The influence of root chilling on the hydraulic characteristics of selected *Eucalyptus* taxa

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Preface

The experimental work described in this thesis was carried out at the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban from January 2004 to November 2008, under the supervision of
Professor N.W. Pammenter.

All contained therein is the original work of the author and has not been submitted, in any form, to another institution. Where the findings of others have been used they have been acknowledged in the text.

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"For there is hope for a tree, if it be cut down, that it will sprout again, and that the tender branch thereof will not cease. Though the root thereof wax old in the earth, and the stock thereof die in the ground; yet through the scent of water it will bud, and bring forth bows like a plant." - Job 14 : 7-10

"Everything considered, we may say that the rise of water is a mystery, provided we do not mean to imply that there is anything mystic about it."

- *The Tree as an Invention* by Charles D. Stewart, 1929

Abstract

The hydraulic conductance of a plant is a significant factor in determining the rate at which transpired water is replenished in the leaves and can therefore limit the leaf water potential that can be maintained. Leaf water potential strongly influences the rate of transpiration and carbon dioxide assimilation, two processes that are cardinal to the growth and development of the plant. In this way whole-plant hydraulic resistance can limit stomatal conductance and photosynthesis as well as the maximum height a tree can attain as stated by the Hydraulic Limitation Hypothesis (HLH). To investigate the effect of such resistances on the hydraulic characteristics of a fast and tall-growing trees it is necessary to manipulate these resistances. Root chilling has been demonstrated to increase hydraulic resistance by influencing suberisation, aquaporin function and water viscosity. Individuals of *Eucalyptus grandis, E. nitens* and *E. grandis* x *nitens* were grown in 15 litre bags and placed in a thermally insulated box with their above-ground parts exposed to the air to ensure optimal conditions for photosynthesis. A soil night/day temperature of 10/15 ˚C was maintained, via a refrigeration unit inside the box, for eight months. It was hypothesised that the chilled plants would show a lower growth rate and whole-plant hydraulic conductance.

A significant negative correlation existed between whole-plant hydraulic resistance and whole-plant biomass in all genotypes. Total biomass was significantly reduced in *E. grandis* and *E. grandis* x *nitens* but not in the cold-tolerant *E. nitens*. Leaf surface area was significantly reduced in all genotypes and in *E. grandis* and *E. grandis* x *nitens* the foliage mass and aboveground

biomass was significantly reduced as well. Height growth was initially significantly reduced in the cold sensitive *E. grandis* but within two months differences were non-significant. Height growth was not affected in the other taxa. Absolute whole-plant hydraulic resistance was significantly increased by root chilling.

The resistance of the soil-to-leaf pathway was significantly lower in chilled *E. nitens* plants and non-significant between chilled and control *E. grandis* or *E. grandis* x *nitens* plants, at the end of the eight month period. The compensation allowing this possibly occurred with the height acclimatisation seen after two months and was most likely the decrease in leaf area.

Only *E. grandis* x *nitens* showed a considerably reduced stomatal conductance and carbon assimilation upon root chilling. Chilled *E. grandis* plants had a considerably higher stomatal conductance and assimilation rate and in *E. nitens* these differences were non-significant. Despite this, chilled *E. nitens* plants had a lower total plant biomass and in *E. grandis* this reduction was significant.

The prediction of the HLH of reduced carbon assimilation via reduced stomatal conductance was therefore not supported by this study. The significant reductions in the transpiration surface and total biomass indicate that the balance between water absorption and loss is indeed a governing factor for plant growth but that plants can compensate to alleviate an increased whole-plant hydraulic resistance by altering, among other things, their biomass partitioning. The significantly reduced total plant biomass in chilled *E. grandis* plants, despite an increased carbon assimilation rate at the leaf level could be due to the non-linear relationship between leaf and canopy carbon assimilation.

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1. Introduction

1.1 The crux of the matter

Growth can be defined as the difference between the amount of carbon fixed through photosynthesis and the amount lost to respiration and litter fall – in other words the net carbon gain. Respiration is a significant sink for photosynthate because for every mole of carbon fixed over the course of a year in a forest, about 0.5 mole must be spent in metabolism (Waring *et al.* 1998). Other than aquatic plants and one or two astomatous plants, such as *Stylites andicola* (Keeley *et al.* 1984) or the shootless orchid *Chiloschista usneoides* (Cockburn *et al.* 1985), atmospheric carbon has to be assimilated through the stomata in the leaves or photosynthetic stems if carbon fixation is to occur. The stomata can be equated to multi-sensory hydraulically-driven valves that integrate a number of stimuli (e.g. vapour pressure deficit, light intensity, internal CO_2 concentration) to maintain an appropriate leaf water balance (Taiz and Zeiger 1998). Stomatal oscillations can be initiated by changes in environmental conditions, such as increases in light intensity or temperature, or may occur spontaneously due to increases in root hydraulic resistances (Meidner and Sheriff 1976). In large trees branches are often separated sufficiently for the leaves to experience different microclimates, causing their stomata to oscillate with different periods or amplitudes (Meidner and Sheriff 1976). Such asynchronous oscillatory behaviour also occurs at the leaf level and is related to the leaf's hydraulic anatomy with patchy stomatal conductance being more common in heterobaric (where the leaf is divided into patches by bundle sheath extensions) than homobaric or monocotyledonous (parallel) vein anatomy (Prytz *et al.* 2003a). Particular conditions may cause the water regulatory system to behave like a mildly chaotic system with complex oscillatory patterns which may be advantageous by "allowing rapid, fine-tuned responses to environmental stimuli" (Prytz *et al.* 2003b).

Carbon assimilation into the leaves is complicated by transpiration. This is the process of water vapour loss through the stomata and is driven by the water vapour pressure difference between the inside and outside of the leaf, which tends to be very high under high light intensities. Transpiration is therefore a necessary evil wherever wet cell surfaces are exposed to the air (Curtis 1926) and is a problem because the diffusion gradient driving water loss is approximately 50 times greater than that driving CO_2 diffusion into the leaf (Taiz and Zeiger 1998). Hence the leaf water potential regularly falls below a critical level causing stomata to close, interrupting or decreasing carbon assimilation but preventing excessive water loss and plasmolysis. The rapidity with which stomata reopen and growth is resumed depends upon the water-supplying ability of the hydraulic architecture of the plant. The resistance to water flow increases with the size and age of a tree and photosynthesis tends to decline with age (Ryan and Yoder 1997). Stomatal conductance is sensitive to changes in the hydraulic resistance and it is therefore the common factor between photosynthesis and hydraulic resistances and explains how hydraulics can limit growth. This is the essence of the Hydraulic Limitation Hypothesis which was formally proposed by Ryan and Yoder (1997).

One way of testing this hypothesis is by manipulating the hydraulic resistances of a plant and to assess the effect on stomatal conductance, which responds almost instantaneously (Sperry 2000). This has been done by a number of researchers; the methods most frequently employed are injecting air into the sapwood (Hubbard *et al.* 2001), cutting notches into branches (Hubbard *et al.* 1999), removing a percentage of the foliage (Hubbard *et al.* 1999; Phillips 1927) or pouring cold water (near 0 ºC) on the soil of a potted plant (Cochard *et al.* 2002)

and measuring the effect on stomatal and hydraulic conductance. The drawback of such treatments are that they are short term, "once-off" manipulations and plants respond relatively quickly to such "insults" by producing more sapwood or foliage. A long term manipulation of the hydraulic resistances could possibly give a more accurate reflection of their importance to stomatal behaviour and growth. By comparison such studies are rare; examples are Chesterfield *et al.* (1991) and Manoharan (Unpublished Ph.D. thesis, 2002) who subjected *Eucalyptus* species to root chilling and found significant reduction in height growth and net CO_2 assimilation respectively.

There are a number of reasons why root chilling is an effective technique for the long term manipulation of whole plant hydraulic resistances. Root chilling has been employed, very often in solution culture, to manipulate hydraulic conductance and to investigate chilling sensitivity. Each plant part has its own resistance to water movement: R_{root}, R_{stem}, R_{branch} and R_{leaf}. These resistances, because they are in series, are additive and thus their sum constitutes the whole plant resistance: R_{plant}. The root system is considered to give a disproportionately large resistance to water movement because it is here that water has to move radially across concentric layers of root tissues (epidermis, cortex, endodermis and pericycle) to enter the relatively low resistance axial pathway in the xylem vessels (Jensen *et al.* 1961; Boyer 1969; Steudle 2001). Another anatomical feature increasing R_{root} is the Casparian band. It consists of suberin (a waxy, waterproof substance) and possibly lignin which is deposited in the cell walls of the endodermis, blocking the inter-microfibrillar pores (Drew 1987). The Casparian band and the protoxylem elements mature simultaneously (Dickison 2000). It constitutes a barrier to water movement through the apoplast, forcing water to use the high resistance symplastic and transcellular routes. Very low soil

water potentials (North and Nobel 2000) as well as chilling temperatures have been found to increase the degree of suberisation in the endodermis, decreasing hydraulic conductance (Lee *et al.* 2005). Chilling temperatures can also increase hydraulic resistance by altering the conformational status of aquaporins, causing them to close (Lee and Chung 2005).

