

Commercial-scale field testing and potential release of five elite advanced rootstocks

November 1st 2019 – October 31st 2022.

Authors

Amber Newsome¹, Matthew Elvena¹, Aidan Shands¹, Benjamin Hoyt¹, Bullo Mamo¹, Adrian Garcia¹, Natasha Jackson^{1#}, Erika Nandankar^{1#}, Benjamin Friedenberg^{1#}, Damaris Godinez-Vidal^{1#}, Nathaniel Von Dolteren^{1#}, Johnny David Rosecrans², Rashaan Topham Souikane², Lauren Garner^{2**}, Mary Lu Arpaia^{3**}, Peggy Mauk^{3**}, Timothy Spann⁴, Ben Faber⁵, and Patricia Manosalva^{1*}.

Affiliations

¹Department of Microbiology and Plant Pathology, University of California, Riverside, CA 92521.

²Department of Plant Sciences, California Polytechnic State University, San Luis Obispo, San Luis Obispo, CA, 93407.

³Department of Botany and Plant Sciences, University of California, Riverside, CA 92521.

⁴California Avocado Commission (CAC), Irvine, California 92618.

⁵UC Cooperative Extension, Ventura, CA 93003.

*Principal Investigator (PI), **Co-PI, #Former personnel at the Manosalva Lab that work on field data acquisition.

Co-PI: Phenotypic characterization of *P. cinnamomi* under greenhouse conditions and PineTree and Bonsall field trials evaluation.

Contributions

AN, ME, AS, BM, EN, BF, DGV, JDR, and RTS assisted in the field surveys, budwood collections, field establishments, and field data collection (ratings and harvest); BH assisted with harvest; BH and AG assisted in the isolation and confirmation of *Phytophthora cinnamomi* incidence from field soil samples; AN, ME, NA MLA, RTS, LG conducted data analyses; TS assisted in the identification, characterization of fields, and selection of field sites for the rootstock trials; BF assisted with the outreaching activities including grower surveys; LG oversees the rootstock trial at San Luis Obispo; MLA oversees the rootstock trials at Pine Tree and Bonsall; PMauk advisory role for field activities; PM oversees, designed, and conducted all the activities of the proposal.

EXECUTIVE SUMMARY:

- Breeding and selection for biotic and abiotic resistant/tolerant avocado rootstocks and scions are needed to combat the increasing harsh environmental conditions worldwide. Avocado is a fruit tree crop that is highly sensitive to water stress. In California (CA), abiotic stressors (i.e., drought, soil/water salinization, and extreme temperatures) in combination with biotic stressors (i.e., phytophthora root rot [PRR], avocado branch canker, and anthracnose) and the risk of the arrival of the lethal laurel wilt (LW) disease to CA, are threatening the production and sustainability of the avocado industry.
- The UCR rootstock breeding program have successfully secured different sources of funding from State, Federal, and other Industry sources including the California Avocado Commission (CAC). In addition, we have established multidisciplinary collaborations with scientists from other US Institutions, International Institutions, and International industries to address all these current and future major avocado production challenges. Our research efforts include fundamental and applied research including the field testing of UCR rootstock: scion combinations at all USA avocado producing states (Florida, Texas, Puerto Rico, Hawaii), especially in Florida where our rootstocks will be tested under PRR, LW, and flooding conditions. In addition, new collaborations with Scientists in Spain will soon allow us to test the UCR advanced rootstocks for resistance to white root caused by *Rosellinia necatrix*.
- Tree overall health, salinity, tree mortality, and harvest data during the last 6 years at small regional field trials (10-25 trees/rootstocks) in CA support the establishment of semi-commercial large-scale field trials of these five UCR advanced rootstocks: PP40, PP42, PP45, PP80, and PP35. These five rootstocks exhibited good performance under high PRR incidence conditions especially in the presence of the more aggressive and potassium phosphite less sensitive *Phytophthora cinnamomi* populations. In addition, some of these rootstocks exhibited salinity tolerance and good performance under clay and high alkaline soils as well heat stress. These large-scale rootstock trials will also allow us to collect more meaningful harvest and packing data supporting their patenting and release (semi-adoption by growers in CA).
- Here, we report and describe the large-scale field evaluation of five UCR-advanced rootstocks grafted to ‘Hass’. The establishment of these trials and field evaluations have been funded by the CAC 2019-2022 funding cycle. This is the first-time UCR rootstocks have been evaluated at large- scale (~100 trees/rootstock) and under different environmental conditions and cultural practices before their commercial release. In addition, we continue the evaluation of these UCR five advanced rootstocks in four small scale regional trials (10-25 trees/rootstock), one in San Diego and three in Ventura counties to obtain more harvest data on these materials.
- The tree performance and harvest data at these rootstock trials indicate that PP40 and PP35 are the best performers in Southern and Northern CA, exhibiting PRR and salinity tolerance and low levels of mortality when compared with Dusa at some locations in San Diego, Ventura, and Santa Barbara. Moreover, both rootstocks are highly productive when grafted to ‘Hass’. At some locations, ‘Hass’ trees grafted on PP35 are small vigorous trees (~half canopy size than Dusa and the other rootstocks) but have the same yield efficiency/canopy volume which make it an excellent rootstock for high density planting in CA.
- Field data collected on PP45 and PP42 at these rootstock trials indicates that these vigorous rootstocks are highly resistance to PRR make them ideal for replanting in areas where PRR incidence is high. PP45 and PP42 exhibited good levels of heat tolerance. PP45 is sensitive to high salinity conditions especially when *P. cinnamomi* is not present, however, PP45 performs good under PRR and salinity conditions combined.

- Limited field data on tree performance and harvest is currently available to draw conclusion on this rootstock, however, preliminary data suggests some salinity tolerance and good performance under salinity and PRR conditions. Collection of more tree performance data and harvest data is necessary to determine if we will pursue the commercial release of this rootstock.
- Under this funding cycle, we successfully trained several people including undergraduate and graduate students, technicians, and junior/senior assistants. We conducted several seminars, courses, and field days to disseminate our findings on the performance of these rootstocks.

TABLE OF CONTENTS

INTRODUCTION	7
GOALS AND OBJECTIVES	12
MATERIALS AND METHODS	13
RESULTS AND DISCUSSION Obj. 1	16
RESULTS AND DISCUSSION Obj. 2	50
RESULTS AND DISCUSSION Obj. 3	54
OUTREACH AND EDUCATION	59
PRODUCTS AND PRESENTATIONS	60
CONCLUSIONS	62
REFERENCES	63

LIST OF TABLES

Table 1. Commercially available rootstocks in California	8
Table 2. List of UCR advanced rootstock	12
Table 3. Amount of budwood collected	13
Table 4. Mother trees to collect budwood	13
Table 5. Field site data collected	14
Table 6. Description of the large-scale rootstock trials in CA	17
Table 7. Tree data at San Luis Obispo trial (Tree Height)	47
Table 8. Tree data at San Luis Obispo trial (Trunk Diameter)	48
Table 9. Tree data at San Luis Obispo trial (Trunk Diameter)	49
Table 10. Active rootstock trials in CA (Small trials)	50
Table 11. Summary of Limoneria 2 size picking January 2022	51
Table 12. Summary of Harvest at Limoneria 2 (2022)	52
Table 13. Summary of Harvest at Gunderson (2022)	54
Table 14. Rootstocks planted at Bonsall and Pine tree trials (2017)	54

LIST OF FIGURES

Figure 1. Grower survey conducted in CA in June 2020 -----	7
Figure 2. Yield and yield efficiency for UCR rootstocks grafted to different scions -----	12
Figure 3. The UCR rootstock scoring system-----	15
Figure 4. Map for Leo McGuire’s 2019 plot -----	18
Figure 5. Trees planted in mounds at Leo McGuire’s 2019 plot -----	18
Figure 6. Data collected at Temecula plot 1 -----	18
Figure 7. Field ratings at Leo McGuire plot (Oct. 2022) -----	19
Figure 8. Leo McGuire showing a Hass trees grafted on PP40 (Nov. 2021) -----	19
Figure 9. Data for PP35 harvest collected from packing house (2022) -----	20
Figure 10. Data for PP40 harvest collected from packing house (2022) -----	21
Figure 11. Planting day of the second plot at Leo McGuire (2021) -----	21
Figure 12. Map of the new planting at Leo McGuire’s plot (2021)-----	22
Figure 13. Field ratings at Leo McGuire plat 2 (October 2022) -----	22
Figure 14. Map for John Lamb’s plot -----	23
Figure 15. PP35 and PP40 ratings in Camarillo two weeks after planting -----	23
Figure 16. PP35 and PP40 ratings in Camarillo four weeks after planting -----	23
Figure 17. Rootstock trial at John Lamb orchard in Camarillo (Sept. 2019) -----	23
Figure 18. Updated map at John Lamb orchard in Camarillo (Sept. 2019) -----	24
Figure 19. Field ratings at John Lamb in Camarillo (Oct. 2022) -----	24
Figure 20. PP35 and PP40 trees at Camarillo Plot 1 (Summer 2022) -----	25
Figure 21: Map for John Lamb plot 2 planted in Camarillo in 2021-----	25
Figure 22. John Lamb plot 2 planted in Camarillo in 2021 -----	25
Figure 23. Field ratings at John Lamb plot 2 planted in Camarillo in 2021 -----	26
Figure 24. Original layout and design for planted discussed -----	27
Figure 25. Planting layout of the plot at Adna Farms, LLC -----	27
Figure 26. Performance of ‘Hass’ trees grafted on Dusa and PP45 at Adna Farms (2021)----	28
Figure 27. Tree performance at Adna Farms, LLC (April 2021)-----	29
Figure 28. Field ratings at Adna Farms, Temecula (Oct. 2022) -----	30
Figure 29: Field ratings at Adna Farms, Temecula (Oct. 2022) cont. -----	30
Figure 30. Map for Chris Sayer trial, Ventura (2020) -----	30
Figure 31. Rootstock trial view at Petty Ranch, Ventura (2020) -----	31
Figure 32. Tree performance at Petty Ranch, Ventura (2021) -----	31
Figure 33. Tree performance at Petty Ranch, Ventura (2022) -----	32
Figure 34. Avocado trees at Petty Ranch, Ventura (2022) -----	32
Figure 35. Alina Ranch top terrace trial design -----	33
Figure 36. Alina Ranch bottom terrace trial design -----	33
Figure 37. Field layout of the rootstock trial design at Alina Ranch (2020) -----	34
Figure 38. Tree performance at Alina Ranch (2021) -----	34
Figure 39 Tree performance at Alina Ranch (2022) -----	35
Figure 40. Tree performance at Alina Ranch (Pictures, 2022) -----	36
Figure 41. Section 1 (S1), Santa Barbara, 2020 -----	36
Figure 42. Section 2 (S2), Santa Barbara, 2020 -----	36
Figure 43. Section 3 (S3), Santa Barbara, 2020 -----	37

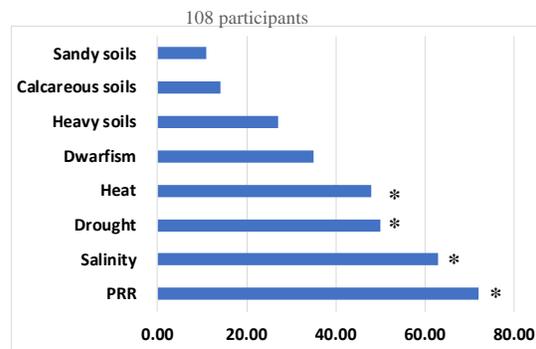
Figure 44. Section 4 (S4), Santa Barbara, 2020 -----	37
Figure 45. Section 4 (S5), Santa Barbara, 2020 -----	37
Figure 46. Tree height (ft) at Pete Miller Ranch (April 2021) -----	38
Figure 47. Tree health score (0 – 5 dead) at Pete Miller Ranch (April 2021) -----	38
Figure 48. Salt damage score (0 – 5 dead) at Pete Miller Ranch (April 2021) -----	38
Figure 49. Heat damage score (0 – 5 dead) at Pete Miller Ranch (April 2021) -----	39
Figure 50. Flush score (0 – 5 best) at Pete Miller Ranch (April 2021) -----	39
Figure 51. Trees at Pete Miller Ranch Section 1 (April 2021) -----	39
Figure 52. Trees at Pete Miller Ranch Section 2 (April 2021) -----	40
Figure 53. Trees at Pete Miller Ranch Section 3 (April 2021) -----	40
Figure 54. Trees at Pete Miller Ranch Section 4 (April 2021) -----	41
Figure 55. Trees at Pete Miller Ranch Section 5 (April 2021) -----	41
Figure 56. Tree Mortality at Pete Miller Ranch (October 2022) -----	42
Figure 57. Overall Tree Health Scores Pete Miller Ranch (October 2022) -----	42
Figure 58. Tree Salinity Damage at Pete Miller Ranch (October 2022) -----	43
Figure 59. Tree Heat Damage at Pete Miller Ranch (October 2022) -----	43
Figure 60. Trees at Pete Miller Ranch Section 1 (October 2022) -----	43
Figure 61. Trees at Pete Miller Ranch Section 2 (October 2022) -----	44
Figure 62. Trees at Pete Miller Ranch Section 3 (October 2022) -----	44
Figure 63. Trees at Pete Miller Ranch Section 4 (October 2022) -----	44
Figure 64. Trees at Pete Miller Ranch Section 5 (October 2022) -----	44
Figure 65. Map and samples collected at Cal Poly field -----	45
Figure 66. Map for the Cal Poly SLO plot -----	45
Figure 67. Pictures at planting day a SLO field trial (2020) -----	46
Figure 68. Tree mortality at Limoneria 2, Santa Paula, Ventura -----	50
Figure 69. Tree performance at Limoneria 2, Ventura (October 2022) -----	51
Figure 70. Cumulative yield/rootstock and yield efficiency at Limoneria 2, Ventura -----	52
Figure 71. Tree mortality at Gunderson, Santa Paula, Ventura -----	52
Figure 72. Tree performance at Gunderson, Ventura (October 2022) -----	53
Figure 73. Cumulative yield at Gunderson, Ventura (October 2022) -----	53
Figure 74. Cumulative yield at Gunderson, Ventura (October 2022) -----	53
Figure 75. Tree mortality at Bonsall and Pine Tree Ranch (2022) -----	55
Figure 76. Overall tree health at Bonsall and Pine Tree rootstock trials -----	56
Figure 77. Percentage of surviving trees that had fruits at harvest time at Bonsall (2022) -----	56
Figure 78. Cumulative yield at Bonsall rootstock trial -----	57
Figure 79. Average of yield influenced by rootstock (2022) -----	57
Figure 80. Average cumulative yield at Pine Tree, Santa Paula -----	58
Figure 81. Average fruit size influenced by rootstock in Santa Paula -----	58

INTRODUCTION

Avocado growers face numerous production challenges including devastating diseases such as Phytophthora root rot (PRR) caused by *Phytophthora cinnamomi* and Laurel Wilt (LW) caused by *Raffaelea lauricola* which in combination with salinity, drought, and heat stress cause severe reduction in fruit yield, quality, and can destroy complete avocado orchards if not managed properly. Resistant/tolerant rootstocks are the most environmentally friendly, sustainable, and effective long-term solution for managing these major biotic and abiotic stressors. By definition, resistance traits reduce the harm caused by the disease by preventing infection or limiting the pathogen growth (reducing pathogen populations) while tolerance traits do not inhibit infection or pathogen populations, but instead reduce or offset its negative fitness consequences by reducing host mortality or restoring the reproductive capacity of infected hosts.

The UCR avocado rootstock breeding program began in the 1950's under the directorship of Dr. George Zentmyer, professor at the Microbiology and Plant Pathology Department. The rootstock breeding program was initiated because of the need for rootstocks harboring resistance to *P. cinnamomi* and it has been continuously funded by the avocado growers through the California Avocado Commission (CAC). In the last decade, the emergence of *P. cinnamomi* populations more virulent and less sensitive to potassium phosphate (PP) applications combined with the decline of water quality and availability have contributed to significant losses in productivity. Avocado is highly sensitive to water stress. Salinity stress is influenced by both cultivar and rootstock. Rootstocks also vary in salt resistance/tolerance, which has been demonstrated in numerous studies. Avocado research priorities for the UCR rootstock breeding program have been identified through communication with avocado growers, some of whom are currently participating in the field evaluation of our advanced *P. cinnamomi* and salinity resistant rootstocks. Our recent rootstock survey conducted in 2020 indicated that avocado growers' major concerns are PRR, salinity, drought, and heat (Fig. 1). These results strongly support our efforts to select and develop rootstocks with resistance/tolerance to these stressors and we will continue conducting surveys to inform us of ongoing stakeholder needs to identify and adjust the program objectives and activities as necessary.

Figure 1. Grower survey conducted in California after the CAS seminar series in June 2020



There are several rootstocks commercially available in California (Table 1). Several of the available rootstocks were developed by the UCR program such as 'Duke 7', 'Thomas', 'Uzi', 'Zentmyer', and 'Steddom'. 'Steddom', a Toro Canyon seedling, is becoming popular among CA growers for its *P. cinnamomi* resistance and salinity tolerance. It has been reported that under certain conditions 'Hass' trees grafted to Steddom rootstocks are smaller than 'Hass' trees grafted to other rootstocks. Other popular rootstocks for their tolerance to salinity are Dusa, Toro Canyon, Day (VC207), Tami (VC801), Miriam (VC218), Ben-Ya' Acov1 (VC66), and Zerala™. ***Even if the UCR rootstock program did not develop this material, the program has evaluated and continue to evaluate some of these material (Dusa, Day, Tami, Miriam) through CA for several years which supported their commercial release in California in the last years.*** Despite the

availability of these rootstocks, the performance under the current pathogen populations of *P. cinnamomi* and their performance under other biotic stressors such as heat, high pH, performance in low drainage soils has not been assessed thoroughly. In addition, their performance when grafted with other commercially available rootstocks has not been tested thoroughly.

Table.1. Commercially available rootstocks in California and their properties. M = Mexican, G = Guatemalan, WI= West Indian, ND = no determined, *based on SNPs markers and comparing >2000 accessions.

Rootstock	Race composition*	Origen	Properties
Duke 7	M x G	UCR/ Zentmyer	Moderate resistant to Phytophthora Root Rot (PRR) and exhibited cold tolerance. Trees are large, vigorous, and good producers. Susceptible to waterlogging. More sensitive to salinity than Dusa and Toro Canyon. High yield efficiency when grafted with Hass, Carmen, GEM, Lamb, and Reed.
Thomas	M	UCR/Coffey Zentmyer	Highly susceptible to PRR, <i>P. citricola</i> , and salinity.
Toro Canyon	M x G	Royden Stauffer	Moderate resistant to <i>P. cinnamomi</i> and <i>P. citricola</i> , exhibited similar salinity tolerance than Dusa. Good productivity under PRR, high salinity conditions, and low temperatures.
Dusa	M x G	UCR/Menge & Douhan	Moderate resistant to PRR and exhibited salinity tolerance. Good productivity under PRR and high salinity conditions. Highly sensitive to waterlogging conditions so it is not good for fields with heavy soils, PRR, and salinity. Susceptible to white root rot (WRR) caused by <i>Rosellinia necatrix</i> . Less yield efficiency compared with Duke 7 when grafted with Hass, Carmen, GEM, Lamb, and Reed.
Uzi	M	UCR/Menge & Douhan	Highly resistant to <i>P. cinnamomi</i> (PRR). Extremely vigorous and fast-growing rootstock. Good producer but susceptible to salinity. Ideal for replanting problems due to high incidence of PRR. Similar yield efficiency as Dusa when grafted with Hass, Carmen, GEM, Lamb, and Reed.
Zentmyer	M	UCR/Menge & Douhan	Highly resistant to <i>P. cinnamomi</i> (PRR). Extremely vigorous and fast-growing rootstock. Good producer but highly susceptible to salinity. Ideal for replanting problems due to high incidence of PRR. Low yield efficiency when grafted with Hass, Carmen, GEM, Lamb, and Reed compared with Duke 7, Dusa, Leola, Steddom, and Uzi.
Steddom	M x G	UCR/Menge & Douhan	Highly resistant to <i>P. cinnamomi</i> (PRR). It is a slow growing rootstock having heavy yield with higher yield efficiency when grafted with Dusa when grafted with Hass, Carmen, GEM, Lamb, and Reed. Exhibited good salinity tolerance, excellent rootstock with small canopy, low vigor which make it desirable for high density or hedge-row avocado planting.
Day (VC207)	WI x G x M	Volcani Center ARO/Ben- Ya'acov1	Moderate resistant to <i>P. cinnamomi</i> (PRR) and highly tolerant to salinity. Large and vigorous trees.
Tami (VC801)	WI x G	Volcani Center ARO/Ben- Ya'acov1	Moderate resistant to <i>P. cinnamomi</i> (PRR) and highly tolerant to salinity. Large and vigorous trees.
Miriam (VC218)	WI x M	Volcani Center ARO/Ben- Ya'acov1	Moderate resistant to <i>P. cinnamomi</i> (PRR) and highly tolerant to salinity. Large and vigorous trees. Exhibit drought resistance, alkaline soil resistance as indicated for data collected in Israel.

Ben-Ya'acov1 (VC66)	WI x G	Volcani Center ARO/Ben-Ya'acov1	Salinity tolerant. Lower tendency towards alternate bearing.
Leola™ (Merensky 6)	ND	Westfalia	Moderate resistance to PRR similar to Dusa. Good productivity when grafted to Hass and GEM. Similar yield efficiency than Dusa when grafted with Hass, Carmen, GEM, Lamb, and Reed, however Duke 7 and Steedom exhibited more yield efficiency when grafted with these scions. This rootstock is sensitive to high salinity.
Zerala™ (Merensky 5)	ND	Westfalia	Moderate resistance to PRR similar to Dusa. Exhibited salinity tolerance. Is highly susceptible to waterlogging conditions.

UCR Rootstock Breeding Program

In March 2015, a 5.5-year rootstock breeding proposal (Contract Number: 65209) was submitted to the California Avocado Commission (CAC). This proposal was funded for 18 months to provide the recently hired PI (Dr. Manosalva) time to better assess the status of the rootstock breeding program and to revise and modify the original proposal according to program needs. In addition to this, an Advisory Breeding Committee was established by the CAC to help the team to evaluate the first-year achievements and plan the future direction for the rootstock breeding program. We revised the activities that were originally proposed in the previous scope of work so that we can better achieve the goal of a breeding program that will result in the timely development of rootstocks with the resistance to *Phytophthora cinnamomi* (*Pc*), the causal agent of Phytophthora root rot disease (PRR), and salinity. We made changes based on the initial program assessment, current status of the program, and the inputs and revisions from the Advisory Breeding. These adjustments allow us to: i) genotype, curate, and assess the genetic diversity and structure of our germplasm, ii) re-plant genotyped material for better maintain ace (50%), iii) establish more efficient breeding blocks to increase the genetic diversity, selection, and genetic analyses, iii) establish more small and large-scale rootstock trials, iv) develop two mapping populations which were genotyped and phenotyped, and iv) improve our greenhouse selection process. The first cycle of CAC funding allows us to assess the phenotypic and genotypic characterization of *P. cinnamomi* regarding virulence and fungicide sensitivity. We demonstrated changes in pathogen populations towards a more aggressive and potassium phosphite insensitive populations. Moreover, in collaboration with Dr. Jim Adaskaveg, we tested the *in vitro* sensitivity of the PRR pathogen against several fungicides with different mode of action (MOA). We conducted fungicide efficacy experiments under greenhouse conditions. Field fungicides trials are being conducted by Dr. Adaskaveg as part of one of our USDA grants to register new fungicides to controls PRR. Under the original CAC funding cycle (2016-2019), it was possible gather the data required so Dr. Jim Adaskaveg could register **Orondis** to control PRR in avocado (**Belisle *et al.* 2019**).

Due to several reasons including extreme heat waves and fires in CA, the California avocado industry faced smallest crop which affected negatively the industry back in 2019. ***Thus, CAC prioritize the rootstock new cycle of funding (2019-2022) to be used only on establishment of large-scale trials with the five UCR advanced rootstocks and the continuation of some small regionals trials to continue the evaluation of these rootstocks for their commercial release in CA. Note that the rootstock program modifications and outputs of the first funding cycle (2016-2019) was instrumental to secure USDA federal and CA state funding to continue the activities of the program especially the continuation of the fundamental research that is critical to speed-***

up the breeding and development of new rootstocks. Currently, our new breeding blocks allowed us to increase the genetic diversity of our germplasm with more admixed rootstocks (combination of races) and selection of highly resistant rootstocks with West Indian (WI) and Guatemalan (G) genetic make-up which is important to confer salinity resistance. These new selections will be soon evaluated in small trials with ‘Hass’ and other scions as part of our new USDA and International industry funding.

