

## **Assimilation Productivity from Canopy to Fruit as Determined by Avocado Tree Physiology**

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### **Benefit to the California Industry**

The results of this research will provide a valuable framework for cultural management decisions and future research on the physiology of the avocado.

A more complete understanding of the tree's response to environmental stresses will be a direct outcome of this research. The results of this research will validate the high temperature sensitivity of 'Hass' and will explain why this variety is not necessarily well adapted in warm inland valleys. The impact of high temperature on carbon assimilation may very well explain the smaller fruit size and lower overall yields experienced in dry and hot inland valleys as compared to coastal environments. This in turn will help define grower best management practices that can be utilized to mitigate stress.

The research results to date have resulted in a better understanding on how the 'Hass' avocado leaf responds to light. The tentative conclusions from this project confirm that low light levels within the tree canopy results in lowered carbon assimilation and that the avocado leaf is slow to respond to sudden bursts of light. These results can guide growers in developing canopy management strategies for their orchards.

### **Project objectives:**

- 1) Refinement of the assimilation-based process model to predict avocado field performance:  
Continue to collect data from controlled environments as well as field measurements.
- 2) Sap flow and the prediction of productivity:  
Continue work with sap flow and confirm whether sap flow can be used as a predictor of tree stress levels such as drought, flooding or salinity. Also this work with sap flow measurements will provided the research community with the tools to examine more critically the manner in which 'Hass' avocado responds to other environmental stresses.
- 3) Leaf production and abscission:  
Continue work examining flush development and leaf abscission.

Fundamentally our data collected this year has not yet been fully analyzed due to time constraints, funding reorganization, and the difficulty in some of the analysis. Merely looking at the general data of water use during a drought condition has not yet shown any real patterns before extreme drought sets in (in the approach to that stage the sap flow lowers over about two days ultimately ceasing<sup>1</sup>). As such we are examining other possibilities of analysis including morning and afternoon sap flow and maximum sap flow near noon each day. We have data from 4 drought experiments, two experiments involving the removal of leaf material, and two field experiments. This last year we increased the number of probes that can be monitored by two fold to 16 and while we now have more data, the analysis has been severely slowed.

Due to the shifts in budget during the year, our process of evaluation of how new flushing alters the leaf abscission of existing flushes has been slowed and remains fragmentary. However, we will continue with the process of evaluation even after this grant period ends and are looking forward to a further report.

### **WORK ON THE PLASTOCHRON INDEX**

The plastochron index is a normalized age function which allows different leaves of similar physiological functioning to be compared. We have spent a great time of time making the system work for avocado and, in this period, continued to push the limits of the process.

#### *VARIED SURROGATES FOR SIZE*

In the past we have shown that area is a good surrogate for the actual functioning weight of a leaf. We wished to have a non-destructive measurement of area and found that we could use length x width of leaf as an accurate measurement for area. Further we found that length<sup>2</sup> could be used but with less precision because width of the leaf was not perfectly correlated with the length. It was faster to measure only the length of many leaves rather than the two dimensions especially for a large number of leaves on a branch.

We did realize that the weight could change with area and the previous determinations of the factor associated with  $\{(\text{length} \times \text{width}) / \text{area}\}$  was done with only mature leaves. Thus, we ran several other experiments in which we gathered small, developing leaves along with larger, nearly fully-developed leaves. The results demonstrated what was observed previously, that length and width can be used as a surrogate for area. Again the fit is best for using both length and width. However, when we plotted fresh weight per unit area (as determined for fully turgid leaves<sup>2</sup>) verses area, we found a near absolute

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<sup>1</sup> Most of these experiments were done in the green house with small trees in pots. The loss of water was dramatic and it required only 3-4 days for the soil water potential to drop below that which lead into a permanent wilting point of the leaves. The shape of the diurnal curve of sap flow has not been greatly analyzed but it appears as though the afternoon sap flow is lowered more than the maximum sap flow.

<sup>2</sup> The leaves were harvested and placed immediately into pre-weighed bags (which allow the determination of fresh weight on the tree, FW). Water was added to the bag and the leaf was allowed to absorb water over night. The leaf was removed and weighed immediate, allowing the determination of full turgid fresh weight (TFW). The leaf was then dried overnight in a 121C oven and the weight was re-determined for the dry weight of the leaf (DW).

direction relation with area (as seen before) except for small leaves (those with an area below about 12-15 cm<sup>2</sup>, for maximum leaves of 75 cm<sup>2</sup>, see Figure 1). The variation in weight per area is small but higher than for mature leaves. For the larger leaves, the weight per area is virtually constant, as found previously. For smaller, developing leaves, there is an increase in weight/area for both TFW and DW for these smaller leaves (see Table 1 for a calculated fit of this increase in weight per area).

Leaves generally begin their expansion as leaves with a highly compact structure. This structure opens up as the leaf develops. This opening up is due to the formation of air spaces within the leaf. The increase in weight per area is about 140% (132% for TFW and 155% for DW); thus, the opening up of the air spaces is about 70% as the leaf grows to about 20% of its maximum size. This corresponds with what is observed in other species.

The reason for this work was to make it still easier to convert the measured sap flow (measured in flow for the entire branch) into transpiration rate (which is dependent upon leaf area). We found that measuring the leaf length for hundreds of leaves on a typical large branch was nearly impossible. It would be easier to strip the leaves off and measure total weight, which could then be converted back into total area (of course that eliminates productivity of that branch). These data above show that there is a small deviation if the branches are developing since they have some of these small leaves but that deviation seems to be small. We are currently working of the actual deviation for varied degrees of flush development.

