HYDRAULIC CHARACTERISTICS AND PHOTOSYNTHETIC CAPACITY OF *Chrysanthemoides monilifera* L. WHEN GROWN IN CONTRASTING ENVIRONMENTAL CONDITIONS

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ABSTRACT

A semi-herbaceous, pioneer plant, *Chrysanthemoides monilifera* was grown under varying environmental conditions in order to assess whether altering environmental variables would affect its hydraulic conductance and photosynthetic rates. The plants were grown under sun and shade conditions, subjected to low and high watering treatments and to two different nutrient regimes. Steady-state gaseous exchange parameters, and whole-plant and leaf hydraulic conductance were measured on plants from each treatment. A key aspect of this study was to investigate how the following leaf components – petiole, major veins, minor veins and extravascular tissue – contributed to the overall resistance to water flow in the leaf (R_{leaf}). Vein orders were cut in specific sequences to interrupt water flow which then allowed the partitioning of leaf hydraulic resistances.

The results showed that the maximum photosynthetic rate, under light saturating CO_2, (A_{max}) was significantly affected by both nutrient and light treatments. Environmental conditions (light, water and nutrient treatments) did not, however, affect the CO_2 compensation point, or dark respiration of the measured $A:C_i$ curves for *C. monilifera*. In terms of whole-plant hydraulic conductance, the shoot, stem and root were not significantly affected by environmental treatments. When investigating R_{leaf}, only the light treatments significantly affected the resistance of the petiole, extravascular tissue, and minor veins. $R_{petiole}$ was found to be positively correlated with R_{leaf} and contributed between 34-59 % of the total leaf resistance. When considering the resistance of the leaf, it was observed that the vascular tissue of the leaf contained up to 90 % of the total leaf resistance.

The results from this study show that the hydraulic conductance of *C. monilifera* was found to be significantly affected by light treatments only. Water and nutrient treatments did not have a substantial impact upon the water flow of the plant. Leaf hydraulic resistance was partitioned differently to that of results from other studies, in that the petiole and major veins contained the majority of the leaf resistance. In retrospect this study would have been more effective if *C. monilifera* treatments were more severe, in terms of water and nutrients. Further studies should focus on a comparison of leaf hydraulic resistance partitioning of other species, across a range of plant types.

DECLARATION

The experimental work carried out in this thesis was performed at the School of Biological and Conservation Sciences, University of KwaZulu-Natal, from November 2005 to October 2007, under the supervision of Professor Norman Pammenter.

I, the undersigned, declare that the work contained in this dissertation is my own original work and has not previously in its entirety, or in part, been submitted at any other university for a degree.

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Date

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LIST OF SYMBOLS / ABBREVIATIONS

Ψ	Water potential(MPa)
Ψ_P	Pressure potential (MPa)
Ψ_{JI}	Osmotic potential
A	Assimilation rate (μmol CO_2 m^{-2} s^{-1})
A_l	Leaf area distal to segment measured (m^2)
A_{max}	Maximum assimilation rate at saturating light (μmol PPFD m^{-2} s^{-1})
A_{sw}	Cross-sectional area of conductive sapwood (m^2)
C_a	CO_2 concentration at ambient conditions
g_s	Stomatal conductance (mol m^{-2} s^{-1})
HPFM	High pressure flow meter
IRGA	Infrared Gas Analyser
J_{max}	Maximum assimilation rate at saturating CO_2 (μmol CO_2 m^{-2} s^{-1})
k_h	Conductance (kg s^{-1} MPa $^{-1}$)
K_h	Conductivity (kg s^{-1} MPa $^{-1}$ m^{-1})
K_{leaf}	Hydraulic conductance of a leaf (kg s^{-1} MPa^{-1} m^{-2})
$K_{lleaves}$	Hydraulic conductance of the leaves normalized by leaf area (kg s^{-1} MPa^{-1} m^{-2})
K_{lroot}	Hydraulic conductance of the roots normalized by leaf area (kg s^{-1} MPa^{-1} m^{-2})
K_{lshoot}	Hydraulic conductance of the shoot normalized by leaf area (kg s^{-1} MPa^{-1} m^{-2})
K_{lstem}	Hydraulic conductance of the stem normalized by leaf area (kg s^{-1} MPa^{-1} m^{-2})
K_s	Specific hydraulic conductivity (kg s^{-1} MPa^{-1} m^{-2})
K_{stem}	Hydraulic conductivity of the stem (kg s^{-1} MPa^{-1} m^{-1})
LSC	Leaf-specific conductivity (kg s^{-1} MPa^{-1} m^{-2})
PAR	Photosynthetically active radiation (μmol m^{-2} s^{-1})
PPFD	Photosynthetic photon flux density (μmol m^{-2} s^{-1})
r	Resistance (MPa s kg $^{-1}$)
R	Resistivity (MPa s m kg^{-1})
$R_{extravascular}$	Hydraulic resistance of the extravascular tissue (MPa s m^2 kg^{-1})

R_{lamina}	Hydraulic resistance of the leaf lamina (MPa s m^2 kg^{-1})
R_{leaf}	Hydraulic resistance of a leaf (MPa s m^2 kg^{-1})
R_{leaves}	Hydraulic resistance of the total leaf area (MPa s kg^{-1})
$R_{lleaves}$	Hydraulic resistance of the leaf area normalized by leaf area (MPa s m^2 kg^{-1})
R_{lroot}	Hydraulic resistance of the roots normalized by leaf area (MPa s m^2 kg^{-1})
R_{lshoot}	Hydraulic resistance of shoot normalized by leaf area (MPa s m^2 kg^{-1})
R_{lstem}	Hydraulic resistance of the stem normalized by leaf area (MPa s m^2 kg^{-1})
$R_{major\ veins}$	Hydraulic resistance of the major veins (MPa s m^2 kg^{-1})
R_{mincut}	Hydraulic resistance after the minor veins have been cut (MPa s m^2 kg^{-1})
$R_{minor\ veins}$	Hydraulic resistance of the minor veins (MPa s m^2 kg^{-1})
$R_{petiole}$	Hydraulic resistance of the petiole (MPa s m^2 kg^{-1})
R_{root}	Hydraulic resistance of the roots (MPa s kg^{-1})
$R_{venation}$	Hydraulic resistance of the venation of the leaf (MPa s m^2 kg^{-1})
$R_{whole\ shoot}$	Hydraulic resistance of the shoot (MPa s kg^{-1})
$R_{tertcut}$	Hydraulic resistance after the tertiary veins have been cut (MPa s m^2 kg^{-1})
SLA	Specific leaf area (m^2 g^{-1})

Chapter 1

INTRODUCTION

1.1 The Cohesion-Tension Theory

Water is the earth's most abundant molecule and yet restricts productivity of terrestrial plants due to its limited availability (Boyer, 1985). Not only does water limit agricultural plant productivity but it also restrains the productivity of natural ecosystems (Boyer, 1985). Agricultural yield losses attributed to water stress, account for more losses than any other biotic and environmental factor combined (Lambers *et al.*, 1998). Understanding how plant water relations function is therefore imperative for not only agricultural and forestry yield productivity but for natural plant productivity patterns too.

The biomass of non-woody tissues is made up of between 80 – 95 % water (Lambers *et al.*, 1998). Plants assimilate 90 % of the nitrogen that is absorbed by the roots, and between 10 – 70 % of the carbon fixed by the leaves, but only about 1% of the water absorbed by the roots is retained. The other 99 % of the water absorbed by plants is lost through the process of transpiration (Lambers *et al.*, 1998). Transpiration is the unpreventable consequence of photosyntheis, whereby plants keep their stomata open to fix carbon from the atmosphere. Transpiration does confer some advantages for the plant, in that nutrients may be transported from the soil to the root surface. The other added advantage is that transpiration allows the plant to be cooled by the transfer of heat to the atmosphere (Lambers *et al.*, 1998). Transpiration is influenced by numerous biotic and abiotic factors, but the process of how the plant pulls up water to lose it is important when assessing plant water relations as a whole.

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The way in which plants pull up water from the roots, to be transpired through the leaves, is explained by the Cohesion-Tension theory. This theory was proposed by Dixon and Joly in 1894, and states that "water ascends plants in a metastable state under tension, with a xylem pressure more negative than that of a perfect vacuum." The driving force for this negative pressure is created by the surface tension at the evaporation surface of the leaf (Tyree and Zimmerman, 2002). This tension at the surface of the leaf is transferred through a water column of a continuous nature, otherwise referred to as the soil-plant-atmosphere continuum (Tyree and Zimmerman, 2002). The surface tension at the leaf is generated by the lowering of the water potential (Ψ) of the stomatal and mesophyll cell walls. This therefore causes the sub-stomatal chambers to have a lower water potential than that of the water vapour in the air, which allows for the evaporation from the stomata (Tyree and Zimmerman, 2002). The lowering of the water potential is a primary result of the lowering of the pressure potential (Ψ_P). The pressure potential is produced from the process of evaporation from the cell walls, whereby the curvature of the menisci of the water in the cell wall microfibrils is changed (Tyree and Zimmerman, 2002). The pressure in the liquid behind the menisci is lowered by the surface tension forces, which facilitates the lowering of the water potential in the adjoining cell walls. Pressure potential is one of the main components driving water potential, with the other being that of solute potential ($\Psi_Л$). The relationship can be described as follows:

$$\Psi = \Psi_P + \Psi_Л$$

This lowering of water potential, and resulting negative pressure, from the stomatal cell walls is actually transmitted from the leaves all the way via the stem down to the roots (Tyree and Zimmerman, 2002). The root cells then have a lower water potential than that of the soil, which causes water to be taken up by the roots eventually replacing that in the leaves of the plant. Van der Honert (1948) proposed an Ohm's Law analogy of the soil-plant-atmosphere continuum, stating that the driving force for the ascent of sap within any given plant is the constant lowering of the pressure potential in the direction of sap flow.

Due to the fact that such a negative pressure is generated in the plant, one has to ask the question of how the water in the plant actually continually stays at that negative pressure. The answer as to how the pressure stays negative in the xylem is found in the term "cohesion" of the Cohesion-Tension theory. Firstly, the walls of the xylem elements have to be bubble-free for the process of cohesive to operate, implying that the roots should absorb water while excluding air bubbles (Tyree and Zimmerman, 2002). The absorbed water within the xylem elements is held together via the cohesion force of the hydrogen bonding of the water molecules. If the water and the walls of the xylem elements are bubble-free, then water within the xylem column does not evaporate under negative tension. There is however a point at which the water in the xylem column is subjected to pressures that are too negative to sustain. When this occurs the xylem column breaks and the breaking of the column is known as a cavitation (Tyree and Zimmerman, 2002).

1.2. Xylem Cavitation

Xylem vessels or tracheids are alive during their period of growth and differentiation, and are filled with water and cellular organelles (Tyree and Zimmerman, 2002). These living xylem elements become functional only once they have matured and died, and are then considered operational conduits. A cavitational event occurs when water vapour forms a void and air within the void forms a radius large enough to create a bubble within the water column. These voids are extremely unstable and can be dissolved by the surface tension in the conduit, or if the negative pressure drops further, the void enlarges (Tyree and Zimmerman, 2002). When the void forms a sufficient radius to completely fill the lumen of a xylem conduit, then the conduit is considered to be dysfunctional and will not be able to conduct the flow of water. Conduits that have experienced an embolism can continue their function once the conduit has been refilled with water (Lambers *et al*., 1998). The refilling of conduits can occur at night under positive pressure or during rain episodes.

Embolized air is prevented at all costs from extending to the neighbouring conduits. This is made possible by the surface tension forces of the air and water meniscus between adjoining conduits, which are connected via pit membrane pores. The pit membrane opens into the lumen of the conduit by means of a pit chamber. The porosity of the pit membrane determines the extent to which embolisms may spread from one conduit to another (Tyree and Zimmerman, 2002). "Safe" pit membrane pores are regarded as those pores of a narrow diameter that essentially prevent the majority of embolisms from spreading to other conduits. The main disadvantage of having smaller pit membrane pores though is that the conduits do not conduct water as effectively. Therefore it is the pores in the pit membrane that ascertain the vulnerability of individual species to cavitation, and not the actual diameter of the conduit itself (Tyree *et al*., 1995). Species-specific differences in vulnerability to cavitation are hence controlled by a strong selective pressure of the genetics of the pit membrane pores (Tyree and Sperry, 1989). The size of the pit membrane pores consequently forces a trade-off between the safety and ultimately the efficiency of flow of the conduit (Sperry, 1995).

Plants operate close to the water potential at which cavitation occurs, and complete cavitation can occur from between -1.0 to -9.0 MPa (Sperry *et al*., 1996). Depending on the environment in which the plant is growing, be it a mesic or xeric environment, the risk of cavitation is inevitable for even slightly water-stressed plants. During any period of drought, cavitation is the most severe cause of productivity loss of any agricultural environment (Lo Gullo and Salleo, 1993). Drought-adapted species will therefore possess smaller pit membrane pores to allow for a higher degree of resistance to cavitation (Lambers *et al*., 1998).

Cavitation may also be caused by freezing and thawing events in colder climates (Sperry, 1995). Gases that have dissolved in sap are insoluble in ice and freeze as bubbles. If the bubbles have a large enough radius, when the sap thaws and tension develops, these bubbles can cause cavitation events (Sperry, 1995). Again, differences in conduit pit membrane pores explain how different species have varied susceptibilities to cavitation in response to freeze-thaw events. Freezing stress that causes cavitation is more pronounced in conduits that are wide and long, as opposed to those that are short and narrow (Lambers *et al*., 1998).

The diameter of xylem vessels determines the rate of water flow through them and the rate of water flow or conductance (K_h) is approximately proportional to the fourth power of the vessel diameter. This proportional relationship is otherwise known as the Hagen-Poiseuille Law. The conductance of a stem with few large vessels is much larger than that of a stem with many narrow vessels according to the law stated above. The size of the vessel diameter is not however the only factor that determines water conductance through a plant. Other factors, including environmental variables *e.g*. light, water supply, nutrient availability and temperature, also affect the hydraulic conductance of any specific species.

1.3. Hydraulic Conductance

When considering plant hydraulic measurements, it is essential that the right nomenclature be used in order to describe parameters that have been or are to be measured. There is a distinct difference between the terms conductance and conductivity when explaining plant hydraulics. Conductivity is the flow rate through a plant per unit pressure gradient (kg s^{-1}MPa m^{-1}) and is given the symbol *K* (Tyree and Zimmerman, 2002). Conductance, on the other hand, does not take into account the length of a conducting system and is instead simply the flow rate per unit pressure drop (kg s^{-1} MPa).

Conductance is denoted by means of *k* (Tyree and Zimmerman, 2002). The inverse of conductivity and conductance are resistivity and resistance respectively. Resistances are said to be additive in series, whereas conductances are additive in parallel.

Hydraulic conductivity can be defined as follows:

$K_h = F\ L / \Delta P$

where F = flow rate (kg s^{-1}); L = length (m); and ΔP = pressure gradient (MPa).

Another means of defining conductivity is to express it in terms of area of conductive sapwood. This measurement is known as specific hydraulic conductivity (K_s) and is a measure of the efficiency of the stems or branches or twigs of a plant to conduct water (Zimmerman, 1978).

$K_s = K_h / A_{sw}$

where A_{sw} = cross-sectional area of the conductive sapwood.

The final manner in which hydraulic conductivity can be expressed is by normalizing by leaf area. This is referred to as leaf-specific conductivity (LSC) and is a measure of the hydraulic sufficiency of a plant to supply water to the leaves at the end of the branch segment (Zimmerman, 1978).

$K_l = K_h / A_l$

where A_l = leaf area distal to segment measured.

When measuring any one of the hydraulic parameters above, it is important to note that observable trends will differ depending on the species, growth conditions and the growth form within a species (Ewers *et al*., 1991; Cochard *et al*., 1997). The fact that the growth form of plants occurs in a segmented manner is extremely important to the hydraulic construction of any perennial plant. Hydraulically speaking the plant can be divided into components, which determine the overall conductance and hence resistance.

These components include the roots, stems and leaves, and the leaves can be further subdivided into petioles, and vein orders. Perennial plants maintain a distinct drop in conductance at the petiolar insertions of the plant, and this allows perennial plants to sacrifice their leaves during winter or water-stressed periods (Tyree and Zimmerman, 2002). There is a trend however to report these plant component measurements in terms of resistance as these are additive in series.

Conductance and resistance can be measured accurately by means of a high pressure flow meter (HPFM). The HPFM forces water into the leaves of a given branch, faster than the plant is transpiring, thereby filling all the leaf air spaces with water (Tyree *et al*., 1995). It can be used to calculate the component resistance of branches of different diameter size classes by measuring the combined hydraulic resistance of the shoot or root vascular pathway (Tyree *et al*., 1995). For example, the resistance of the shoots and roots of *Pinus* species have been found to be roughly equal (Tyree, 1993). When measuring resistance of shoots or roots, the smaller branches and roots have exhibited more vascular resistance than that of larger shoots or roots (Tyree *et al*., 1995). It has been confirmed that non-vascular pathways and resistances do also exist, although the relationship between vascular *versus* non-vascular is relatively unclear (Tyree *et al*., 1995).

1.3.1 Hydraulic conductance and the environment

It has been shown that climatic variation can affect the distribution of hydraulic resistance in plant populations (Maherali *et al.*, 2002). Changes in climate may force strong selective pressures upon plant populations, which have the ability to drive genetic differentiation of traits on plants growing in varying environments (Maherali *et al.*, 2002). Plants do have the capability of acclimating to different environmental variables, a phenomenon otherwise referred to as phenotypic plasticity.

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When water resources are limited, the capacity of the plant to acclimate to low water availability is of utmost importance. It is well known that the accessibility of water in the soil influences the survival and growth of any plant (Drake and Franks, 2003). How plants utilise the water resources provided to them changes across growth forms and within specific growth strategies too (Ehleringer *et al.*, 1991; Meinzer *et al.*, 1999). In whichever way plants utilise their water is ultimately going to have an impact upon the hydraulic functioning of the plant.

When considering drought, there are a number of mechanisms necessary to allow for drought resistance. Plants can be grouped into either the drought-delay or drought-tolerance strategy (Tyree *et al.*, 2002). Drought delay include physiological traits that increase access to water resources, and is limited to mild water stress, rather than severe drought. These traits include deeper roots, stomatal closure and leaf shedding (Tyree *et al.*, 2002). The response of leaf shedding during water stress has been documented in warm, dry sites and results in a reduction in leaf area: sapwood area ratio (Mencuccini and Grace, 1995). Drought tolerance however, involves traits that allow for water transport to continue at lower negative pressures (Tyree *et al.*, 2002). The vulnerability to xylem cavitation is lowered and also permits cells to exist at low water potential values. Tyree *et al.* (2002) showed that drought- tolerance and -delay can be related to hydraulic conductance when concerned with water stress of plants. As desiccation of water stressed plants progresses, the hydraulic conductance of the roots is reduced much slower than that of the shoots. Because leaves are shed from the plant, and below-ground resources per unit leaf area are increased, the hydraulic supply to the shoots is reduced in order to allow the continued survival of the plant.

Reductions in hydraulic conductance due to water stress have been documented for a number of species, including *Euculyptus globulus*, *Pondorosa* pine and *Licania platypus* (Costa E Silva *et al.*, 2004; Maherali *et al.*, 2002; and Tyree *et al.*, 2002).

Drake and Franks (2003) studied five tropical tree species and found that stem and leaf hydraulic conductivity was significantly reduced during the dry season. Similarly, *Pondorosa* pine exhibited a significant reduction in leaf specific conductivity when exposed to drought periods (Maherali *et al*., 2002). Whole-plant hydraulic conductivity of *Pondorosa* pine was however not significantly lower in drought *versus* well-watered plants (Maherali *et al.*, 2002). This lack of response was considered to be caused by the fact that well-watered plants were larger and hence had a higher hydraulic conductance. These findings suggest that hydraulic conductance can be correlated with plant size and that it is complicated by environmental stress.

Leaf specific conductivity (LSC) has been shown to be lower for drought-adapted species, thereby increasing a plants ability to survive prolonged water stress and maintain a favourable water balance (Nardini *et al.*, 1999). This lower leaf specific conductivity is also associated with a different vulnerability to cavitation for similar drought-adapted species and between differing genotypes of the same plant species (Tyree and Ewers, 1991; van der Willigen and Pammenter, 1998). The reduction in LSC is therefore associated with a less gradual decrease in root conductivity, as reflected in terms of increased below-ground resources. The allocation to roots to ensure plant survival during drought allows periods of water stress to be less harmful. The developmental changes in response to water stress are fundamental determinants of plant performance during drought.

There is less information in the literature when assessing the response of hydraulic characteristics to changes in nutrient availability or nutrient quality. Lovelock *et al*. (2004) performed nitrogen and phosphorus enhancement experiments on the dwarf mangrove, *Rhizophora* spp. It was found that fertilization with nitrogen increased stem hydraulic conductance (K_{stem}) by 2.5 times, whereas phosphorus enhancement increased K_{stem} by up to six times (Lovelock *et al*., 2004). LSC was also significantly increased by nitrogen and phosphorus addition, whereas photosynthesis was not.

1.4. Leaf Hydraulic Characteristics

Angiosperms, gymnosperms and ferns employ different strategies regarding how water is transported through a particular leaf. Angiosperms utilise a highly branched reticulate system of leaky veins, which allow for water to be transported to the evaporation sites of the leaf (Zwieniecki *et al*., 2002). Gymnosperms adopt a different water transporting system where a vascular network extends with only one vein per leaf. Ferns can be either branched or highly reticulate in order to irrigate the individual fronds (Zwieniecki *et al*., 2002). It is unclear how these large differences between vascular networks affect the performance of the plants photosynthesis, growth and construction (Brodribb *et al*., 2004). The conductance of the leaf lamina is determined by the vascular and non-vascular pathways of transpiring water (Yang and Tyree, 1994). The flow of water through the leaf starts at the petiole and flows through the vein orders in either a series or parallel pathway (Sack *et al*, 2003a). The water then crosses the bundle sheath, flows into and around the mesophyll and then into the air spaces of the stomatal chamber and is evaporated into the atmosphere (Sack *et al*, 2003a).

The resistance to water flow through the leaf is high, relative to the rest of the plant, and has been measured to be from 25 to 90 % of whole shoot resistance (Sack *et al*., 2003b; Nardini, 2001). This demonstrates that the hydraulic conductance of the leaves has a disproportionate influence over whole plant water relations (Brodribb *et al*., 2004). In terms of leaf resistance, this disproportionate influence is considered the hydraulic bottleneck of the plant, and it often correlates with the structure of the leaf (Sack *et al*., 2003b). The resistance of the leaf and its individual components are one of the most important and yet also least understood processes of whole-plant hydraulic conductance (Sack *et al.*, 2004).

There have been only a handful of studies examining how the components of the water flow pathway through the leaf actually contribute to the total leaf resistance (Sack *et al.*, 2004). These studies have made use of cutting or freezing treatments on individual leaves to determine the resistance of separate water pathway components of the leaf. From these experiments, it has been shown that leaf resistance can be partitioned into four component resistances. These are: resistance of the petiole ($R_{petiole}$), resistance of the major veins ($R_{major\ veins}$), resistance of the minor veins ($R_{minor\ veins}$) and lastly the resistance of the extravascular tissue ($R_{extravascular}$).

Understanding how the leaf resistance components are partitioned and how they respond to changes in stress is essential in crop breeding when conferring hydraulic properties that increase agricultural productivity (Tyree, 2003; Gasco *et al*., 2004). There is however a debate between researchers measuring hydraulic partitioning, where early studies initially showed that most of the hydraulic resistance of the leaf resides outside the vascular pathway of water (Tyree and Cheung, 1977). This pattern of hydraulic resistance partitioning was confirmed by experiments that targeted the removal or bypass of all the resistance that was associated with mesophyll membranes (Tyree *et al*., 2001; Salleo *et al*., 2003).

Sack *et al*.(2004) showed that the majority of the leaf resistance of *Acer saccharum* and *Quercus rubra* was found to be in the xylem or vascular water flow pathway. The vascular pathway encompasses not only the resistance of the petioles, but that of the major and minor veins as well. The contribution of the vascular pathway varied from 69 to 74 % of the total leaf resistance (Sack *et al*., 2004). Zwieniecki *et al*., (2002) also reported that the highest resistance to water flow was found in the leaf venation, rather than in the extravascular tissue of the leaf.

These values are in great contrast with those obtained by Salleo *et al*. (2003) and Yang and Tyree (1994), which showed xylem resistance to be of only 9 to 50 % of the total. Cochard *et al*.(2004) calculated that more than 75 % of the total leaf resistance resided outside the vascular pathway of water. The method described was similar, although not exactly the same as that of Sack *et al*. (2004) and the differences could therefore be because of species specific differences in total leaf resistance partitioning. When relating the vascular *versus* non-vascular partitioning to water potential and gaseous exchange, it would be of a greater advantage for a plant to have most of its resistance located in extravascular tissue (Cochard *et al*, 2004). The alternate theory has also been supported in recent literature though, where if the majority of the resistance was outside the vascular pathway, the xylem hydraulic efficiency of the venation would be lowered (Cochard *et al*., 2004). Should a decrease in hydraulic conductance (*i.e.* an increase in the total leaf resistance) be detected then it would imply that a decrease in leaf gaseous exchange would follow and that the water relations of the plant would be affected (Cochard *et al*, 2004).

Increasing interest is also being directed to the component of the venation, which has the most hydraulic importance for water flow. Two schools of thought exist, the first of which implies that the minor veins are the most hydraulically useful. It is based on the premise that the minor vein network has an excess of parallel water flow pathways throughout the leaf (Plymale and Wylie, 1944). Therefore the major veins are beneficial only for structural support and play no role in hydraulically supporting the plant. More recent publications by Sack *et al*. (2003) show that the major veins of *A. saccharum* and *Q. rubra* are indispensable for continued water transport, as well as for mechanical support. The studies showed that for these particular species, the major veins of the leaf are essential for hydraulic supply. The fact that the major veins are so important indicates that the vascular system of the leaf constrains hydraulic conductance to a certain degree.

Given that changes in leaf resistance affect plant water balance, it is not surprising that the attention being paid to long- and short-term changes of leaf resistance is on the rise. The focus of recent studies has been the response of leaf resistance to environmental and developmental factors, to assess how plants adapt to the changing environment (Nardini and Salleo, 2003; Sack *et al.*, 2003b). Most of this literature is based on the effects of light on leaf resistance, and the partitioning of resistance within leaves. The total leaf resistance measured for a number of different species has displayed that the leaf resistance of certain sun species can be between 15 and 67 % lower than that of measured shade leaves of the same species (Sack *et al.*, 2003b). Sack *et al*.(2003b) have shown that sun leaves of *A. saccharum* and *Q. rubra* had significantly lower lamina resistance (*i.e*. higher lamina conductance) than those of shade leaves of the same species. The conductance of the lamina of sun leaves of *A. saccharum* and *Vitis* spp differed by up to four-fold and was also found to be correlated with petiole conductance. The same trend was found for the resistance of the petiole of the leaf, whereby the petioles of sun leaves showed a lower resistance to water flow than that of shade leaves (Sack *et al*., 2003b). The serial positioning of leaf hydraulic components seen above is related to the process of leaf gaseous exchange, because of the flow of water through the plant by means of xylem and stomatal pathways (Brodribb *et al*., 2004).

