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Avocado Tree Physiology – Understanding the Basis of Productivity

Continuing Project: Year 4 of 5

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Benefits to the Industry

A grower's profit margin is the difference between the input costs to produce a marketable crop and the gross amount of money realized from the sale of the "output", or the production itself. Anything that affects one or both of these 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' production 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 irrigation schedules and tree management strategies. For example, more growers are pruning older trees or considering high-density plantings. Canopy management strategies hinge on effective light management to increase fruit size and production. However, 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 examines, in detail, the response of avocado leaves to light, temperature, and changes in light and temperature by analyzing carbon assimilation (which fuels both tree and fruit growth) and changes in evaporative demand (which governs the amount of water the tree requires). The goal 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 model of total carbon assimilation in the canopy that will predict the effects of changes in relative humidity and temperature upon the assimilation. This research will provide the framework for 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 its relation 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 of this Year's Progress

We have made good progress on many topics with an emphasis on two —flush growth, and optimal light and CO_2. Further, all topics have been better described, leading to much more complete understanding of how we can follow flush development and why extreme California afternoon weather changes are a problem for production. Flush growth has proven to be more complex than we hoped, but we can describe quantitatively its growth and aging. We have emphasized three major points.

1. An understanding of the effects of all the environmental variables (light intensity, air temperature, and air relative humidity) on avocado leaf gas-exchange and carbon assimilation, including high temperature and its reversibility upon assimilation, and the stomata response to sun flecks.

2. An increased understanding of the developmental physiology of avocado leaves and how this relates to canopy management. In particular the manner in which multiple layers of leaves within the canopy will support a positive carbon balance to the plant; how a longer duration of a light period throughout the canopy can maintain a positive carbon balance. The question of how much productivity each layer of leaves gives to the plant when only slightly illuminated or illuminated for a short time is critical.

3. Continued development of a model of carbon assimilation and allocation in avocado that will allow growers to make informed decisions on cultural practices and will aid researchers in developing future research endeavors.

In the long term, we hope to adapt models of conductance and assimilation to predict productivity in the field based upon simple measurements of the environment.

Details

Any work on a canopy model must be based upon an appropriate understanding of leaf processes. Most models are for specific plant systems and have empirical parameters linked to leaf systems. At this stage our model merely groups various research questions into their

suitable positions. Beginning with the fundamental processes of assimilation within the leaf (Figure 1), the critical processes are [1] the gas flows of CO_2 (inward to provide the carbon for growth) and water vapor (outward to carry nutrients up to the leaf and cool the leaf through evaporation), [2] light absorption to provide the needed energy for all metabolism, and [3] the assimilation of the CO_2 into carbohydrates and other metabolites with the movement of those compounds into storage (starch), respiration (to provide energy) and transport (translocation to growing parts of the plant including fruit). All these processes also depend upon the air temperature, air relative humidity, and water within the plant, as well as the developmental age of the leaf.

The main concepts are that [1] since light governs the assimilation rate, light intensity is critical as to how much assimilation occurs; however, [2] temperature has to be taken into account for respiration, total maximum assimilation, and photorespiration; [3] the depression of the ambient CO_2 level as it enters the leaf is critical in setting the total conductance of the leaf's surface which is regulated by the stomata conductance (after modification by the boundary layer conductance govern by the wind speed); and [4] this gas conductance leads to the transpiration rate which ultimately can affect the leaf water potential, leading to stomata closure under some environmental conditions.

Light Intensity and Assimilation

Light drives assimilation directly and as such, the effect of varying light intensity must be understood. While we obtained excellent light curves for assimilation, there were problems which had to be solved, particularly when light intensity was increased. For a given light intensity, the assimilation rate is fixed or balanced by the conductance. As the light intensity rises, the stomata are unable to respond rapidly to allow more CO_2 to enter the leaf, and thus assimilation is limited. A sequence of decreasing light intensities can generate the correct observed assimilation rate; but for an increasing light intensity, we obtain a flattened curve with a great amount of variability due to stomata interference with CO_2 flow[1].

The complication of stomata conductance interference with measurements of assimilation, if the experiments are conducted correctly to remove the stomata interplay, need not concern us at this stage. However, as discussed in the last few CAC yearly reports, assimilation is limited by stomata when there are light flecks within a canopy, especially when the light intensity increases. Thus, we are paying special attention as to how fast the stomata can respond to changing light intensity and under what conditions that speed changes.

Our protocol generated light curves as the light intensity was being decreased (over 6-8 minutes[2]) which then were fit to a linearization of the Michaelis Menten Kinetics[3]:

$$A = A_{max}\, I / (I + K_I) - R_d \qquad [1]$$

where A is the assimilation with A_{max} being the highest level of assimilation, I is the intensity of

[1] This "flatten curve" was first discussed by Blackman in 1906 and is described by a convexity coefficient which alters the shape of the curve.

[2] This time scale does not allow the stomata to respond by very much. Closure is slight and does not limit the assimilation.

[3] This type of kinetics is what is expected by a system of light absorption units (photosystems). For low light, there is a linear relation between CO_2 assimilation and intensity given by a quantum yield (ϕ) relation $\{A + R_d \approx (A_{max} / K_I)\, I = \phi\, I\}$.

light, and R_d is the dark respiration. A and R_d are measured by the Licor 6400. The linearization is the Hanes-Woolf Plot which is:

$$I / (A + R_d) = I (1/ A_{max}) + (K_I / A_{max}) \quad [1a]$$

A plot of $\{ I / (A + R_d)\}$ *verses* intensity (I) generates a straight line which allows a regression to be applied to it. A typical curve generated for an experiment is shown in Figure 2. For each observation/measurement sequence (there were 84 over the course of 4 months) the values for three "constants" were found: maximum assimilation (A_{max}), R_d (assimilation in the dark), and a light constant (K_I). The Licor also generates a value for the internal CO_2, which is calculated as described in earlier CAC reports, as C_i[4]. When this value is subtracted from the external CO_2 (as Co, generally 400 ppm), the difference in CO_2 (outside less inside or ΔC) gives a measure of the driving gradient of CO_2. Also previously discussed, this seems to be relatively constant for a varied light intensities but declines upon increasing temperature. The right side of the figure shows that relationship with light intensity. The data for a single leaf is very precise and fits equation [1a] very well. However, when the data are taken for many leaves in order to observe how the above constants vary with leaf conductance, we obtain scattering plots (see 2005 CAC Midyear report). In order to determine how each constant (A_{max}, K_I, and R_d) varies with the initial stomata conductance (that conductance obtained for the first reading at the highest light intensity), we had to construct a series of bands of initial conductance in order to generate a band average plot as shown in the Figure 3. This allowed relationships to become clearer. Of first note is that the respiration rate (bottom right panel of Figures 3) shows no dependence upon the conductance and has an average of -0.46 ± 0.05 μmoles/m^2 sec for an average air temperature of 25.3 ± 2.2 ^{o} C for all the experiments. This is similar to what was reported last year and demonstrates that under most circumstances the gas flow for respiration is too slow to be impeded by any conductance measured here.

However, this is not true for the light processes. There is a break in the curves for both the maximum assimilation rate (top left panel of Figures 3) and the light constant relation (top right panel of Figures 4), indicating an interaction with the stomata. The linear portion of the maximum assimilation (with conductance) was observed previously and shows a strong, nearly absolute, dependence of the assimilation to the conductance. However, as the conductance becomes higher, the assimilation is more dependent upon light intensity and the absolute dependence upon conductance is broken. It is surprising that the light constant is dependent upon the conductance as that should be totally driven by photochemistry. However, as will be discussed below, that is probably due to photorespiration, that interference of oxygen with the assimilation processes. More will be said about these processes in the Flush Section (and Table 1).

At the same time, another measurement is made for leaf temperature. There were two distinct types of leaf-temperature: one in the full light (full radiation load) and one in the dark (no radiation load). At this stage how the leaf handles the full radiation load is not unambiguous. However, the heat loss from the leaf under no radiation load should depend upon the total gas flow conductance as it is proportional to the evaporative cooling due to transpiration. This loss is dependent upon the stomata conductance and the boundary layer conductance (Grantz and

[4] It is the difference in the inside and outside CO_2 that is calculated as approximately proportional to the assimilation divided by the conductance. This calculation also depends upon the transpiration as the outward flow of water vapor slightly inhibits the inward flow of CO_2. The Licor reports internal CO_2, however.

Vaughn, 1999) and thus can indicate the extent that the boundary layer conductance plays in heat loss. To eliminate any variation of the air temperature, the difference between air and leaf temperature is used as the dependent variable. That difference is shown in the bottom left panel of Figure 3 and is proportional to the stomata conductance. Unfortunately the uncertainty of the data makes the role of heat loss through the boundary layer difficult to determine.

Carbon Dioxide Concentrations within the Leaf

Under most conditions the amount of CO_2 in the air is relatively constant (at about 380-400 ppm[5]); however, in a canopy, active microbes in the soil below will enrich the atmosphere with CO_2. Furthermore, because of air flow restrictions within the canopy, the CO_2 within the canopy will be depleted through assimilation. Thus the canopy structure changes the CO_2 concentrations within it.

The flux of CO_2 into the inside of the leaf (Jc) is given by the conductance of CO_2 through both the boundary layer and the stomate (g_T) and the difference of the CO_2 concentrations (outside minus inside, or [Co] - [Ci], see Figure 1).

$$Jc = g_T \{[Co] - [Ci]\} \quad [2]$$

Under conditions where steady state is roughly maintained, the flux through the stomata must be balanced by the rate of photosynthesis within the leaf; that rate is dependent upon the amount of CO_2 within the leaf (Ci). The assimilation rate (A') is given by:

$$A' = \{Am [Ci] / (K_C + [Ci])\} - R_d \quad [3]$$

Where Am is the maximum assimilation rate at whatever light intensity is present, K_C is the substrate constant (as modified by O_2, see later) and R_d is the respiration value. "Am" is similar to A, defined above in Equation [1].

Again the level of internal CO_2 is critical to the normal functioning of photosynthesis, since it is that level which sets the rate of CO_2 fixation through the enzyme, Rubisco (official name, Ribulose 1,5-bisphosphate-carboxylase/oxygenase). The internal concentration of CO_2 is limited by total gas flow conductance and increased by respiration and other metabolic processes which liberate CO_2, yet it is depleted by photosynthesis and other metabolic processes which use CO_2.

We have shown previously that higher air temperatures induce an increase in Ci, by inhibiting assimilation, so it is close to Co. The temperature at which no net assimilation is occurs is that temperature where the Ci is equal to Co. Another way of expressing this is using the CO_2 difference or deficit ($\Delta C = Co - Ci$). At high non-productive temperatures both (A) and (ΔC) are zero. What makes this concept so useful is that Ci is derived from Co, A, and g_S measurements. This, in turn, suggests that ΔC may be an integrative aspect that allows one parameter to express several physiological measurements.

In order to explore this concept further we made a series of measurements in which we varied Co and found how Ci varied as well as how A and g_S were altered. The LICOR 6400 allows this investigation (called, A-Ci curves) to be carried out rapidly and with some ease. The interesting relation is shown by a plot of both the internal CO_2 and the assimilation rate against the

[5] ppm stands for parts per million in terms of volume. As the volume of CO_2 changes with temperature, the ml of CO_2 per m^3 of air changes likewise with temperature.

conductance (data not shown). The excellent fit duplicates what we have observed before. For example, a typical curve gives the relation of: $A = 12.0\ g_S / (47 + g_S)$ and $Ci = 365\ g_S / (51 + g_S)$, with A in units of $\mu mol/m^2$ sec, Ci in units of ppm, and gs in units of $mmol/m^2$ sec. The values 47 and 51 are the Michaelis-Menten constants (K_g) with units of g_S.