The actual average values of these varied parameters for leaves that are well-watered are also shown in Table 1 and represent data observed previously for avocado and are similar to those for other species. About 25% of the fresh weight is actual dry weight (which represents the material that is actually doing the photosynthesis) and the leaves on the tree are not fully turgid (94% of fully turgid weight) due to their high transpiration rate during the day.

It must be realized that for photosynthesis capacity, the total area of leaves must be used since most of the measurements are made in terms of per unit area. While the capacity does vary somewhat with the structure of the leaf; for example, the margins of the leaves actually have higher capacity due to the lowered boundary layer induced by any wind flow, overall the calculations are best served by an average.

On the other hand, the actual use of photosynthesis is into the production of dry mass of the leaf (due to growth) and so mass actually mirrors real accumulation of leaf material best. The material of photosynthesis not used by the leaf is what goes into translocation to fruit as well as other growing points of the shoot and root. Thus, photosynthetic rate per unit area is still the best marker of potential fruit matter accumulation.

#### *FLUSH DEVELOPMENT UNDER MINIMUM SAMPLING OF LEAVES*

In addition we have been trying to determine the flush initiation and growth rate of a branch with single sampling. Typically we need to sample several times as the maximum size of each leaf on the branch is required to obtain a good value for initiation point and growth. Furthermore, each leaf does not reach the

same maximum size and so there is variation which is difficult to determine. The concept of this work was to be able to sample a tree in the field once only and determine the physiological age of each leaf on that branch.

We are having difficulty in doing this on a routine basis since the maximum size of each leaf has to be guessed at and that places a large variation upon the typical logistics plot (see past CAC reports for this project). For some branches it works quite well but the variation between branches makes the full procedure difficult. We do not yet know how to handle this important process but work is continuing.

#### *EFFECT OF CUTTING PARTS OF LEAVES*

We were interested in further probing the ability to use sap flow under varied conditions. We placed sap flows on several trees with varied root stocks (all with scions of Hass) and then removed varied portions of the leaves to determine how the root stock would respond to an altered amount of leaf area upon the branch being measured.

We measured continuously the sap flow for a branch on each tree and then using the total leaf area, determined at the end of the experiment by weighing all the leaves, to calculate the transpiration rate of each tree. Due to variations in illumination, that transpiration rate varied with each day of the experiment as expected. While we attempt to find branches that were facing in the same direction and illuminated nearly equally, the data demonstrates that is nearly impossible; there is variation for the same root stock. There is a second problem with the data which is difficult to solve. When we use trees in pots, the root mass varies and this causes the transpiration rate to be more or less than expected. Our goal is to use the actual measurements themselves during the day to determine a scaling factor which takes into account both leaf area and relative illumination. We have focused upon two time periods: (1) during the midday when the sap flow seems to be somewhat constant and (2) during the morning when there is a very limited restriction on water flow due to soil water potential).

We used the maximum sap flow (the average flow which occurs from 11AM to 2 PM each day) to normalize the comparisons (see Table 2). This has been arbitrarily set to tree #3 as that seemed to be the most stable. We are hoping to find a better method of handling the environmental variability. As a reminder, both the leaf area and the illumination pattern of the particular branch greatly affect the sap flow rate. Measurement of the leaf area can be done relatively easily if we use a small branch with few leaves; however, in the field, the branches are much larger with many leaves and are often partially shaded by other branches during the day. Measuring the area of these branches is difficult (see below) without a destructive harvest. Thus, we need to find an internally consistent method of measuring the non-restrictive sap flow.

This normalization of the transpiration rate (Table 2) showed an interesting effect (Figure 2). If about a third of the leaves were removed by cutting individual leaves from the branch, the transpiration rate actually increased (compare Figure 2A with Figure 2B for example). The sap flow (in terms of g/hr for the entire branch) did not increase even though it did not decline by

very much. But when the area of remaining foliage on the branch was taken into account, the transpiration rate (so calculated) showed an increase (Figure 2B). On the other hand, taking a series of small area punches from the non-vascular portion of the leaf did not change the transpiration rate (see Figure 2A). Less material was taken from the leaf as it is difficult to remove a large amount of leaf mass without cutting a vascular bundle; however as observed in Figure 2A, the transpiration rate is virtually the same.

Our explanation of this is due to the pre-existing water delivery system of the branch. The vascular system is formed to provide a certain amount of water to the leaves on the branch. That delivery system is not in excess under normal conditions, as in the late afternoon the leaves become limiting for water and the transpiration declines. Removal of some of the leaf material allows the water to be supplied for a longer period of time and at a greater rate. The light can support a greater photosynthesis rate and if the water is not limited, the stomata open more to provide more CO<sub>2</sub> and so the transpiration increases even if the sap flow (total water loss) remains nearly the same (loss of water through the more open stomata). Punching small amount out of the leaf does not change the water flow by much and the same vascular system has the same difficulty in providing water to the branch. Hence, the transpiration rate is not changed. This demonstrates that one should be careful as to how one takes leaf material for varied samples. Punches should be taken rather than a full leaf.