This project is an expansion on the work of Manoharan (unpublished Ph.D. thesis, 2002) using a larger number of replicates, larger bags for the roots to grow in, and running the chilling treatment for longer. The aim is to subject three commercially important *Eucalyptus* taxa – *E. grandis, E. nitens* and *E. grandis* x *nitens* – to a long term root chilling treatment and to assess the influence of this on the hydraulic characteristics and net CO_2 assimilation of the plants. *E. grandis* thrives under warm, humid conditions and is very sensitive to frosts and cold winds when still at the sapling stage (Poynton 1979). *E. nitens* is a cold and frost tolerant species that is grown at higher elevations (Poynton 1979; Swain and Gardner 2004). It is hypothesised that the chilling treatment will increase the hydraulic resistances causing significant decreases in hydraulic conductance. This is expected to cause a cascade of secondary effects such as potentially lower leaf water potentials (and hence lower leaf stomatal conductances) decreasing biomass accumulation and plant height. It is hoped that this study will provide some insight into the influence of hydraulic resistances on the physiology of these quick-growing taxa.

1.2 Literature review

1.2.1 Eucalypts in forestry

Eucalyptus is a relatively large angiosperm genus of approximately 600 evergreen, hardwood species that are native to Australia and Tasmania (Doughty 2000) and are grown extensively in many countries (Wilks 1988). They exhibit great variation in habit and grow well in a wide range of habitats (Cremer 1960), to the extent that they are invasive aliens in some areas like the fynbos of South Africa (MacDonald and Richardson 1986). They are, however, primarily known as straight and fast growing trees grown in the forestry industry. "*Eucalyptus* is the most cultivated tree genus in the world due to its economic relevance and fast growth." (Da Rocha Correa and Fett-Neto 2004). Doughty (2000) mentions that 37.5 percent of all tropical forest plantations consist of *Eucalyptus* species with Brazil being the world's foremost producer of *Eucalyptus* wood. In the warmer regions of the world *Eucalyptus* plantations amount to approximately 10 million hectares (De Paula Lima *et al.* 2003). On the coastal savannahs of the Democratic Republic of the Congo, clonal *Eucalyptus* plantations were established in 1978 and have, since then, increased in extent to about 42 000 ha (Saint-Andre *et al.* 2002). In the Congo, as well as in South Africa, optimal harvesting age is considered to be seven years (Laclau *et al.* 2004; Tyree 2002) as opposed to an 8 to 15 year rotation period of the native hardwoods of North America (Mehra-Palta 1982). In South Africa and Brazil the forestry industry is based upon exotic species and it has been singularly successful because the species are separated from their natural enemies and pathogens. *E. globulus* was the first species to appear in the Cape colony's gardens in 1828 (Poynton 1979) but the first *Eucalyptus* plantation was only established at Worcester in 1876 to stem the growing shortage of timber (Van der Zel and Brink 1980). Eucalypts are the preferred species for forestry areas with a low rainfall (Dye 2000).

1.2.2 Water use efficiency

Biodiversity conservation and sustainable development have become increasingly important to governments and Non-Governmental Organisations worldwide and a frequently mentioned factor in such discussions is water management. The value of *Eucalyptus* plantations is not enthusiastically approved by everyone because their environmental impacts, especially water consumption, are far from being resolved (Doughty 2000; De Paula Lima *et al.* 2003). *Eucalyptus* plantations are known to decrease water levels in catchments (Le Roux *et al.* 1996; Laclau *et al.* 2004) and, with forestry being legally classified as a stream flow reducing activity, it is likely that new afforestation will occur in marginally productive rather than highly productive areas (Dye 2000). Three years after planting, *Eucalyptus* stands caused a significant decrease in stream flow of 300 to 380mm per annum (expressed as a rainfall equivalent) in a Transvaal catchment, South Africa (Van Lill *et al.* 1980). Genotypes that can moderate their water use, allowing them to survive or even grow in dry soils, would therefore be very useful to expand plantation ranges. Under drier conditions optimal water use efficiency is more important for survival than maximum water use efficiency (Dye 2000). In other words plants whose hydraulic architecture is geared to maximal water use efficiency transpire excessive amounts of water, giving them a much increased growth rate but decreased survival probability the moment water becomes limiting. Plants whose hydraulic architecture inadvertently "rations" their water use have a better chance of surviving and growing when water is the limiting factor; however, they are often unable to take adavantage of a sudden increase in water availability (February *et al.* 1995). Water Use Efficiency (WUE) is a measure of the relationship between carbon accumulation and water loss and is expressed as the ratio of moles of carbon dioxide fixed to

moles of water lost (Lajtha and Marshall 1994; Taiz and Zeiger 1998). At the leaf level plant physiologists often define photosynthetic water use efficiency as the photosynthetic rate divided by the water transpired (Calder *et al.* 1993). Whole plant water use efficiency is defined as the change in biomass over a period of time divided by the amount of water transpired in that period as described by the following equation (Pammenter 2002):

$WUE_{plant} = \Delta B/T_r$ …(1)

ΔB represents the increase in biomass over the specified period of time and T_r the amount of water transpired during that period. Generally forests (including *Eucalyptus* stands) transpire considerably more water than agricultural crops and a suggested strategy for recharging the soil water is to rotate *Eucalyptus* stands with a particular crop species; this could also be beneficial because deep-rooted trees have been found to bring nutrients from deep soil layers to the surface (Calder *et al.* 1993)

A high growth rate would increase the biomass and increase WUE if T_r remained unchanged, but high growth rates require an adequate supply of water. Site preparation and management such as weeding are therefore important because they minimise the pathways for water loss other than through the trees of interest. Bird *et al.* (2000) reported that a combination of procedures such as ripping, mounding and fertiliser application improved early tree growth in *E. globulus*. Similarly Calder *et al.* (1993) found fertiliser application to increase the water use efficiency of *Eucalyptus* stands. Biomass partitioning is a malleable characteristic and mechanisms exist that enable plants to regulate the partitioning of photosynthate, between the above and below ground parts, according to the

particular environmental conditions (Persson 2002; Melamy 2005). Working with *E. nitens* plantations Misra *et al.* (1998) found a significant decrease in the amount of below-ground production per unit above-ground production of biomass with increased rates of fertilisation. However, increasing the total biomass is less important in agriculture than increasing that part of the biomass of interest. Genotypes with an partitioning pattern favouring that which is to be harvested (fruit in apple trees, stalk in sugarcane or tubers in potato) are said to have a high Harvestable Index (HI), the ratio of the biomass of interest to total biomass (Awal *et al.* 2003); in forestry this would be the stem. Genotypes that have a high WUE with respect to stem production are therefore highly sought after.

It is not always clear what are the underlying mechanisms that cause changes in the hydraulic architecture in trees. A frequently asked question is: "Are such changes genetically determined or due to environmental factors?". Wilks (1988) concluded that wood anatomy is determined more by genotype than the environment and that there is a "…weak relationship between growing conditions and xylem anatomy in *Eucalyptus*". On the north-coast of New South Wales, Australia, Bamber *et al.* (1982) found that continuous fertiliser application and regular weeding and insecticide treatment of an *E. grandis* plantation significantly increased the growth rate of the trees. With the increase in growth rate was associated a significant decrease in vessel frequency and diameter and an increase in ray volume; the basic wood density and fibre dimensions were unchanged. They conclude that, considering the fact that trees are oversupplied with conducting tissue, the decrease vessel dimensions may have little effect on conductive capacity but that the increased ray colume is indictive of a greater requirement for lateral transport and food storage in rapidly growing trees. Gava

and de Moraes Gonçalves (2008) found a higher soil clay content to decrease the lignin content and cause an exponential increase in holocellulose content in *E. grandis*; wood density was unaffected. Wood production and quality was significantly higher on the clay soils which Gava and de Moraes Gonçalves (2008) ascribed to the soil type's inherently higher water content. February *et al.* (1995) found that water availability had a significant effect on vessel diameter, length and frequency in *E. grandis* and two hybrids *E. grandis* x *camaldulensis* and *E. grandis* x *nitens.* A study on *Combretum apiculatum* and *Protea caffra* populations that span areas differing in rainfall and temperature showed that the hydraulic architecture of both species changed significantly in response to the gradient in environmental factors (February 1993). An extensive survey of commercial wheat varieties by Richards and Passioura (1981) showed vessel diameter to be a more variable trait (than vessel frequency) with high heritability in most populations, making it an important trait for breeding programmes aimed at producing wheat varieties with a more conservative water use.

Amid this ongoing polemic Vander Willigen *et al.* (2000) put forward a new perspective. Comparing four subtropical species of different habit and habitat that were growing together, they concluded that conduit dimensions and pit pore sizes (assessed indirectly as vulnerability to cavitation) were genetically determined while leaf area was the more phenotypically plastic attribute; by changing this factor plants can control transpiration to prevent cavitations. In a comparison of five species of *Acer* (two softwoods, two hardwoods and a ruderal) Woodrum *et al.* (2003) found that, although the hardwoods had a significantly higher density wood than the softwoods, there was no inverse relationship between mechanical strength parameters and conductive efficiency. Woodrum *et al.* (2003) mention that their specimens most likely experienced

similar climatic conditions because they all came from a single 108.7 ha woodlot and that the absence of a trade-off between density and conductance could occur if water transport is not limiting to plant growth. Franks *et al.* (1995) found significant differences in the xylem permeability, embolism susceptibility and stem to branch hydraulic connections in *E. camaldulensis* trees growing on a xeric and mesic site; it is interesting to note that, despite their genetic relationship, a striking difference was observed between their hydraulic architectures when specimens from both sites were planted in the same environment. Among a taxonomically diverse set of Californian shrub species Preston and Ackerly (2003) showed that the shrubs had undergone repeated evolutionary changes in hydraulic architecture and leaf-stem allometry in response to water availability, implying a significant long term effect of the environment.