1.5. Photosynthesis and Hydraulic Conductance

1.5.1. Photosynthesis and the Environment

Photosynthesis is essential for growth and survival of all plants (Lambers *et al*., 1998). The dry mass of a plant consists of approximately 40 % carbon, all of which is fixed in photosynthesis. Light is captured for photosynthesis by the chloroplasts, which are in close proximity with the vascular tissue of the leaf (Lambers *et al*., 1998). Carbon dioxide uptake for fixation during the process of photosynthesis enters through the stomatal pores of the leaf. Ideal conditions incorporate the correct balance of water, nutrients, optimum temperature and light.

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Photosynthesis is a coordinated response of all the component processes and it is affected by any environmental stress/es imposed on the plant (Freeden *et al*, 1991, Chaves, 1991). Photosynthesis in response to light is extremely variable and the degree in terms of quality and quantity of light, is an important determinant of photosynthesis (Lambers *et al*., 1998). Low light limits plants and thus causes slower growth. High light produces a different photosynthetic response. High light can cause denaturation of enzymes (Lambers *et al*., 1998). Plants can be referred to as "sun" or "shade" plants in terms of their response to light. Sun plants are those which grow under high light conditions and do not survive in shaded environments. Sun leaves have a greater capacity for photosynthesis, as they have more photosynthetic machinery per unit leaf area and can maintain a larger number of cells (Lambers *et al*., 1998).

Photosynthesis is dependent upon the availability of soil water, and the ability of the roots to absorb that water. The stomata of the leaves allow for the uptake of carbon dioxide for photosynthesis, but also permit the inevitable process of transpiration (Lambers *et al*., 1998). During periods of water stress, the stomata close to prevent cavitation and desiccation (Flexas and Medrano, 2002). This closure ultimately affects the supply of carbon dioxide and stimulates the plant to down-regulate photosynthesis (Wong *et al*., 1985). The down-regulation of photosynthesis due to water stress is seen as one of the earliest effects of soil drying (Lawlor, 2002, Correia *et al*., 2006). The extent to which photosynthesis is affected by water stress depends on the severity, duration and speed with which the drought occurs (Rouhi *et al*., 2007). Drought also has the ability to cause the reduction of photosynthesis via non-stomatal factors *e.g*. decreased carboxylation effeciency in the mesophyll of the leaf (Ramanjulu *et al*., 1998).

Decreased whole plant photosynthesis is primarily caused by a reduction in leaf area due to leaf shedding (Rouhi *et al*., 2007). Leaf shedding brings about a reduction in water losses via transpiration, although the cost is that photosynthesis will also decline. Characteristics of drought-adapted species include the slow decrease of assimilation rate, together with decreased stomatal conductance (g_s). The correlation of assimilation with g_s has been well documented, and their correlation is considered another feature of drought-adapted species (Rouhi *et al*., 2007). In well-watered plants, higher photosynthetic rates are associated with higher stomatal conductances, reiterating the fact that the two variables are closely related (Franks, 2005).

The interaction of water stress and nutrient deficiency has not been well studied, even though both nutrient deficiency and water stress are important determinants of photosynthesis (Mcdonald and Davies, 1996; Lawlor, 1995). When plants are grown in well-watered conditions, photosynthesis and stomatal conductance are higher in high nitrogen treatments (Shangguan *et al*., 2000). When drought stress is experienced, the effects of nitrogen treatments are reduced in terms of photosynthesis and stomatal conductance. Thus, the reaction to drought differs, depending on the treatments (Shangguan *et al*., 2000).

1.5.2. The Correlation of Photosynthesis and Hydraulic Conductance

The xylem hydraulic characteristics have been shown to influence the form and function of plants (Brodribb and Feild, 2000). The environment influences the hydraulic conductance, which in turn can be constrained by xylem characteristics (Macinnis-Ng *et al*., 2004).

Meinzer *et al*. (1995) have evidence to support the proposal that stomatal conductance can be linked to hydraulic conductance within (Hubbard *et al*., 2001) and across plant species (Santiago *et al*., 2004). Stomatal conductance and transpiration are directly related therefore transpiration rates can be correlated with that of hydraulic conductance (Meinzer et al, 1999). These two parameters may be correlated even though the vascular system of the plant hydraulically constrains maximum transpiration (Andrade *et al*., 1998; Meinzer *et al*., 1999). The same principle can also be applied to photosynthesis and hydraulic conductance, whereby for any given allocation of carbon to a leaf, the photosynthetic potential of the leaf is constrained by the hydraulic conductance of the system (Franks, 2005). Higher assimilation rates correlate with higher stomatal conductances, which are then supported by higher hydraulic conductances (Franks, 2005). Therefore the relationship between photosynthesis and hydraulic conductance is seen to be an indirect one, linked by the correlation of both parameters with stomatal conductance. Ultimately, the hydraulic architecture would be reflected in physiological and anatomical traits of leaf photosynthesis (Brodribb and Feild, 2000).

1.6. Growth and Partitioning in Response to the Environment

Growth can be defined as an increase in dry mass, volume, length, or area, and involves the production, expansion and differentiation of cells (Lambers *et al*., 1998). Growth also incorporates the distribution and partitioning of carbon to plant organs above and below ground. The allocation of resources to plant organs will determine the growth rate, and the growth and allocation of any given plant is dependent not only on the genetics of the plant, but also the influence of the environment. The natural conditions of any environment are rarely conducive for maximal growth, and understanding how growth is influenced by environmental factors is extremely important.

The three main environmental factors that influence plant growth, and which were manipulated in this study, are light, water and nutrient supply. Plants that grow in the sun can attain higher photosynthetic rates, produce thicker leaves and have more layers of palisade cells than that of their shade counterparts (Ogren, 1993). Sun leaves can achieve higher photosynthetic rates because they have more of the components that determine photosynthesis *i.e*. they have more photosynthetic capacity per unit of chlorophyll in the leaf (Ogren, 1993). Shade leaves however allocate more resources to increase leaf area. The leaves of shade plants are relatively thin but maintain a large surface area for capturing the maximum amount of light as possible (Lambers *et al*., 1998). Shade plants exhibit greater stem and petiole elongation, as well as reducing the amount of branching, which reduces shading from other leaves. In terms of allocation of resources, when light limits growth, there will be a greater allocation of resources to the leaves for capturing light. This pattern of resource allocation is opposite for those plants which experience water or nutrient deficiency, whereby plants deficient in water or nutrients will allocate more resources to the roots (Garnier, 1991). Plants have a functional balance between root and shoot activity, in which below-ground resources that are acquired will approximately balance with the above-ground resources acquired by the shoots (Garnier, 1991).

When plants experience water stress, the cell walls of the roots loosen and extend (by both cell extension and production) in order to absorb as much water as possible. This root elongation comes at a cost to the leaves, as leaf expansion decreases, thus allowing for promoted root growth (Saab *et al*., 1990). A similar response can be observed for plants experiencing a nutrient deficiency. Roots will extend during periods of low nutrient availability, thereby investing in the plant part that acquires the limiting resource (Lambers *et al*., 1998). The diversion of resources to plant parts that require it most is considered an imperative growth mechanism, and the response of changing resource allocation will prolong survival of environmental stress.

1.7. *Chrysanthemoides monilifera* (L.) T. Nord.

This study focused on the growth adaptations of *Chrysanthemoides monilifera* when grown under eight different environmental treatments. *Chrysanthemoides monilifera* (L.) T. Nord was named by T. Nordlindh in 1943 and belongs to the daisy family Asteraceae. *C. monilifera* has many common names, the primary ones being bietou bush or bush tick berry (van Jaarsveld, 2001). It is endemic to eastern and southern Africa, and has in recent years become a weed of significant importance in Australia (van Jaarsveld, 2001). *C. monilifera* is a fast-growing, perennial, semi-succulent with fleshy branches that become woody (Palgrave, 2006). It is a pioneer species, capable of withstanding water stress, and growing primarily in full-sun habitats that are well-drained (Palgrave, 2006). The fruit of *C. monilifera* can be used as a food source and the leaves are used as a traditional medicine to treat fevers. *Chrysanthemoides monilifera* is found in fynbos, grassland, coastal dunes and sub-tropical forest margins (van Jaarsveld, 2001). It produces a shallow root system, and although it cannot survive agricultural cultivation, it has been deemed a serious weed of environmental importance because of its invasive nature and ability to out-compete endemic species in Australia. *C. monilifera* is highly tolerant of extreme environmental factors *e.g*. salinity, drought and wind.

C. monilifera was chosen as the experimental plant species for this study because of its ability to respond well to light conditions (i.e. sun or shade). It is a pioneer and has a relatively high growth rate (Palgrave, 2006). *C. monilifera* is also endemic along the south eastern African coast and is therefore an indigenous and traditionally used species of importance in Southern Africa. The basis of this study was to assess the impact that light, water and nutrients would have on hydraulic characteristics of *C. monilifera.* These characteristics would be reflected in photosynthetic performance, and the relationships between physiological adjustment and changes in growth patterns.

Chapter 2

MATERIALS AND METHODS

2.1. Plant Material

On 24th November 2005, 40 *Chrysanthemoides monilifera* seedlings were collected from Silverglen Nursery in Chatsworth, Durban, KwaZulu-Natal and were planted into 20-litre potting bags at the greenhouse at the University of KwaZulu-Natal, Durban. The seedlings were chosen to be of similar height and stem diameter, so that initial variation in the experiment was kept to a minimum.

2.2 Experimental Design

Two sites were used for the experiment: the first was positioned in direct sunlight, and the second was in the shade house. Twenty 20 litre potting bags were placed in the first site in the sun (Figure 2.1) and the remaining twenty bags were placed in the site under the shade-cloth. Each bag was filled with approximately 15-18 litres of mixed river sand and labeled with the appropriate treatment type. All bags had pre-cut drainage holes at the bottom to prevent water retention. Into each bag, one *C. monilifera* seedling was planted, and all bags were watered equally for the first week of the experiment. Experimental treatments were designed to maximize the amount of replication, while including eight different treatments. The first environmental variable that plants were subjected to was either sun or shade treatments.

Figure 2.1: *Chrysanthemoides monilifera* plants grown under shaded conditions.

The second of the environmental conditions that was altered was watering treatments. Of the 20 plants in the sun, 10 received a "high" watering treatment and the remaining 10 were given a "low" watering treatment. The same procedure was repeated with the plants in the shade. The plants were watered to field capacity (*i.e*. until water was seen above the soil) each time a watering treatment was administered. A high watering treatment was administered twice a week for sun plants and once a week for those plants in the shade. A low watering treatment was applied only once a week for those plants in the sun and once every two weeks for plants in the shade.

The last environmental condition that was varied was that of nutrient treatments. The 10 plants that received a high water treatment in the sun were sub-divided again, so that five of those plants were given a "high" nutrient treatment and the other 5 plants were given a "low" nutrient treatment. This application was repeated for the 10 plants in the sun being administered a low watering treatment.

The corresponding application of nutrients was copied in that of the shade plants, which were administered low and high watering treatments. Nutrient treatments were made by using Hoagland's solution (Hoagland and Arnon, 1950) (Table 2. 1) and only the quantity given to the plants allowed for the difference between "low" and "high" treatments. High nutrient treatments were given 1.5 litres of Hoagland's solution, every 3 weeks for the first 8 months of the experiment. The low nutrient treatments received 10 percent of the high treatment – 150 ml at the same time interval as that of high treatments.

Table 2.1: Concentrations (mmol l^{-1}) of macro- and micronutrients used in preparation of Hoagland's solution.

	Salt Solution (g/l)	Final vol added to 1 l solution (ml)	Concentration (mol l^{-1})
Macronutrients:			
KH_2PO_4	136	1.00	0.001
KNO_3	101	5.00	0.005
$Ca(NO_3)_2$	164	5.00	0.005
$MgSO_4$	120	2.00	0.002
Micronutrients:			
H_3BO_3	2.86	1.00	4.61×10^{-5}
$MnCl_{2.4}H_2O$	1.81	1.00	9.19×10^{-6}
$ZnSO_4.H_2O$	0.22	1.00	7.66×10^{-7}
$CuSO_4.H_2O$	0.08	1.00	4.51×10^{-7}
$H_2MoO_4.H_2O$	0.02	1.00	1.09×10^{-7}
FeEDTA	5.00	1.00	8.99×10^{-5}

Therefore a summary of the *C. monilifera* treatments can be made as shown (Table 2.2). In total, eight treatments were conducted for this experiment using *C. monilifera* plants. Each treatment had five *C. monilifera* plants in separate potting bags, thereby allowing for at least five replicates of each treatment type.

Table 2.2: The eight treatments in this study using *C. monilifera* plants.

Treatment	**Description**	**Abbreviation**
1	Sun, high water and high nutrient	SUN HWHN
2	Sun, high water and low nutrient	SUN HWLN
3	Sun, low water and high nutrient	SUN LWHN
4	Sun, low water and low nutrient	SUN LWLN
5	Shade, high water and high nutrient	SHADE HWHN
6	Shade, high water and low nutrient	SHADE HWLN
7	Shade, low water and high nutrient	SHADE LWHN
8	Shade, low water and low nutrient	SHADE LWLN

This study consisted of only one growth trial (from 24th November 2005 to 25th January 2007). Various anatomical and physiological measurements were taken in order to understand the effects of varying environmental conditions on *C. monilifera*. Plant characteristics that were measured during the growth trial were photosynthetic characteristics (light and CO_2 response), leaf hydraulic characteristics, whole shoot and root hydraulics. At the end of the growth trial, while completing measurements of whole plant hydraulic characteristics, accumulated leaf area was measured for each treatment. The plants were harvested and above- and below-ground biomass were measured.

2.3. Plant Measurements - Pre-Harvest

2.3.1. Leaf Gas Exchange

All gas exchange measurements were carried out using the LI-Cor 6400 (Li-Cor, Lincoln, Nebraska, USA) portable infrared gas analyser (IRGA), as a open-flow system. Variability in gas-exchange measurements were minimized by ensuring that *C. monilifera* plants were fully watered and keeping all environmental conditions as standardized as possible. No plant was sampled more than once on a particular day and no leaf was sampled more than once. One leaf per potting bag was measured, so that there were 5 replicates for each treatment. Each leaf was positioned in the 0.25 dm^3 cuvette and the 3rd uppermost leaf (usually the youngest fully expanded leaf) was measured.

For light response measurements, leaf chamber conditions were kept at the following levels: CO_2 concentration 350 µmol mol^{-1} (ambient) and block temperature at 25 °C. Plants were slowly brought to light saturation point at ambient CO_2, over 15-20 minutes using the red/blue LED light source. The light saturation point that was determined for *C. monilifera* was approximately 1500 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD). At this point of the light response measurement, A (assimilation rate) and g_s (stomatal conductance) were noted. The light intensity of the leaf chamber was then increased to 2000 µmol m^{-2} s^{-1}, and A and g_s were measured after 3 minutes response time. Measurements were made after light intensity was decreased by 200 µmol m^{-2} s^{-1} intervals until 400 µmol m^{-2} s^{-1} PPFD. Below 400 µmol m^{-2} s^{-1}, light intensity was decreased in 100 µmol m^{-2} s^{-1} intervals and the last measured point was at 50 µmol m^{-2} s^{-1} PPFD. Net CO_2 assimilation rates (A) with their corresponding light intensities were used to construct a light response curve. If the A and g_s measured at the initial 1500 µmol m^{-2} s^{-1} PPFD were significantly different from the measured A and g_s in the light response curve at 1500 m^{-2} s^{-1} PPFD, the curve was discarded and a new leaf was sampled at a later stage.

Leaf gas exchange can also be measured in terms of an $A:C_i$ curve, where changes assimilation rate in response to an increase in internal CO_2 concentration are measured. For $A:C_i$ measurements, the following leaf chamber variables were controlled: PPFD at light saturation point (1500 µmol m^{-2} s^{-1}) and block temperature at 25 °C. Leaves were exposed to this saturating PPFD and at a CO_2 concentration of 400 µmol mol^{-1} until the photosynthetic rate stabilised, which took between 10 and 15 minutes. A and g_s were recorded after C_a (ambient carbon dioxide concentration) was reduced step-wise by 100 µmol mol^{-1} to 50 µmol mol^{-1}. C_a was then increased to 400 µmol mol^{-1} and A and g_s were remeasured. Thereafter C_a was increased in steps of 200 µmol mol^{-1} to 1000 µmol mol^{-1}. If the value of A at the initial and the second measurement at C_a of 400 µmol mol^{-1} differed widely, the $A:C_i$ curve was discarded and a new leaf was sampled later. The assimilation rate was recorded at each C_a interval and was used to construct an $A:C_i$ curve.

The photosynthetic rates acquired from CO_2 and light response measurements were fitted to an exponential saturation curve. This was done by non-linear regression of the data to the equation:

a) $y = a (1- \exp(b-c*PPFD))$ for light response and

b) $y = a (1-\exp(b-c*C_i))$ for CO_2 response.

The values of the parameters a, b and c were obtained for each individual curve. These parameters were then used to calculate photosynthetic variables which were

For light response curves:

a = A_{max}

b/c = light compensation point

$a*c*e^b$ = initial slope (quantum efficiency on an incident radiation basis)

$a (1-e^b)$ = dark respiration rate

For A:Ci curves:

a = J_{max};

b/c = CO_2 compensation point;

$a*c*e^b$ = initial slope of the curve (the carboxylation coefficient – measure of rubisco activity) and

$a(1-e^b)$ = photorespiration rate.

2.3.2. Leaf Hydraulic Characteristics

Leaf hydraulic parameters were measured using the high pressure flow meter (HPFM) (supplied by Mel Tyree) as described by Tyree (1993). For the purpose of this study, looking specifically at leaf hydraulic properties, leaf hydraulic resistance normalized by leaf area (MPa s m^2 kg^{-1}) was used as the unit of measure. This was done, instead of expressing data in terms of hydraulic conductance, as hydraulic resistances are additive in series. Consequently, measurements on leaves could be partitioned into resistances of particular components.

Chrysanthemoides monilifera leaves have extremely small petioles and can be regarded as apetiolar. This poses a measurement problem when needing to connect the petiole of the leaf to the HPFM compression fitting. This was overcome by cutting the leaf-bearing stem slightly below and above the point of leaf insertion and then using cyanoacrylate glue ("super-glue") to seal the twig above the petiole (Fig.2.2). The cut end below the petiole was attached via a compression fitting to the HPFM and pressurized (0.3 MPa) deionized, filtered water (with 0.1 M HCl) was forced through the leaf, and finally out of the stomata. The pressure transducers of the HPFM allow for the measurement of the pressure drop across the leaf, which is then divided by the flow rate, and normalized by leaf area giving R_{leaf} (MPa kg^{-1} s m^2). A leaf was considered fully hydrated when water droplets were observed on the underside of the leaf surface, and R_{leaf} measurements were made when R_{leaf} values had been stable for between 2 and 5 minutes.

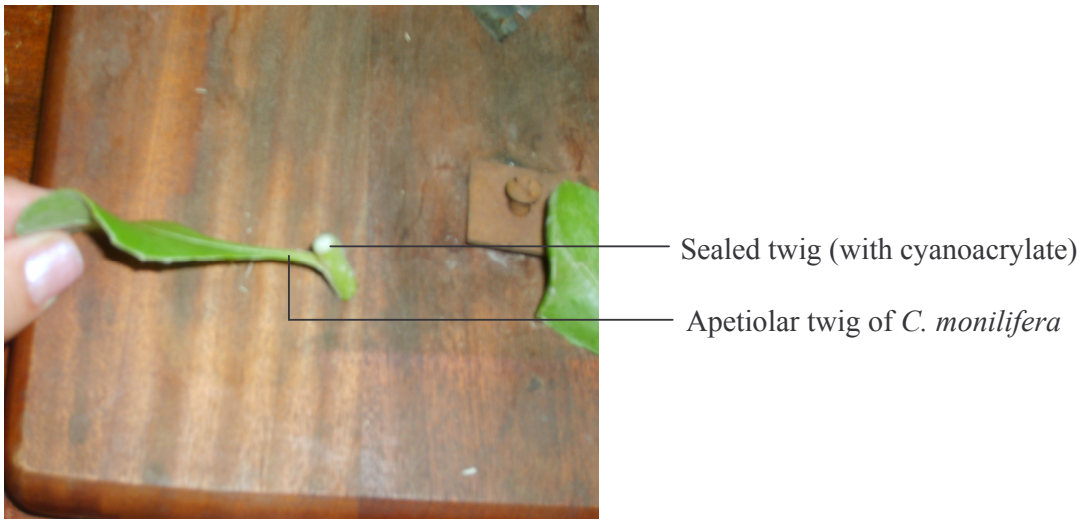

Figure 2.2 : *C. monilifera* leaf cut below and above the petiole, where the end of the twig above the petiole is sealed off with cyanoacrylate.

The first leaf hydraulic measurement made after measuring R_{leaf}, involved severing the connection of the lamina to the petiole. This gave the portion of resistance allocated to the petiole ($R_{petiole}$). R_{lamina} (MPa s m^2 kg^{-1}) was then calculated by subtracting the petiolar resistance from R_{leaf}. This was expressed by the following equation: $R_{lamina} = R_{leaf} - R_{petiole}$.

The remaining partitioning of leaf hydraulic resistances were performed using one of two different vein-cutting techniques as described in Sack *et al*, 2004. In order to determine the resistance of the extravascular tissue, the resistance downstream of the minor veins had to be removed. The minor veins were therefore cut (1.5 – 2 mm) at random locations over the entire lamina with a scalpel (Fig. 2.3). Extreme care was taken not to cut any major veins and between 120-150 cuts were applied to each leaf (until the resistance did not decline any further). The resistance measured after cutting minor veins was regarded as the $R_{venation}$ (the resistance of the venation of the leaf). The resistance of the leaf venation was therefore: $R_{venation} = R_{leaf} - R_{extravascular}$.

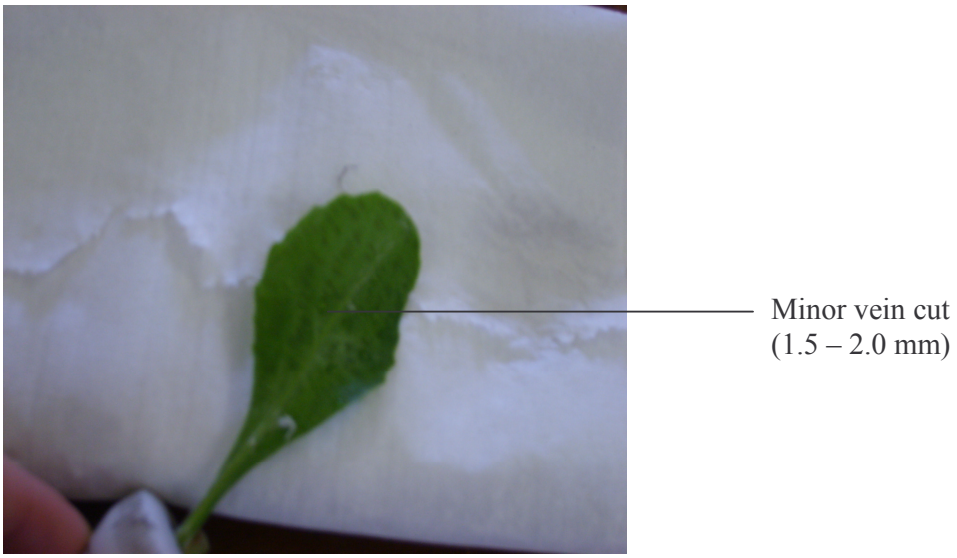

Figure 2.3 : The cutting treatment on the under-surface of the leaf to the minor veins of *C. monilifera*.

For the final set of cutting treatments, leaves were subjected to having all the visible tertiary veins on the lamina surface cut, which were between 15 – 20 tertiary veins. Once the tertiary veins were cut, the remaining hydraulic resistance was that of major and minor veins of the leaf ($R_{major\ veins}$ and $R_{minor\ veins}$ respectively). R_{major} veins was calculated as the difference between the R_{leaf} and the resistance left over after severing the tertiary veins *i.e.* $R_{major\ veins} = R_{leaf} - R_{tertiary\ cut\ veins}$.

The resistance of the minor veins had to be calculated as the percentage proportional difference between $R_{venation}$ and $R_{major\ veins}$ i.e. $R_{minor\ veins}$ (%) = $R_{venation}$ (%) - $R_{major\ veins}$ (%).

For each cutting procedure, three leaves of each treatment (1-8) were used. The following hydraulic resistance parameters were subjected to a 3-way Univariate Analysis of Variance (as parameters were expressed as values in MPa s m^2 kg^{-1}): resistance of the intact leaf lamina, resistance of the petiole, resistance of the lamina with minor veins cut, and finally resistance of the lamina with tertiary veins cut.

Leaf resistance components were then partitioned ($R_{petiole}$, $R_{extravascular\ tissue}$, $R_{major\ veins}$ and $R_{minor\ veins}$) and illustrated in the form of pie charts as percentage values. In order for these values to be statistically compared, the percent values were transformed by the $\overline{\text{function}}$ of $\sqrt{}$arcsin, and then subjected to a 3-way Univariate Analysis of Variance (SPSS, version 13.0).

2.3.3. Whole Plant Hydraulic Properties

Whole plant hydraulic studies, which includes the measurement of the hydraulic conductance of shoots, stems and roots, was also measured with the HPFM (Tyree, 1993). For these measurements however, values were reported in terms of hydraulic conductance (kg s^{-1} m^{-2} MPa^{-1}), unlike that of leaf hydraulics, which was expressed in terms of hydraulic resistance. Three plants from each treatment were selected for whole shoot hydraulic measurements, and subsequently harvested for total leaf area and dry weight measurements. Each plant was transported from the greenhouse to the laboratory, in order to keep other variables *e.g*. temperature, humidity and light intensity, as constant as possible when performing measurements on whole shoots and stems.

A *C. monilifera* plant was initially cut about 10 centimeters above the root base and the shoot was placed in a 0.1 M HCl solution to hydrate. The end of the root was attached to the one end of the HPFM compression fitting, after which it was cut with a sharp razor blade to ensure that the surface was even and that water could be perfused through it. Then the compression tubing was inserted into the other end of the fitting, which was attached to the root and filled with HCl water. Pressurized water was then forced through the root in order to investigate the flow rate, using the transient measurement mode of the HPFM. The flow rate was converted to hydraulic conductance by means of a line-fitting linear regression. The hydraulic conductance of each plant from each treatment was statistically analysed by means of a Univariate Analysis of Variance.

