Avocado Tree Physiology – Understanding the Basis of Productivity

New Project: Year 1 of 5

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Benefits to the Industry

A grower's margin of profit is the difference between the input costs to produce a marketable crop and the output, or the production itself. Any influence that affects one or both of these two items can make the difference between profit and loss. The management of the avocado tree under southern California conditions, which can experience rapid changes in temperature and relative humidity, provides a challenge under the best of conditions. 'Hass' productivity in California tends to be less than in other countries such as Mexico, Chile, New Zealand and South Africa where environmental conditions are less stressful. Additionally, increasing market competition from other countries is pressuring the California grower to become increasingly ingenious in orchard management practices so that profits can be made. . These practices include changes in how irrigation of orchards and management of tree size. Increasing numbers of growers are pruning older trees or considering high-density plantings. Canopy management strategies hinge on effective light management to increase fruit size and production. Unfortunately, the science behind the current strategies used to manage tree canopies and tree water status are poorly understood. We do not understand how the 'Hass' avocado responds to either light or water stress. This project will examine in detail the response of the avocado leaf to light, temperature, and changes in light and temperature according to carbon assimilation (which fuels both tree and fruit growth) and changes in evaporative demand (which governs the amount of water the tree requires). The outcome of this project will be a better understanding of the tree's response to environmental stress. This in turn will allow us to develop a canopy model of total carbon assimilation that will predict the effects of changes in relative humidity and temperature upon the assimilation. This research will provide the framework for predicting tree and canopy management strategies to optimize productivity.

Project Objectives:

1. An understanding of the effects of environmental variables (light, temperature, and relative humidity) on avocado leaf gas-exchange and carbon assimilation, essential for plant growth and fruit production.
2. An understanding of the developmental physiology of avocado leaves and how this relates to canopy management. In particular how many layers of leaves within the canopy will support a positive carbon balance to the plant and how the duration of light flecks through the canopy can induce a positive carbon balance.
3. Development of a model of carbon assimilation and allocation in avocado that will allow growers to make informed decisions on horticultural practices and will aid researchers in developing future research endeavors.

Summary

Assimilation as Affected by Relative Humidity

This year our work centered upon air relative humidity and how changes in it could alter efficient photosynthesis. When the humidity is very low, much more water evaporates out of the leaf through the stomata (transpiration). Under the best of conditions water from the soil flows through the xylem and wall spaces of cells to replace this lost water by transpiration. Unfortunately, often soil water potential[1] is low and that soil's water cannot replace the transpirational loss. Furthermore, resistance to flow within the tree through the xylem system causes a delay in the water movement, and thus the water lost through transpiration cannot be replaced rapidly enough to maintain the water potential of the leaf. The leaf's water potential governs the stomata gas conductance[2] and many metabolic processes so it is critical for the leaf to keep the potential as high as possible. It is our hypothesis that the avocado leaf can support some transpiration loss but as the relativity humidity falls, the tree (and the soil) cannot supply enough water and the water potential of the leaf falls. The immediate consequence is that the stomata close to prevent the high transpirational loss. Stomata closure will limit CO_2 entry, which is the critical metabolite of photosynthesis. These relationships are shown in the next two figures.

Figure 1 shows the water potential of a leaf, when it is held under a constant transpiration stream held for either 1 or 3 hours in cotton. After one hour the minimum leaf water potential is about -520,000 Pascals (about – 5 bars). Even for relatively high transpiration (1.5 mmol/m^2 sec) under these conditions, the water delivery system can maintain the water potential at a reasonable level. However after 3 hours of the high transpiration, the water delivery system (from soil through the xylem system) begins to fail and the leaf water potential begins to decline (reaching nearly –8 bars). Long-term transpiration shows the limitations of the system and the level of the water potential reaches a critical state.