1.2.3 Water transport and hydraulic architecture

A great variety of architectures has evolved to suit different species' circumstances. A number of measures are used to define a hydraulic system and have been reviewed by Tyree and Ewers (1996). The volume of water transported by a branch or stem per unit time per unit pressure gradient is the hydraulic conductivity (K_h), a characteristic largely influenced by the mean xylem vessel diameter and frequency as well as the size of the pit membrane pores through which water flows from vessel to vessel. The frequently used measure of sapwood hydraulic efficiency is the specific conductivity (K_s) which is the ratio of the hydraulic conductivity to sapwood cross-sectional area. Leaf specific conductivity (K_l) is calculated by dividing K_h, of a stem segment, by the leaf area distal to the segment. This then is a measure of the hydraulic sufficiency in terms of supplying water to the leaves.

The structures involved in water transport are collectively called the xylem and consist of various tissues such as tracheids, vessels, fibres and parenchyma cells (Tyree and Ewers 1996). Fibres are mainly involved in mechanical support whereas parenchyma cells are involved in capacitance and food storage (Bamber *et al.* 1982). Tracheids and vessels are the two main conduit types that start life as undifferentiated, expanding parenchymatous cells that are functional only once programmed cell death (PCD) has occurred, leaving hollow cell corpses behind for water conduction (Kozela and Regan 2003). Xylem development, or "xylogenesis", is a complex process involving enzymes, structural proteins and specific ratios of hormone and carbohydrate concentrations (Roberts 1969). Auxin plays a key role in the process but cytokinins and gibberellins are known to act in concert with it (Noel *et al.* 1977). Nitric oxide has recently been identified as a key factor in the mediation of PCD and lignification (Gabaldón *et al.* 2005). Roberts (1969) cited indirect evidence of an unknown "xylogenic factor" that induced xylogenesis in mature vascular tissue. The findings of Savidge and Wareing (1981) also alluded to the presence of a "Tracheid Differentiating Factor" that was distinct from indole acetic acid (IAA) which is the most studied natural auxin (Casson and Lindsey 2003). Tracheids are phylogenetically more primitive than vessels and occur in both angiosperms and gymnosperms whereas vessels are found only in angiosperms and the small gymnosperm group Gnetales (Taiz & Zeiger 1998). Tracheids originate from a single cell and are typically three to five millimetres in length (Milburn 1979). In general they are much shorter and narrower than vessels, making them less efficient at conducting water. However, they serve the dual purpose of water conduction and mechanical support; a high conduit lumen to conduit wall

thickness ratio, such as found in vessels, would make them inefficient for mechanical support (Comstock and Sperry 2000).

Vessels are multicellular in origin and vary greatly in length and diameter. They range from a few millimetres to many metres in length, with the majority being a few centimetres in length and a very small proportion of them spanning the entire length of the tree (Zimmermann and Jeje 1981). Vessels are made up of vessel elements stacked end to end in vertical files; during hydrolysis the end walls of elements are lost or at least perforated, allowing water to pass directly from element to element (Dickison 2000). Vessel elements under 350µm in length are considered short and those over 800µm are long (Dickison 2000). Both tracheid and vessel element walls are decorated with pits through which water moves laterally between conduits. In vessel walls these are depressions that lack the secondary thickening and lignification common to the rest of the wall and the "…thin compound middle lamella of adjacent primary cell walls is modified to form a relatively porous pit membrane" (Sperry and Hacke 2004). In tracheids the pit membrane structure is very different. A solid torus is held in a central position by cellulose strands constituting the membrane, called the margo.

To increase the water conducting capacity of the sapwood, plants can either increase the number of xylem vessels per unit area or increase their efficiency by increasing the vessel diameters. Vessel diameter is by far the most influential for a system's water transporting ability. The volume flow rate through any pipe is directly related to the fourth power of its radius as described by the following equation (Taiz and Zeiger 1998):

$$\text{Volume flow rate} = (r^4\pi/8\eta)/(\Delta P/\Delta x) \qquad \ldots(2)$$

where r is the conduit radius, η is the dynamic viscosity of the fluid and $\Delta P/\Delta x$ the pressure gradient across the length of pipe. This is known as the Hagen-Poiseuille equation and indicates that water flux through a conduit is very sensitive to the conduit radius. If the radius was doubled the resistance to water flow would be one sixteenth of the initial value. The surface area in contact with the pit membranes, determining interconduit pit transport, would increase only linearly, meaning that with increasing conduit diameters pit resistance will become increasingly limiting (Comstock & Sperry 2000). There is a stark contrast in the function of pits: they have to be large enough not to be too limiting to water flow between conduits but small enough to exclude air bubbles that can cause cavitations. Presumably the conduit dimensions have evolved to optimise these conflicting functions "…providing the necessary hydraulic conductivity with the least investment in wall material and at a given safety from air-seeding and implosion"(Sperry and Hacke 2004).

A corollary of the Hagen-Poiseuille equation is that the amount of water flowing through the narrow vessels is insignificant compared to that flowing through the wider vessels. The majority of narrow vessels therefore serve as mechanical support or capacitance. Lianas and vines have been called "structural parasites" (Tyree and Ewers 1996) because of their reliance on trees to reach light. The fact that they have little need of mechanical support, and that they have to compensate for narrow stems, is thought to be the reason why they have significantly larger vessel diameters than tree species (Fisher and Ewers 1995).

Wider conduits therefore have much higher hydraulic conductances. In an in-depth analysis of the mechanics of conduit design Hacke *et al.* (2004) point out

that an increase in diameter has to be accompanied by an increase in conduit wall thickness to keep the walls strong enough to withstand implosion. Pit depressions will therefore be deeper, decreasing their conductivity. However, this decrease in conductance pales in comparison with the increase in conductance due to an increase in length. They also found that the torus-margo design is more efficient at conducting water due to fewer microfibrils in the margo which means larger spaces between them for water to pass through. However, overall tracheid conductance is still significantly less (than that of vessels) due to their length (Hacke *et al.* 2004). The practical limits to minimum and maximum conduit diameters is from 5 – 10µm and 500µm (Tyree 2003).

In addition to this anatomical division another difference of great ecological importance has evolved, namely diffuse-porous and ring-porous xylem. In the ring-porous design conduits of large diameter are produced early in the growing season, forming a distinct ring of large vessels followed by a number of rings of narrow vessels. This is often found in deciduous species which produce a single flush of leaves at the beginning of spring. In diffuse-porous xylem wide and narrow vessels are distributed at random. This is characteristic of deciduous species producing leaves throughout the growing season, and of evergreen species, such as *Eucalyptus* species (Wilks 1988), that retain their canopies throughout the year and therefore always experience a high transpirational demand. To survive dry seasons such species often have higher Huber values (the ratio of the cross-sectional area of a stem to the leaf area supplied by the stem) which in turn gives them higher leaf specific conductivities (LSCs) than deciduous species (Eamus 1999). Higher LSCs enable a plant to maintain the same transpirational flux at a lower pressure gradient from the roots to the foliage (Tyree and Ewers 1991). Pioneer species are characterised by very fast growth

and were found to have significantly higher whole-plant hydraulic conductance than late succession species, which enabled them to colonise gaps and outgrow the shade-tolerant species destined for the next successional stage (Tyree *et al.* 1998; Becker *et al.* 1999). Similarly, in Australian forests and savannahs *Eucalyptus* species are often quick-growing pioneer species that are eventually phased out unless disturbances such as fires occur frequently, earning them the epithet "transient fire weeds" (Cremer 1960).

1.2.4 The mechanism of water transport: opinion is divided

When the Cohesion-Tension theory of sap ascent was proposed it was not widely accepted by all researchers. It was, and remains, an elegant, comprehensive and testable explanation of the mechanism of sap ascent. It assumes that the water columns in plants are continuous from root to leaf regardless of the tree's height. A loss of vapour from the leaf will therefore transmit a tension down the column and water is then drawn up to replace the transpired water. However, ever since its inception "this venerable theory has been beset by seemingly conflicting observations" (Scholander *et al.* 1965). The most notable discrepancy being the presence of water potentials, within physiological range, in foliage of trees near 100 metres tall, yet anyone familiar with plumbing systems knows that it is inadvisable to install a suction pump on a third storey because at best it can lift water 33 feet (Stewart 1929). Although the Cohesion-Tension theory is the most widely accepted model of sap ascent it does require water columns in the xylem to withstand tensions of such magnitude that many researchers are still sceptical about the theory's validity (Steudle 2001), and consequently "the mechanism of axial water transportation in plants is still hotly debated" (Schäffner 1998; see also Zimmermann *et al.* 2004 and Angeles *et al.* 2004). Experiments have been done that attest to the remarkable cohesive forces of water (Briggs 1950; Zheng

et al. 1991; Pockman *et al.* 1995) but Smith (1994) criticised these experiments for using pure water – something rarely found in biological systems – and he stated that cavitations are typically due to adhesive, rather than cohesive failure. Richter (2004) is of the opinion that older publications "…convincingly demonstrated that transport occurs completely without the participation of living cells" which is an important point for the Cohesion-Tension theory because it states that the vapour pressure gradient between the leaf and the air is the sole source of energy for long distance water transport. Water transport is therefore a passive process which is supported by the observation that more than 90 percent of the water taken up by a plant is not directly coupled to growth but is lost via transpiration (Hacke and Sperry 2001).

