reporting and transition (Weeks 11 to 12)

Deliverables are a final report, a research summary and one presentation to the Production Research Committee. The final report includes a plan for transition to a Phase 2 application.

6. Project Management

Dr. Mary Lu Arpaia (PI, UC Riverside) leads the project and is responsible for sampling strategy and accession selection, drawing on her expertise in 'Hass' and avocado research. Dr. Edwin Solares (Co-PI, ESB AI Lab) performs the bioinformatic, AI/ML and computational analysis and selects candidate primers. Dr. Peggy Mauk (Co-PI, UC Riverside) performs DNA extraction, designs PCR primer protocols with EAS and performs in vitro screening and initial testing of primers prior to validation at UCI. Dr. Melanie L. Oakes (UCI Genomics Research and Technology Hub) performs Illumina sequencing, blind PCR validation and validation of the PCR protocol. Existing instrumentation at UCR and the GRT Hub covers all laboratory work, so no equipment is requested. Status notes are provided at Weeks 4 and 8, and the final report at Week 12.

7. Risks and Contingencies

Risk	Assessment	Mitigation
No candidate survives replication and blind validation	On chromosomes 7 and 10 no large copy-number difference is resolved; the signal is sub-window, a 12 to 15 percent dispersion excess with individual 5 kb windows departing at the flowering-associated loci.	The dispersion scan, 2 and 1 kb windows and high-resolution methods target that size class; six independent trees and the complete 'Hass' clonal panel establish replication and specificity. Negatives are reported with power analysis.
'Carmen' proves to be a chimera	A layer-restricted difference appears at reduced allele fraction in leaf DNA.	Candidates are classified by allele fraction and depth as present in all layers or layer-restricted; layer-restricted markers are noted for a future Phase 2 application.
Sub-kilobase candidates cannot be verified, or a candidate is a mapping artifact	The residual signal sits at a size class window statistics cannot localize, and mapping artifact is the dominant failure mode in a comparison this close.	Long reads give base-pair boundaries where they exist; no candidate without a resolved boundary goes to assay design. Artifacts are controlled by zero-depth retention, median window statistics, the 'Hass' null under identical filters, Sanger confirmation and blind validation at UCI.

8. Timeline and Milestones

The schedule is shown in Figure 1.

ID	Milestone and deliverable	Completion
M1	Material received; libraries submitted; analysis applied to all 12 chromosomes	Week 4
M2	Sequence aligned; concordance and empirical null computed genome-wide	Week 6
M3	Origin determination with stated confidence; ranked candidate catalog; primers designed and ordered	Week 8
M4	Primer screening and Sanger confirmation complete; panel and protocol transferred to UCI	Week 10
M5	Blind validation complete; final report with Phase 2 transition plan; PRC presentation	Week 12

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10. Budget

	Amount	Basis
University of California, Riverside		
Salaries and Benefits		
Asst. Project Specialist, R. Li (1.25 months)	\$10,053	PCR Analysis
Fringe Benefits (47.6%)	\$4,785	As mandated by UC Policy
Total Salary and Benefits	\$14,838	
Supplies and Expenses		
PCR and gel supplies for primer screening	\$4,470	Vendor supplies; in-house screening
Sanger confirmation sequencing	\$760	UC pricing
Primer Oligonucleotides (22 nt)	\$1,980	Commercial vendor, UC negotiated pricing
Total Supplies and Expenses	\$7,210	
TOTAL University of California, Riverside	\$22,048	
ESB AI Lab		
Salaries and Benefits		
Edwin Solares, Executive Director (3 months @ 50%)	\$23,125	Computation, analysis, primer design, reporting
Fringe Benefits (21.6%)	\$4,989	
Total Salary and Benefits	\$28,114	
Supplies and Expenses		
Sample procurement, shipping, handling	\$450	UCR and UC field sites
Total Supplies and Expenses	\$450	
Travel		
Travel (sample collection, CAC meeting)	\$800	Based on current US Federal reimbursement rates
Total Travel	\$800	
Services		
Illumina sequencing, 3 'Carmen' and 3 'Hass'	\$2,520	Recharge rate
Blind PCR validation from leaves	\$1,500	Recharge rate
Total Services	\$4,020	
Other		
Contingency reserve	\$2,800	Reruns and iterative validation
Total Other	\$2,800	
TOTAL ESB AI Lab	\$36,184	
Total direct costs	\$58,232	

Budget Justification

University of California, Riverside - \$22,048

Salary and Benefits (\$14,838)

Salary is requested for Assistant Research Specialist Rui Li for 1.25 months at a base salary of \$8,042 per month. Dr. Li will perform the PCR analysis for the project in collaboration with Dr. Solares under the direction of Dr. Mauk.

