Proceedings of the California Avocado Research Symposium, October 30, 2004. University of California, Riverside. California Avocado Commission. Pages 65-88.

Avocado Tree Physiology - Understanding the basis of Productivity

New Project: Year 3 of 5

Primary Researchers: R. L. Heath, M. L. Arpaia
*Cooperator: Michael V. Mickelbart**
University of California
Dept. of Botany and Plant Sciences
University of California
Riverside, CA 92521
* (MVM) Soil, Plant & Ecological Sciences Division
P.O. Box 84, Lincoln University
Canterbury, New Zealand
Phone: 909-787-5925 (RLH); 559-646-6561 (MLA), 64-3-325-2811 ext 8202 (MVM)
FAX: 909-787-4437 (RLH); 559-646-6593 (MLA), 64-3-325-3843 (MVM)
E-mail: heath@citrus.ucr.edu; arpaia@uckac.edu, mickelbm@lincoln.ac.nz

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 irrigation schedule of orchards and management of tree size. Also 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 examines 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 of this Year's Progress

We have made progress on many fronts but two topics—leaf growth and temperature optima—have been largely described, leading to much better understanding of how we can follow flush development and why extreme California afternoons are a problem for production.

We have developed a methodology to measure leaf growth that allows us to place leaves into a coherent physiological development sequence and place branch development into coherent pattern. In other words, a few non-destructive measures on several developing leaves of a branch can give us a time scale of the full branch's development and a measure of the growth rate of that branch. This protocol has been a remarkable development of our measurements and allows for placing each of the flushes into their proper physiological context.

We have a concept of how varied air temperatures affect the rate of photosynthesis, as limited by the stomata conductance, and of dark respiration. It seems that the level of the CO_2 level inside the leaf (internal CO_2) plays an important role in control, in that as the temperature rises, the internal CO_2 becomes closer to the ambient with the assimilation rate leading stomata conductance. This means that photosynthesis *per se* is what higher temperatures are affecting, rather than stomata conductance. This raises two questions: [1] how long of a duration can high temperature be present before damage permanently alters the tissues and [2] does the loss of water and subsequent closure of the stomata, while lowering net productivity, actually help the survival of the tissue. Any field methodology used to change either air temperature or relative humidity, including when to start and how long to maintain, will be set by these findings.

Using a combination of varied measurement technologies, we have made progress in understanding the relative importance of the boundary layer around the leaf and within the canopy to gas exchange. Following of the leaf temperature by an infra-red camera has given us the tool to determine both the boundary layer interaction and the actual leaf temperature with respect to the air temperature. Under many conditions, wind does count in altering the real productive, as controlled by gas exchange.

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

Details

Leaf Development

When we began our work, the community did not have a good method for describing how to measure avocado leaf development. There was no understanding of what measurements to make to determine where the leaf was in its normal physiological process from initiation to senescence and how it fit within the productivity of the branch, which contained it. We suspected that changes occurred in its time scale but did not know if that time scale was predictive in the leaf's productivity. Furthermore, it was not obvious of how a leaf's productivity was linked to the full productivity of the branch and each of its flushes. That total productivity would seemingly be linked to the production of fruit, but we had no simple linkage mechanisms. This is especially true in California, which has two flushes per year, where we suspect that one flush feeds the flower production and the other feeds the fruit production.

We suspected that young leaves were dependent upon mature portion of plant but, under some conditions, very old leaves were a drag upon the plant's efficiency and therefore were prime targets for abscission. To follow both the flushes and abscission efficiency, we needed to be able to determine a leaf's age with a few simple measurements. Then we could follow that age as we measure the efficiency of the leaf and how it relates to each flush on a branch.

A major accomplishment this past year has been to [1] determine a correlation between width and length, and area of the leaf, although it is variety dependent, [2] show that the area can be found accurately by using only length measurement, and [3] find that area can be correlated with leaf age. The leaf position along the flush (leaf number) can be correlated with leaf age and therefore flush age, according to each branch. Surprisingly, once a flush initiates the leaf production, its growth is surprisingly constant. Each leaf develops according to the flush and branch, rather than randomly growing. Most importantly, we have found that we do not have to take many measurements on individual leaves for a single branch. One measurement for every other leaf along the branch once a week is more than adequate to describe the leaf growth and the full flush growth characteristics—including the date of flush initiation and specific growth rate of each leaf.

Leaf Growth

In order to determine how photosynthetic assimilation (in the leaf) changes during leaf and fruit growth, it is important to understand the relationship between leaf physiological and chronological ages. Our major obstacle was the determination of the physiological age of a leaf. It was not possible to do this using a "single" measurement; rather we have resorted to developing a model which estimates an accurate growth curve for all leaves. The literature describes several types of growth functions, many of which only fit over one portion of the growth; however, the most reasonable model was the Logistic function since it duplicates most of the phases of leaf growth. The Logistic function possesses a rapid increase phase when the leaf is very small, followed by a shift to an exponential rise phase to a maximum size. The Logistic function is very similar to the Gompertzian function, which has been previously used for growth of animals and populations. While it seems that those differences are relatively trivial when both are compared, most series of measurements can show that the Logistics curves fit the

real growth more precisely[1]. The Logistics function has been often used in plant research and so we follow that tradition.

Briefly the Logistic function is described by leaf area (A) as follows, in which the growth rate is limited by the maximum size (A_{max}) but has a characteristic rate (κ):

$$dA / dt = \kappa A [(A_{max} - A) / A_{max}] \quad [1]$$

In integrating this equation we obtain a more useful equation ([2]) in which the symbols are given by t = time, A_{max} = maximum size of leaf, with α being an integration constant. If this equation is solved for leaf area, we obtain:

$$A = A_{max} / [1 + \exp (\alpha - \kappa t)] \quad [2]$$

This can be converted into a linear plot easily by some simple algebra and becomes:

$$\text{Ln} [(A_{max} - A) / A] = \alpha - \kappa t = \psi(A) \quad [3]$$

Thus, a plot of $\psi(A)$ verses time (t) yields a straight line from which we can obtain the value of κ (the slope of the plot) and α {= κ t', where t' is the value of time when Ln $[(A_{max} - A) / A] = 0$ or when $A = A_{max} /2$, the half-grown leaf}. Thus, if we have several data points of A during the growth phase and the final size of the area (A_{max}), we can find α and κ for the leaf. The value of "α"gives a measure of when (in time) the leaf is half-grown. If there is a sequence of leaves growing under the same conditions (in which the growth rate, κ, is constant), then the value of "α" increases with leaf number[2].

We have found that under relatively constant conditions (in a growth room, data described in the figures):

[1] the "κ" values for a branch are virtually the same but that the value for each leaf does decreases somewhat with more recently formed leaves on that branch;

[2] the "α" values increase uniformly with leaf number, signifying a set-period between each leaf initiation act;

[3] the maximum size of leaves rises nearly 50% over the first 4 to 5 leaves but that size does vary somewhat over all the following leaves[3];

[4] while each branch has approximately the same "κ" value, the "starting-α" value (the time of initiation of the first leaf) varies between branches and trees.

This analysis can be used in a green house situation in which variability of growth is much greater for measurements that are made only twice a week. In essence, we have found our method for age analysis—a simple method of occasional measurements which gives us the leaf size for any time point and fixes one point of the growth in time (the half-grown size) from which all other events can be measured.

---

[1] In the field the growth conditions is not constant due to variation in the ecological conditions and frequent measurements can show this. Unfortunately that then makes the fitting (described below) of the area to a logistics curve more difficult. Strangely we found that fewer measurements actually leads to the ability to fit a curve which is an average growth rate (in turn an average of the environment). Under these conditions it is difficult to determine the difference in these two curves.

[2] Leaf number is an important factor in this analysis. The numbering system is not arbitrary, but rather leaf 1 must be the first leaf to appear on the branch and the highest leaf number is the last leaf to appear with all others being sequential (see Figure 1).

[3] Later we will show that the morphology of the leaf (length & width) likewise changes from first initiated leaves to the later ones.