Opposition to the Cohesion-Tension theory gained renewed impetus with the development of the pressure probe in the 1970s – an instrument enabling one to measure turgor changes of single cells in real time in intact plants and which was later modified to measure xylem element tensions (Tomos and Leigh 1999). It can accurately measure pressures down to –1 MPa (Zimmermann *et al.* 1995) and using this instrument Zimmermann *et al.* (1994) did not find a tension gradient increasing with one atmosphere with every ten metre increment in height in a tall forest tree, as was predicted by the Cohesion-Tension theory. Zimmermann *et al.* (1994) are adamant that the data they collected demands a re-evaluation of our understanding of pressure bomb measurements and long distance water transport in trees.

A relatively recent observation, made using the technique of cryo-scanning electronmicroscopy (cryo-SEM), is difficult to reconcile with the Cohesion-Tension theory. In this technique petioles, stems or roots are frozen *in situ* on a

transpiring plant, using liquid nitrogen (Cochard *et al.* 2000; Facette *et al.* 2001). Cross-sections of the samples are then viewed using cryo-SEM and those conduits not filled with ice are considered to be embolised. Theoretically this provides snapshots of the water status of the transpiration stream at a particular time of day. Using this technique Canny (1995) showed that the percentage of embolised vessels decreases during the peak period of transpiration of the day. To explain this he proposed a "Compensating Pressure Theory" of axial water movement which asserts that embolised vessels are refilled under a positive, compensating pressure with water from the phloem. According to this theory the water columns are ultrastable and not metastable, as proposed by the Cohesion-Tension Theory. According to Canny (1998) the Cohesion-Tension theory has remained the dominant model for water transport in trees for over a century because of "…a tacit agreement to ignore large bodies of experimental fact about water transport that were once well known and widely believed to be essential".

Cochard *et al.* (2000) found that the freezing rate when using liquid nitrogen was too slow to freeze vessel contents intact when the tension on the water column was greater than approximately -0.1 MPa. They therefore conclude that partially air-filled conduits are artifacts of the cryo-SEM technique and should be reconsidered before being used as arguments against the Cohesion-Tension theory.

1.2.5 Tensions and cavitations

The water columns in the conduits are continuous from root to leaf and hence this path of water movement is termed the Soil-Plant-Atmosphere-Continuum (SPAC). The driving force for water transport is the vapour pressure deficit between leaf and air which is profoundly influenced by both the leaf and air

temperature. As temperature increases the relative humidity decreases, increasing the water-holding capacity of the air which promotes transpiration. This creates a tension drawing water from the soil solution to replace the water lost at the leaves. This tension oscillates in tandem with the transpiration and a clear manifestation of this is the dilation of the tree trunk during a 24 hour period. The tension usually reaches a peak at midday when the stem diameter is at a minimum (Phillips 1927; Federer and Gee 1976). Daum (1967) measured the dilation of branches of a Y-shaped tree and recorded a greater transpiration rate and tension on the east facing branch early in the morning. The rate of transpiration often exceeds that of sap flow; this is due to the resistance to water absorption offered by the roots (Kitano and Iguchi 1989; Coelho Filho *et al.* 2005) – a phenomenon called the "absorption lag" (Meidner and Sheriff 1976). Transpiration can continue because of a buffer-volume of water stored in the stem tissues (Kitano and Eguchi 1988). This volume of water stored in the stem tissues is related to the concept of hydraulic capacitance, which is defined as the mass of water that can be extracted per megapascal change in water potential of the tissue (Tyree and Ewers 1996; Phillips *et al.* 2003). Examples of absorption lag times are ten minutes in cucumber plants (*Cucumis sativus* L.) (Kitano and Eguchi 1988), 30 minutes in navel orange trees (*Citrus sinensis* [L.] Osbeck) (Steppe *et al.* 2006) and 15 to 45 minutes in four to five year old Douglas fir saplings (*Pseudotsuga menziensii* [Mirb.] Franco) (Hinckley 1971).

The resistance to water absorption by the roots and water transport through the vasculature means that water loss at the leaves exceeds water transport to the leaves whenever high vapour pressure deficits develop. This results in large tensions developing in water columns in the xylem vessels. In addition to this the gravitational pull on the water columns increases by 0.1 MPa for every ten metre

increment in height. Using a model plant, starting with one metre of trunk between the roots and leaves, Raven and Handley (1987) calculated that, assuming the biomass partitioning stayed constant, to keep the water potential difference between roots and leaves constant, as the tree increases in height the sapwood cross-sectional area to leaf area ratio would have to increase in direct proportion to the height. Empirically this has been found not to occur and thus it is the water potential difference (tension) instead that has to increase in direct proportion to height, unless of course sapwood conductance can be increased or transpiration decreased (Raven and Handley 1987).

Due to the wick-like absorption of water by the narrow capillaries formed by the cellulose microfibrils of the cell walls and the high cohesive forces among water molecules, the columns remain largely intact, although metastable, allowing water transport to occur. The hydrogen bonding between water molecules confers a high tensile strength upon water which is approximately ten percent that of copper wire (Taiz and Zeiger 1998); aqueous water inclusions in quartz crystals have been found to withstand tensions of up to 140 MPa (Zheng *et al.* 1991). However, under conditions of water stress, water columns do cavitate, which is the sudden breaking of a water column. Initially the conduit will be very close to vacuum conditions but it is soon filled with air that is drawn out of solution from nearby conduits (Tyree and Ewers 1991). The actual rupture of a water column under tension is called cavitation and the resulting gas-filled conduit is called an embolised conduit (Dickison 2000). Roberson *et al.* (1998) mention that gas bubbles expand explosively when the water column's tension is less than the vapour pressure of water and that such expansion is termed "boiling" when it is due to an increase in temperature at constant pressure and "cavitation" when it is due to a hydrodynamically induced pressure reduction at constant temperature.

The most widely accepted hypothesis for embolism formation is the air-seeding hypothesis proposed by Zimmermann (1983). According to this theory, cavitation events occur when an air bubble is drawn through one of the pit membrane pores from an air-filled conduit, which is near atmospheric pressure, to a water-filled conduit. The water-filled conduit is under tension, causing the air bubble to expand explosively upon entry, filling the conduit completely and the tension in the water column relaxes. The vibrations associated with cavitations were first detected acoustically by Milburn and Johnson (1966) using a record player pick-up head connected to an amplifier; they reported a high correlation between the frequency of "clicks" heard and the leaf's water status. This is the principle behind modern instruments that are capable of sensing acoustic emissions in ultrasonic frequencies which enables them to exclude the background noise from extraneous vibrations.

Cavitations decrease the transport efficiency of the whole stem because the cavitated vessel cannot transport water until the air bubble is dissolved. Under sunny, warm conditions the transpirational demand at the leaves can only increase. This increases the tension in the remaining functional water columns, increasing the probability of further cavitation events. Cavitations are therefore "serious dysfunctions to be avoided" by plants because they can give rise to runaway cavitation cycles (Tyree and Sperry 1989; Tyree and Ewers 1991). This can result in the die-back of branches and the eventual death of the tree. When temperatures fall below 0 ºC the water inside vessels often freezes, causing air dissolved in the water to come out of solution. A study by Davis *et al.* (1999) confirmed that narrow vessels are relatively resistant to cavitation by freezing. They found that a modest water stress of -0.5 MPa caused a significant increase in cavitations after one freeze-thaw cycle in vessels with a diameter of 44 µm and

above, whereas conifers and diffuse-porous angiosperms with mean conduit diameters below 30 µm showed no freezing-induced cavitations at the same water stress. The higher hydraulic conductance but lower safety of wide and long conduits gives rise to a "see-saw" relationship which plants need to balance by finding the optimum conduit dimensions matching the water availability in their particular environment. Plants with increasing tracheid diameters are therefore increasingly abundant closer to the equator. In ferns, for example, vessels are thought to have developed first in those populations occurring in areas with a moderate degree of seasonality (Schneider and Carlquist 1998). A plant's hydraulic architecture can therefore influence its distribution range; plants with a high vulnerability to cavitation, whether it is freezing-induced or otherwise, will be restricted to areas with a high average rainfall. Contrary to this elegant explanation, Brodribb and Hill (1999) compared the cavitation thresholds and hydraulic conductivities of stems of ten conifers and found the most cavitation resistant species to have the highest specific conductivity and leaf specific conductivity. As an explanation they state that, assuming xylem vulnerability is related to pit membrane pore diameter, the hydraulic effects of small pore diameters might be offset by an increase in the number of pits per conduit, allowing the two parameters to vary independently of each other.

The vulnerability of particular hydraulic architectures to cavitations can be represented by a "vulnerability curve" which is a plot of the percentage loss in conductivity (PLC) against the water potential necessary to induce the loss. A vulnerability curve provides information on how drought-induced xylem pressure potentials influence hydraulic conductivity (Tyree *et al.* 1991) and its shape is species-specific (Tyree and Ewers 1996). Assuming the air-seeding hypothesis is correct, vulnerability to cavitation is related to the diameter of the largest pit pore

of a vessel rather than vessel dimensions; cavitations at low tensions therefore imply large maximum pore diameters while cavitations at high tensions imply small pore diameters (Pammenter and Vander Willigen 1998). However, because the liquid-gas phase is discontinuous there is an energy barrier to the formation of bubbles large enough to cause cavitions in the xylem and thus the assumption that the presence of the tiniest bubble will induce a cavitation event is incorrect (Oertli 1971; Williams and Williams 2004).

