

PHOTOSYNTHETIC PERFORMANCE OF OVERWINTERED LEAVES

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ABSTRACT

Many avocado trees in New Zealand exhibit alternate bearing, the physiological basis of which is thought to be determined by the levels of starch in the tree which sets the fruiting potential each year. Tree starch levels increase over winter to maximum levels at flowering. Accumulation of starch before spring requires leaves which are photosynthetically active in winter. In New Zealand, winter temperatures are often $<10^{\circ}\text{C}$ which can significantly limit photosynthetic activity and damage photosynthetic apparatus. To determine the minimum temperature for net CO_2 exchange and if there was cold damage to the photosystems in 'Hass' avocado leaves during winter the photosynthetic performance of leaves was measured by regular monitoring of leaf gas exchange and chlorophyll fluorescence. Mean net CO_2 exchange in summer and autumn under saturating light was between 7 to $12 \mu\text{mol m}^{-2} \text{s}^{-1}$. The maximum net CO_2 exchange measured on a warm day in winter was $18 \mu\text{mol m}^{-2} \text{s}^{-1}$. The lowest net CO_2 exchange rates were zero in mid winter coinciding with the coldest temperatures. Leaves on the north side of the tree always had higher net CO_2 exchange than leaves on the south side of the tree. The F_v/F_m ratios were about 0.83 on the south side of the tree and were consistently greater than on the north side of the tree. The leaves on the north side of the tree had F_v/F_m ratios that declined to 0.73 at the time of the minimum net CO_2 exchange of the leaves. The F_v/F_m ratios for the leaves on the north side of the tree fully recovered by the beginning of spring. Stomatal conductance declined during winter closely coinciding with the

changes in net CO_2 exchange. Net CO_2 exchange had the expected light saturation response $1181 \mu\text{mol m}^{-2} \text{s}^{-1}$. Mean net CO_2 exchange increased as daily mean air temperature increased on bright sunny days with net CO_2 exchange positive at air temperatures above 3°C . It was concluded that winter conditions could limit photosynthesis and starch accumulation. The reduction of net CO_2 exchange in winter was not considered to be photoinhibition as the F_v/F_m ratios remained high during winter. Leaves of avocado trees in New Zealand may be acclimated against high light photoinhibition. While winter conditions appear to reduce the assimilation of CO_2 , the tree can maximise photosynthetic productivity on sunny days implying that as many leaves as possible should be exposed to full sunlight through structural pruning or tree thinning. Further research is required to confirm the relationship between temperature and net CO_2 exchange and that avocado leaves in New Zealand are adapted to have low levels of chilling damage.

Keywords: chlorophyll fluorescence, gas exchange, light saturation, stomatal conductance, photoinhibition

INTRODUCTION

Many avocado trees in New Zealand exhibit alternate bearing, with heavy cropping years followed by light cropping years. Inconsistent yields restrict orchard profitability and limit the regular supply of sufficient fruit required to promote growth of new markets for the New Zealand avocado industry. Avocado fruit are energy rich and can contain more than 25% oil as fresh weight (Hofman *et al.*, 2000). To accumulate the oil, it is assumed that the tree needs to produce high amounts of carbohydrate that is then converted to oil. The current theory on the physiological basis of alternative bearing in avocados has as a central theme that starch productivity by the tree determines fruit potential each year. Trunk starch levels are their lowest around May (Dixon *et al.*, 2005) before increasing over the winter months to be at maximum at flowering (Wolstenholme and

Whiley, 1989). In winter, despite the trees having little apparent growth, photosynthetically active leaves are required to accumulate starch. A strong vegetative flush in spring is considered important to ensure there is sufficient flowering wood for the following spring (Dixon, pers. com.). Insufficient starch accumulation in winter, along with heavy flowering is assumed to inhibit the spring shoot flush. A poor shoot flush then leads to poor flowering and fruit set in the following spring, thus perpetuating the alternative bearing cycle.

There has been little research into the photosynthetic capacity of avocado trees under New Zealand conditions and there are only a small number of detailed studies into the gas exchange characteristics of the avocado leaf (Heath *et al.*, 2005; Mickelbart *et al.*, 2002; Schaffer and Whiley, 2002, 2003; Schaffer *et al.*, 1991; Whiley, 1994).

