Chlorophyll $a + b$ Content and Chlorophyll Fluorescence in Avocado

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Abstract
Tonnage (T), Simmonds (S) avocado trees, and TxS crosses were evaluated for differences in chlorophyll content and maximal quantum yield of photosystem II in sun and shade-type leaves. Total chlorophyll content by area (Chl $a+b_a$) ranged from 984 mg m$^{-2}$ in TxS240 to 4320 mg m$^{-2}$ in Simmonds. Chlorophyll $a/b$ ratio (Chl $a/b$) ranged from 9.8 to 5.5 in TxS238 and TxS243, respectively. Tonnage and Simmonds had similar Chl $a/b$ with a wide range in values found among the avocado trees tested. Shade leaves contained more Chl $a$, Chl $b$ and Chl $a+b$ wt than sun leaves. Differences in Chl $a/b$ were insignificant or greater in shade adapted leaves for all trees except TxS238; this did not follow the expected sun/shade pattern. A low chlorophyll $a/b$ ratio indicates more light harvesting proteins and higher stacking of thylakoids. Chl $a+b_a$ indicates Simmonds, Tonnage and to a lesser extent TxS238 had dense packing of chloroplasts in both sun and shade adapted leaves. Shade leaves had more efficient Fv/Fm values than those adapted to sun for all varieties except TxS240. Tonnage had the largest range of total chlorophyll content between shade and sun adapted leaves and likely has the largest genetic variation in its ability to acclimate to changing light intensities. The range in efficiency of photosystem II found between the avocado trees tested indicates a potential for improvements through selective breeding. More research is needed to evaluate the entire USDA avocado germplasm collection for traits associated with photosynthetic efficiency and to determine their heritability.

Keywords: Avocado, Chlorophyll $a/b$ ratio, Chlorophyll fluorescence, Specific leaf area

1. Introduction

1.1 Avocado Production
In the United States, avocados are grown on over 66,000 acres, primarily in three states California, Florida and Hawaii. Almost 300 tons of fresh fruit were produced earning growers $429 million in revenue for the 2009-2010 season (USDA, NASS, 2011). Avocado grows best in full sunlight; with branches facing the sun yielding larger and more abundant fruit than their shaded counterparts. The USDA/ARS is in the process of mapping Florida adapted populations of the avocado genome. To augment this effort a vast amount of data is needed on physical and chemical characteristics associated with producing a marketable item for consumption.

1.2 Photosynthetic Efficiency vs. Yield
Avocado varieties adapted for tropical and subtropical environments produce a wide range in fruit size implying there is potential for yield increases through selective breeding. Differences in photosynthetic efficiency among avocado varieties may be related to these yield differences. Although low reserves of storage carbohydrates are associated with low yield, increasing the amount of stored carbohydrates may not improve yield (Finazzo et al., 1994). From onset of flowering through early stages of maturity, reproductive organ development relies in part upon production of de novo photoassimilates taken directly from the Calvin cycle (Liu et al., 1999a, 1999b; Liu et al., 2002). Varieties with efficient photosynthetic machinery in both leaves on the outer canopy, well exposed to light, and those in the inner canopy adapted to shade, would have an advantage in fruit production.

