Preliminary investigation of water and nitrogen use efficiency in Mango

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Summary

The aims of this preliminary investigation were to identify if natural abundance carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes reflect the dynamics of carbon and nitrogen between mango varieties, canopy function and management practices (fertilization and/or irrigation). This preliminary investigation has identified factors that increase water use efficiency, nitrogen efficiency and tree productivity. The natural abundance of stable isotopes of carbon and nitrogen show promise as new tools to study the drivers of mango productivity efficiency and will be useful in studying mango orchard systems and in selecting and evaluating breeding parents and progeny in mango breeding programs.

Measurement of stable isotopes of carbon ($\delta^{13}C$) in three mango varieties grown under similar irrigation and environmental conditions (in Summer 2015) have shown significantly different WUE between the varieties. These findings were supported by traditional gas exchange analysis, taken during winter 2016 and foliar N concentrations in these varieties.

There were significant relationships, between WUE and foliar N concentrations, in both the heredity and crop load experiments indicating an effect of both genotype and terminal function on terminal WUE and foliar N concentration. Results from the gas exchange assessments supported $\delta^{13}C$ heredity results indicating greater WUE in the Keitt variety when compared to Calypso variety.

The methods used in this project can help us better understand the integrated carbon and nitrogen dynamics of tree canopies and how nitrogen allocation is influenced by nitrogen nutrition. They also help us understand the photosynthetic components that influence light harvesting and electron transport efficiencies and hence contribute to growth and productivity. The report also discuss how gas exchange characteristics between varieties may be used to evaluate the productivity and canopy efficiency of different mango varieties growing in different planting configurations with varying light and shading characteristics.

Finally, when measurements in trees with varying crop load from three sites data where pooled together, the $\delta^{15}N$ was able to identify nitrogen dynamics in high and low nitrogen sites.

This preliminary investigation of the use of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to identify water use efficiency and productivity efficiency's in mango has been successful and has potential application in studying orchard systems and in mango breeding. Application of these methods in future projects would enhance current studies of mango canopy architecture efficiency in the Small tree High productivity initiative and provide a new way of assessing mango breeding lines for production efficiency.

Table of contents

Table of figures

Table of tables

Background

The Mango Industry in Far North Queensland is valued at $51,000,000. Therefore it is an important commodity to the Far North Region's and Queensland economy. Commercial Mango orchards are typically grown in regions highly reliant on irrigation water and fertilizers for nutrition. With ever increasing costs of production there is a real need to help Industry to better understand how water and nitrogen use efficiency can be maximized. There are a number of techniques that can be utilized to assist grower in improving water and nitrogen use in orchards. These methods range in application from soil and plant water monitoring techniques which use instrumentation including soil moisture probes and plant tensiometers to assess soil and plant water status, to more keystone methods such as assessing breeding lines for improved water use efficiency from a physiological perspective.

This project aimed to use carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes to identify how water and nitrogen use efficiency vary in three highly productive commercial mango varieties grown in Far North Queensland. The evaluation of stable isotopes for water use efficiency in this research was supported by leaf N dry matter analysis and instantaneous gas exchange measures using traditional photosynthesis instrumentation which helped identify which physiological characteristics are contributing to improved water and nitrogen use efficiencies in mango varieties.

Currently the DAF mango breeding program is aiming to identify mango varieties with high productivity, low vigor and other desirable phenotypic characteristics such as flavour, disease resistance and fruit quality etc. If using the stable carbon and nitrogen isotope can be used to identify genotypes with superior water and nitrogen efficiency, breeding for productivity efficiency will be enhanced and reduce breeding time frames.

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes could help in the development of more efficient and resilient mango orchards capable of tolerating risks of extreme weather conditions, drier environments and toward expanding the production area in Northern Australia. This type of work can also allow DAF to continue to connect with the Mango Industry and develop innovative approaches to help improve sustainability and to reduce environmental impacts of agriculture to the environment (increasing water and nitrogen use efficiency).