UCR Advanced Rootstocks

In the last decade resistance to salinity and other environmental stressors have been assessed by the UCR breeding program under field conditions. Currently, all UCR rootstocks selections were selected for their high *P. cinnamomi* resistance after GH seedling and clonal trees screening. Currently, we are evaluating ~55 UCR rootstock selections grafted to Hass in 7 active small regional trials in Santa Paula, Temecula, Fallbrook, and Ramona. In addition, we have the most advanced rootstocks grafted with Hass being tested in 9 large commercial trials established in 2019, 2020, and 2021 in Temecula, Camarillo, Goleta, Ventura, and San Luis Obispo (established under this cycle of CAC funding [2019-2022]). These selections are being tested for field performance when grafted to ‘Hass’ regarding tree health, salinity damage, heat damage, cold damage, tree size, tree vigor, canopy size, blooming, flushing, fruit set, and yield. These fields represent different environmental conditions and cultural practices: i) PRR problems, ii) high salinity and chloride toxicity, iii) high pH and alkalinity (as CaCO₃), iv) waterlogging conditions and clay soils, and v) different cultural practices (i.e., organic, mulching, gypsum, high density planting, etc).

Eight years of field data for five UCR advanced rootstocks, PP35, PP40, PP42, PP45, and PP80, supports the continuation of semi-commercial evaluation of these rootstocks grafted with Hass and other varieties as well as their commercial release in California (**Table 2**). In 2022, under a USDA-SCRI funding, these five rootstocks grafted with ‘Hass’ and other scions are being tested for their performance under Laurel Wilt conditions in Florida. In addition, multi state rootstock trials were established with these UCR advanced rootstocks grafted with Hass, Waldin, Lula, Sharwill, GEM, Lamb-Hass, and Reed in Florida, Puerto Rico, Texas, Hawaii, and CA. In addition, one large-scale trial was established in Goleta, Santa Barbara to evaluate PP35, PP40, and PP80 grafted with Hass, GEM, and Lamb Hass as part of a USDA-SCRI grant activity. PP35, PP40, and PP45 is currently being tested by Dr. Mary Lu Arpaia grafted with Hass, Carmen, GEM, Lamb, and Reed in a rootstock trial in Saticoy, Ventura. This plot was established in 2012. Tree health and harvest data collected at this site since 2015 indicate that Duke7, Steddom, PP40, and PP35 exhibited the best yield and yield efficiency when grafted with these different scions (**Fig. 2**). In addition, in collaboration with Dr. Clara Pliego (Malaga, Spain), we will test all these UCR five advanced rootstocks in Spain for resistance to white root rot (WRR) caused by *Rosellinia necatrix* using Eurosemillas S.A funding. Current field data from California support the continuation of the evaluation and data collection for these five UCR advanced rootstocks to gather the most compelling data especially for yield and packing to support their commercial release within the next 3 years.

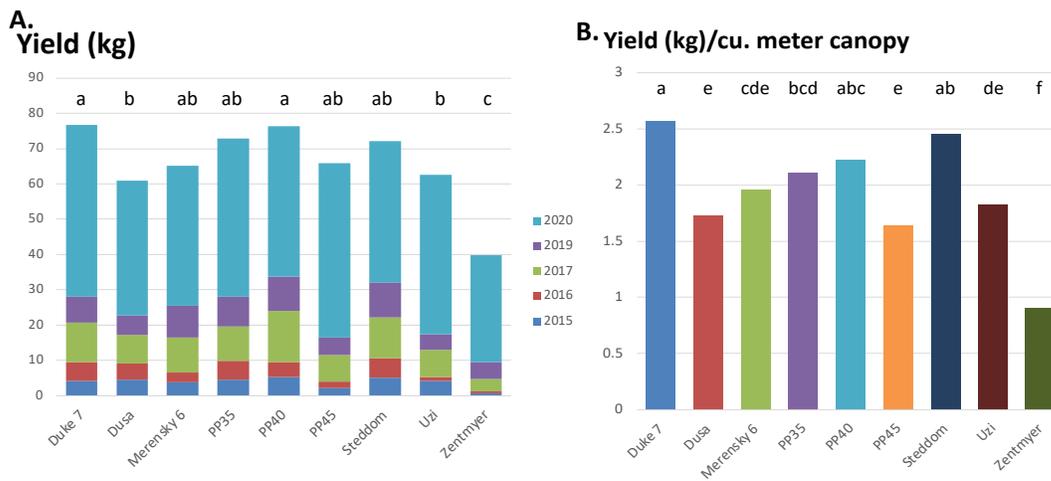
Table 2. List of advanced UCR rootstocks.

Rootstock	Race	Active Fields	Field conditions	Phenotype	Years of tree health and harvest data [#]
PP35	M x G	<p>Small trials Santa Paula (Hass, 2011), Santa Paula (Hass, 2017), Fallbrook (Hass, 2017), Pala (Hass, GEM, Lamb-Hass, Reed, 2022), Saticoy (Hass, Carmen, GEM, Lamb, and Reed, 2012).</p> <p>Large trials Temecula (Hass, 2019), Camarillo (Hass, 2019), Temecula (Hass, 2020), 2 plots in Ventura (Hass, 2020), Goleta (Hass, 2020), San Luis Obispo (Hass, 2020), Goleta (Hass, GEM, Lamb-Hass, 2022).</p>	High PRR incidence, high salinity, high levels of chloride, high pH, alkalinity (as CaCO ₃), and waterlogging conditions.	Good Phytophthora Root Rot (PRR), salinity tolerant, vigorous trees, low tree mortality and some places less than Dusa, some levels of heat tolerance. Good yield similar to Dusa. No strong alternative bearing effect on Hass. In some field growth smaller than Dusa, making it desirable for high density or hedge-row avocado plantings.	8
PP40	M x G	<p>Small trials Santa Paula (Hass, 2006), Santa Paula (Hass, 2017), Fallbrook (Hass, 2017), Pala (Hass, GEM, Lamb-Hass, Reed, 2022), Saticoy (Hass, Carmen, GEM, Lamb, and Reed, 2012).</p> <p>Large trials Temecula (Hass, 2019), Camarillo (Hass, 2019), Temecula (Hass, 2020), 2 plots in Ventura (Hass, 2020), Goleta (Hass, 2020), San Luis Obispo (Hass, 2020), Goleta (Hass, GEM, Lamb-Hass, 2022).</p>	High PRR incidence, high salinity, high levels of chloride, high pH, alkalinity (as CaCO ₃), and waterlogging conditions.	Good Phytophthora Root Rot (PRR), salinity tolerant, vigorous trees, low tree mortality and some places less than Dusa, moderate heat sensitivity. Good yield similar to Dusa and better than Dusa in some fields. No strong alternative bearing effect on Hass.	8
PP80	M x G	<p>Small trials Santa Paula (Hass, 2017), Fallbrook (Hass, 2018), Pala (Hass, GEM, Lamb-Hass, Reed, 2022).</p> <p>Large trials Temecula (Hass, 2021), Camarillo (Hass, 2021), Ventura (Hass, 2020), Goleta (Hass, 2020), Goleta (Hass, GEM, Lamb-Hass, 2022).</p>	High PRR incidence, high salinity, high levels of chloride, high pH, alkalinity (as CaCO ₃), and waterlogging conditions.	Good Phytophthora Root Rot (PRR) similar to Dusa, some levels of salinity tolerance, vigorous trees, good levels of heat tolerance better than Dusa. We need to collect more tree health and yield data since is the most recent selection.	8 ^s
PP42	M	<p>Small trials Santa Paula (Hass, 2006), Santa Paula (Hass, 2017), Fallbrook (Hass, 2017).</p> <p>Large trials Temecula (Hass, 2021), Camarillo (Hass, 2021), Ventura (Hass, 2020), Goleta (Hass, 2020).</p>	High PRR incidence, high salinity, high levels of chloride, high pH, alkalinity (as CaCO ₃), and waterlogging conditions.	Good Phytophthora Root Rot (PRR) better than Dusa, some levels of salinity tolerance, vigorous trees, good levels of heat tolerance. Good yield (similar to Dusa). No strong alternative bearing effect on Hass.	8
PP45	M	<p>Small trials Santa Paula (Hass, 2006), Santa Paula (Hass, 2011), Santa Paula (Hass, 2017), Fallbrook (Hass, 2017), Saticoy (Hass, Carmen, GEM, Lamb, and Reed, 2012).</p>	High PRR incidence, high salinity, high levels of chloride, high pH, alkalinity	Good Phytophthora Root Rot (PRR) better than Dusa, susceptible to salinity , vigorous trees, good levels of heat tolerance better than Dusa. Good yield (similar to	8

		<p>Large trials Temecula (Hass, 2020), 2 plots in Ventura (Hass, 2020), Goleta (Hass, 2020), San Luis Obispo (Hass, 2020).</p>	(as CaCO ₃), and waterlogging conditions.	Dusa). No strong alternative bearing effect on Hass. This rootstock is the best producer in plots with high PRR incidence which is good for replanting under these conditions.	
--	--	--	---	--	--

= data collected since 2015-2022, there is not harvest data in 2015 and 2020 (COVID-19). \$ field data is less for this rootstock since from 2015-2019 was only planted on two plots.

Figure 2. Yield and yield efficiency data for the UCR rootstocks PP35, PP40, and PP45 grafted to Hass, Hass, Carmen, GEM, Lamb, and Reed. A. Yield and B. Yield efficiency.



Overall Goal: The overall goal for this proposal is to continue with the generation and collection of compelling field and horticultural data require to commercially release five of the most promising advanced UCR rootstocks (PP35, PP40, PP42, PP45, and PP80), which are currently under field evaluation in small regional and large-scale trials throughout California.

To address this goal, we have divided this proposal in three objectives:

Objective 1. Establishment of commercial-scale field trials of PP35, PP40, PP42, PP45, and PP80 UCR advanced rootstocks before official release in cooperation with CA growers and Non-Propagation Agreements (NPAs). The objective at the San Luis Obispo (SLO) site was to evaluate the establishment of a long-term commercial planting with statistically valid blocking and replications of ‘Hass’ avocado grafted with the UCR rootstocks in a field with a known history of PRR.

Objective 2. Collection of tree health and harvest data for PP35, PP40, PP42, PP45, and PP80 UCR advanced rootstocks at previously established field trials in Ventura.

Objective 3. Continue the collection of tree health and harvest data for PP35, PP40, PP42, PP45, and PP80 UCR advanced rootstocks, Israeli rootstocks, and South African rootstocks at Pine Tree and Bonsall rootstock trials (established June 2017).

MATERIAL AND METHODS

Establishment of commercial-scale field trials.

The goal of these large trials is to have a better assessment of yield, packing data, and also will be a way to test early adoption of the UCR rootstocks before releasing them. We will test PP35, PP40, PP42, PP45, and PP80 rootstocks at large-scale with California avocado growers under appropriate NPA agreements. Site selection was done in conjunction with Dr. Tim Spann (CAC Research Director). Soil and water analyses were conducted by Fruit Growers Lab and the PRR detection and incidence (amount of pathogen present in the field) were conducted by FGL and the Manosalva Laboratory. Two large-scale field trials were established in collaboration with John Lamb at his ranch located in Camarillo and with Leo McGuire at his ranch located in Temecula. Brokaw Nursery delivered 200 trees of PP35 and 150 trees of PP40 at each ranch and fields were planted in Summer 2019. Five additional large-scale trials of 100 trees per accession (Dusa®, PP35, PP40, PP42, PP45, and PP80) were established in Summer 2020. In summer 2021, two rootstock trials of ‘Hass’ trees grafted with Dusa®, PP42, and PP80 rootstocks were established as an extension of the trials located at Lamb and McGuire rootstock trials in Camarillo and Temecula, respectively.

Budwood for each field trial was collected by members of the Manosalva Lab and personnel from Brokaw Nursery. The amount of budwood provided to the nursery was carefully recorded and monitored through the clonal process since the amount of material for these five UCR rootstock provided to a third party needs to be recorded for the NPA agreements. Brokaw Nursery requires at least two to three times the amount of budwood for the total of tree requested per accession. This also will depend on the tree quality at the time of collection. **Table 3** showed the amount of budwood from all the PP35 and PP40 trees available at AgOPs and SCREC provided to Brokaw for the Spring 2019 order (total of 350 trees). **Table 4** indicates the number of trees available for each rootstock for budwood collection.

Table 3. Amount of budwood collected and required by Brokaw Nursery to clonally propagate 200 PP35 and 150 PP40 field trees to be delivered Spring 2019.

Variety	Rootstock	Stock Grafting								Scion Grafted	Field Planted	Quantity Ordered
		Buds Harvested	Tuesday, Feb. 27th	Thursday, March 1st	Friday, March 2nd	Buds Harvested	Total Harvested	Total Stock Grafted	% Utilization			
Hass	PP35	500	-	310	100	410	910	448	49%	337	-	200
Hass	PP40	-	330	-	240	570	570	424	74%	263	-	150
	Total	500	330	310	340	980	1,480	872	59%	600	-	350

Table 4. Mother trees corresponding to the UCR advanced selections used for budwood collection.

Rootstock	AgOPs_UCR	SCREC
PP40	BB4_2001 (1) , BB10_2018 (5)	F6_1984 (1), F6_2001 (1), F7_2018 (3), F48_2018 (10)
PP35	BB10_2009 (7), BB10_2018 (10)	F6_1985 (1), F7_2018 (3), F48_2018 (10)
PP80	BB10_2018 (10)	F6_2003 (2), F6_2012 (2), F7_2018 (3)
PP42	BB4_2001 (1)	F6_1985 (1), F7_2018 (3), F48_2018 (10)
PP45	BB10_2009 (3)	F6_2001 (1), F7_2018 (3)
Dusa	BB9_2003 (4)	F6_1984 (3), F7_2018 (3)

BB= Breeding block located at AgOPs (UCR). Field =F. Year of establishment is indicated for both (BB and F) and in parenthesis is indicated the number of tree for the specific rootstock at each location.

‘Hass’ avocado trees grafted on Dusa®, PP35, PP40, PP42, PP45, and PP80 were purchased by the CAC. Trees were generated and delivered at each experimental site by Brokaw Nursery. Manosalva and her team conducted several visits to discuss the field design at each experimental field trial locations. Considering that the main objective of these large field trials is to collect harvest and packing data, thus the scientific design was not our top priority. The field design was done to fit the needs of the grower cooperators for an easy harvest and data recording per rootstock accession. At each large-scale rootstock trial, a subpopulation of trees (30-50 /rootstock) per rootstock accession were selected to collect tree measurements and health data. At each field trial a map was done and is being updated regularly by the Manosalva team. In addition, blocks of rootstocks have been marked and painted with different colors as code for easy identification. Trees used to collect tree health data have been paint sprayed and tagged with metal tags. Label replacements are conducted regularly at each trial. The large-scale rootstock trial established at SLO site was conducted by Dr. Lauren Garner and her team. At this site, ‘Hass’ avocado trees grafted on PP35, PP40, PP45, and Dusa® rootstocks were transplanted at Cal Poly San Luis Obispo on 24 June 2020 using a randomized complete block design with 10 replications of 8-10 trees per treatment in 3 blocks for a total of 384 trees. Trees were planted at a 15’ x 20’ tree spacing in a field with a known history of Phytophthora root rot (PRR), and trees exhibited similar size at the time of planting. Data is collected for each tree at this location by Dr. Garner and her research team.

Monitoring and data collection.

All field trials under this funding cycle, were monitored from 3 to 4 times a year. Field data was collected as indicated in **Table 5**. We plan to monitor these sites for another 8 to 10 years. Harvest and packing data will be collected at each and will be discussed with each grower cooperator. At the San Luis Obispo location, all trees were assessed by Dr. Lauren Garner and her team, who evaluated tree height (m), above-graft trunk diameter (mm), and below-graft trunk diameter (mm), in addition to rating salinity damage, heat damage, vegetative flush and bloom on a scale of 0-5. All trees were measured and assessed 2 months after transplanting (August 2020) and during flushing in spring (March 2021 and 2022) summer (July 2021 and 2022) and fall (October 2021 and 2022), with all quarterly assessments being overseen by the graduate students.

Table 5. Field site data collected	
Quarterly	<ul style="list-style-type: none"> a. Observe trees and document any noteworthy events such as excessive bloom, fruit set, fruit or leaf drop, heat damage, etc. b. Discuss with cooperators any concerns and modifications in their cultural management such as pruning and nutritional practices that may influence results. c. Update field maps, landmarks, and re-tag trees for identification as necessary.
Biannually (Spring and Fall)	<ul style="list-style-type: none"> a. Measurements: tree height and canopy size (tree height and width). b. Overall tree health (0 best – 5 dead). c. Leaf necrosis (salinity), heat damage (0 best – 5 dead), flush (0 - 5 best). Blooming (0 - 5 best),

	Fruit set (0=none, 1= <10 fruits/tree, 2= <30 fruits/trees, 3 = > 30 fruits/tree.
Annually	<p>a. Trunk circumference below and above the bud union will be collected in the Fall following the end of the summer flush (approximately October).</p> <p>b. <i>Small regional trials</i>: Individual tree yield data (weight and fruit number). Average fruit size will be calculated from the harvested weight and fruit number. Yield efficiency will be calculated using canopy size.</p> <p><i>Large-scale trials</i>: harvest will be conducted by rootstock accession. Crop will be sent to packing house to obtain total pounds, total fruit count, and size distribution. Harvest will be coordinated between the Manosalva Lab and with grower cooperators.</p>

Scoring systems: All the field trials in this proposal will use the UCR rootstock breeding program scoring system for tree field performance to standardize field data. This scoring system is being used by our collaborators in USA and in other countries where these 5 UCR advanced rootstocks will be evaluated as part of our funding with USDA and Eurosemillas S.A in the coming years. We visually rated the trees for overall tree health using a 0 to 5 scale (**Table 3, Fig. 3**). We rated trees at each site for leaf/steam necrosis/dieback (symptoms of salinity or heat damage) on a 0 best to 5 dead scale (**Table 3, Fig. 3**).

Score	Overall Health	Salinity/Heat
0	Perfect looking tree	0 - 5 % damage, perfect/healthy
0.5	Slightly off (less leaves/small leaves, lack of flush)	5 - 10 %
1	Yellow leaves and or small leaves	11 - 20 %
2	Exposed branches, wilting leaves, small yellow leaves	21 - 40 %
3	Branch dieback, very few leaves remaining, starting to die	41 - 60 %
4	Almost dead, won't last long	61 - 80 %
5	Dead	81 - 100 %



Overall tree health and leaf necrosis = 0 Overall tree health = 4 Leaf necrosis = 0 Overall tree health = 3.5 Salinity damage = 4

Figure 3. UCR scoring system.

We measured tree height, canopy height, and width to calculate canopy volume. Yield efficiency for each rootstock was calculated (lbs. fruit per cubic meter of tree canopy). We measured trunk diameter 6 cm below and above the bud union to calculate the bud union ratios (- 1 = rootstock > scion; 0 = smooth bud union; and 1 = scion > rootstock). We scored flush and blooming using a score of 0= none to 5 = (81-100% of tree) (**Fig. 3**). Fruit set was also recorded using a score system 0 – 3 where 0= no fruits, 1= < 10 fruits/tree. 2= < 30 fruits/tree, 3 = >30 fruits/tree. Harvest and packing data were conducted in coordination with the grower cooperator or manager. Individual tree harvest data/rootstock was collected for small regional trials. The average fruit weight per tree and yield efficiency was also calculated.

Statistical Analyses. Data was analyzed using one-way repeated measures ANOVA and are reported herein. Mean comparison was conducted using the Tukey’s Honest Significant Difference (HSD) test at P = 0.05. Data was not-transformed. The categorical data for the entire data collection period at SLO is being analyzed and preliminary results are reported herein.

RESULTS AND DISCUSSION

Obj. 1. Establishment of large-scale field trials of ‘Hass’ grafted PP35, PP40, PP42, PP45, and PP80 rootstocks and data collection.

Establishment of commercial-scale field trials. A total of nine rootstock trials were established. Dr. Tim Spann and Dr. Manosalva selected the growers and sites for these plantings. Soil and water samples were collected and used for PRR incidence calculation at the Manosalva Lab. Samples were also sent to Fruit Growers Lab (FGL) to conduct soil comprehensive and water irrigation suitability analyses. **Table 6** describes the rootstock accessions planted at each site and the number of trees of each rootstock. Field conditions such as PRR incidence, salinity and soil pH for each site is reported (**Table 6**). Each rootstock accession was planted in a single block to facilitate subsequent harvest data collection with the exception of the plot at San Luis Obispo.

Table. 6. Description of the large-scale trials established in California. Number of trees per rootstock grafted with Hass planted is indicated in parenthesis.

Grower/Manager	City/Cou nty	Year planted	Rootstocks (#s)	Field conditions
Leo McGuire	Temecula/ Riverside	2019	PP35 (102), PP40 (75)	E.C value of 0.86 dS/m, however, the chloride level is slightly high 102 mg/L indicating a possible problem with chloride toxicity. High pH (7.9) and alkalinity (as CaCO ₃). High PRR incidence.
Leo McGuire	Temecula/ Riverside	2021	Dusa (100), PP42 (100), PP80 (100)	E.C value of 0.86 dS/m, however, the chloride level is slightly high 102 mg/L indicating a possible problem with chloride toxicity. High pH (7.9) and alkalinity (as CaCO ₃). High PRR incidence.
John Lamb	Camarillo /Ventura	2019	PP35 (100), PP40 (51)	Normal E.C value of 1.16 dS/m, however, there is a high level of chloride 148 mg/L, indicating problems with chloride toxicity which indicate fairly poor crop suitability even if amendments such as gypsum, sulfuric acid (98%), or if leaching is applied. In addition, the water analyses show problems with high pH (8.7) and alkalinity (as CaCO ₃). <i>Phytophthora cinnamomi</i> was not detected in this field.
John Lamb	Camarillo /Ventura	2021	Dusa (100), PP42 (100), PP80 (100)	Normal E.C value of 1.16 dS/m, however, there is a high level of chloride 148 mg/L, indicating problems with chloride toxicity which indicate fairly poor crop suitability even if amendments such as gypsum, sulfuric acid (98%), or if leaching is applied. In addition, the water analyses show problems with high pH (8.7) and alkalinity (as CaCO ₃). <i>Phytophthora cinnamomi</i> was not detected in this field.

Andrew Gabryzak/Newhouse Green Gold Currently, Adna Farms, LLC.	Temecula/Riverside	2020	Dusa (100), PP35 (116), PP40 (100), PP45 (70)	High chloride levels, high pH, and high alkalinity as CaCO ₃ . High PRR incidence, and possible problem with soil saturation (soil contain high clay composition).
Chris Sayer/ Petty Ranch	Ventura	2020	Dusa (100), PP35 (116), PP40 (100), PP45 (70)	High water salinity (2.3 dS/m), high iron levels, high alkalinity as CaCO ₃ , severe problem of total water hardness. <i>P. cinnamomi</i> was not detected. Soil analyses indicate normal chloride levels and soil salinity, optimum saturation (on the high side, might have some problems in the future). High limestone.
Masood Sohaili & Rick Shade/ Alina LLC Ranch	Ventura	2020	Dusa (61), PP35 (116), PP40 (100), PP45 (100), PP42 (28), PP80 (39)	This field has problems with high PRR incidence (100%) which is a serious problem for replanting. Soil analyses indicate normal chloride and salinity levels, optimum saturation (on the high side, might have some problems in the future). High limestone. Water analyses indicate not problems with salinity.
Pete Miller	Goleta/ Santa Barbara	2020	Dusa (100), PP35 (116), PP40 (100), PP45 (100), PP42 (28), PP80 (39)	Section 1 (S1): 60% of PRR incidence. Chloride is not a problem yet but it is on the high side (eventually will became a problem), high soil salinity (2.71 dS/m), has 99% of saturation, high CEC. Section 2 (S2): 40% of PRR incidence. Soil analyses indicate high chloride levels, high soil salinity (3.65 dS/m), and high % of saturation (66.5%), clay soil. Section 3 (S3): 0% of PRR incidence. No problems with salinity or chloride. Low nitrogen, optimum soil saturation Section 4: 90% of PRR incidence. No problems with salinity or chloride. Optimum soil saturation and pH. Section 5: 50% of PRR incidence. No problems with salinity or chloride. Optimum soil saturation and pH.
Dr. Lauren Garner/ California Polytechnic State University	San Luis Obispo	2020	Dusa (96), PP35 (96), PP40 (97), PP45 (95),	Soil and water analyses does not show major problems with salinity, pH, saturation. A total of 16 soil samples from the field were processed by the Manosalva team using different techniques, however no <i>Phytophthora cinnamomi</i> was recovered. Isolates recovered were Pythium and Phytopythium. We need to conduct this evaluation of roots from trees in the field trial.