We are attempting to determine how the sap flow response during the day, in particular during the morning and afternoon. We have a difficulty in finding a measurement for the morning rise to the peak sap flow and the afternoon decline from the peak sap flow. These values are changing and so averaging will not work. We have tried to represent the rise as a linear increase and so calculate that as a real rise in terms of sap flow increase per hour (either as g/hr hr for sap flow or mmol/m<sup>2</sup> sec hr for transpiration increase). We have found that the rise to the peak in the morning is slightly faster for Duke 7 and perhaps Dusa while the peak is slightly higher for Duke 7 compared to Toro Canyon and Dusa (see Table 3). The decline in the afternoon (mid-day depression) is faster for Dusa compare with Duke 7 or Toro Canyon, yet the peak for Toro Canyon is lower and so the decline is relatively faster. Note that leaves that have material removed via punching have a higher peak with a faster rise to that peak for Dusa, yet the decline is likewise faster in the afternoon. Thus, Hass on Duke 7 gives the longest lasting and largest sap flow, indicating highest transpiration and thus productivity. Hass on Toro Canyon is nearly as good.

Furthermore, we suspected that the rise in the morning and the decline in the afternoon were dependent upon the peak sap flow. This dependence would mean that the morning rise could be used to standardize the sap flow (see above) and that the high peak sap flow would deplete the water from the leaves and cause an increase in a decline in the afternoon (the so-called "mid-day depression", see past CAC reports). The data from the trees that are control or have been punched area removed are shown in Figure 3. We see for all trees (independent of rootstock) there is an excellent correlation between AM-rise and PM-decline and peak sap flow (all measured as

transpiration rate). Thus, we seem to be moving towards an independent measurement of illuminated area by using AM-rise.

None of the trees that had large amounts of material removed by cutting of full leaves from the branch follows these conclusions. For all of the rootstocks, the peaks were much higher and there was not a clear relationship between the morning rise and the afternoon fall. Again this suggests that the water potential balance of the branches have been greatly altered. These results follows the earlier conclusion based upon just the peak transpiration rate.

### **FIELD SAP FLOW DATA**

We repeated the experiment in the field similar to that which we ran last year except that we had a weather station and some soil water potential probes operational (see Figure 4, as to placement of the weather station and the power station). There were two trials, each that ran nearly a month. The first one from mid-April to mid-May seemed to have many spikes of electrical interference which were of unknown origin. These spikes made data interpretation difficult and were thought to be due to wind. When the wind speed was correlated with number of spikes in the full series of probes, this concept was shown to be correct except that the number of spikes rose when the wind speed was smallest and the traces were best when the wind speed was highest. We do not know why this should be. We are still in the process of attempting to analyze these data using techniques given in this text.

The second run (from mid-May to mid-June) yielded excellent data with much fewer spikes and is the basis for the data discussed here. We used two sets of probes (8 each) on a block of Hass (4 rooted and 4 on Duke 7) and on a block of Lamb Hass (3 rooted and 4 on Duke 7<sup>3</sup>), see Figure 4. The mid-day sap flow was very similar for all the trees (see Figure 5), except Hass on Duke 7 gave the highest average rate (200-400 g/hr) compared with Hass on its own roots (150-200 g/hr) or Lamb Hass on Duke 7 (50-80 g/hr) or its own roots (80-200 g/hr)<sup>4</sup>. The variation over the time period is large due to the average illumination and air temperature (data still being evaluated). Under these conditions the standard deviation is large due to the variation between trees and rootstocks.

The variation between trees and day can be more easily observed if we plot the morning rise to the peak for each variety on the two root stocks as shown in Figure 6. When we examine the average mid-morning sap flow, we find very similar flows for all trees except again for Hass on Duke 7. This is what we would expect due to selecting the branches that have similar number of leaves and are facing in the same general direction for illumination. While we believe this morning rate is closer to the unimpeded maximum rate, we do not yet know how to express it. As previously discussed, the morning rate is an increasing sap flow due to the light intensity increase which is opening the

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<sup>3</sup> The Hass block was not the same as was used last year and is separated by 4 blocks from this one. We lost one of the Lamb Hass rooted because we used the wrong tree—the pollinizer.

<sup>4</sup> These data are taken from Figure 5 towards the end of the sampling period. This was during the period of highest illumination for the plot and gives a more realistic peak sap flow.

stomata and increasing photosynthetic rates, however we cannot use directly the solar intensity as each branch is illuminated irregularly due to shading effects<sup>5</sup>. The increase in rate makes it difficult to take an average and thus we are experimenting with calculating the rate of increase of sap flow per hour (in units of g / hr<sup>2</sup>). As seen in Figure 6, this seems to give the best results, but we have to do a complex calculation as the sap flow is increasing.

The water potential gauges indicated that most of the trees were well watered but since the slope of the land is considerable, the water potential around the trunk is not especially uniform (lowest on the up-hill side). We do not have enough data to use this to model how the transpiration is limited by soil water potential as yet but the sap flow throughout our drought treatments may aid in this evaluation.

### **CHANGES IN FRUIT SUGARS**

We had begun the experiments on metabolite transformation within the fruit but due to budgetary problems, we did not complete the work. However, we have taken samples for a full year of fruit growth (Figure 7) and did start some of the analysis for the 7-carbon sugars within the fruit. These early data sets show that as expected the fruit grows and the amount of dry weight per total weight remains nearly the same until the season for harvesting begins. Our original proposal was to track the accumulation and transformation of sugar into lipid. Our results show that as the fruit passes from immature into mature fruit (based upon the %dry-weight of the fruit) the 7-carbon sugars disappear, see Figure 8. During this time period the typical 6-carbon sugars (fructose and glucose) and sucrose remain much lower than the 7-carbon sugars but do not vary with maturity (data not shown). Our plan is to fill in the data between about 19% and 24% with the existing samples and keep the lipid samples until some funds can be found to analyze them.