Project Objectives

1. To use carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes to identify if variety (heredity) influence water and nitrogen use efficiency in three highly productive commercial Mango varieties grown in Far North Queensland.
2. To use carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes to identify if irrigation, management and crop load influence water and nitrogen use isotopes.
3. To carry out preliminary gas exchange analysis on two monoembryonic mango varieties to identify how tree water and nitrogen use efficiency vary in their canopies.

Methodology

Experiment 1 – Crop load

Research question: Does crop load on at the terminal level influence water and nitrogen use in Mango?

Leaf samples from three varieties (NMBP 1243, Calypso™ and Keitt) were collected from terminals with and without fruit (crop load), from mature, fruiting trees for $\delta^{13}C$, $\delta^{15}N$ and foliar N analysis . Each of the varieties, were grown at independent sites across the Mareeba-Dimbulah Mango growing region. The Keitt trees were approximately 26 years old and grown at Southedge Research Station; the National Mango Breeding Program (NMBP) 1243 trees were approximately 5 years old and grown at Walkamin Research Station, while the Calypso™ trees were approximately 10 years old and grown on a commercial farm Dimbulah.

Experiment 2 – Heredity and management

Research question: Does variety and irrigation influence water and nitrogen use efficiency in Mango?

Leaf samples from two varieties (Calypso™ and Keitt) from 2 year old trees in the Mango planting systems experiment at Walkamin Research Station were assessed for water and nitrogen use efficiency using $\delta^{13}C$, $\delta^{15}N$ and N concentration analysis. This experimental site is known as the Mango planting systems trial as a part of the A13000 HIAL Transforming Tropical and Subtropical Tree Productivity Project, and was established to look at the effect of planting density (low - 208 tree / ha, medium - 450 tree / ha and high -1250 tree / ha), training systems (conventional and single leader) and variety (NMBP1243, Calypso™ and Keitt).

In addition, leaf samples from three varieties (NMBP 1243, Calypso™ and Keitt) to assess how irrigation influences $\delta^{13}C$, $\delta^{15}N$ and N concentration analysis, in summer 2015. In the Mango planting systems trial, the 2.5 year old trees from each of the densities are grown under different irrigation systems i.e. high density has drippers at 8L/hr, medium density has 35 L/hr sprinklers and low density has 70L/hr sprinklers hence the comparison was across each variety within the different density to compare if variety showed any variability within the same system of irrigation. Only trees from the conventional training systems trees from each of the densities were used in this experiment.

Experiment 3 – Gas exchange

Research question: How is gas exchange influenced by Mango variety and sampling position?

Daily course measurements

Gas exchange characteristics including photosynthesis, stomatal conductance, transpiration and photosynthetic photon flux density (PPFD) were measured using a Li-6400 (Li-Cor Inc., Lincoln, NE, USA) on fully expanded, most recently expanded and mature, new leaf on the northern side of canopy in Winter 2016. The CO_2 concentration in the leaf chamber (CO_2) was set at 400 mol CO_2 mol^{-1} air provided by a fresh CO_2 cartridge and a relative humidity between 40-60%. The flow rate was 400 ml min^{-1}. For the daily course measurements, leaf exchange parameters were monitored in both Keitt and Calypso™ varieties from a single tree in each of the six blocks from 9:00 am to 17:00 pm. The light source of the Li-6400 was also used and set at a similar level to ambient sunlight during the day.

Light response curves measurements

For measurements of light response curves, responses were determined in each tree from 6 blocks. Leaves were first exposed to 1500 µmol m^{-2} s^{-1} until the steady state was reached (at around 1-2 min) to stimulate stomatal opening, and then the PPFD level was changed from 2000,1800, 1500, 1200, 1000, 800, 300, 100, 50, 10, and 0 µmol m^{-2} s^{-1}. Data were recorded automatically at intervals of 2 min between PPFD levels using automatic programme). When the fluctuation in Pn was less than 0.5 µmol m^{-2} s^{-1}, two repeats at each PPFD level were recorded automatically at 3-s intervals. Nonlinear adjustment of the Pn/PPFD curves followed the model described by Prado and Moraes (1997). This was performed in order to obtain the light-saturated net photosynthetic rate (Pmax, µmol m^{-2} s^{-1}), the light compensation point (LCP, µmol m^{-2} s^{-1}), leaf respiration in the dark (R, µmol m^{-2} s^{-1}), and apparent quantum yield (AQY, µmol CO2 µmol absorbed photon).