To restore its hydraulic conductance a tree can either produce more xylem or "reclaim" embolised elements by dissolving the air bubble occupying it. It is reasonable to assume that the amount of air dissolved in the xylem sap is the maximum concentration at atmospheric pressure (Tyree and Ewers 1996). The pressure on an embolised element will therefore have to exceed atmospheric pressure and become positive, for at least a short time, because the solubility of a gas increases as the gas pressure is increased, a relationship known as Henry's law (Brady and Holum 1981). Transpiration, however, draws the pressure in the water columns below atmospheric pressure and embolisms, once formed, are rarely dissolved (Tyree and Ewers 1996). The positive pressure generated by root pressure should theoretically be dissipated by the gravity gradient and be ineffectual higher than 1.1 metres above ground level (Tyree and Ewers 1996).

For a bubble to be stable the tendency for it to collapse must be balanced by the pressure difference across its surface, which is the difference between the absolute gas pressure of the bubble and the absolute pressure of the surrounding water (Zimmermann and Tyree 2002). Tensions in the water columns tend to increase with transpiration, causing a decrease in xylem pressure potential and hence bubbles can grow to form an embolism in the conduit. Air injection and

dehydration experiments have shown that plants can restore their hydraulic conductance by dissolving the air bubbles while the adjacent water-filled vessels are at tensions below -1 MPa (Salleo *et al.* 1996; Tyree *et al.* 1999). This reclaimation of embolised vessels does not occur in all species and occurs faster when the embolism is artificially introduced by air injection rather than through drought (Hacke and Sperry 2003).

Our current understanding does not provide a satisfactory explanation for this phenomenon (Tyree *et al.* 1999) but other theories have been put forward. Holbrook and Zwieniecki (1999) proposed that solutes are secreted into the embolised vessel by xylem parenchyma cells thus decreasing the osmotic potential and setting up a driving force for water movement into the vessel. The possibility of a positive pressure acting locally requires that the vessel be hydraulically isolated from surrounding water filled vessels that are under tension. According to Holbrook and Ziwieniecki (1999) this is made possible by the hydrophobic nature of the lignified secondary wall and the "inverted funnel" geometry of the bordered pits trap pockets of air that sever any hydraulic connections until the embolism is completely dissolved. This theory has been dubbed the "pit valve hypothesis" by Hacke and Sperry (2003). In support of this theory Vesela *et al.* (2003) have calculated that such a refilling process is indeed physically possible providing that the vessel is hydraulically isolated.
Hacke and Sperry (2003) have proposed the "pit membrane hypothesis" to explain the observed refilling. Here an osmotic gradient is also responsible for pulling water into the embolised vessel, however, it is not necessary for the vessel to be hydraulically isolated. They argue that if the hypothetical osmoticum is larger than the pit membrane pores the membrane will act as a selectively permeable membrane and water can be drawn from the transpiration stream

which is under tension. This mechanism would work only in species whose pit membrane pores are small enough to exclude the osmoticum, exlpaining why refilling does not occur in some species.

1.2.6 The nature of plant hydraulic resistances

Plants exhibit remarkable adaptations not only because they are sessile but because they exist at the interface of the soil and the atmosphere. Therefore there exists a water potential difference between the roots and the aerial parts of the plant. If plants were devoid of hydraulic resistances water would move instantaneously from the point of high to low pressure potential and the water potential would stabilise instantly in the plant (Meidner and Sheriff 1976).

Georg Simon Ohm (1787-1854), a German physicist, postulated that, for a given conductor at a constant temperature, the ratio of the voltage across the ends and the current flowing through the conductor is a constant (The New Encyclopedia Britannica 2003). This ratio is the resistance expressed in the unit Ohm. An analogy between Ohm's law and water transport in plants is particularly apt because water transport through each component – root cells, tracheids and xylem vessels, leaf mesophyll cells and from the intercelullar air spaces to the atmosphere – is governed by a potential difference on either side and a resistance in between (Van den Honert 1948; Richter 1973) If the plant is considered as an electric circuit water transport can be seen as a current passing through a series of conductors; the flux of water from point A to B, F_{AB}, is thus equal to the product of the hydraulic conductance of that region (K_{AB}, $kg.s^{-1}.MPa^{-1}$) and the pressure difference between A and B (ψ_A-ψ_B, MPa) (Tyree and Ewers 1991):

$$F_{AB} = K_{AB}(\psi_A-\psi_B) \quad \ldots(3)$$

The maximum water supply rate of a stem, branch or petiole is therefore the product of its hydraulic conductance and the water potential difference across its ends. The hydraulic conductance (or the reciprocal – its resistance) and pressure gradient are important factors in determining the water potential of the leaves and hence the transpiration rates that can be sustained. If transpiration (E) is defined as the total plant water loss in $kg.s^{-1}$ then

$$E = (\psi_{soil} - \psi_{leaf}).K_{plant} \quad \ldots(4)$$

Leaves need to maintain their water potential above a particular threshold for growth to occur (Bray 2002) and the transpiration rate is an important point of control because it determines the driving force for water transport from the soil to the leaves. By re-arranging equation (4) the leaf water potential can be expressed as the difference between the soil water potential and the ratio of transpiration to plant hydraulic conductance:

$$\psi_{leaf} = \psi_{soil} - (E.K_{plant}) \quad \ldots(5)$$

If resistance is used instead of conductance the equation changes to

$$\psi_{leaf} = \psi_{soil} - R_{plant}.E \quad \ldots(6)$$

In addition, water transport through a plant can be divided into a liquid phase, from the soil to the leaves, and a vapour phase from the evaporating surfaces of

the mesophyll cell walls, through the intercellular air spaces and stomata to the external environment:

Flow in the liquid phase = $(\psi_{soil} - \psi_{leaf})/R_{plant}$ …(7)

Flow in vapour phase = $(VP_i - VP_o)/r_{gas\ phase}$ …(8)

Both operate simultaneously and at steady state their respective flow rates will be the same. The gas phase resistance (r_g), however, is significantly higher than that of the liquid phase because the gradient between leaf and air is much greater than that between soil and leaf. It therefore has a controlling influence on the rate of transpiration (Van den Honert 1948). "The stomata are therefore strategically placed, in the gas phase, making them effective in controlling transpiration" (Weatherly 1976).

The evapo-transpirational demand depends mainly on the combined effect of solar radiation, vapour pressure deficit, temperature and wind speed (Davenport 1967). These variable factors are connected such that a change in one brings about a change in another. With the diurnal variations in these parameters, conditions arise conducive to a departure from steady state, with water being transpired faster than it is being transported to the leaf which gives rise to the already mentioned absorption lag. This causes water deficits that are responsible for the often recorded wilting of leaves (Clements and Marshall 1934). That these water deficits develop in the foliage because transpiration exceeds absorption would be numerically correct but is also an oversimplification (Weatherly 1976). The plant is more complex than a conduit with a porous surface at either end, the one in contact with the soil solution and the other exposed to the air with a high

evapo-transpirational demand. The departure from steady state transpiration is possible because there is in fact another water source, besides the soil solution, that can be tapped – the water contained in the tissues of the leaves and branches and stem. Water deficits arise in the leaves because the water potential of the cells adjacent to the main flow pathway equilibrates with that of the pathway by water moving out of the cells (Jarvis 1976).

This water source stored in the tissues contributes significantly to transpiration and hence photosynthesis (Phillips *et al.* 2003). Plants were probably ecologically sensitive to this because a circadian rhythm has evolved in some species which have root hydraulic resistances two to three times lower during the warmest time of the day than at night (Skidmore and Stone 1964; Parsons and Kramer 1974). The hydraulic resistance of sunflower leaves have also been found to be variable (Black 1979), making the points of water uptake and loss important for regulating the plant's water status.

The root system, stems, petioles and leaves are the basic components of most plants and because they vary greatly in structure and anatomy it is obvious that they would have different resistances to water flow. The sum of their resistances ($R_{root} + R_{stems} + R_{petiole} + R_{leaf}$) equals the whole plant resistance: R_{plant}. Earlier investigations (Jensen *et al.* 1961) showed that these resistances are spread unevenly among the components, with the root system giving the greatest resistance to water movement followed by the leaves. Investigations of the hydraulics of root systems showed that the ability of plants to increase their root permeability with increasing flow rate was a general phenomenon (Aston and Lawlor 1979). Theoretical analyses showed that the relationship between an applied hydrostatic pressure and flow rate was decidedly nonlinear with increases

of five to 20 fold in the slope of the force-flux curve over a pressure range of only 0.2 MPa (Fiscus 1975). Newman (1973) compared the permeabilities of the root systems of five herbaceous species under a range of positive hydrostatic pressures and found their hydraulic resistances very variable, decreasing with increasing pressure. Similarly, when an increasing negative pressure is applied to the transpiration stream the root and leaf resistances are flux-dependent, decreasing to accommodate higher transpiration rates (Black 1979a; Koide 1985). Measuring whole plant hydraulic conductance of a number of crop plants Tsuda and Tyree (2000) found that all plant components varied their conductance in response to increases in temperature and transpiration (possibly due to the activation of aquaporins), often making the relationship between transpiration and conductance non-linear.