1.3 Chlorophyll in Sun and Shade leaves
Sun leaves are known to differ from shade leaves in their composition of photosynthetic pigments, chloroplast ultrastructure, photosynthetic rates, and resistance to light stress (Anderson et al., 1995; Lichtenalther et al., 1981; Lichtenalther et al., 1982; Sarjeva et al. 2007; Lichtenalther et al., 2007a; Sims and Pearsy, 1991). Maple (Acer
pseudoplatanus L.), beech (Fagus sylvatica L.), linden (Tilia cordata Mill.) and fir (Abies alba Mill.) had a higher total chlorophyll content on a leaf dry weight basis in shade adapted than in sun adapted leaves, however, this trend was reversed when sun and shade leaves were compared on a leaf area basis (Lichtenthaler et al., 2007a). Chlorophyll a to b ratio and maximum net photosynthetic CO₂ assimilation at saturating irradiance were higher with sun-adapted leaves. Similar sun/shade leaf chlorophyll adaptations have been found in ginkgo (Ginkgo biloba L.), rice (Oryza sativa) and orchardgrass (Dactylis glomerata L.) (Murchie et al., 2002; Peri et al., 2007; Sarijeva et al., 2007). In three broadleaf tree species, Platanus acerifolia Wild., Populus alba L. and Tilia cordata Mill. chloroplasts from sun leaves had lower amounts of light-harvesting proteins, more reaction center proteins and a greater number of electron transport chain than shade-adapted leaves (Lichtenthaler et al., 2007b). Sun leaves had a higher photosynthetic CO₂ assimilation and stomatal conductance rate. Sun acclimated leaves of Ginkgo were thicker and possessed higher CO₂ assimilation and stomatal conductance than shade leaves (Sarijeva et al., 2007). The high net photosynthesis in sun leaves is attributed to the sun-type structure of the chloroplasts and the high stomatal conductance.

Avocado trees with inner canopy leaves that have more of a sun-type chloroplast structure should have a higher chlorophyll a/b ratio and chlorophyll fluorescence values, and maximal quantum yield of photosystem II in sun and shade-type avocado leaves.

2. Materials and Methods

2.1 Study Area

Trees were sampled from a five-year old avocado orchard located on the USDA-ARS, Subtropical Horticulture Research Station, Miami, FL, USA (25°38´N, 80°17´W). South Florida has a subtropical marine climate with 146 cm mean annual precipitation falling mostly between May and October. Mean daily maximum and minimum temperature range is between 26°C and 17°C for Nov – Apr, and between 31°C and 24°C for May – Oct. Elevation at the site is 4 m above sea level. Soil is classified as a Krome very gravelly loam (Loamy-skeletal, carbonatic, hyperthermic Lithic Udorthents). The soil has a depth of 5 to 25 cm above an oolitic limestone bedrock and is well drained. The trees were planted by auguring holes into the bedrock, inserting the sapling, and backfilling the hole with pulverized rock and mulch.

2.2 Chlorophyll Fluorescence

One tree was selected from each of two avocado varieties: Tonnage (T) and Simmonds (S); with an additional four trees from TxS crosses. Tree height, stem diameter at 25 cm above soil surface, and the number of branches were recorded for each tree. Ten healthy fully mature leaves with a southern exposure in constant daytime sunlight were selected from each tree. In addition, ten shade leaves (north exposure) from the inner canopy of each tree were selected and labeled with a marking pen. Fluorescence readings were taken during January 2010. Between 8:00 and 10:00 A.M. leaf sections were dark adapted for 30 min. Chlorophyll fluorescence readings were taken with an OS-30p portable chlorophyll fluorometer (Opti-Sciences Hudson, NH, USA). Dark-adapted leaves were exposed to saturating actinic light (660 nm) at 1100 μmol m⁻² s⁻¹ intensity. In the fast kinetic region of fluorescence initial (Fo), maximal (Fm) and terminal fluorescence (Ftr) were measured. The maximum quantum efficiency of photosystem II (Fv/Fm = Fm-Fo/Fm and Fv/Fo) was calculated. Leaves exposed to the sun received a light intensity of > 2200 μmol m⁻² s⁻¹ shade exposed leaves received < 148 μmol m⁻² s⁻¹.

2.3 Chlorophyll Extraction

Immediately after fluorescence determination, the leaves were cut off at the petiole, sealed in plastic bags, and placed in a cooler for transport to the laboratory (15 min travel time). Leaf area was determined with a CI-202 portable leaf area meter (CID, Inc.; Vancouver, WA). Five, 29.2 mm² holes were punched through each leaf and a fresh weight determined for excised tissue. The remaining leaf tissue was weighed and oven dried at 60°C; dry weights were recorded when there was no longer weight loss with additional drying. Chlorophyll extraction comprised of placing excised tissue in 50 mL centrifuge tubes containing 20 mL methanol and three balls of stainless steel shot. Tubes were shaken for two hours on a reciprocal shaker set on high. Tubes were centrifuged and the supernatant collected. This procedure was repeated four more times with 10 mL methanol and a 15 min shaking time.

