AVOCADO TREE PHYSIOLOGY – UNDERSTANDING THE BASIS OF PRODUCTIVITY

New Project: Year 2 of 5

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Benefits to the Industry

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

Project Objectives:

1. An understanding of the effects of environmental variables (light, temperature, and relative humidity) on avocado leaf gas-exchange and carbon assimilation, essential for plant growth and fruit production.

2. An understanding of the developmental physiology of avocado leaves and how this relates to canopy management. In particular how many layers of leaves within the canopy will support a positive carbon balance to the plant and how the duration of light flecks through the canopy can induce a positive carbon balance.

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

Summary of this Year's Progress

- ABA-induced responses seem to be normal within the avocado, which indicates that if water stress induced ABA within the root, the conductance of stomata will be influenced and made lower.
- The high evaporative demand induced by high temperature closes the stomata routinely in the afternoon similar to a shift of relative humidity to a lower value. The conductance in the morning influences greatly the change in the conductance in the afternoon.
- Assimilation is highest from 20-24 C and then declines nearly 80% (from 7 to 1 μmoles/m^2 sec, at medium light levels) as the air temperature rises to 38C. Concurrent with the fall in assimilation, the respiration of the leaf rises nearly 5-fold (from 0.2 to 1.1 μmoles/m^2 sec) over the same temperature range. The stomatal response with temperature indicates that it remains the major control of assimilation under relatively high light.
- A series of experiments indicate that the LiCOR porometer measurement does not take into account conductance of water vapor through the boundary layer, which seems to be limiting for the large avocado leaf at the low wind velocity.
- Wind (6 mph) increases the sap flow (and so the transpiration) by 31% within a green house. If the assimilation (limited by the conductance) is increased by the same amount by raising its boundary layer conduction, then the productivity would increase by 31% over midday.
- While the light reflectance of a leaf is slightly different from the top and bottom surfaces (from 400 to 1100 nm) and also differs for varied ages of leaves, the differences is only about 10-15% of the total.
- During the day only a small change in the sap flow between the east and west branch is found (about 10-15%). The environmental data suggests that diffuse light (that light which is reflected and scattered from the environment and tree) is nearly as good at supporting photosynthesis as direct light in Avocado.

Details

Leaf Chamber System

The production of avocado fruit is the *raison d'être* of growers; increased and more efficient fruit production begins with the ability of the plant to assimilate carbon through a process called photosynthesis. The source of carbon comes from the atmosphere as gaseous carbon dioxide (CO_2). Once it is converted into a stable compound (e.g. carbohydrates), the movement and arrangement of carbon can vary, but for efficient productivity, should be directed into the production and growth of fruit. Carbon dioxide enters the leaf through the stomata, which are analogous to tiny pores that have the ability to open and close in response to light and water stress among other factors. For efficient carbon assimilation the stomata must remain fully open during the day so that photosynthetic conversion of CO_2 is maximized at the prevailing light level; open stomata have maximum gas conductance, governing the speed of the gas movement[1]. However, since the stomata also control water loss from the plant, open stomata can lead to excessive water loss that is, unfortunately, harmful to the plant, especially when soil water is limiting. Stomatal physiology is set such that water loss is minimized under most water stress conditions, often at the expense of carbon assimilation. We wish to understand the mechanisms that control the stomata, a sophisticated control system to balance water loss and assimilation—a normal balance designed to maintain the well being of the plant, but not necessarily to maximize fruit production.

In southern California during the spring and summer months which are critical for fruit set and growth, rapid shifts to high temperatures and low relative humidity can occur. This can result in stomata closure early in the day thereby potentially limiting carbon assimilation and ultimately fruit production. This cause and effect is made worse when the relative humidity changes from relatively high (50%) in the morning to very low (<10%) in the afternoon, due to Santa Ana winds, which bring about rapid warming of the air mass around the canopy with concurrently lower relative humidity. It is our belief that high temperatures in the afternoon also detrimentally affect net assimilation and fruit production. Certainly, as the temperature increases, the relative humidity declines driving an increased water loss from the leaf. This water loss will ultimately cause the closure of the stomata, leading to an assimilation fall. Results from the first year of this project have suggested that another process, respiration, (a process that maintains the leaf by burning stored carbon and releasing CO_2) may be in operation. It has been shown in many plants that at higher temperatures the respiration of the leaf increases much more rapidly than does assimilation. Thus, respiration decreases the amount of fixed carbon (both as a percentage and absolute amount) available for translocation to the fruit, which could impact both fruit set and fruit growth. We have learned that although relative humidity is important, air temperature, as it controls the leaf temperature, may be even more important.

In 2001 – 2002 we began a collaborative effort to examine the effects of various environmental conditions and cultural practices on avocado leaf photosynthesis. The goal of this research is to determine how environmental factors such as light and relative humidity affect photosynthesis so

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[1] The nomenclature is: photosynthesis, photosynthetic CO_2 fixation, and (carbon) assimilation are the same and equal to the rate of or amount per unit time of carbon dioxide which is converted into carbohydrate (of all kinds) on the basis of leaf area. Translocation is the amount of carbohydrate moved out of the leaf to some other part of the plant. Transpiration is the rate of water loss out of the leaf and is governed by the opening of the stomata in leaf, which is related to the conductance of water through the stomata.

that growers and researchers alike can understand how orchard management decisions ultimately influence productivity. This year (2002-2003) we are continuing and expanding those finding to begin to generate a good model of how to manage the avocado trees.

Individual Leaf

Our lab has a leaf chamber system in which a leaf disk can be exposed to a mixture of gases that can be rapidly changed within tens of seconds. Rates of carbon assimilation (uptake of CO_2) and transpiration (production of water vapor by evaporation within the leaf) can be measured with two Infra-Red Gas Analyzers (IRGA). The monitoring of these important components of the gas stream and the leaf is carried out simultaneously with gas exchange alterations and give a good measure of CO_2 assimilation and stomata conductance. The cut edge of the leaf disk is sealed in a separate compartment through which flows a water solution. Thus, the water potential of the leaf is maximized since the cells are no further than 3 cm from plentiful water supply.

We proposed, using this equipment with 'Hass' avocado leaves, to examine the temperature dependence of both respiration (the rate of CO_2 production in the dark) and photosynthesis. Unfortunately we had problems with temperature changes between the production of the relative humidity, the chamber and the measuring system. The lower room temperature induces a lowering of the higher air temperatures, which does not harm our measurements as we monitor air temperature carefully. However, the temperature change from the chamber to the IRGA monitors is very detrimental as the measurement of the water vapor and CO_2 depend absolutely upon the temperature in the monitor, which we can't determine. We suspected that this would be a problem and we are trying on more modification to change the leaf temperature without generating artifacts of temperature changes. The technological problems made it impossible to perform the experiments we wished to, but we determined the temperature dependence of assimilation and respiration using the growth chambers and small trees (see later section).