Theoretical Analysis

Our analysis of what the Logistic function signifies mechanistically is based upon Thornley's discussion (1990) in which the foundation of the use of the Logistic plot to describe leaf growth is laid. Thornley uses two interrelated equations to formulate how two variables interact to allow non-linear growth. Equation [4] is based upon productivity of photosynthesis (the "y" variable), which allows the area of the leaf (the "x" variable) to control its net productivity rate. Equation [5] is based upon decreasing the amount of photosynthetic capacity, used for the individual leaf growth; the decrease is due to the development of export capacity of the leaf to other sink regions of the plant[4].

$$dy / dt = \mu_1 x \qquad [4]$$

$$dx /dt = \mu_2 x e^{-b y} \qquad [5]$$

The two constants (μ_1 and μ_2) govern the growth of the leaf and the production of carbohydrate, both based upon the leaf area. The combination of these two equations allows for the defining equation of the logistic equation, as shown in equation [1].

$$d \{(1/x) (dx /dt) + b \mu_1 x\} / dt = 0 \qquad [6]$$

The solution of this equation is:

$$(1/x) dx /dt + b \mu_1 x = \text{constant} \qquad [7]$$

in which the constant should be defined as:

$$\text{constant} = \mu_2 + b \mu_1 x_i \qquad [7']$$

We will return to the x_i definition later. Here when $dx /dt = 0$ or no net change in the area (x), the x (at that point, x_f) is the maximum area of the leaf, and so using equation [7]:

$$(b \mu_1) x_f = (b \mu_1) x_i + \mu_2 \qquad [8]$$

$$x_f = x_i + \mu_2 /(b \mu_1) \qquad [8']$$

Combining all these relationships we obtain:

$$(1/x) dx /dt + b \mu_1 x = b \mu_1 x_f \qquad [7"]$$

$$(1/x) dx /dt = \mu_1 b (x_f – x) \qquad [9]$$

$$dx /dt = \mu_1 b x [x_f – x] = \mu_1 b x_f x [(x_f – x)/ x_f] \qquad [9']$$

which is directly related to the defining equation of the Logistic equation, where:

$$dA / dt = k A [(A_{max} – A) / A_{max}] \qquad [1]$$

Here with A = x, then $x_f = A_{max}$ and $\kappa = \mu_1 b x_f = \mu_1 b A_{max}$.

Results

One of the most important goals is to maintain a labeling sequence for the leaves on a branch. The two (or more) flushes must be separated as shown in **Figure 1**. Furthermore, the leaves

---

[4] Both x and y are in units of g-DW but x stands for total weight with area as the surrogate for weight and y is for assimilation as carbon. Equation [4] represents total assimilation with y in units of g-DW /sec and so μ_1 is in units of sec^{-1}, and is proportional to κ. Equation [5] represents units of g-DW or m^2-area /sec and as such the units of μ_2 is likewise in sec^{-1}. Additionally "b" must be in units of $g\text{-}DW^{-1}$ in order for the exponential to be unit-less.

should be numbered from the first leaf of a flush to the last leaf (larger number, often 12 to 18 in most cases). The point of this exercise is to be able to easily monitor non-destructively the growth of each leaf and relate that to a physiological process. Further this monitoring must be such that missing a few days of observation does not handicap the analysis process.

Firstly we built upon our previous observations that the length and width of a leaf were related. Using a series of leaves from 'Hass' trees (on clonal Duke 7 rootstock) maintained in a green house, we measured the length, width and area[5]. **Figure 2A** demonstrates that the length times the width of a leaf is proportional to the area; this means that length *times* width *times* a constant factor (L *x* W *x* f) is equal to the area of a leaf. Thus, we do not have to scan the full area of the leaf but can use only length and width to obtain a measure of the area. Furthermore, with some small amount of error, only the length can be used to obtain a measure of the area if that length is squared (see **Figure 2B**). Again a nearly constant factor (g) times the length^2 can be used as the area. The error is due to the variation of width divided by length with leaf number. The first leaves tend to be broader than the later leaves, but there is some variability (see **Figure 3**). Thus, for our determinations we used [L *x* W *x* g] as the area.

Typical leaf growth curves are shown in **Figure 4**. These data are from Mickelbart (for growth of branches during 2003 in New Zealand in a controlled growth chamber) for 'Hass' avocado. The area has been determined as above for the first four leaves of Tree 1 & Branch 1. We determined full leaf growth for at least three branches on nine trees. While there is variation in area, the general trend is that each leaf slowly increases its rate of growth until it is about half its maximum size and then the growth rate slows ultimately ceasing when the maximum size of the leaf is reached. The period required for a leaf to reach maturity (maximum size) is about 26-32 days.

If the area data for one leaf is transformed into a Logistic expression $\{\text{Ln}\,[(A_{max} - A)/ A]\}$ is plotted versus the chronological time scale (starting a fixed date as time = 0), we obtain a straight line with a declining in value with time. For each leaf on a branch, the measured area is fit to a logistic curve giving a measure of two parameters (α and κ). In some cases due to insufficient observations we have to estimate the maximum size of the leaf. From a least squares regression fit, an estimate of the time (in terms of days of observation) that the leaf required to reach a 50% size (α / κ, in days) is calculated. That value of time to reach a 50% size is subtracted from the days of observation and denoted as the plastochron day; all leaves reach 50% of their maximum size at zero platochron day. The leaf size is likewise scaled to a percentage of the maximum size (by dividing the maximum area into the observed area). Those calculated data are then plotted for all leaves on a given branch (see **Figure 5A** for one shoot/branch). The data are uniform and seem to follow a single curve with the variation in data (denoted by crosses) being quite small. That total data (all points) are then fit to a branch/shoot Logistic curve (see **Figure 5B** for the linearly transformed data). The constant (α) and slope (κ) for each branch are relatively constant for each branch of the same tree. Further under uniform growth conditions those values are nearly the same for several trees (e.g., the nine that we measured here), although the date of initiation for the first leaf varies with each branch[6].

---

[5] The leaves were scanned and then the area was determined from the number of pixels that the scanned image had. The calibration was with a known area of paper.

[6] The date of initiation of the first leaf of the branch is somewhat arbitrarily denoted as the zero plastochron day of a non-existent zero leaf.

For three separate branches on one tree (#2), the data for these parameters are uniform. In **Figure 6A** the leaf area (given as square dimension) becomes larger with leaf number, reaching a large size after the emergence of about 5-8 leaves. There is some evidence that the maximum size of each leaf varies somewhat for the higher numbers and may even vary between every other leaf from very large to somewhat large. This variation makes mere guessing the final size difficult and thus each leaf should be measured until it reaches maximum size (A_{max}). In **Figure 6B** the number of days to reach 50% of the maximum size of the leaf (zero plastochron day) seems to vary linearly with leaf age. The time between each leaf (the slope of this plot) is constant at 2.3 days, the time between leaf initiations. While we expect the value of κ to be constant, it seems to decline by about 20-30% from the first to the last leaf. The scatter of the data is large and often an average of all the κ values is adequate (see **Figure 6C**). The values of α ultimately rise, which is expected since it should nearly linearly track the increased value of the initiation (to 50% of the maximum size) (**Figure 6D**). We have summarized all the data from this set of experiments (27 branches in all) and the variations of the growth rate and initiation rate of each leaf and branch are very small (less than 15%) under uniform growth conditions, which indicate how well the data fits to the theory.

Based upon this extensive data set we have developed a computer program to carry out the fitting of the data to the Logistic formula easily and have successfully used this technique to obtain growth patterns under several greenhouse conditions. We are currently using it to predict how old (plastochron age) a given leaf, is based upon its measured area (using length times width) in other studies.

This concept was found by studies in a growth chamber in which the amount of light and its directionality is fixed along with the relative humidity and temperature. This, of course, is not a usual situation but allowed for the determination of the relation of leaf growth. We have continued these studies to test their applicability to the "real world" through greenhouse studies (in which the air temperature and relative humidity was held constant) but the light varied normally throughout the day and a retrogressive study of field data collected a few years ago.

The greenhouse data (with constant temperature and variable light) was done with a longer spacing between each time points. Under these conditions, the light variability between days and the assumed difference in growth seems to average out. In other words, the data set can not see day to day variation but the increase in leaf area does fit a logistics curve in which the growth constant is the average over several weeks of growth. The field data collected in Irvine during spring and early summer (*from Xuan and Arpaia, 1998*) was taken every three weeks. Again the number of leaves within a flush and their timing off set gives rises to another possible fit to the logistics curve, but again an average growth rate and initiation time is found. Thus, the system seems to work well regardless of the timing of the data, but under variable environmental conditions, the average values are the best for understanding total productivity and for ease of data collection.