Results

Experiment 1 – Crop load

Two of the varieties investigated for crop load, NMBP 1243 and Calypso™, have shown significant variation in $\delta^{13}C$ between terminals (shoots), with and without fruit on the terminals (Table 1). In addition, the three varieties tested each showed significant differences for foliar N concentrations between terminals with and without crop load (Table 1). When data was pooled and compared using regression analysis, there were significant positive relationships between foliar N concentration and $\delta^{13}C$, which was grouped by variety (r^2= 0.52, p<0.001, n=112) (Figure 1a). Finally, when the three crop load sites where pooled together the $\delta^{15}N$ was significantly different for variety (Figure 1b).

Experiment 2 – Variety and management

When individual varieties were compared across different irrigation systems, there was no significant difference for $\delta^{13}C$, $\delta^{15}N$ nor foliar N concentration, at the planting systems trial. However, when variety was compared within irrigation systems, $\delta^{13}C$ was significant for variety within an irrigation system. $\delta^{15}N$ and foliar N concentration were not significantly different for variety (Table 2). When all data was pooled, there was again a significant positive relationship between $\delta^{13}C$ and foliar N concentration (r^2= 0.58, p<0.001, n=54) (Figure 1c).

Experiment 3 – Gas exchange and variety

The light response curves showed that the photosynthetic capacity (P_{max}) of Keitt was 16.0 µmol CO_2 $m^{-2}s^{-1}$ greater by two-fold (p < 0.01), then that of Calypso (7.3 µmol CO_2 $m^{-2}s^{-1}$) (Fig. 2 and Table 3). Keitt had greater light saturating point (LSP) than did Calypso (p = 0.0293), but presented lower light compensation point (LCP) (p = 0.0284). Although there were no significant differences for apparent quantum yield (AQY) and leaf respiration (R), Keitt had higher light use efficiency (AQY) and lower dark respiration (R), which indicates higher sunlight use efficiency and more instantaneous carbon storage in Keitt compared with Calypso.

Daily course of gas exchange of two mango varieties (Keitt and Calypso).

The results of daily course of gas exchange showed that when Keitt was compared with Calypso, it had higher net photosynthetic rate (Figure 3G) and water use efficiency (Figure 3H). The higher net photosynthetic rate in Keitt was associated with higher stomatal conductance (Figure 3C) and mesophyll conductance (Figure 3D), higher transpiration rate (Figure 3E).

Gas exchange at different sampling positions in Keitt

The light response curves showed that upper new mature leaf had higher photosynthetic capacity (Pmax), light saturation point (LSP) and light compensation point (LCP) than upper old leaf and inner leaf (Fig. 4 and Table 4). Upper old leaf presented slightly higher values of Pmax, LSP and LCP but no significant difference (Table 4). Apparent quantum yield was higher in upper old leaf and inner leaf than in upper new mature leaf. There was no significant difference in respiration between leaf positions. In general, gas exchange measurements in the early morning showed that the photosynthetic rate, stomatal conductance, mesophyll conductance, transpiration rate and water use efficiency followed the order: upper new mature leaf > upper old leaf > inner leaf (Table 5).

Conclusions/Significance/Recommendations

These preliminary results have shown that in some varieties fruit (crop load) can influence shoot WUE and foliar N concentration. This is a significant finding as terminal function has not previously been identified as contributing to WUE and N relations in mango canopies. Other mango agronomic N studies have shown highly variable, canopy nutrition status for foliar N in the canopy, which to date, has reduced the effectiveness of rapid nutrient analysis techniques. In this study, when data was pooled across the three different sites there was a significant relationship between foliar N concentration and WUE at the shoot level when grouped by variety.

In the planting systems trial, varieties grown at the same site and exposed to the same environmental conditions had significantly different $\delta^{13}C$ indicating different varieties show different $\delta^{13}C$ as an index for long-term WUE.