It was becoming clear from the above data sets that another effect, photorespiration, was occurring which influences all the above relationships and suggested that some of the inefficiencies or decline in assimilation observed in older flush leaves may be due to photorespiration. The primary enzyme responsible for assimilation is the first enzyme in the carbon reactions of photosynthesis, the enzyme which "fixes" or integrates CO_2 into the biochemistry of larger carbohydrate molecules (Rubisco, shown below as E). Rubisco is an enzyme which can utilize either CO_2 or O_2, using Ribulose 1,5-bisphosphate (RuBP) and CO_2 to produce 2 molecules of phosphoglycerate (PGA, the carbon assimilation = A_C) or RuBP and O_2 to produce 1 molecule of PGA and 1 of phosphoglycolate (PGO, photorespiration reactions = PRs). The reaction with O_2 is photorespiration because it uses O_2 and results in negative assimilation (using up) of carbon dioxide. In most plants photorespiration represents about 25-35% use of the available light energy and leads to carbon products which, while useful, do not lead into carbohydrate production directly.

$$\begin{array}{l} \qquad\qquad\quad K_C \\ E + RuBP + [CO_2] \leftrightarrow \{ E \cdot RuBP \cdot [CO_2] \} \rightarrow E + 2\,[PGA] \\ \uparrow\downarrow \qquad\quad K_O \\ E + RuBP + [O_2] \leftrightarrow \{ E \cdot RuBP \cdot [O_2] \} \rightarrow E + [PGA] + [PGO] \end{array} \qquad [4]$$

Oxygen is competitive with CO_2 and *vice versa*. What this means is that at high enough concentration of CO_2, O_2 is out-competed by CO_2 and therefore, the inefficiency of photorespiration is not a problem. Similarly if O_2 rises, it becomes a greater competitor of CO_2 and photorespiration decreases. At very low CO_2 concentration, O_2 (even at atmospheric concentration) appears to be higher and so more photorespiration should be apparent.

The process of competition causes both CO_2 and O_2 to look like substrates in enzymatic kinetics (Segel, 1975). Thus we can write:

$$Ac = Ac_{max}\,[CO_2] / (K'_C + [CO_2]) \qquad [5]$$

$$Ao = PRs = Ao_{max}\,[O_2] / (K'_O + [O_2]) \qquad [6]$$

Here the assimilation (which makes carbohydrate) is Ac, with a maximum value of Ac_{max} and an enzyme constant of K'c. The oxygenase reaction, which does not allow carbohydrate metabolism and is wasteful of the light energy, is Ao, with a maximum value of Ao_{max} and an enzyme constant of K'o. Both enzyme constants (K) are not truly constant but have the opposing gas acting as a modifying factor.

One expects the ratio of Ac / Ao to be near 3 at typical atmospheric values of 390 ppm CO_2 and 21% O_2. While it is expected that leaf age should not affect the relationship (as only one enzyme is responsible for the effect), an understanding of this process could be important in the canopy where the concentrations of both gases can be modified by many processes.

These effects are easily seen in a typical A-Ci curve (see Figure 4, left side). Assimilation at low CO_2 is often negative due to respiration and photorespiration. However, correcting for respiration and plotting the curve against the internal CO_2 concentration gives the curve a typical

enzymatic, hyperbolic curve (as for equation [5]). These types of curves yield both dark respiration and kinetic parameters for maximum assimilation (at a given light intensity) and CO_2 (that which is inside the leaf). The point at which the observed assimilation rate is zero is where a balance exists between carbon assimilation, and dark-respiration and photorespiration (called the compensation point, Γ_C). Table 1 shows a summary of some of the data and how much the "constants" vary. At this point we cannot determine how leaf age affects these measurements, although older leaf age leads to lower maximum assimilation. We do not expect any connection to K'_C, as that is a fundamental mechanism of the enzyme.

Unfortunately, Ci does not really trace the external concentration of CO_2, which is what is measured in canopies. While one might expect a direct correlation, there is a marked curvature or a break in the straight line at lower levels of external CO_2 (right side of Figure 4). The curvature of the Ci-Co curve is a result of a "gain" of CO_2 due to the interference of A_C by O_2. At high CO_2, this interference is minimized and so for points taken at higher levels (above 500 ppm), we obtain the expected direct relation curve. For high concentrations, the internal CO_2 concentration tracks the external level; however, at the concentrations for which we are most concerned, that tracking is imprecise due to the use of CO_2 by assimilation. In fact, that "use" can be modeled as shown in the figure. The depression of internal CO_2 follows a hyperbolic relation with a "K" constant and a maximum depression (ΔC_{max}). The value of ΔC_{max} is dependent upon light intensity and temperature (see last year's report) but also relates to the external CO_2 level (as given by the $K_{\Delta C}$ value). Note that $K_{\Delta C}$ is about 400 ppm which the same value is the ambient level of CO_2 and so as the CO_2 about the leaf varies (due to assimilation or soil processes), it affects the ΔC and so alters assimilation. However, these parameters are relatively constant also, as seen in Table 1, and so can be easily modeled.

When the relation of Ac_{max} (from curves such as Figure 4) is examined, photorespiration seems to be relatively constant for three ages of flushes while maximum carbon assimilation declines (Table 2). Photorespiration seems to play a larger role in assimilation in flush ages, but only in comparison to the decline in carbon assimilation. Thus, while we have to be aware of photorespiration, there is very little that can be done about it.

In regard to photorespiration, the three critical items of this study are: [1] it occurs much like for all C3 plants in that Rubisco has oxygenase activity, [2] it is important if the external CO_2 concentration varies, and [3] it seems not to play any role in lowering further the efficiencies of older flushes. Thus, avocado has about 3x more assimilation than photorespiration at 400 ppm and that causes an inefficiency which is expensive to overcome (e.g., by enrichment of the air with more CO_2). Furthermore, the interplay between O_2 and CO_2 alters the assimilation of CO_2 within the canopy (see above) will be important in understanding the relation between canopy structure and photosynthetic efficiency.

Effects of Temperature upon Assimilation

Last year we found a temperature optimum by subjecting a tree to a short period of a different temperature (about 1 hour). While the curves were interesting, one question was whether differences would be observed when the trees were grown at different temperatures. These experiments are technically difficult as we need a growth room at each specific temperature and the temperatures must not be outside the range of the tree. For example, five days at a day time temperature of 35C (95F) causes most of the leaves to abscise from the tree (an accidental

experiment). We had access to two chambers that had approximately the same light intensity and could be maintained at a steady temperature. We picked day time temperatures of 18 and 28 C as the set points (the actual temperatures were held at about 14 and 25 C—*57^o and 82^o F*—below the set) as temperatures which were not excessive and within the optimum of assimilation and stomata conductance (see last year with an optimum of about 22C – 72 *^{o} F*).

We are still working on this aspect of the research but several things correspond to what we have seen with the short term temperature experiments (see Table 3). Assimilation and conductance are both decreased at low temperatures (about 50%) with no change in the calculated internal CO_2 (both were close to 260 ppm, for an external CO_2 of 400 ppm in both chambers). While the light intensities and the radiation loading within the chambers were similar (740 vs. 970 mole/m^2 sec with 18C vs. 28C), the leaf-air difference temperatures were quite different (the leaf temperature was nearly 3 times greater at 18C compared with 28C). This was not due to transpiration (evaporative water cooling) as that was about 3 times as great at 28 (the relative humidity was about the same in both chambers but the water vapor pressure gradient was greater at 28C). We have not yet completed a full analysis of the energy balances but we expect that this difference can be explained by the varied heat losses at different temperatures.

Within the same experiment we examined the light intensity dependence of assimilation for leaves held on the trees maintained at the two temperatures (Table 4). Looking only at the youngest flush (N) we see several relationships. Interestingly the A_{max} at both temperatures were similar but statistically different (the maximum was about 15% higher at 28C). The light compensation point (the intensity at which there is no observable assimilation and which is proportional to R_d, A_{max} and K_I) does not differ between the temperatures. On the other hand, there is a statistical difference between the quantum yields; about 30% higher for trees grown at 28C. As noted previously, the higher temperatures promote high dark respiration (nearly 2 times).

Finally in Table 4 we examined the relationship of light intensity on leaves in two different flush ages. Here what was noted above (less assimilation for older flushes) was duplicated in that the A_{max} was lowered by nearly 40-50% and the quantum yield was lower, especially in the lower temperature. Also the light compensation point fell. Surprisingly, the respiration rate declined in the older flushes, but not to such a large extent as assimilation.

Thus, while temperature affects these photosynthetic processes similarly, there are differences which are difficult to sort out. In particular, we are not yet comfortable with the statistics of these processes; the variability and interactions between environmental parameters tends to confound our results.

Plastochron Index

We continued to determine what varied processes are linked to certain physiological ages by the use of the plastochron (PC). The plastochron day is the actual calendar day (from the start of the experiment, 11/18/2004 for the experiment described here) minus the day at which the leaf has expanded 50% of the maximum value (the zero plastochron day). We measured chlorophyll content of the leaves using a SPAD meter, which measures the red spectra of absorbed light. When we combined all the SPAD data using the plastochron day to normalize the variations in leaf initiations, we obtained a tight scattergram (shown in the 2005 CAC midyear report) in which the position for the accumulation of 50% of the total chlorophyll within the leaf was at the

time of about 8 plastochron day (to be compared with 50% of the area growth at 0 day). We present here (Figure 5) the chlorophyll accumulation for each of the 9 trees, each with 3 measured shoots and each shoot with an average of 11 leaves measured for a total of 60 days. These plots demonstrate the variation of growth and development of each tree and were plotted as individuals against its tree plastochron day. Here relation between the leaf age and chlorophyll content is more obvious. The rise in chlorophyll was nearly linear (with missing points for very young leaves). The maximum value of mg-chlorophyll / dm^2 was about 5.5 to 6.5 and the crossing of the Plastochron Day = 0 occurred at an mg-chlorophyll / dm^2 value of 2 to 3. The slope of the lines (rate of chlorophyll formation per area) was consistent at 0.11 mg-chlorophyll / dm^2 /day.

Translocation of Metabolites

For the most part we have been studying assimilation and gas flow into the leaf as a measure of productivity. Assimilation, while vital, is not the end result of metabolism for the plant. Transformation of the initial products of assimilation is critical to maintain the stable state of the leaf and to allow certain metabolites to flow into developing parts of the plant (growth of new shoots and roots, and into the fruit). As stated in the next year's proposal (for 2005-2006), avocado is unusual in that it produces a large amount of 7-carbon sugar (mannoheptulose) and alcohol (perseitol) in addition to the normally translocated sugar, sucrose (Liu et al., 2002). We don't understand the metabolism of these 7-carbon compounds but it seems that perseitol and sucrose are moved through the vascular system.