We are continuing these studies for flush development. Here the production of a new flush gives rise to a loss of the older flush, but not completely. We seem to have three cases of leaf loss—full loss of leaves, loss of half the leaves, and no loss from the older flush. We do not understand what conditions lead to what types of leaf loss but our fundamental hypothesis is that a balancing act exists between production and the flushes. The older flush "feeds" the younger flush initially but once the younger flush becomes able to contribute enough carbon to the newly growing leaves of that flush, the older flush is not required. However, the older flush does have the

ability to give more carbon to any developing fruit on that branch. It is our hypothesis based upon other work that one specific branch does not easily transport carbon to other branches. The question remains as to what causes the leaves to fall off of older flushes? It is a light limitation as new flushes on other branches shade the older branch or do plant hormones play a critical role in shifting the older flushes efficiency or need for continuation? We simply do not know at this point; however, evidence points to the role of light, intensity and duration.

Interestingly, the conditions in New Zealand can support up to 4 flushes at a time on a small tree. For our study of 12 trees, leaf loss was proportional to total leaf area. An individual branch seemed maintain a constant total leaf area at the expense of older leaves.

The Effect of Temperature on Photosynthetic Efficiency

We believe that temperature plays a large role with the problems associated with carbon assimilation in the afternoon. Our earlier work with humidity showed that low humidity caused excessive water loss from the leaf, especially when the stomata were highly open in the morning. This excessive water loss seemed to lower the water potential of the leaf therefore inducing an early closure of the stomata. This will consequently limit the assimilation of carbon dioxide into carbohydrates and so influences overall tree productivity. Yet when relative humidity declines during many summer afternoons, most of that decline is due to a rising temperature of the air. From a plant perspective this is expected to increase respiration and to lower assimilation. The question we are asking in these studies is while the stomata limit assimilation in avocado under many conditions, does this limitation also hold for higher temperatures?

In order to answer this question we first needed to observe how air temperature affected the normal assimilation, respiration and water vapor exchange of avocado leaves. We have a growth chamber available in which two trees can be maintained for about three weeks. In that chamber we can maintain both a set temperature (from about 21 to 37 C; 70 to 99 F) and a set relative humidity. Typically the chamber is maintained at a day temperature and relative humidity of 28C (77F) /40% and a night temperature and relative humidity of 15C (59F) /80%, with 12 hours of constant light (200-300 μmoles of light /m^2 sec) for the day. Twice a week (Tuesday and Thursday) we subject the trees to a changing temperature program as shown in **Figure 7**, starting at about 10AM. The chamber temperature is raised in steps from 20C (68F) to 36C (97F). After stabilization of air temperature and stomata conductance (requiring about 40 minutes), we measure the assimilation rate and conductance using a Licor 6200 system. We then cover the leaf and Licor 6200 with a dark cloth and measure the assimilation rate and conductance again (however, the assimilation rate is then negative denoting a production of CO_2, the dark respiration rate). We do this for five leaves, measuring each one in triplicate. We then reset the chamber's temperature, ramping up by 4C (7F) (see **Figure 7)**, wait one hour and then repeat the series of measurements. There are two measurements sets in the morning followed by three measurements in the afternoon.

We have run four series of these experiments (each for about 3 weeks) using different trees. The low light in the chamber causes a problem to develop with the trees leading to early abscission after about 4-5 weeks. We started with two experiments were run per week, one following a ramping up and the other, a ramping down in temperature. We are in the process of summarizing all of these runs. The description below is for a typical run with a ramping up in temperature.

The direction of the ramping is critical as we do not obtain exactly the same shape of curve for both directions. The ramping down in temperature leads to lower inhibition of assimilation. We suspect that higher temperatures in the morning (starting at 36C) do not lead to a greater water stress in the afternoon when the temperature and water loss is less (at 20C) when compared with the protocol that leads to a higher temperature in the afternoon. Also the ramping down (starting at 99F and lowering the temperature) created problems with the health of the tree; avocado seem not to do well if the morning temperature is high. For California growers, the ramping up with a high afternoon temperature is more natural and so we are adapting that protocol for further experiments designed to find a recovery threshold for higher temperatures.

Assimilation seems to be highest at about 20-22C (68-72F; see **Figure 8A**); however, the scatter of the data makes it difficult to see a clear temperature peak for many trials. Unfortunately the growth chamber often cannot stabilize below 18C (64F), making many of the lower temperatures unreachable and so we begin our trials at near the optimum temperature for assimilation. Under most conditions and in **Figure 8A**, the assimilation falls to near zero by 36C, certainly the assimilation rate is less than 20% that of the maximum assimilation seen at 21C (70F).

The stomata conductance seems to follow the same trend as assimilation but the relationship is not totally clear (**Figure 8B**). Certainly the conductance is lower at 36C compared with 20C, but there is much variability in the data. We can conclude that conductance does not seem to be the limiting factor at the higher temperatures (see later). While a linear relation between assimilation and conductance occurs as has been previously discussed, the variation of the data makes the relationship unclear. From these data the question remains, at higher temperatures is the stomata conductance forcing the declining assimilation or is the decline in assimilation inducing stomata closure?

Leaf respiration rate increases with increased temperature (**Figure 8C**) and that increase can be fit by a linear curve or an exponential curve. Both work well from 20 to 36 C, except that the linear curve generates a positive respiration rate below 18C, which is not reasonable. The exponential curve possesses a negative rate at all values of temperature above 0C, but rises rapidly from 20 to 36 C. The scatter of the data points makes it impossible to judge which curve is best but from the literature one would suspect that the exponential curve is more realistic. Clearly the respiration rate at 36C is much higher (5-10x) than at 20C.

One concern was that the light intensity was not uniform across the leaves within the growth chamber for our measurements[7]. This seems to be the case (see dotted line in **Figure 9**). Some leaves are closer to the light source than others and their positions cannot be changed. In order to try to correct for any light intensity problems, we decided to use internal CO_2 concentration as a measure of effective photosynthesis (the internal concentration should be lower at more effective photosynthesis). For a more complete understanding, we have added a discussion of this parameter in the following section.

The Effect of Temperature upon Carbon Dioxide Use

The movement of water vapor from inside the leaf to the outside atmosphere (transpiration) is best described by an electrical conductance analog in which the water vapor moves from a

[7] When we perform experiments of changing relative humidity or temperature, we select leaves at the same height within the growth chamber so that the illumination is the same.

highest (chemical) potential down a diffusion gradient from mesophyll tissue to a region of the lowest chemical potential (the atmosphere surrounding the leaf) as illustrated in **Figure 10**. There are three principle conductances of movement (g[8]) in the total pathway: (1) the boundary layer, (2) the stomata pore, and (3) the tissue and leaf air space itself. In the pathway for H_2O, the sites of evaporation for water vapor are cell surfaces very near the stomata pore via either epidermal or mesophyll cells near the guard cells. This means that there are only two conductance pathways for water vapor (number 1 and 2 above).

The stomata govern the rate of gas flow into the mesophyll cells within the leaf, where photosynthesis occurs. Two gases—water vapor and CO_2—are critical to the plant. The water vapor flow out of the leaf is responsible for the movement of inorganic nutrients up from the root zone. However, if water is evaporated from the leaf too rapidly, the water potential of the leaf falls and that alters many processes of metabolism including the ability of the stomata to open fully. Thus, there is a balance between water vapor loss and the conductance through the stomata.

On the other hand, CO_2 movement into the leaf is critical for assimilation and production of carbohydrates. If the light intensity is high enough, the assimilation may still be limited due to the flow of CO_2 into the leaf. The level of CO_2 within the leaf (called the internal CO_2 concentration) is due to a balance between gas flow into the interior and CO_2 assimilation (or uptake) via photosynthesis. In general, the internal CO_2 level is lower than the external concentration by a relatively small amount. The leaf tries to balance the flow in (via conductance) to the use inside (via photosynthesis) to maintain a relatively constant internal concentration. As light intensity rises and photosynthesis increases, the stomata open to allow more gas flow into the interior. Unfortunately, this allows the loss of more water vapor into the exterior, altering the water potential of the leaf.

We now understand that the assimilation rate of avocado leaves is largely governed by the conductance of gas flow, in that assimilation is linearly dependent upon conductance. It must be remembered that conductance is not exactly water vapor flow (or transpiration rate). That is governed by conductance and the gradient of water vapor from the leaf interior to the exterior.