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<sup>5</sup> We have tried very hard to pick only south facing branches, but the size and age of the trees (about 2 m high; 1 ½ years old) does not allow much choice.

## Tables

**TABLE 1. LEAF WEIGHT AS A FUNCTION OF AREA.**

These represent the data to be matched to Figure 1 and 2.

A. Average Leaf Parameters for leaf size 2 cm<sup>2</sup> and up (taken from Figure 1). The RWC is relative water content of the leaf and is the amount of wet weight of the leaf on the tree (obtained immediately after cutting) divided by the wet weight of the leaf fully hydrated (overnight in a sealed plastic bag with added water). The dry weight is obtained after overnight in a 121C oven (and remains constant even after longer time in the oven).

	RWC	FW/DW	mg /cm <sup>2</sup>	FW mg DW /cm <sup>2</sup>
average	93.7%	3.99	31.53	7.37
std dev	13.6%	0.62	6.24	1.32
std error	1.2%	0.06	0.57	0.12
		variation	1.8%	1.7%

B. Best fit of the data of Figure 2 at small leaf areas. The data was fit by eye with the curves as listed below.

$$\text{FW (mg / cm}^2\text{)} = 29 + 22 \times \exp \left[ - \text{area} / (4.8 \text{ cm}^2) \right]$$

with a maximum weight / area of 50.7 mg/cm<sup>2</sup>

$$\text{DW (mg / cm}^2\text{)} = 7 + 4.5 \times \exp \left[ - \text{area} / (4.8 \text{ cm}^2) \right]$$

with a maximum weight / area of 11.8 mg/cm<sup>2</sup>



**TABLE 2. SCALE FACTOR TO MAKE A CONSTANT STARTING TRANSPIRATION RATE.**

It was found that these trees that had no change in leaf area (control) or had a number of small (0.3cm<sup>2</sup>) punches removed from the mesophyll portion of the leaf behaved similarly (as compared with branches with full leaves physically removed). This table tabulates the specific root stock onto which was grafted Hass shoots, each trees treatment, beginning area of the leaf surface, and that removed during the course of the experiment.

In addition, the average maximum sap flow (taken from 11: 00AM to 2:00 PM) was adjusted by the total leaf area of the specific branch. At the beginning of the experiment (June 15) the transpiration rates of all the trees were set to that of tree #3 (100% = 0.120 mmoles/m<sup>2</sup> sec). Each tree had a scale factor is given here that was associated with it. This scale factor is used to “normalize” the specific illumination of each branch and can be tested again the morning and afternoon rates.

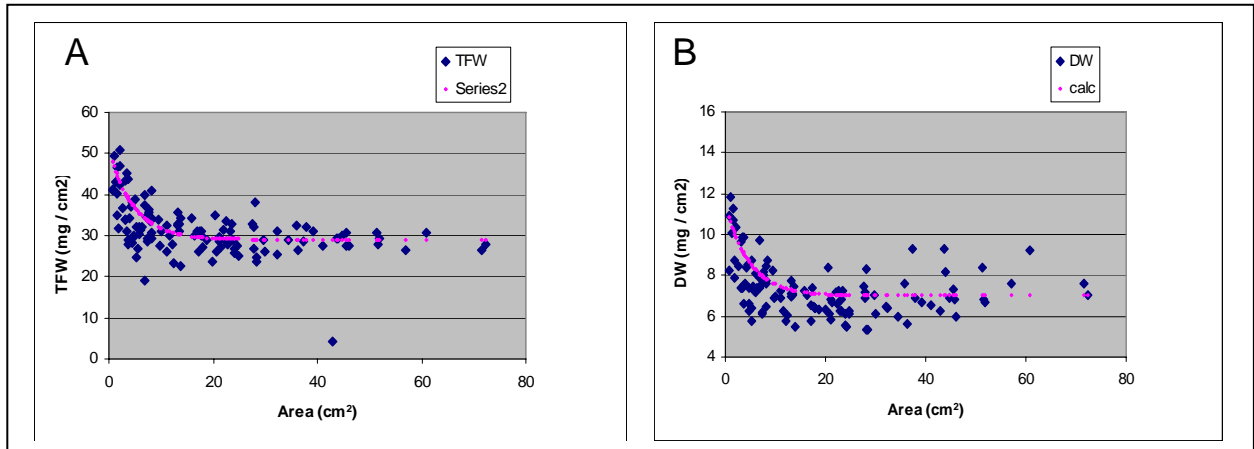
Tree	Root stock	Treatment	Beginning Area (m <sup>2</sup> )	Removed Area	Scale Factor
1	Duke 7	punch	10.31	0.47	55.0%
2	Toro Canyon	control	11.08	0.00	93.0%
3	<b>Toro Canyon</b>	punch	<b>12.85</b>	<b>0.68</b>	<b>100.0%</b>
4	<b>Dusa</b>	cut	3.60	3.60	125.0%
5	Duke 7	control	3.45	3.45	91.5%
6	Dusa	control	7.79	0.00	101.5%
7	Dusa	control	13.5	0.00	94.0%
8	Dusa	punch	12.86	0.90	174.0%
9	Toro Canyon	cut	9.98	3.62	90.0%
10	Dusa	Cut	10.52	10.52	74.5%
	Duke 7	Cut	4.38	4.38	
11					59.0%
12	Dusa	punch	8.16	0.45	70.5%