The maximum net carbon dioxide (CO₂) exchange rates for avocado leaves range from 6 to 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with the highest rates reported in California. Under optimal light and temperature conditions a maximum CO₂ exchange rate of 15 to 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ is possible. The light saturation point for CO₂ exchange rate in avocado leaves is about 20 to 33% of full sunlight for container grown plants (Whiley, 1994) and around 1100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ or over 50% of full sunlight for orchard grown trees, depending on the time of year (Wolstenholme and Whiley, 1999). The light intensity (photon flux density or PFD) required for light saturation decreases by 18% in winter compared to summer in Florida (Schaffer and Whiley, 2002).

Photosynthetic rates of avocado may be significantly affected by slight fluctuations in temperature. For 'Edranol' trees, a Guatemalan hybrid cultivar, in containers, the optimal temperature range for photosynthesis was 20 to 24°C (Bower, 1978). Within plus or minus 5°C of this temperature range, net photosynthesis declined by about 20%. For container-grown 'Fuerte' trees maximum net photosynthetic rates were at temperatures of 28-31°C. The net photosynthetic rates declined by about 33% at

temperatures below 15°C or above 40°C (Scholefield *et al.*, 1980). It was observed (Whiley, 1994) that temperatures lower than 10°C during winter reduced net photosynthesis from 19 to 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in field-grown 'Hass' avocado trees in Florida. In New Zealand, temperatures above 30°C are rare but temperatures below 10°C can be common in winter. The most likely limit to photosynthesis in New Zealand is low temperatures in winter. Therefore it is important to establish the lower temperature limits of avocado net CO₂ exchange over winter. Most studies overseas have concentrated on finding the optimum and maximum temperature for photosynthesis, in New Zealand determining the lowest temperature for photosynthesis is most important.

At temperatures found in New Zealand winters (<10°C) the photosynthetic apparatus could be damaged (Whiley, 1994). This is commonly referred to as chilling injury and is considered to be where photosystem II is damaged. The damage can be measured using a pulse amplitude modulation fluorometer. The ratio of variable chlorophyll fluorescence to maximum fluorescence (F_v/F_m) of undamaged photosystems is about 0.83. Ratios less than 0.83 indicate stress or damage to the photosystem. Ratios of 0.79 to 0.81 have been reported when the minimum temperature was 12.9°C, indicating only slight light stress, falling to 0.41 when minimum temperatures fell below 10°C (Whiley, 1994). The drop in F_v/F_m has been attributed to photoinhibition (Whiley, 1994). In New Zealand, minimum temperatures in winter are often lower than 10°C, raising the possibility of greater damage and a significant limitation to carbohydrate production in winter.

To determine the minimum temperature for net CO₂ exchange and if there was cold damage to the photosystems in 'Hass' avocado leaves in New Zealand during winter, the photosynthetic performance of leaves with either a northern or southern aspect was measured by regular monitoring of leaf gas exchange and chlorophyll fluorescence under different environmental conditions.

MATERIALS AND METHODS

Field measurements were conducted at a single, irrigated avocado orchard southeast of Katikati, in the Western Bay of Plenty, New Zealand (S 37° 35' 23.4", E 175° 56' 0.50"). The orchard is approximately 5m above mean sea level and within one kilometre of the coastline of the Tauranga harbour. The orchard was selected because it has a history of leaf yellowing and for practical considerations such as a power supply to run the scientific equipment and ease of use due to a flat contour. The orchard block comprised of 176 'Hass' avocado trees on 'Zutano' rootstock planted in 1998 that were approximately 6m tall by the winter of 2006. The orchard is managed according to New Zealand industry norms.

Ten exposed leaves, on 5 trees, were tagged on 25/3/2005 for repeated measurements, 5 leaves on the northern side of each tree and 5 leaves on the southern side, between 0.5 and 2.0m above the ground. These were replaced on 1/12/2005 with fully expanded leaves from the spring flush, as the original leaves began to senesce.

A portable, open flow, differential gas exchange measurement system (CIRAS-1, PP Systems, Massachusetts, USA) was used to monitor gas exchange parameters. Measurements were taken between 10.00h and 12.00h to minimise the possible effect of afternoon stomatal closure on gas exchange results. Each leaf was enclosed in an automatic leaf cuvette avoiding the central vein of the leaf and exposed to a saturating light intensity ($1200 \mu\text{mol m}^{-2} \text{s}^{-1}$) from a fan cooled tungsten lamp. Sufficient time was allowed to pass (typically 1 to 2 minutes) until net CO_2 exchange had become stable. Maximum photosynthesis was measured with the leaves under saturating light intensities and maintained at ambient temperature. Measurements were made in spring, 15/11/2005 and 29/11/2005 to investigate leaf photosynthetic performance unaffected by winter chilling. Eight measurements of photosynthetic performance were conducted in winter 2006 from 24/4/2006 to 12/9/2006.