While the water potential difference drives the movement, the conductance provides a measure of the resistance to flow. This formula is linear and follows an equation of flux = conductance times force (for water vapor, wv, and for CO_2, c).

$$j_{wv} = g_T \times \Delta(\text{force})_{wv} \qquad [10]$$

If we define the flux inwards as positive, then the $\Delta(\text{force})_{wv}$ must likewise be defined to the positive when the outside force is higher than the inside force. We will find that this is not the case for water vapor as the water vapor "force" is higher inside, thus this "difference" term is negative and so the flux is negative (from the inside towards the outside). With those definitions, the sign in equation [10] is correct as positive.

This means that the $\Delta(\text{force})_{wv}$ is due to the gradient of water vapor from inside, where it is nearly 100% relative humidity, to the outside, where it is governed largely by the relative humidity of the air. Thus equation [10] can be written (in general terms) as:

[8] Although we speak of conductance to flow, the past mathematical formalism used a resistance to flow similar to Ohm's law for electricity in which the linear relation between a gradient of concentration or of electrochemical potential and flux or flow of material is given by r.

$j_{wv} = g_T \times \Delta\text{relative humidity} = g_T \{RH_{inside} - RH_{outside}\} = g_T \{100\% - RH_a\}$ [10']

There is another way of looking at the relation of assimilation to conductance. It begins with understanding that the flow of CO_2 is governed by the same relationship as water vapor, but in the opposite sense.

$j_{CO2} = g_C \times \Delta(\text{force})_{CO2}$ [11]

The $\Delta(\text{force})_{CO2}$ is the gradient of CO_2 concentration from outside to the inside and is given by the same relationship:

$j_{CO2} = g_C \times \Delta[CO_2] = g_C \{[CO_2]_{out} - [CO_2]_{in}\}$ [11']

The beauty of these relationships is that the conductances are related to each other. The conductance of water vapor is higher due to the lower molecular weight of H_2O, relative to CO_2. The relation is through the Lewis coefficients, so that $g_c = 0.958/1.346\ g_{wv} = 0.712\ g_{wv}$.

The flow or flux of CO_2 is the assimilation rate (A) and so equation 11' becomes:

$A = 0.712\ g_{wv}\ \Delta[CO_2]$ or $A = 0.712\ g_{wv}\ \{[CO_2]_{out} - [CO_2]_{in}\}$

$\Delta[CO_2] = \{A / (0.712\ g_{wv})\}$ [12]

Thus, if the gradient between outside and inside CO_2 (or for a constant external CO_2, the inside CO_2) is held constant, a plot of A against g_{wv} should be a straight line with a zero intercept or a plot of $\{A / g_{wv}\}$ against nearly anything (e.g., conductance, light intensity or temperature) should be constant. That is true only if the "anything" is not altering the basis relation that internal CO_2 is held constant.

Measurement of Internal CO_2 Concentration

Returning to the light intensity problem we calculated (for all temperatures) the average value of A / g_{wv} from the data set (see **Figure 9**). We found that there seemed to be little, if any, light dependence (shown as leaf position). The average value of A / g_{wv} was 1458 ± 262 $\mu mol/m^3$, which for this particular trials translates into an internal CO_2 concentration of 345.0 ± 9.0 ppm (for an external concentration of 395.0 ± 1.1 ppm). Thus, we felt that we could use this measure effectively for determination of the effect of higher temperatures.

From the data set shown in **Figure 8**, the difference in CO_2 concentration (effectively according to equation [12]) can be plotted against an increasing temperature. Under those conditions, we can easily see that the assimilation rate/stomata conductance declines as temperature increases (**Figure 11**). The falling gradient means that the internal CO_2 concentration is becoming closer to the ambient level (since that is constant with temperature, data not shown). It seems that the rise in internal CO_2 is nearly linear with temperature, going from nearly 300 ppm at 20C to nearly 400 ppm (ambient) at 36C (see **Figure 11**). If the stomata were closing prematurely (e.g., a more rapid closure than a lowering of assimilation would support), then the internal CO_2 should fall since the assimilation was using the CO_2 at a more rapid rate than the conductance could support. The rising internal CO_2 suggests that assimilation is being inhibited more completely than the conductance closure, leading to a closer equilibrium between the internal and external CO_2 levels. In many of the experiments, assimilation at 36C was close to zero while a sizable, non-zero stomata conductance remained.

These data suggest that high temperature presents a triple problem for avocado trees. The assimilation rate is becoming inhibited while the stomata remain partially open. That leads to water loss, with little assimilation. Furthermore, the respiration rate is dramatically increasing, leading to a loss of the carbohydrate made earlier in the day. The net carbohydrate within the leaf must be falling due to a decline in assimilation and an increased use of carbohydrate, and a water potential problem is develops within the leaf.

There was another interesting observation from the data set. Under most conditions, the dark period to measure respiration was only a few minutes and the stomata conductance did not change. This was expected from the light pulse experiments on the leaf disks; sun flecks of a few minutes duration do not cause much opening of the stomata. However, when stomata conductance was relatively high in the light, even a few minutes of dark would close the stomata and lower the conductance (**Figure 8B**). This effect was only observed for the higher conductance and suggested that the lack of assimilation (in the dark) would induce a rapid stomata closure. Thus, we would expect a faster closure in the dark if the conductance were high. We are re-examining our earlier data to determine if this is true. This effect has an important consequence on how conductance and assimilation responds when the leaf is shaded after a long period of direct sunlight.

Boundary Layer as Detected by Leaf Temperature

Last year we suspected that boundary layer conductance was influencing how the stomata behaved. For the most part, we measure only stomata conductance by porometry and hope that the boundary layer conductance is very high so that it does not matter. The effect of the boundary layer was suggested by the sap flow measurements, which were lower than that expected by the porometer measurements. Another test of this concept that we are not taking into account of the boundary layer conductance correctly can be seen with an infrared camera, which detects long infra red radiation due to black body emission. This emission is proportional to the temperature of the body and for a leaf, can detect the surface temperature of the leaf. If a leaf is maintained in the light at an air temperature of 28C, its surface temperature reaches about 31.0C (**Figure 12**). This temperature is a steady-state temperature which is the balance between the influx radiation (sun light) and losses of radiation due to a sensible heat loss or convection due to hot air rising and latent heat of evaporation (due to the loss of water through the stomata, see left side of **Figure 12**). The other heat loss is conduction due to air movements or wind. The principle balance to incoming radiation is heat of evaporation through the stomata. If a wind is applied to the leaf, it cools rapidly (ca. 3.0F) due to the loss of heat through conduction and this is directly a measure of the boundary layer. If the wind ceases, the temperature of the leaf returns to the previous value rapidly. The speed of this change is within tens of seconds. The actual calculation of boundary layer conduction is somewhat difficult (see Monteith and Unsworth, 1990), but can be done. Furthermore, if the air flow due to the wind is lamellar, the conduction can be calculated according to theory involving wind speed and leaf dimensions (see Schlichting & Gersten, 2000).

We are currently using this technology with the sap flow experiments to determine if we can show that sap flow measures only the flow of water through the stomata and that the LICOR value of stomata conductance is only part of the story of water flow (the other part is boundary layer conduction).

Canopy Structure

We continue to investigate the role of the canopy in allowing light to penetrate into the internal leaves. This penetration is critical to understanding sun flecks and the full canopy's productivity. Leaves on the outside of the canopy are exposed to at least half of the day length of full sunlight and thus can be as productive as the physiology and other environmental parameters will allow. This is the Layer I of leaves. Leaves deeper within the canopy are not so productivity since the Layer I can shade them for at least part of the day. The fundamental question is how many Layers of leaves can be supported by the normal distribution of leaves.

Experiments along the "sun fleck" line (described in the last years' reports) are continuing. Since the placement of the sensors within the canopy is critical, it was decided to do some modeling with more simplified systems to understand what types of events may be happening. **Figure 13** shows such a model. Here each leaf has the same dimension and orientation (they are "facing" upwards). Each layer of leaves has equal spacing from the one above and the leaves overlay directly the opening in the layers below and the next leaf in the layer two down. This structure allows for a simple model in which the illumination "rises" from the right and "sets" in the left side of the model. The angles (θ) can be related to the time of day and the durations of the illumination of full light on a given layer can be calculated during the day. For a given spacing and leaf size we can obtain the amount of and area illuminated by light for angle (or time of day). There are two points of interest: [1] the amount of light that the third layer receives increases as the spacing of the layers increases (concurrently with a decline in light for the second layer, see **Figure 14A**) and [2] this duration of light is probably the key to understanding whether or not the leaf is a net producer[9].