The actual dependence of the leaf stomatal conductance upon the leaf water potential is shown in Figure 2 for Oleander. There seems to be a threshold from 0 to about –6 bars where the conductance remains high. After the leaf falls below this threshold, the stomata begin to close reaching only 10% of full opening at –18 bars (generally, marked as a wilted leaf). This is true for many other plants but the actual levels of threshold and amount of conductance will vary with species. We don't yet have good numbers for these types of measurements for avocado.

Of course, the stomatal conductance[3] (or aperture) directly affects the photosynthetic productivity since the movement of carbon dioxide into the leaf is directly proportional to the stomatal conductivity as:

Assimilation = conductance *x* (difference in CO_2 from outside to the inside)

The amount of light, which is absorbed and converted into energy to drive photosynthesis, alters the CO_2 level inside the leaf and so affects assimilation, but, with all else being equal, the conductance directly affects the assimilation rate.

Leaf Chamber System

Our leaf chamber system functions by measuring the photosynthetic responses of discs cut from fully expanded leaves. Water surrounds the cut edge of the leaf to provide high water potential such that the leaf should suffer only minimum water potential deficits. These measurements should indicate the maximum efficiency of photosynthesis when water potential is near maximum.

Using the continuous monitoring capability of the leaf chamber, we have been studying the time course for partial closure of the stomata. We have changed the maintenance of our trees from which we collect the leaves for the leaf chamber experiments. These data were taken from leaves from small trees kept within green houses (with

[1] The concept of water potential is a powerful one in plant physiology since it defines the driving force behind water movement. Water potential is a measure of energy with pure liquid water defined as 0 bars or Pascals. Nearly anything done to water (evaporation or the addition of salts) will lower the water potential so that typically soils are –3 bars or for very dry soil, -25 bars. This means that water will tend to flow into that soil and the plant must expend considerable energy to extract water from that soil.

[2] Stomatal conductance is a measure of the flow of gases through the stomata and is generally in units of moles of the gas flowing through a square meter of leaf surface per second. Conductance is low when the stomata are partially closed and when they are fully open, conductance is maximum.

[3] Often stomatal conductance is abbreviated as g_s.

controlled temperature and no injury nor pests). These leaves had stomata conductance and assimilation rates as indicated in Table 1. Before the light illuminates the leaves to drive photosynthesis, respiration can be measured (expressed as a negative number in Table 1). Both the respiration and assimilation are reasonable compared with other tree crops. The stomata are not completely closed even after long periods of darkness, which allows the determination of respiration by measuring the amount of CO_2 released into the gas stream surrounding the leaf disc. We expected to be able to see a difference between younger and older leaves, but the data scatter and the apparent small differences made it impossible to statistically demonstrate any dissimilarity (see Table 1 Section B).

Within the leaf chamber a low relativity humidity rarely affects the stomatal conductance. Once conductance reaches a steady state level, it remains at that level for hours (see Table 2). The conductance rarely declines, even when the relative humidity of the air stream is lowered from the normal of 37% to a very dry 14%. On the other hand the temperature of the leaf rarely rises above 25 C, which is unlike a warm summer day where the air temperature can easily reach 35 C (95 F). So though the relative humidity is very low, the water vapor gradient from the leaf to the air is small because the temperature is low[4].

These are not unexpected results, as water should rarely become a problem in this system. The cells within the leaf disc are not very far from a source of water at the cut edge, and so the leaf water potential should not fall to a low value.

Light Intensity

Light intensity is critical for high levels of photosynthesis to provide metabolites (initially carbohydrates) to the plant and developing fruit. We suspected that in avocado the light dependence of photosynthesis was lower, but needed to demonstrate it.

The methodology to probe photosynthetic light dependence was made available using the leaf chamber. After the leaf within the chamber reached a steady state level of gas exchange after about 50 –60 minutes[5], the light intensity was decreased with neutral density filters[6]. Each level of intensity was held for about 5 minutes, which is long enough such that the assimilation rate can be accurately measured yet short enough to prevent the stomata from closing to limit CO_2 exchange. A typical curve is shown in Figure 3A. This cycle can be repeated if the light intensity is returned to 100% for about 45 minutes in order for the steady state of assimilation to be re-established. Typical curves for young and old leaves are shown in figure 3B. Interestingly the maximum assimilation (A_{max}) is higher for the younger leaf, while the half-saturation (K_I) is lower.