That root permeability is known to be very sensitive to environmental factors suggests that the major component of this resistance resides in a part closely linked to metabolic activity (Newman 1976) and the variability of root hydraulic resistance has indeed been found to be a function of the intact, living root system (Kramer 1940; Stoker and Weatherly 1971). Root puncturing experiments combined with cell pressure probe measurements allowed researchers to evaluate the permeabilities and reflection coefficients of different cell layers and their relative contributions to overall root conductivity (Steudle and Peterson 1998). The results of these experiments indicated that the root did not behave like a perfect osmometer (which would be completely impermeable to solutes) but more like an osmometer with a complex, composite osmotic barrier made up of several cell layers (Steudle and Peterson 1998). Water transport through the different cell layers is thought to occur in three parallel pathways – the apoplastic, symplastic and transcellular paths. Apoplastic transport occurs

through cell walls while transport via the symplast is dominated by plasmodesmata and the transcellular path by aquaporins (Steudle 2001). Experiments have not as yet been recorded that show a conclusive separation between the transcellular and symplastic pathways which are often treated as one cell-to-cell path (Steudle 2001). These pathways have different hydraulic resistances and the amount of water transport through each varies with environmental conditions. Nuclear magnetic resonance and root and cell pressure probe studies suggest that the cell-to-cell pathway is the dominant pathway for radial water transport (De Boer and Volkov 2003). The advantage of this composite transport model is that it provides an explanation for (1) the varying contribution of osmotic and hydraulic water movement through the root, (2) the low reflection coefficients found for root cell membranes and (3) the variation in root hydraulic conductance between species (Steudle 2001).

1.2.7 Hydraulic limitations to growth: ecological and physiological repercussions

Two processes are at work in a growing cell: irreversable cell wall expansion and the inflow of water to occupy the extra volume (Passioura and Fry 1992). The maintanence of turgor pressure above a particular threshold is therefore essential for growth and to maintain an osmotic potential favourable to further growth (Tomos 1985). An adequate water supply to the leaves is thus important for the growth of the individual, but because growth is also dependent upon access to light and soil resources, the growth trajectory is influenced by competition with neighbouring plants (Bond 2000). In a very productive environment a seedling with a high hydraulic conductance would have an advantage above one with an inherently low conductance. Angiosperm seedlings, having broad leaves and xylem vessels, are known to grow much faster than gymnosperm seedlings with

their relatively inefficient tracheids and small leaf surface area. Bond (1989) therefore proposed the "slow seedling" hypothesis to explain why gymnosperms are dominant in very harsh environments and excluded from more productive temperate or tropical regions. This hypothesis was supported by Pammenter *et al.* (2004) who measured hydraulic conductance and assimilation parameters on angiosperms and gymnosperms growing in the same area and conditions; however, they found an inherently higher photosynthetic capacity (higher carboxylation coefficients and RuBP regeneration rates) in angiosperm leaves which could also confer a higher growth rate and competitive ability on a plant.

The stomatal regulation of transpiration, in response to increasing soil-to-leaf resistances, acts to prevent a disproportionate decline in leaf water potential and hydraulic failure (Sperry 2000). Stomatal conductance and hence carbon assimilation are therefore both dependent upon water transport from the soil to the leaves and any changes in the hydraulic conductance of the entire pathway may effect leaf gas exchange (Hubbard *et al.* 1999). As trees grow taller the path water has to travel becomes longer, increasing the resistance to water transport to the leaves. There is a pronounced and predictable trend that after reaching maximum height (maximum tree height is a predictable characteristic depending upon the species and site productivity) there is a marked decline in carbon assimilation (Ryan and Yoder 1997; Bond 2000). Branch junctions have a higher resistance to water flow than the trunk or branches, causing branches downstream to have lower water potentials and which could give older trees a reduced whole-plant conductance (Schulte and Brooks 2003; Zimmermann and Tyree 2002; Tyree and Alexander 1993). In addition, age-related changes in terminal twig morphology and crown architecture have been found to cause a significant decrease in conductance and photosynthesis (Rust and Roloff 2002).

The observations that overall carbon assimilation and photosynthesis decline in older trees and that stomatal behaviour is strongly influenced by the state of the hydraulic pathway in the plant led Ryan and Yoder (1997) to propose the Hydraulic Limitation Hypothesis (HLH). The HLH predicts that the hydraulic resistance of the soil-to-leaf pathway will increase as a tree increases in height and that this will reduce stomatal conductance, photosynthesis and growth as the tree approaches its maximum height (Bond and Ryan 2000). In some cases the HLH has failed to explain the decline in growth observed in some trees (Barnard and Ryan 2003) and this has been ascribed to the trees compensating in some way for the increased resistance. Barnard and Ryan (2003) compared 1 year old (7m tall) and 5 year old (26m tall) *Eucalyptus saligna* trees and found that a rapid increase in the sapwood area:leaf area ratio enabled the older trees to maintain a lower threshold leaf water potential. Alternatively, trees could compensate by increasing their carbon allocation to fine roots thereby decreasing the hydraulic resistance of the root component (Magnani *et al.* 2000). Bond and Ryan (2000) have argued that "…the fact that these compensations occur is evidence that hydraulic limitations must be important to the overall fitness of woody plants". A recent summary of experimental data of the HLH by Ryan *et al.* (2006) showed that, although the hydraulic limitation to leaf gas exchange does operate in many trees, it is not a universal response. They also point out that different sized trees often experience different micro-environments (such as light intensity and soil moisture) and that the link between tree hight growth and tree or stand biomass is tenuous and can vary with stand density and fertility.

1.2.8 Temperature and plant growth

Temperature has a marked effect on plant growth. All factors being constant, plants in warmer climates have higher growth rates than those from colder climates. All living organisms have temperature tolerance ranges within which they grow optimally; above or below this threshold the plant is stressed. Temperature shifts cause a number of physiological changes to occur that are homeostatic changes (temporal changes in the steady-state physiology), allowing the plant to acclimatise and survive in the altered environment. An example is the optimal photosynthetic temperatures tracking the seasonal ambient temperature shifts (Wand *et al.* 2001).

Over evolutionary timespans environmental fluctuations create a selective pressure in favour of genotypically determined traits, termed adaptations, that enhance the environmental fitness of a plant population (Bray *et al.* 2000). A widely used indicator of the thermal dependence of metabolic reactions is the Q_{10} value. It is defined as the relative change in a reaction rate that will parallel a 10 ºC shift in temperature, and is based on the assumption that overall metabolic rate shifts are dependent on overall shifts in the underlying physico-chemical rates (Chaui-Berlinck *et al.* 2001).

At temperatures above the physiological tolerance range proteins, such as enzymes integral to growth, start to denature causing biochemical reactions to cease. In higher plants a short exposure to temperatures of 38-40 ºC is generally sufficient to produce specific heat shock proteins (HSPs) that are involved in protecting cellular protein function from temperature stress (Iba 2002). Their expression has been found to increase in a heat-correlated manner in root extracts from plants growing in geothermal soils near hot springs (Stout and Al-Niemi 2002). Very high temperatures are also associated with high levels of irradiation

often causing excessive amounts of energy that cannot be dissapated by the photosynthetic pathway. This often leads to photo-inhibition. Water stress can increase the susceptability of leaves to photo-inhibition by decreasing transpiration and causing leaf temperature to increase above the optimal range, which appears to have an upper limit at about 37 ºC for *Eucalyptus* species (Ögren and Evans 1992). This would also decrease stomatal conductance, reducing the inward diffusion of CO_2 and this, in turn decreases the amount of energy that can be dissapated by photosynthetic CO_2 reduction. Photo-inhibition can also occur under normal light intensities when low temperatures decrease the photochemical efficiency of photosystem II causing a build-up of excess light energy (Adams and Demmig-Adams 1994). This has been reported for *E. nitens* plantations of south-eastern Australia that are established on areas experiencing mean annual temperatures below 10 ºC (Close *et al.* 2001).

The water relations of a plant can be significantly influenced by temperatures below its optimal growth temperature and water absorption appears to be more affected than transport in the stem. Sap ascent *per se* is relatively unimpeded by low temperatures except below freezing point and ice starts forming in the vessels (Johnston 1959; Zimmermann 1964). However, transpiration, which drives sap ascent, is significantly affected by temperature, decreasing with a decrease in temperature. Water absorption by the root system also decreases considerably at temperatures ranging from 20 to 4 ºC, depending upon the species and genotype (Clements and Martin 1934; Arndt 1937; Aston and Lawlor 1979).

To acclimatise to low ambient temperatures there is a variety of physiological changes plants can make and most result in optimising the source-sink relationship to the new conditions. Carbon is often allocated to the organ that is

responsible for acquiring the most limiting resource and hence this can involve changes at the whole plant level, such as the root:shoot ratio or root surface area:leaf surface area ratio (Russel 1977; Equiza *et al.* 2001). Water absorption is known to be proportional to the size of the root system (Fiscus and Markhart 1979); a plant can therefore either produce more efficient roots or simply increase the root biomass. An increase in the below ground:above ground biomass partitioning is a common response when a plant experiences water stress because it enables the plant to explore a greater soil volume in search of water (Chen and Reynolds 1997). Low temperature acclimatisation is also known to influence processes at the cellular level such as changes in the relative sizes of the soluble and membrane-bound Rubisco pools in leaves (Mercado *et al.* 1997) and increasing the non-structural carbohydrate (NSC) concentration in the plant tissues (Engels 1994; Mercado *et al.* 1997). In examining the low temperature acclimatisation of *Eucalyptus pauciflora*, Atkins *et al.* (2000) found no associated change in leaf soluble sugar concentrations in field-grown plants but only in those grown under controlled environmental conditions. Changes in root anatomy such as decreases in xylem vessel diameter (Haung *et al.* 1991) which can significantly influence hydraulic conductance have also been reported.