Of more interest is the actual light intensity falling on the leaf surface and how that intensity drives photosynthesis. If a leaf is not illuminated directly "face on", it does not receive as much intensity. As the angle varies from the normal (angle goes from 0 to 90^o), the intensity falls as the cosine of the angle; as the intensity falls so does the photosynthetic rate. **Figure 14B** shows actual calculated productivity (per leaf) as the angle varies from 90^o (sunrise) to 0^o (at noon). Layer I receives all the illumination and reaches a maximum at noon. Layer II, partially shaded by Layer I, receives illumination later in the day but reaches maximum at noon (see Figure 13 for the geometry). Its total production will be less than Layer I. Layer III receives some illumination but never is the full leaf illuminated at any time and shading becomes severe at noon. It never becomes fully productive.

Although these are very artificial situations, they do give insight on the orientations that are most critical for leaves and how they may arrange themselves into "correct" layers for efficient productivity.

Continuing Experiments

We continue to test our sap flow measurements against both actual water use from a pot and the total transpiration rate through the leaves as measured by the Licor 1600 steady state porometer. These data sets have yet to be fully evaluated and thus we cannot say as yet what problems may

[9] The total productivity of a leaf is equal to its photosynthesis over the entire area, when the leaf is illuminated less the respiration, which occurs throughout the day regardless of illumination. Thus, there is a minimum duration of light, which can provide enough photosynthetic carbohydrate to balance the loss through respiration.

exist. However, with small trees that have been pruned to a single branch, the water loss by sap flow measurements are within 20% of the water loss measured by water addition. However, it seems that the Licor porometer does not measure the actual water loss very well.

We are continuing our experiments with wind to lower the boundary layer but have not yet obtained reproducible results that are statistically significant. We have traced the problem to the changes in stomata conductance due to high water loss in the afternoon and are modifying the protocol.

We are continuing measurement of the assimilation with varied light intensity and duration by the use of trees within a controlled green house environment in order to better define the rate of response of the stomata to relatively brief illumination time of the leaves.

We continue to develop models of productivity based upon how the microenvironment around the individual leaves affect the stomata conductance and how the conductance and the light intensity alter the leaf's carbon productivity. This model will be a simple spread sheet that can be used to predict how the microenvironment in the field as measured by a few simple instruments can affect carbon fixation. It is hoped that an individual small model can be used as a full tree model to yield predictions that will add to the ability of the grower to understand how certain treatments will affect his/her productivity.

Reference

Monteith, J.L. & M. H. Unsworth (1990) Principles of Environmental Physics, 2nd edition, Edward Arnold Publishers, London.

Schlichting, H. & K. Gersten (2000) Boundary Layer Theory. 8th edition. Springer-Verlag, Berlin.

Thornely J. H. M. (1990) A new formulation of the logistic growth equation and its application to leaf area growth. Annals of Botany, 66, 309-311.

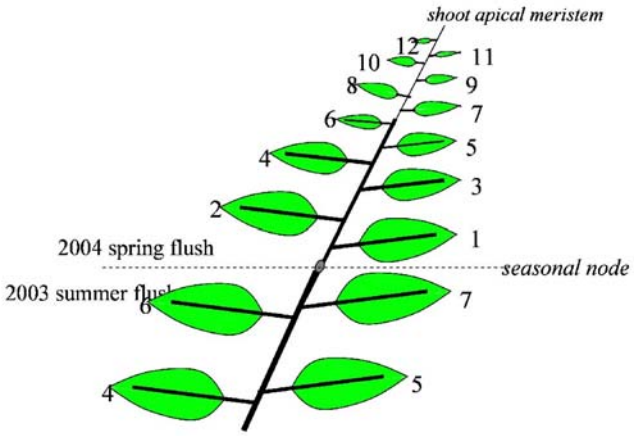


Figure 1. The Numbering Sequence for a Branch. The oldest leaf has the lowest number while the youngest leaf has the highest number. The physiology of leaves, which we wish to investigate, depends upon when the flush begins (denoted as when the first leaf appears) and the time between the initiation points of each leaf.

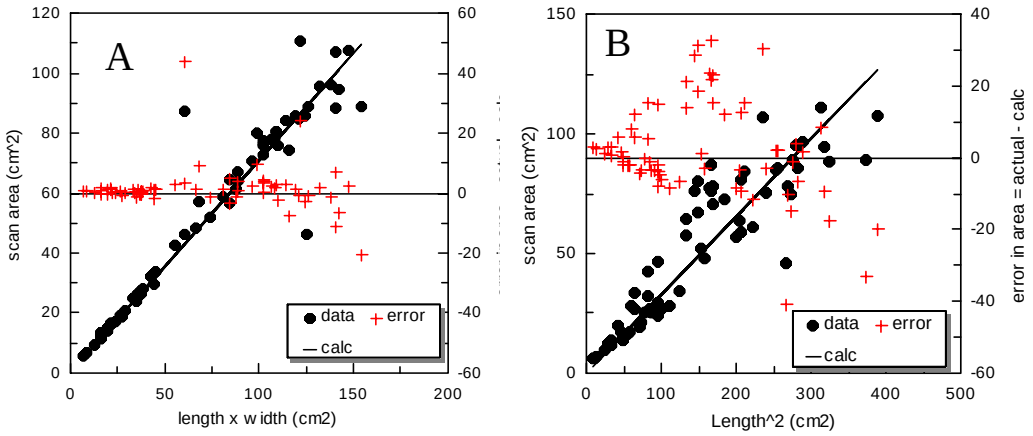


Figure 2. Calibration of Leaf Area by the Linear Dimensions of the Leaf. **A**, Calibration by Length and Width. **B**, Calibration by Length Alone. The scan area was that derived from the use of a Leaf Area meter, which measures the real area of the leaf by passing at a constant speed or by scanning, a leaf over a line of photodiodes, some of which are eclipsed by the leaf. The number of dark photodiodes over the scanned time represents the area. A ruler measured the lengths and widths. The error points (shown in red) are the difference between the actual area and the calculated area. The lines represent (for A) the actual area in cm^2 equal to (for A) f *x* length *x* width or (for B) g *x* $length^2$, with both length and width in cm. Both f and g are scale factors and vary with variety of avocado.

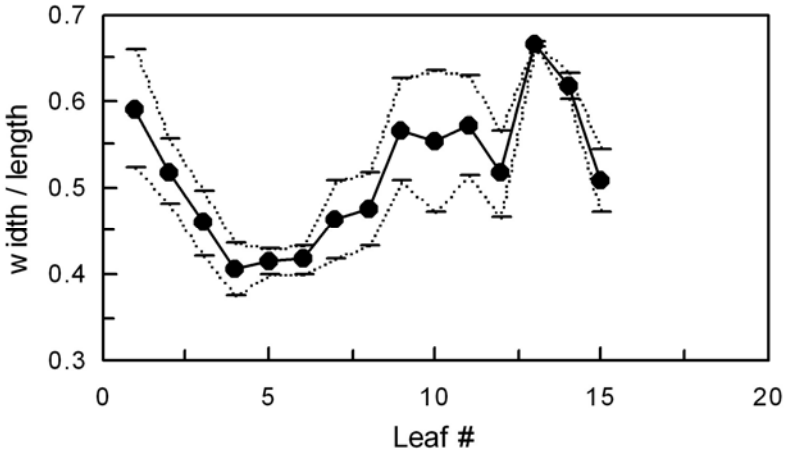


Figure 3. The Variation in the Width/Length Ratio of Avocado Leaves. Leaf number was measured as shown in Figure 1. The bars represent an error measurement (2 *x* standard error of the data, which was the leaves from three branches.)