Using $\delta^{13}C$ as a proxy for water use efficiency was also supported by instantaneous gas exchange measurements. The results presented here have identified the variety Keitt having a higher photosynthetic, stomatal conductance and transpiration rates, as well as a wider range of light use efficiency characteristics. The sampling position experiment also identified different levels of gas exchange occurring in different canopy positions in Keitt conventionally trained trees, which is important information for tree management if we want to lift tree productivity.

Finally, when the crop load data was pooled for three sites, the $\delta^{15}N$ was significantly different. $\delta^{15}N$ varies in soils as a result of N cycling, hence soil $\delta^{15}N$ can be used to investigate soil N cycling dynamics and this is reflected in foliage growing above these soils. While N cycling is dynamic, trees exposed to different N management practices influencing soil N pools will show the long-term $\delta^{15}N$ build up in the foliage. The results presented here show how a site with little recent fertiliser applications demonstrated low $\delta^{15}N$ (reflecting low soil N) while the other two sites demonstrated high $\delta^{15}N$ such as is found typically in N fertiliser, such as is applied in commercial Mango orchards.

Key Messages

This preliminary investigation has identified some important considerations that may influence the future of mango canopy, agronomic sampling and potentially offer a new breeding tool to identify trees with increased water use efficiency (WUE) in tree crop research.

The results show that three monoembryonic mango varieties grown under similar irrigation and environmental conditions, have significantly different WUE as identified by $\delta^{13}C$. These findings were supported by more traditional gas exchange analysis where the Keitt variety was more WUE than the Calypso variety.

Foliar N concentrations were also different amongst varieties grown at the same site while receiving the same management treatments. There were significant relationships, between WUE and foliar N concentrations, in both the heredity and crop load experiments indicating an effect of both variety and terminal function on terminal WUE and foliar N concentration.

Carbon isotope techniques can help study the integrated carbon and nitrogen dynamics of tree canopies and linked to gas exchange measurements we can better understand tree growth and productivity because these measures show how carbon, water and N are allocated between the photosynthetic components that influence light harvesting and electron transport efficiencies. These results identify that Keitt and Calypso mango varieties have different light use efficiencies and hence light distribution studies may be used to inform how tree canopies respond to different light environments.

Where to next

The successful demonstration of the use of stable carbon and nitrogen isotopes to measure mango tree water use efficiency is an ideal platform from which the technique can be incorporated into other ongoing DAF studies on mango physiology, orchard efficiency and the breeding of new well adapted mango varies. Some areas where the techniques could be used are listed in the numbered points below.

1. To continue this work and include a greater volume of commercial mango varieties to scope for superior water and nitrogen use efficiency, high productive, semi-vigorous varieties. (Potential for an ARC grant with Griffith University through Prof. Xu and Dr Chang)

2. To use this preliminary data in physiological models for decision support and crop yield modelling in a collaborative project with other researchers (Potent collaborations with Griffith University, Dr Chang and QAFFI modellers).
3. To apply this information to redesign future agronomy work, including more about how to decipher terminal function, sampling procedure and canopy management and how these factors can influence tree water and nitrogen use efficiency commercial mango varieties in Far North Queensland with the intention to help reduce pollution and increase on-farm water and nutrient use efficiency and profitability.
4. There is also potential for this type of work to be transferred to other tree crops e.g. Tropical fruits, avocado, macadamia.

Budget Summary

a) Research collaborator cost for:

   i. Analysis of 200 foliar $\delta^{13}C$ isotope (‰), foliar N concentration (%) and foliar $\delta^{15}N$ isotope (‰) (all anyltyes completed together @ $20 per sample (budget $4,000).

   ii. Trip 1 for Prof. Zhihong Xu the Director of the Environmental Futures Research Institute and visiting scholar Dr Chengjun Zhang Mareeba as a scoping tour to visit Mango Planting Systems Trial. Prof Xu also gave a presentation to staff at Mareeba on ‘Tipping points of non-linear tree growth responses to climate change in different forest ecosystems from subtropical to boreal regions’. Included 2 nights accommodation in Mareeba and 2 return flights from Brisbane (budget $2, 270.00).

   iii. Trip 2 for visiting scholar Dr Chengjun Zhang Mareeba travel to Small tree High productivity Mango site to complete gas exchange on Mango on the Planting Systems Trial in Walkamin FNQ. Includes 6 night’s accommodation and 1 return flights from Brisbane (budget 2,000.00)

   iv. Transport costs for the LiCor 6400 - Photosynthesis equipment from Griffith University, Brisbane to Mareeba and return from Mareeba to Griffith University.