1.2.9 Root chilling

Today many agricultural crops are cultivated outside of their natural ecological range. This has been made possible by the production of new varieties (mostly through artificial selection) that have increased tolerance ranges for different environmental factors. The temperature range of the new environment intended for crop production is often different from where the crop species originated. Determining temperature tolerance ranges has therefore been (and still is) an important field of investigation. The effect of soil temperatures on plant growth is

important because two major functions of a root system – water and nutrient absorption – are both influenced by temperature (Glinski and Lipiec 1990). In addition, root system expansion is due to the combined effect of root growth (increase in length and diameter) and development (initiation of new roots), both of which are temperature sensitive (Kaspar and Bland 1992).

Each species has its own optimum temperature range for maximum root growth (Seiler 1998). It would appear that low soil temperatures inhibit water absorption in all plants but to a much lesser extent in plants native to temperate regions, where plants experience cold soils for at least part of the year and appear to have an inherent chilling tolerance (Kramer 1942; Kaufmann 1975). Root chilling caused a significant decrease in water transport in maize and sunflower, two warm region plants, but not in barley which is a temperate species (Aston and Lawlor 1979). Similarly, in figleaf gourd – a chilling tolerant species, hydraulic conductance was reduced by a factor of two after one day's exposure to a root zone temperature of 8 ºC but in chilling sensitive cucumber it was reduced by a factor of ten (Lee *et al.* 2005). Chilling injury is a characteristic of tropical and sub-tropical species which usually exhibit physiological dysfunction in the 6 to 10 ºC range (Crawford 1989) whereas temperate species have a lower limit closer to 0 to 4 ºC (Lyons 1973). Chilling tolerant species often adjust root hydraulic conductance to a sudden drop in temperature after a period of acclimatisation that can be as short as 1 to 3 hours for spinach (Fennell and Markhart 1998) or 30 hours for maize (Aroca *et al.* 2001). Upon prolonged exposure to low temperatures a progressive increase in root conductivity has been reported for tolerant genotypes of spinach (Fennel and Markhart 1998), maize (Aroca *et al.* 2001) and figleaf gourd (Lee *et al.* 2005); chilling sensitive genotypes often show a progressive decrease in conductance (Bolger *et al.* 1992; Aroca *et al.* 2001).

The factors monitored to assess the level of acclimatisation to chilling depends upon the research question behind the investigation. If the aim is to assess growth or vigour after chilling, the activities of the foremost photosynthetic enzymes can be measured (Stamp 1980); if it is postharvest fruit quality after chilling storage, parameters such as electrolyte leakage, tissue texture and respiration could be measured (Mercado-Silva *et al.* 1998), although Kolek *et al.* (1981) warn that an estimate of electrolyte leakage may not be a reliable test for cold resistance because it is relative to the amount of electrolytes in the tissue to start with. If the aim is to assess the hydraulic characteristics after root chilling and relate this to growth, then stomatal conductance of the intact plant and the rate of sap flux from the separated root system are two frequently used methods. The strong correlation between root chilling and decreased stomatal conductance has been well documented and is attributed to an increase in the suberisation of the endodermis as well as the deactivation of root aquaporins (Lee *et al.* 2005). Aroca *et al.* (2001) found mercuric chloride treatment to cause a much greater decrease in root conductance in acclimatised maize than before acclimatisation, indicating the upregulation of aquaporins to be a strategy to alleviate water stress.

The increased resistance to radial water movement across roots upon rapid cooling, combined with the increase in the viscosity of water, causes a decrease in water transport to the leaves, which subsequently become water stressed and wilt (Naidoo and Von Willert 1994; Cochard *et al.* 2002). This inhibition of water transport could be due to either changes in the root structure or the cell membrane properties (Drew 1987). Lee *et al.* (2004) found hydrogen peroxide (H_2O_2) production to increase in cucumber root cells upon exposure to chilling temperatures and they suggest that this is responsible for the marked decrease in water transport. Roots of desert succulents grown in a soil with a spatially

variable water content showed a significant increase in suberisation in roots grown in dry sections (North and Nobel 1998). They also act like rectifiers, allowing substantial uptake of water from wet soils but greatly restricting water loss to dry soils and the switching between these properties occurs within hours to days (Nobel and Sanderson 1984). Endodermal suberisation therefore seems to be a response to low water availability; when plants are dependent upon water extracted from deep soil layers, there will be a tendency for the water to follow the water potential gradient out of the root as it moves through drier upper layers (Sharp and Davies 1985).

1.2.10 Hormonal influences

Despite the direct inhibitory effect low temperature has on root growth (Drennan and Nobel 1998; Rodchenko 1981) it also influences plant growth by altering root metabolic activity and influences the signals exchanged between roots and shoots (Cruz *et al.* 2003). Roots are not solely absorbing organs but produce a number of hormones that are important for growth (Russel 1977). Root apical meristems have been identified as the centres of production for cytokinins (Torrey 1976), a group of hormones that play an essential role in initiating the growth of lateral buds (Taiz and Zeiger 1998; Mehra-Palta 1982) and in conjunction with IAA and ethylene, regulating lateral root development (Aloni *et al.* 2006). Root chilling can alter the export of cytokinins from the roots (Skene and Kerridge 1967) and cause hormonal imbalances leading to the loss of apical dominance in some cases (Mercado *et al.* 1997). A form of inter-organ growth control in young maize plants has been reported by Jesko (1981) where zeatin – the most abundant natural cytokinin (Taiz and Zeiger 1998) – inhibits leaf expansion but stimulates photosynthesis and the photosynthate is thus used for root growth at the expense of leaf growth; this inhibition is gradually diminished

as the distance between the root meristematic tips and shoot base increases with time.

Cytokinins are produced in the roots and appear to be transported to the shoot via the transpiration stream; the evidence for this is, however, circumstantial (Taiz and Zeiger 1998). Veselova *et al.* (2005) found that root chilling of wheat caused a significant decrease in stomatal conductance and cytokinin concentration in the shoot, supporting the hypothesis that root chilling inhibits shoot growth via a decrease in cytokinin production or export from the roots. In a study using roses (Dieleman *et al.* 1998) found root chilling to have no effect on shoot cytokinin concentrations, indicating that hormones other than cytokinins might be involved. In a comparison of mutant and wild type pea plants Beveridge *et al.* (1997) also found evidence of root signals other than the balance between the concentration of auxins in the shoot to cytokinins in the root system to regulate branching. The hormonal effects due to root chilling appear intricate and may depend upon species, the developmental stage of the plant, the temperature and duration of the chilling treatment.

Abscisic acid (ABA) plays an important regulatory role in the plant's response to environmental stress as well as interacting with auxins, cytokinins and gibberellins, usually as an antagonist (Little 1975; Taiz and Zeiger 1998). It is also important for seed survival because it is involved in increasing desiccation tolerance of immature seeds (Ooms *et al.* 1994; Taiz and Zeiger 1998). Exogenous applications of ABA often mimic many of the physiological effects of water stress (Jones 1985). The hormone plays a significant part in regulating leaf gas exchange, and an increased abscisic acid concentration is a common response to soil drying or root constriction; low water potentials have been found to

increase the sensitivity of target cells to abscisic acid (Little 1975; Davies *et al.* 1993; Carmi 1995). In addition, abscisic acid can facilitate shifts in the thermal tolerance of root systems (BassiriRad and Radin 1992), and significant increases in root hydraulic conductance, which are much more pronounced at the root cell level, indicating an interaction with aquaporins (Hose *et al.* 2000).

A distinguishing feature between the cold tolerant and intolerant plant is the continued ability to homeostatically regulate its metabolism (Crawford and Huxter 1977). Chilling injury can occur directly (qualitative, "all-or-none" physical damage) or indirectly through changes in metabolism (Levitt 1980). Indirect injury could manifest itself as solute leakage, the inhibition of translocation from sources and sinks, respiratory upset, protein breakdown, biochemical lesions (Levitt 1980) or the production of Activated Oxygen Species (AOS) which may initiate degradative reactions (Kang *et al.* 2003). Pre-treating plants with the hormone-like substance salicylic acid has been found to significantly increase their chilling resistance as well as increasing the activity of H_2O_2 degrading enzymes (Kang *et al.* 2003).

1.2.11 Aquaporins

Aquaporins are integral membrane proteins that significantly increase the passive movement of water across membranes and are open or closed depending upon their phosphorylation state (Taiz and Zeiger 1998). Prior to their discovery most models of water movement across membranes were based on simple diffusion. Aquaporins were first isolated from red blood cells and later from plant tissues and are part of a superfamily of Major Intrinsic Proteins (MIPs) (Agre *et al.* 1998). In plants they are divided into Tonoplast Intrinsic Proteins (TIPs) and Plasmamembrane Intrinsic Proteins (PIPs) (Schäffner 1998). Aquaporin

expression has been detected in virtually all root cell types and may represent critical points where an efficient and spatially restricted control of water uptake can be exerted (Javot and Maurel 2002). They can increase membrane permeability to water 10 to 20 fold and their expression is often simultaneous with transport-demanding processes such as seed germination and fruit ripening where large amounts of osmotically active solutes have to be moved across membranes (Schäffner 1998) as well as guard cell movements (Agre *et al.* 1998).

