Enhancement of Avocado Productivity. Plant improvement - selection and evaluation of improved varieties and rootstocks

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The goal of the avocado scion breeding program is to help maintain and enhance the California avocado industry by introducing consistently heavier producing, high-quality avocado varieties, better pollinizer varieties, and to test improved rootstock hybrids. This can be achieved through identification of material which is less prone to alternate bearing and more tolerant to adverse environmental conditions. Additionally identifying varieties with a more upright tree structure will assist in high density tree management schemes. The goals of this project will be achieved through continued evaluation of new material generated by traditional selection techniques, collaboration with other researchers as they develop refined techniques to increase the efficiency of selection and introduction of new material from other improvement programs. Increasing the genetic diversity of varieties will decrease the risk of major pest and disease invasions on a susceptible monoculture. Our progress from November 2009 to October 2010 is summarized below.

A major change this year is the addition of Dr. Harley Smith as co-PI on this project. Dr. Smith is a member of the Botany and Plant Science Department and the Center for Plant Cell Biology group at UCR. Dr. Smith brings to the project plant molecular and genomic approaches, which will be of great use to the breeding program in future years. His participation will assist us in deploying the marker assisted selection program initiated by Dr. Clegg (UCI) as well as further genetic characterization of our breeding stock.

The bulk of our activities are based at the UC South Coast Research and Extension Center (SCREC) in Irvine where all seedlings are planted and Tier 1 and Tier 2 selections made (Figure 1).

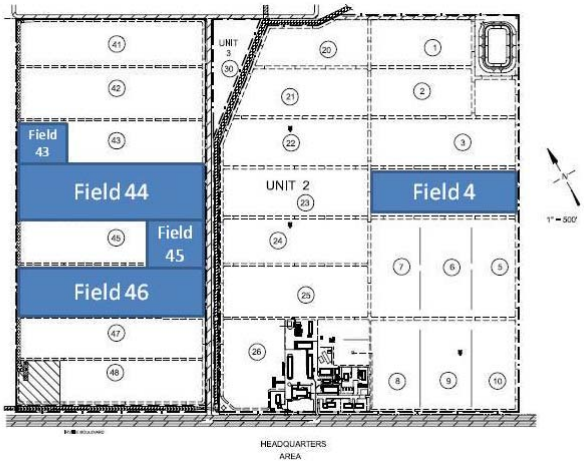


Figure 1. Map of UC South Coast Research and Extension Center in Irvine, CA, the site of the majority of our activities. We have seedlings in Tier 1 evaluation in Fields 44, 45 and 46. Tier 2 activity is centered in Field 4. The 'Hass'/'Lamb Hass' rootstock trial is on the west side of Field 44. The Variety Collection is located on the east side of Field 44. The Persea collection is located on the west side of Field 43.

Seeds for new selections. Last year we began contracting seed propagation/germination to ACW Farms in Fallbrook. This change was necessitated by overall poor germination rates in the SCREC greenhouse, which was likely due to high temperatures in late spring and summer. The SCREC greenhouse is still used to germinate small numbers of SCREC seed and also to hold germinated seedlings from ACW until they are planted in 1 gallon citrus sleeves. We have been collecting seed material from open-pollinated flowers from cultivars of maternal interest as well as the remaining isolation plots at UC Riverside. These are grown and then planted out at SCREC. Seeds have also been collected from 'Sir Prize', 'Reed', 'Nabal' and 'Lamb Hass' from SCREC. In addition, we have collected 100 'Gem' and 100 'BL516' seeds from our UCR isolation plots. We will have a carryover of approximately 700 seedlings to be planted in the field in Spring 2011 from the 2010 seed collection effort (~1,000 seeds).