   Payment .. ……..…………………………………………………………………….$8,270.00

b) Casual labour to assist sample collection and photosynthesis

   Cost……………..…………………………………………………………………….$1,121.00

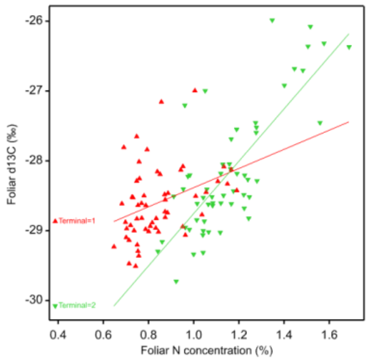
c) Postage for samples and equipment

   - Dried Mango samples to Brisbane Summer 2016
   - Dried Mango samples to Brisbane Winter 2016

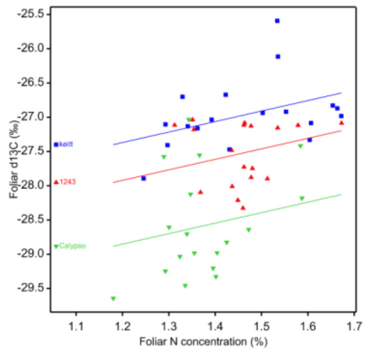
   Cost……………..…………………………………………………………………….$134.00

   Total cost ……………..…………………………………………………………………..$9,614.00

Figure 1. The relationships between foliar δ^{13}C (‰) and N conc. (%) between A) terminals with crop load (1) and without crop load (2) in Calypso^{TM} variety grown in Dimbulah, B) between Keitt, Calypso and NMBP 1243 varieties grown on the same site at Walkamin, and C) the comparison foliar δ^{15}N (‰) from three varieties growing on independent sites with different N fertilization history, in Far North Queensland.



A)



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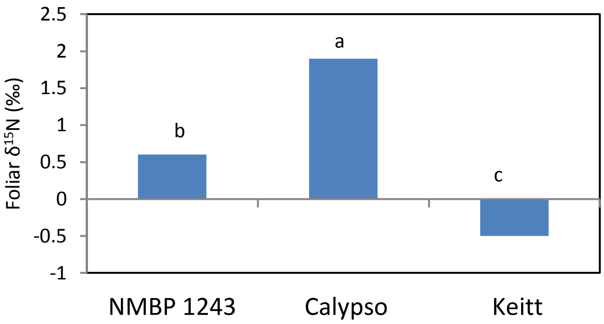

C)

Figure 2. Net photosynthetic rate (Pn) response as a function of photosynthetic photon flux density in mature leaves of two mango varieties, Keitt and Calypso. The curve was obtained by fitting data of six replicates for each variety. R^2 denotes the regression between the observed and the stimulated values.