Continuous, automatic gas exchange measurements were collected using a Compact Minicuvette System (CMS-400) and a climatized cuvette (GK-022) with a custom built leaf chamber (Heinz Walz GmbH, Effeltrich, Germany). The CMS-400 is a mains powered, open flow differential system based on a central control unit. It calculates and records photosynthetic rate, transpiration rate and stomatal conductance. A single, north facing, fully expanded green leaf, 1.5m above the ground was selected and placed within the leaf chamber. Forty-two diel courses (24 hour periods) were recorded between 25/7/2006 and 9/9/2006.

Changes in chlorophyll fluorescence F_v/F_m ratios through the winter of 2006 were monitored with a Pulse Amplitude Modulation fluorometer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany) using the Saturation Pulse Method (Schreiber, 1994). Dark adaptation clips were placed on each leaf, avoiding major leaf veins, for 15 minutes. Each leaf was measured on eight occasions between 18/5/2005 and 12/9/2006.

A repeated measures experimental design was used and gas exchange data was compared to environmental parameters. All results were analysed using Excel 2002 (Microsoft, Redmond, WA, USA), Minitab 13.32 (Minitab Inc., State College, PA, USA) and SigmaPlot 8.0 (Systat Software Inc., San Jose, CA, USA) software programs. A saturation light curve was fitted with a Smith curve, using data collected every minute during daylight.

RESULTS

Net CO_2 exchange under saturating light intensities for leaves on both the northern and southern side of the tree was between 7 to $12 \mu\text{mol m}^{-2} \text{s}^{-1}$ until 21/4/2006. After this time leaves on the south side had lower net CO_2 exchange. The net CO_2 exchange of leaves on the north and south sides of the tree then declined to the lowest reading on 28/6/2006 of less than $0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, and then increased to be almost at typical summer values on

12/9/2006 (Figure 1, upper panel). The lowest net CO₂ exchange rates were zero, indicating no net carbon gain from the leaves in mid winter and coincided with the coldest temperatures during the year. The low-point of net CO₂ exchange was at the same time for both sides of the tree, but the leaves on the north side of the tree always had higher net CO₂ exchange than leaves on the south side of the tree. Leaves on the south side of the tree had an earlier reduction in net CO₂ exchange and were slower to return to typical values compared to leaves on the north side of the tree.

The leaves on the south and north sides of the tree had different chlorophyll fluorescence (F_v/F_m) ratios (Figure 1, lower panel). The F_v/F_m ratios were about

0.83 on the south side of the tree, consistently greater than on the north side of the tree. North facing leaves had a larger decline in F_v/F_m ratios during the coldest days, reaching as low as 0.73 on the 19/8/2006 and 0.75 on 28/6/2006. The latter low ratio coincided with the minimum net CO₂ exchange of the leaves. The F_v/F_m ratios for the leaves on the north side of the tree had fully recovered by 12/9/2006. The F_v/F_m ratios for the leaves on the south side of the tree declined very slightly during the winter but were largely unaffected.

The pattern of F_v/F_m ratios changed when measured over a single day on 27/3/2005 when the northern leaves were exposed to full sunlight