1). Leo McGuire plot 1, Temecula, (2019). A total of 102 PP35 and 75 PP40 trees grafted to ‘Hass’ were planted in Temecula on June 14, 2019. Trees for each rootstock were arranged as rootstock per raw in the field (**Fig. 4 and Fig. 5**). Trees were planted into the top of mounds at a 15 x 20 ft tree spacing. A subset of 30 trees (highlighted in green) were selected and tagged by spraying color paint and tagged with metal tags to collect tree health data. Tree health data has been collected since 2019 until 2022 as indicated in **Table 5**. Trees were rated on June 27 (almost 2 weeks after planting). We did not find statistically significant differences between PP35 and PP40 regarding tree height. However, we did find significantly differences in trunk diameter above

and below union as well as diameter ratio (above versus below the bud union) between these two rootstocks for this plot (**Fig. 6**). Mean separations were performed using Student's t-test analyses.

Figure 4. Map for Leo McGuire's 2019 plot

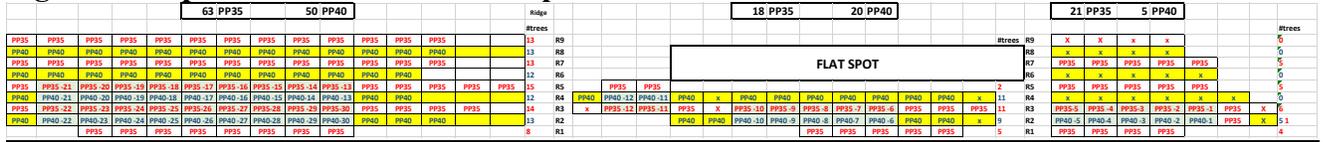


Figure 5. Trees planted in mounds at Leo McGuire's plot



Figure 6. Data collected from a subset of 30 trees per rootstock at Temecula plot. SE=standard error.

Rootstock	Tree height (in)		Diameter above bud union (mm)		Diameter below union (mm)		Ratio (above/below)		Student's t-test			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Tree height	Above bud union	Below bud union	Ratio (above/below)
PP35	43.50	0.54	16.01	0.23	16.87	0.26	0.95	0.01	A	A	A	A
PP40	41.37	0.54	14.84	0.23	13.82	0.26	1.08	0.01	A	B	B	B

Tree performance at Leo McGuire Plot 1, Temecula (2022 field data)

Thirteen PP40 (17% tree mortality) and 10 PP35 (9.8% trees mortality) trees died and were replaced with extra trees in July 2020 (trees have been labeled). In October 2022, no significant differences were found between PP35 and PP40 at this location regarding overall tree health, salt damage, heat damage, flushing, and blooming scores. All trees grafted on PP35 and PP40 trees exhibited heavy flush and blooming.

‘Hass’ trees grafted on PP40 rootstocks exhibited more tree height, more canopy volume, and more fruit set than trees grafted on PP35 (**Fig.7, Fig. 8**). Interestingly, at this location, ‘Hass’ trees grafted on PP35 exhibited significant less trunk diameter above and below union when

compared with trees grafted on PP40, however PP35 and PP40 ratios of trunk diameter above and below the graft union is 1.

Figure 7. Field ratings at Leo McGuire’s plot in Temecula (October 2022)

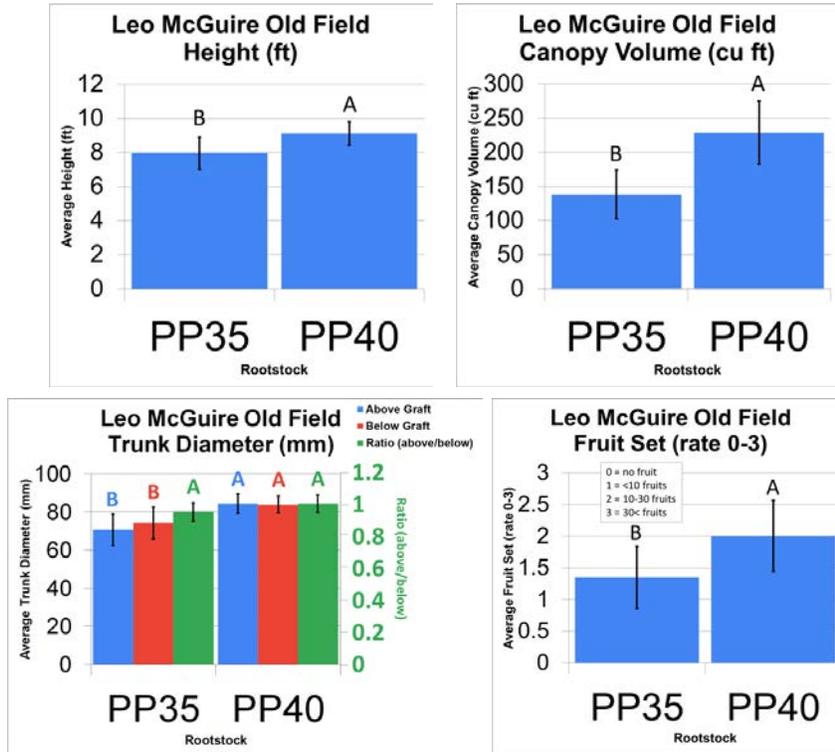


Figure 8. Leo McGuire showing a ‘Hass’ tree grafted on PP40 (Nov. 2021)



Harvest data at Leo McGuire Plot 1, Temecula (2021-2022). The first harvest of this plot was conducted on April 2021. A total 95 fruits were collected for a total weight of 53.7 pounds (lbs) for PP35 (0.56 lb/PP35 fruit). A total of 13 fruits were collected from PP40 producing a total weight of 7.1 lbs (0.54 lbs/PP40 fruit). The second harvest was conducted in this plot on January 26 (2022) and crop was sent to packing house by Leo McGuire who provide the data presented in this report. Amber Newsome from the Manosalva lab supervised the harvest at this plot. Trees in this plot were planted in June 2019. From 95 trees of PP35 trees grafted with ‘Hass’ we obtained 3820.57 average fruit count and a total of 1,718 lbs (marketable fruit) from a total 1756 lbs. including culls. The average fruit number per tree was 39.39 and the average weight (oz)/fruits was 7.19 oz. Majority of the crop for PP35 was marketable sizes: 37.24% (48) and 36.05% (60) (Fig. 9). We obtained 2937.37 average fruit count and a total of 1,404 lbs (marketable fruit) from a total 1449 lbs. including culls from 75 ‘Hass’ trees grafted on PP40. The average fruit number per tree was 39.16 and the average weight (oz)/fruits was 7.65 oz. Majority of the crop for PP40 was marketable sizes: 50.7% (48) and 23.9% (60) (Fig. 10).

Figure 9. Data for PP35 harvest collected from packing house (2022), Temecula.

Bin # 62078			Approximate Fruit Counts By Weight			
Fruit Quality #	Size	Weight (lbs)	Min Fruit Count	Max Fruit Count	Avg Fruit Count	% of Net
1	96	2	8.53	9.85	9.19	0.114
1	84	47	158.32	200.53	179.42	2.677
1	70	136	348.16	458.11	403.13	7.745
1	60	633	1350.40	1620.48	1485.44	36.048
1	48	654	1101.47	1395.20	1248.34	37.244
1	40	116	161.39	195.37	178.38	6.606
1	36	19	24.32	26.43	25.38	1.082
1	32	10	11.43	12.80	12.11	0.569
1	All combined	1617	3164.02	3918.77	3541.40	92.085
2	84	29	97.68	123.73	110.71	1.651
2	70	10	25.60	33.68	29.64	0.569
2	60	51	108.80	130.56	119.68	2.904
2	48	6	10.11	12.80	11.45	0.342
2	40	5	6.96	8.42	7.69	0.285
2	All combined	101	249.15	309.20	279.17	5.751
Marketable (1+2)	All combined	1718	3413.17	4227.97	3820.57	97.836
Culls	All combined	38				2.164
Total	All combined	1756				100

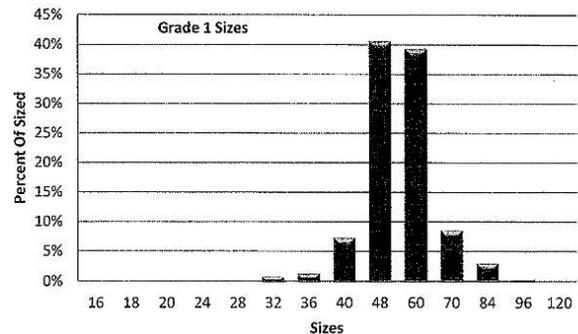
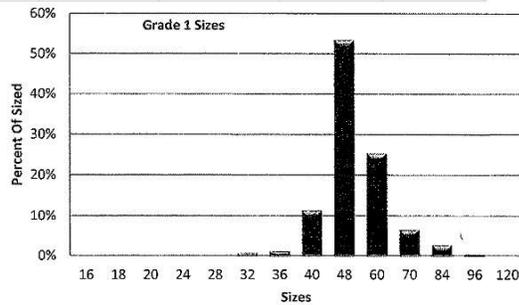


Figure 10. Data for PP40 harvest collected from packing house (2022), Temecula.

Bin # 62078			Approximate Fruit Counts By Weight			
Fruit Quality #	Size	Weight (lbs)	Min Fruit Count	Max Fruit Count	Avg Fruit Count	% of Net
1	96	2	8.53	9.85	9.19	0.138
1	84	34	114.53	145.07	129.80	2.346
1	70	87	222.72	293.05	257.89	6.004
1	60	347	740.27	888.32	814.29	23.948
1	48	734	1236.21	1565.87	1401.04	50.656
1	40	154	214.26	259.37	236.81	10.628
1	36	13	16.64	18.09	17.36	0.897
1	32	7	8.00	8.96	8.48	0.483
1	All combined	1378	2561.16	3188.57	2874.86	95.1
2	84	5	16.84	21.33	19.09	0.345
2	60	11	23.47	28.16	25.81	0.759
2	48	6	10.11	12.80	11.45	0.414
2	40	4	5.57	6.74	6.15	0.276
2	All combined	55.98	69.03	62.50	1.794	
Marketable (1+2)	All combined	1404	2617.14	3257.60	2937.37	96.894
Culls	All combined	45				3.106
Total	All combined	1449				100

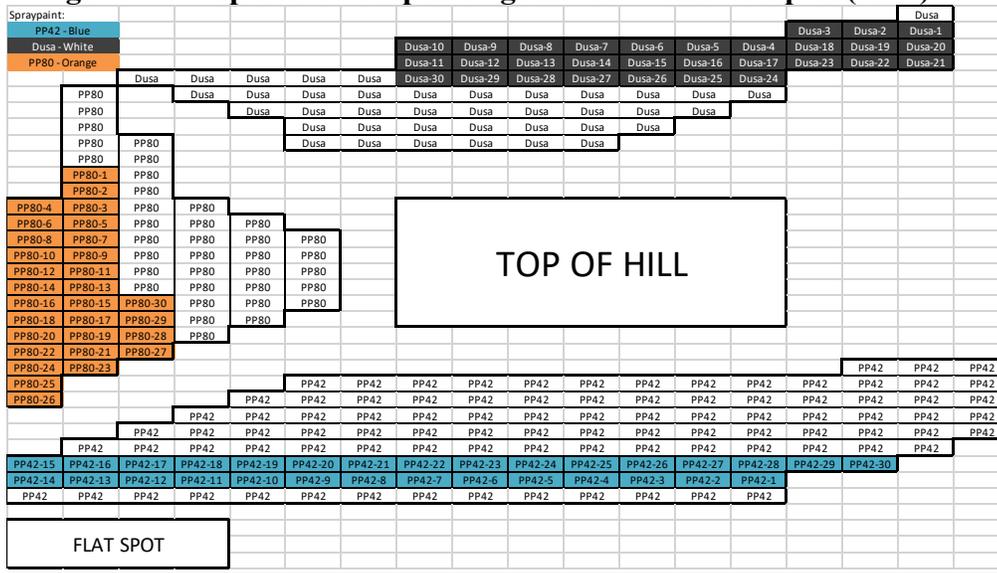


2). Leo McGuire plot 2, Temecula, (2021). In August 2021, this plot was expanded and we planted 100 Dusa, 100 PP80, and 100 PP42 rootstocks grafted with Hass. We selected a subset of 30 trees for each rootstock to collect field data (Fig. 11, Fig. 12). Trees were planted in blocks and each block was landmarked with spray paint and the 30 trees for data collection were tagged with metal tags for tree identification. Trees were planted into the top of mounds at a 15 x 20 ft tree spacing.

Figure 11. Planting day of the second plot at Leo McGuire Ranch in Temecula (2021).



Figure 12. Map of the new plantings at Leo McGuire's plot (2021)



Tree performance at Leo McGuire Plot 2, Temecula (2022 field data). No significant differences were found regarding tree height. Dusa® and PP80 exhibited the best rates for overall tree health, salt, and heat damage. PP80 exhibited significant more flushing followed by PP42 and Dusa®. There are significant differences regarding trunk diameter below and above the graft union and their ratio. Dusa® has significant higher ratio between graft union (1.1) when compared with PP42 and PP 80 (0.9) (**Fig. 13**). These preliminary results suggest the good performance of PP80 under high PRR incidence and elevated levels of chloride.

Figure 13. Field ratings at Leo McGuire's plot in Temecula planted in 2021 (October 2022)

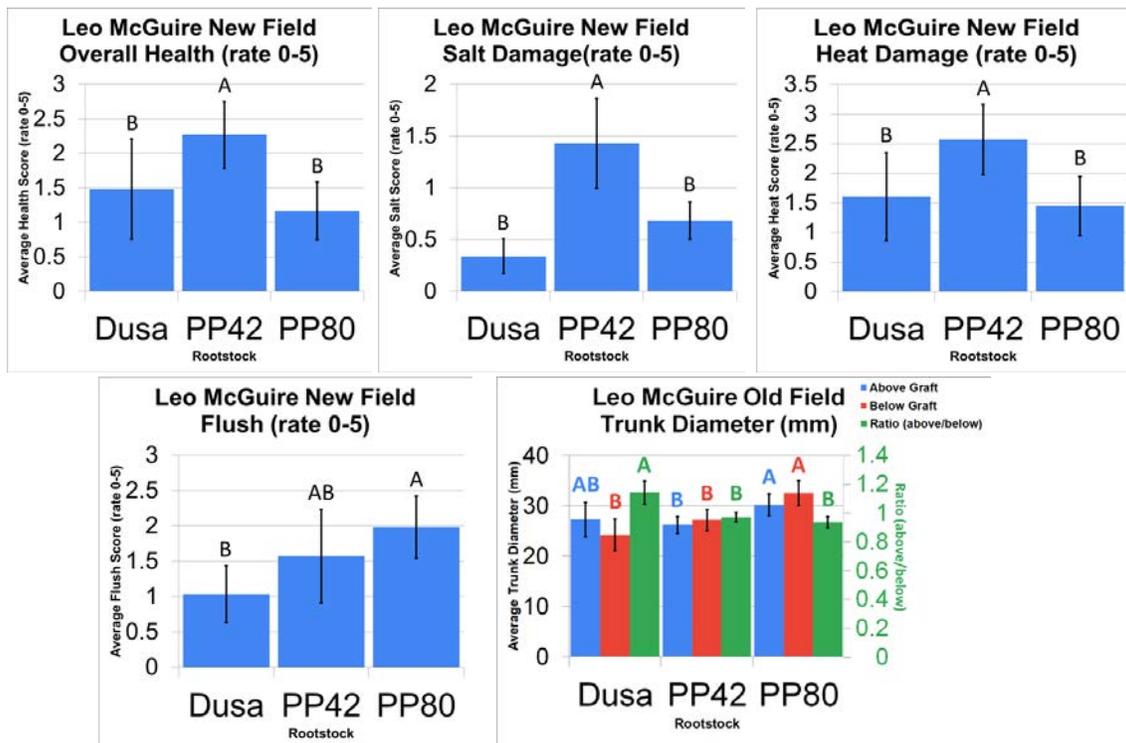


Figure 20. PP35 and PP40 trees at Camarillo Plot 1 (Summer 2022).

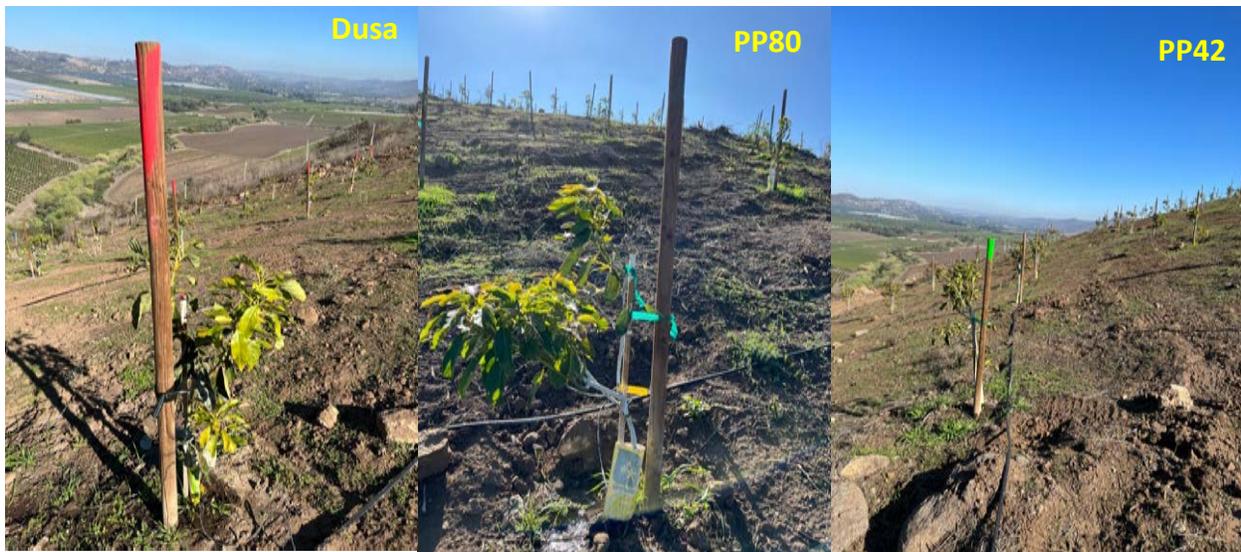


4). John Lamb plot 2, Camarillo (2021). In August 31th 2021, this plot was expanded and we planted 100 Dusa, 100 PP80, and 100 PP42 rootstocks grafted with Hass at 18' x 18' tree spacing. We selected a subset of 30 trees for each rootstock to collect field data (Fig. 21). Trees were planted in blocks and each block was landmarked with spray paint and trees being evaluated were tagged with metal tags (Fig. 22).

Figure 21. Map for John Lamb plot 2 planted in Camarillo in 2021. Trees highlighted are being evaluated.

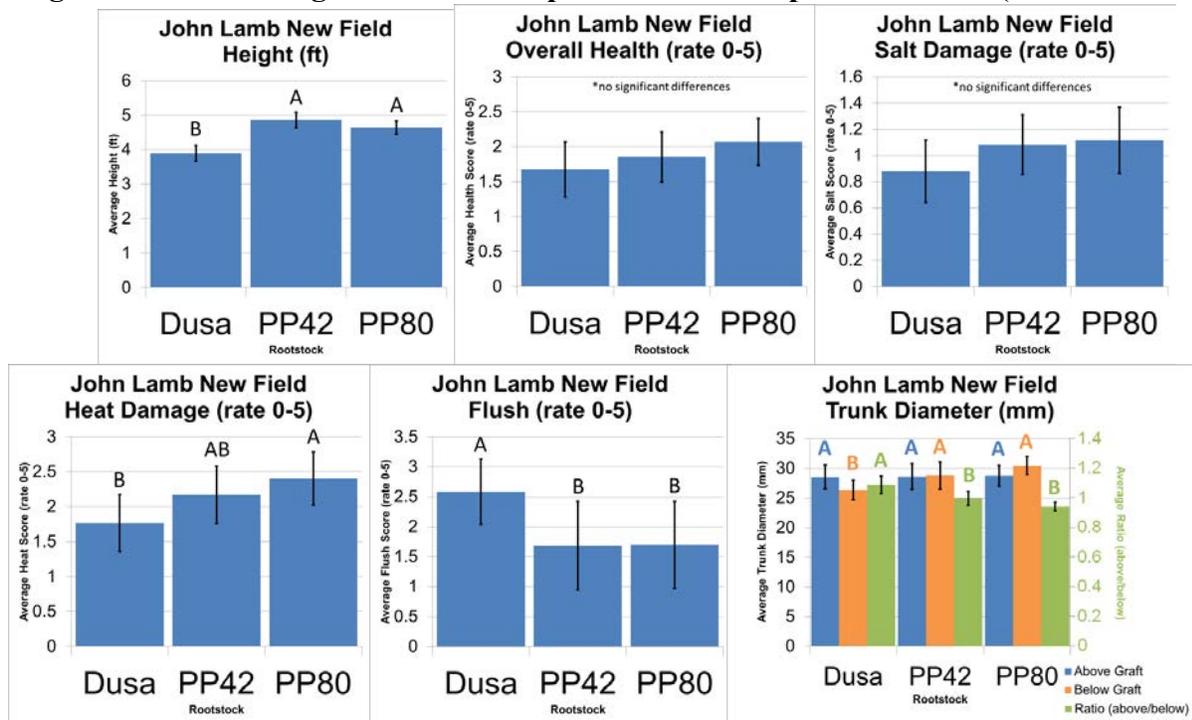


Figure 22. John Lamb plot 2 planted in Camarillo in 2021.



Tree performance at John Lamb Plot 2, Camarillo (2022 field data). Dusa exhibited less tree height than PP80 and PP42. No significant differences were detected among the rootstocks regarding overall tree health, and salinity damage. Dusa and PP42 exhibited similar scores of heat damage and PP80 seems more sensitive to heat damage in this location compared with Dusa. Dusa exhibited the best flushing scores. PP42 and PP80 exhibited similar trunk diameter above and below the union and 0.9 ratios. Similar to the replicated field in Temecula, Dusa has a ratio >1 on trunk diameter (**Fig. 23**).

Figure 23. Field ratings at John Lamb plot in Camarillo planted in 2021(October 2022).



5. Newhouse Green Gold Galen Newhouse (2020-2021)/Adna Farms, LLC (2022), Temecula (planted June 18-19, 2020).

Managers from 2020-2021: Andrew Gabryszak and Nick Lahr (WestPak Avocado).

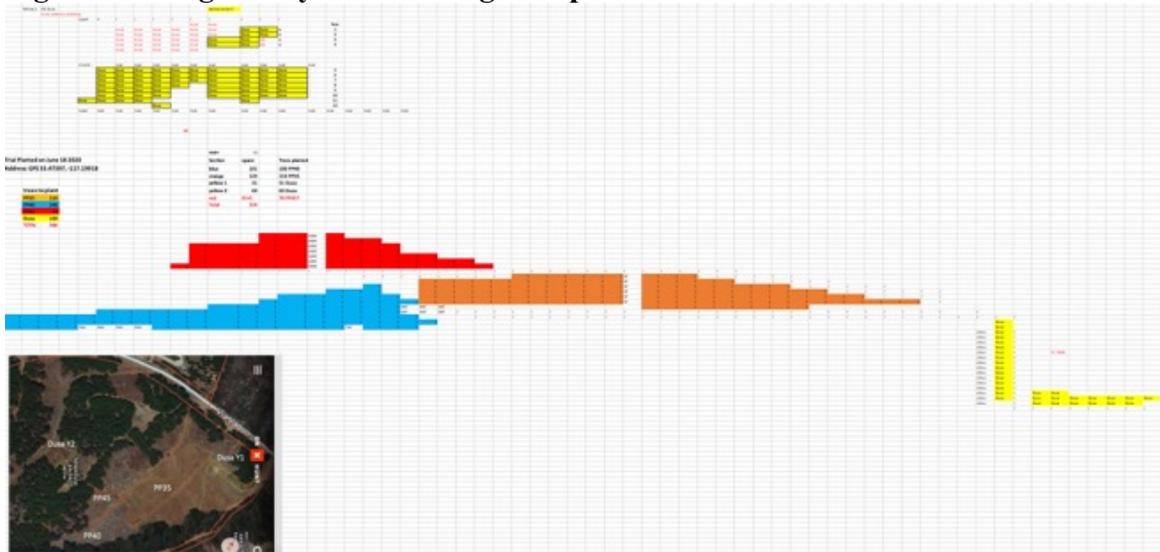
Current owners (2022): Adna Farms, LLC.

Note: Recently (2022), this land was bought by Adna Farms, LLC. They are interested on the continuation of this rootstock trial. Dr. Manosalva and her team met Grace Marcellina and CEO Adriadi Ang to discuss further the continuation of this collaboration.