We proposed several studies to measure how metabolites moved from the point of assimilation to fruit—how much of these compounds are produced and where they move. We are just beginning these studies but it seems that perseitol concentration is highest in the leaf when it is young, and although it declines during growth the concentration remains high. Sucrose, on the other hand, is totally absent in young leaves but its concentration rises as the leaf matures. Mannoheptulose is present at a low level in younger leaves but its concentration rises dramatically as the leaf matures (Figure 6). As seen in the figure, these studies can now be linked to the plastochron index (the methodology was described in last year's CAC report, 2003-2004). In fact, these relations, described above, could not be determined without linking the sugar measurements to plastochron index as we have sampled many leaves at different times to obtain them. The plastochron allows the linkage to a generalized leaf growth. We are currently measuring sugars within the leaves from very young (plastochron index of -15 to -20 days, size about 5% of the maximum size) to old leaves from last year's flushes and will have a complete leaf developmental picture of these important metabolites.

Leaf and Flush Development

Our program has been concerned with leaf and flush development based upon the following: there are at least two flushes per season and we don't yet understand 1) how they affect each other, 2) under what conditions are the leaves of the older flush abscised, and 3) how much energy does each provide the growing and developing fruit. We suspect that the leaves of the older flush become inefficient eventually, but do provide important resources during the development of the new flush. These efficiencies are critical in terms of total carbohydrate that can reach each fruit to maintain it on the tree and to increase its size. Also, each of these

questions is linked to determination of how to shape the canopy; what is the best method to prune?

As observed under different CO_2 concentrations and temperatures, the maximum assimilation rate and respiration are modified by flush age (see Table 5). The youngest flush has highest assimilation and respiration rates. The light intensity at which the rate is ½ the maximum rate is not significantly changed by flush age as it is dependent upon the fundamental mechanism of light reactions. The efficiency of the process is affected by a change in maximum assimilation.

Our working hypothesis is that there are at least two triggers: phytohormonal and illumination. To develop this hypothesis, we have been examining how new flush development and old flush abscission are linked. Our analysis is not yet complete due to the complexity of the data set and the difficulty in proceeding with experimentation. However, we do have some concepts that seem to be useful.

Firstly, the growth, in terms of area or weight, of the new flush is not easily described. The growth of the flush is made up of a sum of a series of leaves, each of which are initiated in a timed sequence and grow logistically (see previous reports). The flush growth (the youngest flush, #4, in Figure 7) can be described by an exponential increase for the first 5 to 7 days, leading into a nearly straight line increase for the next 7 to 21 days, followed by a cessation of growth after an exponential type leveling off. Although a logistics curve can be fitted to this, it does deviate from it in two portions of the growth period (see Figure 8) due to the long period of linear rise in total flush area/weight. We have modeled flush expansion based upon normal observed leaf growth (see previous reports) and, for sparsely-spaced data (as in Figure 7), a logistics growth curve does appear to be a close fit with the observed shoot growth. While not perfect, it does allow us to tabulate relatively easily a growth rate and a flush "plastochron age" (see the right columns in Table 6).

While the new flush growth is understandable, the abscission of the leaves from the old growth is not. Seemingly the abscission process falls into three categories (see Figure 7 and Table 6): total abscission, no abscission and partial (about 50%) abscission. If abscission occurs, its beginnings are observed within the early stages of the new flush growth curve. We are trying to link these two distinct processes by a plastochron type analysis.

We have examined a series of flushes on Hass trees and found that the number of leaves remains constant for each flush (see Table 7A). More importantly when we look at three flushes that still remain on the tree, the youngest (N) and next-to-youngest (N-1) flush have about the same level of photosynthesis (ca. 8 μmole/m^2 sec) but the oldest flush (N-2) has a significantly lower rate (ca. 5 μmole/m^2 sec, Table 7B), again similar to that found above. While this decline is not great, when it is coupled to the total leaf dry weight (which is proportional to leaf area), the oldest flush has less than half of the dry weight (Table 7C). Thus, the total productivity of the oldest flush has declined by nearly 70%, loss of assimilation rate per unit area and loss of total area. Interestingly, significant difference remains when the data are expressed as dry weight/leaf (specific weight); the specific weight of leaves of the oldest flush are less than half that of the younger flushes. This may be due to nutrient mobilization out of the older leaves, a phenomenon previously reported for other plant species.

We have also found that the xylem water potential is lower in the older flushes (Table 7D). While the lower potential is not detrimental *per se*, it would affect how much transpiration each leaf could maintain throughout the day before the water potential within the leaf would decrease

and so cause a stomata closure. One would expect that the older flush would have a mid-day closure of stomata earlier in the day and this would also limit assimilation and productivity.

That area loss was obtained in another experiment (Table 6) in which three flushes are again compared. The total areas of leaves for the younger flushes (N-1 and N) are about the same but the areas for the older flushes (N-3 and N-2) are reduced about 70%. Also the amount of leaf abscission is variable from 0 to 99%, with an average of about 50%. Using the logistics growth curve for the growth of the full flush, we obtain a rate of growth (negative slope) of about the same as for individual leaves (0.27 cm^2-increase in area/ cm^2-area / day) with a period of 34 days (from the start of the experiment) to reach a plastochron day of 0 (where the total area of the leaves of the flush are 50% of the maximum).

There are a few relationships that can be extracted from these tables. For example, there is a relation between leaf drop and the total area on the branch (Figure 9). If the leaf area of the branch is high enough (total area of 1000 cm^2), then abscission seems not to occur. This would suggest a required level of assimilatory support from all flushes on the entire branch.

While we still do not know what actually induces the initiation of the flush or the abscission of leaves in the older flush, total productivity is considerably lower in the oldest flush. Also, while photosynthesis has declined it is still above the rate of respiration[6].

We still believe that the best model for flush development is: [1] an initiation trigger is provided by the plant (presumably auxin) which stimulates flush production and sets the potential for leaf abscission of the older flush and [2] a secondary trigger, such as reduction in light or a decline in total production from the entire branch, must be provided to actually cause leaf abscission. Once both triggers have been received by the older flush, nutrients are mobilized out of the leaf and the level of assimilation falls due to inefficient photosynthesis rates.

Monitoring Sap Flow

In principle, the sap flow monitoring system is an ingenious device. Wrapped about a small trunk or branch, it heats a small section of the branch with a known amount of energy input (see Figure 10). That heat energy moves up and down the branch and is detected by thermocouples placed a short distance away from the heated section. With knowledge of the temperature that heat flow can be calculated. As the transpiration stream through the xylem increases upward, more heat is carried up than down and that change in temperature is registered by the second thermocouple. By calculating the difference in heat at the two sites, the xylem flow can be calculated. With readings every 15 minutes, the sap flow monitor can show the total transpiration stream through the branch.

While seemingly easy, there are some concerns with the system (Grime & Sinclair, 1999). For one, it does not operate as well on a small branch because the heat flow is not very well defined in terms of volume of tree affected (see Tatarinov et al., 2005); thus some of our earlier work, while adequate, is not sensitive enough to give us good indication of minor changes in sap flow. Another problem relates to comparisons between two different branches. The sap flow is absolutely dependent upon the number of leaves carrying out transpiration. Generally two

[6] While respiration is typically 0.5 -1.0 μmole /m^2 sec and so much lower than assimilation (5 μmole /m^2 sec) for the oldest flush, respiration continues during the full day (24 hours) while high assimilation occurs for less than 4-6 hours, dropping the total carbon assimilation to 108 mmole /m^2 with a total loss of about 80 mmol/m^2, nearly equivalent.

branches have different numbers of leaves and that gives different flows. Finally, there is a critical calibration which must be carried out when the sap flow is zero, generally at night, which gives a measure of how heat flow occurs within that particular branch. For crop plants, the stomata are closed at night and water status of the whole plant reaches equilibrium in the early morning before sunrise (e.g., no sap flow at 5AM).

We are now using larger branches or trunks to eliminate the first problem. By calculating ratios of flow during the highest point of the day or later in the afternoon and comparing that to flow earlier in the morning after the light of day is well established (e.g., 10AM), we can compare what is happening to the flow of an individual trunk, regardless of number of leaves on it. Finally, to our surprise, we have determined that sap flow of avocado is lowest and perhaps close to zero during late evening (from 11:00 to 12:30 PM) and thus we have used that time period to calibrate our sap flow. These improvements have increased our confidence of the sap flow monitors and, as described in the next section, allow us to compare the flow of sap in varied trees.

Because we carried out long term monitoring of these trees, with monitors placed on the trunk and on selected branches, we discovered another problem with sap flow monitoring. After monitoring for about 2 ½ to 3 weeks, the restrictive nature of the monitoring probe and its related insulation bands cause damage to the bark and an alteration in the general state of the branch/trunk. It seems that the higher temperature of the monitor (generally about 2-3 C above ambient) and restrictive nature of gas flow to the shoots seems to be the source of the problems. Relocation of the monitor probe solves the problem and allows recovery but it means that we have a different recording position in need if a re-equilibration time period. We are involved in re-analyzing the earlier data in order to extract changes, if present, or set limits on those potential changes, if we do not observe them.

Furthermore, in this investigation, we also found that the conductance of avocado leaves continues during the night; the stomata do not completely close. While the residual conductance is small (probably less than 10% of the day), it is not zero. Often the night relative humidity is high, near 80-90% and so the water vapor gradient between the leaf and air is small, thus making the transpiration rate low (meaning a very low sap flow). While this is an unimportant phenomenon for most growers, a very dry night will generate water flow and thus the water status of the tree will not reach equilibrium during the night. Thus, the tree would be expected to have a lower water potential at the start of the day and so any water deficit problems would begin earlier in the day. These events would tend to close the stomata earlier in the day and so assimilation would be reduced.

Rootstock

We have found that the water delivery system of the roots and vascular system within the shoot can be compromised during the afternoon; in the sense that water from lower in the plant cannot replace water lost through transpiration of the leaf. This imbalance of water movement causes a decline in the water content of the leaf (lowered water potential) causing the stomata to close and the stomata conductance to decrease. In the avocado the assimilation is tightly linked to the conductance and that linkage would limit the assimilation and productivity. Anything interfering with the plant's water system will make matters worse. We have been working with 'Hass' grafted onto clonal Duke 7 rootstock (Figure 11). We became concerned that the graft union between the Duke 7 and the Hass may be impeding water flow. This concern is due to reports by

other researchers that water flow within the avocado plant may be influenced by rootstock due to differences in the size of the vascular cells between rootstock and scion. It may be that the junction limits the ability of water to move into the shoot and leaves.

In order to probe that potential problem, we are using the sap flow meter to monitor the rate of xylem water movement during the course of the day in six trees—three with no junction (all clonally rooted 'Hass' trees) and three with a clonal rootstock (Duke 7). We have found that the flows are similar but differences within the trees, canopy and placement of the trees cause variations within the sap flow measurements. The absolute sap flow rate is not directly comparable as the number of leaves on each tree is not the same; the trees are of similar size but are not identical. Further, the grafted trees have a sharper peak of sap flow and its total water use (shown as diamonds) is much more variable than the non-grafted tree.

While comparisons between trees are difficult, we have been able to observe difference by comparison of flows during the day of the same tree, as seen in the bottom panel of Figure 11. The ratio of sap flow in the afternoon compared with the morning is very different for each type of tree. The sap flow in the non-grafted tree is more stable over the course of the day and does not decline as early in the afternoon as does that of the grafted tree. This ratio system eliminates the problem regarding the number of leaves on the tree. Additionally, it is more directly related to our previous data in which the Hass Avocado suffered from a "mid-day depression" of stomata conductance, which was accentuated by low afternoon humidity. This earlier afternoon closing of the stomata and decline in sap flow suggest that assimilation, and thus, productivity is being affected. Now, sap flow can be used to directly monitor this phenomenon.