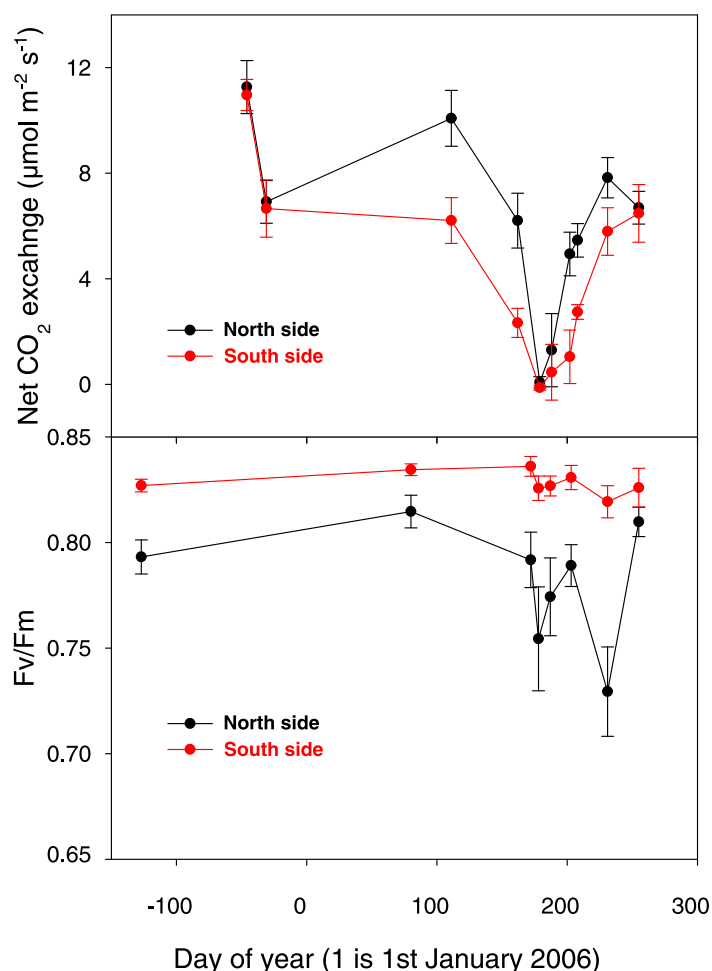


Figure 1. Comparison of leaves on north and south sides of avocado trees through the investigation period. Upper panel: Net CO₂ exchange under saturating light conditions. Lower panel: F_v/F_m ratios. Vertical bars are standard error of the mean, where n=25.

(Figure 2). Leaves on the southern, shaded side had near optimal ratios for F_v/F_m (around 0.83) for the measured daylight hours. Leaves on the northern side declined during the morning, from 0.83 to 0.79 at 13.30h. The F_v/F_m ratio then recovered to about 0.80 by 18.00h.

The pattern of change in stomatal conductance was very similar to that found for net CO_2 exchange. Both north and south facing leaves had a decline in stomatal conductance values during the winter which coincided closely with the decline and recovery in net CO_2 exchange (Figure 3). On 28/6/2006 the stomata were effectively closed during the day. Leaves on the south side of the tree had an earlier decline in stomatal conductance and later recovery than leaves on the northern side. Neither the north facing nor the south facing leaves recovered their stomatal conductance to the pre-winter values by 12/9/2006.

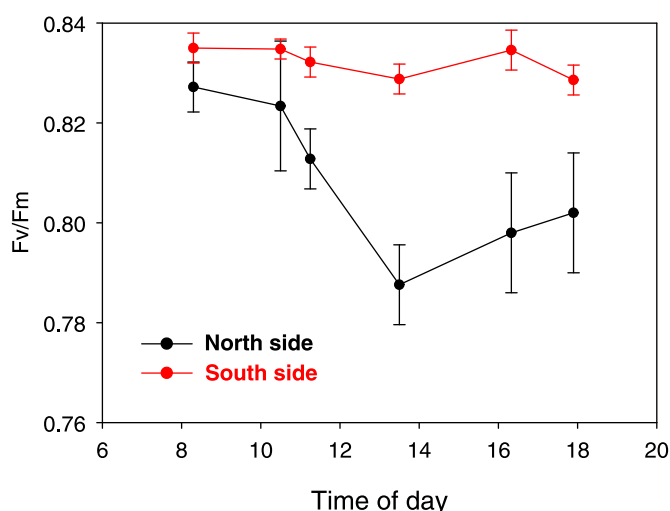


Figure 2. Diurnal pattern of F_v/F_m ratios of leaves on the north and south side. Vertical bars are standard error of the mean, where $n=25$.

Typical daily gas exchange parameters over a single day (3/8/2006), with a mean temperature of 12.1°C , maximum 20.7°C and minimum 7.3°C are shown in Figure 4. The daily maximum net CO_2 gas exchange of $18 \mu\text{mol m}^{-2} \text{s}^{-1}$ was at 12.00h, when the daily maximum photo flux density (amount of

sunlight) or PFD at the leaf surface was $1280 \mu\text{mol m}^{-2} \text{s}^{-1}$. Maximum stomatal conductance was $190 \text{ mmol m}^{-2} \text{s}^{-1}$ at 13.00h and the minimum net CO_2 gas exchange or dark respiration rate was $-1.2 \mu\text{mol m}^{-2} \text{s}^{-1}$. After midday, the net CO_2 exchange was reduced to around half of the daily maximum, despite the temperature remaining around 15°C . This was due to cloud cover reducing incident light (PFD) interception at the leaf.