2.4 Specific Leaf Area and Leaf Weight

Leaf fresh and dry weights were used to calculate leaf relative water content (RWC) on a fresh weight basis (fresh wt – dry wt/ fresh wt). Additional calculations were made for specific leaf area (cm² leaf area g⁻¹ dry weight) and specific leaf weight (mg leaf tissue cm⁻² leaf area).
2.5 Quantification of Chlorophylls A and B

Chlorophyll $a$ and $b$ (chl $a$ and chl $b$) were determined from leaf tissue by the method of Edelenbos et al. (2001). A Dionex DX 500 HPLC system equipped with an AD-20 UV-Vis detector operating at 440 nm and an AS-40 auto sampler were used for analysis. Separations were performed on an Agilent Zorbax ODS column (5 μm; 250 x 4.6 mm i.d.) protected with an Agilent Zorbax ODS guard cartridge (5 μm; 12.5 x 4.6 mm i.d.). The column temperature was maintained at 30 °C and the mobile phases consisted of solvent A (Methanol), solvent B (H2O), and solvent C (Ethyl Acetate). Separations were performed by the following solvent gradient: 0 min (64% A, 16% B, and 20% C), 2.5 min (62% A, 16.5% B, and 22.5% C), 20-22.5 min (40% A, 10% B, and 50% C), 24-26 min (16% A, 4% B, and 80% C), 31-34 min (100% C), 42-47 min (64% A, 16% B, and 20% C). All increases of solvent were linear programmed. The flow rate was 1 mL per min and the injection volume 25 μL. Chlorophyll standards were purchased from Sigma Aldrich (Chlorophyll A C5753-1MG, Chlorophyll B C5878-1MG). Retention times for Chl $a$ and Chl $b$ were 26.5 and 20.5 minutes, respectively.

2.6 Statistical Analysis

Analysis of variance was performed using the GLM procedure of Statistical Analysis System (SAS Inst. 1999). Separation of least square mean estimates was accomplished using linear contrasts with significance at the $P < 0.05$ level unless otherwise specified.

3. Results

3.1 Avocado Tree Size

Table 1 gives size information for avocado trees planted in the USDA orchard. Individual trees sampled for Simmonds and for Tonnage were 1.34 m and 1.50 m tall, respectively. Tonnage x Simmonds crosses were 3 – 4.5 m tall. The orchard sits on thin, very rocky soil formed from oolitic limestone on the Miami Ridge. Small inclusions of loamy to sandy material fill cavities and solution holes formed in the limestone. This provides a supplemental nutrient source for plants. Trees tended to grow larger where their roots could access these cavities. Very little limestone scarification providing access to cavities occurred in areas planted with Simmonds and Tonnage varieties; consequently, after 5-years growth, these trees were smaller than crosses.

<Table 1>

3.1.1 Chlorophyll Content

Chlorophyll $a$ and total chlorophyll on a weight basis (Chl $a+b_{wt}$) were highest in Txs238 and lowest in the three other crosses, Txs239, Txs240 and Txs243 (Table 2). There were no significant differences in Chl $a$ between Tonnage and Simmonds trees in 2010. High values for Chl $b$ were found in Txs243, Tonnage and Txs238 while Txs239 had the lowest Chl $b$ value. Intermediate amounts of Chl $b$ were found in Simmonds and Txs240. However, Tonnage did contain 1.2x more Chl $a$ and 1.1x more Chl $b$ than Simmonds. On a leaf area basis the pattern of total chlorophyll content (Chl $a+b_{ar}$) between trees was slightly different from that found for Chl $a+b_{wt}$. Simmonds contained the most Chl $a+b_{ar}$ and the remaining trees followed the order Tonnage > Txs238 > Txs243 > Txs239 > Txs240. Differences in chlorophyll content on an area basis (Chl $a+b_{ar}$) between Tonnage and Simmonds are likely different from what the January 2010 data indicate. During summer and fall 2009, seven individual Simmonds trees were compared to 10 individual Tonnage trees and Tonnage produced significantly more (Chl $a+b_{ar}$) than Simmonds. In November 2009, cold weather caused some death and complete leaf loss on most other trees, therefore no data were collected that winter for comparison to winter 2010 data presented here. Chlorophyll $a/b$ ratio was significantly higher in Txs238 than any other variety tested. There was no difference in Chl $a/b$ between Tonnage and Simmonds trees. Tonnage had the highest RWC and Txs243 and Txs239 the two lowest. Specific leaf area followed the order Tonnage > Txs240 = Txs238 ≥ Simmonds ≥ Txs239 = Txs243.