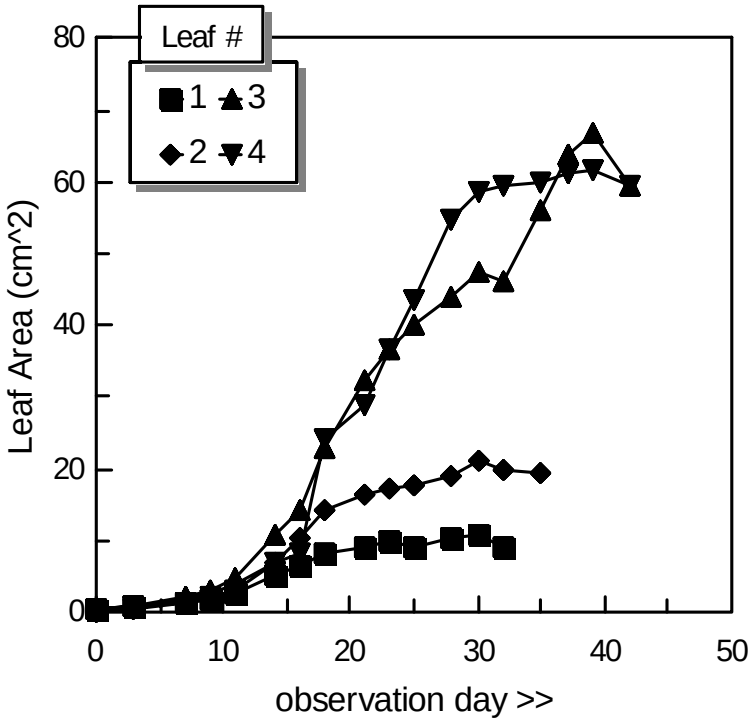


Figure 4. Growth Curve for a Few Typical Avocado Leaves. The Leaf Area was calculated from the length and width of the individual leaf. The "zero observation day" was Nov 13, 2003, when the experiment was started, and is arbitrary. One of the critical elements of the growth curve is the maximum size that the leaf reaches, as it is not absolutely the same for each leaf.

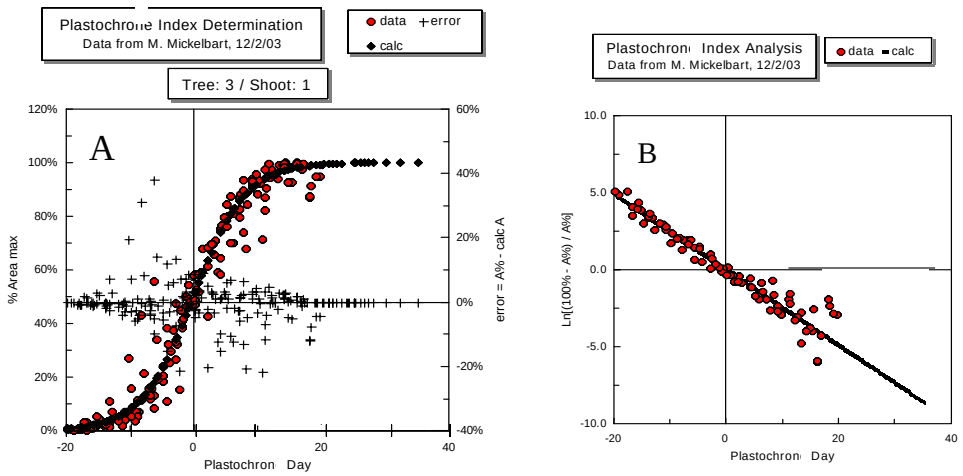


Figure 5A. Combined Leaf Measurements from One Shoot/branch of one Tree. The leaf area was scaled by the maximum size of the leaf and the zero plastochron day was set when the area was 50% of the maximum area (see text for more details.). Here 15 leaves from one branch were used, each leaf had a different time shift (which was equivalent to when the zero plastochron day occurred. **Fig. 5B**. The Logistic linear transformation of the leaf growth curve. Some of the data was taken from Figure 4 and transformed by Equation [3], see text.

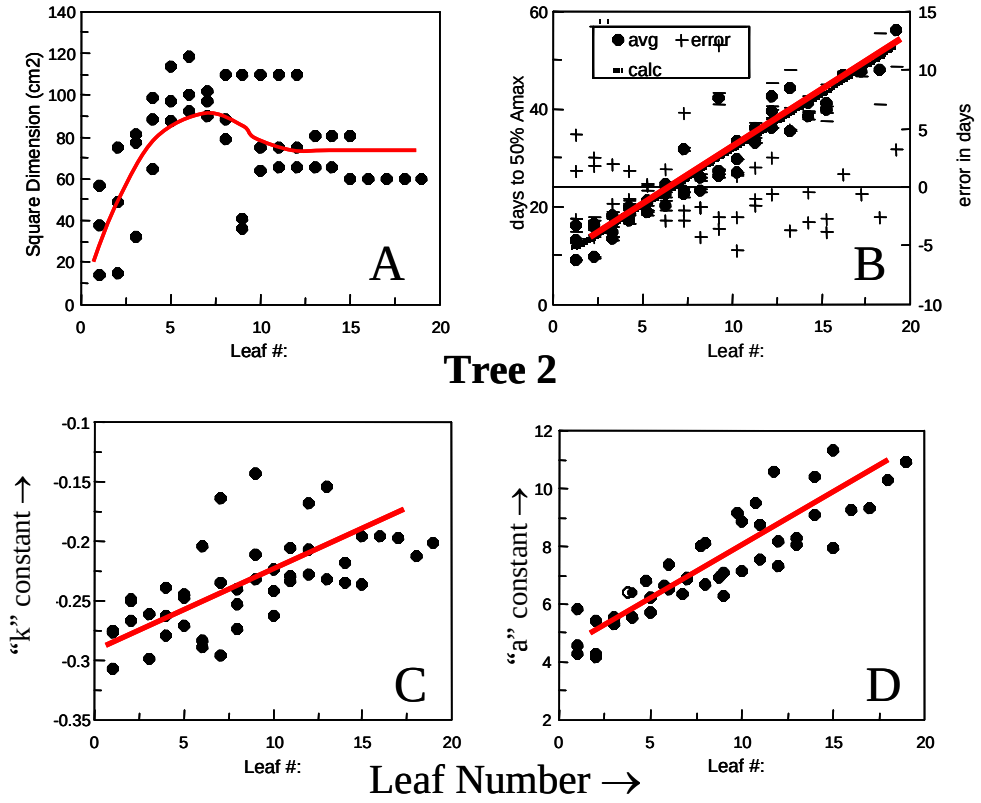


Figure 6. Logistic Parameters from One Tree. The various parameters for the Logistic formulation are shown in the above panels. They have been determined as described in Equation [3] and Figure 5B for three branches of one tree. **A**. The maximum size of each leaf. **B**. The time required to reach 50% of the maximum size, as measure of the plastochron day. The slope of the line represents the average time between initiation of each leaf and for this tree the time is 2.3 days between the initiation of each leaf on the three branches. However, each branch begins its initiation at a slightly different day (about 3-6 days variation). **C & D**. The two constant parameters of the Logistic growth curve—growth rate (κ) and point of initiation (proportional to α).