On another note, conductance of leaves can be changed by exposure of the cells to abscisic acid (ABA). This phytohormone is produced when the tissue water potential drops to a low value due to a loss of water from the tissue; ABA is the signal to the stomata to close and so to conserve water. Typically the roots produce ABA due to the depletion of water within the soil; the ABA then flows to the leaf via the transpiration stream. To ascertain that the avocado stomata responded normally to ABA, we infiltrated the leaf with ABA (at 20-50 μM) in the water solution bathing the cut edges of an avocado leaf (**Figure 1**). ABA caused a drop in the conductance within 5-10 minutes and assimilation followed that drop, again demonstrating that the assimilation under these conditions was governed by the conductance (a finding of last year). The ABA infiltration was stopped after an hour (when the conductance decline leveled off), but the leaf did not recover since the ABA was still within the leaf. It generally requires an hour or two to metabolize the ABA to a form that it does not trigger the closure response; we are investigating that. Thus, ABA-induced responses seem to be normal within the avocado, which indicates that if water stress induced ABA within the root, the conductance of stomata will be influenced and made lower. This is not especially surprising but it is important to demonstrate that our thoughts about avocado's physiology are correct.

Growth Chamber

Our large growth chamber, which has good temperature and humidity control, can hold about 4 small trees. While the light intensity is lower than full sunlight, this chamber can be used to shift the relative humidity and temperature of the air rapidly. We are currently running experiments in

which the air temperature was shifted to a higher level in the afternoon (much like an afternoon in southern California), but it required some work to settle on a temperature/humidity protocol that yielded a reproducible closure in the afternoon. In later experiments we maintained the relative humidity while shifting the temperature. The evaporative demand (which is linked to the actual water vapor pressure in the air and drives water loss from the leaf) is linked to both relative humidity and air temperature, but shifts upwards with a simple air temperature change. We are still evaluating the data but it appears that the curves look similar to those reported last year. Thus far, it appears that the high evaporative demand produced by high air temperature closes the stomata routinely in the afternoon similar to a shift to a low relative humidity. Interestingly, the conductance in the morning greatly influences the change in the conductance in the afternoon. Low conductance in the morning gives rise to a very small amount of lowering in the afternoon, while a high conductance in the morning gives rise to a very large amount of lowering of conductance in the afternoon. The high rate of water loss in the morning makes it critical that the stomata close in the afternoon. As we discussed last year, the concept of a "set-point" for the optimal stomata conductance seems to be the best way to think of how the stomata change. Unfortunately for ease of prediction, the water loss of the day before seems to influence the current day's response. We have no clear picture of how to understand this yet.

We decided to change how we were measuring the leaves' responses and began to use a LICOR 6200 system, which measures both assimilation and conductance, in the growth chambers. The machine is larger and harder to use in the chambers but it gave us the opportunity to determine how assimilation (measured by CO_2 uptake in the light) and respiration (measured by CO_2 release or "negative assimilation" in a dark period) operated at varied temperatures. These data allowed us to by-pass the problems that we face with the individual leaf chamber and allow us to develop a relationship of chamber temperature *verses* respiration (CO_2 use in the dark, obtained by covering the leaf for a few minutes) and photosynthesis (the assimilation). Since we also measured the stomata conductance by changes in relative humidity, we developed a relation of temperature and conductance. These measurements are shown for a typical run in **Figure 2**. The protocol of how the experiment was done is shown at the bottom of the figure. It required about an hour to develop a stable temperature in the growth chamber (after a shift), which is about the same time required for stomata to change conductance to a stable value (see last year's report). We then spent about 15 minutes setting up the system to take the assimilation (or photosynthesis) measurements. A black cloth was draped over the leaf and Licor 6200 and after several minutes, respiration measurements were taken. This was repeated for about 3-4 leaves on 3 branches. Experiments were performed with an increase in chamber temperature over a day and with a decrease in temperature over a day. While there is variation from day to day and leaf to leaf, the general observation shown in the figure hold true for both an increasing and a decreasing air in chamber temperature. Assimilation is highest from 20-24 C and then declines up to 38C (our last temperature). It is noted here that that decline is nearly 80% (from 7 to 1 μmoles/m^2 sec). Concurrent with the fall in assimilation, the respiration of the leaf rises nearly 5-fold (from 0.2 to 1.1μmoles/m^2 sec). Indeed, at the highest temperature measured here, the respiration rate is nearly that of the assimilation rate[2]. There are two points about the stomata conductance: [1] it

[2] There is some question of how to express this because respiration is occurring during the assimilation measurement. For this particular graph, at 24 C photosynthesis is actually measured assimilation + respiration or (6.8 + 3.0) = 9.8 μmoles/m^2 sec while at 38 C photosynthesis is actually (1.3 +1.1) = 2.4 μmoles/m^2 sec or a decline from the maximum of 1 – (2.4 /9.8) = 75.5%.

does not change by much (generally about 3-6%) in our short dark period and [2] it appears to be still limiting the photosynthesis as we have observed before (a plot of assimilation *verses* conductance is nearly linear). We have not completed all the evaluations for all the experiments, but point [1] is absolutely true, while some of the limitation indicated in [2] may not be completely true for all cases of branches/leaves.

Branch Water Use With Sap Flow System

The change of water flow through a branch gives a measure of conductance of all the leaves upon that branch. The carry out this measurement we are using a sap flow system on the branch for a continuous measurement of transpiration (**Figure 3A**). This sap flow system is placed upon the bark of the branch and a small amount of heat is applied within a insulated container. The temperature near the point of heating is monitored on both sides. The heat is lost by movement through the bark up and down but a sap flow through the branch increases the heat loss on the upwards side of the branch and so the temperature on that side is higher (**Figure 3B**). With correct calibrations we can measure total transpiration of that branch.

We can set the system up on a branch and let it run for at least two weeks. Once the heating is adjusted so that no injury occurs to the branch, but the heat is enough to be measured by the thermocouples within the system, the system works very well. The data is collected every 15-30 minutes into a data logger. After down-loading the data, the sap flow can be calculated on a spread sheet. A typical experiment is shown in **Figure 4**. It does vary during the day (3-10 grams/hour flow) due to temperature and light. It ceases during the night and takes time to rise to a high level in the morning, much like is measured for individual leaves. Thus, the system works very well and is stable. The leaves of the branch seem to be unaffected by the monitor clamped to the branch. In addition, the branch is smaller and more easily manipulated than a small tree.

Two major experiments upon a tree in the green house resulted in what were thought initially disappointment, as shown in **Table 1**. We found that the sap flow measurement of a branch is much lower than that calculated from the total transpiration of each leaf measured by a LiCOR 1600 porometer, which measures the actual stomatal transpiration. We felt that this may be due to two problems: [1] the LiCOR porometer measurement does not take into account conductance of water vapor through the boundary layer, which may be very high for the large avocado leaf and the low wind velocity in the green house, and/or [2] the portion of the branch measured (it is really measuring only a portion of a small branch) is not feeding water to all leaves on the branch. In other words, the sap flow through the branch is asymmetric. With other data (as discussed below) the concept has developed that the LiCor system does not measure the real movement of gas through the leaf. In other words, the LiCor by stirring the measurement chamber vigorously does measure the actual stomatal conductance[3]. However, the real gas flow is due to a flow through the stomata after moving through the boundary layer, which is the

[3] The actual Licor 1600 system takes the measurements by clamping small chamber on the bottom surface of the leaf. There is a small fan to rapidly circulate the air within the small chamber and the humidity of the chamber is measured. As the humidity of the chamber changes due to transpiration, the rate of change is used to calculate the transpiration rate (as a function of vapor pressure deficit) and the conductance.

unstirred layer surrounding the leaf. That movement is characterized by the boundary layer conductance[4].