**TABLE 3. AVERAGE SAP FLOW OF VARIED ROOT STOCKS DURING DIFFERENT PERIODS OF THE DAY.**

These data are from the trees described in Table 2. Hass trees were maintained in a green house and were well watered. These trees have nearly intact leaves. The table describes the variation of the average peak sap flow (in terms of  $\text{mmol/m}^2 \text{ sec}$ , from 10:30 AM to 1:30 PM), the morning rise (in terms of  $\text{mmol/m}^2 \text{ sec}$  transpiration per hour) from 6:30 to 8:30 AM, and the afternoon decline (in similar terms to the morning rise) from 4:00 to 6:00 PM.

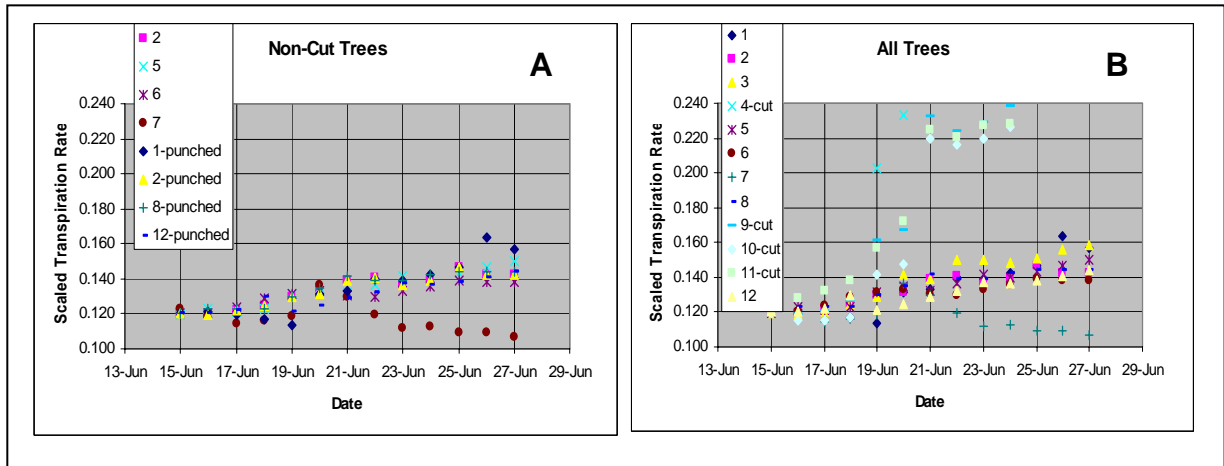
Root stock	Peak Range	AM Rise	PM Decline
Duke 7	0.13 – 0.30	0.48	-0.22
Toro Canyon	0.12 – 0.16	0.38	-0.23
Dusa	0.12 – 0.15	0.43	-0.28
Dusa Punched	- 0.17 – 0.20	0.79	-0.36