The measurement of the roots of *C. monilifera* was problematic, because the roots of the shade treatments were often extremely small and prone to being pulled out of the soil easily. The stems just above the root base of the sun treatments were often highly branched and cutting the bottom of the stem 10 centimeters above the roots was not always possible. For the Sun LWLN treatment it was not possible to measure any root hydraulic conductance because the base of the stems were so highly branched that it was not possible to get an accurate measurement from the 3 replicates.

The hydrated *C. monilifera* shoot was measured by the quasi-steady state method of the HPFM. The compression fitting was attached to the cleanly cut end of the shoot and water was then forced through the tubing under pressure. The hydraulic conductance of the shoot was recorded once the shoot had filled up, seen as water droplets forming on the under-surface of the leaves. The conductance had to have been stable for a minimum of 2 minutes before a reading was taken. Following this, the leaves of the plant were removed from each branch until only the side branches and main stem were left. Once the hydraulic conductance had stabilised again and remained at the same value for more than 2 minutes, the value was recorded and considered the hydraulic conductance of the stem.

The total leaf hydraulic conductance could only be calculated by using the resistances of the components of the whole shoot. This follows the same principle of that of the leaf hydraulic studies, where resistors are additive in series, and thus can be applied to the resistances of the whole shoot. Therefore the total leaf hydraulic resistance is the difference between the shoot resistance and the hydraulic resistance of the stem i.e. $R_{total\ leaf} = R_{shoot} - R_{stem}$. $R_{total\ leaf}$ is then converted back to hydraulic conductance ($R_{leaf\ total} = 1/_{Kleaf\ total}$) and all hydraulic conductance parameters were normalized by their corresponding leaf area. The values for each replicate for each treatment were compared among treatments for significance.

2.4. Plant Measurements – Post-Harvest

Immediately after the hydraulic conductance measurements of the whole shoot were completed, the *C. monilifera* plants were harvested and the leaves, stems and roots for each plant were collected separately. The accumulated leaf area of the leaves which were removed from the shoot during the measurement of K_{stem}, were measured using the CI - 202 leaf area meter (CID Inc, Camas, USA).

Leaves, stems and roots from each plant were dried separately in a drying oven for 48 hours at 80ºC. The final dry weights were measured to the milligram level and the biomass ratios for each treatment were examined. The final stem diameter of shoots for each plant were recorded and later compared among treatments.

2.5. Statistical Analysis

All the statistical analyses for this study were considered significant at the $p < 0.05$ level, by using the program SPSS (version 13.0). The photosynthetic and light response data were initially analysed using a non-linear regression to obtain the values of the parameters of the line-fitting equation from each photosynthetic measurement. The derived parameters used to calculate J/A_{max}, CO_2 light compensation point, initial slope and photo/dark respiration were tested for significance among treatments by means of a 3-way ANOVA or Univariate Analysis of Variance. The assumptions of these tests (for both light and CO_2 response measurements) require normality of the residuals of the ANOVA and the residuals to have equal variance. The normality of the residuals were analysed by means of a non-parametric Kruskal-Wallis (K-S) test, and the equality of variance of the residuals was analysed by Levene's test of equality.

If either of these assumptions were violated, the data in question were log transformed and checked again. Subsequent to the ANOVA, a post-hoc LSD test was performed, which allowed each treatment to be analysed with every other treatment.

The measured values of leaf hydraulic resistances (intact leaf lamina, petiole, lamina with minor veins cut, and lamina with tertiary veins cut) were subjected to a 3-way ANOVA with the same procedure and testing of assumptions. The components of the leaf hydraulic resistance reported as percentages ($R_{petiole}$, $R_{extravascular\ tissue}$, $R_{major\ veins}$ and $R_{minor\ veins}$) were first $\overline{\sqrt{\text{arcsin}}}$ transformed and then also subjected to a 3-way ANOVA to compare significant differences between treatments.

The whole shoot hydraulic conductance measurements ($R_{total\ leaf}$, R_{stem} and R_{shoot}), biomass measurements (dry weights of leaves, stems and roots), accumulated leaf area, stem diameter and biomass allocation were all also subjected to a 3-way ANOVA and its related assumptions.

Finally, a Pearson correlation was performed to investigate the relatedness of certain variables measured in this study, the principle ones being A_{max} and K_h. The normality of distribution of the variables considered for the Pearson correlation were tested using a K-S test to ensure the correlation was correct. The values generated from the correlations were summarized and correlation graphs were shown only for those variables that had a significant correlation, whether positive or negative.

Chapter 3

RESULTS

3.1. Leaf Gas Exchange

3.1.1. Light Response

Figures 3.1.1 and 3.1.2 show representative examples of light response curves of *C. monilifera* plants shown in the sun and shade respectively. It is apparent that the sun plants showed higher maximum (light-saturated) photosynthetic rates. This was confirmed by the statistical analysis of the data in Table 3.1. High nutrient treatments showed a higher photosynthetic rate than that of low nutrient–treated plants and it was found that the photosynthetic rate saturated at approximately 1500 μmol PPFD m^{-2} s^{-1}. The A_{max} (maximum photosynthetic rate) values for the sun plants varied substantially more among water and nutrient treatments (22.5 – 27.4 μmol m^{-2} s^{-1}), when compared to shade plants light response (18.9 – 21.8 μmol m^{-2} s^{-1}).

The maximum photosynthetic rate of *C. monilifera* plants grown in the eight treatments outlined ranged from 18.9 to 27.4 μmol CO_2 m^{-2} s^{-1}. A_{max} was found to be significantly higher in sun and high nutrient treatments (p=0.000; p=0.005 respectively) (Table 3.1; Fig. 3.1.3 (A)). *C. monilifera* showed no significant differences for light compensation point and dark respiration rate within and between environmental treatments (Table 3.1; Fig. 3.1.3.(B) and 3.1.3 (C)). Water treatments had no significant effect on *C. monilifera* plants in terms of maximum photosynthetic rate. The initial slope (which is a measure of quantum efficiency) was significantly higher in sun *versus* shade plants (p=0.001). Values ranged from 0.061 to 0.70 μmol μmol^{-1} for sun plants and shade plants exhibited values between 0.033 to 0.055μmol μmol^{-1}. There was a difference between water treatments, but this difference was not found to be significant (p=0.078) (Table 3.1; Fig. 3.1.3 (D)).

Stomatal conductance (g_s), when measured at maximum photosynthetic rate (A_{max}), was relatively higher in low nutrient treatments although not significantly so (p=0.057) (Table 3.1; Fig. 3.1.4). Stomatal conductance of plants grown under shade was higher than that of *C. monilifera* plants grown in full sun, although the variation within treatments was higher in that of the sun plants (p=0.018) (Table 3.1.; Fig. 3.1.4). Water treatments did not significantly affect stomatal conductance. Stomatal conductance was found to extend between 0.167 and 0.384 mol m^{-2} s^{-1} for all eight treatments. The interaction between all three environmental treatments (light, water and nutrients) was significant for stomatal conductance only (p=0.031) (Table 3.1).

The light response of stomatal conductance of *C. monilifera* plants grown in the sun, differed among treatments without any apparent trend (Fig. 3.1.5 (A)). For both sun and shade plants, high water, low nutrient plants had the highest stomatal conductance (0.407 and 0.384 mol m^{-2} s^{-1} respectively). Stomatal conductance (for most but not all treatments) was also seen to decrease at the highest light intensity, supporting the observation that 1500 µmol m^{-2} s^{-1} was the optimum light intensity for *C. monilifera* plants to photosynthesize (Fig.3.1.5 (B)).

Table 3.1 Light Response photosynthetic parameters A_{max}, light compensation point, dark respiration and initial slope for *C. monilifera*, derived from an univariate analysis of variance.

Parameter	Treatment	Mean ± SEM	p-value			
			light	water	nutrient	interaction
A_{max} (µmol m^{-2} s^{-1})	SunHWHN	27.4 ± 5.3[a]				
	SunHWLN	21.2 ± 3.2[b]				
	SunLWHN	24.5 ± 2.3[ab]				
	SunLWLN	22.5 ± 3.6[b]				
	ShadeHWHN	21.8 ± 3.5[b]				
	ShadeHWLN	19.0 ± 2.9[b]				
	ShadeLWHN	20.0 ± 1.9[b]				
	ShadeLWLN	18.9 ± 1.8[b]				
			0.000	**0.389**	**0.005**	**0.323**
Light Comp Point (µmol mol^{-1})	SunHWHN	-71.8 ± 199.7[a]				
	SunHWLN	-0.17 ± 34.6[a]				
	SunLWHN	32.3 ± 74.6[a]				
	SunLWLN	15.3 ± 28.9[a]				
	ShadeHWHN	-20.5 ± 49.1[a]				
	ShadeHWLN	7.2 ± 19.5[a]				
	ShadeLWHN	5.4 ± 25.8[a]				
	ShadeLWLN	-0.92 ± 24.9[a]				
			0.877	**0.175**	**0.449**	**0.295**
Dark Respiration (µmol CO_2 m^{-2} s^{-1})	SunHWHN	-0.65 ± 5.1[a]				
	SunHWLN	-0.09 ± 2.1[a]				
	SunLWHN	-1.36 ± 4.2[a]				
	SunLWLN	-2.04 ± 3.5[a]				
	ShadeHWHN	0.64 ± 1.5[a]				
	ShadeHWLN	-0.38 ± 1.1[a]				
	ShadeLWHN	-0.49 ± 1.4[a]				
	ShadeLWLN	-0.12 ± 1.1[a]				
			0.298	**0.332**	**0.832**	**0.925**
Initial Slope (µmol $µmol^{-1}$)	SunHWHN	0.70 ± 0.06[a]				
	SunHWLN	0.061 ± 0.01[a]				
	SunLWHN	0.066 ± 0.02[a]				
	SunLWLN	0.123 ± 0.04[b]				
	ShadeHWHN	0.033 ± 0.01[ab]				
	ShadeHWLN	0.055 ± 0.01[a]				
	ShadeLWHN	0.054 ± 0.01[a]				
	ShadeLWLN	0.044 ± 0.01[a]				
			0.001	**0.078**	**0.112**	**0.144**

Table 3.1 continued: Stomatal conductance(G_s) of *C. monilifera* measured at maximum photosynthetic rate (A_{max}), derived from an univariate analysis of variance.

Parameter	Treatment	Mean ± SEM	p-value			
			light	**water**	**nutrient**	**interaction**
g_s (mol m^{-2} s^{-1})	SunHWHN	0.181 ± 0.05^{a}				
	SunHWLN	0.407 ± 0.17^{b}				
	SunLWHN	0.263 ± 0.21^{ab}				
	SunLWLN	0.167 ± 30.05^{ac}				
	ShadeHWHN	0.294 ± 0.06^{ab}				
	ShadeHWLN	0.384 ± 0.06^{ab}				
	ShadeLWHN	0.324 ± 0.05^{ab}				
	ShadeLWLN	0.370 ± 0.08^{ab}				
			0.018	**0.318**	**0.057**	**0.031**

P-values indicate light, water and nutrient main effects on *C. monilifera* and their interaction together (light*water*nutrient). Mean values followed by different letters are significantly different from one another.

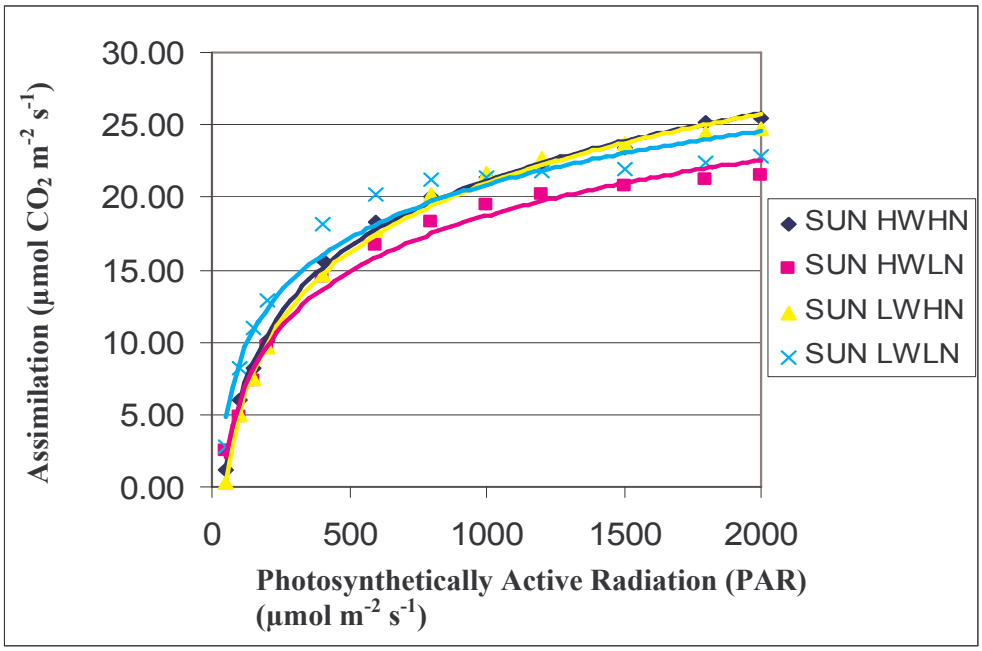

Figure 3.1.1: Light response curve of sun-grown *C. monilifera* plants (n = 5 per treatment). Legend reflects treatment type of sun plants *i.e.* H/LW – high/low water and H/LN – high/low nutrient treatment.

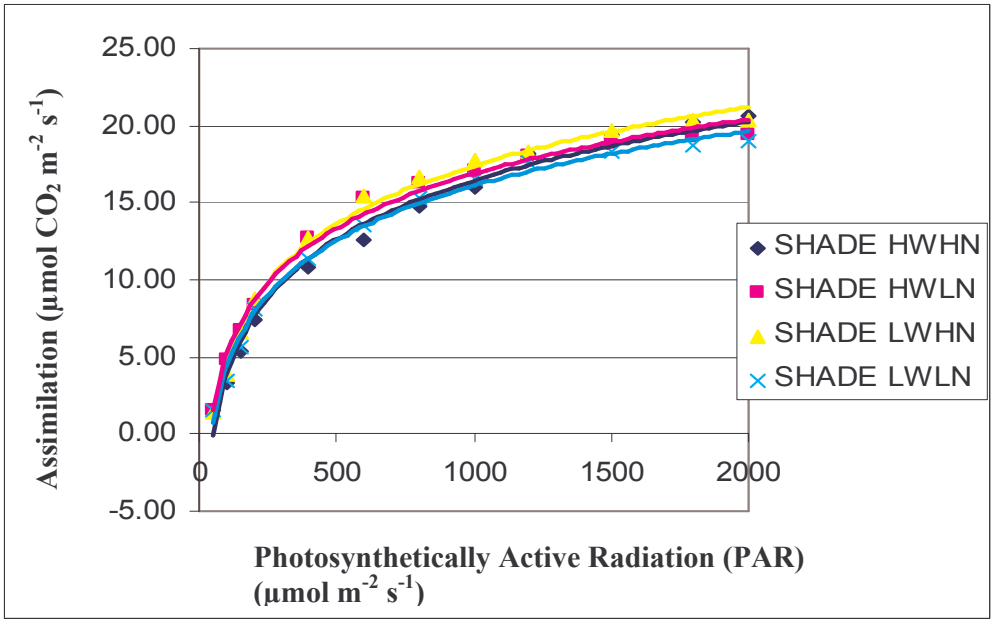

Figure 3.1.2: Light response curve of shade-grown *C. monilifera* plants (n = 5 per treatment). Legend reflects treatment type of shade plants *i.e.* H/LW – high/low water and H/LN – high/low nutrient treatment.

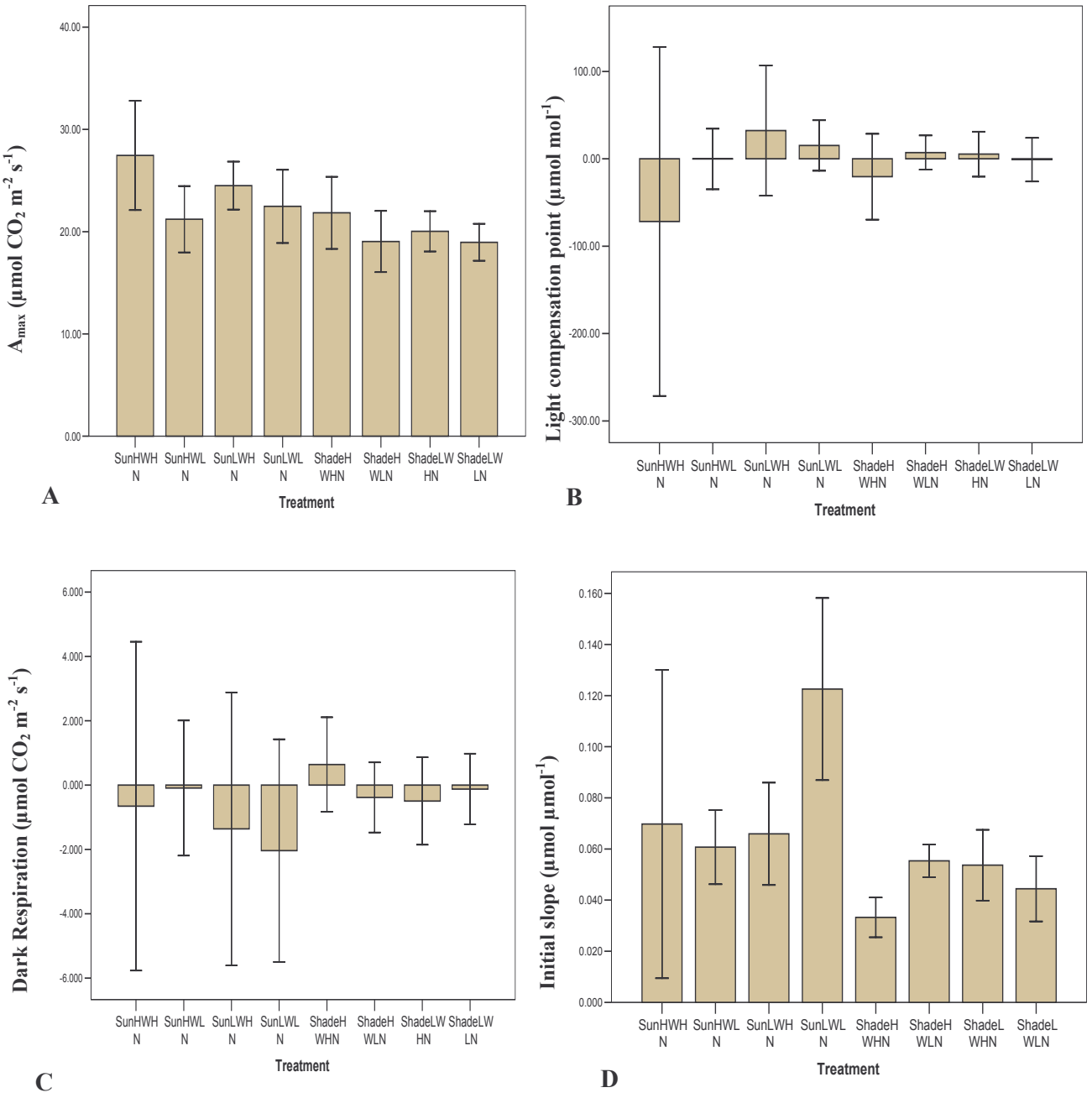

Figure 3.1.3: Light response photosynthetic parameters of *C. monilifera* grown in 8 environmental treatments (n=5 per treatment). (A) A_{max}; (B) Light compensation point (C) Dark Respiration and (D) Initial slope. Bars represent mean value ± standard deviation. Treatment type represents - H/LW – high/low water and H/LN – high/low nutrient treatment.

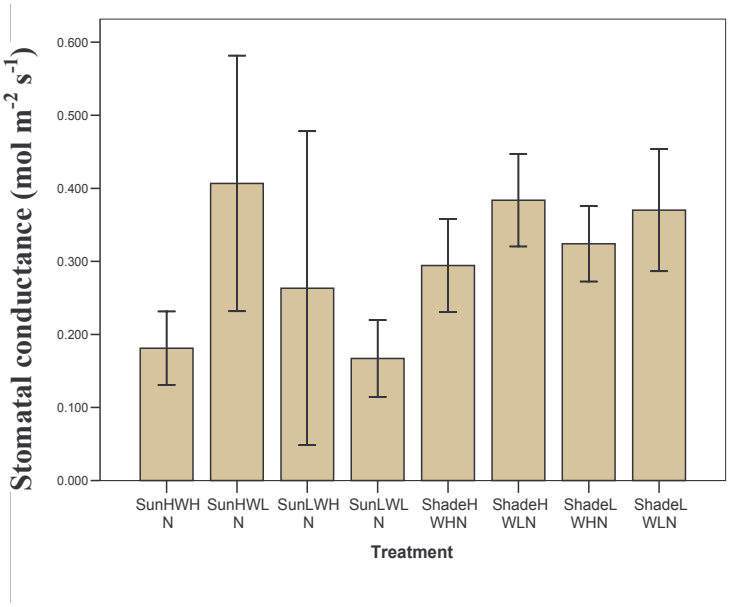

Figure 3.1.4: Stomatal conductance (g_s) of *C. monilifera* at saturating light intensity A_{max} (n=5 per treatment). Bars represent mean value ± standard deviation. Treatment type represents - H/LW – high/low water and H/LN – high/low nutrient treatment.

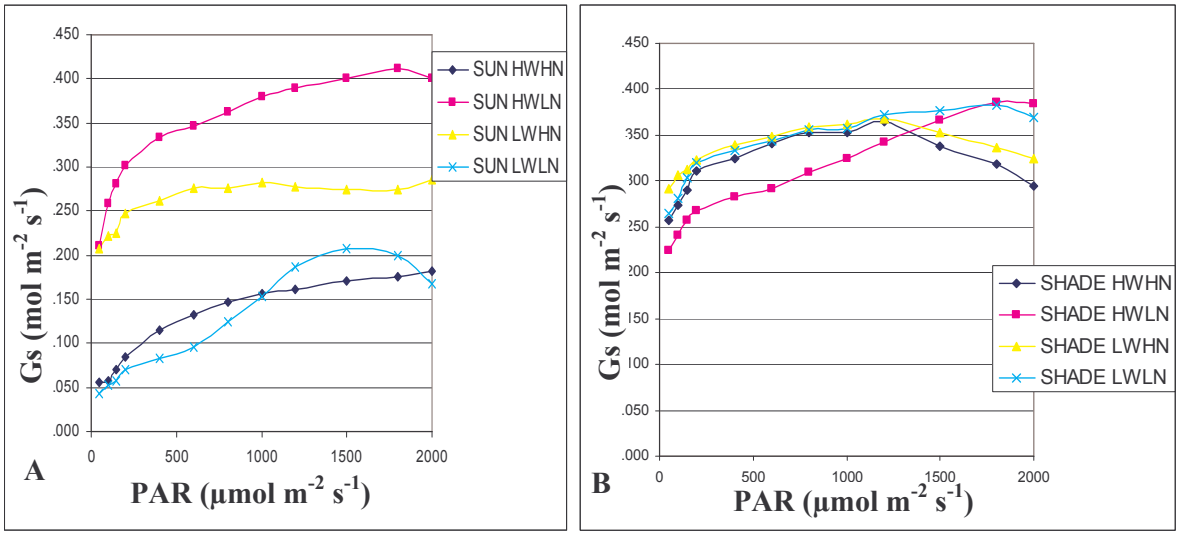

Figure 3.1.5: Stomatal response of sun (A) and shade (B) treated *C. monilifera* plants when measured under varying light intensity (n = 5 per treatment). Legend reflects treatment type *i.e.* H/LW – high/low water and H/LN – high/low nutrient treatment.

3.1.2. CO_2 Response

The $A:C_i$ curves show that for both sun and shade treatments, high water, high nutrient treated *C. monilifera* plants had the highest photosynthetic rate (Fig. 3.1.6 and 3.1.7). Differences between and within treatments are more marked for the $A:C_i$ curves compared to that of the light response curves. The slope of the $A:C_i$ curves are also shown to be steeper, whereas the light response curves had a considerably more gradual slope. All eight $A:C_i$ curves measured for *C. monilifera* did not fully saturate at 1000 µmol mol^{-1} CO_2, even though the light intensity was at an optimum level.

The results, in terms of significance of environmental treatments, for the CO_2 response of *C. monilifera* were extremely similar to that of the light response results. Maximum assimilation rate at saturating CO_2 (J_{max}) differed in that it ranged from 46.7 – 66.8 µmol CO_2 m^{-2} s^{-1} (Table 3.2). J_{max} was significantly higher in sun and high nutrient treatments (p=0.000; p=0.018 respectively) (Table 3.2; Fig. 3.1.8 (A)). The CO_2 compensation point and photorespiration rate (like that of light response) were not significantly affected for *C. monilifera* (Table 3.2; Fig. 3.1.8(B) and 3.1.8 (C)). As with light response, water treatments did not show an effect on *C. monilifera* plants with regard to maximum photosynthetic rate. The initial slope (carboxylation efficiency) was again the only measured parameter significantly affected by water treatments (p=0.005) (Table 3.2; Fig. 3.1.8 (D)). The carboxylation efficiency of *C. monilifera* was generally higher in high water treatments than in low water treatments (Fig. 3.1.8 (D)).

The stomatal conductance (g_s) of *C. monilifera* at J_{max}, was in general higher in high water and high nutrient but not significantly so (Table 3.2). It was also observed that the variation within treatments of g_s measurement was relatively high (Figure 3.1.9). The g_s values were similar to that observed for g_s of light response, and they were between 0.086 – 0.363 mol m^{-2} s^{-1}. No treatment of gs for CO_2 response was considered significantly different from another, unlike that of g_s in response to light.

The stomatal conductance of shade-treated plants (maximum was 0.363 mol m^{-2} s^{-1}) was generally higher than that of sun-treated (maximum 0.291 mol m^{-2} s^{-1}) *C. monilifera* plants when observing the stomatal conductance curves (Fig. 3.1.10 (A) and 3.1.10 (B)). The high water treatments of shade plants responded more than that of the low water treatments when exposed to CO_2. Stomatal conductance exhibited a great decrease between 50 and 400 μmol mol^{-1} CO_2, and was saturated between 400-600 μmol mol^{-1} CO_2. Down-regulation was exhibited to a small extent at very high C_i (800 μmol mol^{-1} CO_2), which has been found to occur in C_3 plants.

Table 3.2 CO_2 response photosynthetic parameters J_{max}, CO_2 compensation point, photorespiration and initial slope for *C. monilifera*, derived from an univariate analysis of variance.