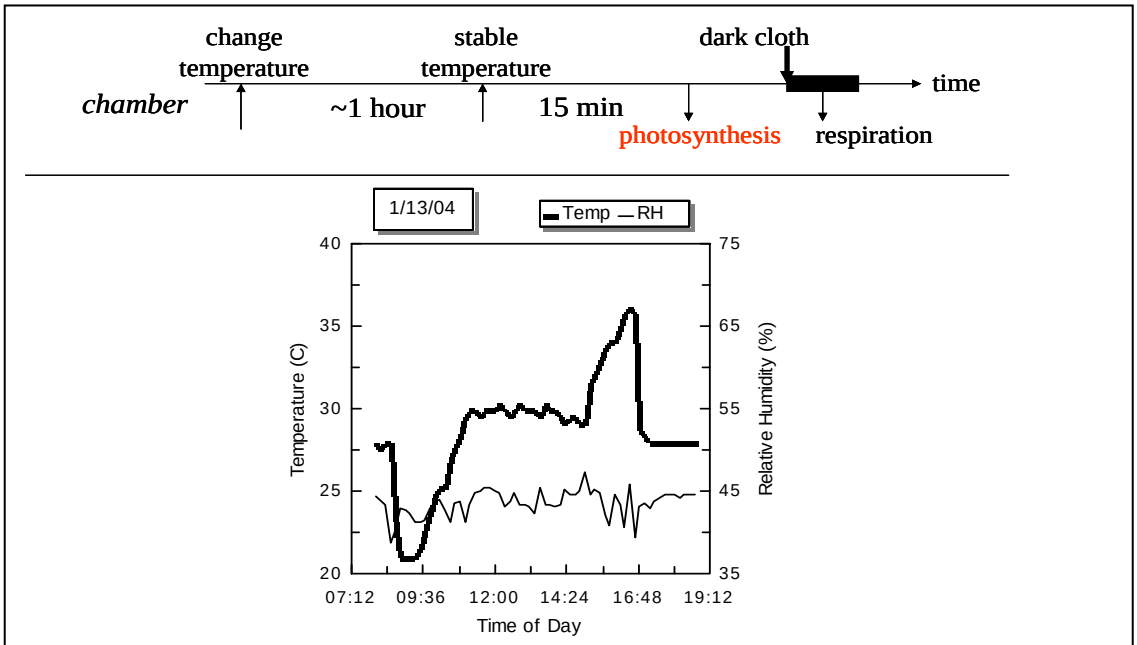


Figure 7. The Protocol for Growth Chamber Shifts in Temperature. These data were taken from a HOBO automatic sensing device placed within the growth chamber at the leaf level. It represents temperature and relative humidity change during the course of a day-experiment when the temperature was being ramped up from 20C (68F) to 37C (97F). Note the lack of change of relative humidity. Respiration is measured within a few minutes of the leaf begin place in the dark (under a black cloth), see Figure 6.

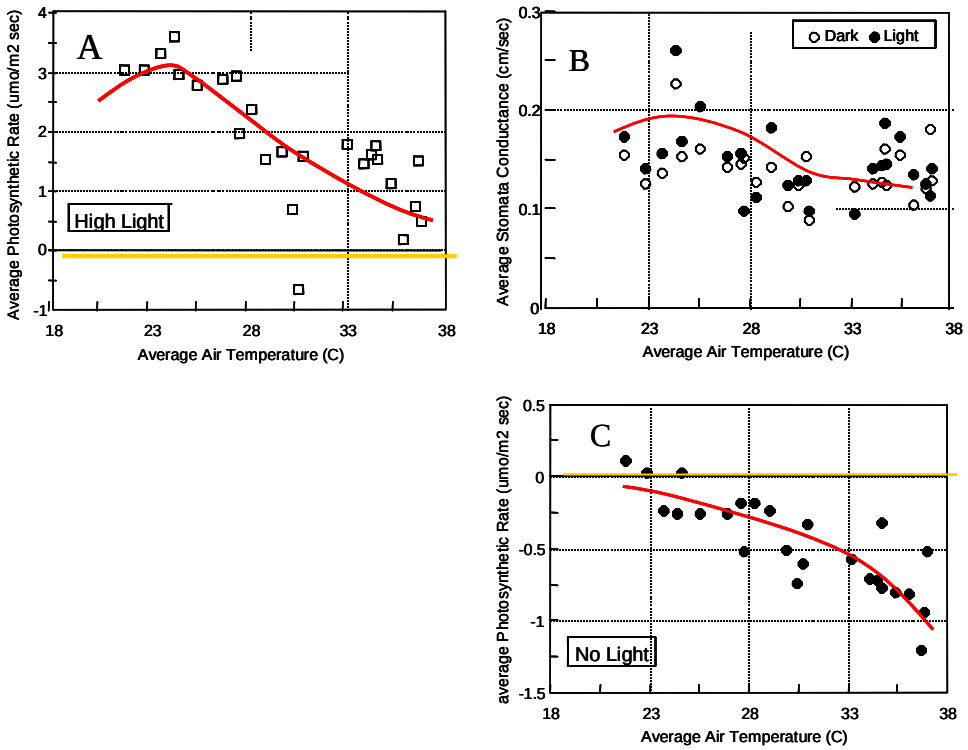


Figure 8. Effect of Temperature upon the Photosynthetic Rate, Stomatal Conductance and Respiration Rate of avocado leaves. This is an example of a typical experiment in which 5 leaves are measured on two trees. The measurements were by a Licor 6200 system. The data points are an average of three measurements on one leaf for each temperature. The yellow line represents zero (which is off-set in some plots) and the red line represents a "best-guess" line for the average of the data. **A**, Average Photosynthetic rate. **B**, Average Conductance Rate. **C**, Average Respiration Rate.

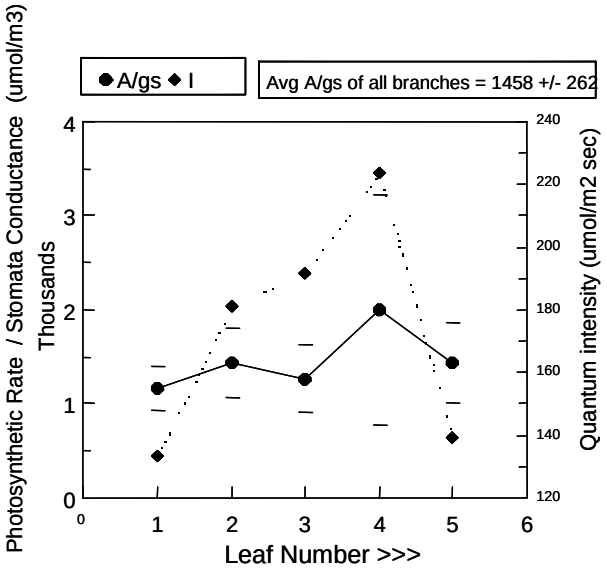
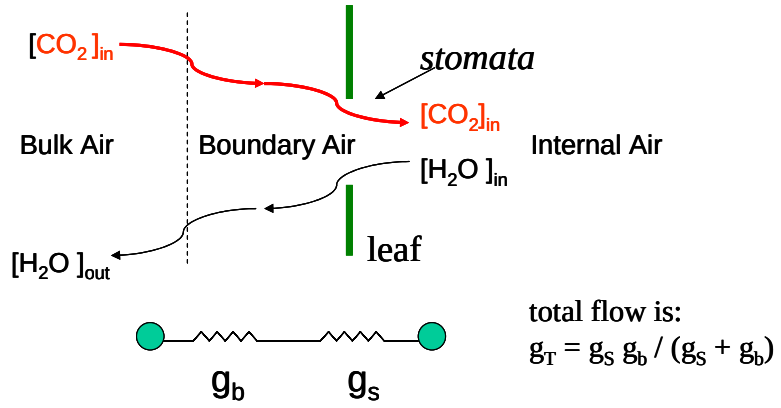


Figure 9. Light Intensity at Each Leaf and Internal CO_2 Concentration for Each Leaf in a Typical Experiment. Here the leaf number is arbitrary and represents merely a different leaf. The internal CO_2 concentration (as given by assimilation divided by the conductance, vertical axis) is averaged over all temperatures for these experiments. These measurements are done within a growth chamber and thus each leaf is at a slightly different level (distance from lights) and so experience different light intensities (as given by the dotted line).



$$\text{Photosynthesis rate} = g'_T \{[CO_2]_{out} - [CO_2]_{in}\}$$

$$\text{Transpiration rate} = g_T \{[H_2O]_{in} - [H_2O]_{out}\}$$

Figure 10. Movement of water vapor from inside to outside the leaf. The varied transition zones are indicated by name and conductance (g_i) as the top two lines, while the regions of "constant" gas concentration are shown as the third line. The bottom line indicates a possible concentration of the gas within each region. CO_2 movement is shown by the red line and equation while H_2O movement is shown by the green line and equation.