<Table 2>

3.1.2 Chlorophyll Content sun vs. Shade Leaves

The overall distribution of chlorophyll followed expected patterns of shade adapted leaves, that is, shade leaves contained more Chl $a$, Chl $b$ and Chl $a+b_{act}$ on a dry weight basis than sun adapted leaves (Table 3). However, individual trees did not always follow this pattern. Where differences were significant, Tonnage, Txs239, Txs240 and Txs243, shade leaves contained more Chl $a$ and Chl $a+b_{act}$ than sun. There were no significant differences between shade and sun leaves for Simmonds and Txs238, never-the-less sun leaves contained more Chl $a+b_{act}$. On a leaf area basis, significantly greater Chl $a+b_{ar}$ were found in shade, only for Tonnage and Txs243. Differences in Chl $a/b$ were insignificant or greater in shade adapted leaves for all trees except Txs238.
The opposite was found for Acer, Fagus, Tilia and Abies (Lichtenthaler et al., 2007) and with Ginkgo and Fagus (Sarijeva et al., 2007) where sun leaves had the highest chlorophyll $a$ to $b$ ratio. In contrast, *Dipteryx odorata* (Aubl. Wild.) contained a higher chlorophyll $a$ to $b$ ratio in shade leaves (de Morais et al., 2007). *D. odorata* is a canopy-emergent tree native of tropical South America, Central America and the Caribbean. Significant differences were found in SLA for Tonnage, TxS239, TxS240 and TxS243. SLA pointed to a more compact arrangement of cells in sun adapted than shaded leaves. There were no significant sun/shade differences in SLA for Simmonds and TxS238. Analysis of combined data for all avocado trees showed significantly higher Chl $a$, Chl $b$, Chl $a+b_{st}$ and Chl $a/b$ in shade than sun leaves.

<Table 3>

3.2 Chlorophyll Fluorescence

Chlorophyll fluorescence values and maximal photosystem II quantum yield of dark-adapted samples (Fv/Fm) are given in Table 4. There were no differences in initial fluorescence (Fo) between sun and shade leaves in four of the six trees or in the combined data. Where differences in Fo existed, sun leaves had higher values than shade. A higher maximal fluorescence (Fm) occurred in shade than sun-adapted leaves. As expected shade leaves had significantly higher Fv/Fm and Fv/Fo values than those adapted to sun for all varieties except TxS240. In shade leaves, highest Fv/Fm values were recorded for TxS238, TxS239 and TxS243 and in sun leaves TxS238 and TxS240 had the highest Fv/Fm.