Table 1. The branch used had 15 and 16 leaves on it and each leaf was measured by the porometer. The leaf area was estimated by measurements of length and width and the total conductance was calculated. Each conductance was summed to provide the total LiCOR transpiration. The sap flow meter was calibrated as described by the methods provided by the distributor.

Trial	LiCOR Transpiration (grams/hour)	Sap Flow (grams/hour)
A	200	1.4
B	143	3.5

Again in Table 1 we test the real flow of the sap flow by measuring the measured stomata conductance with the sap flow. We find that the value is nearly 20-100 times lower. This suggests that the sap flow meter is incorrect or that the LiCor transpiration rate does not measure the actual transpiration stream. If the measurement systems are correct, the only interpretation is that we have not taken into account the boundary layer correctly. Unfortunately, this is suspected to be true by many researchers, but that boundary layer is very difficult to calculate or measure.

Tests of the Boundary Layer Limitation Concept

Our basic concept to date is that the stomata of the avocado provides the major limitation to gas flow into the leaf. With a gas flow limitation, light may not provide the measure of the maximum productivity of the leaf and anything that interferes with the stomata will limit CO_2 fixation, photosynthesis, and so limit productivity. In fact, our concept is that by measuring stomata conductance we can measure the productivity of the tree and our goal would be to maximize stomata conductance, either by manipulation of the microenvironment or by altering the tree's physiology.

Under that concept we re-examined some earlier data obtain under field conditions. In **Figure 5** we plot the data collected by Xuan, Mickelbart and Arpaia (unpublished). While these data were collected under very different conditions in the field, they fit a scatter plot of photosynthetic assimilation verses stomata conductance. The conductance measured in the field was very large under some conditions but so was the assimilation. **Figure 5A** show how well the data fit a enzymatic mechanism. While the data show a scatter (there is a large variability of the microenvironment—light intensity, relative humidity and air temperature—and of the leaf age), it does fit well an enzymatic concept, as given below.

$$A = - R + A_{max} \; g / (g + K_g)$$

[4] The schematic of the movement of gases is shown in **Figure 4**. CO_2 is higher on the outside of the leaf (since it is being fixed within the leaf and so its concentration is lower there). Thus the flow is from outside to in and is govern by the resistance to flow and the gradient of concentration. Water vapor flow similarly except it is from inside (at 100% RH) to outside (bulk atmosphere). Both conductances are similar, except for the mass of the gas involved, and so CO_2 and H_2O move under similar driving forces. There are however two resistances, stomata (r_s) and boundary layer (r_b).

where A = Assimilation, R = respiration, A_{max} = maximum assimilation, g = conductance and K_g = a constant governing the shape of the curve. This indicates that there is a maximum assimilation possible even when the conductance is very large.

The respiration measured here is similar to what is obtained under relatively high air temperature (see Figure 2). The conductance is higher than what was normally obtained under lower light conditions (high light has been correlated with higher conductance). Thus, here the highest assimilation rate would be about 54 μmoles/m^2 sec with a respiration of about 16% of the maximum at a field air temperature of 30-35C. Note that under most conditions, these data could be fit to a linear relation (assimilation varies linearly with conductance), indicating that the stomata limit assimilation.

Interestingly these data also yield a measure of how higher assimilation rate depress the internal CO_2, as expected (**Figure 5B**). The depression of CO_2 due to fixation causes the internal concentration to be lowered to about 200 ppm (with an external concentration of about 380-400 ppm). Thus once the internal CO_2 reaches 200 ppm, maximum assimilation is reached, suggesting the mechanism of feedback control on the avocado. Also shown in **Figure 5C** is the variation of conductance with a water vapor pressure deficit or differential (VPD) between the air and the interior of the leaf (there is a threshold before depression occurs of about 2 kPa and then conductance ceases when the VPD reaches 3.5 kPa).

Another test of the concept that we are not taking into account of the boundary layer conductance correctly can be seen with the sap flow monitor and an artificial wind (use of a fan). In the experiments shown in **Figure 6**, a fan blows air on a portion of a small tree (wind at 3 m/sec = 6.5 mph, onto gauges 1 and 3). On day 1 no wind is used but on day 2 wind is used on the branches that are monitored by gauges 1 and 3 (gauges 2 and 5 are on a portion of the tree not reached by the wind). The rate of sap flow is higher on the branches that are affected by the wind (the wind lowers the boundary layer resistance and thus should allow a faster gas flow and a higher level of transpiration and sap flow). The peak is higher under the wind but a better measurement can be made by integrating the total flow of sap during the period of wind (from 11AM to 5PM). If we add the increase on the wind gauges divided by the sap flow on the control (no wind) branches, we obtain a wind effect of 31%. In other words, the wind increases the sap flow by 31%. If the assimilation (limited by the conductance) is increased by the same amount (by lowering the boundary layer conduction), then the productivity would increase by 31% over the 6 hours of wind. More experiments are in progress but it is clear that measuring the stomata conductance by the LiCor which does not take into account the boundary layer and so does not measure the true gas exchange[5]. Furthermore, rapid mixing of the air above the leaf by wind increases the assimilation, at least under high light conditions.

Light Intensity

Another factor, which can greatly influence productivity, is light. Light provides the energy source that drives carbon assimilation in the leaf. Avocado trees, 'Hass' in particular, can develop a very large umbrella-like canopy that is largely empty inside. There is only a relatively

[5] Licor claims that boundary layer is considered. It is in the actual measurement as a value is given to the program calculating the conductance which is governed by the rapid movement of air by the fan in the chamber. It is not this that is the problem. It is the lack of measurement of the boundary layer within the field that gives the wrong indications. In fact, it is very difficult to measure this on one leaf. It really takes an integrated measurement of the flow of transpiration stream (sap flow) that we feel is the correct measurement.

thin layer of leaves on the outer part of the canopy. It appears that light can be fully absorbed by this layer of leaves, leaving no light available for interior layers of leaves. This is a typical observation in physiological terms for other plant species, but it is quite striking in avocado. Once the light intensity drops, photosynthesis drops. If the photosynthetic rate is too low, it cannot compensate for respiration.. Under these conditions the leaf has a negative carbon balance; the leaf uses more carbon than it produces and is therefore a net drain on the plant. This can lead to leaf abscission. A large, somewhat empty canopy and leaf abscission is not a problem for the large-canopied tree *per se* but it is a problem for the grower since yield efficiency (the amount of fruit produced per cubic foot of tree) is greatly reduced. At this stage we do not understand the relationship between light intensity over a day and the potential for a leaf to abscise.