These data are taken under conditions where the stomata do not limit CO_2 exchange. These are the maximum extrapolated values for photosynthetic assimilation and may not be reached when the stomatal limitation is factored in. In fact, under our conditions after the light illuminates the leaf disc, the assimilation rate tracks the opening of the stomata, and under most conditions the stomata remain the limiting step for assimilation. This may explain why we did not see any statistical difference in stomatal conductance between younger and older leaves when the leaf disc was under high light (see the last section). The stomata conductance was altering the possible higher photosynthetic rate and thus both types of leaves responded similarly.

Relative Humidity

We have had considerable success in our measurements of stomatal conductance of leaves which remain on trees kept in the growth chambers (where the relative humidity can be precisely controlled). We measure the conductance on a series of leaves in the morning and in the late afternoon. After examination of the data, we found that relationships are more easily seen if we examine the change or difference in the conductance from the afternoon to the morning, as $\{g_s\text{-PM}\} - \{g_s\text{-AM}\}$ as Δg. This difference is correlated to the conductance in the morning. If the morning conductance is high, the subsequent water loss generally leads to a closure of the stomata and so the

[4] The amount of water vapor that the air can contain rises exponentially with air temperature and so a low relative humidity at high temperature places a much greater force to remove water from the leaf than the same relative humidity at a lower temperature.

[5] Under these conditions the assimilation rate is often limited by stomatal conductance and, more importantly, any decline in light intensity will be ultimately balanced by a partial closure of the stomata to re-establish that balance.

[6] Neutral density filters are constructed such that the light quality or dependence upon wavelength or color of light is not altered as the intensity is uniformly decreased. The intensity of the light was lowered but the general color of the light was not altered.

afternoon conductance is quite a bit lower. However, if the morning conductance is low, the lower water loss leads to maintenance or even an increase of the conductance in the afternoon. Typical data are plotted in Figure 4. The relationship described above seems to hold very well for the varied experiments.

We have developed a model for this and are currently validating it (see Figure 5). More importantly, one would predict that a higher water loss (that is, for a given conductance, a lower relative humidity of the ambient air) would lead to a lower conductance in the afternoon, which would increase the slope of the lines in Figure 4. The parameters presented above are the least squares regression of the lines[7] in Figure 4. Generally these are for 12-18 leaves in each trial. In most case, the constant of the regression declines slightly, while the slope increases up to nearly two-fold. This means that for a drier atmosphere in the afternoon, the conductance (compared with the morning value) falls further than for a wetter atmosphere, leading to a larger impairment of the assimilation capacity of the leaf. Since it appears that assimilation is limited by the stomatal conductance under most cases, a drier atmosphere in the afternoon would have striking effect on productivity.

Other Areas

We are beginning the work that will allow us to measure total leaf conductance of a branch. We have purchased and are using on trees in green houses a sap flow monitoring system. It is a highly sophisticated system, which requires a great deal of understanding to make it work properly. Yet the data thus far gathered suggests that this measurement of sap flow will allow us to test our concepts in the field with changes in relative humidity induced by spraying water into the air around trees. The data will be incorporated into a model that will allow us to predict what we expect the stomata to be doing from simple micrometerological data, such as air temperature, relative humidity, light intensity and wind speed. Once we can predict stomatal conductance, we should be able to predict photosynthetic assimilation and productivity.

References

Bunce, J.A. (1978) *Effects of Shoot Environment on Apparent Root Resistance to Water Flow in Whole Soybean and Cottom Plants.* Journal of Experimental Botany, **29**: 595-601.

Brinckmann, E., et al., *Effects of Atmospheric and Soil Drought on Leaf Water Status and Stomatal Response.* pp 135-140. Cram,WJ, K Janácek, R Rybová, K Sigler[502.], 1984.