<Table 4>

4. Discussion

Relative water content and SLA are considered an indication of the leaf structure and morphology produced by the differences in light intensity under which a given leaf develops (Lichtenthaler et al., 2007). A low light environment will produce leaves with a higher RWC and SLA with a lower chlorophyll $a/b$ ratio than leaves adapted to a high light environment (Gilmore et al., 1995; Lichtenthaler et al., 2007; Peri et al., 2007). A low chlorophyll $a/b$ ratio is indicative of shade-type chloroplasts, with more light harvesting proteins and a higher stacking degree of thylakoids than sun-type chloroplasts (Lichtenthaler et al., 1981; Lichtenthaler et al., 1982; Sarijeva et al. 2007). The photosynthetic machinery of shade-adapted leaves is more efficient at harvesting light but will assimilate less CO$_2$ than sun-leaves. Avocado species that feature high chlorophyll $a/b$ ratios, low RWC and SLA should have a high CO$_2$ assimilating sun-type leaf structure. In the present study, no tissue analysis was performed to confirm the association of RWC and SLA with light adapted chloroplast structure. However, total chlorophyll content on an area basis indicates Simmonds, Tonnage, and to a lesser extent TxS238 had dense packing of chloroplasts in both sun and shade adapted leaves. These trees contained between 4 and 12% chlorophyll on a dry weight basis compared to 1 to 3% for the other trees tested. In addition, chlorophyll $a/b$ ratio was highest in TxS238, Tonnage and Simmonds with high values recorded for both sun and shade leaves. For most trees, chlorophyll $a/b$ ratio was higher in shade leaves. This result was unexpected. The five year-old trees in this orchard were relatively small (1 – 4 m) with a more-or-less open canopy. During a portion of each day, enough light could penetrate the canopy to confound the degree of low light acclimation shade leaves could attain. Leakey et al (2003) and Yin and Johnson (2000) reported fluctuating light levels affected the acclimation response of plants grown in shade and subsequently exposed to sunlight. Since most leaves on trees used in our study were newly developed after a frost the year before, any acclimation response of shade leaves was likely muted. In addition, a light intensity of 148 umol m$^{-2}$ s$^{-1}$, measured at the darkest point under the canopy, still may have been above a transition point where a strong response to shading would begin. Sun / shade characteristics of the Chl $a/b$ ratio in avocado were similar to those reported for *D. odorata* by de Morais et al. (2007). *D. odorata* is a tropical tree that grows at the top layer of the forest. In this setting, it would receive a large portion of the incident radiation. It is possible that plants like avocado that evolved under conditions of intense year-round solar radiation will have a low capacity to adapt (or a slow response) to changes from sun to shaded conditions.

Chlorophyll $a/b$ ratio is a measurement of the proportion of light harvesting complex to other chlorophyll components. Plants with a sun type adaptation tend to have a higher chlorophyll $a/b$ ratio, implying a lower amount of light-harvesting proteins and a higher amount associated with the reaction center complex (Leong and Anderson, 1984). An increase in the Chl $a/b$ ratio under high light has a concomitant decrease in photosystem 11 and an increase photosystem 1 chlorophyll. Plant species tend to have a range of light intensities over which an acclimation process will progress (Bailey et al., 2004). Murchie and Horton (1997) attributed large variation in light saturated photosynthetic rates and accompanying chlorophyll content to two strategies for diversity in photosynthetic acclimation: changes in chlorophyll on a leaf area basis associated with photosynthetic capacity and changes in chlorophyll $a/b$ ratio and photosynthetic rates associated with alterations of chloroplasts. Among
the avocado trees studied, Tonnage had the largest range of total chlorophyll content between shade and sun adapted leaves and the second highest chlorophyll a/b ratio. Therefore, Tonnage likely has the largest genetic variation in its ability to acclimate to changing light intensities. Chlorophyll density on an area basis was greatest in Tonnage. High total chlorophyll content and high density imply tightly packed layers of chloroplasts as you move through the leaf from top to bottom. This would provide a range of high and low adapted chloroplast at different depths allowing Tonnage to exploit better a variety light conditions.

Avocado is a tropical plant that is productive only in full sun. Growth comes in several flushes during warm weather and leaves will remain on the tree for two to three years. Although tree pruning is for ease of access in the orchard, the open canopy that results has a positive affect on yield. Trees with a wide range in their shade adaptation response can effectively utilize changing light intensities as new leaves form and are then shaded in subsequent flushes. During reproductive growth, inner canopy leaves that assimilate high levels of CO₂ can enhance yield. In this study, chlorophyll distribution (Chl a+b) in avocado was similar in both sun and shade leaves. However, shade leaves had the unexpected combination of a higher chlorophyll a/b ratio and an approximately 7% greater photosystem II efficiency (Fv/Fm). This implies that the inner canopy contains a variety leaves covering a wide range of light harvesting characteristics. Trees that favor this arrangement should provide more carbohydrate for fruit production than those with a more typical shade-type, low chlorophyll a/b ratio.