Yet an understanding of the developmental physiology of avocado leaves and how this relates to canopy management depends upon how the layers of leaves within the canopy can 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. We now believe that light flecks of less than 10-20 minutes do not contribute to the overall productivity of the tree. It is rather the amount of prolonged periods of light that each leaf intercepts which drives the productivity and longevity of the leaf. The question of how long during the day each layer of leaves can be illuminated seems to be the more important point.

Initially we tested the movement of light through a leaf by applying the measurement of light reflected from and transmitted through a leaf using the Licor 1850 spectroradiometer (see **Figure 7A**). The measurements are light intensity at each wavelength into the surface of the leaf (I_{in}), the light intensity reflected from the leaf surface (I_{ref}), and the light transmitted through the leaf (I_{trans}). The difference of the amount in *verses* the amounts through and reflected is the absorption of the pigments within the leaf (see **Figure 7B**). It is that which is absorbed in the visible range (wavelengths of 400-700 nm) that drives photosynthesis and is critical for productivity. On the other hand, that which is absorbed in the IR range (700-1100 nm) alters the temperature of the leaf by heating. While the reflectance is slightly different from the top and bottom surfaces and those same parameters differ between the ages of each leaf, we see only about 10-15% total differences. These differences are too small to affect any productivity differences between different aged leaves or orientation and to affect greatly the leaf temperature. To be sure, under certain conditions of modeling they should be taken into account, but they are small shifts in light absorption and use and should not greatly affect productivity. It would be interesting to see certain differences between varieties as there are differences in wax composition/structure over the varieties and that might indicate how waxes affect the light absorbed by the leaf and so affect productivity. However, at this stage we feel that the effects are small (5-10%).

We are concerned about the amount of light which is intercepted by the leaves. There are two types of light: direct (that falling directly on the leaf from the sun) and diffused or indirect (that which is reflected from the sky and other surfaces). Direct is most intense and drives photosynthesis but diffuse can give rise to many effects of physiology and does provide enough energy to generate some photosynthetic products. In particular, avocado seem to carry out photosynthesis under relatively low light intensity, which is that of diffuse light. We wished to tested the notion that diffuse light maintained the stomata in an open conditions. Using the sap flow monitor, but applying separate monitors to two branches on opposite sides of the tree, east

and west facing, we followed sap flow during the course of the day. We expected that the full sunlight on the east side in the morning would support more open stomata on the leaves on that branch with a higher sap flow, while the opposite should occur in the afternoon—the sap flow of the branch on the east side should be lowered while the sap flow on the west side branch should increase. Unfortunately that was not exactly the case (see **Figure 8**). There was a small change in the sap flow between the east and west branch during the day but it was small (about 10-15%). In part we feel that is true because conditions change during the day as to the condition of the tree and the environment near the branches. But we also suspect that diffuse light (that light which is reflected and scattered from the environment and tree) is nearly as good at supporting photosynthesis as direct light. This has profound impact on how we model any canopy—it is not simply the direct light that causes productivity and so the side of the tree that is illuminated may not be very important in productivity calculations.

We tried to obtain an idea of the amount of light intensity that we have to be concerned with by measuring the light intensity in different directions at 8 AM in the morning (**Figure 9**). Naturally the sun was low in the east and the maximum intensity was measured in that direction (1650 μmol of photons /m^2 sec). Yet because of atmospheric scattering light was observed in every direction with about 200 μmol of photons /m^2 sec towards the west. This may be important since the dependence of photosynthesis in avocado seems to be saturated as relatively low light intensity, or at least that intensity is where the stomata limitation is the greatest and so higher light may not add much to photosynthesis. We are beginning a series of more controlled experiment in which the local environment on both sides of the tree are heavily monitor and more branches are monitored (as we have bought more branch sap flow monitors) to see how great this effect is.

An understanding of the developmental physiology of avocado leaves and how this relates to canopy management depends upon how the layers of leaves within the canopy can 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. While we continue to be concerned with light flecks of less than 10-20 minutes which do not contribute to the overall productivity of the tree, we don't understand how these light flecks interact with the tree to allow it to use diffuse light more effectively. **Figure 10** illustrates a typical light exposure experiment conducted by the Mickelbart's group at Lincoln University. One-year-old potted 'Hass' avocado trees are maintained in a growth room with light levels around 1000 μmol of photons/m^2/s (about half of full sunlight on a cloudless day). Individual leaves are exposed to about 200 μmol of photons/m^2/s for a period of time before increasing the light level to close to 2000 μmol of photons/m^2/s (roughly full sunlight). We see variable photosynthetic responses in the leaves, both during light transitions and after. There are potentially several reasons for this. First, we may not be adapting the leaf to the lower light level for long enough. We are currently testing this theory. Second, we believe that the response will be dependent on leaf age and, more specifically, the flush in which the individual leaf was produced. We are currently setting up a series of experiments to test these theories and hope to have a working model for light response of individual leaves by the end of next year's work. At the moment, we believe it is the amount of prolonged periods of light that each leaf intercepts which drives the productivity and longevity of the leaf, rather than its ability to respond to short periods of intense light exposure. However, this needs to be rigorously tested before any conclusions can be made. The question of how long during the day each layer of leaves can be illuminated seems to be the more important point.

Productivity Model

We are developing a model 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. However, it seems that the data obtain above are critical to understand which parameters of such measurements are important and how to integrate these measurements into the simple model. 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.

Figure 1. Alteration of conductance by application of ABA. The gas flow across a cut leaf was measured as described in the previous report, but the leaf water potential was maintained at normal by solution of water flowing across its cut circumference. The data shown here are from a leaf that has been illuminated for about 1 ½ hours to reach stable state of assimilation and conductance. ABA solution (25 μM) was added at the arrow. Without ABA the assimilation and conductance remained the same (as shown). At the next arrow the ABA solution was replaced by a water solution. The steps down to zero for both traces were due to automatic zeroing of the IRGA used to measure the CO_2 and water vapor and, while necessary, were not part of the actual measurements of the leaf.