Water analyses of this location indicate problems with high chloride levels, high pH, and high alkalinity as CaCO₃. *Phytophthora cinnamomi* (Pc), the causal agent of phytophthora root rot (PRR) was detected in the soil using traditional approaches involving pathogen isolation and morphological identification (50% PRR incidence). Soil analyses were conducted by Fruit Growers Lab (FGL). Soil comprehensive analyses were done and indicate possible problems with soil saturation (46%). Trees were planted at 20' x 15' tree spacing and all trees exhibited similar size at the time of planting (June 2020). As discussed with field manager on August 18th at the plot, our original designed plot consisted in planting the trees as blocks per rootstocks to facilitate harvest and individual yield and packing data for each rootstock (**Fig. 24**, original discussed

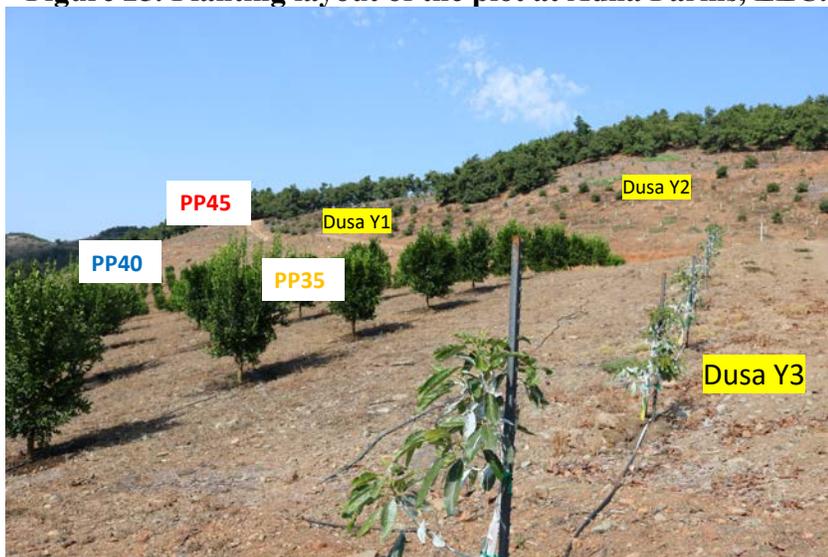
layout), however, 69 Dusa trees were interplanted with older grower's Dusa trees in one block, which will create complications for harvest and obtaining the data for Dusa. This problem was discussed with the manager on August 17th in order to be sure that they will be able to record harvest data per rootstock.

Figure 24. Original layout and design for planted discussed



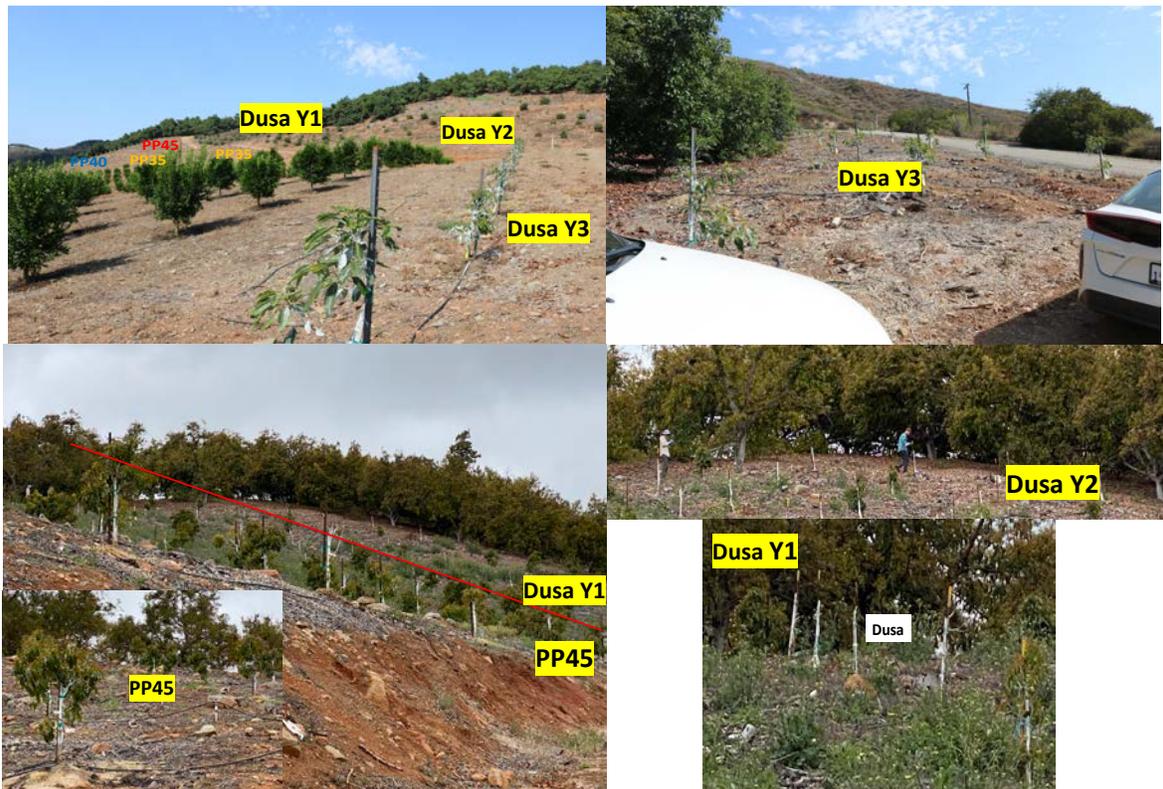
Two yellow sections for Dusa trees (Dusa Y1, 31 trees and Dusa Y2, 69 trees), one red section for PP45 trees, orange section for PP35 trees, and the blue section for PP40 trees. With the exception of the Dusa Y2 section containing 69 trees, all the other rootstocks were planted as described in **Fig. 25**. Unfortunately, due to COVID-19 restrictions we visited the plots before the planting (one day before) or the same day to discuss and landmark the fields but we did not stay for the complete planting because it was too much risk with so many people in the field.

Figure 25. Planting layout of the plot at Adna Farms, LLC.



At this plot in April 2021, ‘Hass’ avocado trees grafted on Dusa rootstocks exhibited the highest mortality (~80%). Majority of trees in the areas Dusa Y1 and Dusa Y2 indicated in **Fig. 25** died. We believe that the combination of high temperatures in July 2020, the soil structure (clay), and high PRR incidence was probably the cause of this high Dusa tree mortality at this location. The tree health data collected from Dusa corresponds to the 29 survivor trees in Y3 section (**Fig. 26**). Interestingly, ‘Hass’ trees grafted with PP45 rootstocks planted next to trees grafted on Dusa on the same hill showed 0% mortality and exhibited good performance (**Fig. 26**). PP45 exhibited better performance than Dusa under these conditions (high heat combined with heat exposure, heavy soil, and PRR). This better performance of PP45 over Dusa rootstock also has been observed in some plots at Ventura having similar growing conditions. We are starting conversations with Adna Farms to transplant the Dusa Y1 and Y2 areas for another commercially available rootstock as control (Israeli and South African rootstock controls).

Figure 26. Performance of ‘Hass’ trees grafted on Dusa and PP45 rootstocks at Adna Farms, LLC (April 2021).



In April 2021, 3/100 PP40 and 11/116 PP35 trees died at this location. No significant differences were observed among the rootstocks regarding overall tree health, and heat damage. The Dusa survivor trees in Y3 section exhibited less tree height. Dusa survivors, PP40, and PP45 exhibited less salinity damage when compared to PP35. PP40 exhibited less flushing when compared with other rootstocks (**Fig. 27**).

Figure 27. Tree performance at Adna Farms, LLC (April 2021).

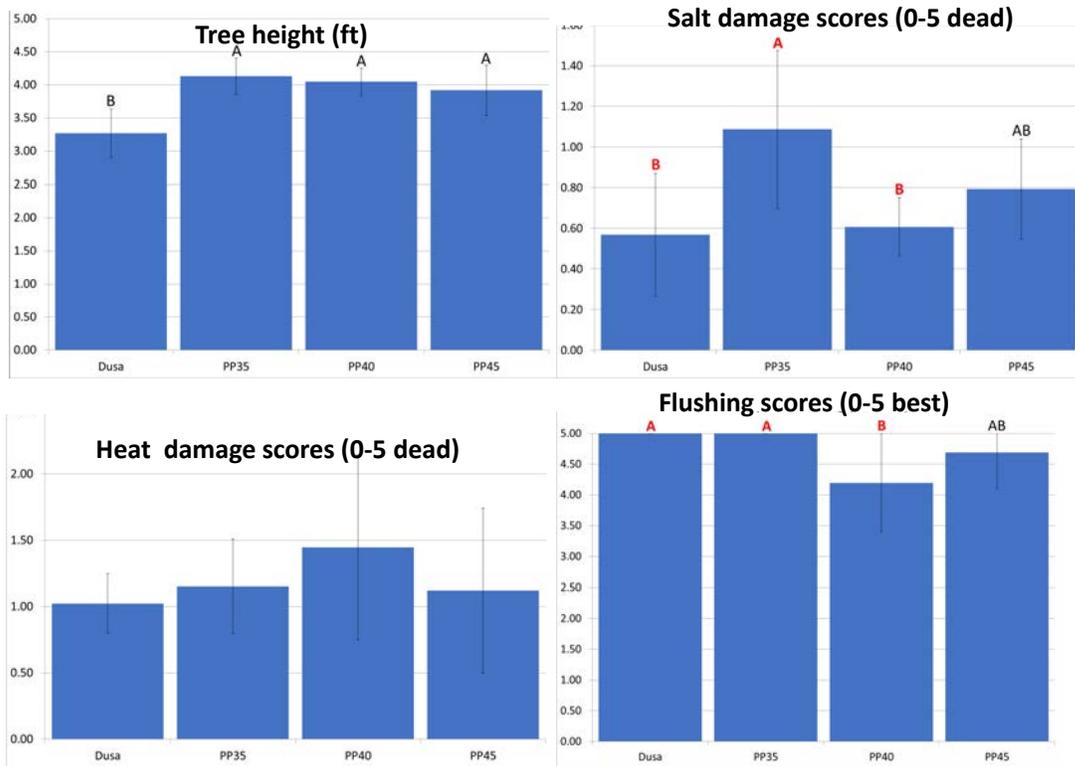
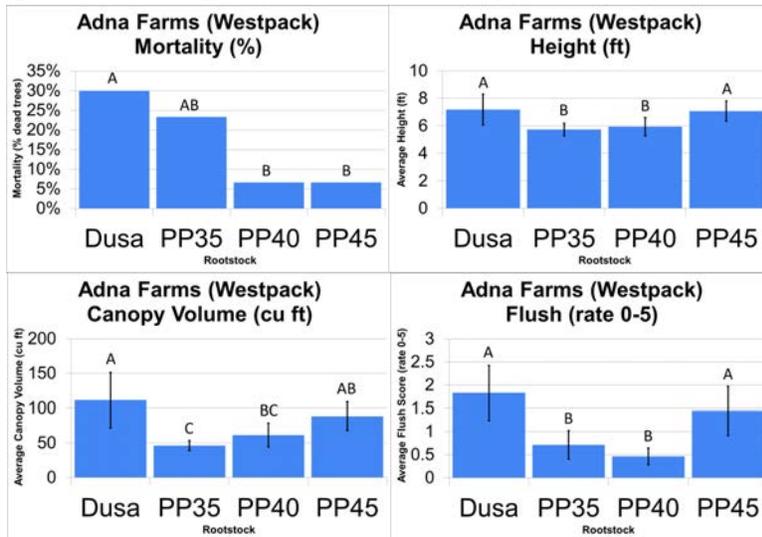


Figure 28. Field ratings at Adna Farms (Temecula) planted in 2020 (October 2022).

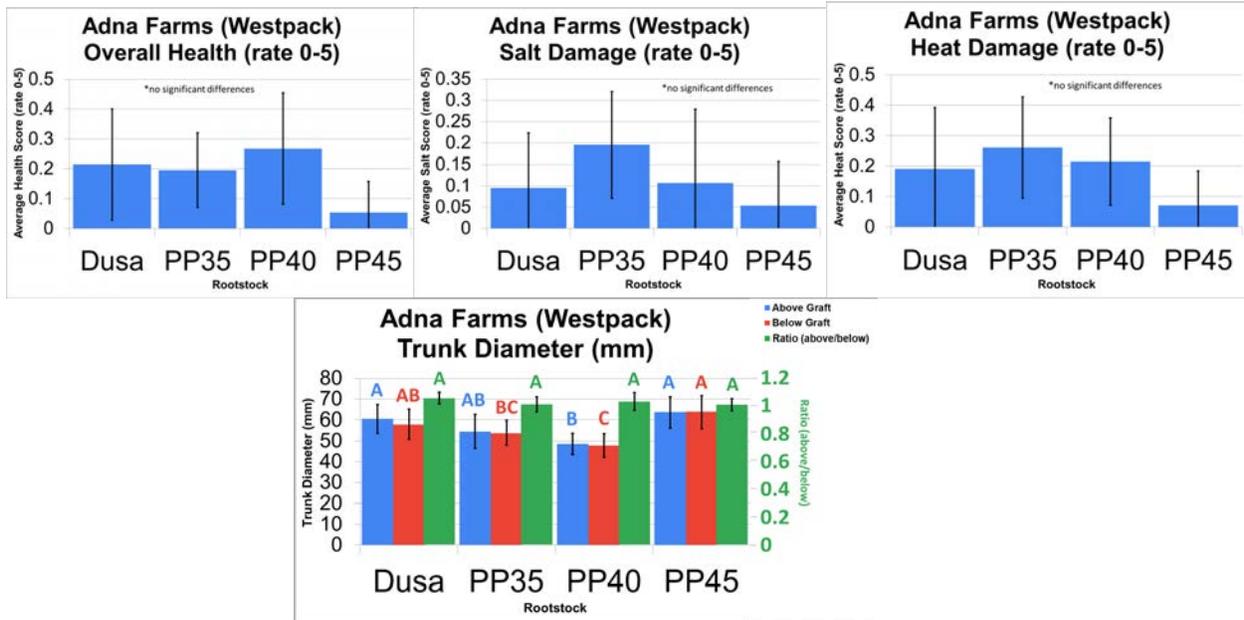


Tree performance at Adna Farms, Temecula (2022 field data). NOTE that the data presented here correspond to the Dusa trees alive out of 100 trees planted at this location (n=21/100). Based on data of the subset of 30 trees/rootstock being analyzed and 21 Dusa survivor trees, Dusa still has the highest mortality at this location followed by PP35. PP40 and PP45 have significant less mortality (Fig. 28). Dusa and PP45 are the tallest trees, have bigger

canopy size, and have better flushing scores followed by PP40 and PP35 (Fig. 28). PP35 exhibited less canopy value at this location but we know PP35 is a small rootstock but is vigorous and highly productive. Moreover, PP35 overall health, salinity damage, heat damage, and trunk diameter are similar than the other rootstocks. No significant differences were detected among rootstocks regarding the ratio of trunk diameter above/below the graft union. In addition, Dusa, PP35 and

PP40 have similar fruit setting scores (Fig. 29). We expected to conduct the first harvest in this location in 2023.

Figure 29. Field ratings at Adna Farms (Temecula) planted in 2020 (October 2022).



6. Petty Ranch, Chris Saver, Ventura (planted June 16, 2020). The grove is located in Ventura. This location has problems with elevated water salinity (2.3 dS/m), high iron levels, high alkalinity as CaCO₃, severe problem of total water hardness. *Phytophthora cinnamomi* was not detected. Soil analyses indicate normal chloride levels and soil salinity, optimum saturation (on the high side, might have some problems in the future). High limestone. As discussed with Chris Saver during our visits to the trial, trees were planted as blocks per rootstocks to facilitate harvest and individual yield and packing data for each rootstock (Fig. 30). Trees were planting at 20' x 15' spacing and trees exhibited similar size at time of planting. A subset of 30 trees per rootstock were selected for ratings and labeled with metal tags. Chris Saver labeled each block with a wooden stick at the limit of each block indicating the rootstock name for easy identification (Fig. 31).

Figure 30. Map for Chris Saver trial planted in Ventura (2020). Trees highlighted in yellow are being evaluated.

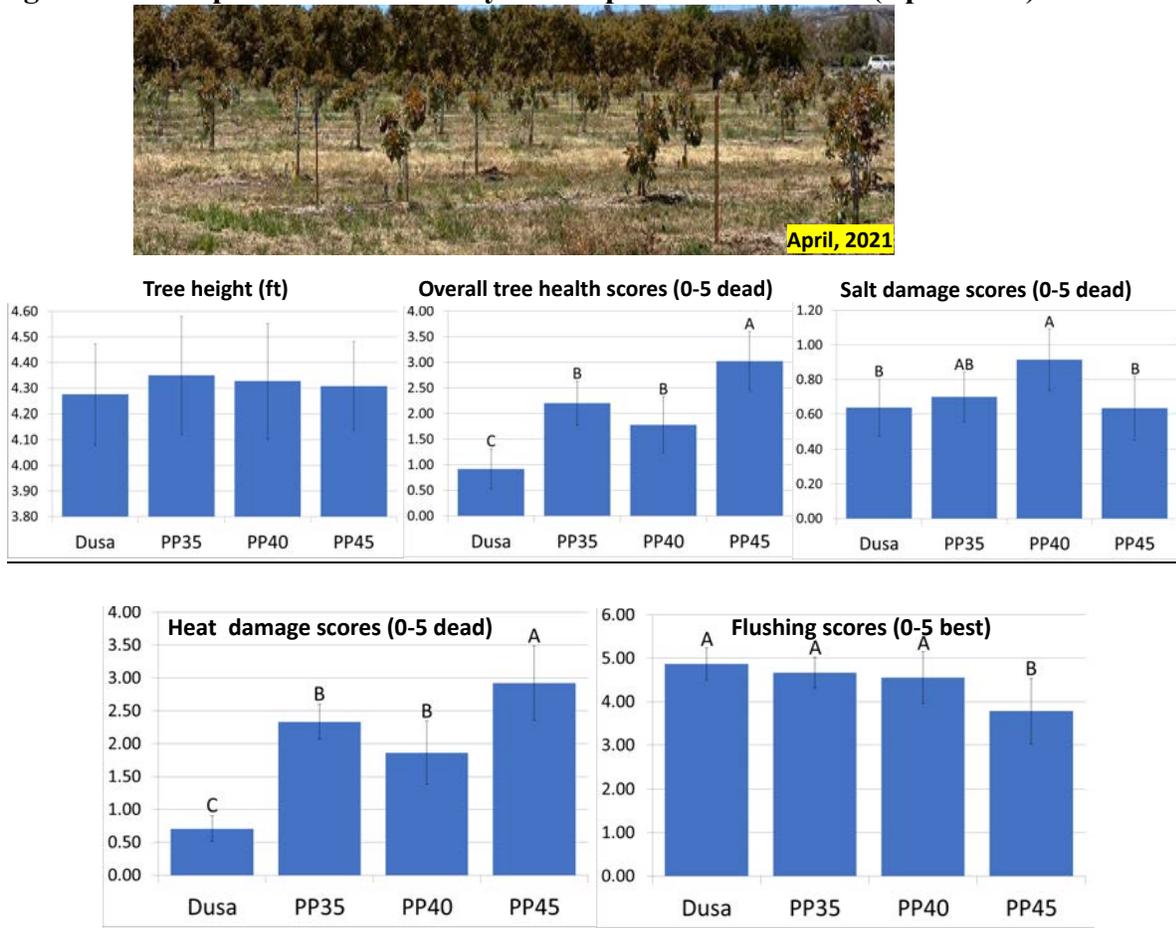
		Spacing 20' ↔																	15' V	1 Rows					
		24	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
		Dusa	Dusa	Dusa	Dusa	Dusa	PP40	PP45	PP45	PP45	PP45	PP45	PP45	PP35	PP35	PP35	x	x	x						
Hass		Dusa-25	Dusa-26	Dusa-27	Dusa-28	Dusa-29	Dusa-30	PP40-25	PP40-26	PP40-27	PP40-28	PP40-29	PP40-30	PP45-25	PP45-26	PP45-27	PP45-28	PP45-29	PP45-30	PP35	PP35	PP35	PP35	PP35	PP35
Hass		Dusa	Dusa	Dusa	Dusa	Dusa	PP40	PP45	PP45	PP45	PP45	PP45	PP45	PP35	PP35	PP35	PP35	PP35	PP35						
Hass		Dusa-19	Dusa-20	Dusa-21	Dusa-22	Dusa-23	Dusa-24	PP40-19	PP40-20	PP40-21	PP40-22	PP40-23	PP40-24	PP45-19	PP45-20	PP45-21	PP45-22	PP45-23	PP45-24	PP35-25	PP35-26	PP35-27	PP35-28	PP35-29	PP35-30
Hass		Dusa	Dusa	Dusa	Dusa	Dusa	PP40	PP45	PP45	PP45	PP45	PP45	PP45	PP35	PP35	PP35	PP35	PP35	PP35						
Hass		Dusa	Dusa	Dusa	Dusa	Dusa	PP40	PP45	PP45	PP45	PP45	PP45	PP45	PP35	PP35	PP35	PP35	PP35	PP35						
Hass		Dusa	Dusa	Dusa	Dusa	Dusa	PP40	PP45	PP45	PP45	PP45	PP45	PP45	PP35	PP35	PP35	PP35	PP35	PP35						
Hass		Dusa	Dusa	Dusa	Dusa	Dusa	PP40	PP45	PP45	PP45	PP45	PP45	PP45	PP35	PP35	PP35	PP35	PP35	PP35						
Hass		Dusa-13	Dusa-14	Dusa-15	Dusa-16	Dusa-17	Dusa-18	PP40-13	PP40-14	PP40-15	PP40-16	PP40-17	PP40-18	PP45-13	PP45-14	PP45-15	PP45-16	PP45-17	PP45-18	PP35-19	PP35-20	PP35-21	PP35-22	PP35-23	PP35-24
Hass		Dusa	Dusa	Dusa	Dusa	Dusa	PP40	PP45	PP45	PP45	PP45	PP45	PP45	PP35	PP35	PP35	PP35	PP35	PP35						
Hass		Dusa	Dusa	Dusa	Dusa	Dusa	PP40	PP45	PP45	PP45	PP45	PP45	PP45	PP35	PP35	PP35	PP35	PP35	PP35						
Hass		Dusa-7	Dusa-8	Dusa-9	Dusa-10	Dusa-11	Dusa-12	PP40-7	PP40-8	PP40-9	PP40-10	PP40-11	PP40-12	PP45-7	PP45-8	PP45-9	PP45-10	PP45-11	PP45-12	PP35-13	PP35-14	PP35-15	PP35-16	PP35-17	PP35-18
Hass		Dusa	Dusa	Dusa	Dusa	Dusa	PP40	PP45	PP45	PP45	PP45	PP45	PP45	PP35	PP35	PP35	PP35	PP35	PP35						
Hass		Dusa	Dusa	Dusa	Dusa	Dusa	PP40	PP45	PP45	PP45	PP45	PP45	PP45	PP35	PP35	PP35	PP35	PP35	PP35						
Hass		Dusa-1	Dusa-2	Dusa-3	Dusa-4	Dusa-5	Dusa-6	PP40-1	PP40-2	PP40-3	PP40-4	PP40-5	PP40-6	PP45-1	PP45-2	PP45-3	PP45-4	PP45-5	PP45-6	PP35-7	PP35-8	PP35-9	PP35-10	PP35-11	PP35-12
Hass		Dusa	Dusa	Dusa	Dusa	Dusa					PP40	PP40	PP40	PP40											
																					PP35-1	PP35-2	PP35-3	PP35-4	PP35-5
																					PP35	PP35	PP35	PP35	PP35

Figure 31. Rootstock trial view at Petty Ranch planted in Ventura (2020).



On April 26th (2021), the trees evaluated were tagged as need it and the wooden sticks were spray painted for easy identification of the blocks and trees. At this location, Dusa, PP45, PP35, and PP40 were painted as yellow, pink, orange, and blue respectively. No significant differences were detected for tree height among rootstocks. Dusa was the best performer followed by PP35 and PP40. The majority of Dusa, PP35, and PP40 trees exhibited heavy flush. PP45 is the rootstock with less new vegetative tissue. The majority of Dusa and PP45 trees were blooming followed by PP40 and PP35. PP45 is the worst rootstock at this location and exhibited the highest mortality (11 trees) followed by PP40 (2) and Dusa (1) (Fig. 32).

Figure 32. Tree performance at Petty Ranch planted in Ventura (April 2021).