Modeling Assimilation and Transpiration in Avocado

To place our research investigations of leaf processes into their proper perspective, our current canopy model taken from Su et al. (1996) must be discussed. The overall experimental model is diagrammed in Figure 12. The top portion of the figure shows the process boxes for what we have investigated and are currently working on (similar to Figure 1). The stomata conductance seems to control the assimilation rate under most conditions, but because of the stomata response to the environment, assimilation seems independent of the stomata only when the light intensity declines rapidly. We still believe that the boundary layer plays an important role in gas exchange, in leaf cooling (leaf energy balance), and assimilation. The relationship of wind, light, air temperature and relative humidity is important as a linking into the canopy model (shown at the bottom of the figure). Here the same processes occur but are modified by the canopy, in particular, the generation of varied gradients within the canopy. These gradients are generated by global conditions within the canopy while the local values of the varied environmental parameter control the leaf processes.

Assimilation (A) is the result of CO_2 fixation due to photosynthetic activity of the leaf and is dependent upon many aspects of leaf physiology that we have been investigating. The critical aspects for rapid assimilation are: 1) light intensity absorbed by the leaf, 2) dependence upon the stomata conductance (g_S) or aperture, 3) dependence upon boundary layer of the leaf (g_B) and canopy, 3) leaf temperature (T_L), 4) evaporative demand due to atmospheric relative humidity, and 5) internal CO_2 concentration (Ci). Many of these aspects have an integrative nature such as loss of water vapor through transpiration and control of the stomata conductance which are

linked through the leaf's water potential. Thus, changing one aspect influences another but often that linkage is not useful when attempting to increase individual leaf assimilation (Heath, 1991).

Fundamental Aspects of Potential Model

Any model must be built upon environmental data that comes into the system from simple environmental sensors. Typically these sensors are 1) a HOBO sensor to monitor the air temperature and the relative humidity and 2) a LICOR 1000 light sensor to monitor the light intensity from several directions. This model is in the beginning stages of development as we are determining where to place the sensors, how many to use, and are linking experimental measurements of the tree to the predicted values of water flow—the transpiration rate.

While the transpiration of the stomata can be checked on an individual leaf basis with a Licor 1600 Steady State Porometer, this value may not be the total transpiration of the individual leaf on the tree, as the boundary layer may alter its actual value. The transpiration rate is, of course, what the Sap Flow Monitor measures as the water flows from the roots to the leaves (Wullschleger et al., 2001). From the total actual water flow, a measure of potential water loss from the leaf can be estimated. If the roots can supply that loss during the day, the water potential should be maintained and all will be well; however, above a certain amount the conductance will be lowered and mid-day depression of assimilation will occur.

These predictions of the sap flow will allow an estimation of total assimilation and with an understanding as to what percentage of the total assimilates used for fruit production, we can predict what effect various horticultural practices will have on that productivity.

Summary

In the final year of this project, we would like to achieve:

1. An understanding of the effects of all the environmental variables (light intensity, air temperature, and air relative humidity) on avocado leaf gas-exchange and carbon assimilation, including how high temperature reverses assimilation, and how the stomata respond to sun flecks.

2. A continuing and advancing understanding of the developmental physiology of avocado leaves and how this relates to canopy management. In particular, we'd like to understand the manner in which multiple layers of leaves within the canopy support a positive carbon balance to the plant and how a longer duration of a light period throughout the canopy can maintain a positive carbon balance. The question of how much productivity each layer of leaves contributes to the entire plant when only slightly illuminated is critical.

3. Continued development of a model of carbon assimilation and allocation in avocado that will allow growers to make informed decisions on cultural practices and will aid researchers in developing future research endeavors.

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Figures

Figure 1. Schematic of the Movement of CO_2 into the Site of Assimilation within a Leaf Cell. The first step of assimilation is due to the enzyme Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase (Rubisco). The assimilation material can be used for growth of the individual cell, or respiration to generate needed energy, or translocation to move the material to another tissue.

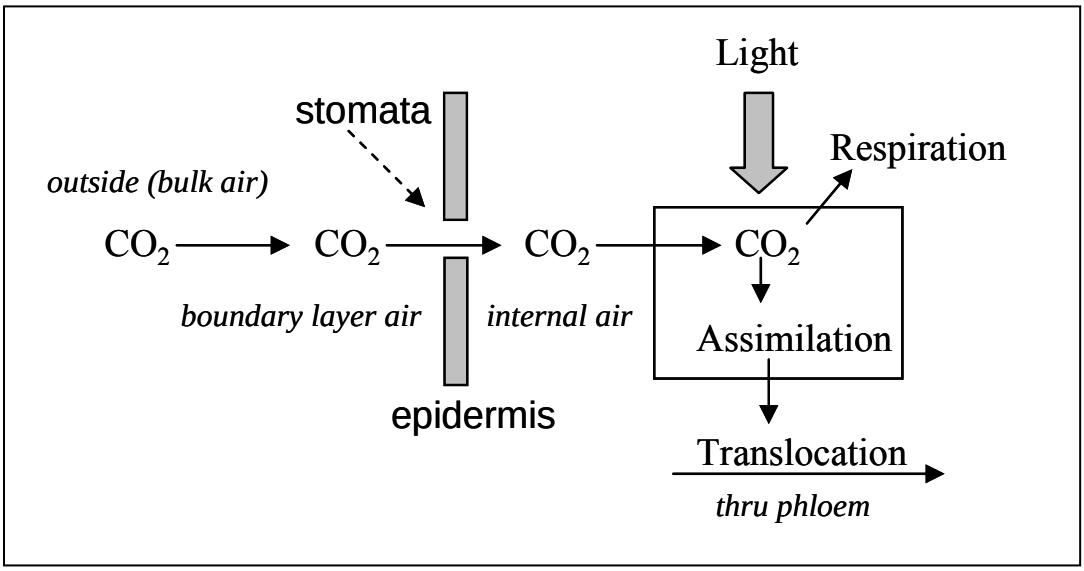


Figure 2. Typical Light Dependence Curve for Hass Avocado Leaves. The data have been gathered for a single mature leaf from a typical tree using a LICOR 6400 porometer.. The other environmental conditions were matched to the greenhouse in which the tree was growing. The air temperature was 26.2C with a relative humidity of 56.8% and a light intensity near that at the maximum given below on the x-axis. The first data point was taken at the maximum intensity and then the intensity was decreased in steps as described in the text. At the start, the stomata conductance was 110 mmol/m^2 sec which declined somewhat as the light intensity was reduced.. The curve was fitted by least squares regression to a hyperbolic curve with A_{max} = 9.95 $\pm$ 0.4 µmol/m^2 sec and K_I = 108 $\pm$ 10 µmol/m2 sec, with a respiration of -0.54 µmol/m^2 sec. The CO2 Deficit is $[CO_2]_{out}$ - $[CO_2]_{in}$, with the external (out) CO_2 of 400 ppm.

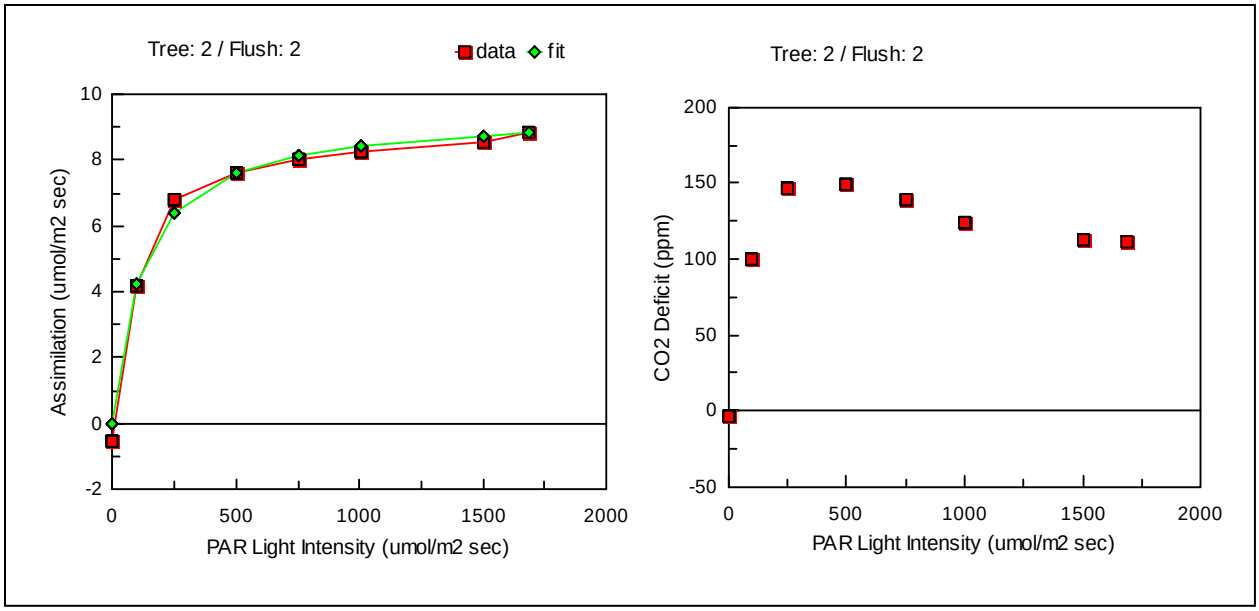


Figure 3. Dependence of the Parameters Governing the Light Intensity for Assimilation upon Initial Stomata Conductance. The data were placed into seven bins of approximately equal number of conductance observations. The average values of the y-axis data with the standard deviations are shown. These curves are of the "Constants" Generated by fitting assimilation to intensity governed by $A = A_{max}\ I / (K_I + I) - R_d$. The data were fit to the linear equation of the previous equation of: $\{I / (A+R_d)\}$ vs. I, the so-called Hanes-Woolf Plot, using Least Squares Regression. For more details see text. Top left panel is for maximum assimilation, top right panel is for the light constant (K_I), bottom left panel is for non-loaded (by radiation) {leaf – air} temperature, and the bottom right panel is for the respiration rate.