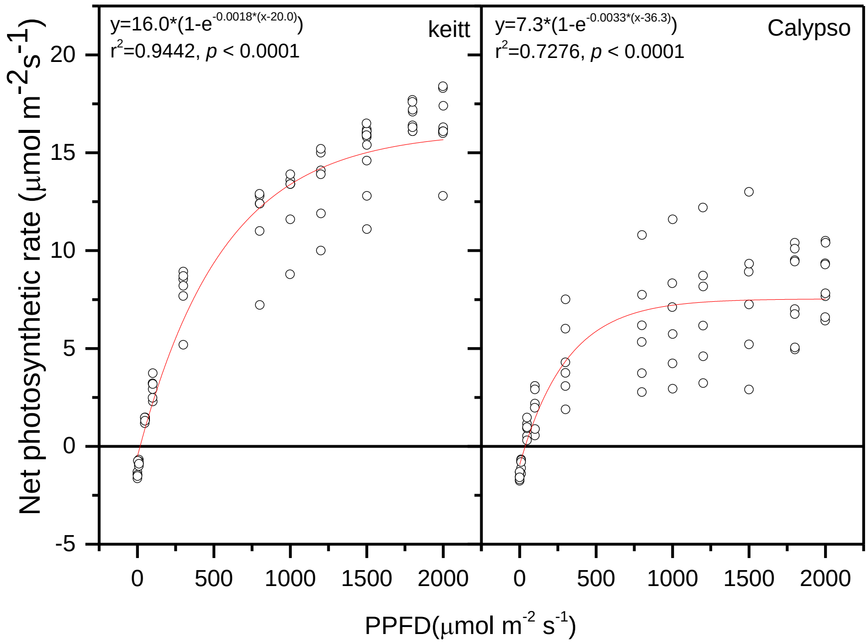

Figure 3. Daily course of A) PPFD (photosynthetic photon flux density, µmol m^{-2} s^{-1}), B) air temperature (°C), C) stomatal conductance (gs, mmol m-2 s-1), D) mesophyll conductance (mol m^{-2} s^{-1}), E) transpiration rate (mmol m^{-2} s^{-1}), F) vapour pressure deficit (VPD, kPa), G) net photosynthetic rate (Pn, µmol m^{-2} s^{-1}) and H) water use efficiency (mmol mol^{-1}) of two mango varieties (Keitt and Calypso). The different uppercase letters indicate significant difference during the whole daily course for Keitt, lowercase letters for Calypso. * indicates there is significant difference between two mango varieties at a given time ('' 0.001, '**' 0.01, '*'0.05). The vertical bars are standard errors (n=30). Water use efficiency= Pn/gs, mesophyll conductance = Pn/intercellular CO2 concentration).***

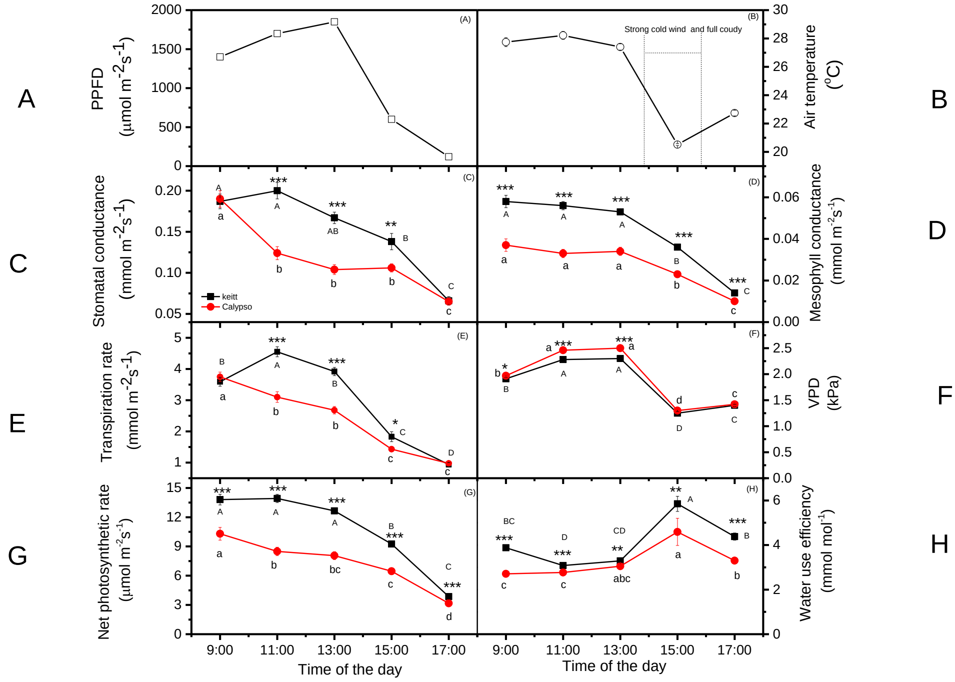

Figure 4. Net photosynthetic rate (Pn) response as a function of photosynthetic photon flux density in leaves of mango variety (Keitt) at different canopy layers (upper new mature leaf, upper old leaf, and inner leaf). The fitting curve was obtained by fitting data of three replicates for each variety. R^2 denotes the coefficient of correlation between the observed and the stimulated values.