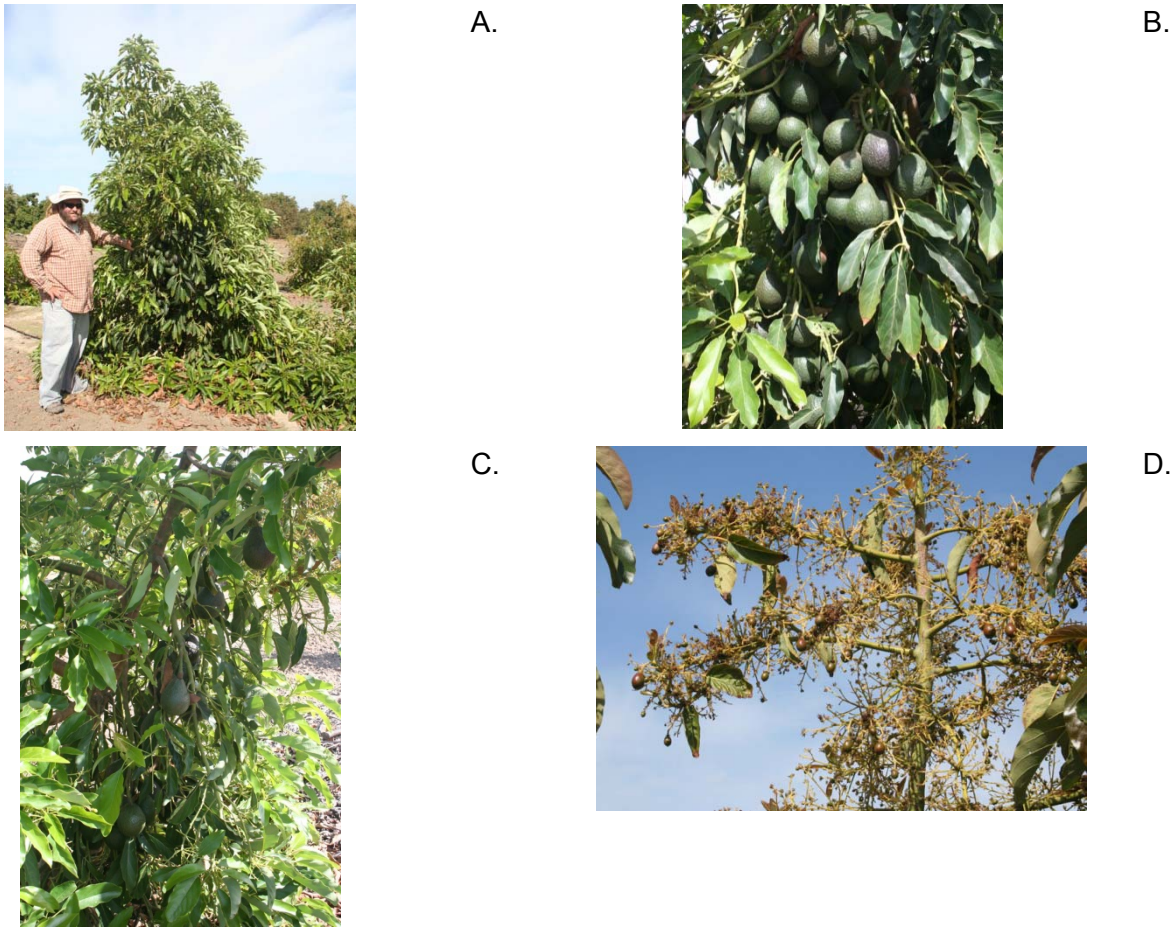


Figure 2. Pictures from Field 4 at SCREC in Irvine, CA. Photographs taken in May 2010. **A.** Tree of a Tier 2 selection, 465202-99, showing tree shape and relative size for a 4 year old tree on Duke 7 rootstock. **B.** Close up of current year crop load on 465202-99. **C.** 2010 fruitlets/2011 fruit on a tree of Tier 2 selection, 465418-99. **D.** Initial 2010 fruit set on selection 465418-99, a selection being propagated for Tier 3 trials. Information regarding these selections has been provided in our Annual Reports and to the CAC-PRC Breeding and Genetics Subcommittee. Tier 1 selections are from seedling material, Tier 2 selections are those seedlings which have passed initial scrutiny and have been propagated for further evaluation.

Fruit evaluations from seedling plantings (Tier 1 selection). The seedling plantings from the breeding program have continued yielding fruit for evaluation this year. This is the third year we have seen fruit on seedlings planted in 2003 and 2004, and the fruit set this year was the heaviest yet. Since November, Eric Focht (Staff Research Associate) has identified 3 interesting selections from this seedling population which will be evaluated further. In addition, he is closely following another 4

selections. If these trees produce sufficient fruit, they are sampled for dry weight and fruit quality approximately every 4 to 6 weeks over the season. Fruit of interest are also being photographed during the evaluation process. Percent dry weights are sampled using the CDFA approved coring method of fruit sampling.

Planting of 2009 seedling material. We concentrated our efforts on planting seedling material on the west side of SCREC. In Field 46, 441 trees planted in spring of 2009 occupy all the space. Plantings in Field 44 from 2003 – 2005 have performed quite well. In spring of 2010, we expanded into the east side of Field 45 (previously planted to citrus) with a planting of 970 trees from seed collected in 2009. We are looking into using this field for fall 2010 planting, as well as for spring 2011 planting.