## FIGURES



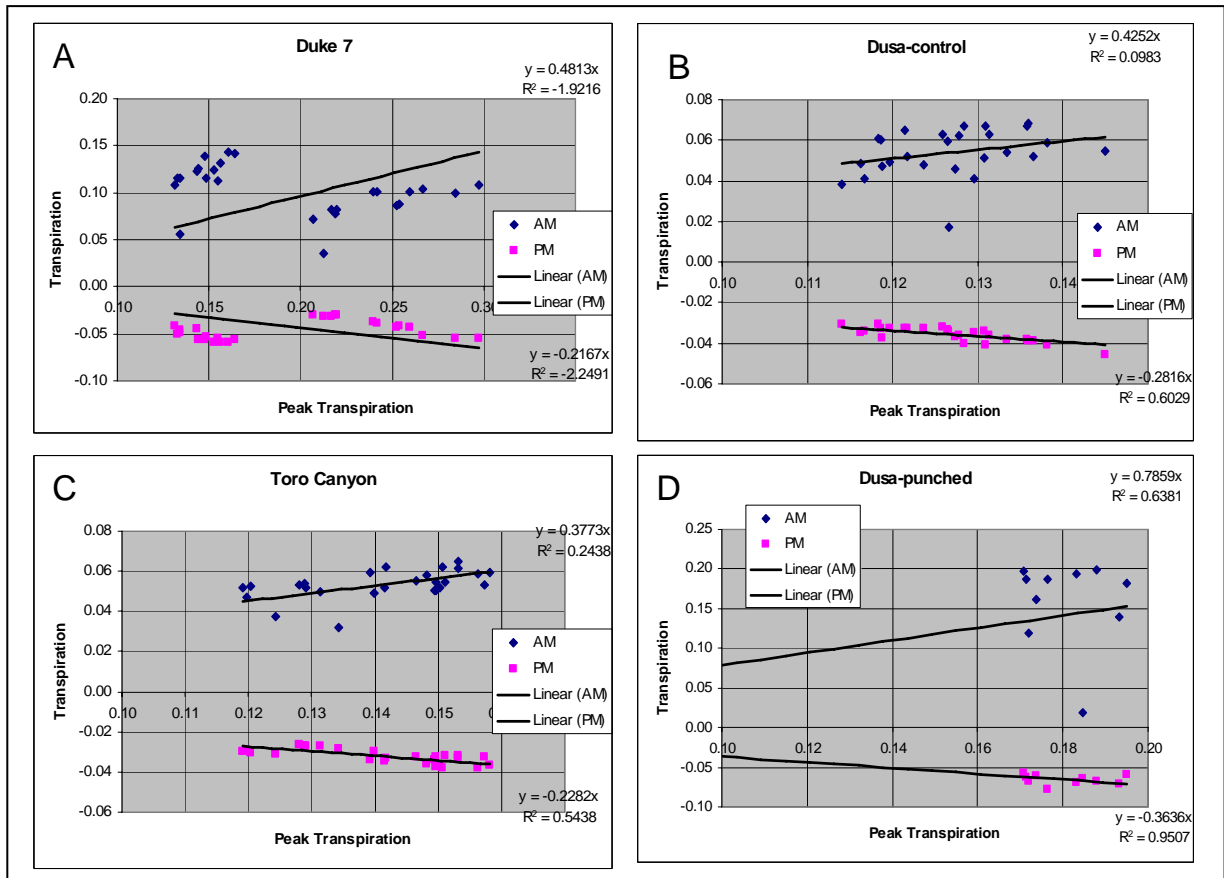
**FIGURE 1. VARIATION IN LEAF WEIGHT / AREA WITH SIZE OF LEAF.**

These results were obtained from several different flushes from Hass trees grown in the Green House during the spring. The turgid fresh weight (TFW) was obtained by allowing freshly cut leaves to equilibrate with water in a plastic bag overnight. The dry weight (DW) was obtained by drying the leaves in a paper bag for 24 hr at 121C. The area was measured from photographs of the leaf as described in last year's CAC final report (2006) and used a software program (JImage). The red curves have been fitted by eye and their parameters are tabulated in Table 1.



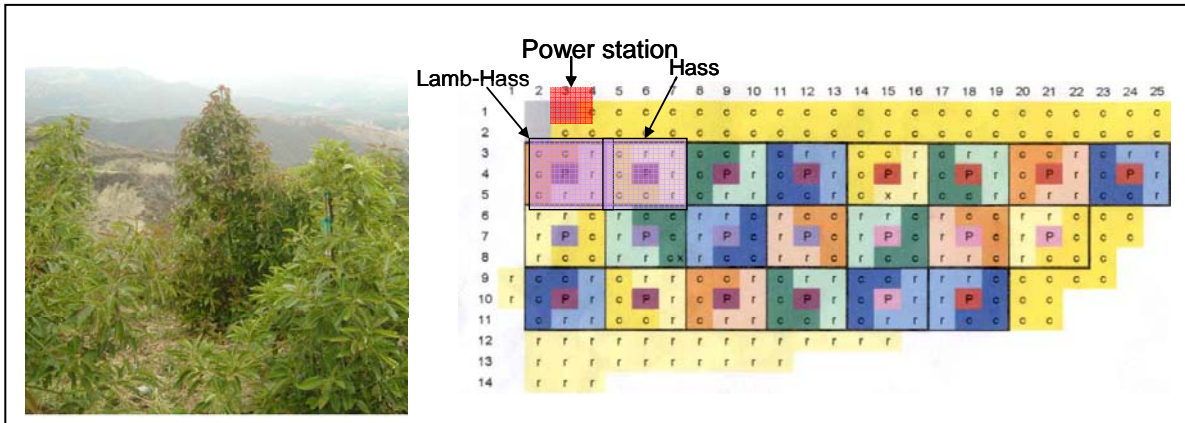
**FIGURE 2. TRANSPIRATION RATE OF HASS TREES GROWN IN A GREEN HOUSE OVER TWO WEEKS**

The sap flow monitored by normal methods (see last few CAC final reports) for each tree was converted to transpiration rate by using the actual leaf area on the branch. To see the variation of each tree, all were normalized to 0.120 mmol/m<sup>2</sup> sec (see Table 2). The “non-cut” trees (A) were either controls (no cutting) or lightly cut using punches (see Text). All trees (B) were presented to demonstrate the change induced by relatively severe cutting (one third of the areas of the trees were removed by cutting off some of the leaves) during 6/18 and 6/20. After the cutting, the sap flow did not change by much and since the area was then reduced, the net transpiration rate rose significantly.