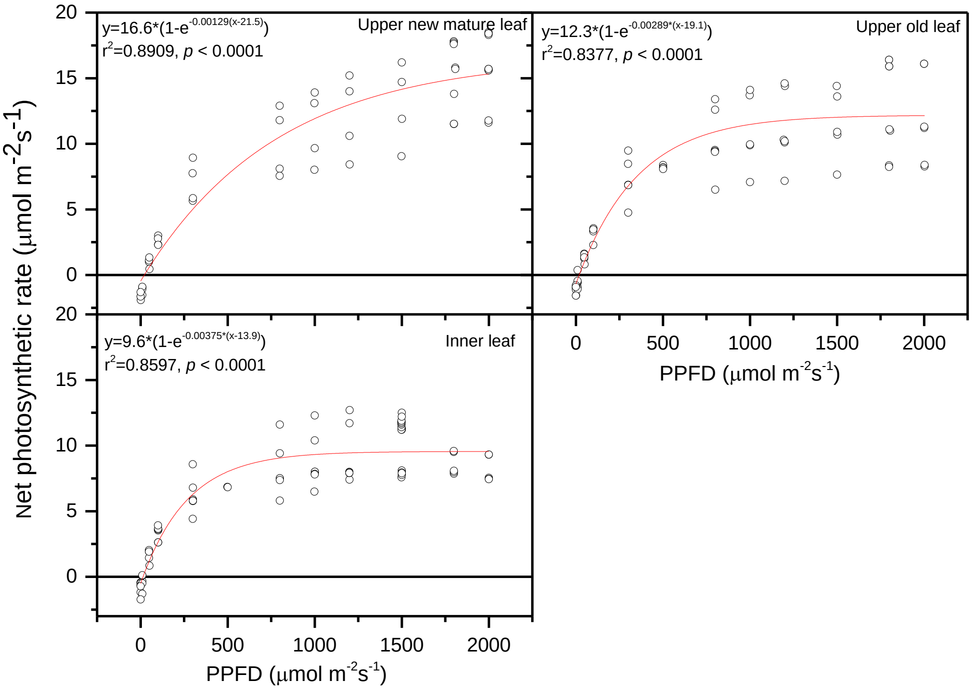

Table 1 ANOVA analysis of the foliar $\delta^{13}C$ isotope (‰), foliar N concentration (%) and foliar $\delta^{15}N$ isotope (‰) of terminals with and without crop load, in three varieties of Mango (Keitt, NMBP 1243 and Calypso TM) at different orchards in Far North Queensland (n=16).

Variety	Terminal treatment	Foliar $\delta^{13}C$ (‰)	Foliar N conc. (%)	Foliar $\delta^{15}N$ (‰)
Keitt	**Crop load**	-29.0	0.7 b	-0.4
	No crop load	-29.0	1.0 a	-0.6
	p-value	*0.937*	*<0.001*	*0.557*
	sed	*0.17*	*0.03*	*0.26*
NMBP 1243	**Crop load**	-28.2 b	1.0 b	2.7
	No crop load	-27.1 a	1.4 a	1.5
	p-value	*<0.001*	*<0.001*	*0.62*
	sed	*0.27*	*0.05*	*2.34*
Calypso TM	**Crop load**	-28.6 b	0.8 b	2.0
	No crop load	-28.2 a	1.1 a	1.9
	p-value	*0.017*	*<0.001*	*0.083*
	sed	*0.14*	*0.03*	*0.15*

Table 2 ANOVA analysis of the foliar $\delta^{13}C$ isotope (‰), foliar N concentration (%) and foliar $\delta^{15}N$ isotope (‰) in three varieties of Mango (Keitt, NMBP 1243 and Calypso ™) under different irrigation (8, 35 and 70 L/hr) system, in orchards in Far North Queensland (n=6).