Between the two varieties, Simmonds and Tonnage: Tonnage had a greater change in chlorophyll content in response to shading. Simmonds had a greater increase in photosystem II efficiency in response to shading. The range in efficiency of photosystem 1 and shade response found between the avocado varieties tested indicates a potential for improvements in efficiency of sun-type leaves through selective breeding. More research is needed to evaluate the entire USDA avocado germplasm collection for traits associated with photosynthetic efficiency and to determine their heritability.

References


Table 1. Mean height, number of branches and stem diameter at 23 cm above the soil surface for Simmonds (S) and Tonnage (T) avocado varieties and TxS crosses

<table>
<thead>
<tr>
<th>Variety</th>
<th>Height (m)</th>
<th>Branches</th>
<th>Stem diameter (cm)</th>
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<tr>
<td>TxS*</td>
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<td>21.5 ± 2.5</td>
<td>7.90 ± 0.1</td>
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<tr>
<td>Simmonds</td>
<td>1.48 ± 0.29</td>
<td>21.0 ± 5.3</td>
<td>4.02 ± 0.95</td>
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<tr>
<td>Tonnage</td>
<td>1.11 ± 0.27</td>
<td>18.0 ± 9.6</td>
<td>3.26 ± 0.81</td>
</tr>
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</table>

* Based on the mean of four trees from each variety measured in 2009.
Table 2. Mean values for chlorophyll $a$ and $b$ and total chlorophyll content by weight (Chl $a$, Chl $b$, Chl $a+b_{wt}$), total chlorophyll by area (Chl $a+b_{ar}$), chlorophyll $a/b$ ratio (Chl $a/b$), relative water content (RWC), and specific leaf area (SLA) for ten sun and ten shade leaves from six avocado trees sampled winter 2010

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<th>TxS243</th>
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<th>Tonnage</th>
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<td>59.4 cd</td>
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<td>45.6 c</td>
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<td>RWC</td>
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<td>SLA (cm$^2$ g$^{-1}$ DW$^+$)</td>
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<td>179 b</td>
<td>135 c</td>
<td>160 bc</td>
<td>217 a</td>
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* Means in each row followed by a different letter are significantly different at $P = 0.05$.
† leaf area per unit dry weight.

Table 3. Mean values for chlorophyll $a$ and $b$ and total chlorophyll content by weight (Chl $a$, Chl $b$, Chl $a+b_{wt}$), total chlorophyll by area (Chl $a+b_{ar}$), chlorophyll $a/b$ ratio, and specific leaf area (SLA) in sun and shade leaves from six avocado trees sampled in winter 2010

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* Means for an individual variable for each avocado variety followed by a different letter are significantly different at $P = 0.05$.
† leaf area per unit dry weight.
Table 4. Differences in initial chlorophyll fluorescence (Fo), maximal fluorescence (Fm), maximum quantum efficiency of photosystem II (Fv/Fm and Fv/Fo), and terminal fluorescence (Ftr) between sun and shade for six avocado trees sampled in winter 2010

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<td>Fv/Fo</td>
<td>2.97 a</td>
<td>1.96 b</td>
<td>3.15 a</td>
<td>2.33 b</td>
<td>3.99 a</td>
<td>3.07 b</td>
<td>3.94 a</td>
</tr>
<tr>
<td>Ftr</td>
<td>347 a</td>
<td>216 b</td>
<td>411 a</td>
<td>283 b</td>
<td>584 a</td>
<td>350 b</td>
<td>526 a</td>
</tr>
</tbody>
</table>

* Means for an individual variable for each avocado variety followed by a different letter are significantly different at P = 0.05.