Fringe Benefits calculated at a rate of 47.6% as mandated by University of California.

Supplies and Expenses (\$7,210)

Supplies are requested for conducting DNA extraction, PCR analysis, gel electrophoresis and development of markers. Estimate based on assumption that not all primers will yield useable markers.

ESB AI Lab - \$36,184

Salary and Benefits (\$28,114)

Salary is requested for Executive Director Edwin Solares. Dr. Solares will oversee the project activities in collaboration with Drs. Arpaia and Mauk.

Fringe Benefits calculated at a rate of 21.6%.

Supplies and Expenses (\$450)

The reference assemblies, the resequencing panel of Solares et al. (2023), the unreleased HiFi assembly and sequencing data on 'Hass' clonal selections, and computation on EAS's compute cluster, NSF ACCESS allocation MCB180035 and NAIRR Pilot resources contributed at no cost to the project.

Travel (\$800)

Travel will include travel to UC South Coast REC in Irvine, CA, UC Riverside and UC Irvine in order to collect samples and interact with the research team and other service providers. Travel also included covering transportation costs to CAC meetings. Mileage, lodging and meals calculated using current Federal mileage rates for Southern California.

Services (\$4,020)

Sequencing covers six independent trees, three 'Carmen' and three 'Hass', which two are also added to the blind validation set as controls. Full-time staff support is added to test a large panel of primers and confirm them by Sanger sequencing. Oligonucleotides are budgeted at published University of California vendor pricing; DNA extraction, Illumina sequencing and Sanger sequencing at UCI Genomics Core internal rates.

Blind validation of multiple trees for a subset of markers is performed at the UCI GRT Hub at UC recharge rates.

Other (\$2,800)

A contingency of approximately 5 percent of direct costs covers sequencing reruns and iterative primer redesign.

11. Biographies

Mary Lu Arpaia, PhD, Lead Principal Investigator, University of California, Riverside

Subtropical horticulturist and lead of the UC avocado breeding program. Responsible for sampling strategy and accession selection, and provides authenticated plant material from the UCR germplasm collection. Co-author on Solares et al. (2023), Kuhn et al. (2019) and Groh et al. (2026), and collaborator with EAS on PacBio HiFi resequencing of 'Hass' clonal selections.

Edwin A. Solares, PhD, Co-Principal Investigator, ESB AI Lab

Founder and Executive Director, ESB AI Lab. Lecturer, Halicioğlu Data Science Institute, University of California, San Diego. PhD, University of California, Irvine. UC President's Postdoctoral Fellow, University of California, Davis, Departments of Evolution and Ecology and of Computer Science. First author of Solares et al. (2023), which produced the 'Gwen' avocado reference genome and the 34-accession resequencing panel underpinning this proposal. Co-author on Groh et al. (2026), which mapped synchronized dichogamy in avocado to SDMYB, and on Zhou et al. (2019), which cataloged structural variation across 69 grapevine cultivars and wild accessions. First author of HapSolo (Solares et al. 2021), a method for resolving alternative haplotype contigs in highly heterozygous diploid assemblies. Co-author on the quantification of short-read SNP call error against maize NAM founder assemblies (Ross-Ibarra et al. 2022), which sets the filtering thresholds in Task 3. Holds active federal funding as PI, including a USDA NIFA Western Regional Aquaculture Center award. Built and executed the preliminary framework described in Section 3.4.

Peggy A. Mauk, PhD, Co-Principal Investigator, University of California, Riverside

Director of Agricultural Operations and Subtropical Horticulture Cooperative Extension Specialist, Department of Botany and Plant Sciences. PhD, Plant Pathology, University of Arizona. Specializes in citrus, avocado and date crop production management. Designs PCR primer protocols with EAS and performs in vitro screening and initial testing of primers prior to validation at UCI.

Melanie L. Oakes, PhD, Collaborator, University of California, Irvine

Manager, Genomics Research and Technology Hub, Department of Biological Chemistry. Performs Illumina sequencing, blind PCR validation and validation of the PCR protocol on a recharge basis.

12. Other CAC Support

Investigator	Project	Sponsor	Amount	Status
Arpaia	Does artificial pollination improve yield of 'Hass' and 'GEM' avocado? (65325-00-00)	California Avocado Commission	\$172,825	Current
Solares	None			
Mauk	None			

Conflict of interest. The investigators declare no financial, professional, or personal relationship with any commercial party with an interest in the propagation or marketing of the varieties studied here. MLA holds current research support from the Commission, listed under Other Support.