[7] Regression is for: $[\{g_s\text{-PM}\} - g_s\text{-AM}] = \text{constant} + \text{slope } x \{g_s\text{-AM}\}$, as is plotted in Figure 3.

Table 1 Part A. The Response of Gas Exchange to Illumination. The data were collected in the Leaf Chamber, before and after the leaf disc was illuminated by 1550 μmole light/m^2 sec (about ¾ of sunlight). The assimilation before illumination was actually a measure of respiration. The data are for a total of 5 older leaves and 6 younger leaves.

	No Light (Respiration)		Light (Steady Photosynthesis)	
	Conductance (mmol/m^2 sec)	Assimilation (μmol/m^2 sec)	Conductance (mmol/m^2 sec)	Assimilation (μmol/m^2 sec)
Average	7.13	-0.13	15.43	1.43
Std. Dev.	8.88	0.23	4.52	0.58

Part B. The Response of Gas Exchange to Illumination for Older and Younger Leaves. The data were collected as above, but the analysis was on the basis of younger (but fully expanded) and older leaves, as judged by the collector of the leaves. There is no difference (to within 5%) by the Student t-test of older and younger leaves, in respiration or photosynthesis.

	No Light (Respiration)		Light (Steady Photosynthesis)	
	Conductance (mmol/m^2 sec)	Assimilation (μmol/m^2 sec)	Conductance (mmol/m^2 sec)	Assimilation (μmol/m^2 sec)
Older Leaves				
Average	2.92	-0.02	16.04	1.69
Std. Dev.	2.12	0.31	3.41	0.61
Younger Leaves:				
Average	9.93	-0.20	14.93	1.21
Std. Dev.	10.43	0.12	5.22	0.46

Table 2. The Stability of Leaf Discs to Longer Periods of Illumination under Varied Relative Humidity. Data were collected as described in the text and in Table 1. These data are for leaves that had reached a constant transpiration and were exposed to light for 3 more hours, with relative humidity either at 37% (no change) or dropped to 14% (indicated as shift in relative humidity). There was no change in either the conductance or in the assimilation observed from the rate data throughout the test or as tested by Students t-test to 5% significance.

	No change in relative humidity		Shift in relative humidity	
	Conductance (mmol/m^2 sec)	Assimilation (μmol/m^2 sec)	Conductance (mmol/m^2 sec)	Assimilation (μmol/m^2 sec)
Average	14.9	1.32	16.6	1.86
Std. Dev.	4.8	0.75	6.0	1.13

Table 3: Dependence of assimilation rate upon the light intensity. The experiments were performed as described in the text and Figure 3, where the stomata were not limiting. The assimilation-intensity relation was used to determine the kinetic parameters, in which: assimilation rate followed Michaelis-Menten Kinetics or rate = maximum assimilation x intensity / (intensity + half-saturation_intensity).

Leaf	Maximum Assimilation (umol/m^2 sec)	Half-Saturation Intensity (μmoles of photons /m^2 sec)
Young	2.07 $\pm$ 0.17	270 $\pm$ 20
Old	1.70 $\pm$ 0.59	560 $\pm$ 160

Figure 1. Changes in the leaf water potential with varied amount of transpirational water loss. (shown to the right)

The data are taken from Bunce (1979) for cotton. The stomatal conductance is measured for the plant as transpiration rate, which is the actual amount of water being lost by the plant. The leaf water potential is then measured for each rate. There are two experiments here: open circles, 1 hour after transpiration is constant and darkened square, 3 hours after the indicated transpiration has been constant.