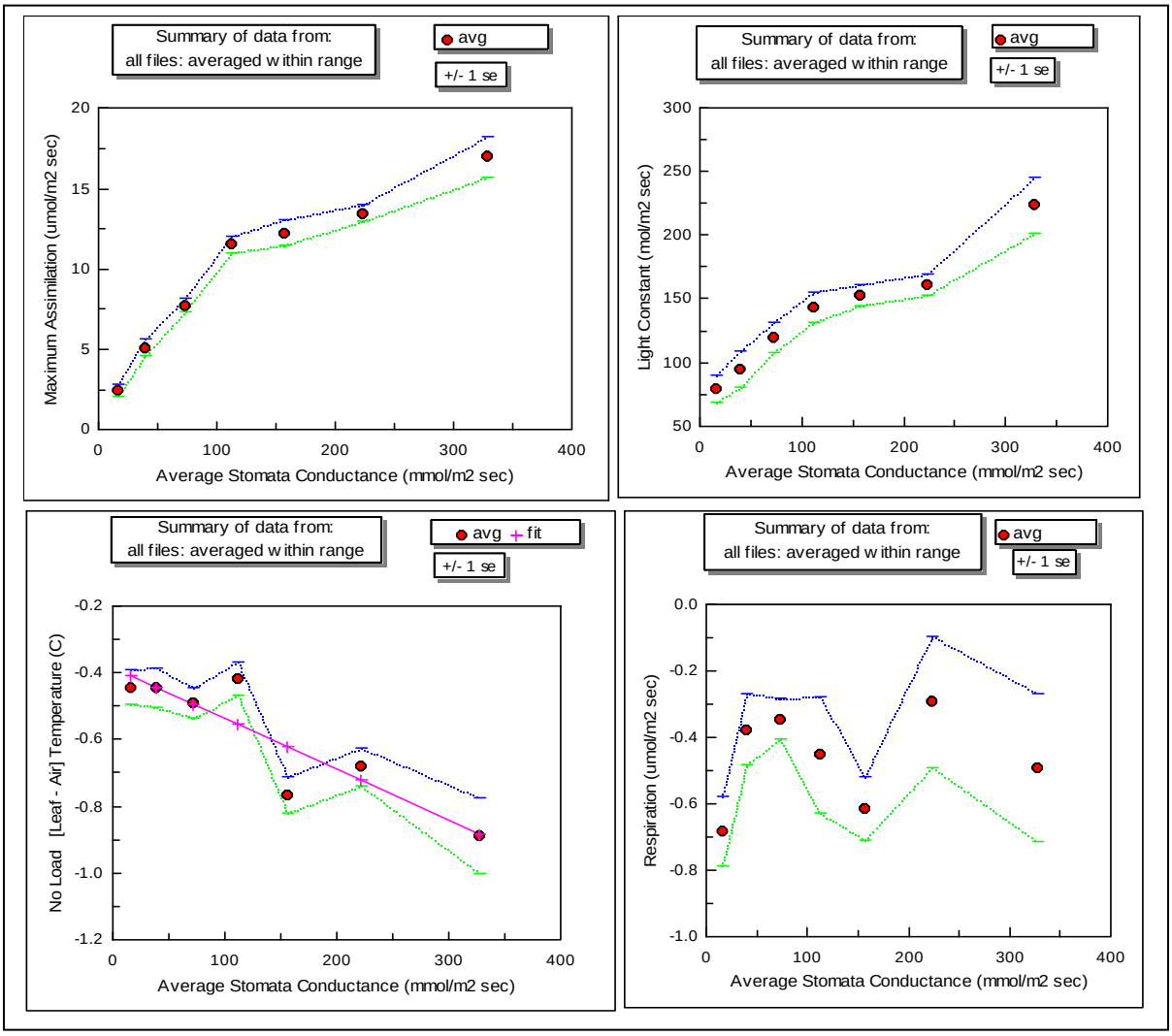
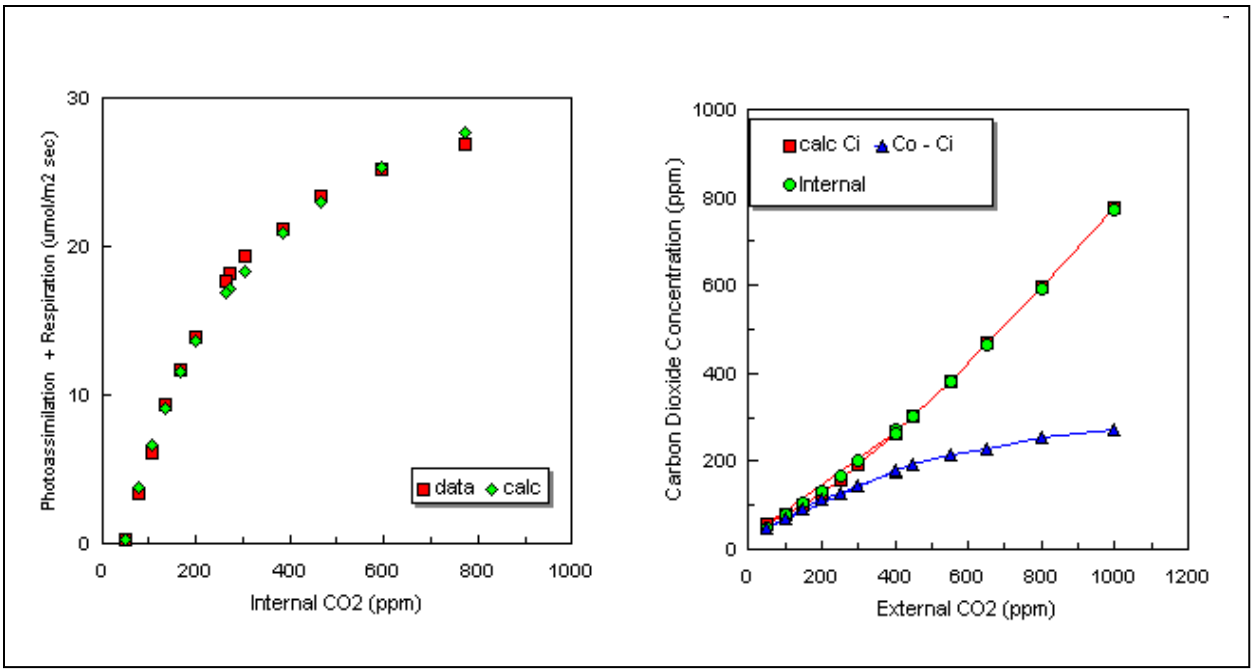


Figure 4. Dependence of Assimilation upon the CO_2 Concentration. Data were taken with a LICOR 6400 set to vary the external CO_2 level within the cuvette chamber, starting with 400 ppm declining to 50 ppm, then raising the level to 400 ppm and increasing to 1000 ppm. The conductance of the leaf changed during the process (taking about 15 minutes). These curves are typical curves for various trees, flushes, and leaf age. **Left Side**: Dependence of Assimilation on Internal CO_2. The calc curve is for $A = A_{max}\ [Ci] / (K'_C + [Ci])$. **Right Side**: Dependence of Internal CO_2 on the External CO_2. The saturating curve (Co-Ci) is the difference of measured



outside CO_2 less internal CO_2 and the fit curve is for: $\Delta C = \Delta C_{max}\ [Co] /(K'_\Delta + [Co])$.

Figure 5. The Chlorophyll Content of Leaves as Correlated with the Plastochron Index. The plastochron index was measured as described in last year's report and is based upon the day that the leaf reaches 50% of its maximum size. The SPAD data are based upon red light absorption by the leaf and is correlated with chlorophyll content. One SPAD unit is equal to 1 mg-chloro/ dm^2-leaf area = 100 mg-chloro/ m^2-leaf area. These data for 9 trees are an average of 3 branches each with an average of 11 leaves. The data were taken over 60 days, every 4-6 days.

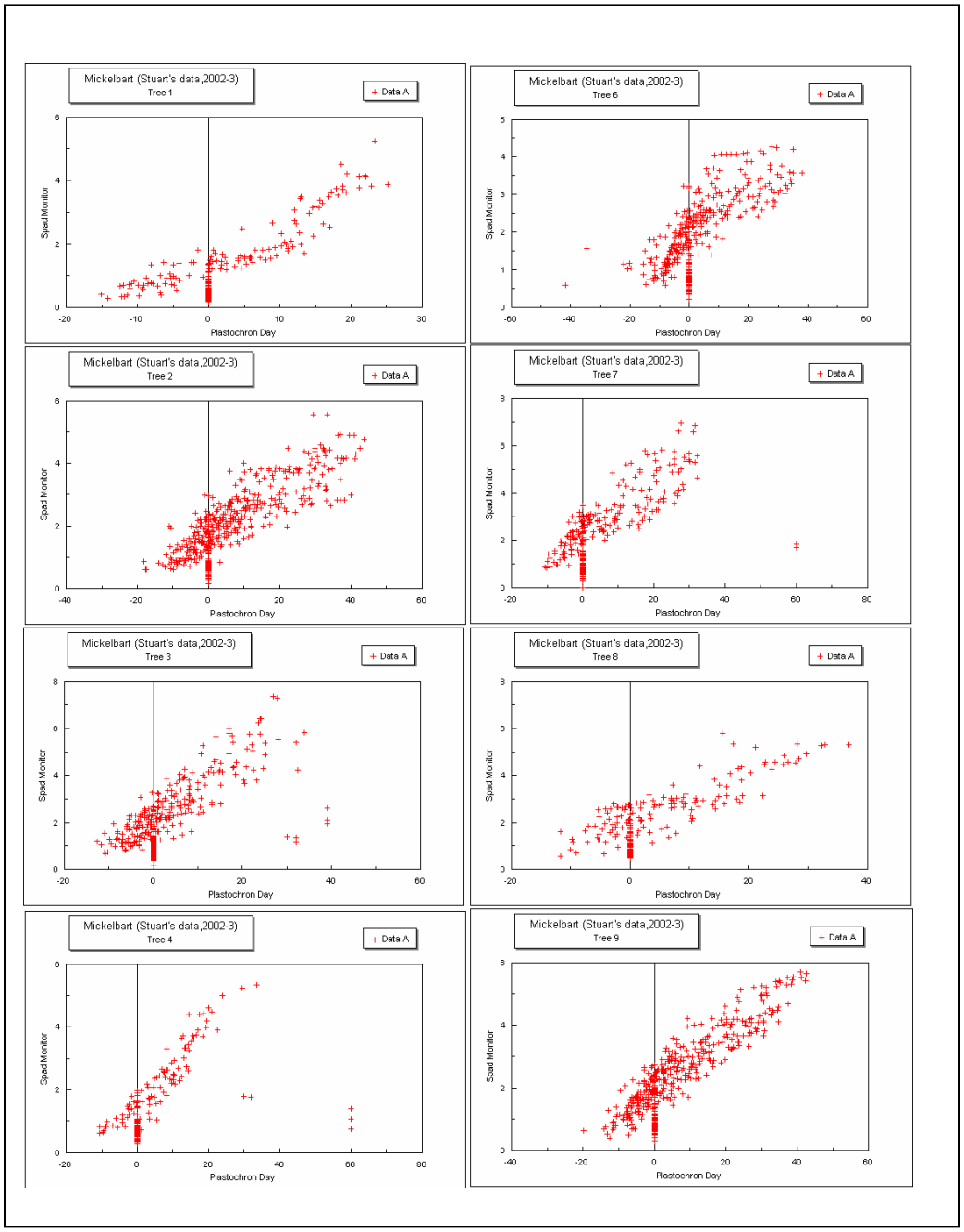


Figure 6. Sugar Content of Leaves**.** The content of varied leaves was determined from a small leaf punch taken from about 10 leaves on different 3 branches on Hass Avocado over 3 weeks. The plastochron day was determined from the areas of the leaves (as measured by its length times width), as previously described. The disc was freeze-dried and ground to a powder in a mortar and pestle. The powder (100 mg) was extracted with 5 ml 80% ethanol by boiling for 1 minute followed by heating at 65˚ C for 30 min. The ethanol solution was dried in a speed vacuum, the pellet was suspended in 1 ml water and applied to two ion exchange columns set up in tandem, one ml each of AG 50W-8X, 200-400 mesh, H form and AG1-8X, 200-400 mesh, formate forms (from Bio-Rad). The column was washed with 10 ml water. The eluate was dried in a speed vacuum. The pellet was suspended in 100 µl water, filtered through 0.45 µm filter, and injected in the HPLC. The rise in the sucrose and mannoheptulose occurs at about -20 plastochron day is when the leaf is switching to an exporter.