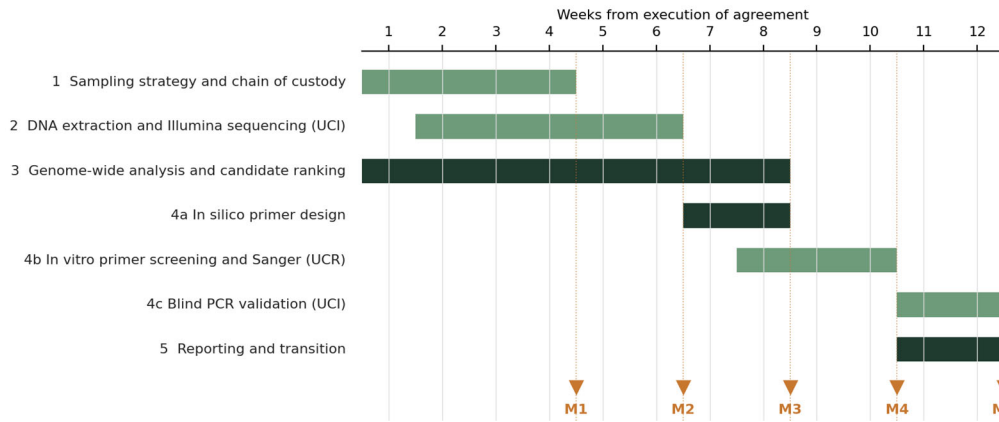


Figure 1. Phase 1 schedule. Dark bars are computational tasks; light bars are laboratory tasks at UC Riverside and the UCI GRT Hub. Milestones M1 to M5 are marked below the chart.

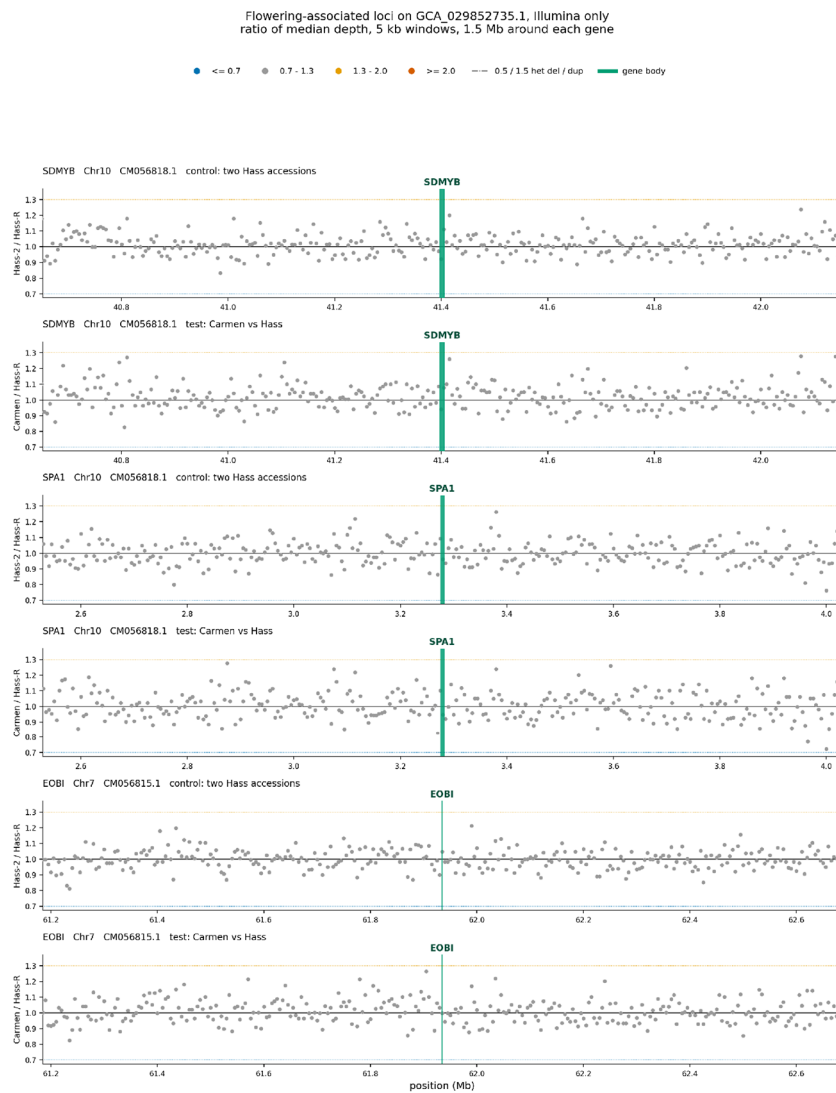


Figure 2. Flowering-associated loci on the Nath et al. (2022) 'Hass' assembly. Ratio of median depth, Illumina only, 5 kb windows across 1.5 Mb flanking each gene, control contrast above test contrast. A heterozygous deletion is expected at 0.5 and a duplication at 1.5, so windows departing from 1.0 mark a copy-number change spanning those windows. No large event is resolved, but individual windows reaching 1.2 and falling below 0.5 are consistent with structural variants below roughly 1 kb.