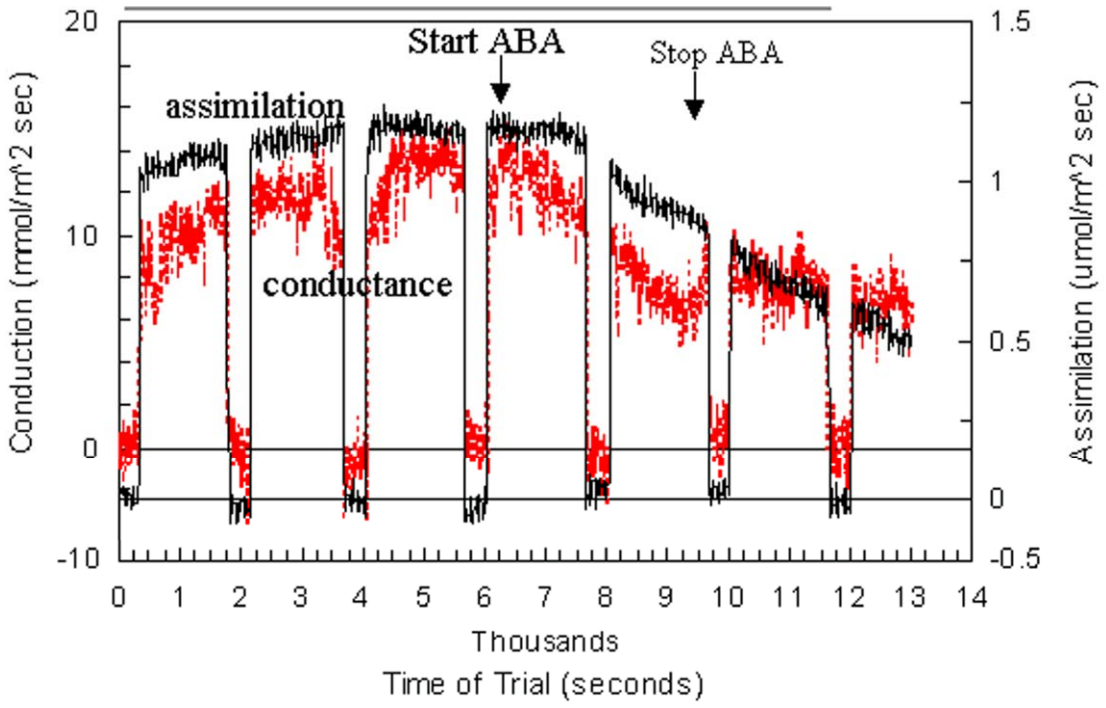

Figure 2. Temperature dependence of assimilation and respiration of leaves on a tree. The growth chambers were as used before (see previous report) and maintained at a constant relative humidity (43%). The air temperature of the chamber was shifted up (from 20 to 36C, as shown here) or down (from 36 to 20C, data not shown). The conductance and CO_2 assimilation of individual leaves (10 on 2-3 different branches of three small trees) were measured by a Licor 6200 system. CO_2 fixation (assimilation) or uptake (respiration) was measured in the light or dark (see Figure 2A) for varied chamber temperatures (average of 10). In 2B, the conductance of each leaf was measured in the light or dark. The sequence of the measurements for each chamber temperature was shown in Figure 2C. In all cases stable chamber temperature and stomata conductance was reached before any measurement were made. During the dark period the stomata begin to close but the measurements were made before any sizable change was measured (less than 10%).

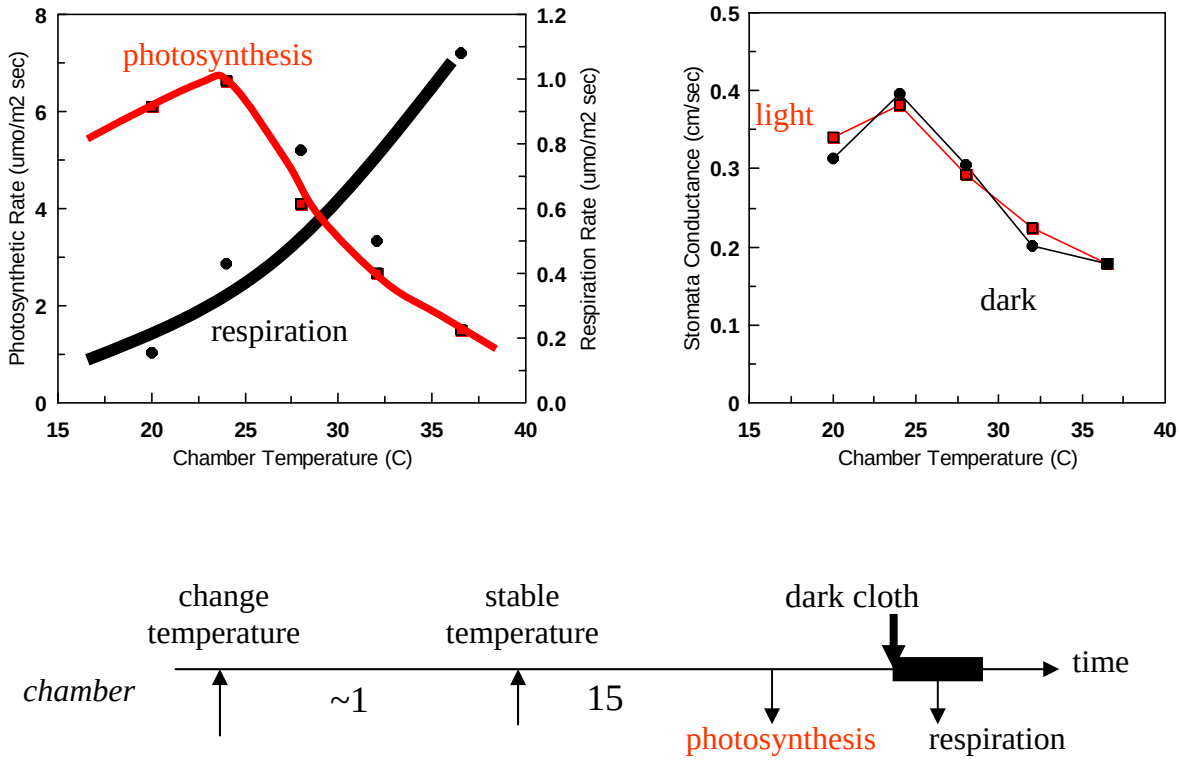

Figure 3. Sap flow measurement system. In figure 3A, the sap flow meters on the branches are shown within the insulation to slow heat flow out of the system. In figure 3B, the schematic of the heat flow within the system is shown and indicates how the measurements were made. A constant amount of heat is put into the branch and thermocouples measure heat flow up and down and outward to the atmosphere. The difference between in and out (up and down) was used to calculate the flow of transpiration upward, since that flow of essentially water carried heat up the branch. In figure 3C, a typical heat flow (dT) and actual sap flow (flow) is shown over the course of a week. The actual flow varied due to the microenvironment of the leaves on each branch. At night the heat flow was small and the calculation induced a large amount of inaccurate variability. Thus, the flow at night was set to zero. The time is 24-hour Pacific Standard Time.