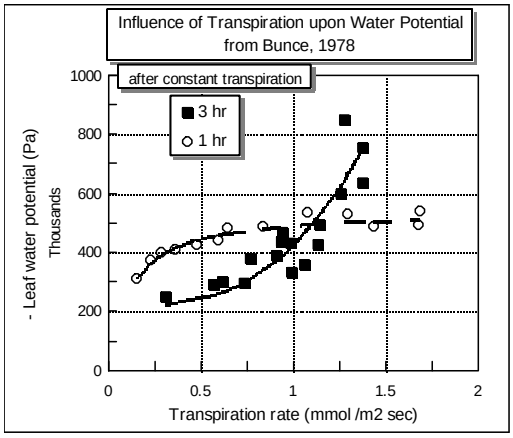


Figure 2. Dependence of Leaf Stomatal Conductance Upon the Leaf Water Potential**.** (shown to the right)

Data are taken from Brinckmann et al., 1984, for *Nerium oleander*. The leaf water potential was altered by allowing the soil to dry out naturally over 7 to 8 days. The water vapor deficit gradient (dependent upon the relative humidity of the air) was held constant at 10 mBar / Bar air pressure during the measurement of the leaf conductance.

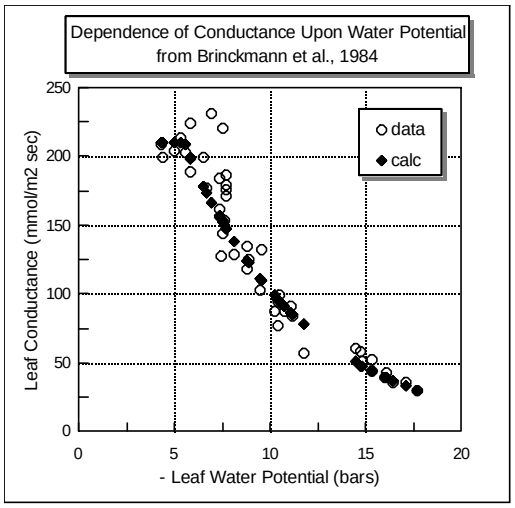
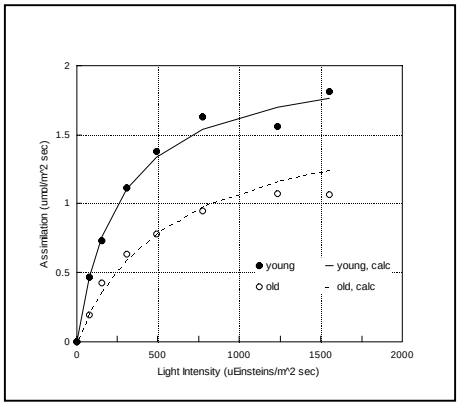


Figure 3. Typical Experiment for the Determination of the Assimilation Dependence upon Light Intensity.

After steady state was reached (under full illumination, generally about 1500 μEinsteins/m^2 sec of white light, from a tungsten filament source), neutral density filters were placed in the light path as indicated on the right hand figure [at the top figure]. These values are in optical density (OD) and are converted to intensity (% of full) by 10^{-OD}. The figure below shows the converted assimilation and intensity relationship for younger and older leaves.



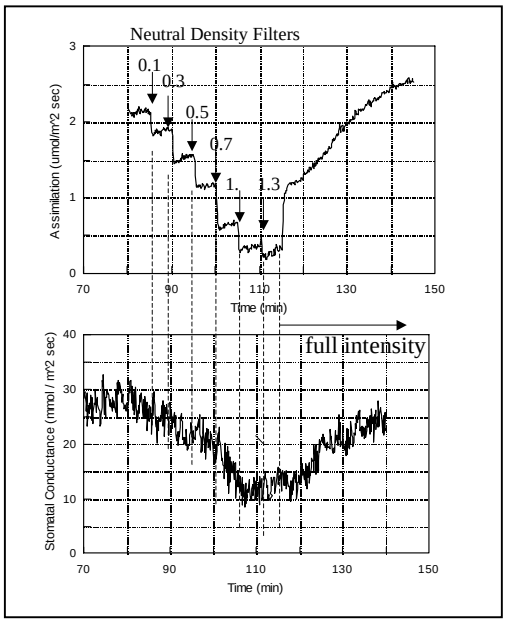
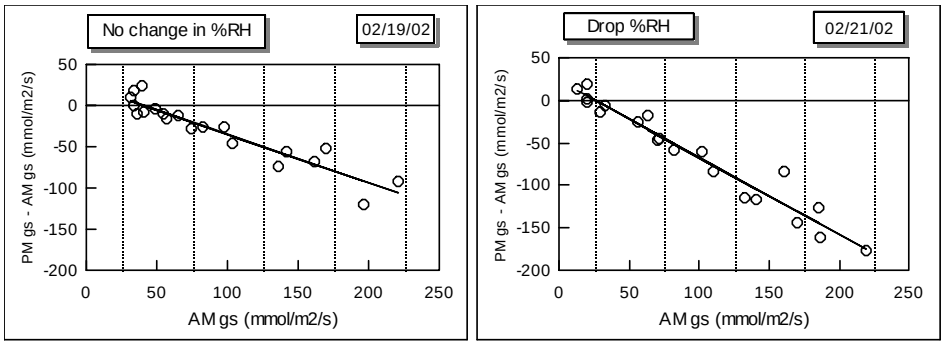