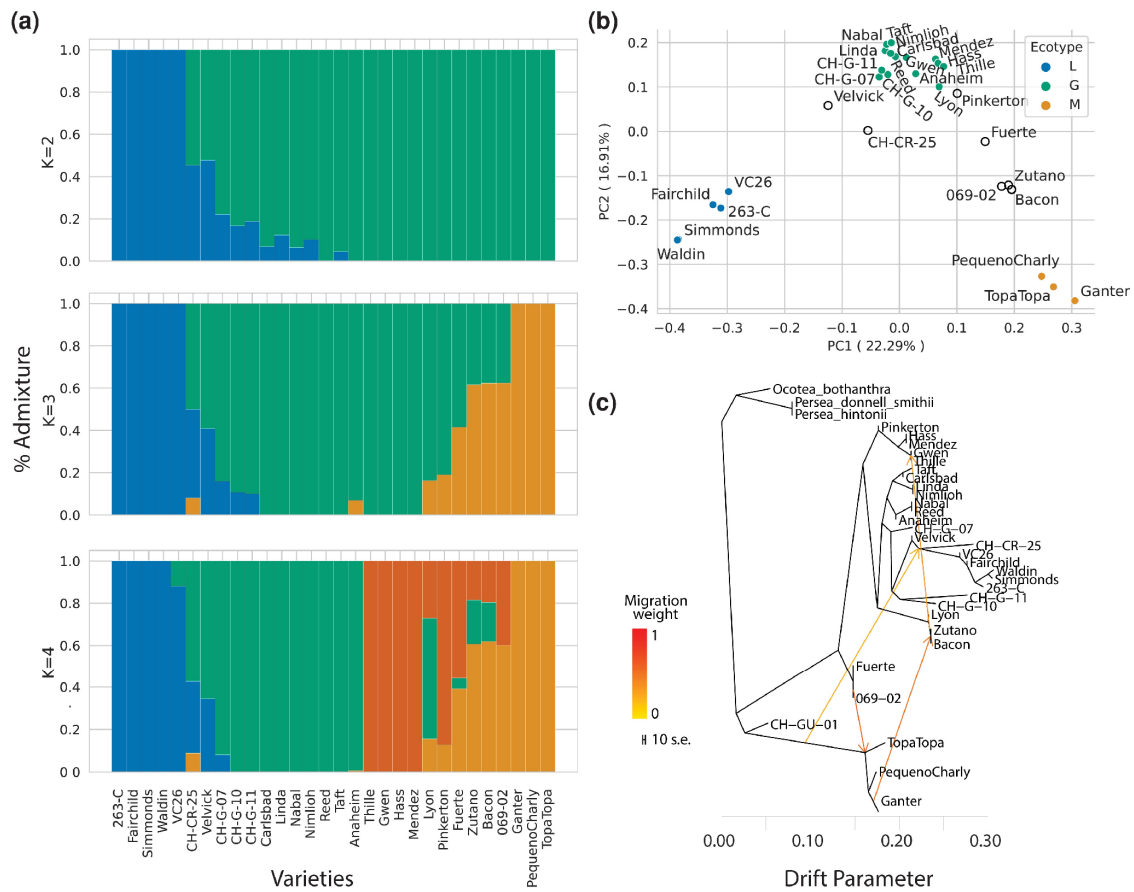


Figure 3. Population structure of the 34-accession panel of Solares et al. (2023). (a) ADMIXTURE proportions at $K = 2, 3$ and 4. (b) Principal component analysis, PC1 22.29 percent and PC2 16.91 percent, colored by ecotype. (c) TreeMix graph with migration edges. 'Hass' and 'Mendez ('Carmen')' are adjacent in every panel and carry indistinguishable ancestry proportions, which is the resolution limit of population-scale analysis rather than evidence that no fixed difference exists.