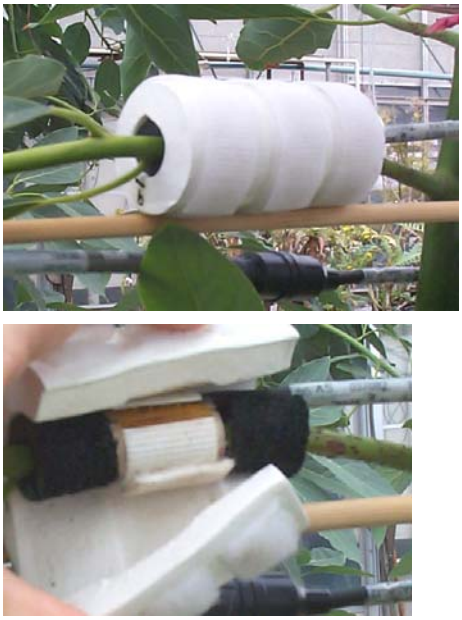

Figure 3A

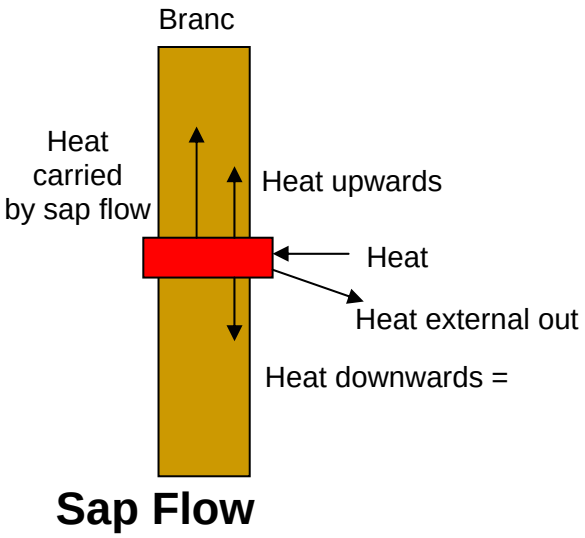

Figure 3B

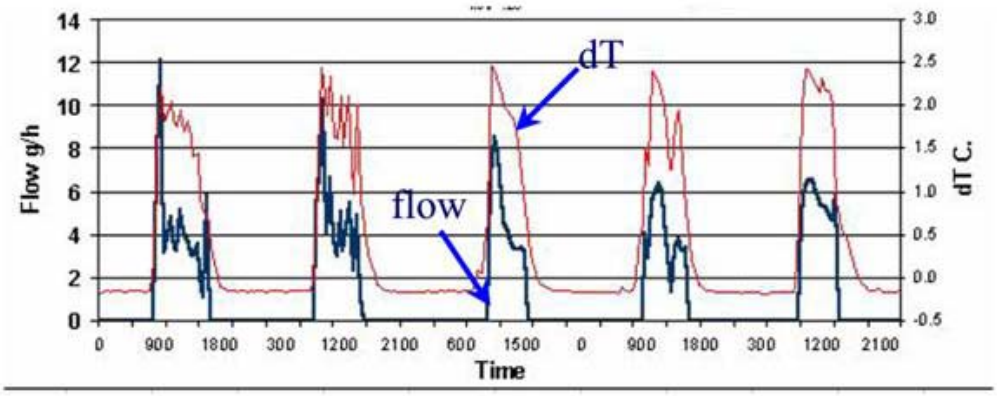

Figure 3 C

Figure 4. The schematic of the gas flow into/out of a leaf. See text for the description but the flux of the gas is proportional to the difference in concentration between inside and out regions and the total conductance of that flow. A better method of thinking about the flow is that the pathway gives a resistance to the flow. The total resistance is due to the sum of a boundary layer (b) and a stomata resistance (s). Then the conductance is just the inverse of the total resistance.

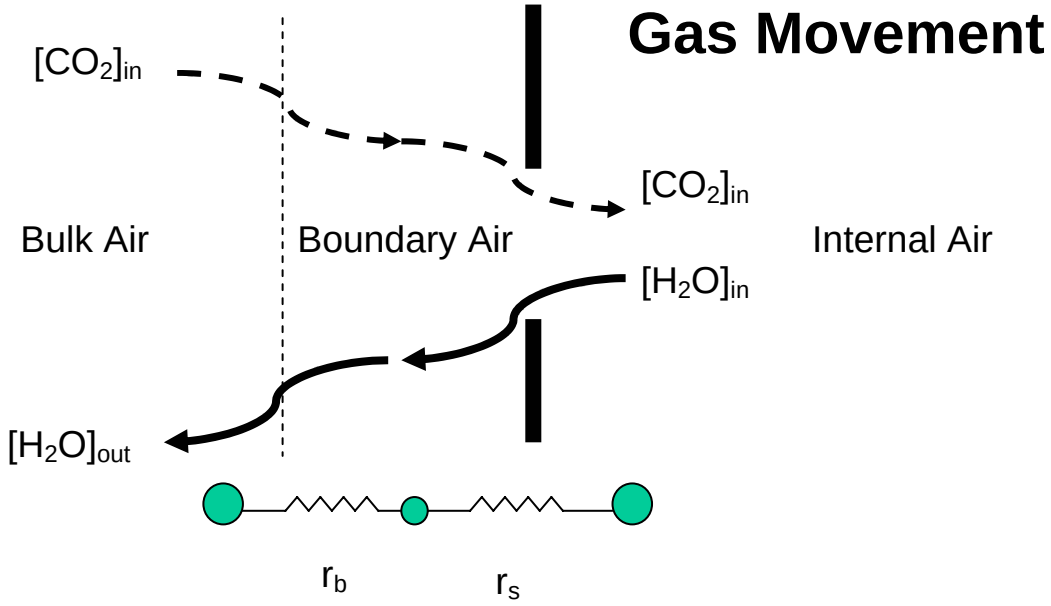

Figure 5. Gas flow from Avocado leaves. **A**. Data were taken from Xuan, Mickelbart and Arpaia, using a LiCor 6200 porometer on Hass Avocado trees. The data of assimilation and conductance is indicative of the field in Riverside over a wide range of microenvironments and leaf age and was fit to the enzymatic formulation of dependence of assimilation on conductance (see equation in text). The scatter of the data fits a Gaussian curve with a standard deviation of 3.5 μmoles/m^2 sec over all the assimilation data points, as an indication of variation. **B**. The dependence of the calculated CO_2 level within the leaf upon assimilation. As the assimilation increases, the internal concentration of CO_2 declines reaching a constant level of about 55-60% of the external level. **C**. The dependence of the conductance upon the leaf vapor pressure deficit (which is calculated as the difference between the internal water pressure of the leaf at 100% and the external water pressure of the atmosphere below 100%).