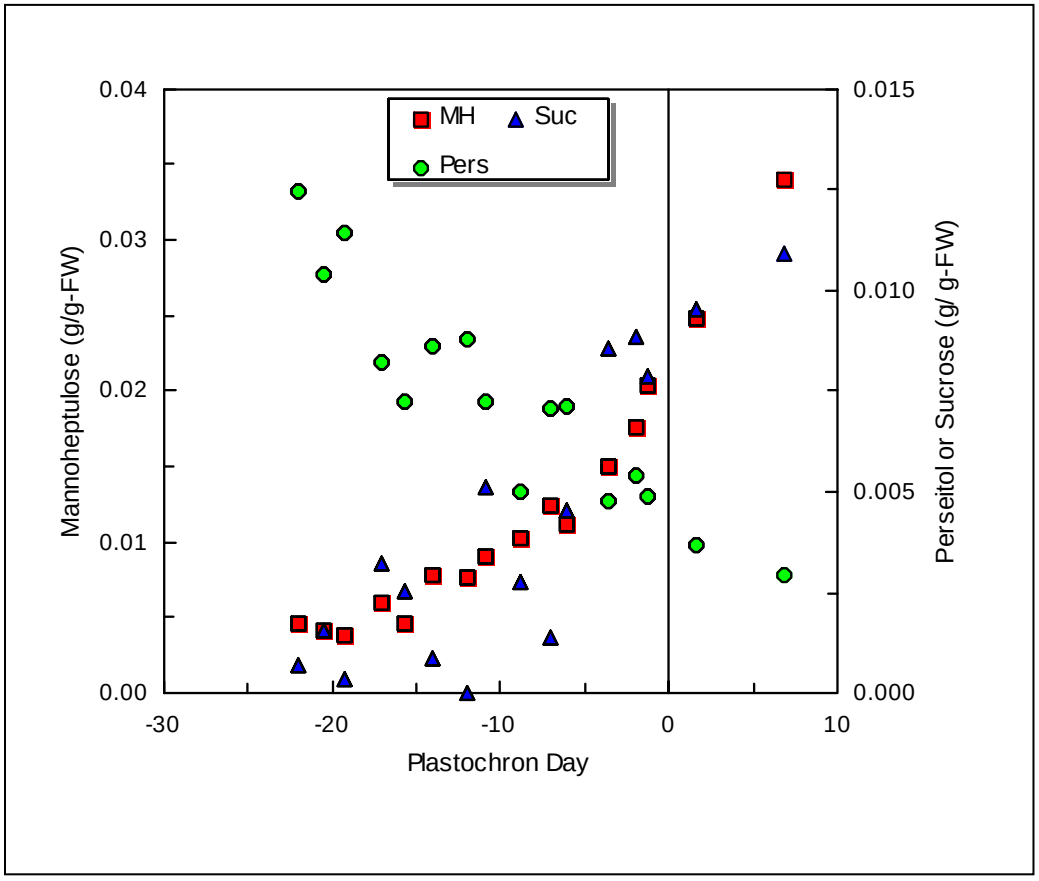


Figure 7. The Change of Total Leaf Area on Varied Flushes. A Hass Avocado Tree was used that possessed four independent flushes, starting with 1 (oldest) to 4 (youngest). The data are for the total areas of all the leaves upon the flush. Note the difference in scales (2x) between the youngest flushes (left side, 1 and 2) and oldest (right side, 3 and 4).

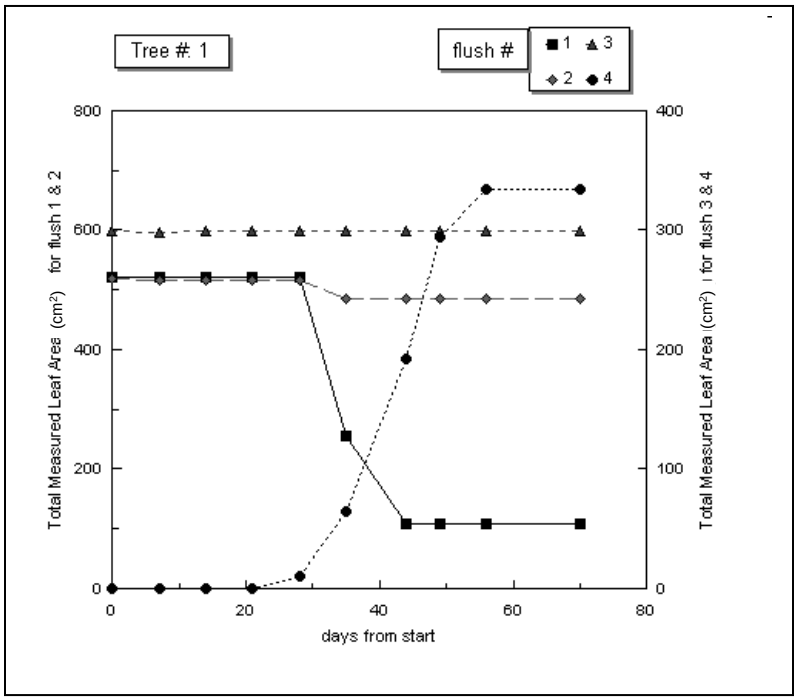


Figure 8. Logistic Equation and the Growth of the Leaves on a Flush. This model was made by using 16 leaves each with a growth rate of 0.23 cm^2-added-area/ cm^2-present-area /day and an initiation period for each leaf of 2.5 days. The maximum leaf areas were similar to what has been observed previously for normal Hass avocado flush growth with the first 3 leaves being smallest and the area of the remainder of the leaves alternating between 100 and 120 cm^2. The red line (crosses) represents the actual model of growth while the green (light crosses) is the best "fit" of a Logistic Curve to the data. The top two figures represent the fit (left side) and the error in the fit (right side). The bottom two figures are the center portion of the actual curve (the long linear region) and the earlier portion of the actual curve (which is following a near exponential growth curve, see axis).

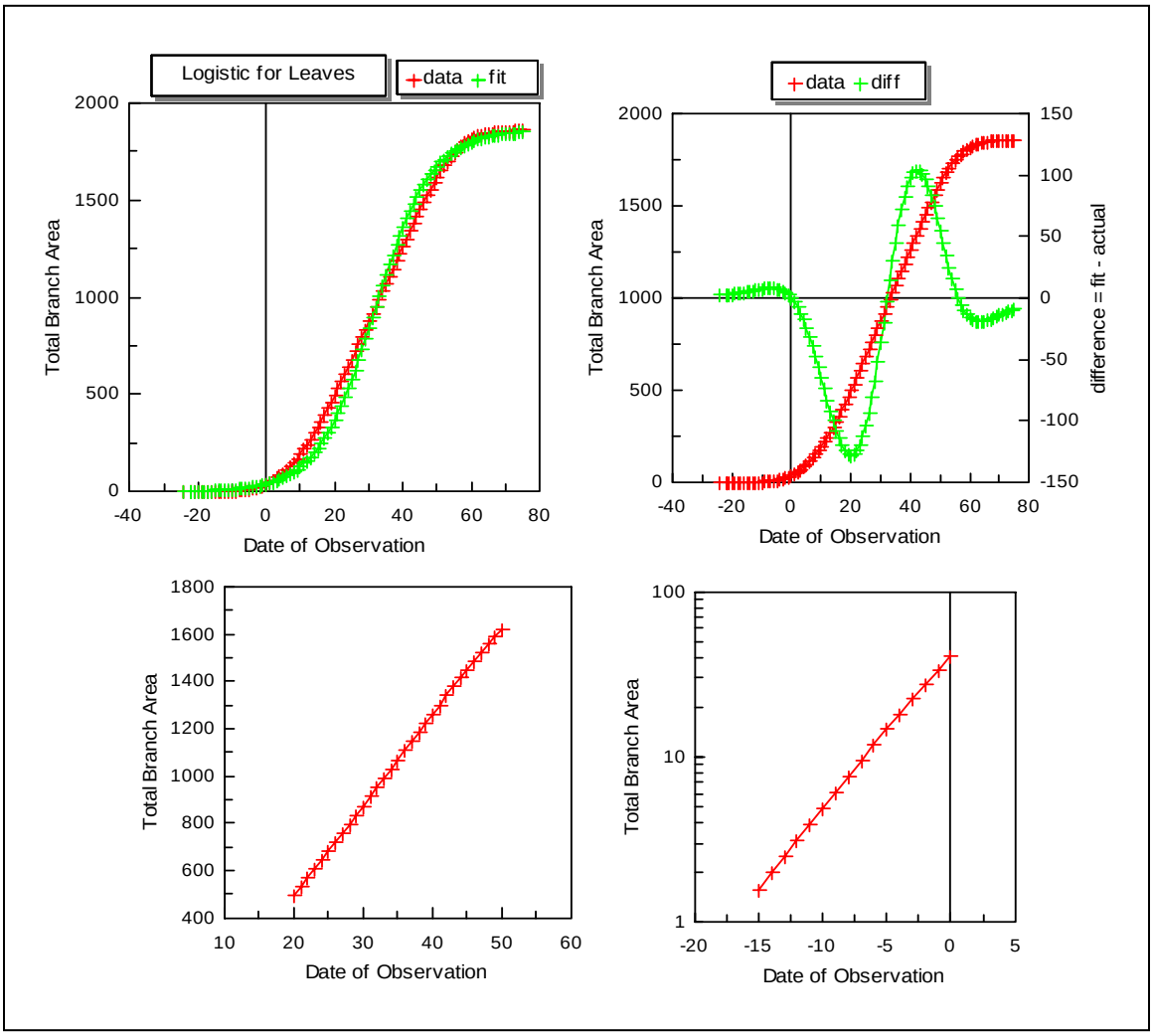


Figure 9. The Dependence of the Amount of Leaf Drop of an Older Flush upon Area on Branch or Flush. The square (red) symbols represent actual data for 10 trees based upon total area on Branch (three flushes) while the diamond (green) symbols are a linear fit to the data. This is for the previous flush (N-1).

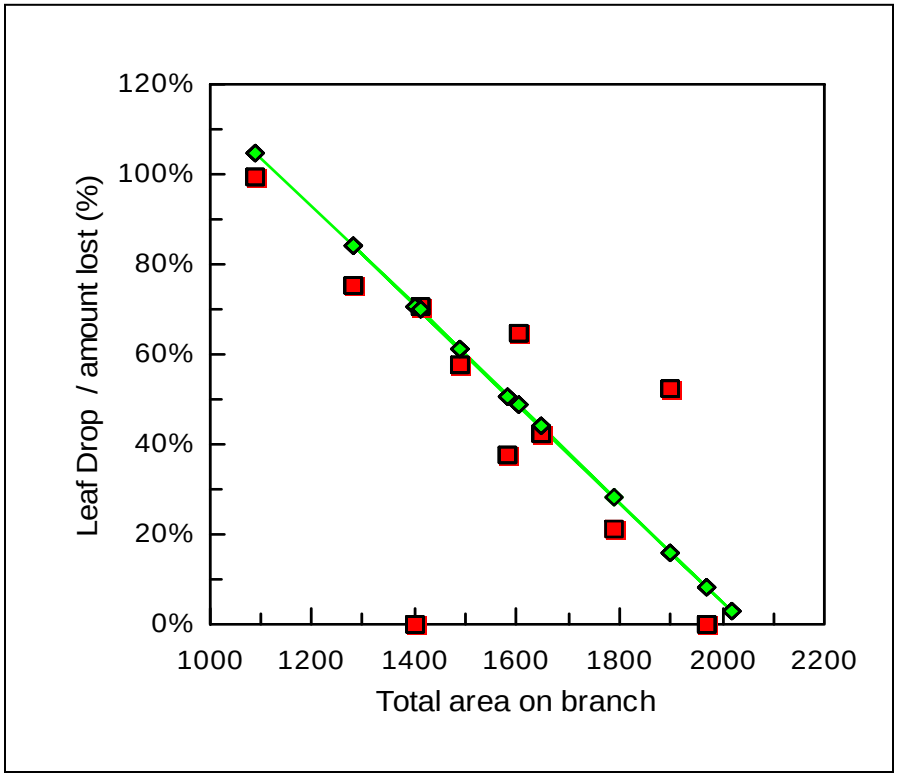


Figure 10. Schematic of Sap Flow Monitor. This system works by application of a heat load to the center of the system and monitoring temperatures of the outer portion of the center and the bark of the branch on both sides of the center. A. Fundamental construction of the monitor. B. Idealized plot of typical temperatures without and with sap flow.