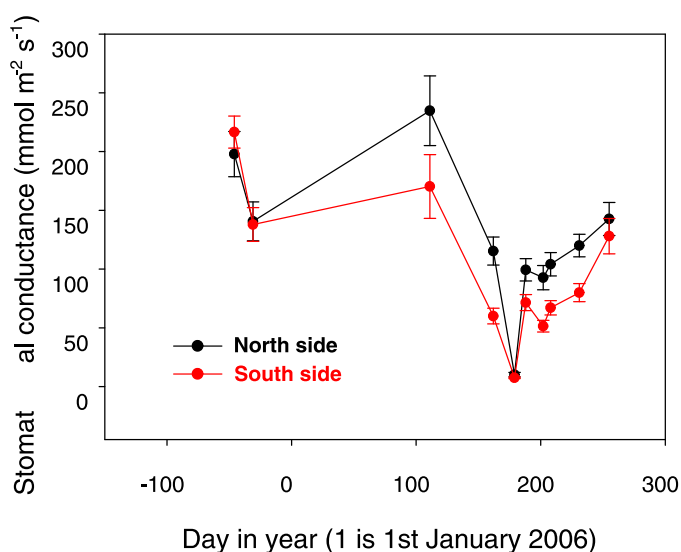


Figure 3. Pattern of stomatal conductance for leaves on the north and south side of the tree. Values represent 5 leaves on each side of 5 trees. Error bars are standard error of the mean, where $n=25$.

Net CO_2 exchange had the expected saturation response to increasing PFD with saturation reached at $1181 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 5). The good fit of the data shows that there was a tight linkage between net CO_2 exchange and PFD ($r^2 = 0.97$, $p < 0.0001$)

There was a positive linear relationship between mean net CO_2 exchange and daily mean air temperature on bright sunny days (optimal PFD) from 25/7/2006 to 16/8/2006 (Figure 6). Mean net CO_2 exchange on cloudy days (where maximum PFD was less than $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$) was less than on bright sunny days. The intercept value from the