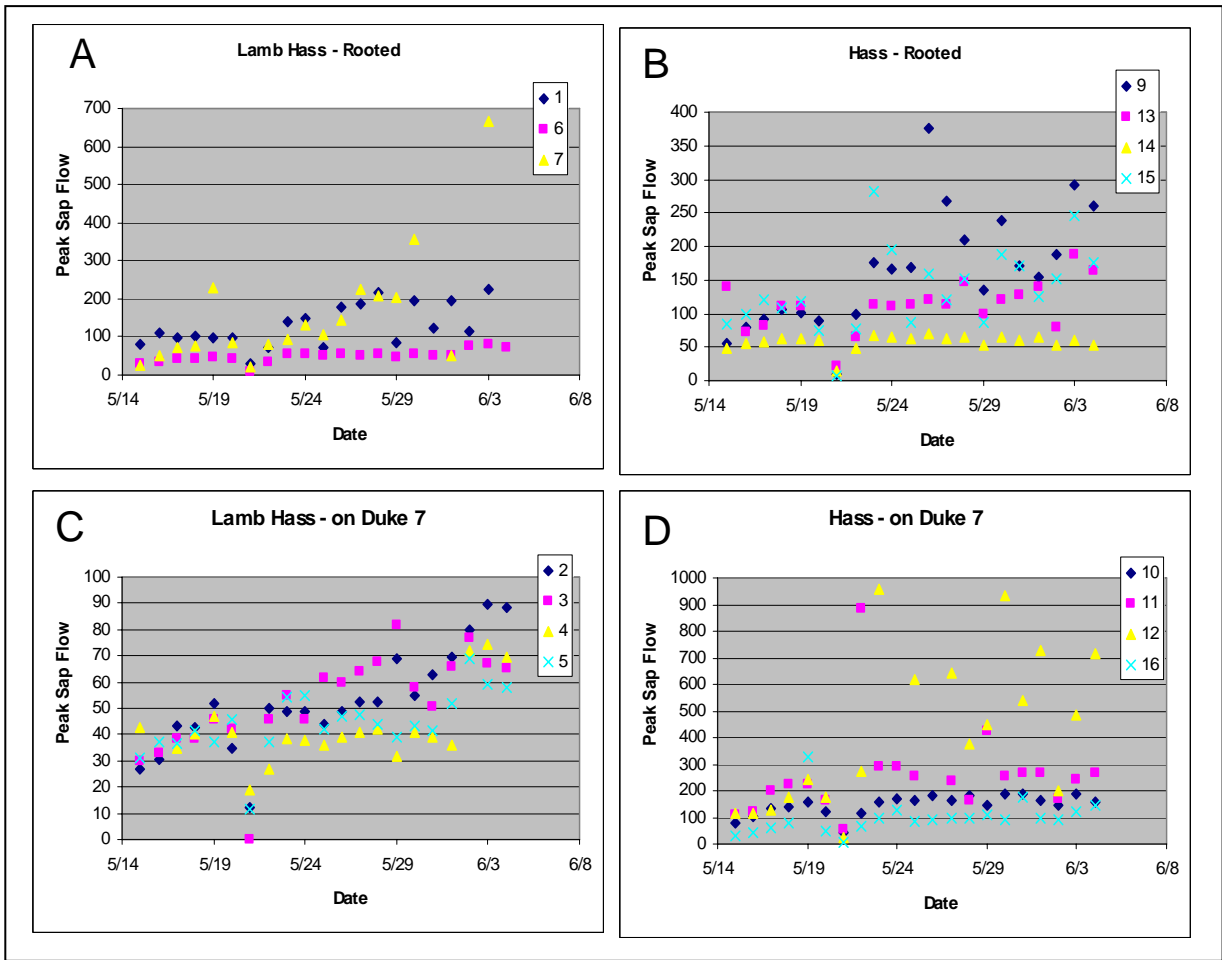
**FIGURE 3. CORRELATION OF PEAK TRANSPIRATION RATE WITH MORNING RISE OF TRANSPIRATION AND AFTERNOON DECLINE IN TRANSPIRATION.**

The data were taken from the Hass trees grafted upon different rootstocks and grown in a green house. The calculations were performed as described above and used the actual leaf area of each monitored branch. See Table 3 for more details.



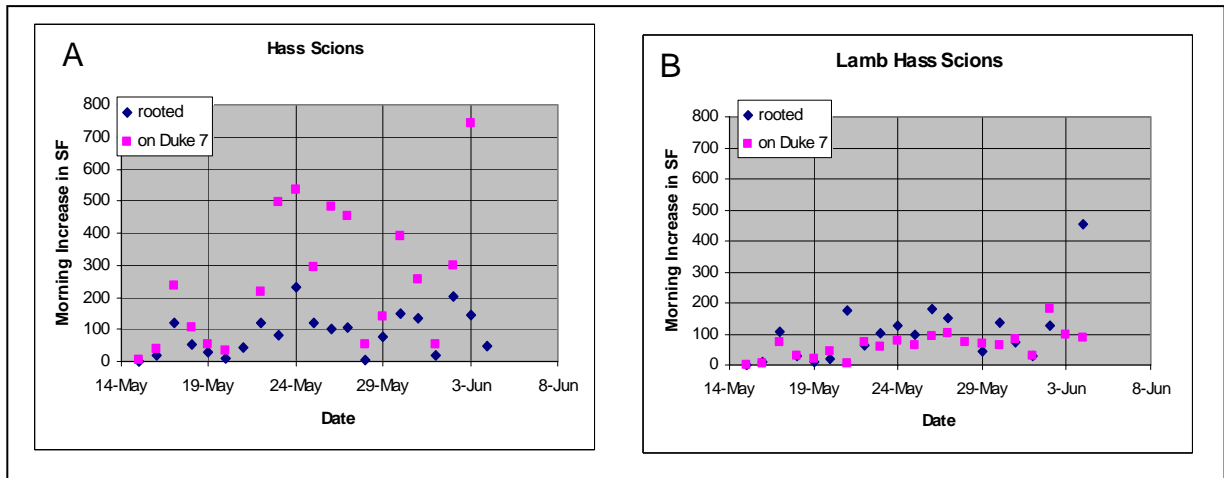
**FIGURE 4. THE FIELD PLOT USED FOR THE MONITORING OF THE ENVIRONMENT AND SAP FLOW OF SELECTED TREES.**

A photo of the trees that were planted about 1 ¼ years ago at ACW. This plot has been previously described in 2005 and 2006 CAC final reports. Each tree is individually water three times per week and is on a NE facing slope. The trees in the experiments described below are shown in the plot and consist of a block of Lamb-Hass and Hass trees, either on their own roots (rooted) or grafted to Duke 7 rootstock. The power station consists of 4 solar panels that charge two batteries used to power the weather station and the sap flow monitors.