Further evaluations of new selections using Field 4 at SCREC (Tier 2 selection). We continue to convert Field 4 in order to produce seed from desirable parentage for the creation of new selections. This is an ongoing project and involves the removal of older trees in combination with a mix of replanting and topworking of different germplasms into the field. As per the 2005 recommendations of Dr. Chaparro (University of Florida, plant breeder of citrus and stone fruit), this planting serves a two-fold purpose of providing pollen for cross pollination with our seed-producing trees while allowing us to closely monitor selections of interest for fruit production, quality, seasonality and other favorable features. We have completed the removal of all undesirable material from the field and are currently topworking the stumps (seedling Mexican race rootstock of unknown origin) to create material of interest for breeding stock. At this point 80 Duke 7 trees were planted in this field and we have begun topworking 40 of these. Varieties topworked in this planting include both released and unreleased material from previous breeding programs and the public domain. In addition, we continue to topwork our promising new selections as well as some selections noted primarily for specific traits (such as two selections noted solely for their very early high dry matter). The availability of the Duke 7 rootstocks for topworking is of great benefit in speeding up our propagation process as we no longer need to send budwood off site for propagation. In spring of 2010, we began filling in spaces in rows where some trees died or topworking (by the UC volunteers) did not take; the replacement trees are all Duke 7 rootstock.

Many of the newest selections located in Field 4 have been propagated for further evaluation and these trees are entering full production (Figure 2). In addition to serving as pollen donors and seed to our breeding program; these trees provide fruit for taste evaluations (both formal and informal), a more complete dry weight tracking (every 4 to 6 weeks), yield, and bloom data. Budwood from some of these Tier 2 selections was harvested and will be used in our Tier 3 trials up and down the state. These trees are being propagated by Brokaw Nursery and should be ready for field planting in the spring of 2011.

An informal 'take home' fruit evaluation system has been developed that makes use of volunteer evaluators from within the UCR community. Results from the 2010 taste evaluations will be used to select material for future Tier 3 plantings. Results from this project are being collected and tabulated; varieties sampled include released varieties, heritage varieties, and our newest selections. Moreover, we conducted sensory analysis at the UC Kearney Agricultural Center's sensory facility in Parlier, CA in June, August and November using a mixture of promising selections, released varieties (to be determined), and Hass.

Overall status of selections of interest. During the current funding cycle, we selected an additional 3 seedlings for further evaluation. We have also eliminated 2 seedlings, thus far, and are likely to eliminate another 1 or 2 in 2011, after further evaluation. This makes for a total of 12 current selections from seedlings planted since 2000. (Additional information on these selections is available upon request and was shared with the PRC Genetics Subcommittee in June 2010.)

Statewide testing of promising material (Tier 3 selection). In March 2010 we placed an order with Brokaw Nursery to begin propagation of trees for a statewide trial on promising selections. The plan for this trial is to identify 5 locations statewide where we can further evaluate selections from the breeding program that show promise. We will have trials in San Diego county (Farm ACW confirmed), Orange County (UCSCREC), Ventura county (Brokaw Ranch, Santa Paula), San Luis Obispo (site still to be

determined) and Tulare county (UC Lindcove Research and Extension Center). The exact number of trees that will be planted each year will vary and will depend on the number of promising selections that are available the year prior to planting. All trees will be on clonal rootstock. For the first 3 years of the project, trees will be propagated on the Dusa rootstock, currently the number 1 rootstock in California. We will assess the status of Dusa after the third year to decide whether we should proceed further with this rootstock or select an alternative. Each selection will be replicated 4 times in the planting. Each replicate will consist of 3 trees per selection for a total of 12 trees. These will be planted in a randomized block design. For the 2011 planting we will have 5 selections: 'Hass' (the control), 465518-99, 465418-99, 'Flavia' and 'Eugenin'. The latter two selections are Hass-like selections from Chile that are here under test agreement. We intend, for the sake of space to only plant one replicate of Hass in years 2 and 3 but then will plant 4 replicates in year 4 when we re-evaluate the rootstock to be used. We will repeat this cycle as planting progress. We will collect data (individual tree) on yield and tree vigor. We will collect pooled data from each replicate on flowering, maturity and postharvest quality.

Introduction of new plant material. We have not introduced any new material this year.

Sunblotch Testing. Leaf samples continue to be collected on a routine basis from Fields 4, 44 and 46 at SCREC. Indexing is done by Dr. Deb Mathews in the UCR Department of Plant Pathology and Microbiology. We have found no positives in our samples from the period September 2009 through October 2010. New additions to our collection are tested prior to planting or grafting at SCREC. Our current focus in testing is to screen all new selection seedling plantings using batch sampling.

Rootstock Evaluation Trial. We continued to collect data from the 'Hass'/'Lamb Hass' trial planted in 1999 at the SCREC. This site has moderate temperature conditions being located within 15 miles of the coast. The site has been tested for the presence of avocado root rot, and although present, the disease is not an important factor since this site is considered to have suppressive soils (John Menge, UCR Professor Emeritus). The trial is planted as a randomized block design with both 'Hass' and 'Lamb Hass' intermixed within each block. Tree spacing is 19 x 20 feet. The 'Hass' portion of the trial was harvested in April 2010 and the 'Lamb Hass' trees were harvested in July 2010. This was our final year of data collection from this trial since we have collected 8 years of harvest data.