	Foliar $\delta^{13}C$ (‰)			Foliar N conc. (%)			Foliar $\delta^{15}N$ (‰)		
Variety	Irrigation rates (L/hr)								
	8	35	70	8	35	70	8	35	70
Keitt	-27.2 a	-27.0 a	-26.7 a	1.5	1.5	1.4	1.9	2.5	2.1
NMBP 1243	-27.2 a	-27.5 b	-27.8 b	1.4	1.5	1.5	1.8	2.8	1.8
Calypso	-28.7 b	-28.5 c	-28.6 b	1.3	1.4	1.4	2.1	2.3	3.2
p-value	*< 0.001*	*0.002*	*<0.001*	*0.67*	*0.561*	*0.414*	*0.727*	*0.846*	*0.383*
sed	*0.34*	*0.35*	*0.35*	*0.07*	*0.06*	*0.06*	*0.034*	*0.9*	*1.02*

Table 3 Photosynthetic capacity (Pmax, µmol CO_2 $m^{-2}s^{-1}$), apparent quantum yield (AQY, µmol CO_2 µmol absorbed photon), light saturation point (LSP, µmol photon $m^{-2}s^{-1}$), light compensation point (LCP, µmol photon $m^{-2}s^{-1}$) and leaf respiration in the dark (R, µmol CO2 $m^{-2}s^{-1}$) in two mango varieties (Keitt and Calypso). These values were calculated from light response curves (n=6).

Variety	Pmax	AQY	LSP	LCP	R
Keitt	16.0	0.034	1176	20.0	0.67
Calypso™	7.3	0.025	826	36.3	0.78
LSD	3.0	0.010	308	22.1	0.31
p-value	6.36e-05	0.0992	0.0293	0.0284	0.453

Table 4 Photosynthetic capacity (Pmax, µmol CO_2 $m^{-2}s^{-1}$), apparent quantum yield (AQY, µmol CO2 µmol absorbed photon), light saturation point (LSP, µmol photon $m^{-2}s^{-1}$), light compensation point (LCP, µmol photon $m^{-2}s^{-1}$) and leaf respiration in the dark (R, µmol CO_2 $m^{-2}s^{-1}$) in mango variety Keitt at different canopy layers (upper new mature leaf, upper old leaf, inner leaf). These values were calculated from light response curves (n=4). The different letters in the column indicate significant difference between leaf positions.

Variety	Pmax	AQY	LSP	LCP	R
Upper new mature leaf	16.6^{a}	0.021^{b}	1850^{a}	21.5^{a}	0.46^{a}
Upper old leaf	12.3^{b}	0.037^{a}	830^{b}	19.1^{ab}	0.68^{a}
Inner leaf	9.6^{b}	0.038^{a}	650^{b}	13.9^{b}	0.51^{a}
LSD	4.2	0.014	427	6.2	0.23
p-value	0.00296	0.0141	3.47e-05	0.0162	0.0405

Table 5 Net photosynthetic rate (Pn, µmol CO2 $m^{-2}s^{-1}$), stomatal conductance (gs, mmol $m^{-2}s^{-1}$), mesophyll conductance (gm, mmol $m^{-2}s^{-1}$), transpiration rate (Tr, mmol H_2O $m^{-2}s^{-1}$) and Water use efficiency (WUE, mol CO_2 mol^{-1} H_2O) in leaves of mango variety Keitt at different canopy layers (Upper new mature leaf, upper old leaf, inner leaf). The different letters indicate significant difference between three leaf positions (n=30).

Variety	Pn	gs	gm	Tr	WUE
Upper new mature leaf	15.7^{a}	0.27^{a}	0.058^{a}	4.42^{a}	3.63^{a}
Upper old leaf	13.2^{b}	0.22^{b}	0.049^{b}	3.80^{b}	3.51^{a}
Inner leaf	9.6^{c}	0.19^{c}	0.033^{c}	3.16^{c}	3.12^{b}
LSD	1.2	0.03	0.004	0.51	0.31
p-value	<2e-16	5.67e-08	<2e-16	2.3e-07	0.000304

Notes: gm=Pn/Ci (Ci-intercellular CO2 concentration), WUE=Pn/Tr.

References

Prado, C. H. B. A. & Moraes, J. A. P. V. (1997). Photosynthetic capacity and specific leaf mass in twenty woody species of cerrado vegetation under field conditions. *Photosynthetica* 33: 103-112.