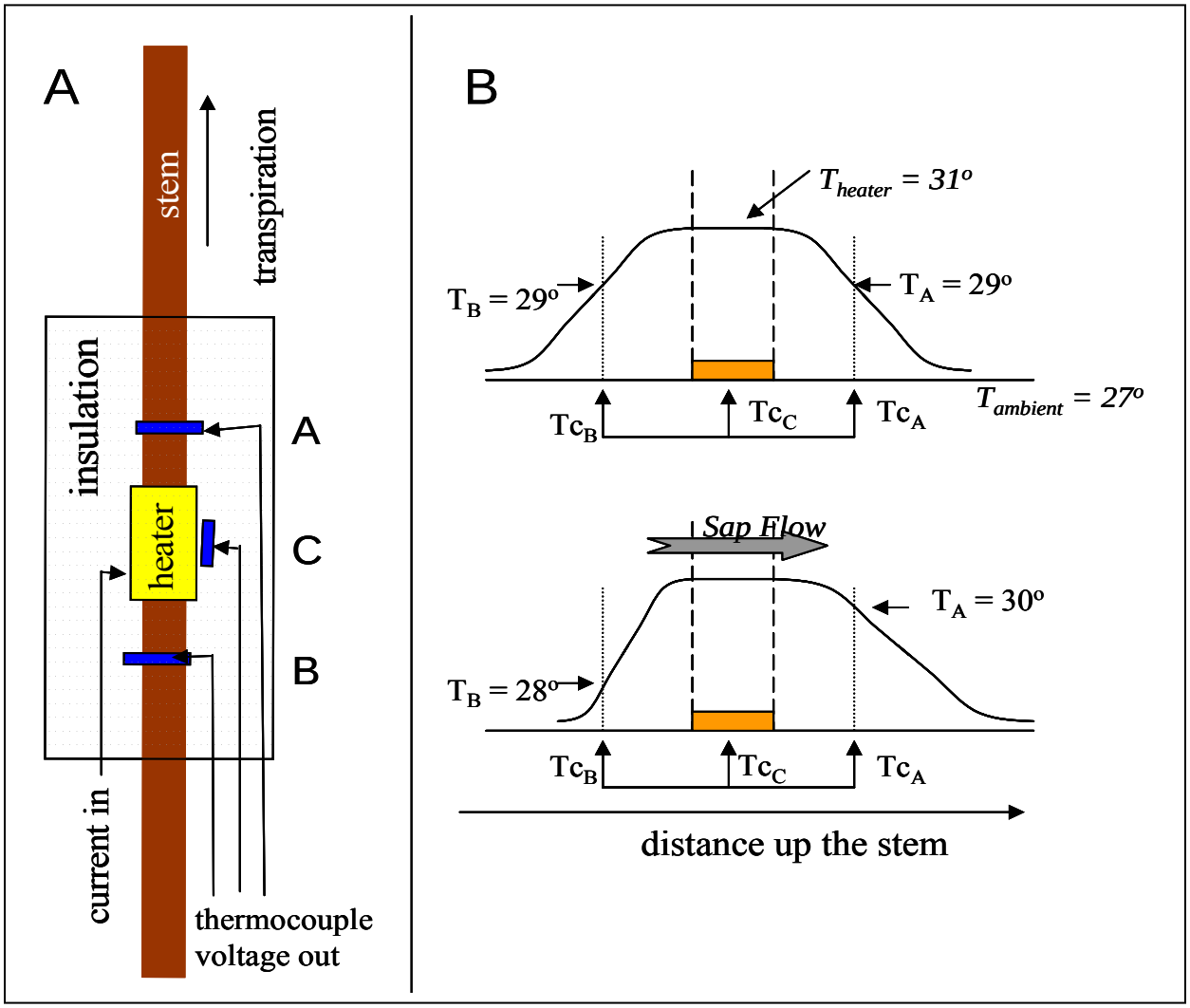
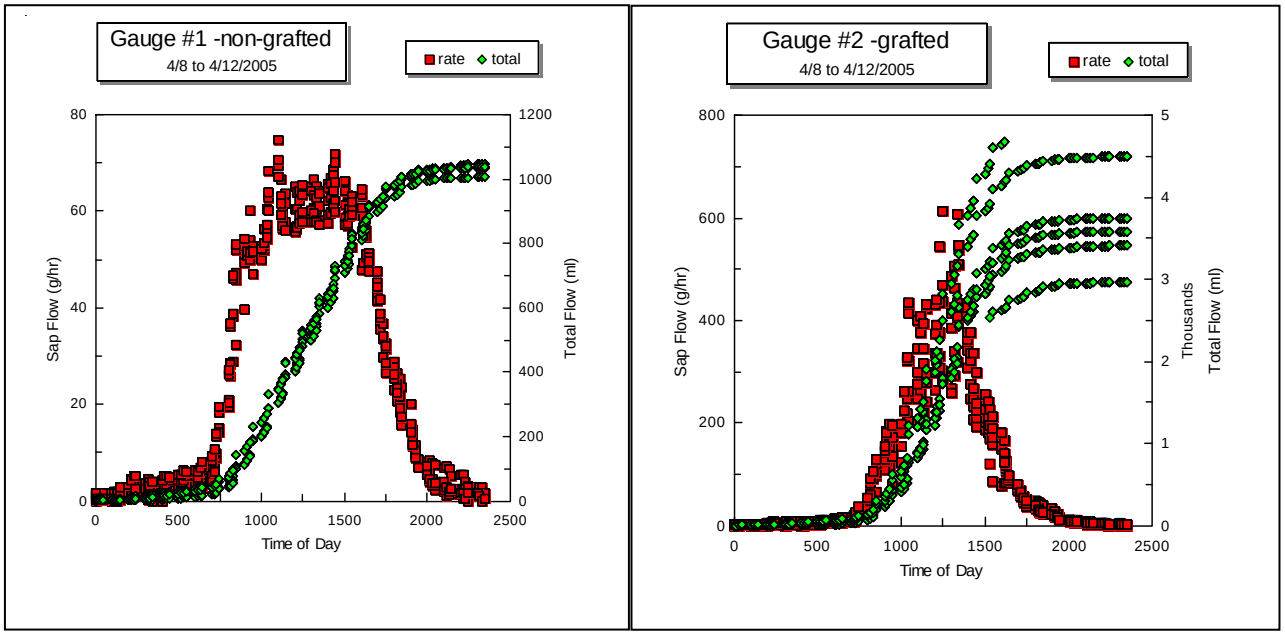


Figure 11. Daily Sap Flow through the Trunks of Two Hass Avocado Trees. The grafted tree is on Duke 7 root stock. The trees were about 2 years old and maintained in a green house in 3-gallon pots. They were watered twice a day by an irrigation system. The top curves are averages of five days of monitoring. The squares represent a sap flow rate while the diamonds represent total water flow over the course of a day. Note the difference in scales between the two plots. The bottom panel represents the average ratio of sap flow at different times in the afternoon divided by the sap flow of the same tree in the morning (10AM). At this time the flow was not at its highest value.



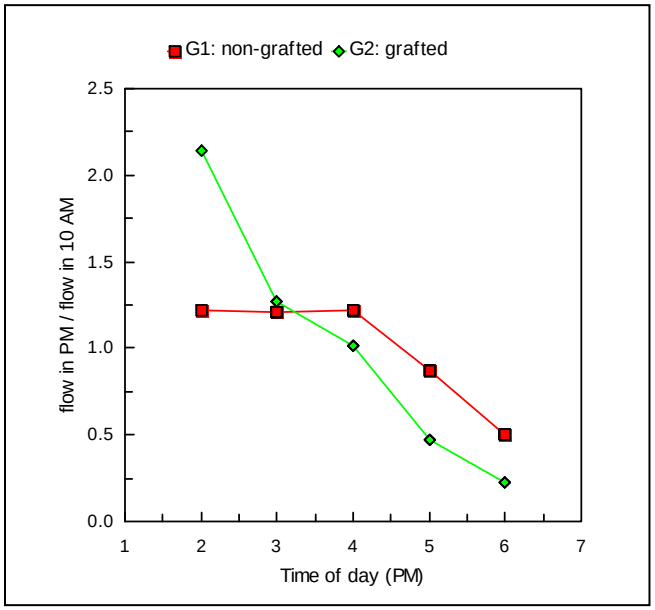
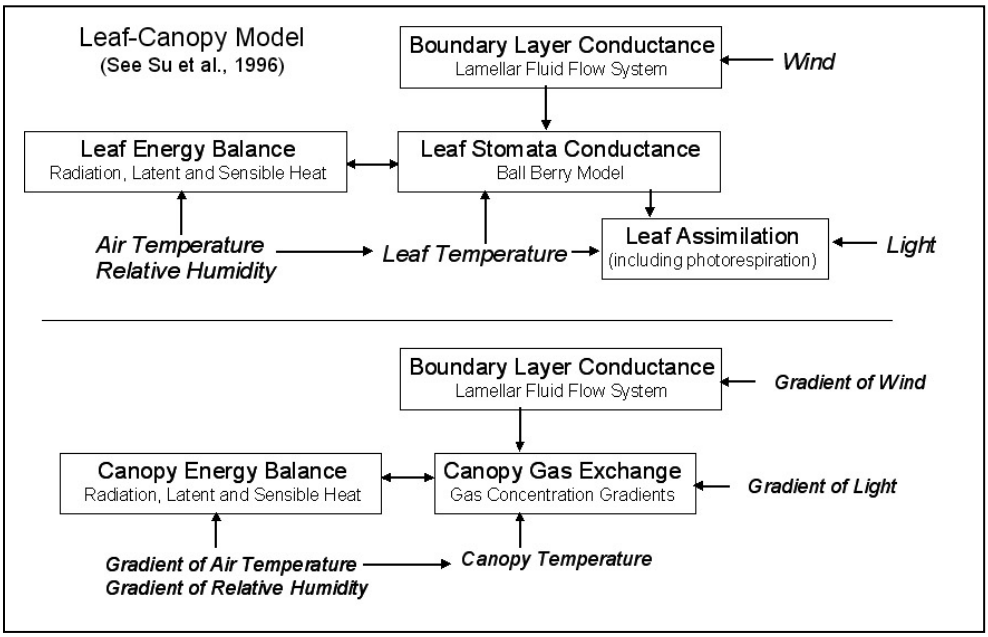


Figure 12. Schematic of Leaf Canopy Model. The fundamental processes are diagrammed in order to focus attention to important details of research. For more details, see the text.