Changes in tree canopy volume are illustrated in Figure 3. The trees were not pruned during the course of the study, rather they were allowed to grow to their full potential. For the 'Hass' portion of the trial (Figure 3A), trees on the Zentmyer and Thomas rootstocks produced consistently the largest trees. The smallest trees were on the Partida, Spencer and G755A rootstocks. The remaining 5 rootstocks were intermediate in size. Figure 3B presents the results for 'Lamb Hass'. Thomas again produced a large tree as did Evstro. Canopy volumes for these two rootstocks grafted to 'Lamb Hass' were significantly greater than those on the other 3 rootstocks. The smallest 'Lamb Hass' tree was those on the Duke 7 rootstock.

Figure 4A presents the cumulative yield for the 'Hass' portion of the trial. Note that the Zentmyer was the highest yielding rootstock over the course of the 11 years of data collection although not statistically different from the yields on Evstro or Dusa rootstocks. The Zentmyer yielded significantly more fruit than the remainder of the rootstocks. Yield on Duke 7 and Toro Canyon were not significantly different from Evstro or Dusa. The 'Hass' yields on the remaining 5 rootstocks were significantly lower than the top 5 producing rootstocks. The cumulative yield for 'Lamb Hass' is shown in Figure 4B. Cumulative fruit production on the Evstro rootstock was significantly higher that the remaining 4 rootstocks, Thomas, Toro Canyon, Duke 7 and Day.

The tendency towards alternate bearing is illustrated in Figure 5. The 'Hass' trees went through 4 distinct alternate bearing cycles since their planting (Figure 5A). This trend is less clear in the 'Lamb Hass (Figure 5B). Using this data it is possible to calculate the alternate bearing index, which is the degree of alternation in yield from one year to the next. The value for the alternate bearing index ranges from 0 to 1. A value of 0 would mean that there is no alternation from year to year whereas a value of 1 would mean that there is complete alternation from one year to the next. The alternate bearing index for this trial was quite high, higher in fact than the previous rootstock trial we conducted at SCREC. In this trial, the alternate bearing index ranged from 0.75 (Thomas) to 0.91 (Evstro) as

compared to values 0.33 to 0.56 in the first rootstock trial. The Duke 7, G775A, Thomas and Toro Canyon were included in the first trial and have approximately twice the alternate bearing index value in this trial. The reason for this change in alternate bearing tendency could be caused by several reasons.