Tree performance at Chris Sayer ranch, Ventura (2022 field data). At this location, PP45 is the least performer and also the rootstock exhibiting the highest mortality. Dusa and PP40 have similar tree height and canopy size followed by PP35 and PP45 (**Fig. 33**). As reported in other trials, PP35 is significant smaller tree and with less canopy volume however trees look vigorous as Dusa and PP40 (**Fig 34**). Dusa, PP35, and PP45 exhibited similar scores for salinity damage. No significant differences were detected among the rootstocks for overall tree health and heat damage scores. All rootstocks have similar ratios for trunk diameter below and above the graft union (0.9-1) (**Fig. 33**). This rootstock trial, Chris Sayer, and The Manosalva lab was featured in a BBC documentary: Follow The Food Series 3 Ep 6 - The endangered food list (<https://www.youtube.com/watch?v=Pi42DQuuxbc>).

Figure 33. Tree performance at Petty Ranch planted in Ventura (October 2022).

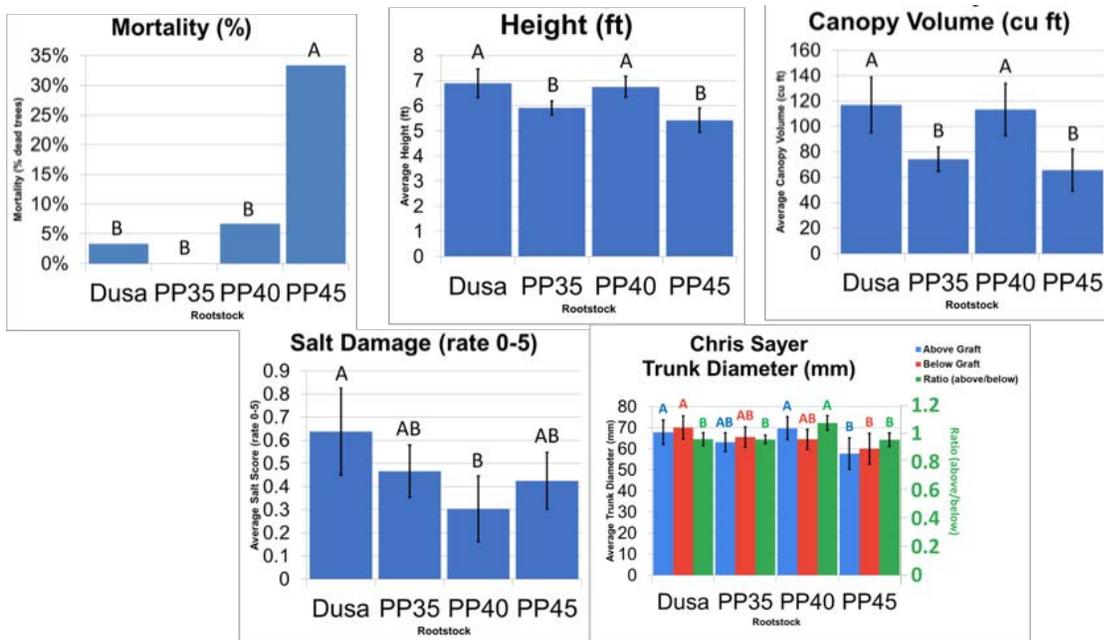


Figure 34. ‘Hass’ avocado trees grafted to Dusa, PP40, PP35, and PP45 rootstocks at Petty Ranch planted in Ventura (October 2022).



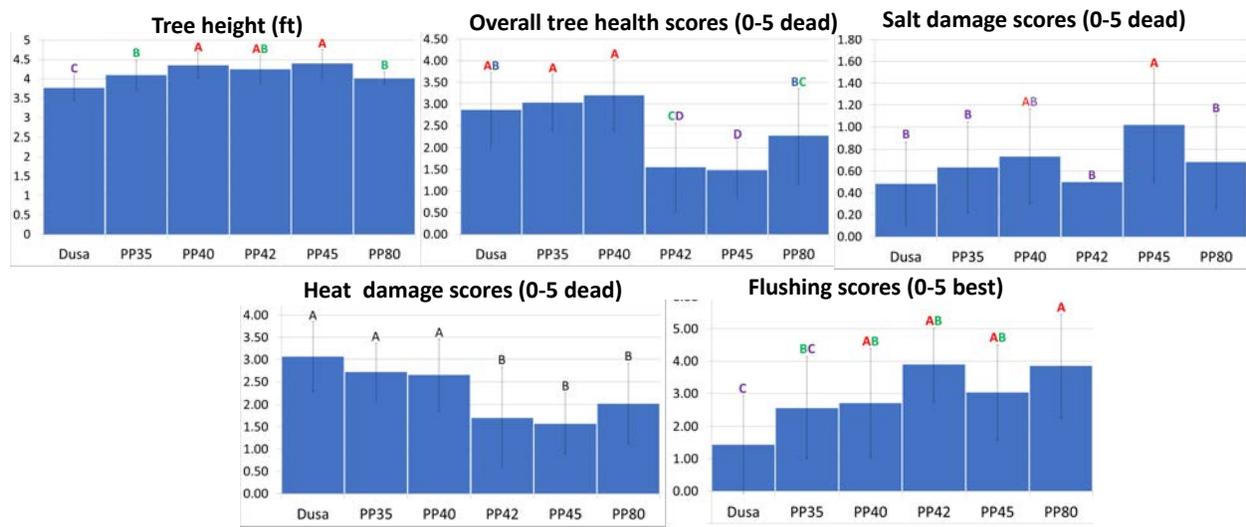
the majority of PP35s (~100) planted in a single block, while 50% of the 28 trees for PP42 are planted with older trees (Fig. 37, Fig. 38). A subset of 30 trees per rootstock were selected with the exception of PP42, labeled with metal tags, and rated on August 25th 2020.

Figure 37. Field layout of the rootstock trial at Alina Ranch LLC, Ventura (August, 2020).



On April 2021. Approximately, 10% of Dusa trees died but none of the trees corresponding to the UCR rootstocks died at this location. PP40, PP45, and PP42 were the bigger trees followed by PP35, PP80. At this location, ‘Hass’ trees grafted on Dusa were significant smaller compared with other rootstocks. Trees grafted on Dusa, PP35, PP40, and PP80 exhibited significant less salinity damage. PP45 exhibited more salinity damage, however PP45 and PP42 are the best performers at this location having the best overall tree health scores and less heat damage (Fig. 38). At this location at this time of rating exhibited the most mortality and was the least performer.

Figure 38. Tree performance at Alina Ranch LLC, Ventura (April 2021).



Tree performance at Alina Ranch LLC, Ventura (2022 field data). At this location, PP80 is the least performer and also the rootstock exhibiting the highest mortality followed by Dusa and PP40. Dusa, PP35, and PP80 have the smallest tree height followed by PP40, PP45, and PP42. **Trees grafted on PP45, PP42, and PP40 UCR rootstocks are the tallest and have the best canopy size when compared with Dusa and PP35. This plot has 100% of PRR incidence and the grower has problems for replanting with trees like Dusa. As expected, the best performers at this location are PP42 and PP45 (Fig. 39. Fig. 40).** PP45 and PP42 are highly resistant to *P. cinnamomi*, the causal agent of PRR, when compared with Dusa in our greenhouse screening using several isolates of the pathogens. **PP45 and PP42 exhibited the best overall tree health and heat damage at this location. Dusa, PP35, and PP80 are the least performers at this location (Fig. 39).** At this location most of the PP45 trees have fruits. Dusa exhibited significant differences regarding the ratio of the trunk diameter below and above the graft union (>1) when compared with PP35, PP80, PP45, and PP42. PP40 and PP35 have ratios of 1 while PP80, PP45, and PP42 less 1(0.96-0.93) (Fig. 40). PP45, PP42, and PP40 exhibited heavy blooming (scores of 4-5). Dusa has significantly less blooming than the other rootstocks. PP45 is the rootstock with the best fruit set at this location (Fig. 40). Based on our observations we expected to harvest PP45, PP42 and PP35 at this location. We will discuss with the ranch manager in the possibility to replace **Dusa and PP80 rootstocks** that failed at this location for other commercially available rootstocks like Tami (VC801) or Zerala.

Figure 39. Tree performance at Alina Ranch LLC, Ventura (October 2022).

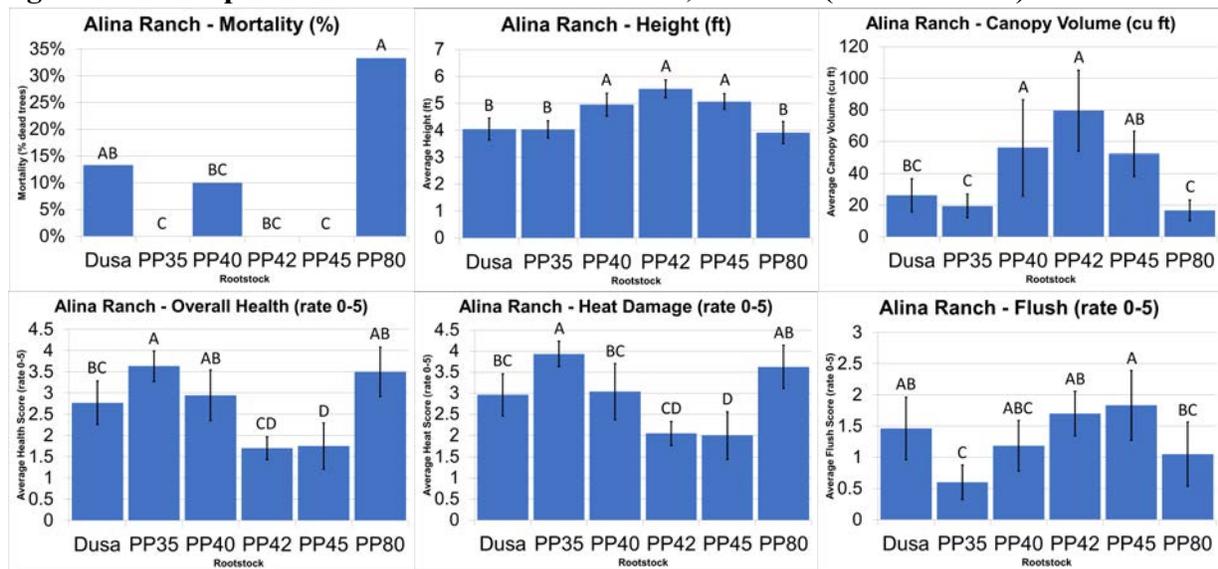
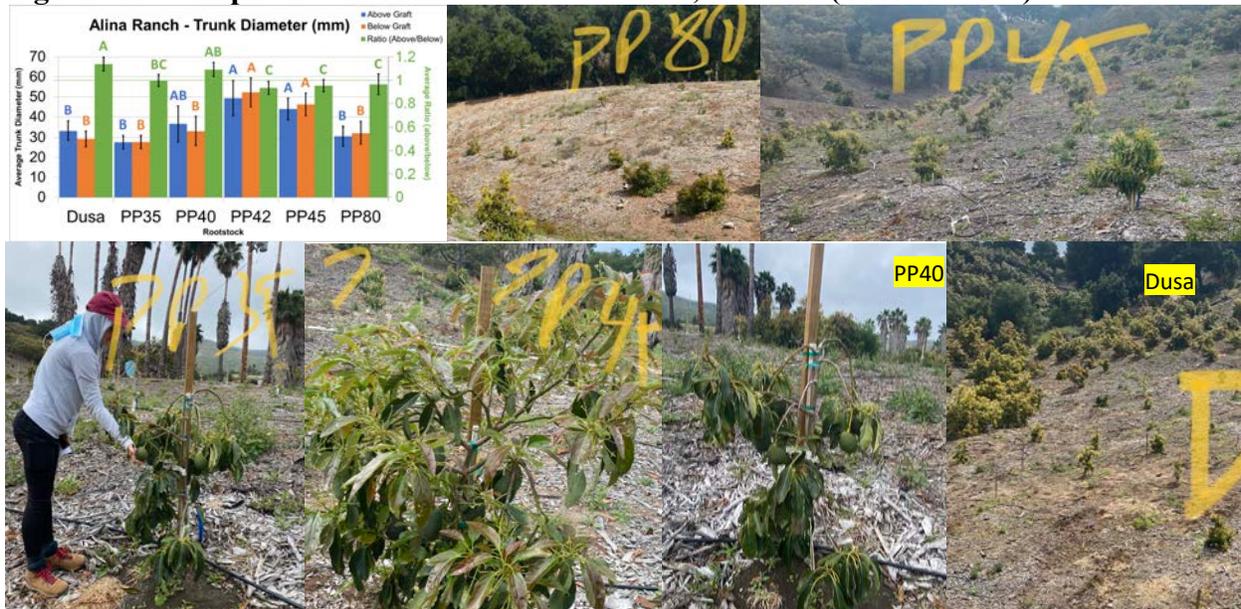


Figure 40. Tree performance at Alina Ranch LLC, Ventura (October 2022).



8). Pete Miller, Santa Barbara, (June 2020). At this location, trees were planted at a 15' x 15' tree spacing and all trees exhibited similar size at the time of planting (June 2022). Soil and water analyses were done in each section and layout, design, and the plot landmark was done with the grower, his manager Agustin, and Dr . Manosalva on June 11th and 12th. Trees were planted in 5 sections (S1- S5) having different soil characteristics and conditions. All sections with the exception of section 3 have from 40 % -90% Phytophthora root rot (PRR) incidence. Sections 1 and 2 in addition to high PRR incidence exhibited high soil salinity, high chloride levels and high saturation. A subset of 10 trees per rootstock (highlighted in green in the maps) at each section were selected and labeled with metal tags to collect tree health data. These trees will be utilized as reference data trees for the duration of the project.

Figure 41. Section 1 (S1), Santa Barbara, 2020.



Section C (S1): 60% of PRR incidence. Chloride is not a problem yet but it is on the high side, high soil salinity (2.71 dS/m), has 99% of saturation, high CEC.

Rows	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	#trees
1	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	Dusa	19
2	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	19
3	PP80	PP80	PP80	PP80	PP80	PP80	PP80	PP80	PP80	PP80	PP80	PP80	PP80	PP80	PP80	PP80	PP80	PP80	PP80	14
4	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	7
5	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	PP40	7
6	PP40-1	PP40-2	PP40-3	PP40-4	PP40-5															4
7	PP40-1	PP40-2	PP40-3	PP40-4	PP40-5															4
8	PP40-1	PP40-2	PP40-3	PP40-4	PP40-5															4
9	PP40-1	PP40-2	PP40-3	PP40-4	PP40-5															3
10	PP45	PP45	PP45																	3
11	x	x																		2

Figure 42. Section 2 (S2), Santa Barbara, 2020.



Section A (S2): 40% of PRR incidence. Soil analyses indicate high chloride levels, high soil salinity (3.65 dS/m), and high % of saturation (66.5%), clay soil.

Rows	1	2	3	4	5	6	7	8	9	10	11	12	13	14	#trees planted
1	PP45	PP45													2
2	PP45	PP45	PP45	PP45											4
3	PP45	PP45	PP45	PP45	PP45										5
4	PP45	PP45	PP45	PP45	PP45										6
5	PP40	PP40	PP40	PP40	PP40	PP40									6
6	PP40								8						
7	PP40							9							
8	PP35			11											
9	PP35		12												
10	Dusa	x	x	x		10									
11	Dusa		13												
12	PP80		14												
13	PP80	PP80	PP80	PP80	x	x									4

Figure 43. Section 3 (S3), Santa Barbara, 2020.



Section B (S3): 0% of PRR incidence. No problems with salinity or chloride. Low nitrogen, optimum soil saturation.

Rows	1	2	3	4	5	6	7	8	9	10	11	12	trees planted
1	PP35			7									
2	PP35			10									
3	PP35			10									
4	Dusa			10									
5	PP45			10									
6	PP42	x	x	10									
7	PP40			10									

Figure 44. Section 4 (S4), Santa Barbara, 2020.



Section 4 (S4): 90% of PRR incidence. No problems with salinity or chloride. Low nitrogen, optimum soil saturation.

Rows	1	2	3	4	5	6	7	8	9	10	11	trees planted
1	PP42			9								
2	PP40		10									
3	PP40	11										
4	PP35	11										
5	PP35		7									
6	PP45	PP45	x	PP45	PP45	PP45	PP45	x				10
7	PP45		10									
8	PP45		10									
9	Dusa	11										
10	Dusa					7						
11	Dusa	Dusa	Dusa	Dusa								4

Figure 45. Section 5 (S5), Santa Barbara, 2020.



Section 5 (S5): 50% of PRR incidence. No problems with salinity or chloride. Optimum pH and soil saturation.

Rows	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	trees planted
1	PP45	x	x	x	PP45				0									
2	PP35				12													
3	PP35				14													
4	Dusa	x	x	x	x	x				9								
5	Dusa	17																
6	PP45	x	x	x	11													
7	PP45	16																
8	PP40				13													
9	PP40				14													
10	PP45				9													

On April 2021, there were significant differences among rootstocks for all the data collected at this plot among all sections. At this plot, Dusa trees from S4 were significantly different than PP45 and PP42 trees in S3, PP45 trees at S5, and PP80 trees at S2 regarding tree height. PP45 trees from S3 exhibited the highest tree height. With the exception of PP35 trees from S5 and Dusa S4, all rootstocks planted in each section were not significantly different on tree height (Fig. 46). Trees from Dusa in S1, PP35 in S1, and PP40 in S4 exhibited the best health scores. All other rootstocks performed similar at each section (Fig. 47). In the section 1 (S1), PP45 and Dusa were significant different from each other in terms of salinity damage. As expected, PP45 is more salinity sensitive than Dusa at this section. All the rootstocks perform similar for salt damage in all the sections. In S2 that has similar conditions than S1 but less PRR incidence and soil saturation no significant differences were observed among rootstocks (Fig. 48). PP35 trees at S1 was significantly different from PP40 trees at S5 regarding heat damage being PP35 at S1 the best performer. No significant differences were detected for heat tolerance among all the other rootstock accessions planted at all sections in this location (Fig. 49). PP35 and Dusa trees in S1 were the best performers. Majority of rootstocks in all the sections exhibited new vegetative growth being PP40 trees in S2 and S3 the trees exhibiting less flush (Fig. 50). All trees in all sections were blooming and bearing fruits. All trees in section 1 for all rootstocks evaluated did not have any fruits.

Figure 46. Tree height (ft) at Pete Miller Ranch (April 2021)

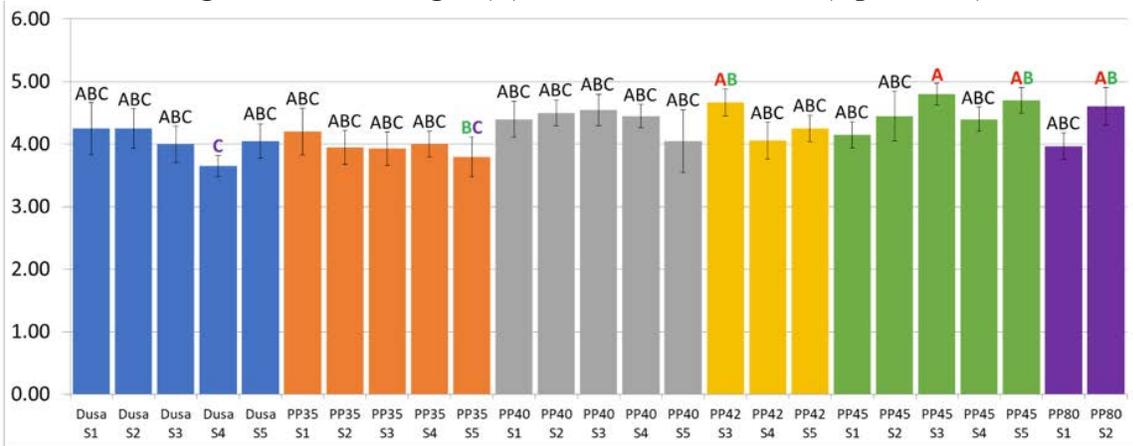


Figure 47. Tree health score (0 – 5 dead) at Pete Miller Ranch (April 2021)

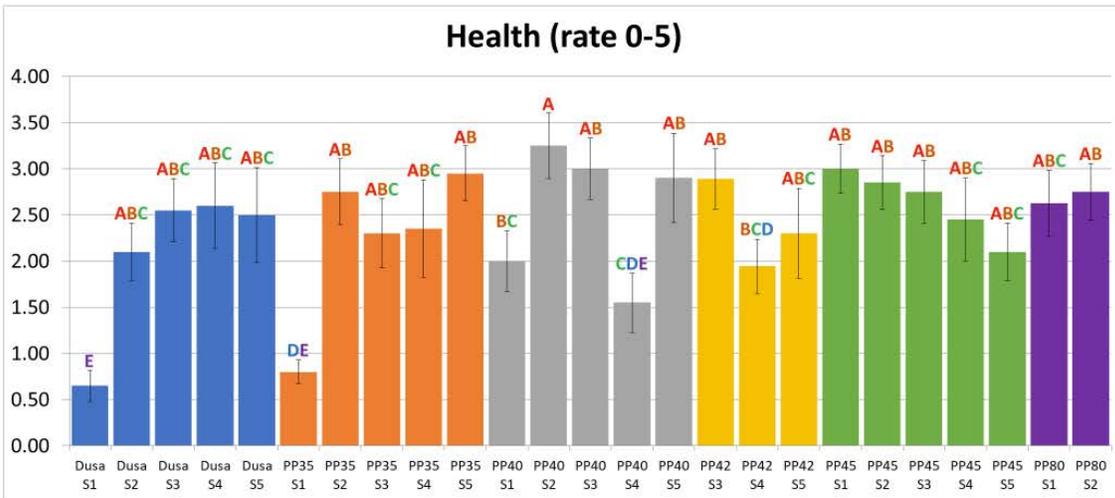


Figure 48. Salt damage score (0 – 5 dead) at Pete Miller Ranch (April 2021)

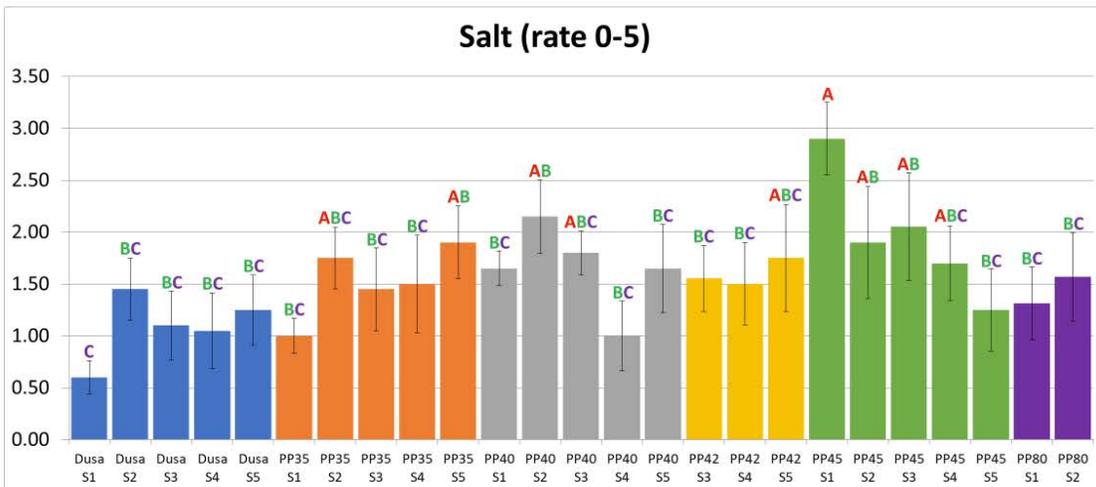


Figure 49. Heat damage score (0 – 5 dead) at Pete Miller Ranch (April 2021)

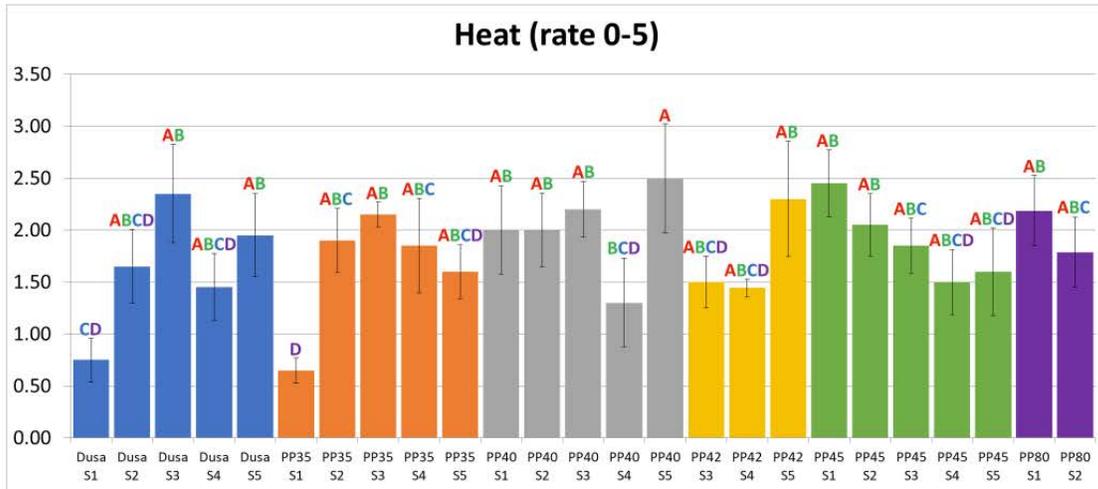


Figure 50. Flush score (0 – 5 best) at Pete Miller Ranch (April 2021)