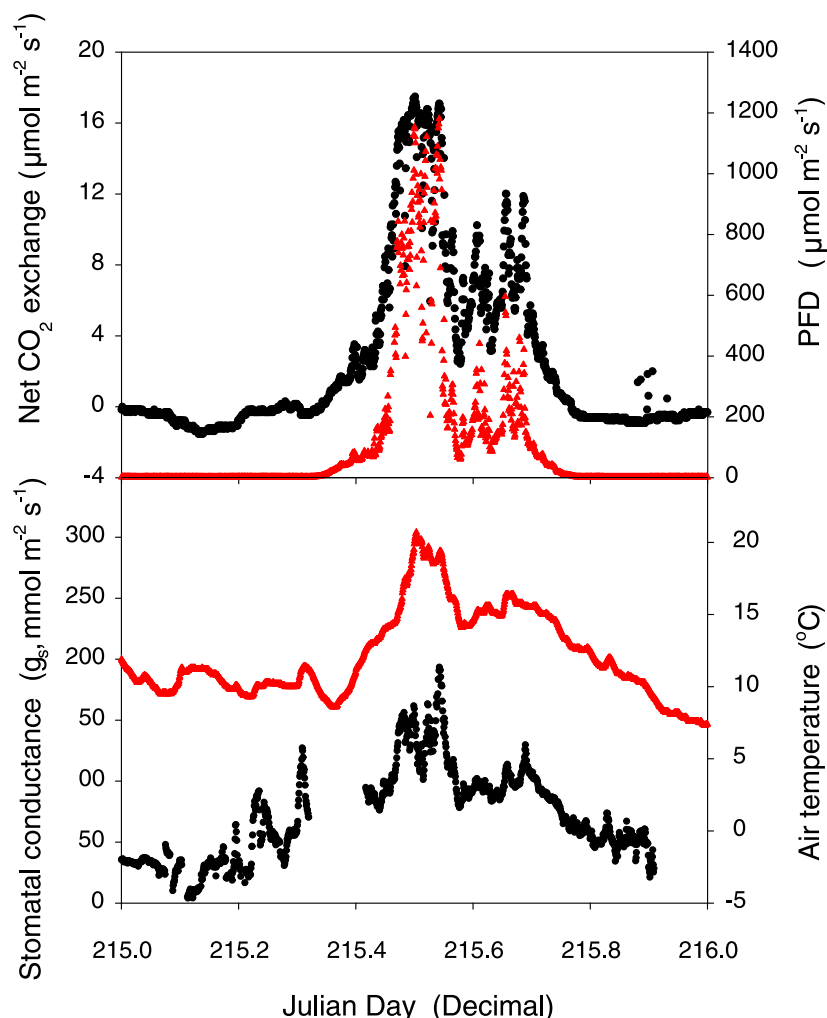


Figure 4. Patterns of gas exchange, air temperature and photon flux density (PFD) for day 215 (3/8/2006), a warm winters day; mean temperature 12.1 °C, maximum 20.7 °C and minimum 7.3 °C. Upper Panel, Net CO₂ exchange, and PFD; Lower Panel, stomatal conductance to water vapour (g_s) and air temperature.

regression function calculates that CO₂ exchange will be positive at mean air temperatures above 3°C.

DISCUSSION

The maximum net CO₂ exchange measured in winter at 18 μmol m⁻² s⁻¹ was similar to the maximum values found by other researchers for field grown avocados (Whiley *et al.*, 1999; Heath *et*

al., 2005). Minimum net CO₂ exchange under saturating light conditions was zero at the coldest time of year. Therefore winter conditions can be expected to limit carbohydrate assimilation and by implication, starch accumulation. There was a capacity for leaves in winter to achieve near summer net CO₂ exchange values if light levels were saturating and the temperature was around 20°C. Although the weather in winter is often not conducive to allowing maximum net CO₂ exchange

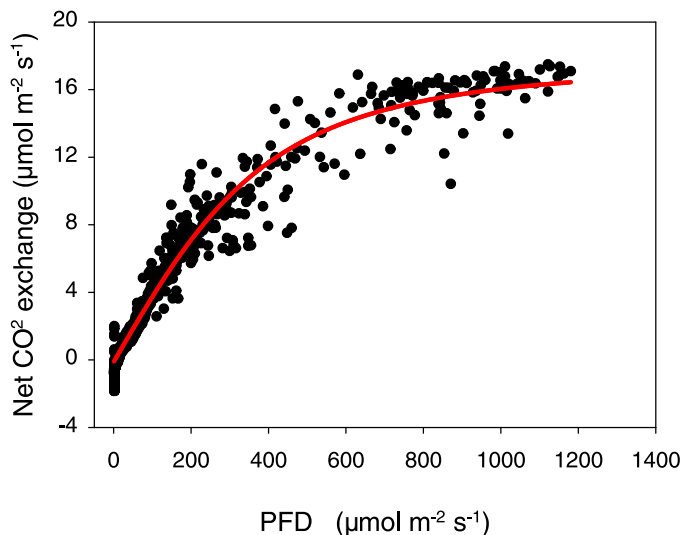


Figure 5. Response of net photosynthesis to PFD for Day 215 (3/8/2006), a warm, winter day; temperatures were, maximum, 20.7 °C, minimum, 7.3 °C and mean, 12.1 °C. Fitted line is a Smith curve ($r^2 = 0.97$, $p < 0.0001$).

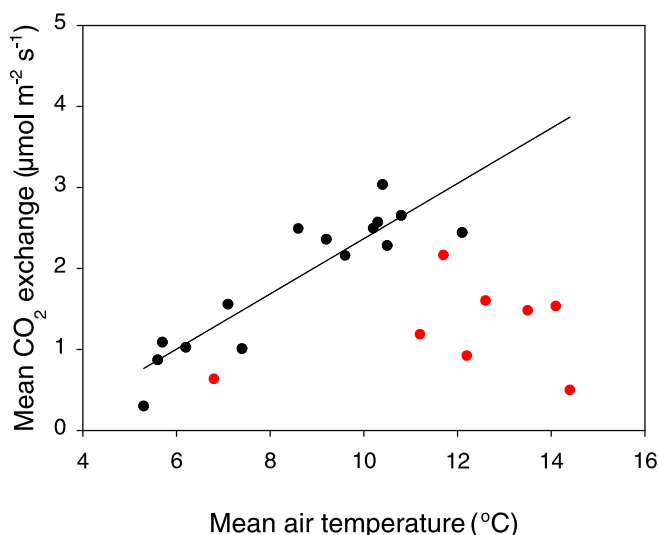


Figure 6. The relationship between mean CO₂ exchange rate and mean air temperature for the period 25/7/2006 to 16/8/2006. The black symbols are for values obtained with high light days with maximal PFD over 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and the red symbols for values from cloudy days with maximal PFD below 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The regression line is fitted only to days with PFD exceeding saturating levels for net CO₂ exchange ($p < 0.0001$, adjusted $r^2 = 0.81$).