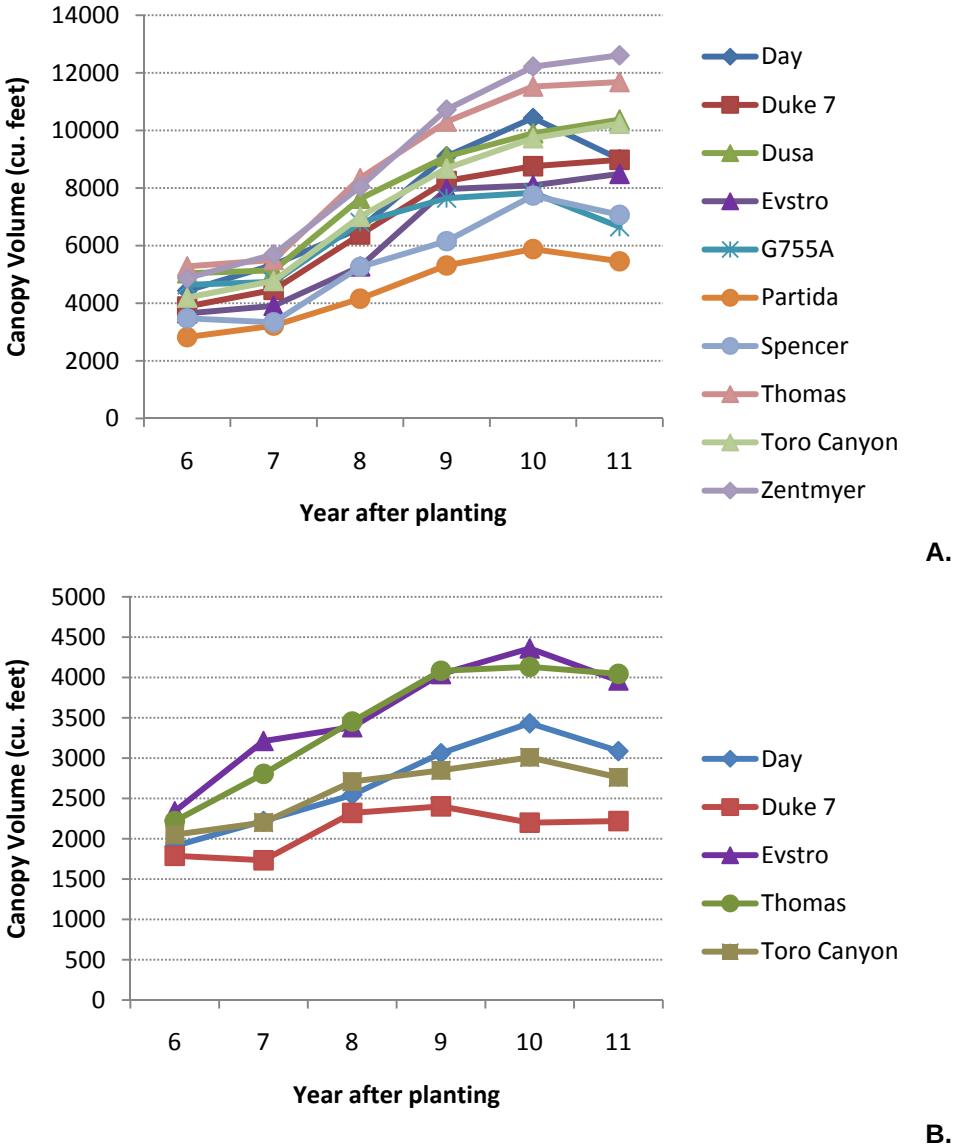


Figure 3. Changes in canopy volume for trees in the scion:rootstock trial at SCREC in Irvine, CA from year 6 to 11 after planting. **A.** 'Hass' trees. **B.** 'Lamb Hass' trees. Canopy volume for 'Hass' calculated as a ½ oblate sphere ($CV = 4/3\pi ab^2$) where the tree is wider than high where a is the tree height and b is the tree width radius. Canopy volume for 'Lamb Hass' calculated as a ½ prolate sphere ($CV = 4/3\pi a^2 b$) where the tree is taller than wide.

Tables 1 and 2 present the comparison between for 'Hass' and 'Lamb Hass' for the trial for tree size and yield characterstics. This comparison is possible since both these varieties were planted on 5 of the rootstocks; Day, Duke 7, Evstro, Thomas and Toro Canyon. The 'Hass' trees were significantly larger than the 'Lamb Hass' in terms of tree height, tree width and canopy volume. The 'Hass' trees were approximately 3 times larger than the 'Lamb Hass' trees in terms of canopy volume.

The 'Lamb Hass' produced significantly larger fruit over the course of the 8 years of data collection but this larger fruit size translated into significantly fewer fruit produced on a per tree basis (Table 2). Cumulative yield was also significantly lower with the 'Lamb Hass'. There was no difference between

the 2 varieties in terms of the alternate bearing although the 'Lamb Hass' was numerically lower, meaning slightly less alternate bearing. The yield efficiency, the amount of fruit produced per cubic foot tree canopy, was twice as high with the 'Lamb Hass' as compared to 'Hass'. This is due to the fact that the trees were considerably smaller than the 'Hass' trees (Table 1). These results corroborate the idea of planting the 'Lamb Hass' at higher tree densities per acre. If this approach was taken it is conceivable that the per acre production would be higher in 'Lamb Hass'.