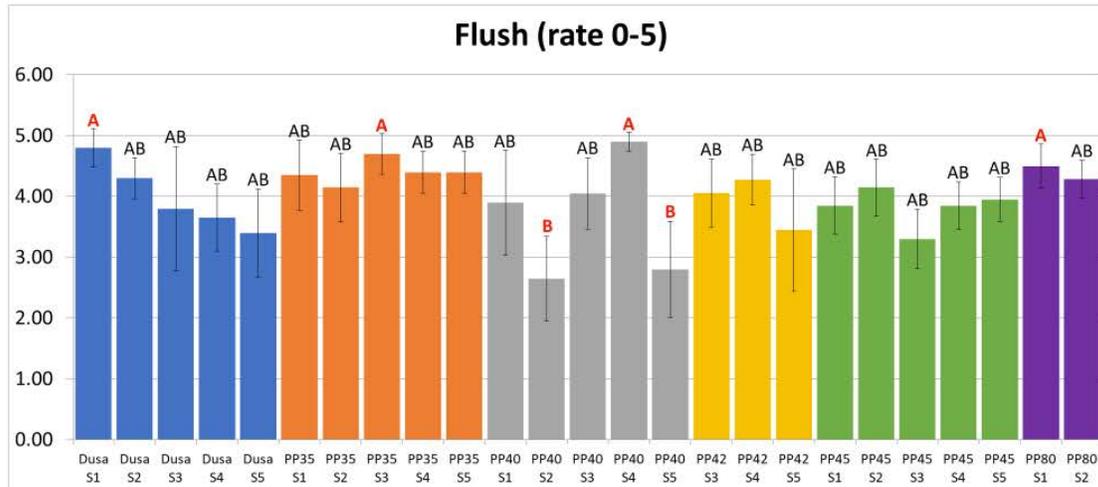


Figure 51. Trees at Pete Miller Ranch Section 1 (April 2021)



Figure 52. Trees at Pete Miller Ranch Section 2 (April 2021)



Figure 53. Trees at Pete Miller Ranch Section 3 (April 2021)

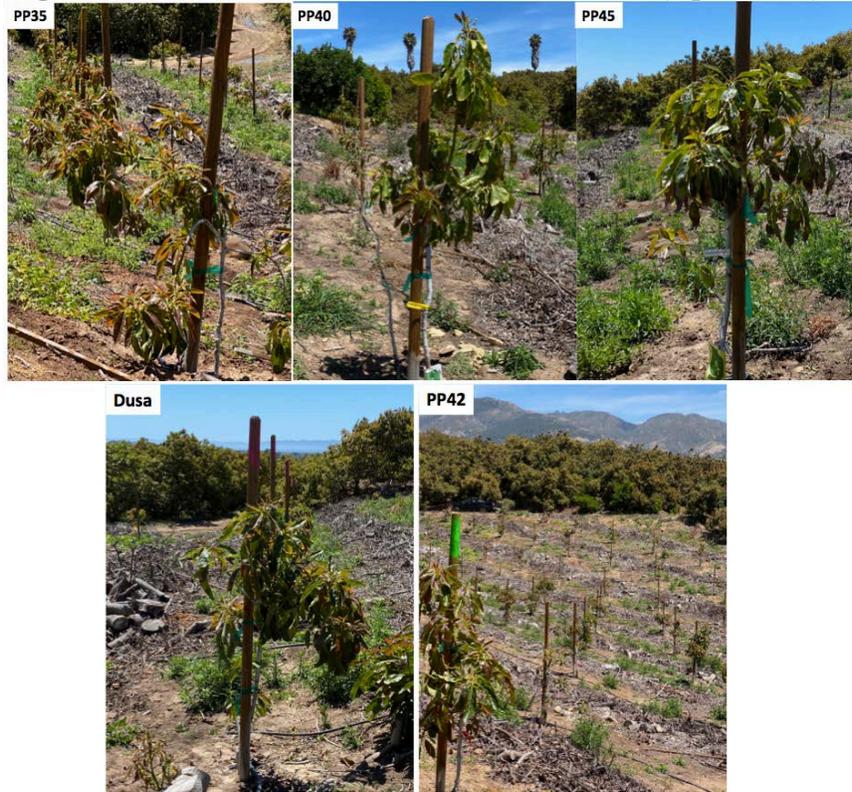


Figure 54. Trees at Pete Miller Ranch Section 4 (April 2021)

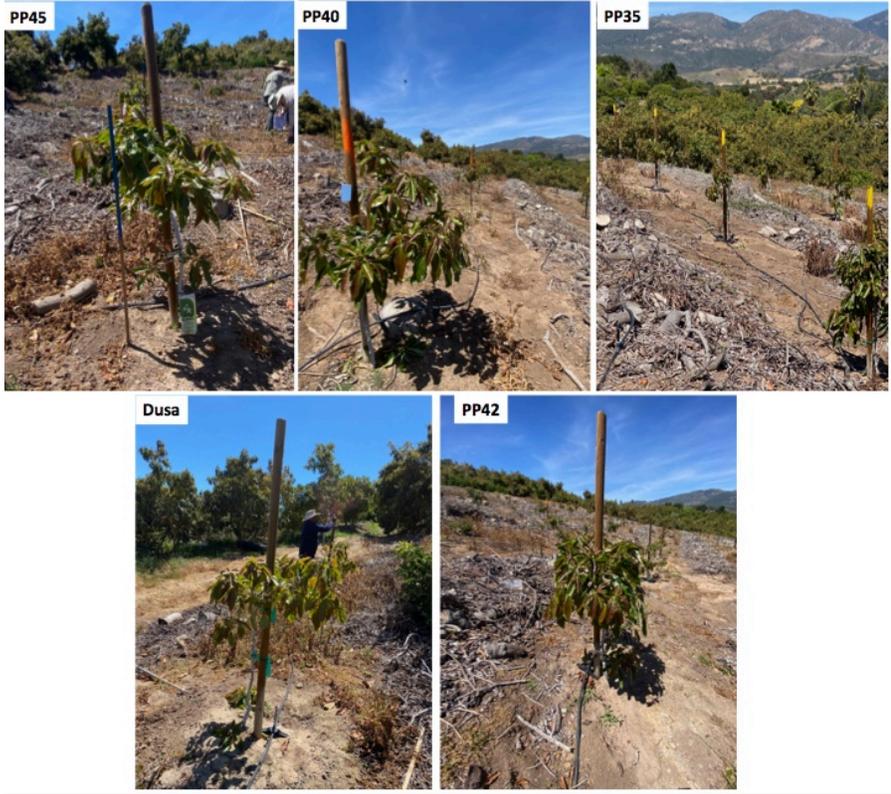
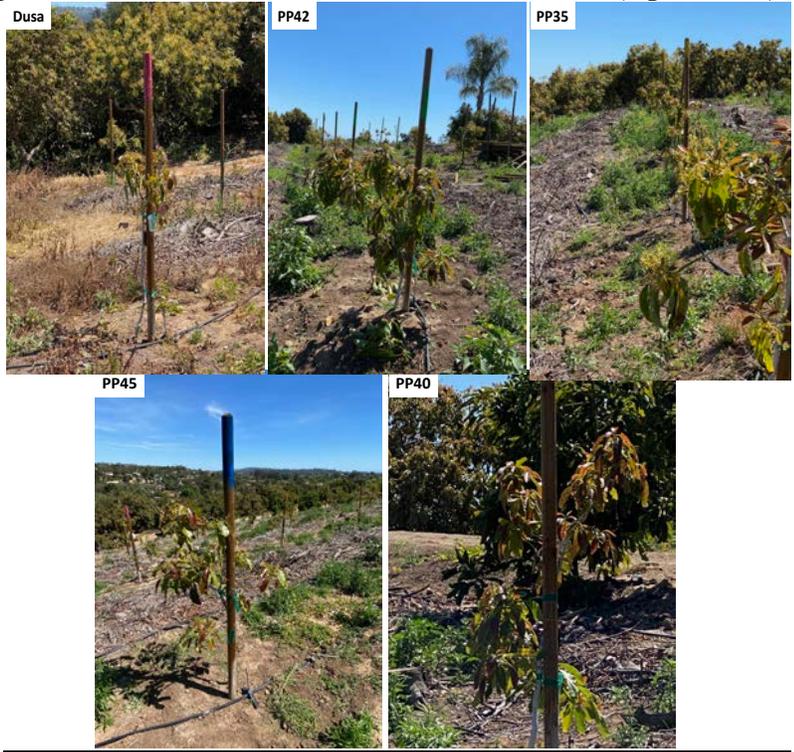
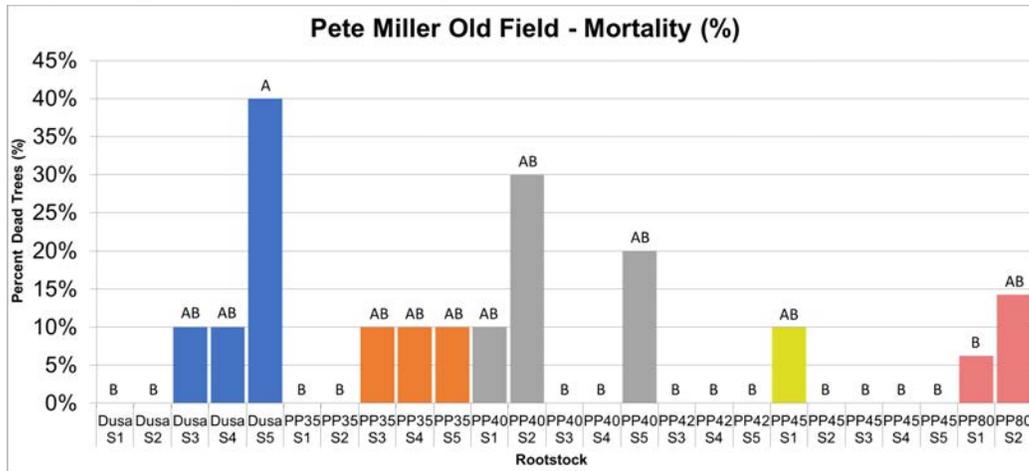


Figure 55. Trees at Pete Miller Ranch Section 5 (April 2021)



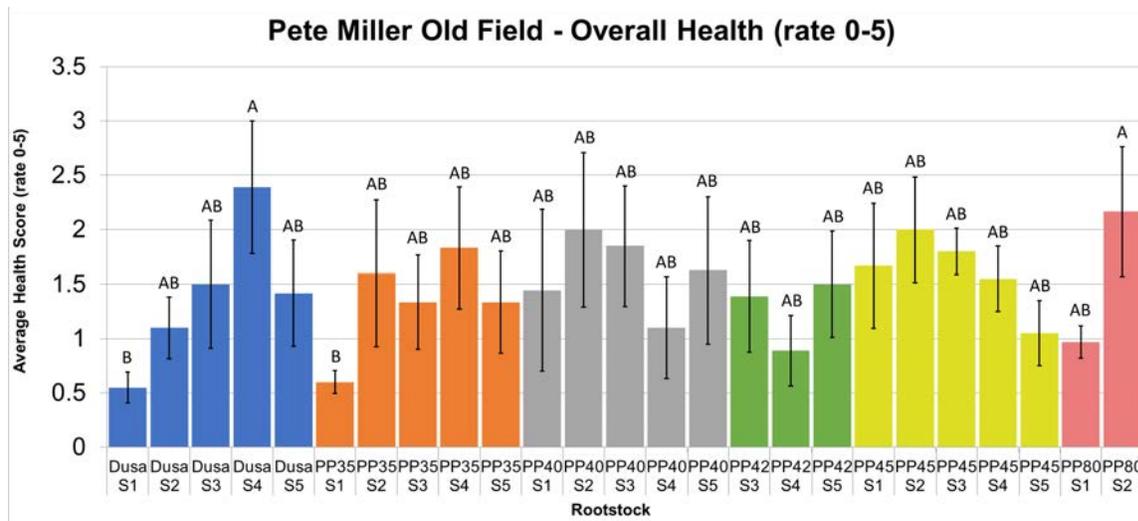
Tree performance at Pete Miller Ranch, Santa Barbara (2022 field data). Dusa is the rootstock with the highest mortality (3/5 sections), followed by PP40 and PP35 (3/5 sections). PP42 exhibited the less mortality, followed by PP45 and PP80 (Fig. 56).

Figure 56. Tree Mortality at Pete Miller Ranch (October 2022)



For overall tree health: Majority of rootstocks exhibited similar overall tree health among all sections. The best scores are those from Dusa and PP35 in section 1 (S1) and where significant different to Dusa in S4 and PP80 in S2 (Fig. 57).

Figure 57. Overall Tree Health Scores Pete Miller Ranch (October 2022)



For salinity damage: When compared by rootstocks, PP45 as expected exhibited more salinity damage than other rootstocks across all sections but has the best performance in S5. The best performers are Dusa and PP35 from S1 (PRR, salinity, and chloride) followed by PP40 in sections 5, 1, and 4 (Fig. 58). No significant differences were found among all rootstocks when compare within sections for salt and heat damage. **For heat damage,** the majority of rootstocks perform similar but Dusa and PP35 in S1 are the best rootstocks and are significant different with Dusa S4 and PP80 S2 (Fig. 59). Dusa and PP35 exhibited the best fruit setting scores.

Figure 58. Tree Salinity Damage at Pete Miller Ranch (October 2022)

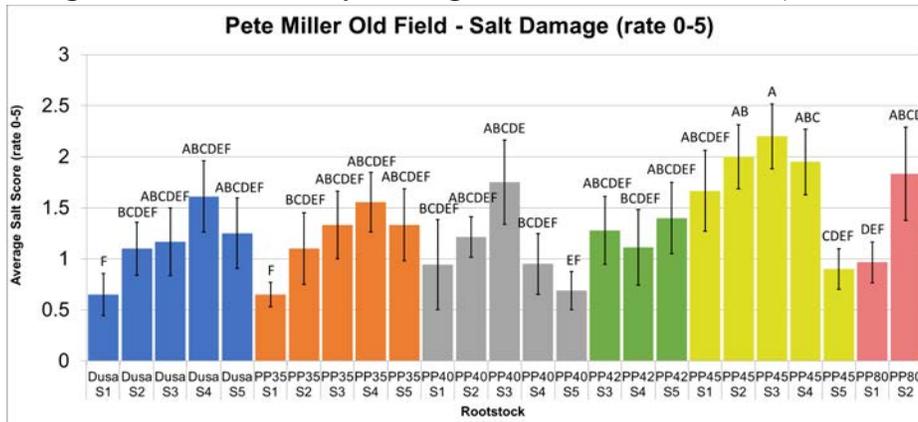


Figure 59. Tree Heat Damage at Pete Miller Ranch (October 2022)

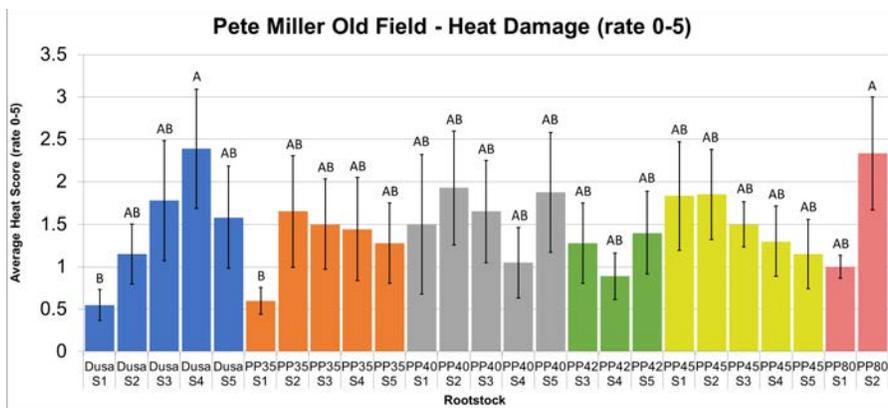


Figure 60. Trees at Pete Miller Ranch Section 1 (October 2022)



Figure 61. Trees at Pete Miller Ranch Section 2 (October 2022)



Figure 62. Trees at Pete Miller Ranch Section 3 (October 2022)



Figure 63. Trees at Pete Miller Ranch Section 4 (October 2022)

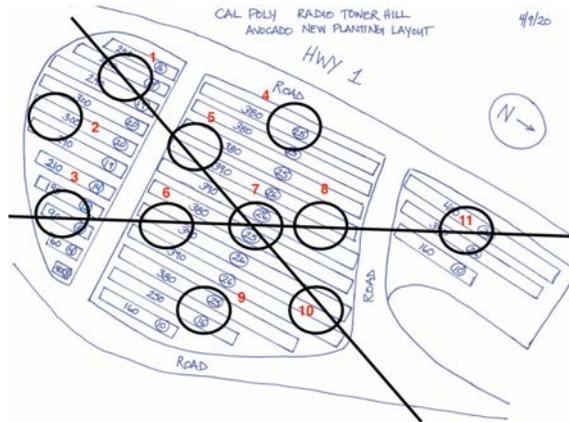


Figure 64. Trees at Pete Miller Ranch Section 5 (October 2022)



9). **San Luis Obispo Plot (planted June 23-24, 2020).** This report was prepared by Dr. Garner and Rashaan Souikane. Trees were planted in Cal Poly Radio Tower Hill in San Luis Obispo (SLO). Soil and water analyses were done before planting in the 3 blocks or sections in the plot (Fig. 65)

Figure 65. Map and samples collected at Cal Poly field.



Soil and water analyses does not show major problems with salinity, pH, saturation. As discussed with Dr. Garner, this will be more like a research plot and layout is indicated in Fig. 66. On August 27th 2020. The Manosalva team visited the rootstock trial to train Dr. Garner's team for ratings and measurements (Fig. 67).

Figure 66. Map for the Cal Poly SLO plot

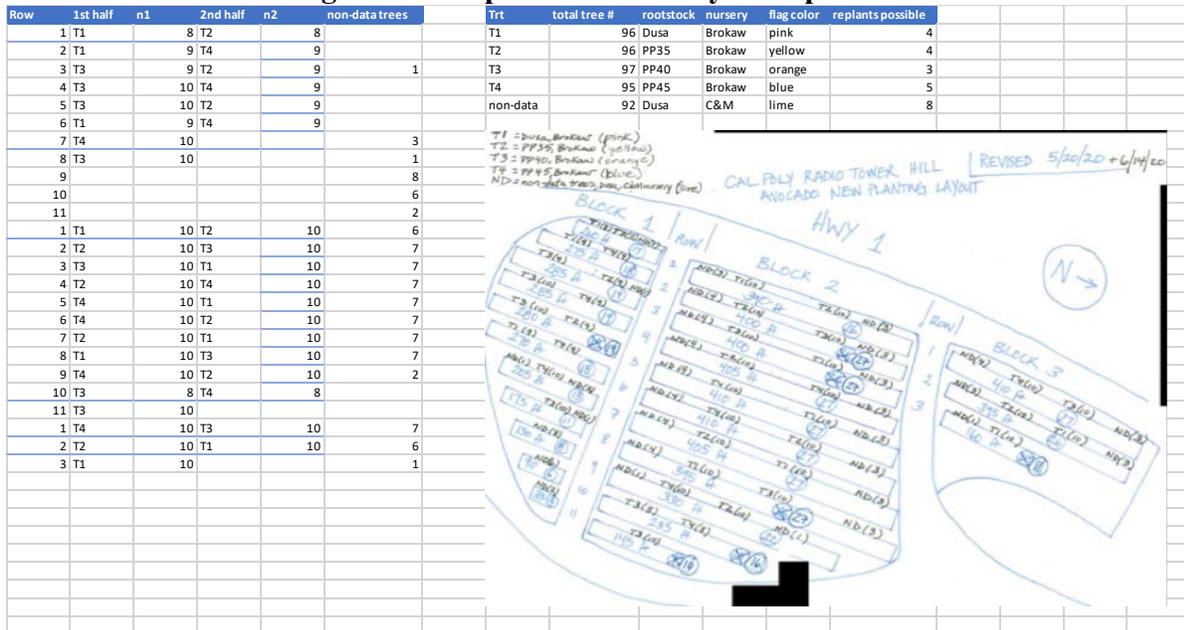


Figure 67. Manosalva and her student Aidan Shands visited the rootstock trial on planting day and on August 2020 to train Dr. Garner’s team on tree ratings.



At the San Luis Obispo site, post-hoc comparisons using Tukey HSD test indicated that mean height of ‘Hass’ trees on ‘PP45’ were statistically greater (mean = 1.865 m; standard error (SE) = 0.029) compared to the other three rootstocks ($P < 0.001$; **Table 7**). ‘PP45’ rootstocks also had the greatest mean trunk circumference (mean = 228.105; SE = 2.284), indicating that the ‘PP45’ rootstock is more vigorous than the other rootstocks, at least during the early growth and establishment of the trees (**Table 8**). ‘Hass’ scion had the greatest mean trunk circumference (mean = 217.976; SE = 2.840) when grafted on ‘PP45’ ($P < 0.001$; **Table 9**). All rootstock treatments resulted in an average above-graft union to below-graft union trunk circumference ratio below or near 1. The majority of trees received a score of 0 for overall health, heat damage and salinity damage, indicating that the trees are visibly healthy and displayed little to no heat or salinity damage. Scion grafted on to ‘PP45’ appear to have a greater mean bloom rating compared other rootstocks, but further analysis is required. Differences in vegetative flush rating among rootstock treatments were typically inconsistent across rate dates and rootstocks.

Table 7. Mean height (m), standard error (SE) and letter plots of four avocado rootstocks ('Dusa', 'PP35', 'PP40', 'PP45') collected 2 months after transplant and subsequently during the spring (3/18/2021, 3/4/2022), summer (7/17/2021, 7/23/2022), and fall vegetative flush (10/22/2021) at the research plot in **San Luis Obispo**, CA. Means labeled with different letters within a rate date are significantly different ($P \leq 0.05$) based on Tukey's HSD test; n = 10.

Rate Date	Rootstock	Mean Height (m)	SE
8/27/2020	Dusa	0.961 C	0.012
	PP35	1.029 B	0.011
	PP40	1.090 A	0.010
	PP45	1.023 B	0.012
3/18/2021	Dusa	1.070 C	0.012
	PP35	1.131 B	0.011
	PP40	1.200 A	0.011
	PP45	1.159 AB	0.013
7/17/2021	Dusa	1.172 C	0.014
	PP35	1.236 B	0.013
	PP40	1.328 A	0.015
	PP45	1.351 A	0.017
10/22/2021	Dusa	1.398 C	0.018
	PP35	1.429 BC	0.016
	PP40	1.529 AB	0.016
	PP45	1.561 A	0.019
3/4/2022	Dusa	1.390 B	0.018
	PP35	1.410 B	0.017
	PP40	1.532 A	0.017
	PP45	1.570 A	0.020
7/23/2022	Dusa	1.633 BC	0.029
	PP35	1.579 C	0.021
	PP40	1.743 B	0.025
	PP45	1.865 A	0.029

Table 8. Mean below-graft union trunk circumference (mm), standard error (SE) and letter plots of four avocado rootstocks ('Dusa', 'PP35', 'PP40', 'PP45') collected 2 months after transplant and subsequently during the spring (3/18/2021, 3/4/2022), summer (7/17/2021, 7/23/2022), and fall vegetative flush (10/22/2021) at the research plot in **San Luis Obispo**, CA. Means labeled with different letters within a rate date are significantly different ($P \leq 0.05$) based on Tukey's HSD test; n = 10.

Rate Date	Rootstock	Mean Below-Graft Union Trunk Circumference (mm)	SE
8/27/2020	Dusa	59.376 B	0.741
	PP35	65.490 A	0.770
	PP40	57.394 B	0.556
	PP45	64.651 A	0.757
3/18/2021	Dusa	86.865 B	1.156
	PP35	89.419 B	1.222
	PP40	86.271 B	1.197
	PP45	100.707 A	1.401
7/17/2021	Dusa	105.162 C	1.527
	PP35	118.623 B	1.470
	PP40	113.094 BC	1.514
	PP45	140.734 A	1.543
10/22/2021	Dusa	140.291 C	2.026
	PP35	151.227 B	1.599
	PP40	142.298 BC	1.687
	PP45	176.554 A	1.624
3/4/2022	Dusa	164.252 B	2.391
	PP35	167.560 B	1.816
	PP40	165.323 B	2.215
	PP45	203.484 A	2.180
7/23/2022	Dusa	188.593 B	3.010
	PP35	191.241 B	1.879
	PP40	185.561 B	2.438
	PP45	228.105 A	2.284

Table 9. Mean above-graft union circumference (mm), standard error (SE) and letter plots of four avocado rootstocks ('Dusa', 'PP35', 'PP40', 'PP45') collected 2 months after transplant and subsequently during the spring (3/18/2021, 3/4/2022), summer (7/17/2021, 7/23/2022), and fall vegetative flush (10/22/2021) at the research plot in **San Luis Obispo**, CA. Means labeled with different letters within a rate date are significantly different ($P \leq 0.05$) based on Tukey's HSD test; n = 10.