Parameter	Treatment	Mean ± SEM	p-value			
			light	water	nutrient	interaction
J_{max} (µmol m^{-2} s^{-1})	SunHWHN	66.8 ± 0.8[a]				
	SunHWLN	58.8 ± 13.6[a]				
	SunLWHN	66.3 ± 10.7[a]				
	SunLWLN	50.5 ± 7.3[ab]				
	ShadeHWHN	48.2 ± 12.5[b]				
	ShadeHWLN	46.7 ± 6.0[b]				
	ShadeLWHN	52.6 ± 3.5[b]				
	ShadeLWLN	40.6 ± 6.0[b]				
			0.000	**0.358**	**0.018**	**0.250**
CO_2 Comp Point (µmol mol^{-1})	SunHWHN	134.6 ± 67.1[a]				
	SunHWLN	80.3 ± 48.8[a]				
	SunLWHN	105.3 ± 31.3[a]				
	SunLWLN	91.7 ± 6.6[a]				
	ShadeHWHN	78.0 ± 12.6[a]				
	ShadeHWLN	86.2 ± 11.5[a]				
	ShadeLWHN	77.3 ± 0.2[a]				
	ShadeLWLN	96.2 ± 8.9[a]				
			0.267	**0.575**	**0.517**	**0.289**
Photo Respiration (µmol m^{-2} s^{-1})	SunHWHN	-9.87 ± 4.9[a]				
	SunHWLN	-8.85 ± 4.6[a]				
	SunLWHN	-7.82 ± 4.6[a]				
	SunLWLN	-6.96 ± 1.6[a]				
	ShadeHWHN	-9.32 ± 1.2[a]				
	ShadeHWLN	-9.50 ± 1.6[a]				
	ShadeLWHN	-7.11 ± 1.2[a]				
	ShadeLWLN	-9.09 ± 1.6[a]				
			0.432	**0.234**	**0.917**	**0.697**
Carbo-xylation efficiency (mol m^{-2} s^{-1})	SunHWHN	0.087 ± 0.07[a]				
	SunHWLN	0.108 ± 0.03[a]				
	SunLWHN	0.066 ± 0.02[b]				
	SunLWLN	0.072 ± 0.02[b]				
	ShadeHWHN	0.110 ± 0.01[a]				
	ShadeHWLN	0.100 ± 0.01[a]				
	ShadeLWHN	0.086 ± 0.01[b]				
	ShadeLWLN	0.085 ± 0.01[b]				
			0.139	**0.005**	**0.666**	**0.990**

Table 3.2 continued: Stomatal response of *C. monilifera* at maximum photosynthetic rate (Ci = 1000 μmol mol^{-1}) in response to CO_2, derived from an univariate analysis of variance.

Parameter	Treatment	Mean ± SEM	p-value			
			light	**water**	**nutrient**	**interaction**
g_s (mol m^{-2} s^{-1})	SunHWHN	0.086 ± 0.05[a]				
	SunHWLN	0.291 ± 0.29[a]				
	SunLWHN	0.186 ± 0.08[a]				
	SunLWLN	0.147 ± 0.05[a]				
	ShadeHWHN	0.363 ± 0.22[a]				
	ShadeHWLN	0.254 ± 0.08[a]				
	ShadeLWHN	0.275 ± 0.13[a]				
	ShadeLWLN	0.181 ± 0.09[a]				
			0.212	**0.219**	**0.688**	**0.549**

P-values indicate light, water and nutrient main effects on *C. monilifera* and their interaction together (light*water*nutrient). Mean values followed by different letters are significantly different from one another.

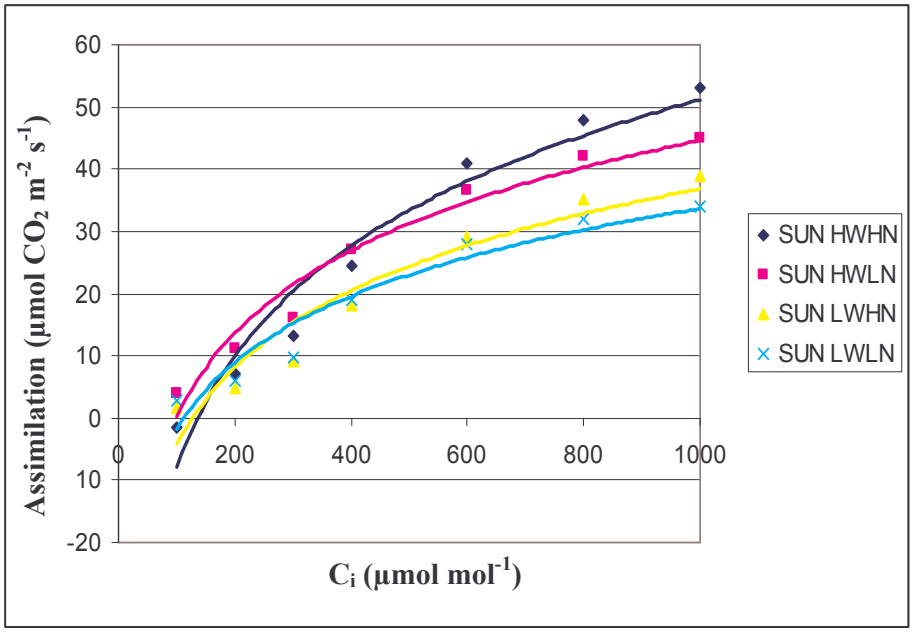

Figure 3.1.6: A:C_i curve of sun-treated *C. monilifera* plants (n= 5 per treatment). Legend reflects treatment type i.e. H/LW – high/low water and H/LN – high/low nutrient treatment.

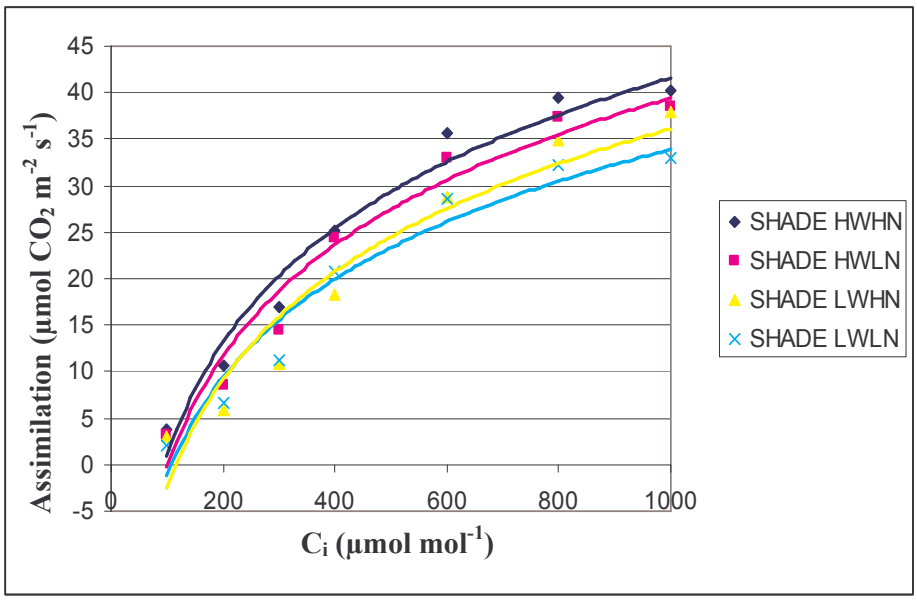

Figure 3.1.7: A:C_i curve of shade-treated *C. monilifera* plants (n= 5 per treatment). Legend reflects treatment type i.e. H/LW – high/low water and H/LN – high/low nutrient treatment.

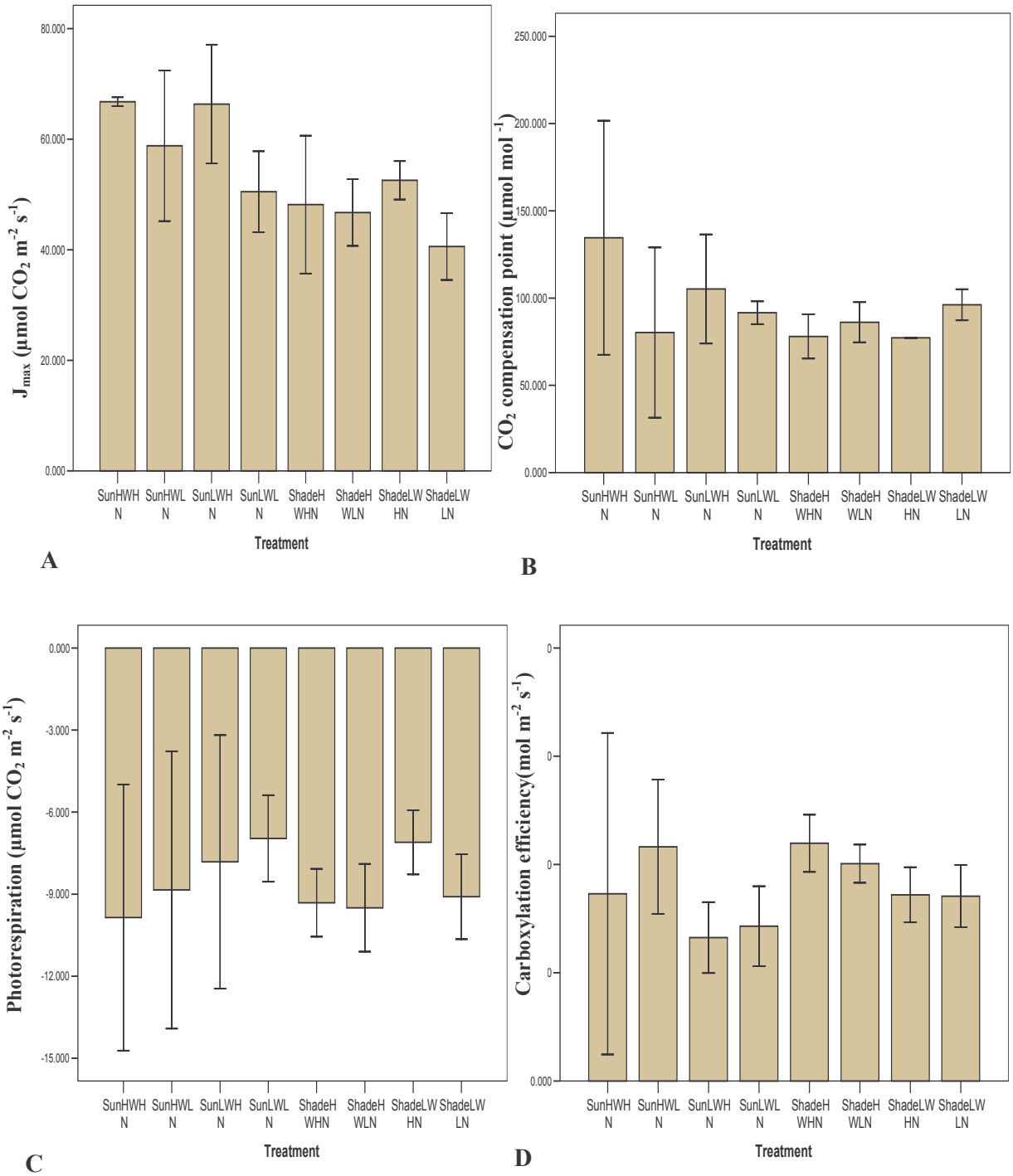

Figure 3.1.8: CO_2 response photosynthetic parameters of *C. monilifera* grown in 8 environmental treatments (n=5 per treatment). (A) J_{max}; (B) CO_2 compensation point (C) Photorespiration and (D) Carboxylation efficiency. Bars represent mean value ± standard deviation. Treatment type represents - H/LW – high/low water and H/LN – high/low nutrient treatment.

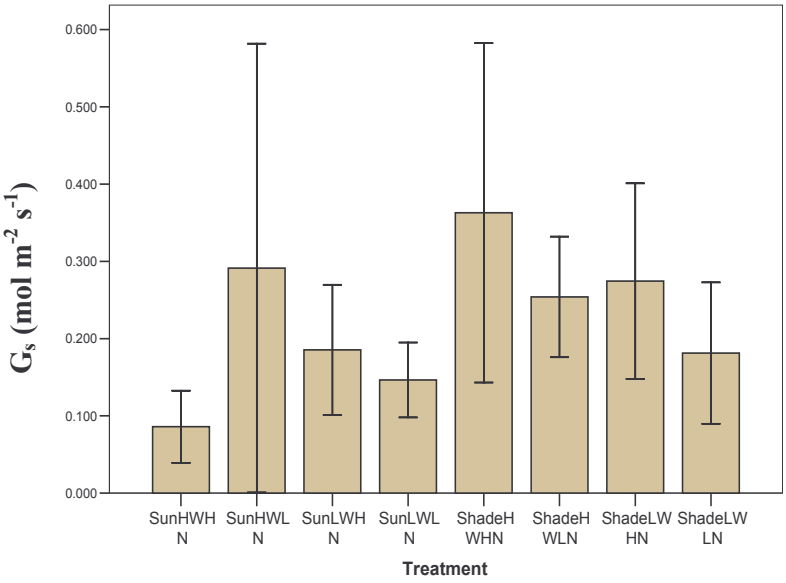

Figure 3.1.9: Stomatal conductance of *C. monilifera* at maximum photosynthetic rate (A_{max}) (n = 5 per treatment). Bars represent mean value ± standard deviation. Treatment type represents - H/LW – high/low water and H/LN – high/low nutrient treatment.

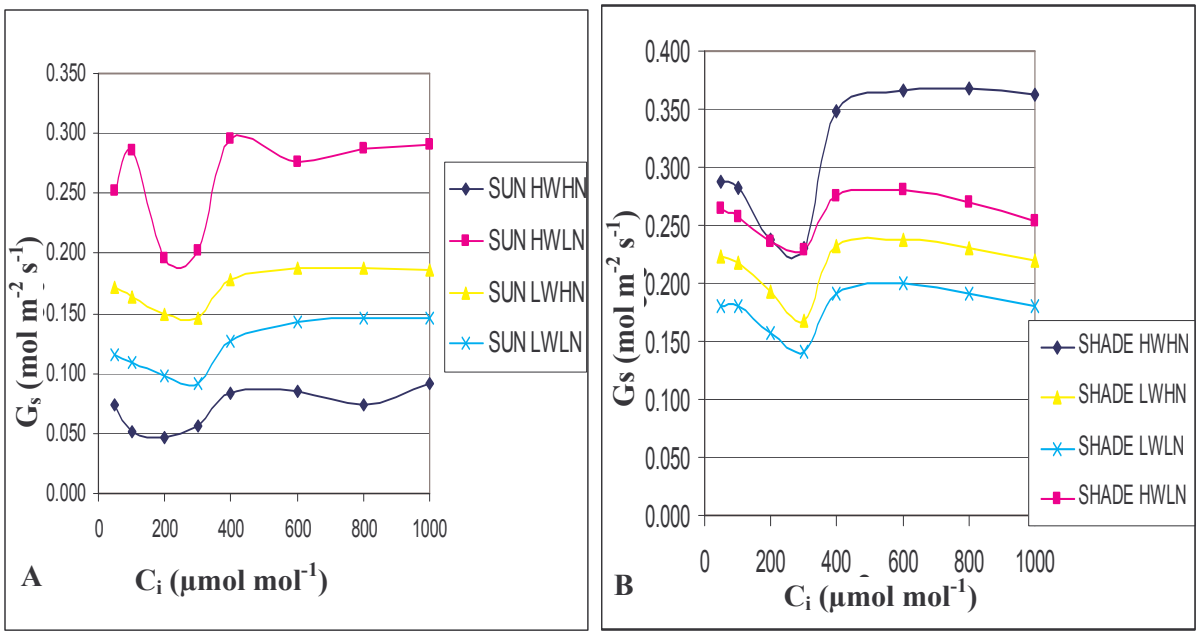

Figure 3.1.10: Stomatal response of sun (A) and shade (B) treated *C. monilifera* plants when measured under different CO_2 concentrations (n = 5 per treatment). Legend reflects treatment type i.e. H/LW – high/low water and H/LN – high/low nutrient treatment.

3.2 Hydraulic Characteristics

3.2.1 Whole Plant Hydraulic Characteristics

All the hydraulic values reported were normalized by leaf area (m^2) and were therefore denoted K_{lshoot}, K_{lstem} *etc*. *C. monilifera* was not significantly affected by any treatments for any measured general hydraulics conductance parameter (Table 3.3). K_{lshoot} and K_{lstem} showed a large amount of overlap between treatments and the sun treatments were seen to be only slightly (but not significantly) higher than that of shade treatments *e.g*. sun values ranged from 1.27 to 8.53 x 10^{-4} kg s^{-1} m^{-2} MPa^{-1}; whereas shade values were 1.30 – 4.19 x 10^{-4} kg s^{-1} m^{-2} MPa^{-1} (Table 3.3; Fig. 3.2.1 (A) and 3.2.1 (B)). $K_{lleaves}$ was also relatively higher in sun treatments of *C. monilifera* but again there was significant overlap between treatments that caused the difference to be non-significant (p=0.114) (Fig. 3.2.1 (C)). K_{lroot} showed the opposite effect to that of the other three hydraulic parameters, being higher in shade treatments, rather than in sun treatments, although the difference was not significant (p=0.089) (Table 3.3; Fig. 3.2.1 (D)). The mean K_{lroot} of Sun LWLN treatment was not displayed because the roots were immeasurable for various anatomical reasons (Table 3.3). It is important to note that root size has a considerable effect on root hydraulics. Larger, more dense roots will have a larger K_{lroot} due to size alone.

Values were also expressed as resistance (R = 1/K), as resistances are additive in series. R_{lshoot} (like K_{lshoot}) was found to show no significant differences among environmental treatments (Table 3.3). However, the effect of watering treatments approached significance (p=0.066) (Table 3.3). Figure 3.2.2 (A) shows that plants subject to high water treatments had a higher resistance to water flow in the shoots than that of plants grown in the low water treatments.

For the parameter shoot resistance it was found that water treatments had considerably more effect than that of light treatments. R_{lstem} did not show any significant differences among treatments (Table 3.3; Fig. 3.2.2 (B)). $R_{lleaves}$ was significantly affected by light, plants in the shade treatments having higher total leaf resistance than that of sun *C. monilifera* plants (p=0.011) (Table 3.3; Fig. 3.2.2 (C)). Lastly, the resistance of the roots (R_{root}) was higher in sun treated plants relative to shade plants (p=0.000) (Table 3.3; Fig. 3.2.2 (D)).

Table 3.3 Hydraulic conductance of various plant organs of *C. monilifera* grown under 8 different treatments (n=3 per treatment). All hydraulic conductance parameters were reported in kg s^{-1} m^{-2} MPa^{-1} x 10^{-4}.

Parameter	Treatment	Mean ± SEM	p-value			
			light	water	nutrient	interaction
K_{lshoot} (whole plant)	SunHWHN	1.27 ± 0.8[a]				
	SunHWLN	8.53 ± 12.5[a]				
	SunLWHN	4.66 ± 4.6[a]				
	SunLWLN	3.44 ± 1.6[a]				
	ShadeHWHN	1.34 ± 0.9[a]				
	ShadeHWLN	1.30 ± 0.9[a]				
	ShadeLWHN	4.19 ± 3.6[a]				
	ShadeLWLN	2.24 ± 1.1[a]				
			0.298	**0.950**	**0.651**	**0.622**
K_{lstem}	SunHWHN	2.74 ± 2.8[a]				
	SunHWLN	12.16 ± 17.6[a]				
	SunLWHN	6.24 ± 4.9[a]				
	SunLWLN	5.9 ± 0.9[a]				
	ShadeHWHN	2.27 ± 1.2[a]				
	ShadeHWLN	3.14 ± 0.9[a]				
	ShadeLWHN	5.22 ± 2.9[a]				
	ShadeLWLN	3.22 ± 0.64[a]				
			0.270	**0.900**	**0.542**	**0.679**
$K_{lleaves}$	SunHWHN	4.07 ± 1.5[a]				
	SunHWLN	28.95 ± 43.3[a]				
	SunLWHN	22.03 ± 26.9[a]				
	SunLWLN	9.58 ± 6.9[a]				
	ShadeHWHN	1.11 ± 0.6[a]				
	ShadeHWLN	1.84 ± 0.7[a]				
	ShadeLWHN	2.15 ± 1.6[a]				
	ShadeLWLN	3.66 ± 1.8[a]				
			0.114	**0.943**	**0.630**	**0.645**
K_{lroot}	SunHWHN	5.20 ± 0.7[a]				
	SunHWLN	4.97 ± 0.1[a]				
	SunLWHN	9.58 ± 6.9[a]				
	SunLWLN	-				
	ShadeHWHN	13.29 ± 0.6[a]				
	ShadeHWLN	58.8 ± 64.6[a]				
	ShadeLWHN	14.21 ± 6.3[a]				
	ShadeLWLN	55.30 ± 45.6[a]				
			0.089	**0.977**	**0.191**	**0.977**

P-values indicate light, water and nutrient main effects on *C. monilifera* and their interaction together (light*water*nutrient). Mean values followed by different letters are deemed significantly different.

Table 3.3 continued: Whole Plant Hydraulics (resistance of plant parts) of *C. monilifera* grown in 8 different treatments (n=3 per treatment). All hydraulic resistance parameters were reported in MPa s m^2 kg^{-1} x 10^3

Parameter	Treatment	Mean ± SEM	p-value			
			light	water	nutrient	interaction
R_{lshoot}	SunHWHN	1.00 ± 0.66[a]				
	SunHWLN	0.93 ± 1.21[a]				
	SunLWHN	0.42 ± 0.41[a]				
	SunLWLN	0.33 ± 0.15[a]				
	ShadeHWHN	1.03 ± 0.77[a]				
	ShadeHWLN	1.01 ± 0.53[a]				
	ShadeLWHN	0.38 ± 0.33[a]				
	ShadeLWLN	0.52 ± 0.23[a]				
			0.884	**0.066**	**0.974**	**0.973**
R_{lstem}	SunHWHN	0.74 ± 0.75[a]				
	SunHWLN	0.63 ± 0.82[a]				
	SunLWHN	0.24 ± 0.19[a]				
	SunLWLN	0.17 ± 0.03[a]				
	ShadeHWHN	0.51 ± 0.27[a]				
	ShadeHWLN	0.34 ± 0.11[a]				
	ShadeLWHN	0.23 ± 0.12[a]				
	ShadeLWLN	0.32 ± 0.06[a]				
			0.529	**0.141**	**0.770**	**0.627**
$R_{lleaves}$	SunHWHN	0.19 ± 0.33[a]				
	SunHWLN	0.30 ± 0.39[a]				
	SunLWHN	0.18 ± 0.22[a]				
	SunLWLN	0.15 ± 0.13[a]				
	ShadeHWHN	0.90 ± 0.04[b]				
	ShadeHWLN	0.63 ± 0.33a[b]				
	ShadeLWHN	0.63 ± 0.46[ba]				
	ShadeLWLN	0.31 ± 0.12[ac]				
			0.011	**0.096**	**0.223**	**0.464**
R_{lroot}	SunHWHN	0.19 ± 0.03[a]				
	SunHWLN	0.20 ± 0.0[a]				
	SunLWHN	0.14 ± 0.10[ab]				
	SunLWLN	-				
	ShadeHWHN	0.08 ± 0.0[b]				
	ShadeHWLN	0.04 ± 0.03[b]				
	ShadeLWHN	0.08 ± 0.03[b]				
	ShadeLWLN	0.03 ± 0.03[b]				
			0.000	**0.343**	**0.509**	**0.796**

P-values indicate light, water and nutrient main effects on *C. monilifera* and their interaction together (light*water*nutrient).

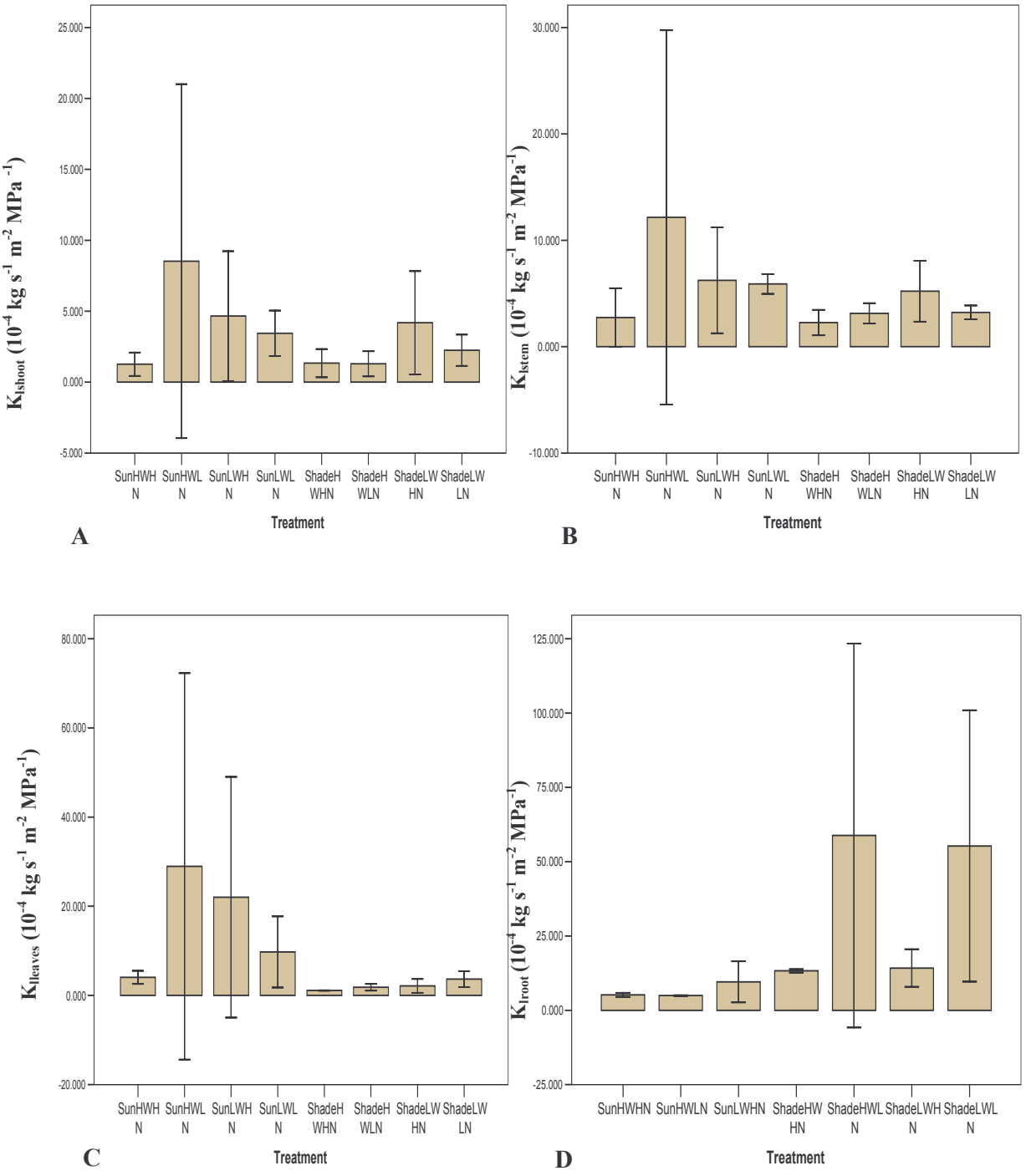

Figure 3.2.1: Whole-shoot hydraulic conductance parameters of *C. monilifera* when grown under 8 environmental treatments (n = 3 per treatment). (A) K_{lshoot} - whole-shoot conductance; (B) K_{lstem} - stem conductance; (C) $K_{lleaves}$ – conductance of the total leaf area and (D) K_{lroot} – root conductance. Bars represent means ± standard deviation. Treatment type represents - H/LW – high/low water and H/LN – high/low nutrient treatment.