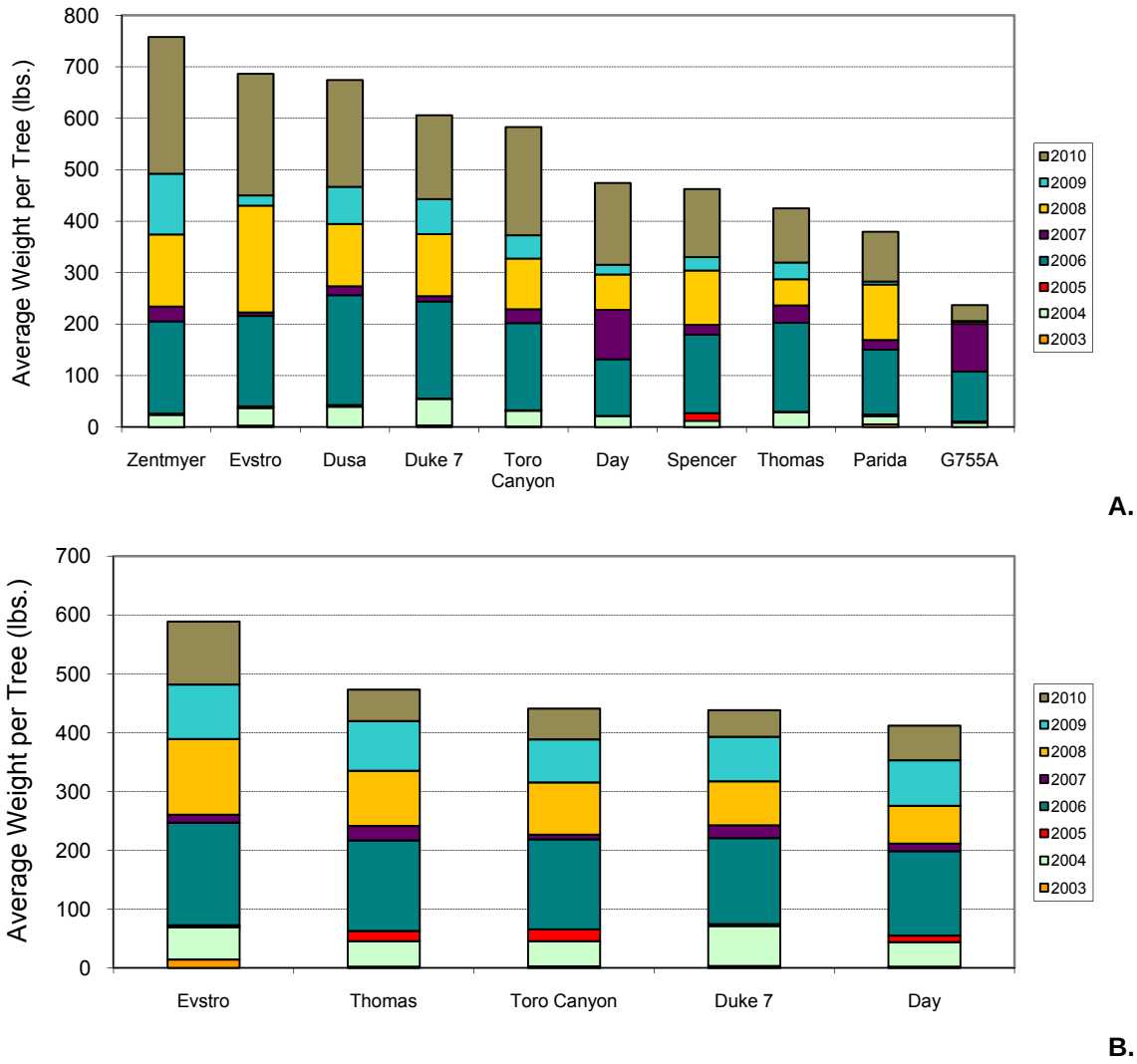
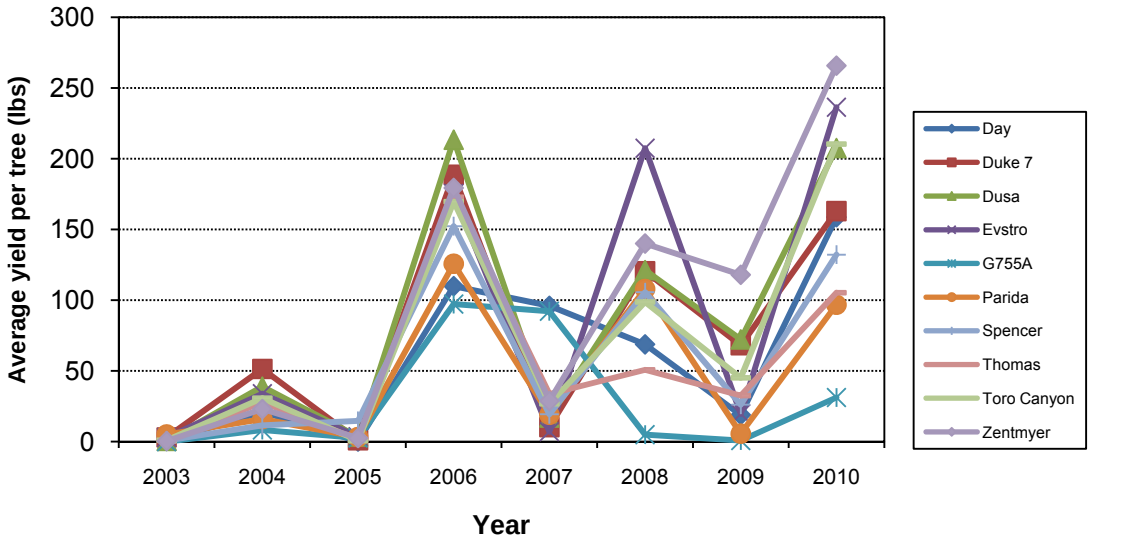


Figure 4. Cumulative yield per tree for trees in the scion:rootstock trial at SCREC in Irvine, CA. **A.** 'Hass' trees harvested in April-May each year. **B.** 'Lamb Hass' trees harvested in July each year.

Trees have been ordered for a new scion/rootstock trial with Brokaw Nursery in February 2010 with anticipated planting date of April 2011. This new trial will hopefully be located in Ventura County. The scions to be included in this trial are 'Hass', 'Carmen Mendez', 'Lamb Hass', 'Gem' and 'Reed'. The rootstocks to be used for the trial were compiled in consultation with Greg Douhan and Larry Rose and include Dusa, Duke 7, RO.O6 (a promising experimental rootstock from South Africa), Zentmyer, Uzi, Steddom, Brandon, Eddie and Anita.