Rate Date	Rootstock	Mean Above-Graft Union Trunk Circumference (mm)	SE
8/27/2020	Dusa	56.524 A	0.528
	PP35	58.343 A	0.578
	PP40	57.290 A	0.503
	PP45	58.305 A	0.578
3/18/2021	Dusa	80.965 B	1.015
	PP35	84.251 B	1.093
	PP40	84.075 B	0.999
	PP45	91.197 A	1.307
7/17/2021	Dusa	101.731 B	1.539
	PP35	109.598 B	1.483
	PP40	111.633 B	1.455
	PP45	129.745 A	1.750
10/22/2021	Dusa	138.802 B	1.879
	PP35	144.837 B	1.960
	PP40	144.862 B	1.854
	PP45	170.369 A	1.668
3/4/2022	Dusa	160.080 C	2.259
	PP35	154.095 C	1.923
	PP40	172.649 B	2.246
	PP45	194.471 A	2.271
7/23/2022	Dusa	177.377 B	2.717
	PP35	173.620 B	1.929
	PP40	191.571 B	2.557
	PP45	217.976 A	2.840

Discussion. At the **San Luis Obispo** site, the results suggest that 'Hass' scions grafted on PP45 demonstrate higher vigor in vegetative growth compared PP35, PP40 and 'Dusa'. The results also suggest that none of the rootstocks in the trial had negative influences on tree establishment or development.

Obj. 2. Collection of tree health and harvest data for PP35, PP40, PP42, PP45, and PP80 UCR advanced rootstocks at previously established field trials in Ventura. In 2015, we conducted an intensive review of all the active field trials that were established under the tenure of J. Menge and G. Douhan. Under the current CAC funding, we have two active field plots being evaluated containing Dusa and the 4 UCR advanced rootstocks that we are focusing on this proposal (PP35, PP40, PP42, and PP45) (**Table 10**). We have conducted soil and water analyses and evaluated each plot for the presence of *P. cinnamomi* using traditional root pathogen isolation and bating soil techniques (**Table 10**). These plots have been properly monitored since 2016, tree health and harvest data has been collected. This data is providing important information regarding the performance of these five rootstocks under these field conditions in Santa Paula at Ventura County under PRR, salinity, and high pH conditions (**Table 10**).

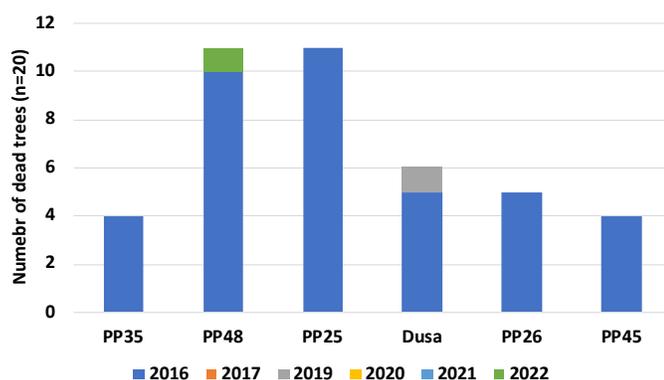
Table 10. Active rootstock field trials containing Dusa, PP35, PP40, PP42, and PP45.

Plot Name	Rootstock varieties	Status	Year Planted
Gunderson, Santa Paula	Dusa , PP#'s 18, 21, 22, 40, 42, 45 , 56, 58, 56, 58, 63, SA-1 Lansfield, and Thomas	This is the oldest plot and was the first plot established at Limoneria Ranch. No harvest records were found before 2015. Harvest data has been collected since 2016. <i>Phytophthora cinnamomi</i> has been confirmed. Water analyses (FGL) shown problems with high pH (7.9) and alkalinity (as CaCO₃) , and possible salinity problem E.C. 1.44 dS/m.	2006
Limoneria Ranch #2, Santa Paula	Dusa , PP#'s 25, 26, 35, 45 , and 48	Good plot, well designed. Trees looks nice. <i>Phytophthora cinnamomi</i> has not been detected by any methods. Water analyses (FGL) indicated problems with high pH, E.C. 1.6 dS/m, and severe problem of alkalinity (as CaCO₃) .	2011

Limoneria 2, Santa Paula. The previous manager Andy Coker is no longer working at Limoneira. We have been communicating and working with the new managers: Mr. Edgar Gutierrez (Vice President of Farming Operations) and Mr. Vince Giacolo ne (Director of Southern Management Operations). Five UCR rootstock selections including the advanced rootstocks, PP35 and PP45, have been evaluated in this field plot established in 2011. In this plot, rootstocks are

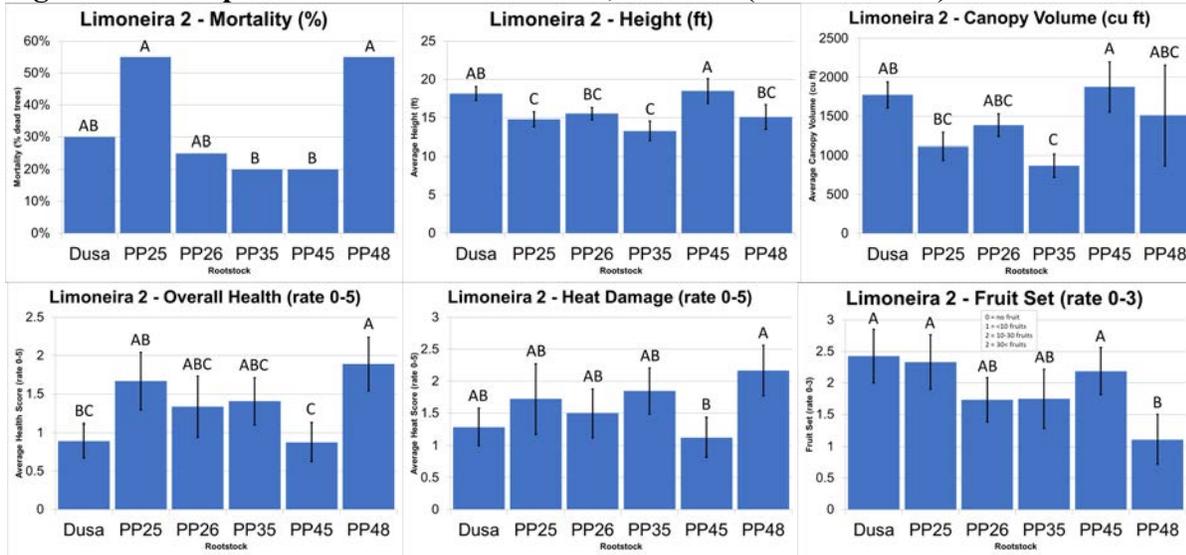
being tested under high pH and high alkalinity conditions. Salinity based on our water and soil analysis indicated a possible salinity problem (**Table 10**). At this site, PP25 and PP48 have the highest tree mortality (~55 %). PP45 and PP35 exhibited the least mortality (20%) (**Fig. 68**).

Figure 68. Tree mortality at Limoneria 2, Santa Paula, Ventura.



At this location no significant differences were found among all rootstocks for salinity damage and flushing scores. PP45 is the rootstock with the higher tree height and canopy size followed by Dusa. PP35 is the smallest rootstock with less canopy size and significantly different than Dusa and PP45. All trees also were heavily blooming at this location. PP45 in this location was the most vigorous and with the most vegetative growth at this location.

Figure 69. Tree performance at Limoneira 2, Ventura (October 2022).



This plot was harvested by 48 plus size picking (7.5 – 9.5 oz) on January 31 (2022). **Table 11** showed the amount of fruit collected for that size. PP45 was the rootstock that produced more total pounds and fruits.

Table 11. Summary of Limoneira 2 size picking January 2022.

Date Harvested	Field	Rootstock	# of Trees	Total Fruit #	Total Weight (lbs)	Avg weight (oz)/fruit	Avg fruit #/Tree
1-31-22	Limoneira 2	Dusa	14	1472	788.28	8.57	105.14
1-31-22	Limoneira 2	PP25	7	597	318.56	8.54	85.29
1-31-22	Limoneira 2	PP26	15	1902	1055.38	8.88	126.80
1-31-22	Limoneira 2	PP35	15	998	542.91	8.70	66.53
1-31-22	Limoneira 2	PP45	15	2199	1214.72	8.84	146.60
1-31-22	Limoneira 2	PP48	6	732	381.84	8.35	122.00

We collected 6 years of harvest data (2016-2022). PP45, Dusa, and PP26 are the best producers at this site. *Note that PP45 was the best producer in 2022. PP35 is a small tree but a good producer. PP35 trees yield half of the total pounds when compared with Dusa and PP45, however PP35 has half of the canopy volume when compared with Dusa and PP45 (Fig. 69, Fig. 70, Table 12).* Dusa, PP35, and PP45 have similar yield efficiency (Fig. 70). These results argue that PP35 in some locations are small but good producers having similar yield efficiency than Dusa highlighting the importance of PP35 for high density planting.

Figure 70. Cumulative yield/rootstock and yield efficiency at Limoneria 2, Ventura.

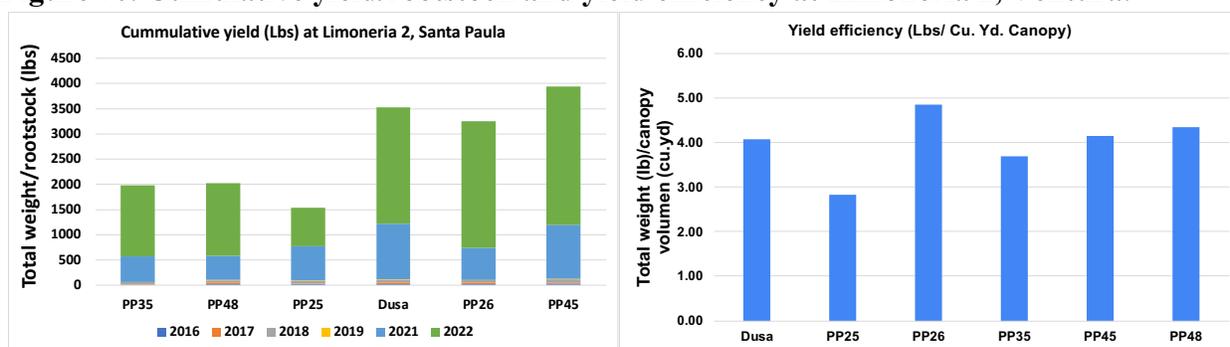
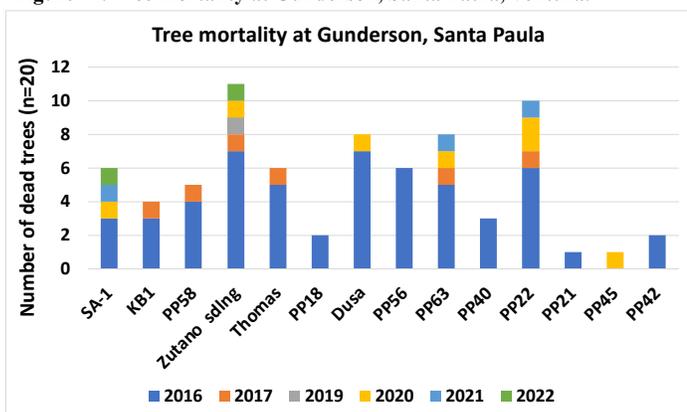


Table 12. Summary of Limoneria 2 harvest in 2022 (January and April).

Field	Rootstock	# of Trees	Total Fruit #	Total Weight (lbs)	Avg weight (oz)/fruit	Avg fruit #/Tree	Avg weight (lbs)/tree
Limoneira 2	Dusa	14	4438	2310.47	8.33	317.00	165.03
Limoneira 2	PP25	9	1534	765.53	7.98	170.44	85.06
Limoneira 2	PP26	15	4668	2509.66	8.60	311.20	167.31
Limoneira 2	PP35	15	2707	1408.45	8.32	180.47	93.90
Limoneira 2	PP45	15	4767	2744.12	9.21	317.80	182.94
Limoneira 2	PP48	9	2921	1433.58	7.85	324.56	159.29

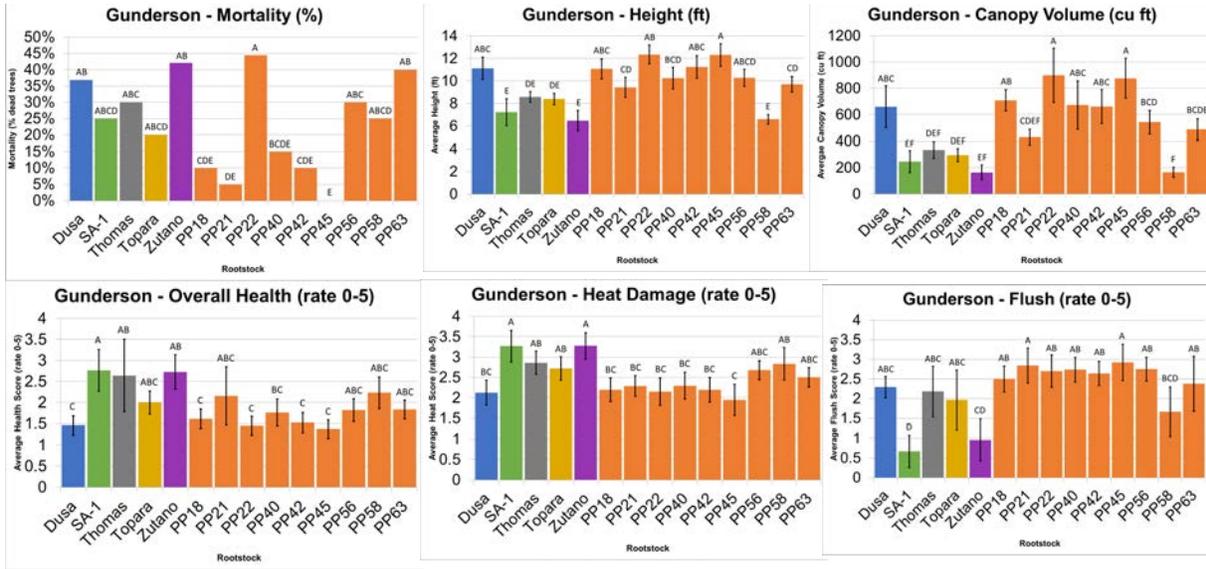
Gunderson, Santa Paula. We have been communicating and working with the new managers: Mr. Edgar Gutierrez (Vice President of Farming Operations) and Mr. Vince Giacolone (Director of Southern Management Operations). Eleven UCR rootstock selections including the advanced rootstocks, PP40, PP42, and PP45, have been evaluated in this field plot established in 2006. In this plot, rootstocks are being tested under PRR, high pH and high alkalinity conditions. Salinity based on our water and soil analysis indicated a possible salinity problem (Table 10). At this site, PP22 and Zutano seedlings have the highest tree mortality (> 50 %). PP45, PP42, PP40, PP21, and PP18 exhibited the least mortality (~10%) (Fig. 71).

Figure 71. Tree mortality at Gunderson, Santa Paula, Ventura.



Zutano seedlings, SA-1 PP58, RO.54 (Topara), and Thomas are the smaller trees at this location. The advanced UCR rootstocks PP45, PP42, and PP40 have similar tree height and canopy size as Dusa at this location. At this location, all rootstocks with the exception Zutano seedlings, SA-1, and Thomas have similar overall tree health. In addition, no significant differences were detected for salinity damage. For heat damage, Zutano seedlings, and SA-1 were the rootstocks exhibiting more heat damage and less flushing scores (Fig. 72). PP22 and PP45 exhibited the best fruits set scores followed by PP18, PP42, and PP56.

Figure 72. Tree performance at Gunderson, Ventura (October 2022).



We collected 6 years of harvest data (2016-2022). PP40 is the best producer in this location, followed by PP42, PP45, PP21, and Dusa (Fig. 73, Table 13). Similarly, PP40 is the rootstock with the best yield efficiency per canopy volume followed by Dusa and PP21 (Fig. 74).

Figure 73. Cumulative yield at Gunderson, Ventura (October 2022).

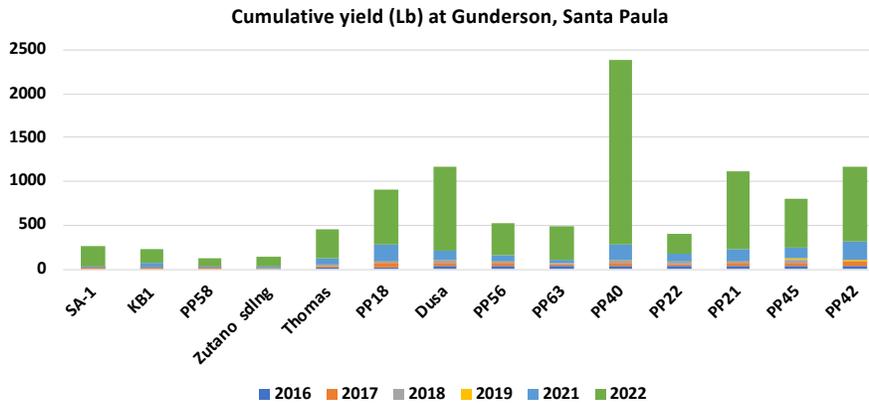


Figure 74. Yield efficiency at Gunderson, Ventura (October 2022).

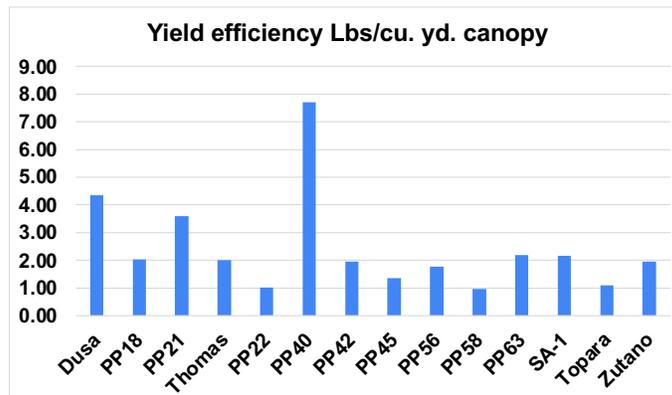


Table 13. Summary of harvest at Gunderson, Santa Paula (2022)

Rootstock	Total # of Alive Trees	Total Fruit #	Total Weight (lbs)	Avg Weight (oz) / Fruit	Avg # Fruit / Tree	Avg Yield (lbs) / Tree	# of Alive Trees w/ No Fruit	Avg # Fruit / Tree	Avg Yield (lbs) / Tree
Dusa	12	2091	959.52	7.34	174.25	79.96	1	190.09	87.23
PP18	18	1357	628.15	7.41	75.39	34.90	3	90.47	41.88
PP21	19	2425	889.35	5.87	127.63	46.81	0	127.63	46.81
Thomas	14	864	332.18	6.15	61.71	23.73	1	66.46	25.55
PP22	10	542	225.60	6.66	54.20	22.56	1	60.22	25.07
PP40	17	5688	2100.68	5.91	334.59	123.57	0	334.59	123.57
PP42	18	1982	846.62	6.83	110.11	47.03	2	123.88	52.91
PP45	19	1136	544.22	7.67	59.79	28.64	2	66.82	32.01
PP56	14	947	370.96	6.27	67.64	26.50	1	72.85	28.54
PP58	15	199	78.62	6.32	13.27	5.24	4	18.09	7.15
PP63	12	996	368.40	5.92	83.00	30.70	2	99.60	36.84
SA-1	15	639	223.50	5.60	42.60	14.90	4	58.09	20.32
Topara	16	421	163.04	6.20	26.31	10.19	2	30.07	11.65
Zutano	11	200	92.66	7.41	18.18	8.42	5	33.33	15.44

The results from tree health and harvest collection at these two plots in Santa Paula support the commercial release of PP40, PP35, PP42, and PP45. These trees perform in some locations and years better or similar than Dusa.

Obj. 3. Continue the collection of tree health and harvest data for PP35, PP40, PP42, PP45, and PP80 UCR advanced rootstocks, Israeli rootstocks, and South African rootstocks at Pine Tree and Bonsall rootstock trials (established June 2017). These two field sites are overseen by Co-PI Dr. Mary Lu Arpaia. Tree health and harvest data collection is conducted by Dr. Arpaia and the Manosalva lab assistants (Amber Newsome and Matthew Elvena).

Comments of the site and overall tree mortality. Two identical trials were planted in June 2017 either in San Diego County or Ventura County. The list of rootstocks included in the trial is presented in **Table 14**. Each site is planted in a randomized block design.

Table 14. Rootstocks grafted to ‘Hass’ included in 2017 rootstock trial planted at 2 sites. Site 1 is near Bonsall, CA and site 2 is near Santa Paula, CA. Both sites planted in June 2017.	
Commercially Released	Dusa, Leola™ (Merensky 6), Steddom, , Topara (RO.54), Toro Canyon, Uzi, Zentmyer, Zerala™ (Merensky 5)
UC Selections from J. Menge Program	PP35, PP40, PP42, PP45, PP50, PP51, PP52, PP80
UC Selections from G. Douhan Program	GD3, GD4, GD5, GD6, GD10, GD11, GD19, GD20
South Africa Selections from WTS	R106, RO.15, RO.17, RO.18
Israel Selections from B. Ya’acov Program	AB20 (VC802), AB22 (VC804)

The San Diego County site is located near Bonsall, CA. This site is farmed as an organic grove. Testing prior to planting showed that the site has *P. cinnamomi* and saline irrigation water. The site is irrigated using well water. The San Diego site was planted on June 28, 2017. The trees are spaced 10 x 10 feet. The trees received an approximated 6-inch application of mulch at the time of planting. The replicated blocks at the Bonsall site were designed to take into account the slope of the field. In recent years, the trees have suffered from a lack of general nutrition and have had “see-through” canopies and overall poor color. In April 2022, the owner applied mineral nutrition to the site and the general appearance of the trees are greatly improved; fruit set looks reasonable for 2023. The owner plans to prune the trees in Summer 2022.

The Ventura County site is located near Santa Paula, CA at the California Avocado Commission demonstration site at the Pine Tree Ranch. This site is managed as a conventional grove. Testing prior to planting showed low levels of *P. cinnamomi* present. The site was planted on June 13, 2017. The grove is irrigated with district water and is of good quality. The trees are planted on berms (approximately 2 feet in height and 3 feet width at base) with a tree spacing of 15 x 15 ft. The site was not mulched at the time of planting; mulch was only applied in September 2018, approximately 16 months after planting. The replicated blocks were laid out across the irrigation rows. After having a difficult 2 to 3 years becoming established the trees now look uniformly good and have very good color. The trees were pruned lightly in Summer 2021.

At the time of harvest for both sites in Spring 2022 a few additional dead trees were noted: 1 tree at the Santa Paula site and 6 trees at the Bonsall site. This brings to a total of 30 trees or 10% of the total planted at the Santa Paula site and a total of 75 trees (25%) at the Bonsall site. Tree deaths are spread across all rootstocks (**Fig. 75**) with high tree mortality ($\geq 50\%$ of trees) for Uzi, PP45, PP80 at the Bonsall site and GD5 at the Santa Paula site. Since we visit the site only periodically, it is nearly impossible to discern the original cause of tree death. However, at the Bonsall site, several trees were originally lost in the early part of this study due to cold and wet soil conditions. Overall tree health scores were higher (lower scores) in Bonsall site (Fallbrook) compared with Pine tree trial. Trees at Bonsall exhibited thinner density canopy and poor leaf color (**Fig. 76**).

Figure 75. Tree mortality by rootstock at each experimental site as of May 2022. At each site, 10 trees for each rootstock were planted.