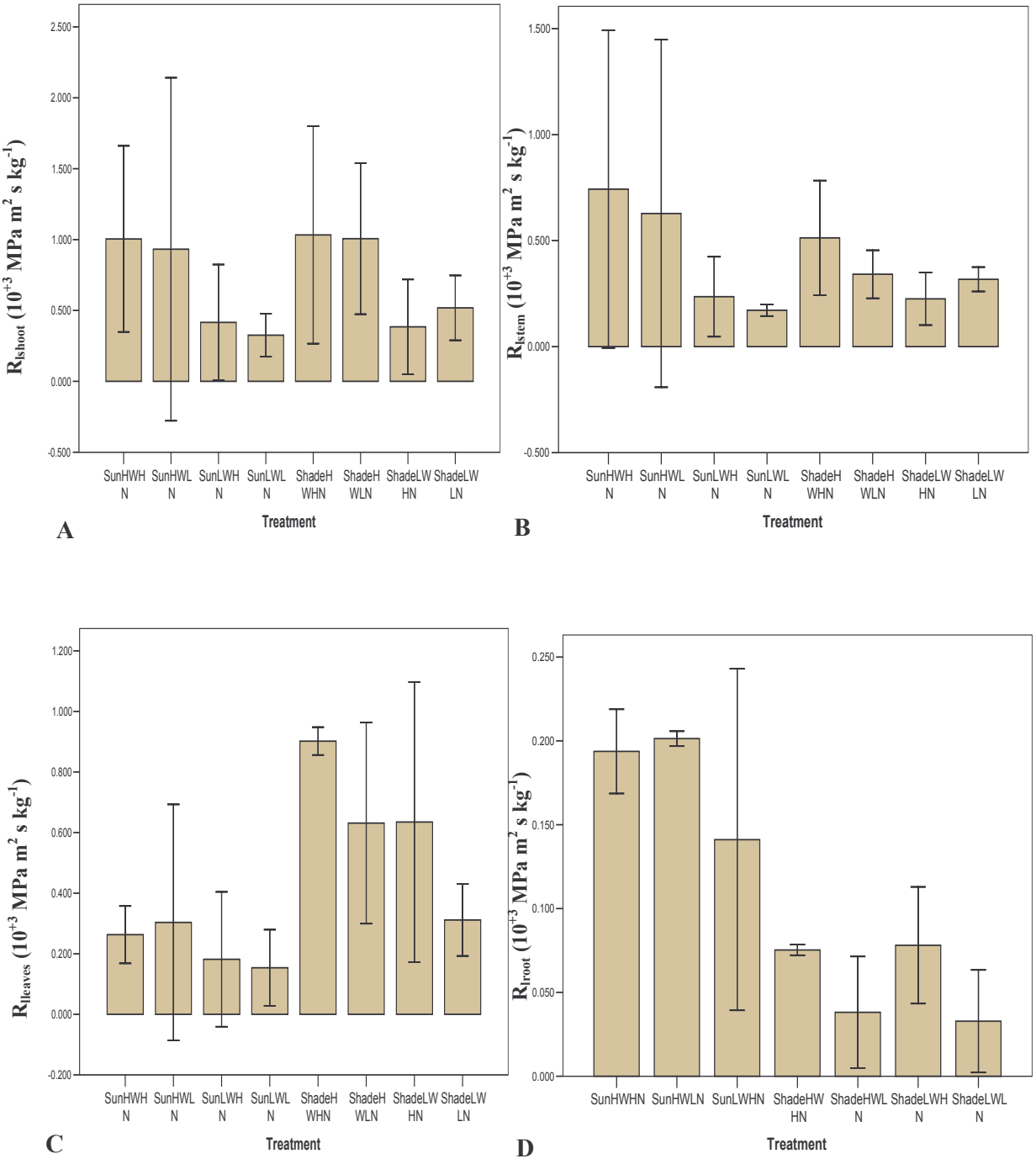

Figure 3.2.2: Whole-shoot hydraulic resistance of *C. monilifera* when grown under 8 environmental treatments (n = 3 per treatment). (A) R_{shoot} – resistance of the whole shoot; (B) R_{stem} – resistance of the stems; (C) R_{leaves} – resistance of the total leaf area and (D) R_{root} – root resistance. Bars represent means ± standard deviation. Treatment type represents - H/LW – high/low water and H/LN – high/low nutrient treatment

3.2.2 Hydraulic Characteristics of Leaves

From the numerous parameters measured for leaf hydraulic characteristics, only two out of seven were found to show significant differences among treatments (Table 3.4). R_{leaf} (total overall resistance of the leaf and inverse of K_{leaf}) ranged from 2.03 – 3.89 x 10^3 kg^{-1} s m^2 MPa (Table 3.4). No significant differences among treatments occurred in R_{leaf}, with relatively high standard deviation within treatments (Table 3.4). High nutrient treatments were relatively higher in terms of R_{leaf} of *C. monilifera* plants but not significantly so (p=0.079) (Fig. 3.2.3 (A)). K_{leaf} (hydraulic conductance of the leaf) did not show significant differences between treatment types (Table 3.4; Fig. 3.2.3 (B)). $R_{petiole}$ of *C. monilifera* was significantly affected by light treatment, where $R_{petiole}$ of shaded plants was higher (p=0.042) than of sun exposed plants (Table 3.4; Fig. 3.2.3 (C)). As seen with R_{root} being higher in shaded plants, the fact that the petioles of shaded plants were longer could have caused the resistance to be higher. Plants under high nutrient treatments also showed higher $R_{petiole}$, although the difference was not significant (p=0.094) (Table 3.4; Fig. 3.2.3 (C)).

R_{lamina} and $R_{tertcut}$ (resistance after the tertiary veins were cut) were not found to be affected by any treatment (Table 3.4; Fig. 3.2.4 (A) and 3.2.4 (D) respectively). R_{mincut} (resistance after the minor veins were cut) tended to be higher in shade plants but the effect was not significant (p=0.061) (Table 3.4; Fig. 3.2.4 (B)). $R_{extravascular}$ (resistance of the extravascular tissue), like $R_{petiole}$, was found to be significantly affected by light treatments (p=0.039) (Table 3.4), with higher values in sun grown plants than those grown in the shade (Fig. 3.2.4 (C)).

Table 3.4 Leaf hydraulic characteristics of *C. monilifera* grown under 8 different treatments (n=3 per treatment) All leaf hydraulic resistance parameters were reported in MPa s m^2 kg^{-1} x 10^3.

Parameter	Treatment	Mean ± SEM	p-value			
			light	water	nutrient	interaction
R_{leaf}	SunHWHN	3.34 ± 1.7[a]				
	SunHWLN	2.04 ± 1.3[a]				
	SunLWHN	2.14 ± 0.4[a]				
	SunLWLN	2.03 ± 0.3[a]				
	ShadeHWHN	2.87 ± 1.5[a]				
	ShadeHWLN	2.17 ± 0.7[a]				
	ShadeLWHN	3.89 ± 1.7[a]				
	ShadeLWLN	2.49 ± 0.3[a]				
			0.332	**0.943**	**0.079**	**0.671**
K_{leaf} (kg s^{-1} m^{-2} MPa^{-1})	SunHWHN	3.54 ± 1.7[a]				
	SunHWLN	7.90 ± 7.4[b]				
	SunLWHN	4.76 ± 0.9[a]				
	SunLWLN	4.98 ± 0.7[a]				
	ShadeHWHN	2.08 ± 1.1[a]				
	ShadeHWLN	3.97 ± 0.2[a]				
	ShadeLWHN	3.01 ± 1.6[ab]				
	ShadeLWLN	4.04 ± 0.4[a]				
			0.085	**0.879**	**0.109**	**0.509**
$R_{petiole}$	SunHWHN	1.16 ± 0.7[a]				
	SunHWLN	0.76 ± 0.5[a]				
	SunLWHN	0.94 ± 0.2[a]				
	SunLWLN	0.92 ± 0.2[a]				
	ShadeHWHN	1.65 ± 0.9[a]				
	ShadeHWLN	1.15 ± 0.4[a]				
	ShadeLWHN	1.99 ± 0.9[b]				
	ShadeLWLN	1.15 ± 0.6[a]				
			0.042	**0.785**	**0.094**	**0.979**
R_{lamina}	SunHWHN	2.18 ± 1.1[a]				
	SunHWLN	1.27 ± 0.9[a]				
	SunLWHN	1.22 ± 0.4[a]				
	SunLWLN	0.77 ± 0.3[b]				
	ShadeHWHN	1.22 ± 0.8[a]				
	ShadeHWLN	1.01 ± 0.4[a]				
	ShadeLWHN	1.90 ± 0.8[a]				
	ShadeLWLN	1.36 ± 0.7[a]				
			0.980	**0.684**	**0.067**	**0.133**

P-values indicate light, water and nutrient main effects on *C. monilifera* and their interaction together (light*water*nutrient). Mean values followed by different letters (a,b etc.) are considered significantly different.

Table 3.4 continued: Leaf hydraulic characteristics of *C. monilifera* grown in 8 different treatments (n=3 per treatment). All leaf hydraulic resistance parameters were reported in kg^{-1} s m^2 MPa x 10^3.

Parameter	Treatment	Mean ± SEM	p-value			
			light	water	nutrient	interaction
R_{mincut}	SunHWHN	1.98 ± 1.0[a]				
	SunHWLN	1.28 ± 0.7[a]				
	SunLWHN	2.06 ± 0.8[a]				
	SunLWLN	1.95 ± 0.8[a]				
	ShadeHWHN	2.83 ± 1.1[a]				
	ShadeHWLN	2.59 ± 1.3[a]				
	ShadeLWHN	2.38 ± 0.6[a]				
	ShadeLWLN	2.56 ± 1.3[a]				
			0.061	**0.866**	**0.583**	**0.746**
$R_{extra-vascular}$	SunHWHN	3.31 ± 4.2[a]				
	SunHWLN	2.22 ± 2.4[a]				
	SunLWHN	0.86 ± 0.3[a]				
	SunLWLN	0.72 ± 0.1[a]				
	ShadeHWHN	0.79 ± 0.4[a]				
	ShadeHWLN	0.57 ± 0.2[a]				
	ShadeLWHN	0.74 ± 0.6[a]				
	ShadeLWLN	0.51 ± 0.3[b]				
			0.039	**0.155**	**0.508**	**0.740**
$R_{tertcut}$	SunHWHN	1.09 ± 0.6[a]				
	SunHWLN	1.47 ± 0.7[a]				
	SunLWHN	2.11 ± 1.1[a]				
	SunLWLN	1.81 ± 0.9[a]				
	ShadeHWHN	1.91 ± 0.3[a]				
	ShadeHWLN	2.89 ± 3.5[a]				
	ShadeLWHN	1.93 ± 0.8[a]				
	ShadeLWLN	1.70 ± 1.3[a]				
			0.414	**0.939**	**0.719**	**0.539**

P-values indicate light, water and nutrient main effects on *C. monilifera* and their interaction together (light*water*nutrient). Mean values followed by different letters are significantly different.

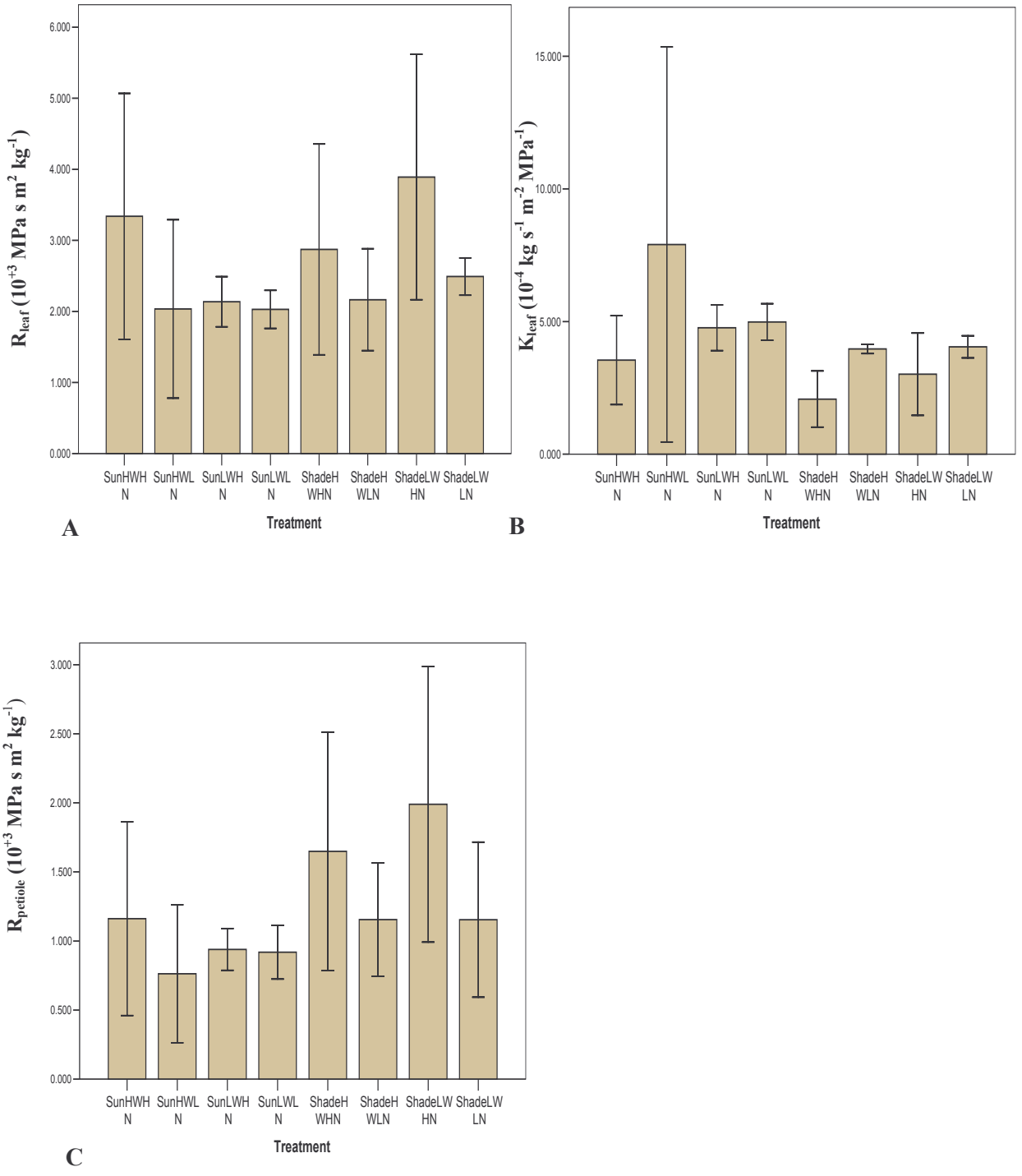

Figure 3.2.3: Single leaf hydraulic resistance/conductance of *C. monilifera* when grown under 8 environmental treatments (n = 3 per treatment). (A) R_{leaf} – resistance of an individual leaf; (B) K_{leaf} – leaf conductance; (C) $R_{petiole}$ – resistance of an individual petiole of R_{leaf}. Bars represent means ± standard deviation. Treatment type represents - H/LW – high/low water and H/LN – high/low nutrient treatment.

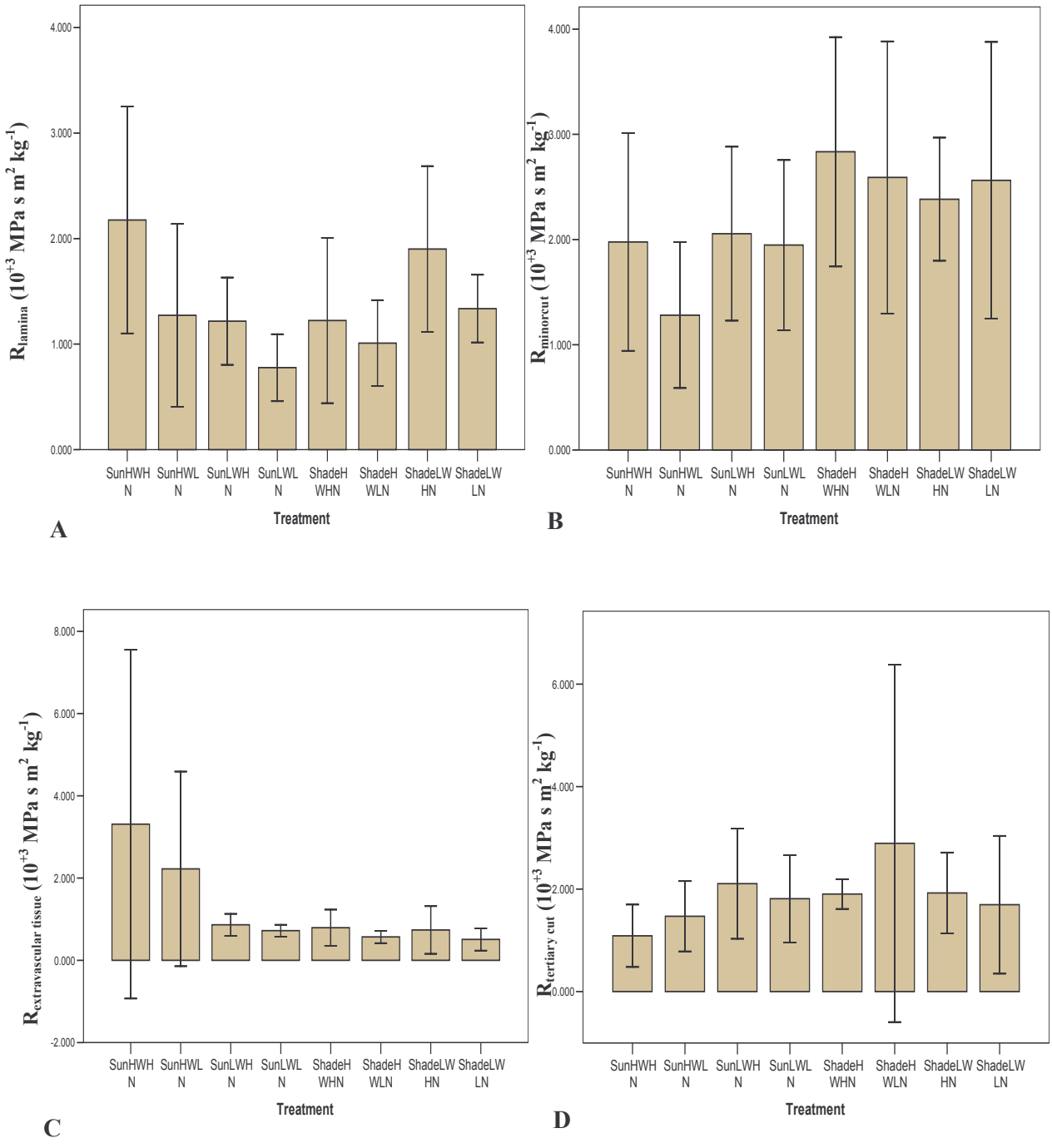

Figure 3.2.4: Single leaf hydraulic resistance of *C. monilifera* when grown under 8 environmental treatments (n = 3 per treatment). (A) R_{lamina} – resistance of the leaf lamina; (B) $R_{minor\ cut}$ – resistance after minor veins severed; (C) $R_{extravascular}$ – resistance of the extravascular tissue and (D) $R_{tertiary\ cut}$ – resistance after the tertiary veins were severed. Bars represent means ± standard deviation. Treatment type represents - H/LW – high/low water and H/LN – high/low nutrient treatment.

3.2.3 Leaf Hydraulics: Location of Resistance

The hydraulic resistance of a leaf can be partitioned amongst four components. These components are firstly the petiole, and secondly the leaf lamina, which is made up of: the extravascular tissue of the lamina, the major veins, and the minor veins of the leaf. The contribution of the petiole was the highest, with values ranging from 34 – 59 % of total leaf resistance (Table 3.5). Second in terms of contribution to total resistance were the major veins, which exhibited values from 25 – 44 %. Resistance contributed by the extravascular tissue was third, and the minor veins showed the lowest contribution. Within the leaf lamina, the major veins contributed the most to overall resistance.

C. monilifera showed significantly higher proportional resistance of the petiole in shade than in sun plants ($p=0.027$) (Table 3.5; Fig.3.2.5). Water and nutrient treatments did not significantly affect the contribution of resistance of the petiole (Table 3.5). Of the three leaf lamina components the contribution of $R_{extravascular}$ was highly affected by light treatments ($p=0.000$) (Table 3.5). Allocation of resistance to the extravascular tissue was double in sun plants as compared with shaded *C. monilifera* plants (Table 3.5; Fig. 3.2.5). Resistance contributed by the major veins was the only leaf component found to show no significant difference between all treatments (Table 3.5). Plants subjected to high water treatments showed higher contribution of major veins to overall resistance but the difference was not deemed statistically significant ($p=0.09$) (Table 3.5; Fig.3.2.5). The contribution of the minor veins was significantly affected by all three treatments (Table 3.5). Sun, high water and high nutrient *C. monilifera* plants all showed significantly increased the resistance as a result of the contribution of the minor veins (Table 3.5). This was surprising as the contribution of $R_{minor\ vein}$ was the lowest of all the leaf components.

Table 3.5 Partitioning of leaf hydraulic resistance of *C. monilifera* among petiole, major veins, minor veins and extravascular tissue as influenced by water, light and nutrient treatments (n=24)

Parameter	Treatment	Mean ± SEM	p-value			
			light	water	nutrient	interaction
$R_{petiole}$ (%)	SunHWHN	34.4 ± 7.4[a]				
	SunHWLN	42.6 ± 16.1[a]				
	SunLWHN	44.8 ± 9.7[ab]				
	SunLWLN	45.5 ± 8.5[ab]				
	ShadeHWHN	59.4 ± 11.6[b]				
	ShadeHWLN	53.6 ± 7.7[a]				
	ShadeLWHN	50.8 ± 5.3[a]				
	ShadeLWLN	45.2 ± 17.1[a]				
			0.027	**0.775**	**0.878**	**0.148**
$R_{extra-vascular}$ (%)	SunHWHN	20.5 ± 2.0[a]				
	SunHWLN	20.8 ± 4.3[a]				
	SunLWHN	16.4 ± 4.2[a]				
	SunLWLN	15.4 ± 4.2[ab]				
	ShadeHWHN	8.8 ± 5.3[b]				
	ShadeHWLN	8.7 ± 1.9[b]				
	ShadeLWHN	10.5 ± 4.8[ab]				
	ShadeLWLN	9.2 ± 3.3[b]				
			0.000	**0.249**	**0.727**	**0.319**
$R_{major veins}$ (%)	SunHWHN	30.8 ± 4.4[a]				
	SunHWLN	28.6 ± 7.1[a]				
	SunLWHN	31.6 ± 11.0[a]				
	SunLWLN	34.5 ± 10.4[a]				
	ShadeHWHN	25.1 ± 10.6[a]				
	ShadeHWLN	35.7 ± 6.6[a]				
	ShadeLWHN	36.7 ± 7.6[a]				
	ShadeLWLN	43.8 ± 14.2[a]				
			0.290	**0.09**	**0.225**	**0.436**
$R_{minor veins}$ (%)	SunHWHN	14.1 ± 4.8[a]				
	SunHWLN	8.0 ± 6.0[ab]				
	SunLWHN	7.2 ± 4.8[b]				
	SunLWLN	4.8 ± 2.4[b]				
	ShadeHWHN	6.6 ± 4.5[b]				
	ShadeHWLN	1.9 ± 1.0[b]				
	ShadeLWHN	2.1 ± 1.2[b]				
	ShadeLWLN	1.9 ± 0.4[b]				
			0.001	**0.018**	**0.032**	**0.206**

P-values indicate light, water and nutrient main effects on *C. monilifera* and their interaction together (light*water*nutrient). Mean values followed by different letters are deemed significantly different.

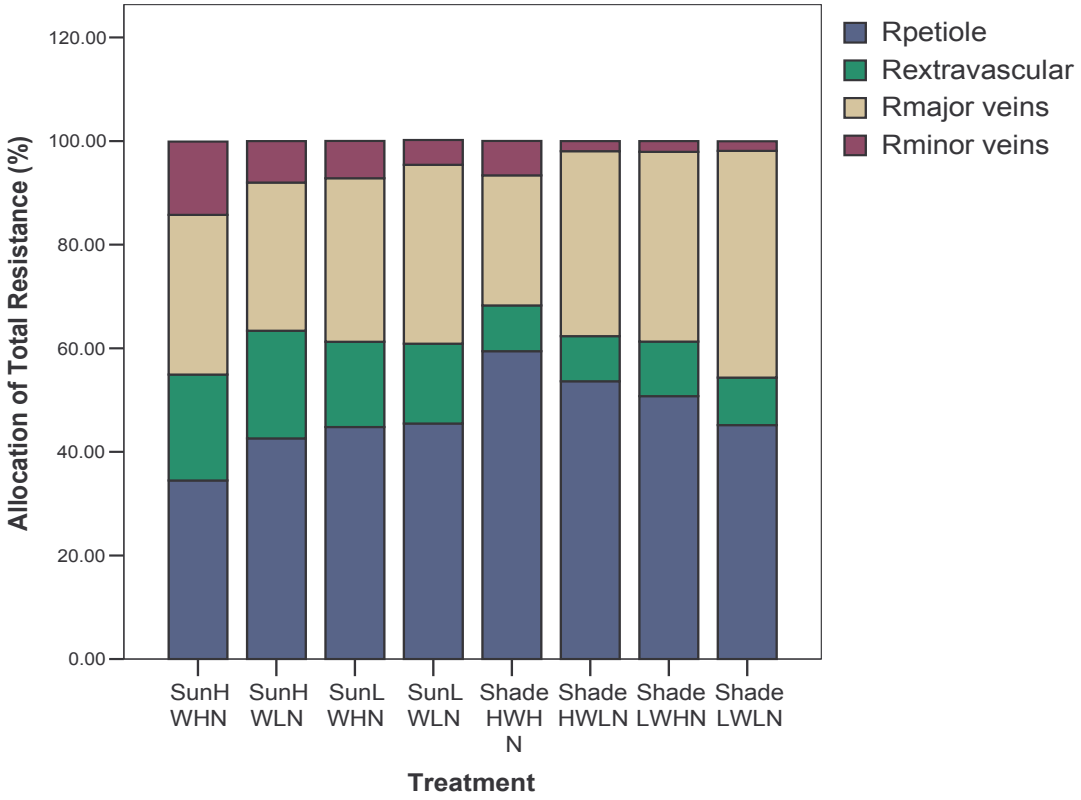

Figure 3.2.5: Allocation of mean leaf resistance to petiole, extravascular tissue, major and minor veins of *C. monilifera* grown under different treatments. Mean values were obtained from individual leaves (n=3 per treatment). Treatment type represents - H/LW – high/low water and H/LN – high/low nutrient treatment.