to occur frequently, the leaves appear to be sufficiently responsive to fully utilise clear sunny days to obtain maximum net CO₂ exchange. This suggests that while winter conditions appear to be detrimental for the overall assimilation of carbohydrate the tree can make the most of any good weather, *i.e.* sunny days, that occur. This is likely to explain the observations that starch accumulates in the tree (Dixon, unpublished data; Whiley and Wolstenholme, 1990), the fruit grow and increase in dry matter (Unpublished data, AIC early maturity monitoring programme) and root growth occurs (Dixon *et al.*, 2006) over winter.

In this study, the reduction of net CO₂ exchange over winter is unlikely to be due to photoinhibition of the photosystems induced by high light intensity and cool/cold temperatures. The F_v/F_m ratios remained relatively high throughout the year, indicating only a negligible and transient inhibition of photosynthesis in winter. The lowest F_v/F_m ratio was 0.73 for sun exposed leaves when the minimum temperature was 5°C. This is a significantly greater ratio, despite being much cooler (5°C vs 10°C), than reported in Australia (Whiley, 1994). It is possible that the leaves of avocado trees grown in New Zealand are more acclimated against high light photoinhibition of photosynthesis than trees in warmer climates.

There was a close coupling of net CO₂ exchange to incident light intensity throughout the day. In other words, a bright sunny day leads to the highest net CO₂ assimilation possible at each temperature. Avocado leaves in New Zealand have a light saturation value during winter of greater than 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ or 50% full sunlight in agreement with previous studies (Wolstenholme and Whiley, 1999). There was also a difference in net CO₂ exchange between the leaves on the north face (high light conditions) and the south face (low light conditions) of the tree. The north facing leaves generally had greater photosynthetic rates than the south facing leaves but the north facing leaves also appeared to be more susceptible to reduced F_v/F_m ratios. High light saturation values indicate that to

ensure maximum photosynthetic productivity of a tree in winter as many leaves as possible are exposed to full sunlight. Shaded leaves are likely to be unproductive and the tree should be managed to avoid shading the canopy. Hence, the need for structural pruning or tree thinning to ensure maximum light exposure to leaves. The different net CO₂ exchange rates for leaves at different aspects of the tree imply the tree shape to capture full sunlight is important for maximising photosynthetic capacity. Further study is needed to confirm if the difference in photosynthesis is fundamentally different at each facing or it is an uneven exposure to saturating light levels that gave different net CO₂ exchange rates. Along with managing the trees to have high light levels it is also important for the tree to have good water status, especially during the spring and summer months, to maximise photosynthetic capacity. Net CO₂ exchange is closely associated with stomatal conductance confirming previous studies (Schaffer and Whiley, 2003). Even when there is sufficient incident light, water stressed plants are likely to restrict gas exchange through the stomata earlier in the day than plants without water stress, leading to a reduction daily carbohydrate accumulation. Therefore under dry conditions CO₂ assimilation can be lower than expected on warm, bright sunny days.

In this study, a lower temperature limit of a mean daily air temperature of about 3°C for avocado photosynthesis was determined on the orchard used for this study. This study is the first report of the lower temperature limit of photosynthesis of avocado leaves, although the minimum temperature may be unique to New Zealand grown 'Hass' avocado trees. The relationship between the daily mean air temperature and net CO₂ exchange was linear and allows photosynthetic activity to be estimated over winter. Both temperature and light intensity influence net CO₂ exchange. For example, during winter a sunny day with a daily mean air temperature of 7°C has the same mean net CO₂ exchange as a cloudy day with a daily mean air temperature of 13°C. To estimate

net CO₂ exchange during winter weather data will also need to be collected including light intensity so that the effect of cloudy days on CO₂ assimilation can be estimated. Further research is required to confirm the relationship between temperature and net CO₂ exchange and that avocado leaves in New Zealand are adapted to have low levels of chilling damage.