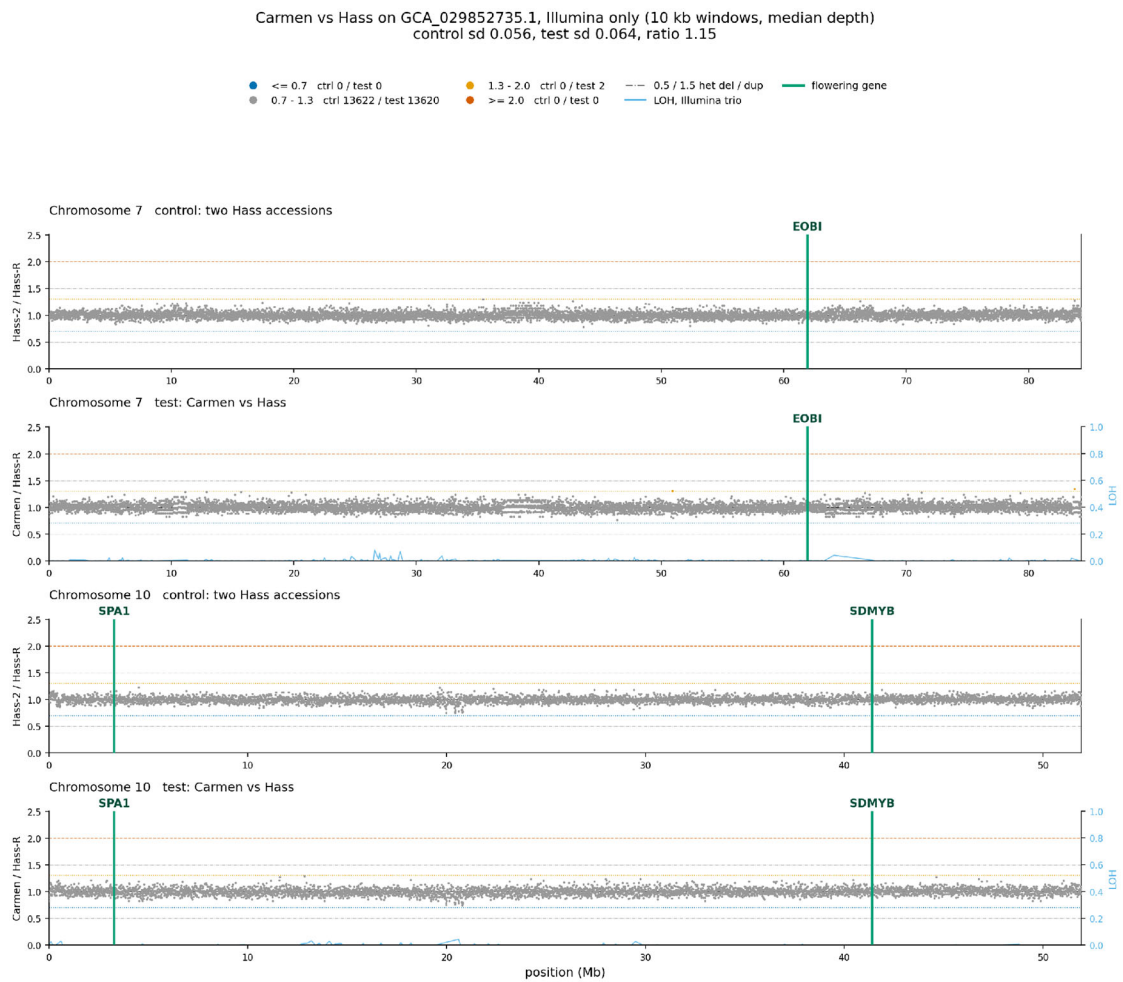


Figure 4. Carmen-to-Hass depth ratio in 10 kb windows on chromosomes 7 and 10 using publicly available Illumina data. Control contrast above each test contrast, windowed LOH trace in blue. Control sd 0.056, test sd 0.064. Of 13,620 test windows, 13,618 lie within 0.7 to 1.3 and none reaches 2.0.

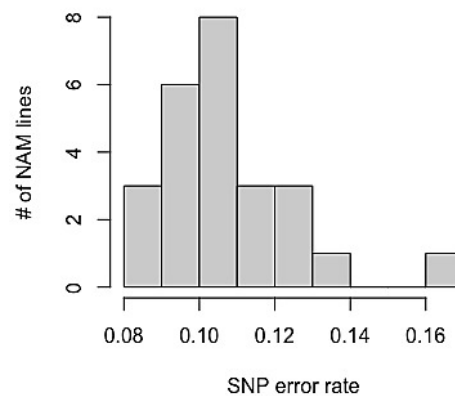


Figure 5. Short-read SNP call error across the maize NAM founder lines, from Ross-Ibarra et al. (2022). Error rates run 8 to 17 percent with a mode near 10 percent, the basis for the thresholds in Task 3.1.