3.3 Biomass and Growth

The diameter of *C. monilifera* plants differed significantly among light treatments (p=0.000), where the diameter of the sun plants (1.6 – 2.4 cm) was at least double that of shade plants (0.9 – 1.2 cm) (Table 3.6; Fig.3.3.1 (A)). No other treatments had any significant effect on diameter (Table 3.6). The same statistical observations were noted for total leaf area, leaf dry weight and specific leaf area (SLA), which showed that only light significantly affected *C. monilifera* plants (Table 3.6; Fig. 3.3.1 (B), Fig. 3.3.1 (C), Fig. 3.3.2 (A) respectively). Leaf area and leaf dry weight were significantly higher in sun than in shade plants of *C. monilifera* (Fig. 3.3.1 (B); 3.3.1 (C)). Leaf area was between double to 10 times higher in sun *C. monilifera* plants compared with that of shade plants. Specific leaf area was higher in shade plants (Fig. 3.3.2 (A)). Stem dry weight, root dry weight and total biomass were unaffected by water and nutrient treatments, whereas all were significantly affected by light (p=0.000; Table 3.6) (Fig. 3.3.2 (B); 3.3.2 (C) and 3.3.2 (D)). The total biomass of sun plants was between 11 and 15 times higher than that of *C. monilifera* shade plants.

Proportional biomass partitioning to leaf, stem and root biomass (expressed as a percentage of total biomass) is shown in Fig. 3.3.3. Partitioning to stem was the highest of all three plant parts in each treatment (36.4 – 70.0 % of the total biomass) (Table 3.7; Fig. 3.3.3). Partitioning to stems was higher in shade plants by between 25 – 35 %. Allocation to leaf was not found to be significantly affected by any treatment but was marginally higher in sun plants (p=0.09) (Table 3.7). Partitioning of biomass to roots was however, significantly higher in sun treatments (p=0.000) (Table 3.7). Allocation to roots in shade plants of *C. monilifera* was extremely small between 9.1 – 15.5 % of the total biomass (Fig.3.3.3). Partitioning to roots under high nutrient treatments was shown to be slightly lower (1-5 % lower) compared with low nutrient treatments.

Table 3.6 Biomass growth parameters of *C. monilifera* plants when grown under 8 different environmental treatments (n=3 per treatment).

Parameter	Treatment	Mean ± SEM	p-value			
			light	water	nutrient	interaction
Diameter (cm)	SunHWHN	1.9 ± 0.6[a]				
	SunHWLN	1.6 ± 0.3[a]				
	SunLWHN	1.9 ± 0.1[a]				
	SunLWLN	2.4 ± 0.0[ab]				
	ShadeHWHN	1.1 ± 0.1[ab]				
	ShadeHWLN	0.9 ± 0.4[b]				
	ShadeLWHN	1.2 ± 0.3[ab]				
	ShadeLWLN	0.9 ± 0.2[b]				
			0.000	**0.447**	**0.361**	**0.314**
Total Leaf Area (m^2)	SunHWHN	0.49 ± 0.3[a]				
	SunHWLN	0.27 ± 0.1[a]				
	SunLWHN	0.24 ± 0.2[a]				
	SunLWLN	0.81 ± 0.1[b]				
	ShadeHWHN	0.12 ± 0.01[ab]				
	ShadeHWLN	0.06 ± 0.01[ab]				
	ShadeLWHN	0.13 ± 0.06[ab]				
	ShadeLWLN	0.07 ± 0.03[ab]				
			0.001	**0.301**	**0.676**	**0.033**
Leaf Dry Weight (g)	SunHWHN	90.7 ± 81.2[a]				
	SunHWLN	36.8 ± 18.7[b]				
	SunLWHN	26.8 ± 21.9[b]				
	SunLWLN	79.4 ± 4.9[ab]				
	ShadeHWHN	7.5 ± 0.7[b]				
	ShadeHWLN	3.5 ± 0.6[b]				
	ShadeLWHN	9.1 ± 5.4[b]				
	ShadeLWLN	4.9 ± 2.6[b]				
			0.003	**0.903**	**0.742**	**0.214**
Specific Leaf Area (m^2/g)	SunHWHN	0.007 ± 0.003[a]				
	SunHWLN	0.007 ± 0.001[a]				
	SunLWHN	0.009 ± 0.001[a]				
	SunLWLN	0.01 ± 0.001[a]				
	ShadeHWHN	0.05 ± 0.002[b]				
	ShadeHWLN	0.016 ± 0.002[b]				
	ShadeLWHN	0.014 ± 0.001[b]				
	ShadeLWLN	0.015 ± 0.002[b]				
			0.000	**0.678**	**0.377**	**0.196**

P-values indicate light, water and nutrient main effects on *C. monilifera* and their interaction together (light*water*nutrient). Mean values followed by different letters are significantly different.

Table 3.6 continued: Biomass growth parameters of *C. monilifera* plants when grown under 8 different environmental treatments (n=3 per treatment).

Parameter	Treatment	Mean ± SEM	p-value			
			light	water	nutrient	interaction
Stem Dry Weight (g)	SunHWHN	98.6 ± 73.9[a]				
	SunHWLN	38.7 ± 10.2[b]				
	SunLWHN	92.5 ± 0.7[ab]				
	SunLWLN	138.8 ± 43.8[ac]				
	ShadeHWHN	28.4 ± 15.5[b]				
	ShadeHWLN	10.3 ± 0.9[b]				
	ShadeLWHN	31.3 ± 20.7[b]				
	ShadeLWLN	11.8 ± 0.7[b]				
			0.000	**0.081**	**0.251**	**0.073**
Root Dry Weight (g)	SunHWHN	46.3 ± 1.3[a]				
	SunHWLN	33.6 ± 8.1[b]				
	SunLWHN	48.7 ± 13.5[a]				
	SunLWLN	44.7 ± 0.4[a]				
	ShadeHWHN	3.8 ± 2.0[c]				
	ShadeHWLN	1.7 ± 0.6[c]				
	ShadeLWHN	2.9 ± 1.1[c]				
	ShadeLWLN	3.3 ± 2.0[c]				
			0.000	**0.161**	**0.107**	**0.590**
Total Biomass (g)	SunHWHN	235.8 ± 153.8[a]				
	SunHWLN	109.0 ± 36.5[bc]				
	SunLWHN	168.1 ± 36.3[a]				
	SunLWLN	262.8 ± 38.5[a]				
	ShadeHWHN	39.7 ± 18.3[c]				
	ShadeHWLN	15.5 ± 0.2[c]				
	ShadeLWHN	43.4 ± 24.9[c]				
	ShadeLWLN	20.9 ± 5.1[c]				
			0.000	**0.307**	**0.352**	**0.135**

P-values indicate light, water and nutrient main effects on *C. monilifera* and their interaction together (light*water*nutrient). Mean values followed by different letters are significantly different.

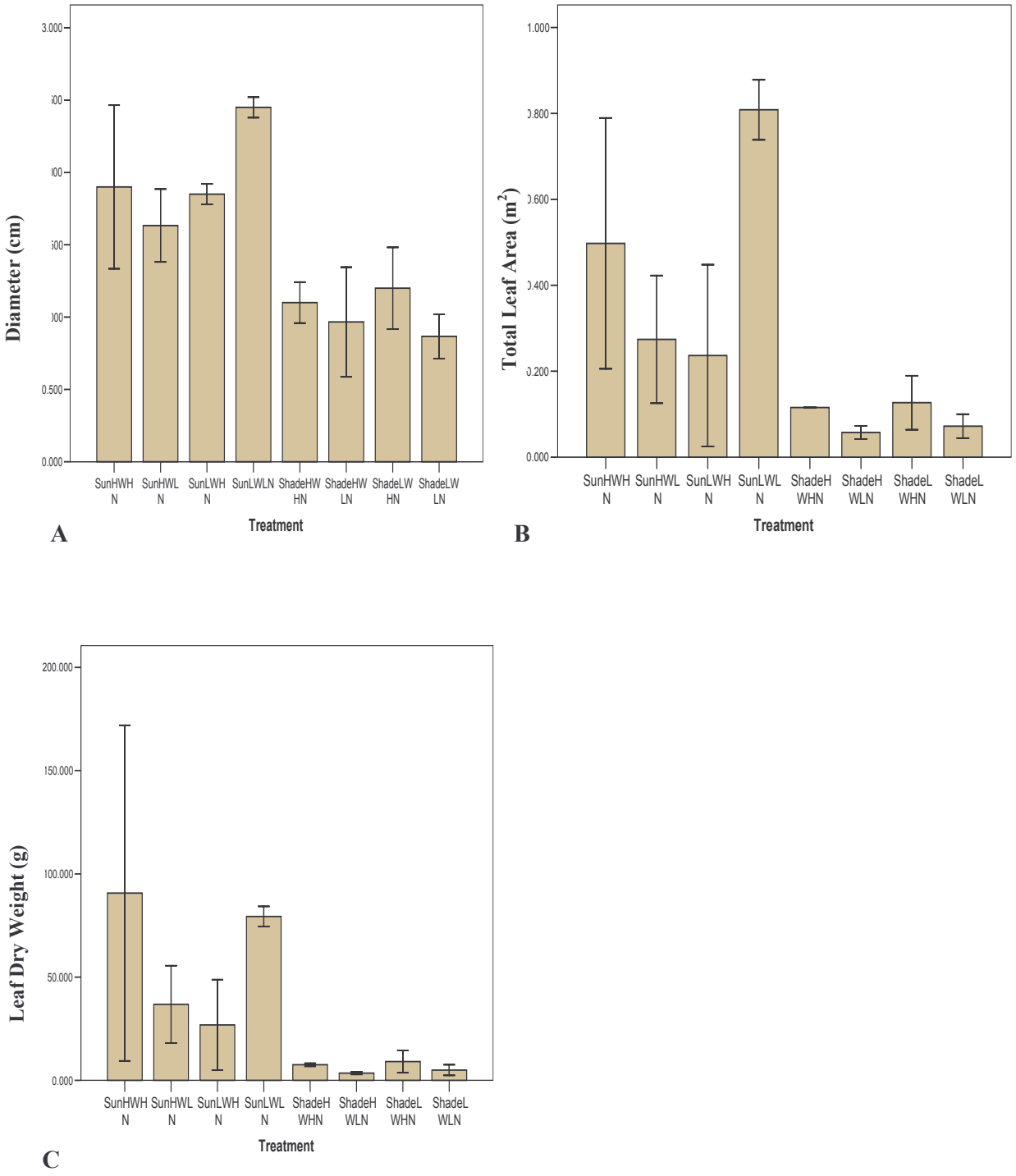

Figure 3.3.1: Biomass and growth parameters of *C. monilifera* when grown in 8 different environmental treatments (n = 3 per treatment). (A) Diameter of stems; (B) Total leaf area; and (C) Leaf dry weight of total leaf area. Bars represent means ± standard deviation. Treatment type represents - H/LW – high/low water and H/LN – high/low nutrient treatment.

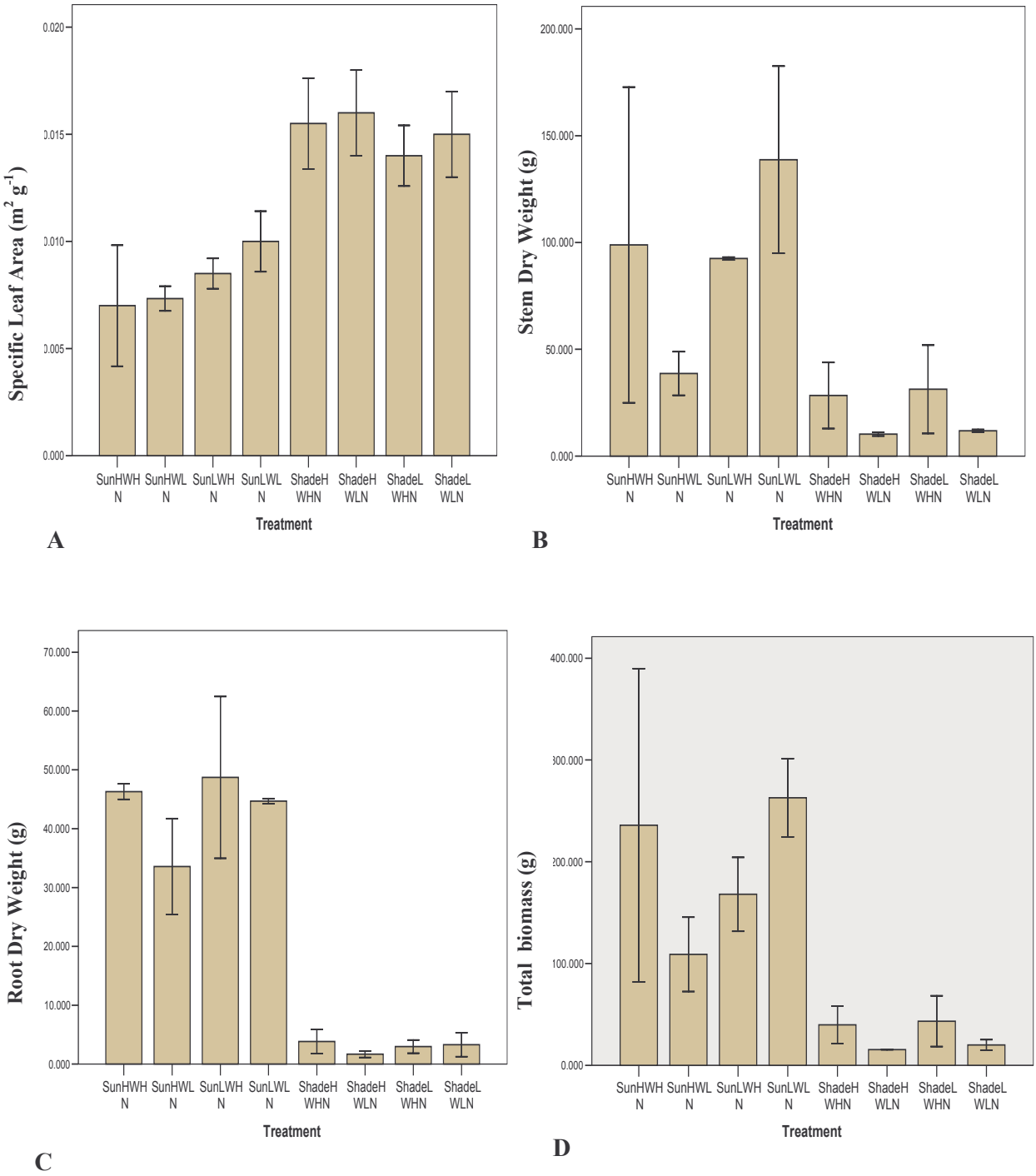

Figure 3.3.2: Biomass and growth parameters of *C. monilifera* when grown in 8 different environmental treatments (n = 3 per treatment). (A) Specific Leaf Area; (B) Stems Dry Weight; (C) Root Dry Weight; and (D) Total biomass. Bars represent means ± standard deviation. Treatment type represents - H/LW – high/low water and H/LN – high/low nutrient treatment.

Table 3.7 Allocation of resources to leaves, stems and roots for *C. monilifera* plants grown under 8 different environmental treatments (n=3 per treatment).

Parameter	Treatment	Mean ± SEM	p-value			
			light	water	nutrient	interaction
Leaf Ratio (%)	SunHWHN	34.6 ± 11.9[a]				
	SunHWLN	32.0 ± 8.3[a]				
	SunLWHN	14.9 ± 9.8[b]				
	SunLWLN	30.7 ± 6.3[ab]				
	ShadeHWHN	20.8 ± 7.7[a]				
	ShadeHWLN	22.7 ± 8.7[a]				
	ShadeLWHN	20.9 ± 0.5[a]				
	ShadeLWLN	23.8 ± 6.3[a]				
			0.097	**0.230**	**0.259**	**0.317**
Stem Ratio (%)	SunHWHN	40.3 ± 5.1[a]				
	SunHWLN	36.4 ± 4.9[a]				
	SunLWHN	56.3 ± 11.8[ab]				
	SunLWLN	52.1 ± 9.8[ab]				
	ShadeHWHN	69.8 ± 6.9[b]				
	ShadeHWLN	66.5 ± 6.2[b]				
	ShadeLWHN	70.0 ± 7.4[b]				
	ShadeLWLN	60.1 ± 10.6[b]				
			0.000	**0.161**	**0.130**	**0.087**
Root Ratio (%)	SunHWHN	25.2 ± 16.9[a]				
	SunHWLN	31.6 ± 3.7[ac]				
	SunLWHN	28.8 ± 1.9[a]				
	SunLWLN	17.2 ± 2.7[ab]				
	ShadeHWHN	9.4 ± 0.8[b]				
	ShadeHWLN	10.9 ± 3.6[b]				
	ShadeLWHN	9.1 ± 7.8[b]				
	ShadeLWLN	15.5 ± 6.0[ab]				
			0.000	**0.641**	**0.674**	**0.178**

P-values indicate light, water and nutrient main effects on *C. monilifera* and their interaction together (light*water*nutrient). Mean values followed by different letters are considered significantly different.

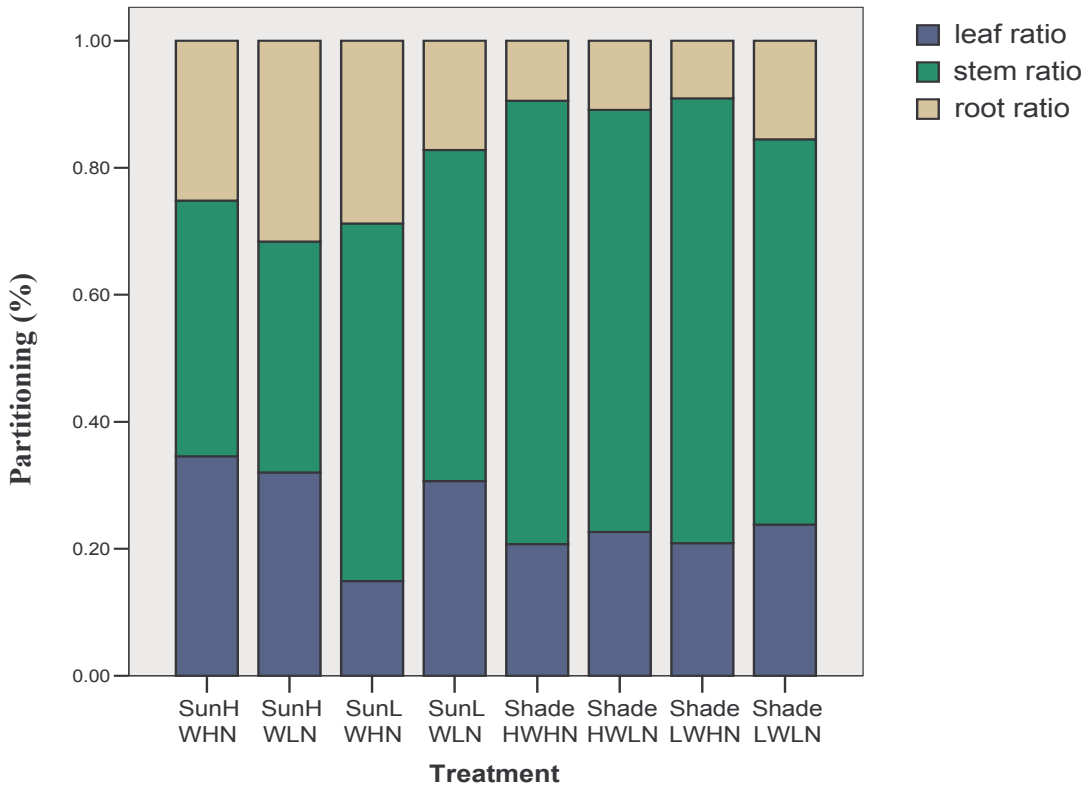

Figure 3.3.3: Partitioning of biomass to plant parts (leaf, stem and root %) of *C. monilifera* when grown in 8 different environmental treatments. Each bar represents allocation of resources to leaf, stem and root biomass as a percentage of total biomass harvested (g). Treatment type represents - H/LW – high/low water and H/LN – high/low nutrient treatment.

3.4. Correlations

Correlation analyses were performed amongst parameters to assess their relevance to either photosynthetic or hydraulic studies and the relationship that exists between the two. Table 3.8 shows that of the 12 correlations performed, only three were found to be statistically significant.

J_{max} (or maximum assimilation rate, A) was not found to be significantly related to any plant hydraulic parameter. In general, all the relationships between J_{max} and hydraulic conductance parameters were positive, although not significant, with the exception of R_{leaf} which showed a negative non-significant correlation. When examining J_{max} and stomatal conductance (g_s), there was a negative, significant correlation between J_{max} and g_s in response to both CO_2 concentration and light intensity ($p=0.035$ and $p=0.05$ respectively). Figure 3.4.1 (A) and 3.4.1 (B) illustrate the negative relationship that was found between J_{max} and g_s, and A_{max} and g_s, and show that J_{max} and g_s in response to CO_2 concentration had a steeper gradient than that of the response to light intensity. Stomatal conductance did not however show any correlation with K_{leaf}, when assessed in terms of CO_2 response and light intensity.

$R_{petiole}$ and R_{leaf} exhibited a positive, significant correlation where for each unit change in R_{leaf}, $R_{petiole}$ increased by 0.866 ($p=0.000$) (Table 3.8). Figure 3.4.1 (C) shows the relationship had the best R^2 value compared with that of all the other correlations ($R^2 = 0.751$). R_{leaf} and $R_{extravascular}$ did not show any significant correlation, but the relationship was in general a positive one (Table 3.8).

The final correlations performed were done to assess relationships between diameter and hydraulic conductance of the shoot and stem. Both correlations ($LogK_{lshoot}$ and $LogK_{lstem}$) were found to have a general positive relationship between Log diameter, although neither of which was significant.

Table 3.8: Pearson Correlation statistics for measured parameters of *C. monilifera* plants when grown in 8 different environmental treatments. Statistics include R^2, p-value to denote significance* (p cut-off = 0.05) and the correlation of the relationship (positive or negative correlation).

Parameters	R^2	p-value	Relationship(+/-)
A vs g_s (CO_2 response)	0.16	0.035*	-ve (1:-0.400)
A vs g_s (light response)	0.106	0.050*	-ve (1:-0.319)
g_s vs K_{leaf} (CO_2 response)	0.049	0.320	-ve
g_s vs K_{leaf} (light response)	0.039	0.354	-ve
A vs K_{leaf}	0.115	0.122	+ve
A vs R_{leaf}	0.119	0.116	-ve
A vs $K_{lshoot\ (plant)}$	0.061	0.314	+ve
A vs K_{lstem}	0.074	0.261	+ve
$R_{petiole}$ vs R_{leaf}	0.751	0.000*	+ve (1:0.866)
$R_{extravascular}$ vs R_{leaf}	0.117	0.101	+ve
LogK_{shoot} vs Log diameter	0.046	0.379	+ve
LogK_{lstem} vs Log diameter	0.031	0.470	+ve

A: Assimilation; g_s: Stomatal conductance; K_{leaf}: Leaf conductance; R_{leaf}: leaf resistance; $R_{petiole}$: resistance of the petiole; $R_{extravascular}$: resistance of the extravascular tissue; K_{shoot}: conductance of the total overall shoot area; K_{stem}: conductance of the stem area.Relationship: where * denotes significance, a relationship gradient is provided i.e. For A vs Gs – for every unit that Assimilation increases, G_s will decrease by 0.400 (1:-0.400).

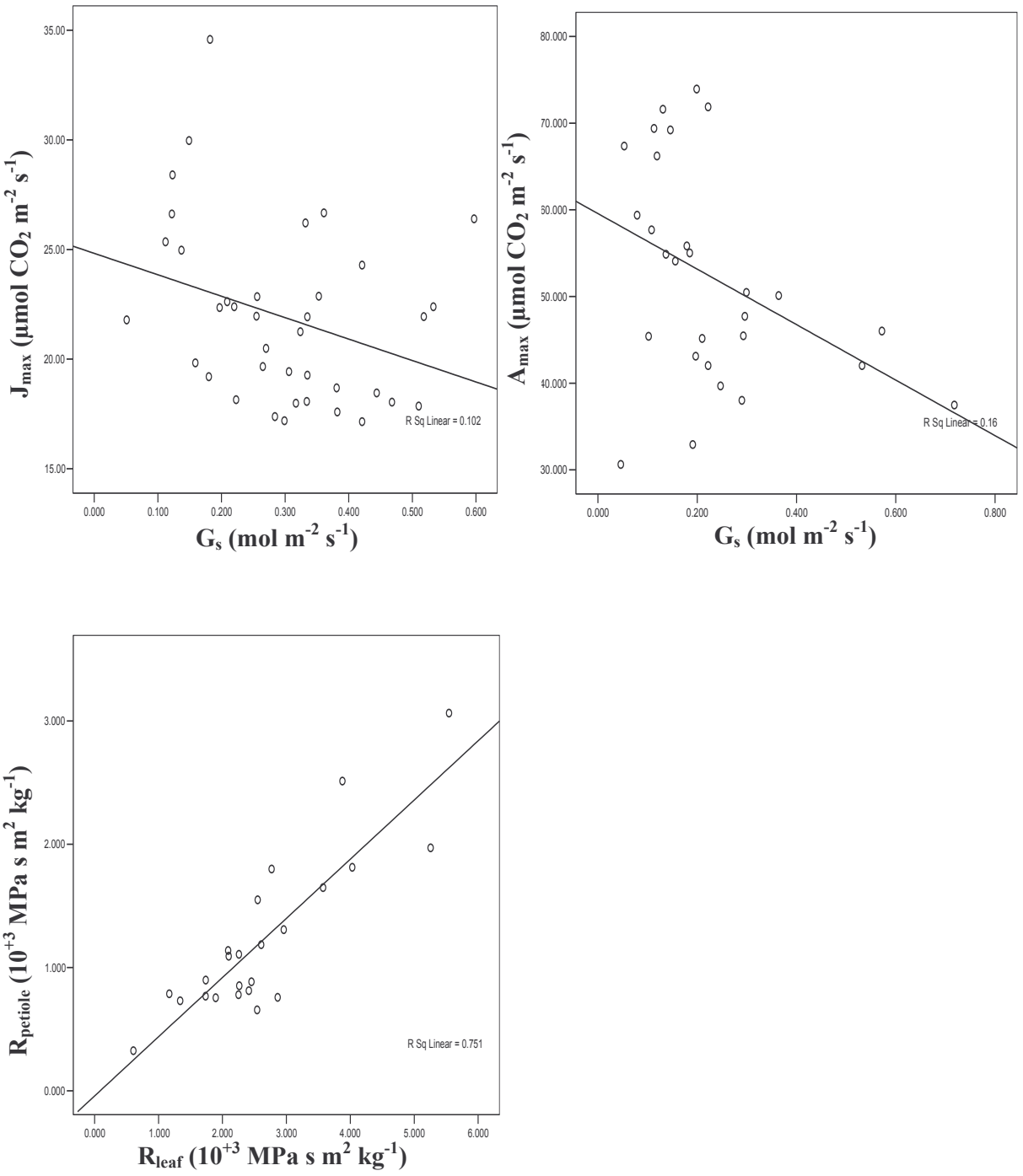

Figure 3.4.1: Scatter plots showing the significant negative/positive relationship between various parameters tested for a correlated relationship of *C. monilifera* when grown in 8 environmental treatments. (A) J_{max} versus stomatal conductance in response to changing light response; (B) A_{max} versus stomatal conductance in response to changing CO_2 concentration; and (C) $R_{petiole}$ versus R_{leaf} of hydraulic resistance of individual leaves.