Tables

Table 1. Variation of Light Dependence of Assimilation Parameters and Internal CO_2. A series of leaves were selected to test their assimilation response to external CO_2 (see left side of Figure 2). The conditions were stable at: air temperature = 24.45C $\pm$ 0.13; leaf temperature = 25.02C $\pm$ 0.02; PAR light intensity =699.7 $\pm$ 0.2 μmoles /m^2 sec, with a relative humidity which varied from 18-36% for tree 10 to about 55% for tree 5. The units of respiration and maximum assimilation were μmoles /m^2 sec and Γ_C was the compensation point, or the internal CO_2 concentration for zero assimilation in Figure 2. The two most right-most columns were determined as in the right side of Figure 2 and were the deviation of internal CO_2 with changes in external CO_2. This type of experiment was done over six weeks about twice a week during the New Zealand Summer with very similar results.

Tree	Leaf #	Γ_C (ppm)	Respiration	Maximum Assimilation	K'c (ppm)	max ΔCO_2 (ppm)}	K_{EC} (ppm)}
5	7	46.0	0.25	38.4 + 0.9	234 + 21	400 + 38	484 + 43
5	3	47.0	0.20	50.7 + 3.0	406 + 43	299 + 38	353 + 40
5	5	45.5	0.15	58.1 + 2.9	482 + 31	293 + 43	329 + 38
5	5	43.5	0.15	36.9 + 1.7	222 + 45	306 + 32	373 + 39
10	6	48.5	0.70	34.7 + 0.6	182 + 15	511 + 38	597 + 39
10	9	46.0	0.40	36.2 + 1.5	264 + 36	444 + 41	543 + 46
	average	46.1 + 1.5	0.31 + 0.19	42.5 + 8.7	298 + 108	376 + 83	446 +101

Table 2. Dependence of Assimilation upon Carbon Dioxide Concentration. The data were collected by a Licor 6400 porometer and evaluated as discussed in Figure 2 and the Text. The compensation point due to photorespiration was subtracted from the measured assimilation data before application of the Hanes-Woolf Plot to linearize the data to determine the enzymatic kinetics. The ratio (end column) was on the basis of maximum assimilation rate divided by the tabulated photorespiration. The flush # corresponds to recently-matured, fully-expanded leaves (Flush N), and leaves from the previous (older) flush (Flush N-1 & the oldest, N-2)

Flush #	Maximum Assimilation (μmole/m^2 sec)	Photorespiration at 400 ppm CO_2 (μmole/m^2 sec)	K_C' (ppm) half saturation of assimilation rate	$\frac{\text{Activity of } CO_2}{\text{Activity of } O_2}$
N-2	26.3 $\pm$ 4.4	- 9	184 $\pm$ 25	2.9
N-1	31.5 $\pm$ 4.0	-8	227 $\pm$ 25	3.9
N	33.0 $\pm$ 3.5	-8	181 $\pm$ 16	4.1

Table 3. The Temperature Environment on Photosynthesis and Stomata Conductance. Hass avocado trees on clonal Duke 7 rootstock were raised in two separate growth chambers maintained at difference temperatures (set at 18 and 28, but having actual temperatures of 14 and 25C, respectively). Measurements made at a light intensity of 1610 $\pm$ 50 μmol /m^2 sec and a relative humidity in both chambers of 47 $\pm$ 2 %.

Within the Leaf Chamber

Chamber / Set Temperature		Photosynthetic Assimilation (μmol/m^2 sec)	Stomata Conductance (mol/m^2 sec)	Transpiration (mmol/m2 sec)	Vapor Pressure Deficit (kPa)	Temperature Difference (Leaf - Air)
18 Cabinet	Average	8.94	0.146	1.84	1.35	1.24
	Std. Dev.	1.32	0.057	0.62	0.08	0.30
	Std. Error	0.27	0.012	0.13	0.02	0.06
28 Cabinet	Average	15.58	0.251	4.83	2.06	0.41
	Std. Dev.	1.74	0.049	0.92	0.19	0.30
	Std. Error	0.35	0.010	0.19	0.04	0.06
difference (28-18)		6.64	0.105	2.99	0.71	-0.83
	t-test	0.000%	0.000%	0.000%	0.000%	0.000%

Outside the Leaf Chamber

		Actual Air Temperature (C)	PAR Intensity (outside)	Internal CO_2 (ppm)	Air Water Vapor (kPa)	Relative Humidity (%)
18 Cabinet	Average	14.00	743.7	263.2	13.22	49.6
	Std. Dev.	0.09	180.7	42.6	0.45	1.66
	Std. Error		36.9	8.7	0.09	0.3
28 Cabinet	Average	25.00	966	258.2	21.59	46.5
	Std. Dev.	0.06	204	21.0	3.07	6.3
	Std. Error		42	4.3	0.63	1.3

T**able 4**. Quantum use efficiency (ϕ), maximum photosynthetic rate (A_{max}), light compensation point (LCP), and dark respiration rate (R_d) of recently-matured, fully-expanded leaves (Flush N), and leaves from the previous (older) flush (Flush N-1). The quantum use efficiency (ϕ) is really the A_{max} / K_I and so K_I is derived from those calculations.

		ϕ	A_{max}	K_I	LCP	R_d
18°C	Flush N	0.060	21.89[z]	365	14.31	0.85
	Flush N-1	0.046	12.19	265	8.74	0.42
28°C	Flush N	0.081	26.48[z]	360	19.21[z]	1.57[z]
	Flush N-1	0.070	15.54	222	9.66	0.68
Significance	Temp	***	**	n.s.	n.s.	**
	Flush	**	***	**	***	***
	Temp*Flush	n.s.	n.s.		n.s.	n.s.

[z] Values for flushes within a temperature regime are significantly different from each other.

Table 5. Dependence of Parameters Governing Light Driven Assimilation with Flush Age. Flush # correspond to recently-matured, fully-expanded leaves (Flush N), and leaves from the previous (older) flushes (Flush N-2 is oldest)

units of μmol/m2 sec

Flush #			Respiration Rate	Maximum Assimilation	Intensity at ½ Saturation
N-2		Average	-0.91 ± 0.18	17.3 ± 2.7	243 ± 26
N-1		Average	-0.69 ± 0.07	15.79 ± 2.1	219 ± 27
	t-test	N-1 vs N-2	11%	48%	30%
N		Average	-1.54 ± 0.21	30.1 ± 6.0	563 ± 401
	t-test	N vs N-2	**0.82%**	**1.53%**	22%
		N vs N-1	**0.06%**	**0.81%**	19%

Table 6. Loss and Growth of Leaf Area in Recent Flushes. Flush N-2 is the oldest flush while the youngest (newest) is flush N. The maximum area of each flush is the total of all the leaves on the flush after they reach maximum size. The total is the sum of the areas of all the flushes on a branch. The leaf drop of the oldest flush (generally flush 1) was not total and varied a great deal. The growth of Flush N was fit with a logistic curve and although the fit was not perfect, the average regression for all trees was 0.9845 $\pm$ 0.0038 (se).

Tree #	Maximum area (cm^2)				Leaf Drop		Logistics growth	
Flush # >	N-2	N-1	N	Total	**Flush #**	Area lost	Growth (/day)	days @ PC=0
1	141	783	721	1645	**N-2**	42%	-0.233	24.6
2	179	869	556	1604	**N-2**	65%	-0.383	44.5
3	197	848	539	1584	**N-2**	38%	-0.260	30.5
4	60	617	726	1403	**N-2**	0%	-0.247	30.4
5	41	720	1209	1969	**N-2**	0%	-0.235	32.9
6	174	1096	631	1901	**N-2**	53%	-0.195	19.8
7	191	998	831	2019	**N-3**	0%	-0.248	40.6
8	75	624	581	1280	**N-1**	75%	-0.255	24.3
9	124	781	583	1488	**N-2**	57%	-0.287	43.2
10	271	761	380	1411	**N-3**	71%	-0.289	14.9
11	140	170	780	1091	**N-1**	99%	-0.293	55.7
Average	155	710	678	1598		47%	-0.265	34
Std. Error	21	78	60	82		9%	0.013	3

Table 7. Fundamental Parameters of Photosynthetic Rate, Area, Dry Weight and Water Relations of Leaves and Flushes on Avocado Trees. Each flush is given at the top of each table with the nomenclature of N (youngest flush) and older flushes counting back (e.g., N-1).

A. Number of leaves of each flush for a single shoot per tree. Bold numbers indicate flushes that developed during the experiment. First number is total number of leaves and the second is the number of leaves at the end of the experiment.

Tree	N-2	N-1	N
1	7/6	10/10	**9/9**
2	11/7	**9/9**	**5/5**
3	4/2	9/9	**9/8**
4	3/1	10/10	**8/8**
5	2/0	10/10	**9/9**
6	1/0	13/12	**12/12**
7	10/9	9/9	**7/7**
8	8/3	**11/11**	**10/10**
9	11/9	8/8	**7/7**
10	4/3	**9/8**	**7/6**
11	9/1	10/9	**6/6**
12	5/0	9/9	**10/10**
13	11/10	**9/9**	**12/11**
14	11/10	**9/9**	**6/5**
Average	6.9	9.6	8.4
Std. Dev.	3.6	1.2	2.1

B. Photosynthesis of a single leaf of each flush in a single shoot per tree. Bold numbers indicate flushes that developed during the experiment. Net CO_2 assimilation ($\mu mol \cdot m^{-2} \cdot s^{-1}$)

Tree	N-2	N-1	N
1	1.19	**6.35**	**8.0**
2	8.15	**10.1**	**9.38**
3	3.37	**6.83**	**8.03**
4	7.59	**13.2**	**11.2**
5		**10.2**	**12.8**
6			**2.21**
7	3.83	**6.09**	**6.45**
8		**6.28**	**7.23**
9	9.41	**14.0**	**9.42**
10	4.02	**6.51**	**9.37**
11		**10.2**	**11.2**
12	8.29	**3.53**	**8.18**
13	0.90	**1.06**	**2.15**
14	0.52	**0.79**	**1.06**
Average	4.73	7.32	7.62
Std. Dev.	3.20	3.96	3.45

C. Total leaf dry weight (in grams) of each flush in a single shoot per tree. Bold numbers indicate flushes that developed during the experiment.

Tree	N-2	N-1	N
1	4.20	9.60	**10.60**
2	11.30	**12.66**	**3.31**
3	2.14	12.48	**8.88**
4	0.89	11.18	**7.54**
5	0	8.54	**10.75**
6	0	2.17	**19.84**
7	13.53	9.53	**11.56**
8	0.28	**14.33**	**13.10**
9	6.55	7.54	**8.84**
10	1.41	**10.12**	**8.14**
11	0.06	10.77	**5.33**
12	1.08	9.67	**10.30**
13	14.50	**12.29**	**16.45**
14	5.40	**9.61**	**6.20**
Average	5.11*	10.03	10.02
Std. Dev.	5.05	2.79	4.18

D. Xylem potential (in MPa) of a single leaf of each flush in a single shoot per tree. Bold numbers indicate flushes that developed during the experiment.

Tree	N-2	N-1	N
1	-0.33	-0.40	**-0.24**
2	-0.47	**n/a**	**-0.25**
3	-0.42	-0.17	**-0.20**
4	-0.85	-1.18	**-0.16**
5		-0.20	**-0.40**
6		-0.60	**-0.24**
7	-0.67	-0.41	**-0.43**
8	-0.86	**-0.90**	**-0.45**
9	-0.41	-0.37	**-0.35**
10	-0.32	**-0.32**	**-0.26**
11	-0.5	-0.45	**-0.25**
12	-0.4	-0.28	**-0.17**
13	-0.4	**-0.20**	**-0.22**
14	-0.2	**-0.29**	**-0.16**
Average	-0.49**	-0.44	-0.27
Std. Dev.	0.20	0.28	0.09