A greater understanding of the ecophysiological limitations of 'Hass' avocado under New Zealand conditions can potentially lead to further understanding of the causes of inconsistent cropping. There is increasing interest in the carbon budget of horticultural fruit crops for the purposes of life cycle analysis in relation to carbon emission research. Further research that investigates whole tree productivity for the entire year is needed to further define the amount of CO₂ avocado trees absorb. This study has identified one important factor previously not appreciated by the New Zealand avocado industry, that 'Hass' avocado trees are active all year including winter and there is no temperature induced "dormancy or quiescent" period over winter. Active tree management all year offers possibilities for overcoming inconsistent cropping through a solid grounding in understanding the basic ecophysiology of the avocado tree.

CONCLUSIONS

'Hass' avocado leaves in New Zealand have similar photosynthetic performance and responses to light intensity as avocado leaves in other countries. The avocado leaves in New Zealand appear to be adapted for cool/cold temperatures and showed only small amounts of chilling damage to the photosynthetic apparatus. The average daily temperature would need to be below 3°C for no CO₂ assimilation to occur. High rates of photosynthesis also require the tree to have a good availability of water at all times. Based on this study there is no temperature induced "dormancy or quiescent" period over winter and the trees should be actively managed all year.

REFERENCES

- Bower, J.P. (1978). Ecophysiological studies of three cultivars of *Persea americana* (Mill.) emphasising photosynthesis and internal water relations. Unpublished M.Sc. Thesis, University of Natal, Pietermaritzburg, South Africa.
- Dixon, J. Elmsly, T.A. and Smith, D.B. (2005). 'Hass' avocado tree phenology in 2004 for the Western Bay of Plenty. *New Zealand Avocado Growers' Association Yearbook* **5**: 13-26.
- Dixon, J., Elmsly, T.A., Dixon, E.M., Fields, F.P., Greenwood, A.C. and Smith D.B. (2006). 'Hass' avocado tree phenology 2004-2006 in the Western Bay of Plenty. *New Zealand Avocado Growers' Association Yearbook* **6**: 1-12.
- Heath, R., Arpaia, M.L. and Micklebart, M. (2005). Avocado tree physiology – Understanding the basis of productivity. *Proceedings of the California Avocado Research Symposium* pp. 65-88.
- Hofman, P. J., Jobin-Décor, M. and Giles, J. (2000). Percentage of dry matter and oil content are not reliable indicators of fruit maturity of quality in late-harvested 'Hass' avocado. *HortScience* **35**: 694-695.
- Mickelbart, M. V. and Arpaia, M. L. (2002). Rootstock influences changes in ion concentrations, growth, and photosynthesis of 'Hass' avocado trees in response to salinity. *Journal of the American Society for Horticultural Science* **127**: 649-655.
- Schaffer, B. and Whiley, A. W. (2002). Environmental physiology. In: *The Avocado: Botany, Production and Uses* (Whiley, A.W., Schaffer, B. and Wolstenholme, B.N., Eds.). CAB International, Wallingford, Oxon, UK. pp.135-160.
- Schaffer, B. and Whiley, A. W. (2003). Environmental regulation of photosynthesis in avocado trees - A mini-review. *Proceedings of the V World Avocado Congress* **I**: 335-342.
- Schaffer, B., Whiley, A. W. and Kohli, R. R. (1991). Effects of leaf age on gas exchange characteristics of avocado (*Persea americana* Mill.). *Scientia Horticulturae* **48**: 21-28.
- Scholefield, P.B., Walcott, J.J., Kriedemann, P.E. and Ramadasan, A. (1980). Some environmental effects on photosynthesis and water relations on avocado leaves. *California Avocado Society 1980 Yearbook* **64**: 96-106.
- Schreiber, U., Bilger, W. and Neubauer, C. (1994). Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: *Ecophysiology of photosynthesis*. New York: Springer. pp. 49-70.
- Whiley, A. W. (1994). Ecophysiological studies and tree manipulation for maximisation of yield potential in avocado (*Persea americana* Mill.). Unpublished PhD Thesis, University of Natal, Pietermaritzburg, South Africa.
- Whiley, A.W. and Wolstenholme, B.N. (1990). Carbohydrate management in avocado trees for increased production. *South African Avocado Growers' Association Yearbook* **13**: 25-27.
- Wolstenholme, B. N. and Whiley, A. W. (1989). Carbohydrate and phenological cycling as management tools for avocado orchards. *South African Avocado Growers' Association Yearbook* **12**: 33-37.
- Wolstenholme, B. N. and Whiley, A. W. (1999). Ecophysiology of the avocado tree (*Persea americana* Mill.) tree as a basis for pre-harvest management. *Revista Chapingo Serie Horticultura* **5**: 77-88.

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