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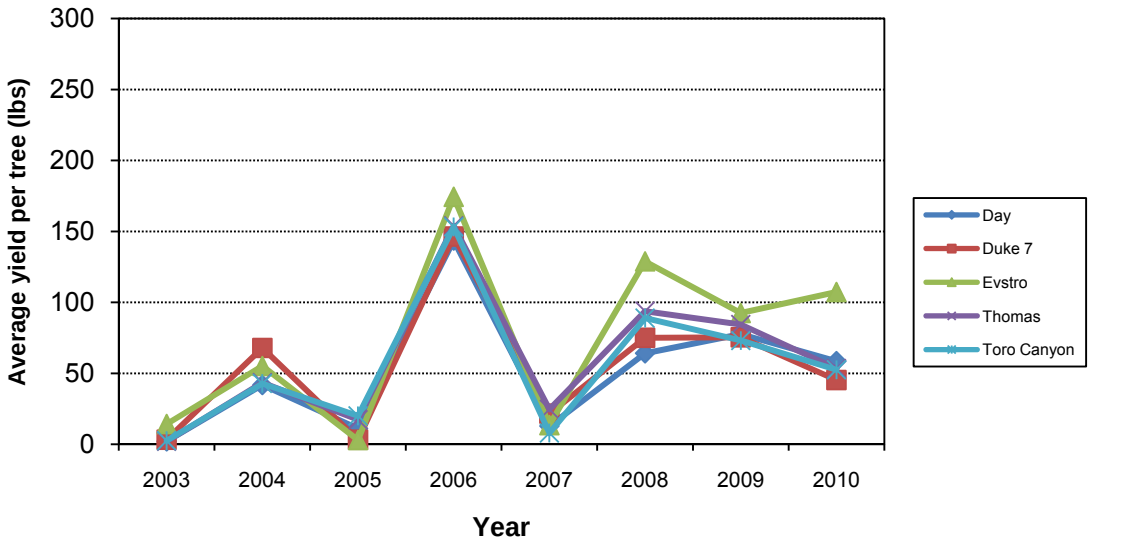

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Figure 5. Annual production per tree for trees in the scion:rootstock trial at SCREC in Irvine, CA. **A.** 'Hass' trees harvested in April-May each year. **B.** 'Lamb Hass' trees harvested in July each year.

Table 1. 'Hass' and 'Lamb Hass' 2010 tree height, width and canopy volume across all rootstocks from scion:rootstock trial at SCREC in Irvine, CA. Trees planted in April 1999. Harvest data collected in April/May for 'Hass' and July for 'Lamb Hass' each year. This trial is maintained with no chemical control for *Phytophthora cinnamomi*, disease pressure is minimal at this site.

Variety	Tree Height (ft)		Tree Width (ft)		Canopy Volume (ft^3)	
Hass	15.8	a	16.6	a	9542	a
Lamb Hass	11.9	b	10.1	b	3220	b
Significance	*0.0000* [z]		*0.0000*		*0.0000*	

[z] Denotes the level of significance between means following statistical analysis. Mean separation for cumulative yield calculated using LSD test at P = 0.05.

Table 2. 'Hass' and 'Lamb Hass' fruit size (all years), cumulative fruit count and weight (2003-2010), alternate bearing index and yield efficiency across all rootstocks from scion:rootstock trial at SCREC in Irvine, CA. Trees planted in April 1999. Harvest data collected in April/May for 'Hass' and July for 'Lamb Hass' each year. This trial is maintained with no chemical control for *Phytophthora cinnamomi*, disease pressure is minimal at this site. [z]

Variety	Average fruit size (oz.)	Cumulative fruit count	Cumulative weight per tree (lb.)	Alternate Bearing Index (0-1)	Yield Efficiency (lb/ft^3)
Hass	7.13 b	1292 a	560.7 a	0.82 a	0.013 b
Lamb Hass	8.80 a	855 b	471.2 b	0.80 a	0.026 a
Significance	*0.0000* [y]	*0.0000*	0.0021	0.0957	*0.0000*

[z] Alternate Bearing index value of 0 denotes no alternate bearing, a value of 1 denotes complete alternate bearing. Yield efficiency calculated for each year (2005-2010) by dividing yield by canopy volume.

[y] Denotes the level of significance between means following statistical analysis. Mean separation for cumulative yield calculated using LSD test at P = 0.05.

Carmen Mendez Evaluation. Within the current rootstock trial at SCREC we have a row of Carmen Mendez planted on the border. Carmen Mendez is a 'Hass'-like selection from Mexico most notable for its strong tendency for a "flor loca" off-bloom in the Fall. The tree displays a compact by spreading phenotype compared to 'Hass'. Wood produced by the Fall Flush is quite stiff and less pliable than 'Hass'. We initiated a bloom evaluation of Carmen Mendez beginning in September 2008 and continued regular evaluation of flowering status through April 2009. In September 2009, we continued this evaluation and will soon have 2 complete cycles of off-bloom data over 4 years. Due to the long term, continuous, and gradual nature of the off-bloom through this period, we developed a different system of rating and evaluation. The trees of the regular crop of 'Carmen Mendez' was harvested in July 2010.