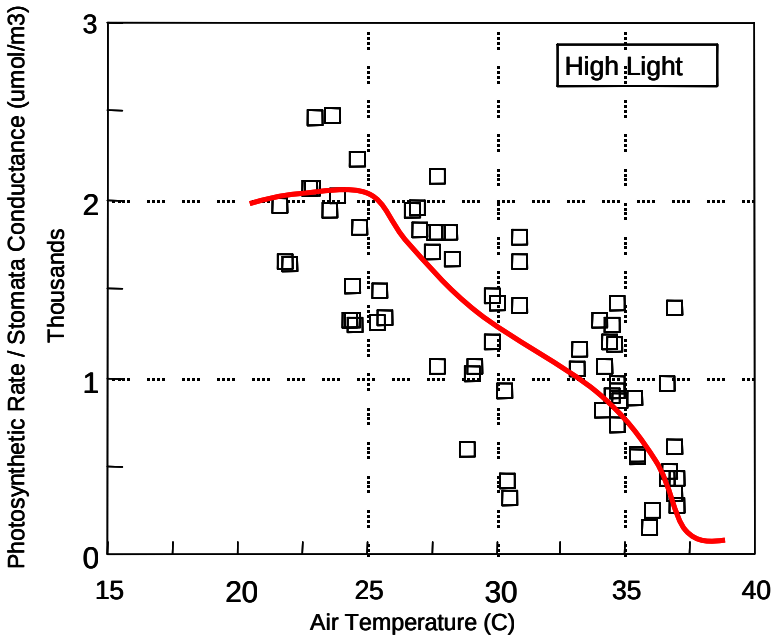


Figure 11. The Dependence of Internal CO_2 Concentration Ipon the Air Temperature. The internal CO_2 is calculated as described in Figure 10 and by Equation [12] in the text. The air temperature is that temperature measured next to the leaf that is being measured by the Licor. Here the zero point of the vertical axis (photosynthetic rate / stomata conductance) is equal to the ambient concentration of CO_2 (generally near 380 ppm). This value is the lowering of the ambient CO_2 due to the uptake of CO_2 due to photosynthesis. Note that by 37 C (98F) there is little or no photosynthesis occurring.

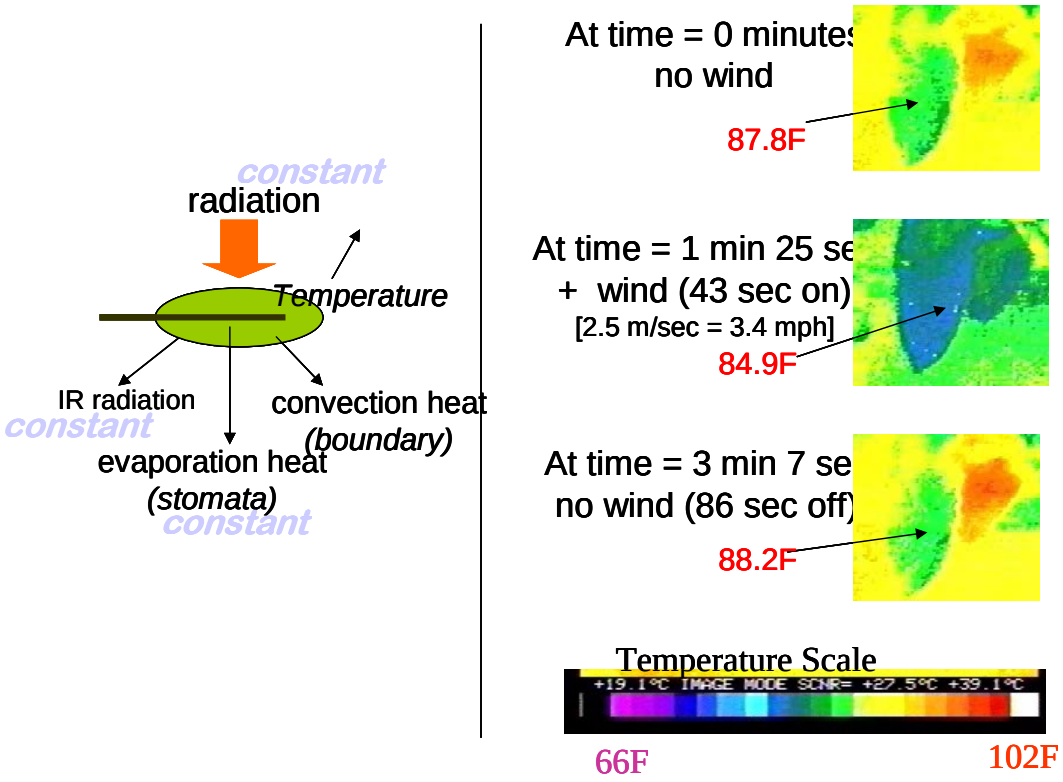


Figure 12. Leaf Surface Temperature as Measured by an Infra-Red Camera. An Inframetrics IR camera was used to record the surface temperature with the image being captured with a VCR. The image was captured using Dazzle Digital Video Creator Image capture interface and software. A Hass avocado leaf was used in a green house at an air temperature of about 28 C at 10AM.

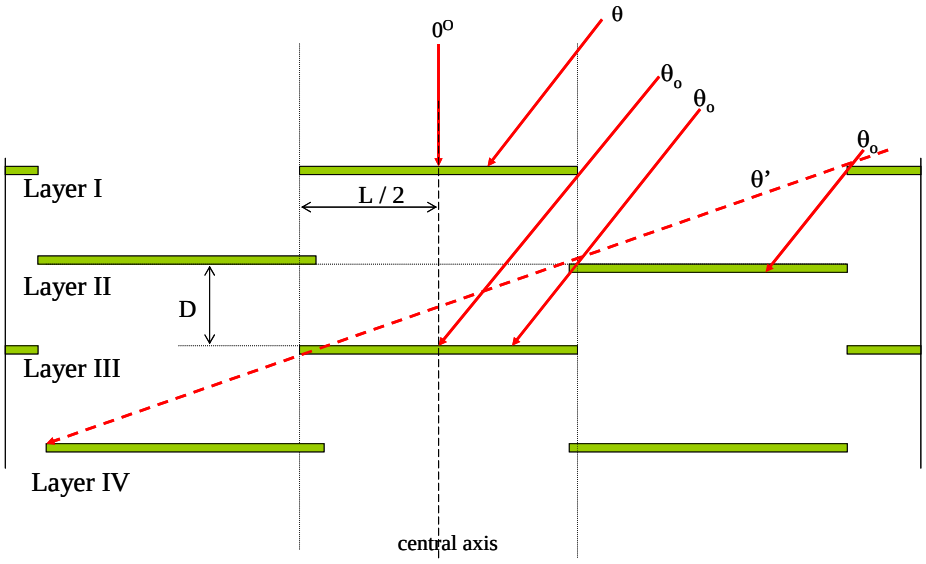


Figure 13. Two Dimensional Model of a Canopy Structure θ_o = arctan (2D / L) θ' = arctan(D/L) with D. The intensity of light (I) is equal to the intensity at the maximum (from the top) *times* the Cos(θ). For more details see text.

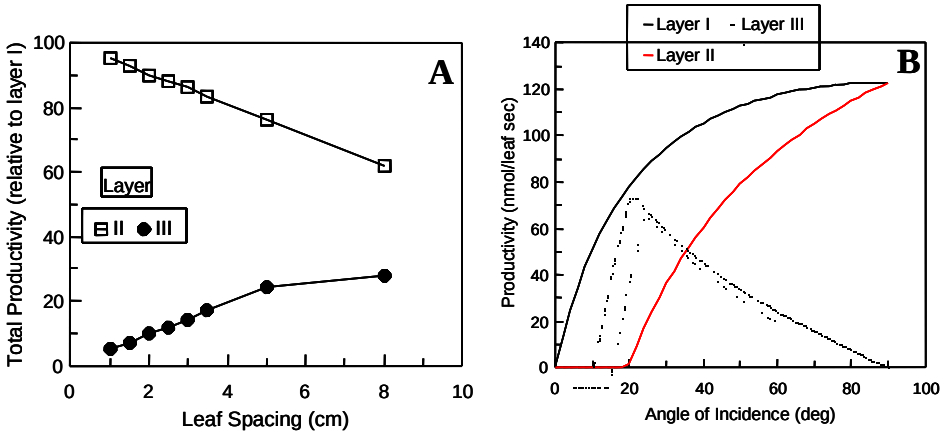


Figure 14. Data from the Model of Figure 13. **A**. Dependence of Productivity Upon Leaf Layer Spacing. Amount of illumination is with respect to layer I. The size of the leaf was constant at 10 cm. **B**. Dependence of Productivity Upon Illumination Angle. The leaf was taken as 10 cm on the side (L) with a spacing (D) of 3 cm. The photosynthetic productivity was calculated as $A = A_{max}\,[I] / \{K + [I]\}$, where A = assimilation rate and A_{max} = maximum rate at 20 μmoles/cm^2 area sec. The surface intensity (I) is in units of μmoles /m^2 sec with its maximum being full sunlight and K is a half-saturation coefficient of about 20% full sunlight.