3.5. Growth form of *C. monilifera*

3.5.1. Whole-shoot growth form

Sun-treated plants of *C. monilifera* differed significantly in growth form from that of shade-grown plants. Firstly, sun plants showed a great investment in stem thickness, as well as producing a large amount of leaf on each stem, thereby increasing total leaf area (Figure 3.5.1 and 3.5.2). Sun plants exhibited leaves that had grown to form tight clusters, and individual leaves were seen to be apetiolar (Figure 3.5.3 and 3.5.4). Differences among sun treatments were not highly observable, where size of the individual plants was greatest for sun, high water and high nutrient treatments (Figure 3.5.1). This did not however imply that the overall biomass was the highest for Sun HWHN, but only that the general size was larger.

Shade-treated *C. monilifera* plants showed stem etiolation, whereby the stems of shaded plants were extremely elongated and fleshy (Figure 3.5.5 and 3.5.6). These elongated stems were not as woody as that of the sun plants, and many of the shade-treated plants also showed light tropism (Figure 3.5.7 and 3.5.8). When observing the leaves of shade-treated *C. monilifera* plants, it was noted that the area of individual leaves was greater than that of sun leaves, although the whole shoot had far fewer leaves on their stems. The leaves (although apetiolar) did show an elongation of the basal portion of the leaf and the leaves on the stems were highly spaced such that light capture was maximised (Figure 3.5.6).

3.5.2. Root growth form

Roots of *C. monilifera* sun plants were approximately 10 times larger in biomass than those of shade plants (Figure 3.5.9 versus 3.5.11). Rooting characteristics of shade plants showed that the roots were extremely shallow and had numerous adventitious roots, rather than one main anchoring tap root (Figure 3.5.12). Sun plants had highly branched adventitious roots, and one or two main anchoring roots (Figure 3.5.10). Within sun or shade treatments, the same occurrence was found, whereby high nutrient treatment plants showed a smaller root than that of plants exposed to low nutrient treatments. The low nutrient treated plants had larger anchoring roots (in sun plants) and had many more adventitious roots overall, for both sun and shade plants of *C. monilifera* (Figure 3.5.9 - 3.5.12).

Figure 3.5.1: *C. monilifera* when grown in sun, high water and nutrient treatments.

Figure 3.5.2: *C. monilifera* when grown in sun, high water and low nutrient treatments.

Figure 3.5.3: *C. monilifera* when grown in sun, low water and high nutrient treatments.

Figure 3.5.4: *C. monilifera* when grown in sun, low water and low nutrient treatments.

Figure 3.5.5: *C. monilifera* when grown in shade, high water and high nutrient treatments.

Figure 3.5.6: *C. monilifera* when grown in shade, high water and low nutrient treatments.

Figure 3.5.7: *C. monilifera* when grown in shade, low water and high nutrient treatments.

Figure 3.5.8: *C. monilifera* when grown in shade, low water and low nutrient treatments.

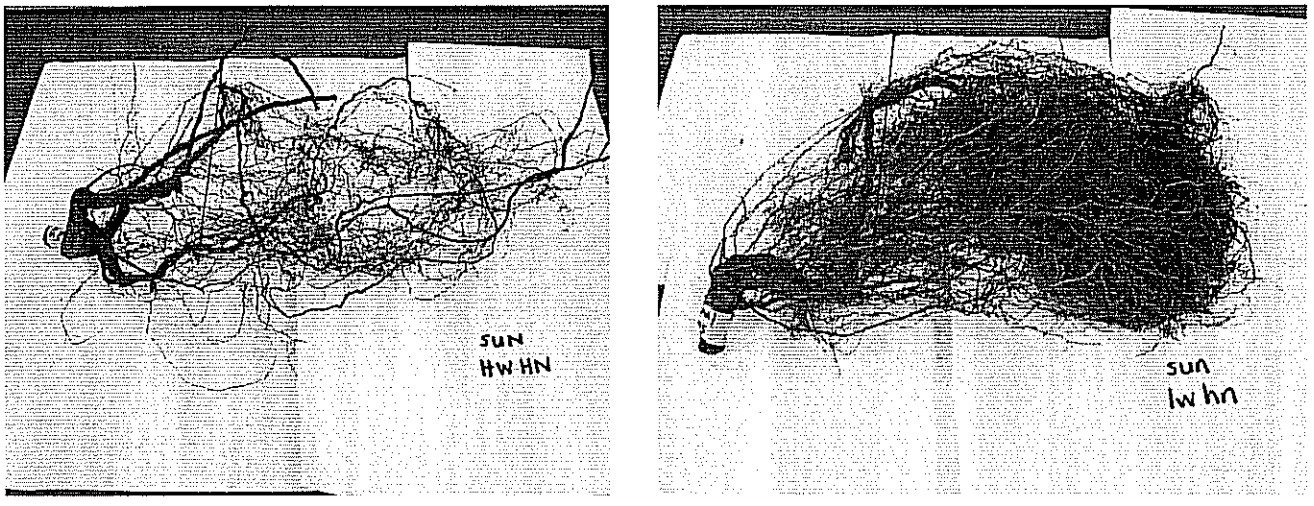

Figure 3.5.9: *C. monilifera* roots of plants grown in sun high water, high nutrient (right) and sun, high water, low nutrient treatment (left).

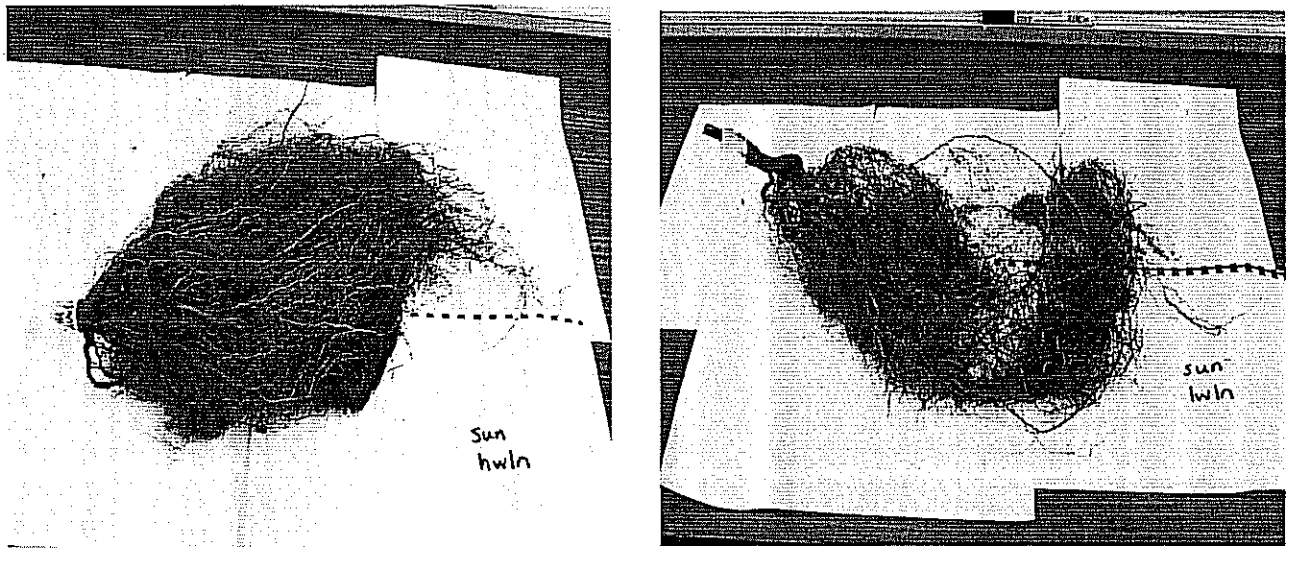

Figure 3.5.10: *C. monilifera* roots when grown in sun, low water, high nutrient (right) and sun, low water, low nutrient treatment (left).

Chapter 4

DISCUSSION

This study attempted to investigate the hydraulic characteristics and photosynthetic capacity of *C. monilifera* when grown in contrasting environmental conditions. Light, water and nutrient treatments were manipulated to assess the extent to which environmental conditions affect hydraulic properties of this specific species. The relationship between photosynthetic capacity (in terms of maximum photosynthetic rate at saturating CO_2 and light) and hydraulic properties of the whole plant, roots and leaves was also examined. Ultimately the impact of altering environmental conditions was considered in terms of the investigation of the correlation between photosynthesis, hydraulic properties and growth patterns of *C. monilifera*.

4.1. Photosynthetic Response

The maximum rate of photosynthesis can differ several-fold over many species, even in those that grow in similar environments (Korner, 1994). Maximum photosynthetic rates of *C. monilifera*, when measured in response to changing light and CO_2 concentrations, were found to be significantly affected by light and nutrient treatment, but not water supply (Table 3.1 and 3.2). Sun and high nutrient treatments enhanced gaseous exchange significantly, a result which has been well documented (Shangguan *et al*, 2000, Lambers *et al*, 1998). The influence of high and low watering treatments was found to have no significant effect on *C. monilifera* in terms of maximum photosynthetic rate (Table 3.1 and 3.2). The observation that water stress did not affect photosynthetic rate was in contrast to literature that supports that A_{max} down-regulates when water stress is experienced

(Lambers *et al*, 1998). It may be possible that the semi-succulent nature of *C. monilifera* "decouples" the response to water stress treatments. The effects of water stress on plants depend upon the severity and the extent to which the drought is experienced (Rouhi *et al.*, 2005). Thus, with regard to A_{max} of *C. monilifera*, perhaps the severity of the low watering treatments was not severe enough. The CO_2 compensation points (a measure of both the initial slope and photorespiration) of C_3 species is generally within the range of 30 – 70 μmol mol^{-1} CO_2 (Salisbury and Ross, 1992). *C. monilifera*, while being a C_3 plant, achieved much higher values for CO_2 compensation points (Table 3.1. and 3.2).

Environmental stress can increase the photorespiration, thereby affecting the CO_2 compensation point (Salisbury and Ross, 1992). There was, however, no significant effect on photorespiration of *C. monilifera*, when grown in light, water and nutrient treatments. Dark respiration can be observed to be influenced by light treatments, whereby sun leaves tend to exhibit higher rates of dark respiration (Lambers *et al.*, 1998). These higher rates are due to the fact that there is a greater demand for respiration energy for maintenance of more and/or larger cells. In general, dark respiration of *C. monilifera* was higher in sun plants, but this difference was not found to be significant (Table 3.1). It can be concluded that the environmental treatments utilised did not affect a dark respiration or photorespiration on *C. moilifera*.

Although watering treatments were found not to influence A_{max} or J_{max}, the initial slope (light response) and carboxylation efficiency (CO_2 response) of *C. monilifera* were significantly affected by watering treatments. Plants subjected to high water treatments showed an enhanced rate of carboxylation efficiency, which can be expressed as a measure of rubisco activity within the plant (Salisbury and Ross, 1992).

The initial slope of the light response measurements (taken as a measure of quantum efficiency on an incident radiation basis) was also found to be significantly higher in sun treatment plants of *C. monilifera*. During periods of water stress, plants can respond in terms of either stomatal or non-stomatal factors (Ramanjulu, 1998). *C. monilifera* showed a relative decrease in g_s in the low water treatments, which is the response one would expect in plants exposed to water stress. Rather than completely closing their stomata during the slight water stress of the low water treatments, the carboxylation efficiency of rubisco decreased. Therefore the water stress response of *C. monilifera* plants was in fact non-stomatal, but it did not affect the net photosynthetic rate (at saturating CO_2 concentrations).

The information observed about photosynthetic traits is important when understanding the effects that global environmental change will have on plants. *C. monilifera* is a weed of serious environmental importance in Australia and therefore understanding how it responds in terms of environmental stress is essential for preventing its further spread.

4.2 Hydraulic Characteristics

4.2.1 Whole-plant hydraulic characteristics

Differences in environmental conditions have been shown to result in different hydraulic conductance in plants (van der Willigen and Pammenter, 1998, Nardini *et al.*, 1999; Tyree and Ewers, 1999; Costa E Silva *et al.*, 2004; Lovelock *et al.*, 2004). *Pinus ponderosa*, *Licania platypus*, *Eucalyptus* species and many drought-adapted species have exhibited lower leaf, stem or root hydraulic conductance when exposed to periods of water stress (Maherali *et al.*, 2002; Tyree *et al.*, 2002; Costa E Silva *et al.*, 2004).

C. monilifera, however, showed no significant differences in terms of the following four parameters: K_{lshoot}, K_{lstem}, $K_{lleaves}$, K_{lroot} (Table 3.3). Each parameter was normalized by leaf area in order to prevent the leaf area of the individual plants from influencing the results.

The values obtained were highly variable within and among treatments of *C. monilifera*, and establishing any kind of environmental trend was not possible for K_{lshoot} and K_{lstem}. $K_{lleaves}$ was somewhat higher in sun treatments of *C. monilifera,* although the effect was not significant, thus showing that the leaves of sun plants may attain higher hydraulic conductance than that of shade plants. This is agreement with studies performed on individual sun leaves, which attain higher rates of photosynthesis and hydraulic conductance than shade leaves (Nardini *et al.*, 2005).

The roots of *C. monilifera* exhibited higher conductance (per unit leaf area) in shade plants than in, although this difference was not significant (Table 3.3). When assessing the roots of *C. monilifera* in terms of hydraulic resistance (inverse of hydraulic conductance), the sun plants showed a significantly higher hydraulic resistance than that of the shade plants. Even though root resistance was normalized by leaf area, the size and weight of the roots from different treatments, was extremely different (Table 3.6; Fig. 3.5.9-12). Hydraulic resistance can be correlated with plant size, and can further be confounded by environmental stress (Maherali *et al.*, 2002). For *C. monilifera*, the extensive branching of the roots of the sun plants would have caused the hydraulic resistance to have been higher because of the increased amount of vascular tissue.

R_{shoot} and R_{stem} were not affected by any environmental treatment, but R_{leaves} was significantly higher in shade *C. monilifera* plants than in sun plants. The shade leaves had a larger specific leaf area and a lower photosynthetic rate (Table 3.6 and 3.1).

The fact that sun leaves had a higher hydraulic conductance may be of primary importance though, rather than viewing the difference in terms of resistance. These measurements were whole-plant measurements, therefore the total leaf canopy (leaves) had a higher conductance in sun-grown plants, but a significantly higher resistance in shade-grown *C. monilifera* canopies.

Climatic variation has the ability to exert great selective pressure on plant species, and plants with phenotypic plasticity have an advantage for the continuation of the species. Phenotypic plasticity allows plants to respond to developmental or environmental stress without being detrimentally affected, due to the adaptive nature of their genetic material (Maherali *et al.*, 2002). The lack of response observed in *C. monilifera* in terms of whole-plant hydraulics could be explained by suggesting that *C. monilifera* may have a degree of phenotypic plasticity in reponse to environmental stress.

4.2.2 Leaf Hydraulic Characteristics

An increasing amount of attention is being paid to leaf hydraulic properties and how these properties of the leaf change in response to environmental and developmental factors (Nardini and Salleo, 2003). The response of plants to environmental variation can be used as an important determinant of environmental adaptation (Sack *et al.*, 2003). The resistance of the leaves is considered a significant hydraulic bottleneck (Sack *et al.*, 2003). Determining component resistances of an individual leaf has been found to promote understanding in crop breeding, thereby contributing to increased productivity (Tyree, 2003). R_{leaf} of *C. monilifera* was not significantly affected by the environmental treatmentsnto which it was exposed (Table 3.4).

Plants subjected to shade treatments had only slightly higher resistances than plants in full sun. This means that K_{leaf} (inverse of R_{leaf}) was slightly higher in sun leaves, which has been observed for red oak, sugar maple and grapevines (Schultz and Matthews, 1993; Sack *et al.*, 2003). Hydraulically, the sun leaves would have had higher conductance to water flow, which could be advantageous in terms of leaf gaseous exchange.

Even though R_{leaf} was relatively higher in shade treatments, statistically there was no significant difference in R_{leaf} and K_{leaf} of *C. monilifera* (Table 3.4). This result (*i.e*. the lack of significance in K_{leaf}) has been observed in other studies when measuring R_{leaf} of six different sun and shade species (Nardini *et al.*, 2005). $R_{petiole}$ of *C. monilifera* was significantly higher in shade leaves than in sun leaves (Table 3.4). Sun leaves of *A. rubrum* exhibited higher petiole resistance than shade leaves, as did that of grapevine (Schultz and Matthews, 1993; Sack *et al*, 2003). The petioles of shade-grown *C. monilifera* leaves were seen to be longer than that of sun leaves due to etiolation and that may have influenced $R_{petiole}$. All shade-grown *C. monilifera* plants had longer stems and basal leaf portions than sun plants to enhance light capture. Larger petioles also maintain a larger petiolar leaf surface area, causing a higher resistance to water flow in *C. monilifera* plants.

R_{lamina}, $R_{tertiary\ cut}$ and $R_{minor\ cut}$ were all unaffected by environmental treatments in *C. monilifera* (Table 3.4). The measured parameters had very slightly higher resistance in shade leaves, which implies that the sun leaves were relatively more conductive. The resistance of the extravascular tissue ($R_{extravascular}$) of *C. monilifera* was, however, significantly affected by light treatment only (Table 3.4). Sun leaves showed a greater extravascular resistance than that of shade leaves. Sun leaves were thicker, and this confers to the leaves a higher mesophyll cell surface area per lamina area (James *et al.*, 1999).

More mesophyll area causes there to be more resistance to water flow in the sun leaves of *C. monilifera* because there is more cell packing. This shows that, although sun leaves exhibited a slightly higher conductance in *C. monilifera*, the extravascular tissue showed greater resistance. This was probably because of the denser nature of the extravascular tissue of the sun leaves compared to the shade leaves.

4.2.3 Allocation of Leaf Resistance

Leaf resistance of the leaf was partitioned among components $R_{petiole}$, $R_{extravascular}$, $R_{major\ veins}$ and $R_{minor\ veins}$, and these were calculated as a percentage of total leaf resistance. Only a few studies have determined how the components of the leaf water pathway contribute to total leaf resistance (Yang and Tyree, 1994; Salleo *et al.*, 2003; Sack *et al.*, 2004; Cochard *et al.*, 2004). The main source of contention in the leaf component studies is whether the majority of the leaf resistance resides in the vascular or in the extravascular (mesophyll) pathway.

In *C. monilifera* the majority of leaf resistance resided in the vascular pathway (Table 3.5). The petioles were shown to have the highest resistance to water flow, and had significantly higher resistance in the shade leaves of *C. monilifera* than in sun leaves. The extravascular tissue made up only approximately one tenth to one fifth of the total leaf resistance. These results are in agreement with those produced by Sack *et al.* (2003), which found that in sugar maple and red oak leaves, the majority of the leaf resistance was located in the vascular pathway. In terms of leaf venation, the major veins of the leaves had much larger resistance to water flow than did the minor veins (Table 3.5). The resistance of the minor veins were the only component to be significantly affected by light, water and nutrient treatments.

This result implies that while the minor veins are phenotypically plastic in response to environmental stress, the major veins are not. The minor veins also represented the smallest proportion of the leaf hydraulic resistance. Because R_{leaf} was unaffected by environmental treatments, it can be suggested that the minor veins do not have a significant influence on the total leaf resistance of *C. monilifera*, despite the impact that environmental treatments had on the minor veins.

These data show that the vascular water pathway is the main resistance to water flow in *C. monilifera* leaves. Cochard *et al.* (2004) showed that the major resistance to water flow in leaves occurs in the extravascular tissue, and that this confers more efficient leaf gaseous exchange rates. In contrast to this literature suggests that having the majority of the resistance in the vascular tissue would cause the hydraulic efficiency of the xylem to be lowered (Cochard *et al*, 2004). According to the theory presented, *C. monilifera* would have less efficient gaseous exchange rates and a lowered hydraulic conductance of the xylem. The J_{max} of sun plants of *C. monilifera* can reach up to 66 μmol CO_2 m^{-2} s^{-1}, and with such a high rate of gaseous exchange, it is unlikely that photosynthesis is being constrained. The large difference exhibited among species in terms of vascular: non-vascular resistance can be attributed to species-specific partitioning of hydraulic resistance within individual leaves.

4.3 Biomass, Growth and Growth Form

Biomass and growth parameters of *C. monilifera* were significantly affected by light treatment only (Table 3.6). The fact that light treatment affects *C. monilifera* in this manner is unsurprising, as higher irradiance is known to enhance plant growth (Ogren, 1993). Sun plants of *C. monilifera* were shown to have a larger diameter; greater total leaf area, stem dry weight, leaf dry weight and root dry weight than shade plants (Table 3.6). Leaves of sun plants are thicker because they have taller palisade cells and more layers of cells, than shade leaves (Lambers *et al.*, 1998). An increase in the number of palisade cells increases the amount of photosynthetic machinery which in turn determines photosynthesis, thereby allowing sun plants of *C. monilifera* to achieve higher rates of A_{max}. Shade-grown plants of *C. monilifera* exhibited a significantly higher specific leaf area than sun-grown plants (Table 3.6). Plants grown in shaded environments tend to invest their resources in leaf area in order to maximize light capture (Lambers *et al*, 1998). In contrast to sun leaves, the shade leaves of *C. monilifera* were thinner and showed much higher specific leaf area.

C. monilifera can be categorized as an obligate sun plant or shade-avoider. The large differences in growth form and photosynthesis were observed in terms of light treatment only. Examining the growth form of *C. monilifera*, it can be seen that the different light treatments caused two distinct growth forms (Fig. 3.5.1 – 3.5.8). Sun plants were larger, highly branched and had woody stems. Shade plants exhibited stem etiolation, larger leaves and less branching than sun-grown *C. monilifera* plants.

The roots of *C. monilifera* were much larger in sun plants (Fig. 3.5.9 – 3.5.12). Shade plants did not develop deep or highly branched roots, and this may be as a result of diversion of resource to the leaves. There were no obvious differences in root size or structure in low *versus* high water treatments. Low nutrient-treated roots of *C. monilifera* were perhaps only slightly larger than that of high nutrient-treated plants. *C. monilifera* responded to the differences in environmental conditions by changing resource allocation. Sun plants show higher partitioning to roots and stems, but significant differences in resource allocation in water and nutrient treatments did not occur (Table 3.7). Nutrient and water treatments did not affect any biomass parameter, either statistically or in general growth form. These results are unusual, especially the lack of response to nutrient enhancement. It may be possible that *C. monilifera* requires only very low soil nutrient content and water in order to survive. It can therefore be concluded that in terms of biomass and growth, the environmental water and nutrient treatments were not sufficiently different enough to produce a morphological response in *C. monilifera*.

4.4 Correlations

At A_{max}, maximum g_s was reduced to prevent excessive transpiration in *C. monilifera* (Fig. 3.1.5. (A) and (B)). The relationship between A_{max} and g_s (in terms of light and CO_2 repsonse) was a significant, negative one (Table 3.8). Stomatal conductance decreased at high photosynthetic rates, which shows that the optimum photosynthetic rate and stomatal conductance were achieved before either 1000 μmol mol^{-1} CO_2 or 2000 μmol PPFD m^{-2} s^{-1} (Fig. 3.1.3 and 3.1.8). Franks (2005) states that higher photosynthetic rates are sustained by higher g_s, but this was not observed for *C. monilifera*. This does not, however, change the fact that A_{max} and g_s are closely correlated for *C. monilifera*, and that the correlation between the two parameters is usually found for C_3 plants (Wong *et al.*, 1979).

There was no significant correlation found between K_{leaf} and g_s in *C. monilifera* (Table 3.8). However, numerous studies have reported that there is a close link between g_s and K_{leaf} (Meinzer *et al.*, 1995; Aasamaa *et al*, 2001; Sack *et al,* 2003a). The link between the two parameters was not exhibited in *C. monilifera* and there could be many explanations for this. One reason could be that because K_{leaf} showed no response to any environmental treatment, and g_s was affected by light treatments, this then caused the overall relationship to be non-significant. The lack of response of K_{leaf} would then, by the same argument, also cause the relationship between K_{leaf} and A_{max} not to be significant. The studies that have tried to correlate and quantify the relationship between K_{leaf} and A_{max}, all operate on the theory that because A_{max} is related to g_s, and g_s is related to K_{leaf}, therefore A_{max} and K_{leaf} are related (Brodribb *et al.*, 2004). For *C. monilifera* this was not the case, and thus it cannot be directly stated that the photosynthesis of the leaf was constrained by hydraulic conductance. This poses another question in terms of the semi-succulence of *C. monilifera* and whether capacitance has a role to play in semi-succulent plant types.

Hydraulic conductance of the shoots and stems of *C. monilifera* was not correlated with diameter of the stems (Table 3.8), even though the diameter was significantly higher in sun treatments (Table 3.6). The only other hydraulic parameters that were found to be significantly correlated were $R_{petiole}$ and R_{leaf} (Table 3.8; Fig. 3.4.1 (C)). This result has been reported for *A. saccharum* and *Q. rubra* (Sack *et al.*, 2003b). Because $R_{petiole}$ contributes up to 69 % of R_{leaf}, it is understandable that these two parameters are closely related. These correlations, especially the lack of significant correlation between A_{max} and K_{leaf}, suggest that the hydraulic and gaseous exchange capacities of *C. monilifera* are not at their optimum.