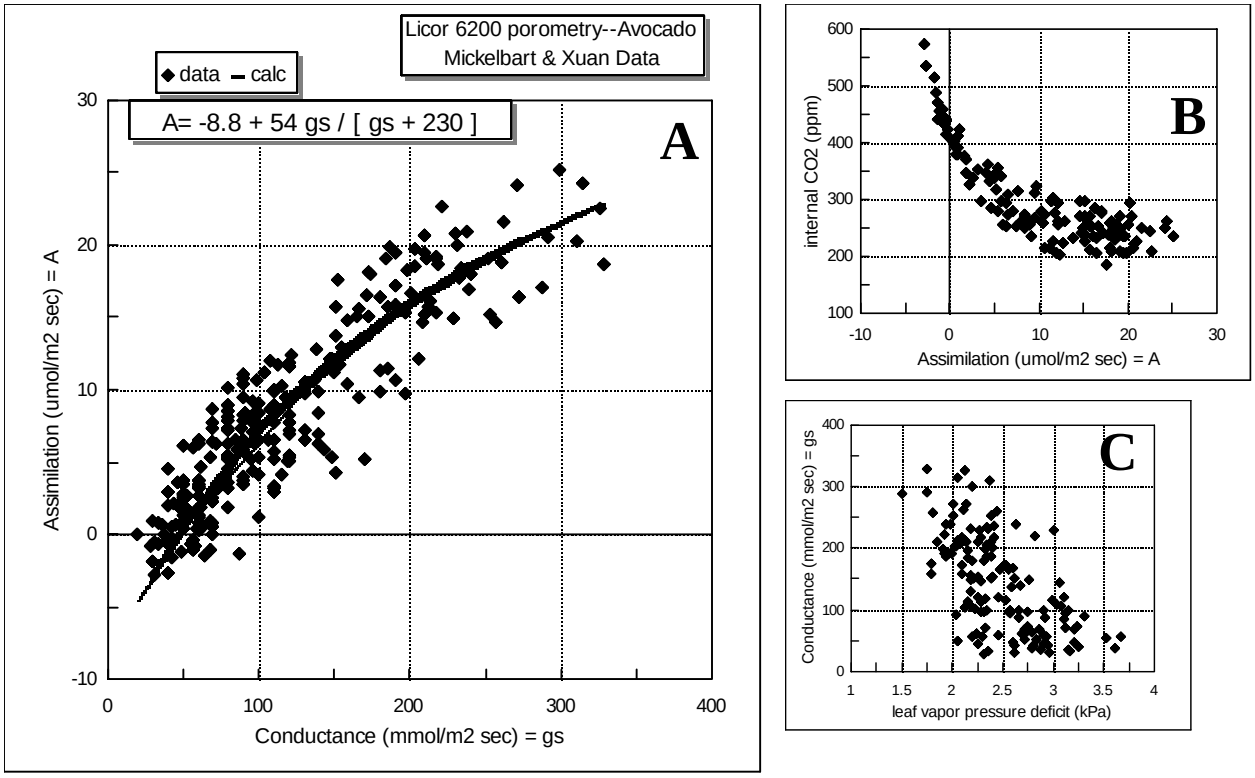

Figure 6. Effect of wind upon the sap flow of branches. Sap flow was measured by the sap flow monitor (see Figure 3). Four gauges were fitted on four separate branches of a single tree. Wind was blown on the one portion of the tree (see Figure 6A) from 11:00AM to 5:00 PM on day 2, while no wind was blown on day 1, see Figure 6B for the data.

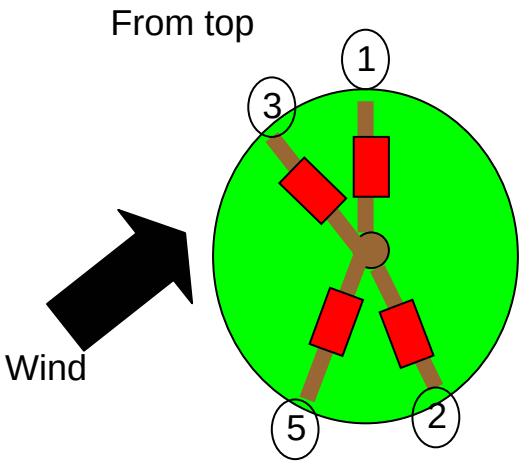

Figure 6A

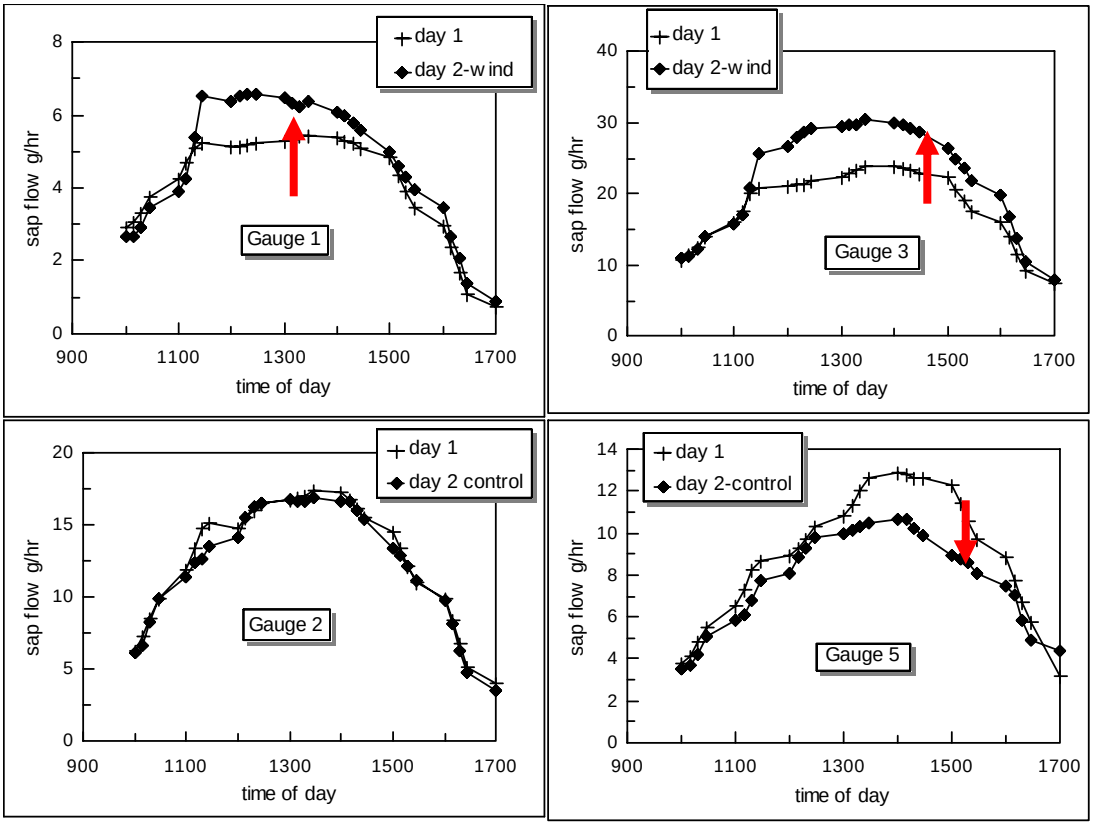

Figure 6B

Figure 7. Light absorption by avocado leaves. **A**. The distribution of light upon a leaf. I represents the intensity of light at a certain wavelength over a given band width of light intensity, where in = incident, ref = reflectance, trans = transmitted, and abs = absorbed calculated as [in – ref – trans]. **B**. Typical light parameters of an avocado leaf from a wavelength of 400 to 1100 n, in 5 nm intervals. The leaf is a mature leaf from a Hass avocado.