Figure 4. Part A. Conductance Changes Between Morning (AM) and Afternoon (PM) Induced by Relative Humidity of Chamber.

The conductance was measured using the LICOR 1600 Porometer in the morning or in the afternoon over a 2 hour period. Between the measurements (just before noon) the relative humidity within the Growth Chamber was either not changed (morning RH was about 45%) or lowered to approximately 20%. This plot emphasizes the change in the conductance in the afternoon by subtracting the morning conductance from it. This is a typical curve found during one week experiment. The slopes of the line are indicative of how greatly the afternoon conductance depends on the morning conductance .



Part B. Summary.

The experiments were done as above. Fifteen weeks of measurements on 8 different trees generally gave the results as above, but experimental scatter makes it difficult to express. Here we took averages for each week for several trees and many leaves and then plotted the results. The data are similar to above; the slope of the line is greater under a shift in RH from AM to PM.

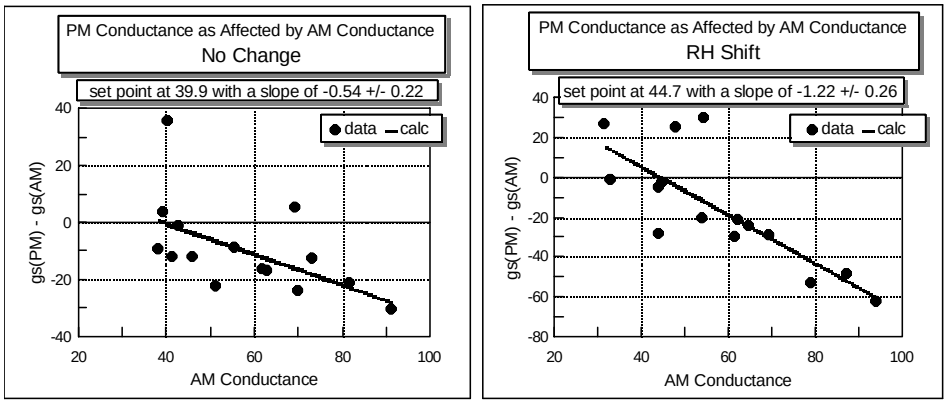


Figure 5. Model of water flow.

A model of water flow in Avocado was constructed in which the conductance was governed by a curve that looked much like the one of Figure 2 and is shown to the right. The loss of water was calculated over a 4-hour period (from noon to 4 PM) with a resistance to water flow from the soil was integrated into the total water picture (called xylem flow). If the soil and xylem couldn't deliver the water to the leaf, the leaf's water potential would fall and that would change the conductance of the leaf (and thus slow water loss). Important in this model is that the lowering of the conductance will lower assimilation and productivity. The curves for the change of conductance from morning to afternoon are shown for the final PM relative humidity (left side, below) and xylem "resistance" to water flow (right side, below).