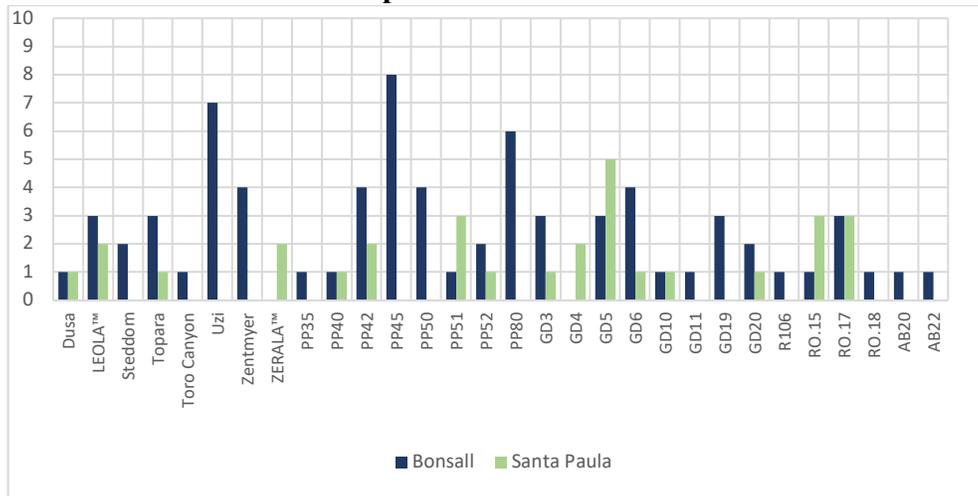
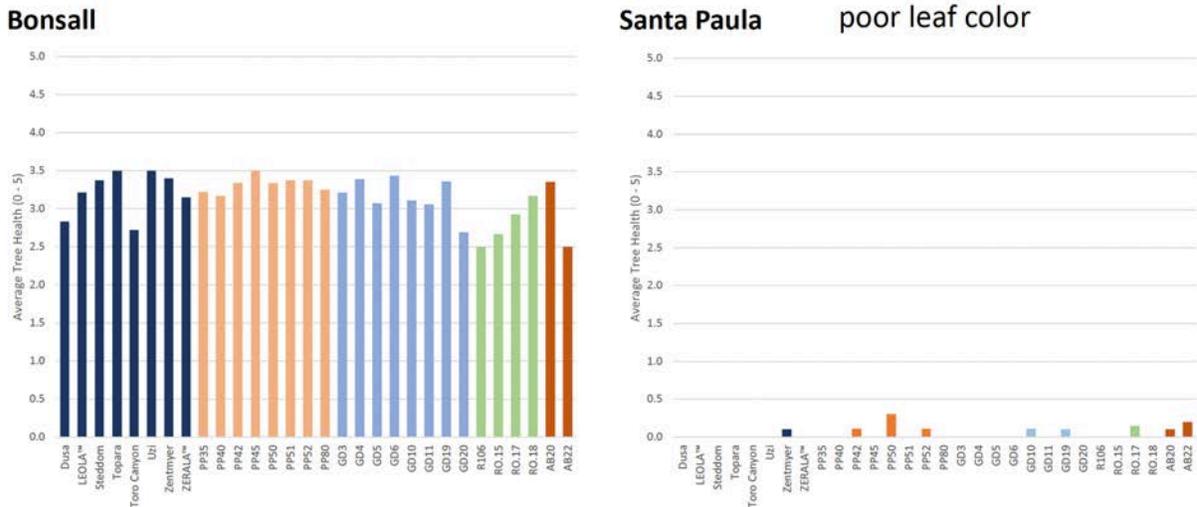


Figure 76. Overall tree health at Bonsall and Pine Tree rootstock trials.

Tree Health (0 = Healthy; 5 = Dead)

Higher ratings (lower health) in Bonsall reflects thinner density canopy and poor leaf color



2022 Yield Data

Bonsall Site, San Diego

The Bonsall site was harvested on May 13, 2022. The yield was exceedingly low with an overall average yield of 0.97 kg/tree. Only 16% of the trees had any fruit and on some rootstocks none of the surviving trees had any fruit (Fig. 77; Leola, Steddum, Topara, Uzi, Zentmyer, PP42, PP45, PP50, GD3, GD4, GD5, GD6, RO.17). In fact, over the course of this study, no fruit have been harvested from PP45 or GD6. Figure 78 presents the cumulative yield data for the trial. Fruit count data shows a similar trend and is not presented. ‘Hass’ on AB22 is the leading rootstock in this trial with a cumulative average total of 35.2 kg/tree; this is significantly greater than the remaining rootstocks. R106 with a cumulative average total of 19.7 kg/tree is the second highest yield rootstock in the trial and is significantly higher than the remaining rootstocks in the trial. There are no significant differences due to rootstock in the cumulative average yield which ranges from 9.97 kg/tree (AB20) to 0.0 kg/tree (PP45, GD6). Average fruit size, with the exception of ‘Hass’ on Uzi where only 1 fruit (745 g) has been harvested in the 4 years, is between 176 g/fruit (PP35) to 318 g/fruit (RO.18)

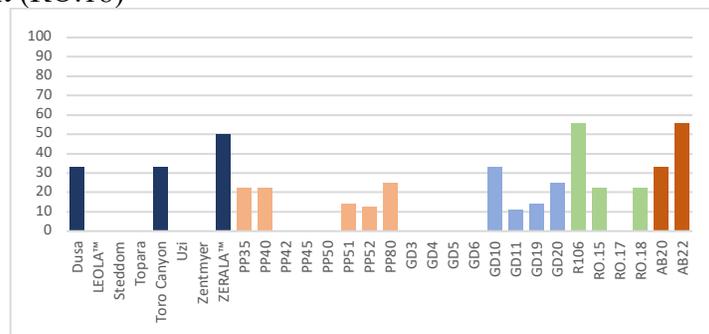
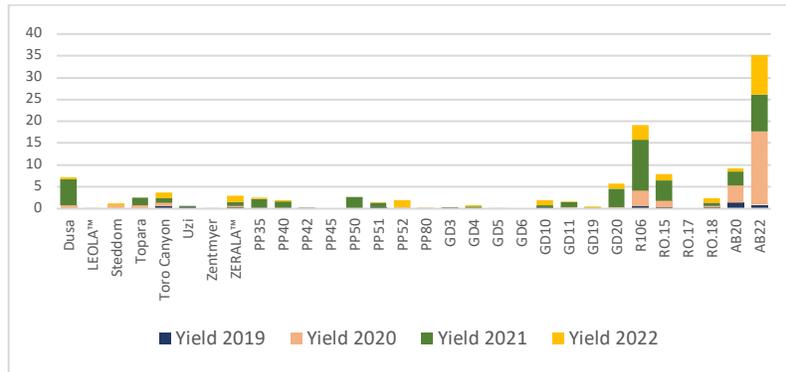


Figure 77. The percentage of surviving trees that had fruit for the May 2022 harvest at the Bonsall rootstock trial.

Figure 78. Average cumulative yield (kg/tree) of ‘Hass’ influenced by rootstock in Bonsall, CA from 2019 through 2022. Trees planted in June 2017.



Santa Paula Site

The Santa Paula site was harvested on April 2, 2022. Yield was good with an overall average yield of 32 kg/tree (151 fruit/tree) at the site. Ninety-eight percent of the surviving trees had fruit. Trends in the yield data whether by kg/tree or fruit/tree were similar. Yield per tree ranged from a high of 56.2 kg/tree (RO.15) to a low of 14.7 kg/tree (PP52) (Fig. 79). The two highest yielding rootstocks, RO.15 and GD10, were statistically higher ($P \leq 0.05$) than PP50, RO.18 and PP52, the three lowest yielding rootstocks. PP45 trees, the third highest yield rootstock, were statistically higher than PP52 trees in terms of yield. There were no other statistically significant differences detected.

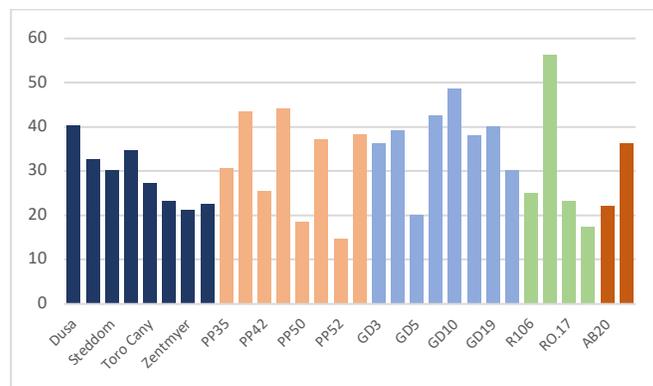


Figure 79. Average kg/tree yield of ‘Hass’ influenced by rootstock. Trees harvested April 2, 2022.

In terms of cumulative yield, results were similar for both average kg/tree or by average fruit count/tree. In both instances, the top 2 performing rootstocks were RO.15 and GD10. Average cumulative kg/tree (Figure 80) ranged from 63.9 kg/tree (RO.15) to a low of 18.6 kg/tree (PP52). RO.15 had statistically higher yield ($P \leq 0.05$) in terms of kg/tree compared to the 10 lowest yielding rootstocks (R106, Uzi, PP42, RO.17, PP50, Zerala, Zentmyer, GD5, RO.18 and PP52). GD10 differed significantly ($P \leq 0.05$) from the 2 lowest yielding rootstocks, RO.18 and PP52. Fruit size trends (g/fruit), whether examined on an annual basis or as the average fruit size over the 3 years of yield data were similar. In both instances the largest fruit have been from the RO.17 and RO.18 trees which tend to have lower overall yields. The smallest fruit has been obtained

from the RO.15 trees, which are the highest producers in the trial. GD10, the second highest producing rootstock in the trial both in terms of kg/tree and fruit/tree is intermediate regarding fruit size (Fig. 81).

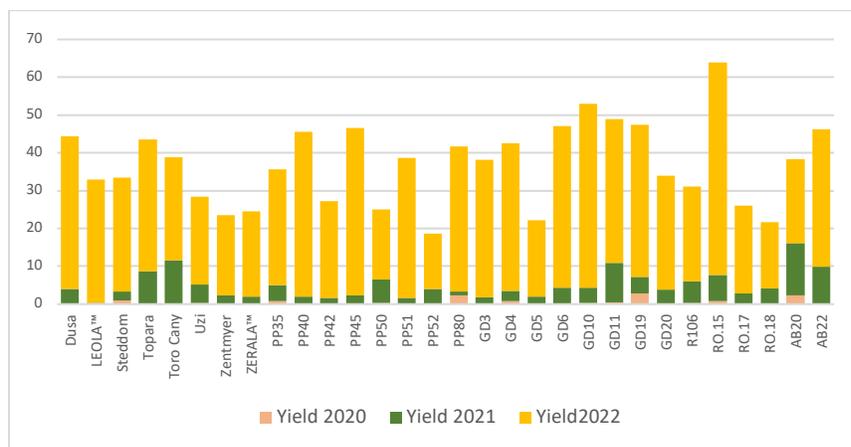


Figure 80. Average cumulative yield (kg/tree) of ‘Hass’ influenced by rootstock in Santa Paula, CA from 2020 through 2022. Trees planted in June 2017; trees had no yield in 2019.

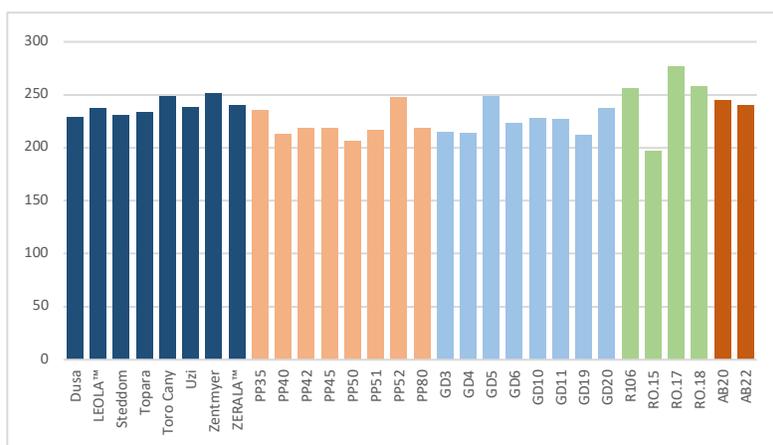


Figure 81. Average fruit size (g/fruit) of ‘Hass’ influenced by rootstock in Santa Paula, CA from 2020 through 2022. Trees planted in June 2017; trees had no yield in 2019.

The results from tree health and harvest data from all the rootstock trials presented above established at Southern and Northern CA under different environmental conditions and cultural practices support the commercial release of PP35, PP40, PP45, and PP42. More data is required for PP80. The UCR team will continue periodically visiting the site and will notify ranch management prior to each visit. The UCR team will discuss any problems with ranch management but the general care of the trees including nutrition, irrigation and pest control will be the responsibility of the grower cooperator. We will compare rootstocks accessions within individual field sites, across sites (when possible), and across years of evaluation for each set of data. Linear mixed models are being used to test if rootstock, location, and the rootstock x location have a significant effect on the phenotypic data collected in the field. Rootstock, location, and their

interaction are being treated as fixed factors, while field will be treated as a random factor in the linear mixed models. We plan to monitor these sites for 8 to 10 years following planting.

Commercial release of PP35, PP40, PP45, and PP42 UCR advanced rootstocks in CA. The release of these rootstocks will be done through UCR. Manosalva's team will gather all the information regarding: greenhouse data, regional and multistate field data grafted with Hass and other scions, yield in CA from the past years, and other relevant information regarding their field performance under different conditions (most of the data is currently available). In addition, we will record horticulture trait data such as tree height and canopy size of the ungrafted trees. We will take photographs of the tree, branches, flowers, and fruits for each rootstock since all this information is required to fill out the patent paperwork within the next 3 years.

PROGRAM OUTREACH AND EDUCATION. During the time for this project, the PI, Co_PIs, and members of the UCR research team gave several oral and poster presentations as well participated during field dates, seminars, courses organized by CAC and the California Avocado Society (CAS). Several undergraduate students, graduate students, junior, and senior scientist were trained (*see below*). The results of this projects were also disseminated at International Meetings (*see below*).

Personnel trained under this project:

- 1- Brandon McKee (Former SRA, field and greenhouse assistant).
- 2- Dr. Nilwala Abeysekara (Former Assistant Specialist, fundamental and field research).
- 3- Dr. Erika Nandankar (Former Postdoc, Breeding and field activities).
- 4- Dr. Damaris Godinez-Vidal (Former Assistant Specialist and field assistant).
- 5- Nathaniel Von Doltoren (Former Jr. Specialist, Field activities).
- 6- Benjamin Friedenber (Former Jr. Specialist, Field activities).
- 7- Dr. Natasha Jackson (Former Ph.D Student, Summer 2022, Field activities support).
- 8- Amber Newsome (Jr. Specialist, Field and greenhouse assistant).
- 9- Matthew Elvena (Jr. Specialist, Field and greenhouse assistant).
- 10- Dr. Bullo Mamo (Associate Specialist, Breeding and Field activities support).
- 11- Aidan Shands (Ph.D Student, Spring 2023, Field activities and field training).
- 12- Benjamin Hoyt (Ph.D Student, Field activities support).
- 13- Rashaan Topham Souikane (Dr. Garner's Graduate Student).
- 14- Johnny David Rosecrans (Dr. Garner's Field Assistant).
- 15- Adrian Garcia (Undergraduate Student Support).

Dr. Lauren Garner's Products: field dates, posters, publications etc.

Since the establishment of the **San Luis Obispo** site, Garner submitted and was approved for a change of scope to an already awarded grant from the Agricultural Research Institute (ARI), which added more than \$85K in funding for this project. This funding has allowed us to support key aspects of the plot's management and most importantly, all Cal Poly students and faculty working on the project have been solely funded by Garner's Agricultural Research Institute grant, for a which a no-cost extension was secured through June 2023. Rashaan Souikane, the graduate student on this project, presented a poster of our work as first author at the annual conference of the American Society for Horticultural Science (ASHS) in August 2022. He is working with a Cal Poly statistician to analyze the data so that he can finish and defend his thesis in early 2023. His thesis will serve as the basis for a peer-reviewed publication that Rashaan will coauthor with

Garner and Manosalva. The **San Luis Obispo** trial was among the topics of the April 14, 2021 [California Avocado Growers Seminar/Webinar](#), "Virtual Avocado Field Day at Cal Poly," hosted by the CAC, CAS, and UCCE. During the project period, 12 senior projects were completed by 9 Cal Poly undergraduate students with projects directly overlapping with the objectives of this project. Additionally, 2 students completed senior projects exploring possible effects of the rootstock on the incidence of suckering above and below the graft union, and 1 student studied the effect of the rootstock on the incidence of perseá mite damage.

Dr. Patricia Manosalva's Products: field dates, posters, publications, etc.

PUBLICATIONS

(Peer-Reviewed Journal Articles) **denotes corresponding authorship*

1. Mondragon-Flores, A., **Manosalva, P.**, Ochoa-Ascencio, S., Diaz-Celaya, M., Rodriguez_alvarado, G., and Fernandez-Pavia, S. 2022. Characterization and fungicide sensitivity of *Phytophthora cinnamomi* isolates causing avocado root rot in Zitacuaro, Michoacan. Revista Mexicana de Fitopatologia, Vol 40, N°1, Pp.1-23.

INVITED PRESENTATIONS

- P. Manosalva. 08/19/2021. Avocado Production Course for New Growers. Avocado Rootstocks and The UCR Avocado Rootstock Breeding Program. University of California Cooperative Extension.
- P. Manosalva. 04/19/2021. North Carolina State University (NCSU). Plant Pathology Department Seminar Series. Towards resistance against Phytophthora species: from model plants to crops.
- P. Manosalva. 12/14/2020. University of California, Davis. Plant Breeding Center. Plant Breeding Annual Retreat Breeding for Resilience. UCR Avocado Rootstock Program: Towards Developing the Next Generation Rootstocks.
- P. Manosalva. 11/11/2020. Second Avocado International Congress. Topic: Rootstocks and their tolerances. UCR Avocado Rootstock Program Update: Towards Developing Disease and Salinity Resistant Rootstocks. *International Venue.
- P. Manosalva. 06/10/2020. Avocado Growers Meeting. UCR Avocado Rootstock Program Update: Towards Developing Disease and Salinity Resistant Rootstocks.
- P. Manosalva. 09/24/2019. IX World Avocado Congress. Medellin, Colombia. Next generation of rootstocks developed at UCR to meet major avocado production challenges. *International Venue.

- P. Manosalva. 08/26/2019. XXI International Congress/ XLVI National Congress Mexican Phytopathology Society. Morelia, Michoacan, Mexico. Reducing losses to Phytophthora root rot by improving resistance selection and disease management. *International Venue.

OTHER PRODUCTS

- A virtual and in person grower survey was conducted in 2022 in California during the Avocado Growers Meeting organized by the California Avocado Society, Ben Faber, our Co-PI assisted in conducting the survey (Manosalva and Faber).
- Conducted several interviews for several press releases featuring the UCR Avocado Rootstock Breeding Program and my recent grant funding. A was interviewed for a documentary by the BBC World News 'Follow the Food' featuring one of my UCR Avocado Rootstock Trials in Ventura.

<https://www.youtube.com/watch?v=Pi42DQuuxbc>

<https://www.highlandernews.org/81426/ucr-is-given-over-4-million-in-grants-for-avocado-preservation/>

<https://www.thedenverchannel.com/news/national/as-avocado-crops-face-serious-threat-team-of-scientists-trying-to-save-orchards>

<https://www.wired.com/2017/01/long-lonely-quest-breed-ultimate-avocado/>

<https://kesq.com/news/2020/12/10/uc-riverside-receives-more-than-4-million-for-avocado-anti-fungus-research/>

<https://www.californiaavocadogrowers.com/sites/default/files/documents/13-Dr-Manosalva-Awarded-Grants-for-Rootstock-Breeding-and-Organic-Disease-Management-Projects-Winter-2020.pdf>

GRANT AWARDS

- | | | |
|--|-------------|-------------------------|
| 1- PI: Manosalva | \$268,884 | 11/01/2022 - 10/31/2025 |
| Co-PIs: M.L. Arpaia, Lauren Garner, and P. Mauk. | | |
| Agency: California Avocado Commission (CAC). | | |
| Title: Commercial-scale field testing and potential release of five elite advanced rootstocks
(current, renewal). | | |
| 2- PI: Manosalva | \$4,401,036 | 09/30/2020 - 09/29/2024 |
| Co-PIs: J. Adaskaveg, A. Mulchandani, H. Kim, J. Jifon, L. Cano, M. Tian, A. Bombarely, R. Gazis, K. Garrett, B. Schaffer, J. Crane, and R. Goenaga. | | |
| Agency: USDA-National Institute for Food and Agriculture (NIFA)-Specialty Crop Research Initiative (SCRI). | | |

Title: Reducing avocado losses to major challenges by improving resistance selection and disease management using next generation technologies. Project No: CA-R-MPP-5217-CG (**current**).

- 3- **Co-PI: Manosalva** \$1,999,317 09/01/2020 - 08/31/2024
PI: Ali Sarkhosh
Agency: USDA-National Institute for Food and Agriculture (NIFA)-Organic Agriculture Research and Extension Initiative (OREI).
Title: Plant safety, horticultural benefits, and disease efficacy of essential oils for use in organically grown fruits crops: from the farm to the consumer. Proposal No: 2020-02141 (**current**).
- 4- **PI: Manosalva** \$2,249,999 03/01/2020 - 02/28/2023
PIs: Mary Lu Arpaia and Peggy Mauk
Agency: Eurosemillas S.A. (Spain).
Title: Support Avocado UCR Variety and Rootstock Breeding Programs (<https://news.ucr.edu/articles/2020/06/09/uc-riverside-and-eurosemillas-partner-bring-next-generation-avocados-market>) (**current**).
- 5- **PI: Manosalva** \$200,000 06/15/2020 - 06/14/2023
Agency: USDA-National Institute for Food and Agriculture (NIFA). Program: Pests and Beneficials in Agricultural Production Systems.
Title: Exploiting genomic resources to understand *Phytophthora cinnamomi* genetic diversity, virulence, fungicide resistance, and host adaptation. Project No: CA-R-MPP-5202-CG (**current**).
- 6- **PI: Manosalva** \$349,934 11/01/2019 - 10/31/2022
Co-PIs: M.L. Arpaia, Lauren Garner, and P. Mauk.
Agency: California Avocado Commission (CAC).
Title: Commercial-scale field testing and potential release of five elite advanced rootstocks (**completed**).
- 7- **PI: Manosalva** \$25,000 07/01/2019 - 06/30/2021
PI: Sylvia Fernandez-Pavia
Agency: UC-MEXUS-CONACYT Grants for Collaborative Projects.
Title: Genotypic and phenotypic characterization of *Phytophthora cinnamomi* populations from Mexico and California associated with avocado root rot (**completed**).

CONCLUSIONS

- Tree health data, salinity data, tree mortality, and harvest data during the last three years at 9 active large-scale field trials and 4 small regional trials support the pursuit of releasing PP40, PP42, PP45, and PP35. These rootstocks show promise to meet the needs of avocado growers at different conditions in California.
- Current field data suggests that PP80 rootstocks has potential to be used in locations with moderate PRR incidence, salinity, and warmer temperatures. More data is need it especially harvest data to support its commercial release in CA.
- PP40 and PP35 exhibited good performance across all field trials in CA. Both have desirable *P. cinnamomi* resistance, heat and salinity tolerance. Both are highly productive trees compared with Dusa and better than Dusa at some locations.
- ‘Hass’ trees grafted on PP35 rootstock are small, vigorous, and good producers at several locations in Southern and Northern CA. PP35 is a great rootstock for high density planting.
- PP45 and PP42 are vigorous trees that are highly resistant to *P. cinnamomi* making them ideal for locations with high incidende of PRR. ***Note that PP45 is salinity sensitive rootstock but it is a great rootstock at locations with high PRR and salinity combined, In addition PP45 have good levels of heat tolerace and are good rootstocks for locations with clay soils, high PRR, and salinity.***
- At the San Luis Obispo site, the orchard is now ~2.5 years old, and has provided a statistically valid site to evaluate and compare the effects of PP35, PP40, PP45, and ‘Dusa’ rootstock on early establishment and growth of ‘Hass’ avocado. Additionally, this site allows for long-term evaluation of the rootstock in continued studies and future grower field days in Growing District 5.
- Under this funding cycles, we successfully trained several undergraduate students and graduate students as well as junior and senior personnel. We conducted several seminars and field days to disseminate our findings. Also the support from the CAC was fundamental for secure federal, state, and international industry funding to continue the fundamental and applied research.

REFERENCES

1. Belisle, R.J., McKee, B., Hao, W., Crowley, M., Arpaia, M.L., Miles, T.D., Adaskaveg, J., Manosalva, P*. 2019a. Phenotypic characterization of genetically distinct *Phytophthora cinnamomi* isolates from avocado. *Phytopathology* 109:384-394. doi.org/10.1094/PHYTO-09-17-0326-R.
2. Belisle R., Hao W., McKee B., Arpaia M., Manosalva P*., and Adaskaveg J*. 2019b. New Oomycota fungicides with activity against *Phytophthora cinnamomi* and their potential use for managing avocado root rot in California. *Plant Disease*. <https://doi.org/10.1094/PDIS-09-18-1698-RE>.