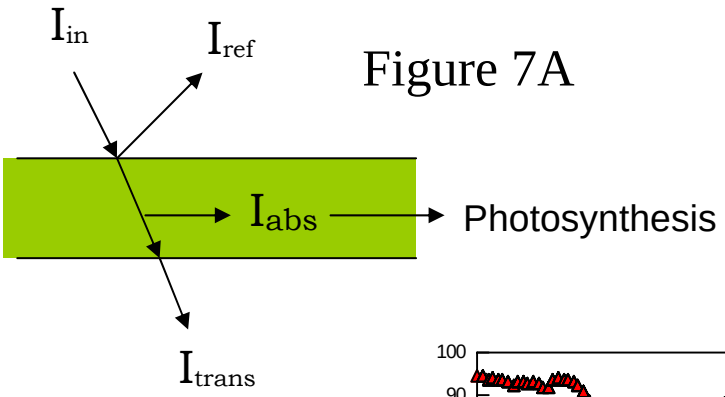

Figure 7A

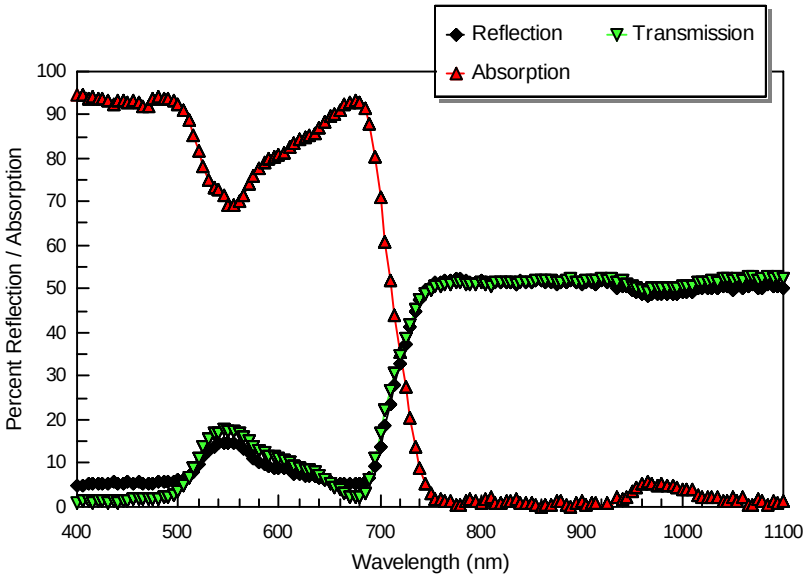

Figure 7B

Figure 8. Sap flow from a tree with sap flow monitors on branches separated by 180°. A comparison of a west and east facing branch on a Hass avocado for two days. Each day varied in its air temperature and light intensity but the sap flow on each branch has been normalized to 100% (while each were similar, they were: maximum flow on the east branch = 6.37 g/hr and on the west branch = 8.86 g/hr).

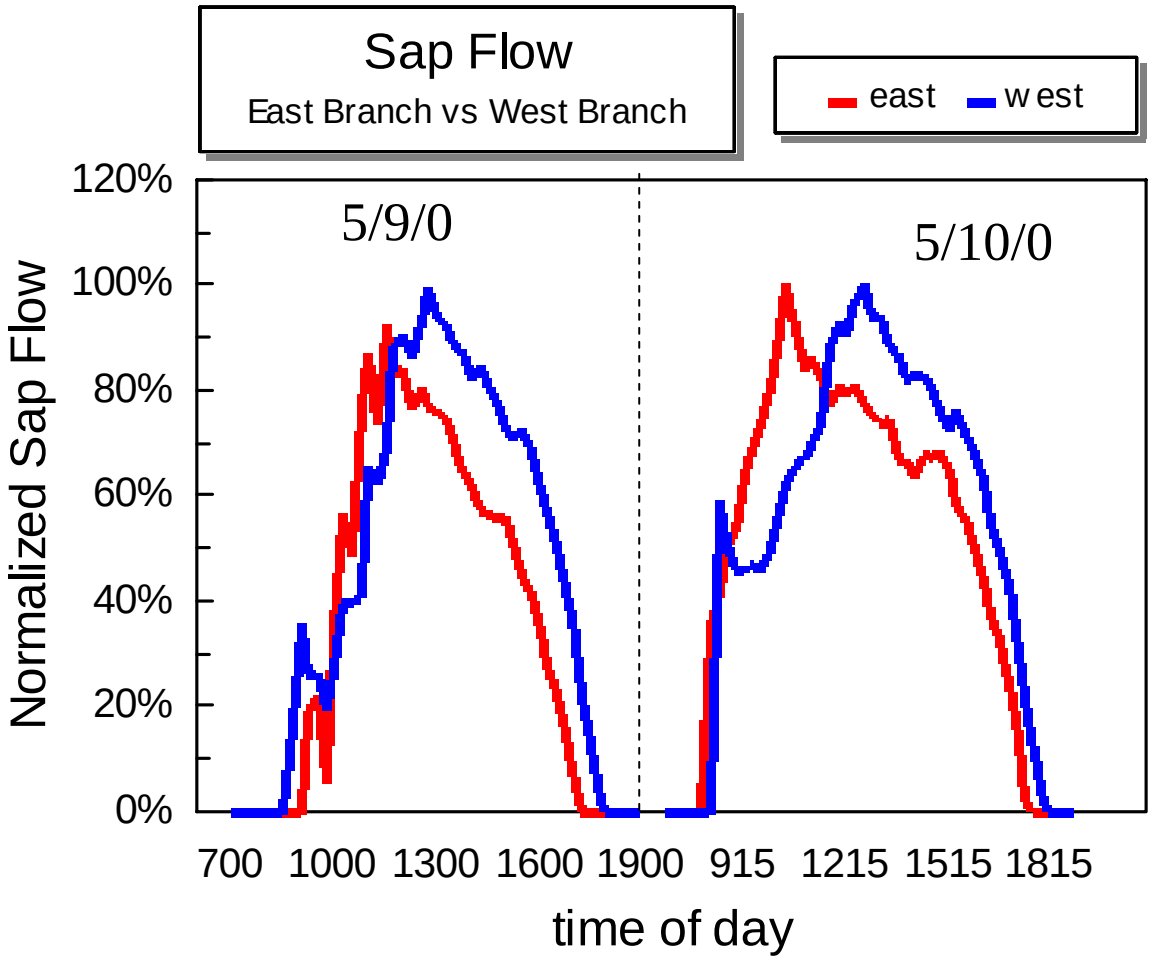

Figure 9. Directionality of sun light. The intensity of sun light in the summer as measured with a Licor 1850 spectroradiometer. The band measured was Photosynthetically Active Radiation from 400-700 nm. The region measured was at 45^{o} from the horizon at 8 AM in the morning.

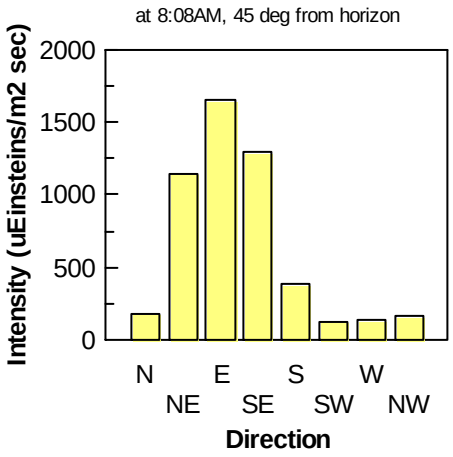

Figure 10. Leaf response to sudden changes in light (simulated "sunflecks"). The data were obtained with a Licor 6400 system to measure the assimilation rate. The gray line (denoted by "light") shows the light level the leaf was exposed to, and the black line (denoted by "assimilation") shows CO_2 assimilation rate. See text for more details.