Preliminary studies into the molecular basis of alternate bearing. Experimental studies indicate that the developing fruit produces a mobile signal that inhibits flowering at the apex of the growing shoots. As a first step in the identification of the anti-flowering signal, we performed a proteomics analysis comparing the sap from "on" and "off" trees during the reproductive transition. Preliminary studies showed that the "off" sap contained proteins that were homologous to auxin response and ubiquitination (proteolysis) proteins. In addition, a large subset of proteins in both the "on" and "off" samples did not match any protein in the Green Plant Database. These "proteins of unknown function" may represent novel proteins that repress reproductive development in biannual bearing crop trees. Funding from the Hofshi Foundation will be used to complete this study. In addition, we are initiating a sequencing and gene expression profiling experiment using high throughput DNA sequencing. The sequencing project will allow us to create an avocado expression database for leaves and apices for "on" and "off" trees. We will also, generate a gene expression map for fruit and seeds. Our short term goal is to develop molecular markers for alternate bearing which will be used to select for low biannual bearing trees in breeding programs. The long term goal is understand the molecular basis of alternate bearing.

Heritage varieties for nurseries: We make selections in our heritage collection available to nurseries. Since September 2009, however, there have been minimal requests for budwood which consisted mainly of Duke 7. Due to cost increases in labor and water, we have begun the process of moving any and all heritage and other varieties of interest out of Field 46 and into our germplasm collection in Field 44. In the future, our germplasm collection will be more geographically concentrated and thus more easily managed.

Collaboration with Domestic Researchers. We have provided sections of wood, or 'bolts' from our *Persea* relatives collection to Dr. Jorge Peña of the University of Florida in Homestead, FL as part of his host preference studies for the Ambrosia Beetle, the vector of Laurel Wilt, a lethal disease affecting members of the Lauraceae in the Southeastern US. In addition, we provided budwood of several heritages and released varieties to Pine Island Nursery in Florida as part of Dr. Randy Ploetz's (University of Florida, Homestead, FL) study on host susceptibility to Laurel Wilt.

Collaboration with Dr. Raymond Schnell (USDA-ARS, Miami. As outlined in our proposal in 2008-09 we initiated a collaborative effort with Dr. Schnell. Based on our plan, we collected a mixture of 'Bacon' and 'Hass' seeds from Limoneira Ranch in Ventura County in February 2009. The seeds were collected from the same field where Dr. Schnell collected seed in 2008 for a mapping population, which he has already planted in Florida. The seeds collected in 2008 were primarily found to be hybrids between the two varieties.

We contracted with Farm ACW in Fallbrook, CA to propagate these seeds for establishment of a mapping population at SCREC that would be used to generate a genetic linkage map for avocado that identifies QTLs controlling key horticultural traits (precocity, productivity, fruit quality, tree architecture, and cold/heat/salinity tolerance) via genetic marker assays and phenotypic evaluation of the 'Bacon' x 'Hass' cross. Beginning in April 2009, Mr. Rodrigo Iturrieta, a visiting researcher from Chile, isolated genomic DNA from the germinated seedlings. He completed this task in November 2009 and the DNA material was sent to Dr. Schnell in January 2010. We received the results of this testing in late September 2010. Of the 242 samples analyzed only 78 of these were Hass x Bacon hybrids. There were 89 selfed Bacons, 64 selfed Hass and 11 off-types. Seventy-eight individuals for a mapping population is considered to be too low of a number for gaining useful information so we have decided to not plant the trees as originally planned and will rather concentrate our efforts collaborating with Dr. Clegg's group and their collection of information from their mapping populations.

Overseas co-operation. We have answered several questions regarding exchange of material with potential overseas cooperators but currently nothing is in progress. In addition, in spring 2010 we continued our collaboration with the Plant and Food Research in New Zealand. This project is looking at carbohydrate and boron partitioning in the phloem sap and floral structures as influenced by alternate bearing. This collaboration is part of a research project funded by the New Zealand Avocado Growers' Association to shed light on the physiological factors involved in alternate bearing. Understanding the physiological pathways that influence to alternate bearing will be an important step in characterizing the molecular basis of alternate bearing.

Website, e-mail and other outreach activities. Mr. David Stottlemyer has continued to assist us in maintaining our website, www.ucavo.ucr.edu. He has updated the website with an improved search function in the variety database. He also modified some of the information to better reflect current knowledge of varieties. We are currently adding more information on the Carmen Mendez variety. Mr. Stottlemyer has set the system up for remote login so that he is able to update and modify the website from his home in Idaho. As pictures, video, and data become available, he is able to add them to the website.