4.5 Conclusion

C. monilifera is a fast-growing, sun-obligate, C_3 plant species. *C. monilifera* was primarily affected by light treatments in this study, where high light conditions enhanced *C. monilifera* both photosynthetically and morphologically. Nutrient and water treatments affected *C. monilifera* on a much smaller scale than that of the light conditions, and very few measured parameters were significantly influenced by water and nutrient supply. Whole-plant hydraulic conductance was unaffected by environmental treatments, and no relationship was found between K_{leaf} and A_{max}. The most reasonable explanation for these results is that *C. monilifera* was unaffected by the extent to which the watering and nutrient treatments were varied under the experimental conditions. Thus, the severity of the contrasting environments for this study were not severe enough for both water and nutrient treatments.

Hydraulic resistance partitioning of leaf components was dominated by vascular resistance. The correlation of R_{leaf} and $R_{petiole}$ confirmed this result, and thereby show that the extravascular tissue of *C. monilifera* does not significantly contribute to overall leaf resistance. The mechanism by which plants distribute leaf resistance to either the vascular or extravascular pathway remains unknown. Even though K_{leaf} and A_{max} were not correlated in *C. monilifera*, this does not imply that there will be no relationship for other species. Photosynthesis takes place within the mesophyll cells of the leaf lamina, and the contribution of hydraulic resistance to the mesophyll may affect the outcome of carbon fixation. Studies that can establish the link between K_{leaf} and A_{max} will not only be beneficial for agricultural productivity, but also for understanding natural environmental communities experiencing global climate change.

Recommendations for future work (in terms of *C. monilifera* specifically) would be to enhance the water- and nutrient-stressed treatments for this species. Further investigations should include more detailed studies of leaf resistance components over a broad range of species from different habitats. Vein-cutting experiments and measurements of whole-plant hydraulic conductance are relatively uncomplicated, and can provide comprehensive understanding of how individual plant parts contribute to the hydraulic pathway. Ultimately the challenge that lies ahead for leaf hydraulic resistance studies is to establish clear trends in how plant species distribute their resistance. Whether the distribution of vascular versus non-vascular tissue resistance is species, family or community specific, remains to be shown.

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APPENDIX

Appendix 1 – Photosynthetic Data

Light Response Data derived from gaseous exchange measurements for *C. monilifera.*
All values are mean assimilation rates (μmol PPFD m^{-2} s^{-1})

PAR	SUN HWHN	SUN HWLN	SUN LWHN	SUN LWLN	SH HWHN	SH HWLN	SH LWHN	SH LWLN
50	1.18	2.56	0.33	2.73	1.50	1.54	1.37	1.55
100	5.95	4.85	4.98	8.19	3.25	4.75	3.91	3.52
150	8.24	7.31	7.53	10.99	5.43	6.68	6.56	5.59
200	10.06	9.67	9.60	12.91	7.45	8.42	8.79	8.13
400	15.70	14.44	14.61	18.12	10.76	12.72	12.76	11.36
600	18.23	16.66	17.78	20.18	12.56	15.38	15.52	13.58
800	20.04	18.26	20.26	21.20	14.78	16.26	16.62	15.26
1000	21.40	19.42	21.66	21.42	15.96	17.06	17.72	16.58
1200	22.18	20.22	22.64	21.80	18.12	18.06	18.28	17.70
1500	23.38	20.78	23.70	22.02	19.38	18.86	19.62	18.32
1800	25.14	21.20	24.42	22.36	20.26	19.34	20.30	18.72
2000	25.48	21.44	24.74	22.78	20.60	19.44	20.42	18.96

CO_2 Response Data derived from gaseous exchange measurement for *C. monilifera*.
All values are mean assimilation rates (μmol CO_2 m^{-2} s^{-1})

Ca	Sun HWHN	SUN HWLN	SUN LWHN	SUN LWLN	SH HWHN	SH HWLN	SH LWHN	SH LWLN
100	-1.56	4.11	1.6525	2.8525	3.792	3.12	3.08	2.06
200	7.09	11.32	4.97	5.8925	10.64	8.604	5.86	6.65
300	13.16	16.08	9.19	9.6775	16.94	14.44	10.745	11.302
400	24.5	27.04	18.225	19.05	25.14	24.46	18.25	20.72
600	40.8	36.62	29.05	27.95	35.64	32.96	28.7	28.54
800	47.8	41.98	35.225	32.125	39.38	37.34	34.85	32.16
1000	53.1	44.84	38.95	34.15	40.18	38.42	37.95	32.92

Light response data for *C.monilifera*

Experiments planted Nov 05 data collected March/April

								b/c	a(1-eb)	a.c / a.c.eb
	Light	**Water**	**Nutrient**	**Pot number**	**A/Jmax**	**B**	**C**	**Comp pnt**	**Respir dark**	**Initial slope**
1	Sun	High water	High nutrient	1	34.58	-.1800	.0042	-42.8571429	5.70	.145
2	Sun	High water	High nutrient	2	21.96	.0710	.0016	44.375	-1.62	.035
3	Sun	High water	High nutrient	3	28.4	-.0250	.0015	-16.6666667	.70	.043
4	Sun	High water	High nutrient	4	29.97	-.0420	.0005	-84	1.23	.015
5	Sun	High water	High nutrient	5	22.35	.3290	.0046	71.52173913	-8.71	.103
6	Sun	High water	Low nutrient	1	26.4	.0710	.0022	32.27272727	-1.94	.058
7	Sun	High water	Low nutrient	2	18.04	.1000	.0037	27.02702703	-1.90	.067
8	Sun	High water	Low nutrient	3	19.2	-.0830	.0021	-39.5238095	1.53	.040
9	Sun	High water	Low nutrient	4	20.49	-.1410	.0040	-35.25	2.69	.082
10	Sun	High water	Low nutrient	5	21.94	.0380	.0026	14.61538462	-.85	.057
11	Sun	Low water	High nutrient	1	22.39	.2950	.0019	155.2631579	-7.68	.043
12	Sun	Low water	High nutrient	2	26.21	-.0330	.0023	-14.3478261	.85	.060
13	Sun	Low water	High nutrient	3	24.97	.1140	.0026	43.84615385	-3.02	.065
14	Sun	Low water	High nutrient	4	21.79	.0210	.0020	10.5	-.46	.044
15	Sun	Low water	High nutrient	5	27.15	-.1380	.0041	-33.6585366	3.50	.111
16	Sun	Low water	Low nutrient	1	22.39	.1200	.0035	34.28571429	-2.85	.078
17	Sun	Low water	Low nutrient	2	26.62	.2130	.0047	45.31914894	-6.32	.125
18	Sun	Low water	Low nutrient	3	18.15	.1960	.0074	26.48648649	-3.93	.134
19	Sun	Low water	Low nutrient	4	25.35	-.0880	.0038	-23.1578947	2.14	.096
20	Sun	Low water	Low nutrient	5	19.83	-.0401	.0062	-6.46774194	.78	.123
21	Shade	High water	High nutrient	1	22.62	-.1220	.0013	-93.8461538	2.60	.029
22	Shade	High water	High nutrient	2	17.38	-.0910	.0027	-33.7037037	1.51	.047
23	Shade	High water	High nutrient	3	26.67	.0390	.0011	35.45454545	-1.06	.029
24	Shade	High water	High nutrient	4	22.87	-.0250	.0012	-20.8333333	.56	.027
25	Shade	High water	High nutrient	5	19.66	.0210	.0020	10.5	-.42	.039
26	Shade	High water	Low nutrient	1	17.15	-.0240	.0027	-8.88888889	.41	.046
27	Shade	High water	Low nutrient	2	24.29	.0480	.0021	22.85714286	-1.19	.051
28	Shade	High water	Low nutrient	3	18.46	.0890	.0029	30.68965517	-1.72	.054
29	Shade	High water	Low nutrient	4	17.19	.0190	.0033	5.757575758	-.33	.057
30	Shade	High water	Low nutrient	5	18.07	-.0520	.0036	-14.4444444	.92	.065
31	Shade	Low water	High nutrient	1	22.85	.0173	.0021	8.238095238	-.40	.048
32	Shade	Low water	High nutrient	2	19.27	.1010	.0035	28.85714286	-2.05	.067
33	Shade	Low water	High nutrient	3	21.25	-.0620	.0019	-32.6315789	1.28	.040
34	Shade	Low water	High nutrient	4	18.69	.0820	.0029	28.27586207	-1.60	.054
35	Shade	Low water	High nutrient	5	18.11	-.0160	.0027	-5.92592593	.29	.049
36	Shade	Low water	Low nutrient	1	17.59	.0090	.0020	4.5	-.16	.035
37	Shade	Low water	Low nutrient	2	17.99	-.0690	.0021	-32.8571429	1.20	.038
38	Shade	Low water	Low nutrient	3	19.43	.0530	.0031	17.09677419	-1.06	.060
39	Shade	Low water	Low nutrient	4	21.93	-.0340	.0017	-20	.73	.037
40	Shade	Low water	Low nutrient	5	17.86	.0720	.0027	26.66666667	-1.33	.048

CO2 response data for *C.monilifera*

Experiments planted Nov 05 data collected March/April

								b/c	a(1-eb)	a.c / a.c.eb
	Light	**Water**	**Nutrient**	**Pot #**	**A/Jmax**	**B**	**C**	**Comp pnt**	**Respir dark**	**Initial slope**
1	Sun	High water	High nutrient	1	105.57	.158	.0090	17.55555556	-18.070	.950
2	Sun	High water	High nutrient	2	66.21	.183	.0021	87.14285714	-13.296	.139
3	Sun	High water	High nutrient	3	67.35	.091	.0005	182	-6.416	.034
4	Sun	High water	Low nutrient	1	59.38	.153	.0014	109.2857143	-9.817	.083
5	Sun	High water	Low nutrient	2	69.21	.119	.0015	79.33333333	-8.746	.104
6	Sun	High water	Low nutrient	3	71.87	.006	.0017	3.529411765	-.433	.122
7	Sun	High water	Low nutrient	4	37.48	.313	.0041	76.34146341	-13.775	.154
8	Sun	High water	Low nutrient	5	56.04	.186	.0014	132.8571429	-11.456	.078
9	Sun	Low water	High nutrient	1	73.93	.182	.0012	151.6666667	-14.757	.089
10	Sun	Low water	High nutrient	2	50.47	.096	.0010	96	-5.085	.050
11	Sun	Low water	High nutrient	4	71.6	.078	.0009	86.66666667	-5.808	.064
12	Sun	Low water	High nutrient	5	69.39	.078	.0009	86.66666667	-5.629	.062
13	Sun	Low water	Low nutrient	2	43.11	.159	.0019	83.68421053	-7.429	.082
14	Sun	Low water	Low nutrient	3	57.68	.104	.0011	94.54545455	-6.322	.063
15	Sun	Low water	Low nutrient	4	55.83	.089	.0009	98.88888889	-5.197	.050
16	Sun	Low water	Low nutrient	5	45.41	.179	.0020	89.5	-8.901	.091
17	Shade	High water	High nutrient	1	32.92	.240	.0028	85.71428571	-8.930	.092
18	Shade	High water	High nutrient	2	54.08	.181	.0019	95.26315789	-10.730	.103
19	Shade	High water	High nutrient	3	42.02	.163	.0026	62.69230769	-7.439	.109
20	Shade	High water	High nutrient	4	46.01	.188	.0026	72.30769231	-9.516	.120
21	Shade	High water	High nutrient	5	65.79	.141	.0019	74.21052632	-9.962	.125
22	Shade	High water	Low nutrient	1	38.02	.213	.0026	81.92307692	-9.025	.099
23	Shade	High water	Low nutrient	2	54.87	.157	.0017	92.35294118	-9.328	.093
24	Shade	High water	Low nutrient	3	47.71	.167	.0019	87.89473684	-8.671	.091
25	Shade	High water	Low nutrient	4	45.46	.166	.0024	69.16666667	-8.209	.109
26	Shade	High water	Low nutrient	5	47.68	.229	.0023	99.56521739	-12.270	.110
27	Shade	Low water	High nutrient	3	55.02	.108	.0014	77.14285714	-6.275	.077
28	Shade	Low water	High nutrient	4	50.11	.147	.0019	77.36842105	-7.935	.095
29	Shade	Low water	Low nutrient	1	42.03	.199	.0021	94.76190476	-9.254	.088
30	Shade	Low water	Low nutrient	2	45.16	.158	.0018	87.77777778	-7.730	.081
31	Shade	Low water	Low nutrient	3	39.68	.257	.0026	98.84615385	-11.628	.103
32	Shade	Low water	Low nutrient	4	30.63	.231	.0021	110	-7.959	.064
33	Shade	Low water	Low nutrient	5	45.41	.179	.0020	89.5	-8.901	.091

Stomatal Conductance of *C. monilifera* in response to changing light intensity.
All values are mean g_s values ($\mu mol\ mol^{-1}$)

PAR	SUNHWHN	SUNHWLN	SUNLWHN	SUNLWLN	SHHWHN	SHHWLN	SHLWHN	SHLWLN
2000	.181	.401	.286	.167	.294	.384	.324	.370
1800	.176	.411	.274	.200	.318	.385	.336	.383
1500	.171	.400	.274	.207	.338	.366	.353	.377
1200	.161	.389	.278	.187	.365	.342	.369	.373
1000	.157	.380	.282	.154	.353	.324	.361	.357
800	.147	.362	.276	.124	.353	.309	.359	.355
600	.132	.346	.277	.096	.342	.292	.349	.344
400	.115	.333	.261	.083	.325	.283	.340	.333
200	.084	.301	.248	.071	.311	.268	.323	.320
150	.071	.280	.225	.058	.291	.256	.312	.304
100	.058	.258	.222	.053	.274	.241	.307	.281
50	.055	.210	.208	.043	.257	.224	.292	.265

Stomatal Conductance of *C. monilifera* in response to changing CO_2 concentrations.
All values are mean g_s values ($\mu mol\ mol^{-1}$)

Ca	SUN HWHN	SUN HWLN	SUN LWHN	SUN LWLN	SH HWHN	SH HWLN	SH LWHN	SH LWLN
50	0.074	0.252	0.172	0.116	0.288	0.264	0.223	0.181
100	0.051	0.286	0.163	0.109	0.283	0.257	0.218	0.180
200	0.047	0.196	0.149	0.099	0.237	0.236	0.193	0.158
300	0.056	0.202	0.146	0.091	0.231	0.229	0.168	0.142
400	0.083	0.296	0.178	0.127	0.349	0.274	0.233	0.192
600	0.085	0.276	0.188	0.143	0.367	0.280	0.238	0.200
800	0.073	0.287	0.188	0.147	0.368	0.269	0.230	0.191
1000	0.092	0.291	0.186	0.147	0.363	0.254	0.219	0.181

Appendix 2 – Whole Plant Hydraulics

Root and shoot Hydraulic Conductance and Root and shoot conductance normalized by leaf area.
All values are actual measurements (kg s^{-1} MPa^{-1} / kg s^{-1} m^{-2} MPa^{-1})

Hydraulic Conductance - Whole Plant											
General conductance measurements											
For Chrysanthemoides monilifera exp											
Light	Water	Nutrient	Kroots(e-4)	Klroots (e-4)	Kshoots (e-5)	Kstem (e-5)	Kleaves (e-4)	Klshoots (e-4)	Klstem (e-4)	Klleaves (e-4)	Leaf area
Sun	High	High	1.376	4.729	1.880	2.170	1.410	.681	.786	5.100	.291
Sun	High	High	4.000	5.682	12.550	31.900	2.060	1.850	4.690	3.030	.704
Sun	High	Low	2.008	5.096	8.183	12.470	2.390	2.260	3.440	6.610	.394
Sun	High	Low	1.572	4.913	1.385	2.048	.429	.433	.640	1.340	.320
Sun	High	Low	.529	4.898	24.800	35.000	8.530	22.900	32.400	78.900	.108
Sun	Low	High	1.811	4.692	5.463	10.460	1.140	1.420	2.710	2.950	.386
Sun	Low	High	1.257	14.465	6.860	8.480	3.570	7.890	9.760	41.100	.087
Sun	Low	Low	-	-	39.300	56.300	13.200	4.580	6.560	15.400	.858
Sun	Low	Low	-	-	17.500	39.800	3.130	2.310	5.240	4.120	.759
Shade	High	High	1.574	13.687	2.341	3.580	.124	2.040	3.110	1.070	.115
Shade	High	High	1.496	12.897	.552	1.234	.100	.635	1.420	1.150	.116
Shade	High	Low	2.068	30.102	.042	1.570	.113	.909	3.230	2.330	.069
Shade	High	Low	.857	13.582	.424	1.350	.062	.673	2.140	.986	.063
Shade	High	Low	5.350	132.754	.931	1.627	.089	2.310	4.040	2.220	.040
Shade	Low	High	1.665	9.737	2.750	5.460	.555	1.610	3.190	3.250	.171
Shade	Low	High	1.528	18.680	5.531	5.920	8.470	6.760	7.240	1.040	.082
Shade	Low	Low	6.158	104.728	.971	1.460	.289	1.920	2.880	5.710	.059
Shade	Low	Low	.784	14.820	1.873	2.100	.014	3.470	3.970	2.720	.053
Shade	Low	Low	4.820	46.346	1.052	2.210	.200	1.340	2.820	2.550	.104

Appendix 3 – Leaf Hydraulics

Leaf conductance and Resistance, as well as resistance of leaf components before and after cutting treatments.
All values are actual measurements (kg^{-1} s MPa / kg^{-1} s m MPa)

Light	Water	Nutrient	Rleaf	Kleaf	Rpetiole	SLA	Lamina area	Dry weight	SDW
Sun	High	High	1.894	5.25	0.755	0.0135	0.00128	0.095	74.219
Sun	High	High	2.864	3.48	0.758	0.0136	0.00117	0.086	73.504
Sun	High	High	5.257	1.9	1.971	0.0153	0.00281	0.184	65.48
Sun	High	Low	2.542	3.83	0.656	0.0158	0.00156	0.099	63.462
Sun	High	Low	0.606	16.5	0.325	0.0142	0.00202	0.142	70.297
Sun	High	Low	2.959	3.38	1.307	0.0193	0.00231	0.12	51.948
Sun	Low	High	2.413	4.14	0.812	0.0165	0.00135	0.082	60.741
Sun	Low	High	2.258	4.4	1.107	0.0206	0.00171	0.083	48.538
Sun	Low	High	1.739	5.75	0.898	0.0215	0.00131	0.061	46.565
Sun	Low	Low	1.736	5.75	0.767	0.0098	0.00126	0.128	101.587
Sun	Low	Low	2.089	4.78	1.138	0.0125	0.00103	0.083	80.583
Sun	Low	Low	2.263	4.42	0.852	0.0231	0.00081	0.035	43.21
Shade	High	High	3.876	2.58	2.513	0.0314	0.00154	0.049	31.818
Shade	High	High	1.168	0.856	0.787	0.0227	0.00129	0.057	44.186
Shade	High	High	3.574	2.79	1.648	0.023	0.00154	0.067	43.506
Shade	High	Low	2.606	3.94	1.185	0.029	0.00168	0.058	34.524
Shade	High	Low	2.551	3.81	1.549	0.0244	0.00246	0.101	41.057
Shade	High	Low	1.338	4.15	0.73	0.0232	0.00241	0.104	43.154
Shade	Low	High	2.098	4.76	1.092	0.0259	0.00101	0.039	38.614
Shade	Low	High	4.031	2.48	1.813	0.0245	0.00135	0.055	40.741
Shade	Low	High	5.544	1.8	3.064	0.028	0.0015	0.054	36
Shade	Low	Low	2.251	4.44	0.779	0.0192	0.0015	0.078	52
Shade	Low	Low	2.769	3.61	1.799	0.0223	0.00145	0.065	44.828
Shade	Low	Low	2.453	4.08	0.884	0.0208	0.00185	0.089	48.108

Leaf conductance and Resistance, as well as resistance of leaf components before and after cutting treatments.
All values are actual measurements (kg^{-1} s MPa / kg^{-1} s m MPa)

Light	Water	Nutrient	Rlamina	Rmincut	Rextravas	Rtertcut
Sun	High	High	1.139	1.316	0.66	0.858
Sun	High	High	2.105	3.17	1.07	1.781
Sun	High	High	3.286	1.446	8.2	0.632
Sun	High	Low	1.886	1.006	4.92	2.091
Sun	High	Low	0.281	2.071	1.253	0.735
Sun	High	Low	1.651	0.77	0.497	1.591
Sun	Low	High	1.66	3.008	1.015	2.99
Sun	Low	High	1.152	1.516	1.019	0.911
Sun	Low	High	0.841	1.644	0.552	2.421
Sun	Low	Low	0.969	1.05	0.596	0.959
Sun	Low	Low	0.951	2.17	0.879	1.813
Sun	Low	Low	0.411	2.622	0.684	2.667
Shade	High	High	1.363	1.715	0.286	1.741
Shade	High	High	0.381	3.892	0.982	2.242
Shade	High	High	1.927	2.896	1.105	1.733
Shade	High	Low	1.421	1.764	0.398	0.729
Shade	High	Low	1.001	1.926	0.625	6.916
Shade	High	Low	0.608	4.08	0.683	1.037
Shade	Low	High	1.006	2.881	1.389	2.575
Shade	Low	High	2.218	2.53	0.548	1.048
Shade	Low	High	2.479	1.739	0.277	2.153
Shade	Low	Low	1.471	4.082	0.816	0.486
Shade	Low	Low	0.97	1.789	0.319	1.461
Shade	Low	Low	1.569	1.819	0.385	3.143

Appendix 4 – Leaf Hydraulics – Allocation of resistance

Leaf resistance allocation to leaf components. All values are represented in percentages.

Allocation to Resistance - Leaf R							
C. monilifera experiments - 8 treatments							
Light	Water	Nutrient	Rpetiole %	Rlamina %	Rextravasc %	Rmajor vein %	Rminor vein %
Sun	High	High	0.410	0.645	0.202	0.298	.104
Sun	High	High	0.263	0.833	0.188	0.366	.197
Sun	High	High	0.384	0.675	0.228	0.277	.126
Sun	High	Low	0.261	0.836	0.247	0.356	.150
Sun	High	Low	0.616	0.436	0.161	0.211	.052
Sun	High	Low	0.458	0.592	0.221	0.305	.039
Sun	Low	High	0.344	0.725	0.168	0.457	.054
Sun	Low	High	0.512	0.535	0.206	0.273	.035
Sun	Low	High	0.543	0.504	0.122	0.238	.126
Sun	Low	Low	0.458	0.592	0.203	0.336	.026
Sun	Low	Low	0.576	0.472	0.131	0.253	.074
Sun	Low	Low	0.387	0.673	0.129	0.474	.044
Shade	High	High	0.705	0.360	0.050	0.198	.105
Shade	High	High	0.740	0.332	0.066	0.185	.077
Shade	High	High	0.479	0.569	0.149	0.383	.017
Shade	High	Low	0.472	0.576	0.100	0.434	.025
Shade	High	Low	0.654	0.403	0.096	0.292	.008
Shade	High	Low	0.578	0.471	0.065	0.372	.026
Shade	Low	High	0.548	0.500	0.156	0.293	.034
Shade	Low	High	0.466	0.584	0.098	0.456	.013
Shade	Low	High	0.586	0.463	0.061	0.380	.015
Shade	Low	Low	0.353	0.713	0.109	0.560	.014
Shade	Low	Low	0.706	0.359	0.053	0.278	.021
Shade	Low	Low	0.368	0.694	0.112	0.532	.021

Appendix 5 – Biomass and Growth Data

Biomass measured parameters of *C. monilifera* when grown in 8 different environmental treatments.

Light	Water	Nutrient	Diameter	Leaf area	Leaf dry wt	SLA	SDW	Stem dry wt	Root dry wt
Sun	High	High	1.5	0.291	33.21	0.009	114.124	46.6	47.24
Sun	High	High	2.3	0.704	148.13	0.005	210.412	151.11	45.36
Sun	High	Low	1.9	0.394	49.33	0.008	125.203	48.78	39.7
Sun	High	Low	1.6	0.32	45.77	0.007	143.031	38.88	36.68
Sun	High	Low	1.4	0.108	15.31	0.007	141.759	28.32	24.33
Sun	Low	High	1.8	0.386	42.35	0.009	109.715	92.96	58.44
Sun	Low	High	1.9	0.087	11.34	0.008	130.495	92.03	39
Sun	Low	Low	2.4	0.858	75.92	0.011	88.485	169.77	44.38
Sun	Low	Low	2.5	0.759	82.82	0.009	109.117	107.79	45
Shade	High	High	1.2	0.115	8.07	0.014	70.174	39.33	5.27
Shade	High	High	1	0.116	7.02	0.017	60.517	17.42	2.38
Shade	High	Low	0.7	0.069	3.75	0.018	54.585	9.35	2.32
Shade	High	Low	0.8	0.063	3.96	0.016	62.758	10.33	1.39
Shade	High	Low	1.4	0.04	2.81	0.014	69.727	11.14	1.33
Shade	Low	High	1.4	0.171	12.94	0.013	75.673	45.91	2.17
Shade	Low	High	1	0.082	5.283	0.015	64.584	16.65	3.75
Shade	Low	Low	1	0.059	3.99	0.015	67.857	11.44	1.58
Shade	Low	Low	0.7	0.053	3.05	0.017	57.656	11.43	2.75
Shade	Low	Low	0.9	0.104	7.88	0.013	75.769	12.59	5.55

Appendix 5 continued..
Biomass measured parameters of *C. monilifera*.

Light	Water	Nutrient	Total biomass	Leaf ratio	Stem ratio	Root ratio
Sun	High	High	127.05	0.261	0.367	0.372
Sun	High	High	344.6	0.43	0.439	0.132
Sun	High	Low	137.81	0.358	0.354	0.288
Sun	High	Low	121.33	0.377	0.32	0.302
Sun	High	Low	67.96	0.225	0.417	0.358
Sun	Low	High	193.75	0.219	0.48	0.302
Sun	Low	High	142.37	0.08	0.646	0.274
Sun	Low	Low	290.07	0.262	0.585	0.153
Sun	Low	Low	235.61	0.352	0.457	0.191
Shade	High	High	52.67	0.153	0.747	0.1
Shade	High	High	26.82	0.262	0.65	0.089
Shade	High	Low	15.42	0.243	0.606	0.15
Shade	High	Low	15.68	0.253	0.659	0.089
Shade	High	Low	15.28	0.184	0.729	0.087
Shade	Low	High	61.02	0.212	0.752	0.036
Shade	Low	High	25.683	0.206	0.648	0.146
Shade	Low	Low	17.01	0.235	0.673	0.093
Shade	Low	Low	17.23	0.177	0.663	0.16
Shade	Low	Low	26.02	0.303	0.484	0.213