**FIGURE 5. THE PEAK SAP FLOW OF TREES AT THE ACW FIELD STATION.**

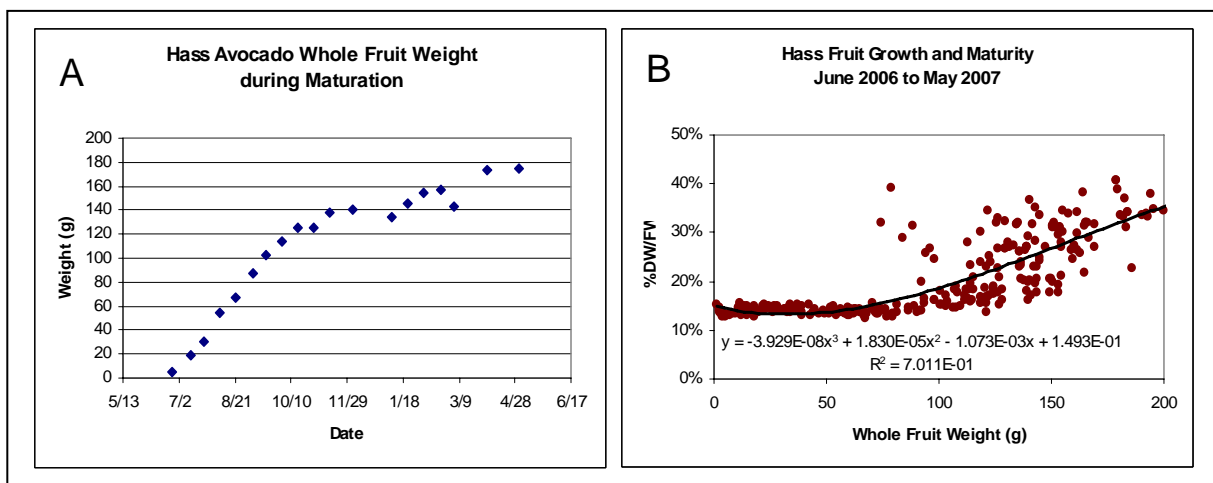
The sap flow was averaged over a two hour period (see Table 3) in the field station described in Figure 3). The illumination and air temperature varied during the course of the experiment and that caused a variation in the peak sap flow in each tree. Examining the maximum averaged peak flow (from 5/24 to 6/3), it is clear that Hass on Duke 7 consistently gives the highest flow rate.



**FIGURE 6. THE SAP FLOW INCREASE IN THE MORNING FOR THE TREES AT THE FIELD STATION.**

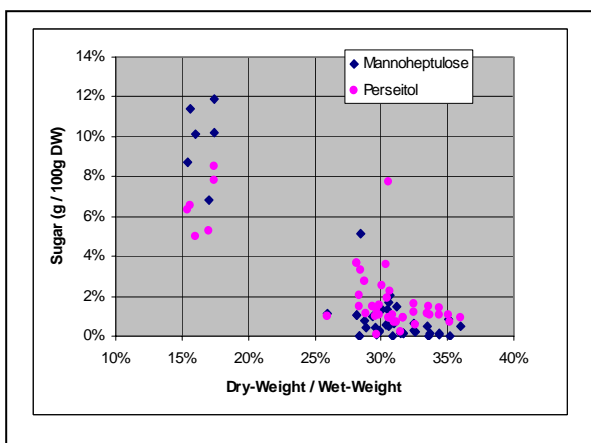
Trees in the field (see Figure 3) were measured for Sap Flow during the morning (see Table 3). Rather than averaging a changing rise in flow, we fit the data to a straight line over 2 hours, obtaining an increase in sap flow (in terms of  $[g/hr] / hr$ ). We hope to use these types of calculations to normalize the sap flow to the amount of foliage (area) illuminated, which will be used to standardize the peak sap flow and the flow in the afternoon. It is believed that during this time, there is minimum interference with soil or leaf water potential, as they have reached a high point during the night.





**FIGURE 7. SAMPLES OF HASS FRUIT TAKEN DURING THE FULL SEASON OF GROWTH (2006-07).**

A set of samples from five distinct Hass avocado fruit was taken from trees maintained in UC Southern Field Station each two week period. A. Weight of fruit during the season. This is an average of fresh weight of five fruit. B. Percentage of dry weight as a function of fruit weight during the growing season. Dry weight to wet weight was measured as described for fruit maturity and was fit to a complex equation. The fruit %DW begins to change at a fruit weight of about 75 g (corresponding to a date of about mid September).



**FIGURE 8. AMOUNTS OF 7-CARBON SUGARS PRESENT IN FRUIT.**

The % Dry Weight/Wet Weight was determined as in Figure 7. The sugar content was found to be best represented by per dry weight, although a similar plot is obtained using wet weight. The sugars are determined after extraction from the fruit samples by HPLC (for method, see the 2006 CAC Proposal).