Aquaporins, therefore, play a central role in plant water relations and especially a plant's response to water stress (Tyerman *et al.* 2002). Plants in which aquaporin expression is suppressed, recover significantly more slowly from a soil water deficit upon rewatering, than plants with unsupressed expression. Mutant *Arabadopsis* plants with a reduced expression of PIP1 and PIP2 aquaporins regained only 52 percent of their initial level of root hydraulic conductance and under water-sufficient conditions they produced more roots to compensate for the decreased root conductance (Martre *et al.* 2002). Also using *Arabadopsis*, Martinez-Ballesta *et al.* (2003) found exposing roots to various NaCl concentrations caused a significant decrease in hydraulic conductance via the downregulation of PIP aquaporin expression. Mercuric chloride ($HgCl_2$) has been shown to be an effective blocker of aquaporins in a range of plants, causing a significant reduction in water transport, but this inhibition can be largely reversed by the addition of β-mercaptoethanol (Maggio and Joly 1995; Javot and Maurel 2002). Aquaporins have also been isolated from xylem parenchyma cells (Barrieu *et al.* 1998), and might be involved in the refilling of embolised vessels (Holbrook and Zwieniecki 1999; Vesala *et al.* 2003).

$HgCl_2$ also inhibits leaf photosynthesis which suggests that CO_2 transport across the plasma membrane of the mesophyll cells could be facilitated by mercury-sensitive aquaporins (Terashima and Ono 2002); this is supported by the finding that this transport process has a Q_{10} of about 2.2 indicating that an enzyme or protein-facilitated process is involved (Bernacchi *et al.* 2002). A mercury insensitive aquaporin has been identified but its physiological function is, as yet, undefined (Knepper 1994).

Aquaporins are also temperature sensitive, closing under low temperatures and causing significant decreases in cell membrane water permeability and consequently root pressure and water uptake (Lee *et al.* 2004; Lee and Chung 2005; Lee *et al.* 2005). Accordingly an acclimatisation strategy of chilling tolerant species is to increase their aquaporin expression in response to low temperatures (Aroca *et al.* 2001).

Root systems, therefore, grow in a demanding medium that is, more often than not, very heterogeneous with respect to its physical and chemical characteristics. Roots have in turn evolved a myriad ways to compensate and still perform their vital function in a changing environment. An important factor in root growth is the simultaneous solution to the equations for root elongation as a function of temperature and the the rate of temperature change with soil depth (Kaspar and Bland 1992).

Soil temperature is thus of paramount importance to root water acquisition and root chilling is expected to decrease water absorption. This would have a significant impact on plant water status and presumably growth because the average daily leaf water potential would be lowered in chilled plants as opposed

to control plants growing under similar conditions. The inhibitory effect of root chilling on hydraulic conductance and growth in chilling sensitive species has been well established over periods of days to weeks. The plant genotype, however, has been likened to "…a constrained repertoire of environmentally contingent and intelligent processes" (Trewavas 2003). They, therefore, can "make informed decisions" based on the continual analysis of their environment and have a number of physiological and physical changes they can undergo to maintain a functional equilibrium between the absorptive and evaporative surfaces.

1.3 This study

Competition between plants for resources is a well known phenomenon and to compete successfully requires a strategy. To maintain a functional equilibrium implies that achieving steady-state water uptake and loss is a goal in itself. Some plants have a "go for broke" strategy and they consequently have a low water use efficiency but it makes them vigorous competitors because they use large quantities of water, at their neighbours' expense, set seed and ensure the presence of a next generation. *E. grandis* is a species that has an extremely high growth rate (in excess of 3 metres per year for the first decade) and is well suited to low altitude, warm sites with good rainfall (Doughty 2000). *E. nitens*, in contrast, grows very well on high altitude sites prone to extreme cold and frost conditions (Kelly 1978; Swain and Gardner 2004). Considering that the responses of plants to short-term increases in hydraulic resistance have been studied and the general consensus is that they have significant effects on transpiration, leaf surface area and biomass allocation, it would be interesting to know if these alterations are long-term or temporary changes. *Eucalyptus* taxa were chosen because of the rapid growth, minimising the length of the experiment. Seedlings generally have

a very different rooting pattern to plants derived from cuttings. Where seedlings form a single, seminal taproot, plants derived from cuttings develop relatively large lateral roots growing in multiple directions. The hybrid plants, *Eucalyptus grandis* x *nitens*, used were from cuttings and their different hydraulic architecture might make them respond differently to the treatment.

Previous work has established that a root chilling period of three months caused significant alterations in height and carbon assimilation among *Eucalyptus* species and clones (Chesterfield *et al.* 1991; Manoharan, unpublished Ph.D. thesis 2002). This study also examines this question by chilling the roots of *E. grandis*, *E. nitens* and their hybrid *E. grandis* x *nitens* and extending the period of chilling to eight months. The chilling temperature chosen was a day/night temperature of 15/10 ºC. Long-term exposure to temperatures below 10 ºC cause irreversible root damage to *E. grandis* (Manoharan, unpublished Ph.D. thesis 2002), however, root chilling should still be effective as a tool to manipulate conductance considering that the optimum root growth temperature for eucalypts is between 23.8 and 29.4 ºC (Pierce 2005). Data collected on the hydraulic conductance of below and aboveground parts, the photosynthetic ability of the leaves as well as biomass allocation patterns will be combined to attempt an answer to the question that sparked this investigation: What is the long-term effect of increased whole plant hydraulic resistance on growth, what are the processes by which this effect is mediated and what is the nature of the compensatory responses, if any?

2. Materials and methods

2.1 Selection of plant material

Thirty, 3.5 month old seedlings each of *E. grandis* and *E. nitens* that had been allowed to take root in a mixture of pine bark (*Pinus patula* and *P. eliotii*) were bought from Top Crop nursery. Thirty three-month old rooted cuttings of *E. grandis x nitens* were obtained from Mondi Business Paper's nursery, Mountain Home Research Centre, based in Hilton. The seedlings and cuttings were brought to the greenhouse complex of the School of Biological and Conservation Sciences of University of KwaZulu-Natal, Durban and placed in a mistbed for two days. The mistbed was set to give a burst of water once a minute. Seedlings used in the study were selected using the first random number table in the appendix of Fischer and Yates (1963). Seventeen *E. grandis* and *E. nitens* seedlings and 18 *E. grandis x nitens* cuttings were selected and planted into 15 litre plastic bags (Figure 2.1). Eight individuals of each taxon were selected to form the control and treatment groups. The box could accommodate 25 plants therefore nine *E. nitens* individuals were selected. The bags had been filled with soil a week before and watered daily to accelerate the settling of the soil.

Figure 2.1: A sample of the 3.5 month old seedlings planted out into 15 litre bags, after spending two days in a mistbed.

A specific watering regime was not adhered to; throughout the experiment the plants were watered to prevent them experiencing water stress. This meant watering them every third or fourth day. As they grew larger they were watered more frequently; at the age of four months they were watered once daily, except if it had rained the previous day. They were given enough water to saturate the soil, which was about one litre. Granular fertilisers were dissolved in the irrigation water (to a one gramme per litre concentration) every third time they were watered. The seedlings were watered with a Nutrifert Natgro solution immediately after being planted out. The fertilisers used in the experiment were

- Nutrifert Natgro
- Plant Ca
- Mondi blue
- Mondi orange

Micronutrients and fungicides were administrered as foliar sprays. Micronutrients were administered once a month as an aqueous solution of “Trelmix” from the horticultural company Grovida at a 2.5 ml per litre concentration. The fungicides Bravo (1 ml/L) and Mancozeb (2 g/L) were obtained from the same company and applied as a preventative measure fortnightly. Administering foliar sprays at midday can result in the evaporation of water and the concentration of salts which can damage the leaf surfaces (Taiz & Zeiger 1998). The plants were therefore sprayed in the late afternoon. The elemental composition of the different fertilizers is listed in Table A6.1 in appendix 6.

2.2 Refrigeration unit design

To manipulate the soil temperature a thermally insulated box was made to house the treatment plants for the duration of the experiment. The box was constructed using 18 mm thick shutterboard and was 3.8 metres wide, 4 metres long and 0.5 metres high. A polyeurathane layer, 18mm thick, was glued to the inner surface of the box and placed on the floor of bare soil. A two metre-long blower unit with four fans was placed inside to circulate the air inside the box (Figure 2.2); the blower was connected to a compressor and heat exchange unit, sitting outside the box (Figure 2.3). The thermostat was situated in a distribution box mounted externally next to the compressor. Whenever the internal air temperature rose above the set temperature the compressor started up, cooling the air. Two thermometers were installed, each in a diagonally opposing corner, to indicate the internal air temperature. A steel framework was welded using 10 mm square tubing and placed inside the box to make it sturdy enough for one to walk on it. To insert the 25 treatment plants into the box 300 mm wide circular discs were cut in the lid (Figure 2.4). A 40 mm hole was cut in the centre of each disc through which the plant stem would protrude. Each disc was then halved to make it easier to fit them around the stems of the treatment plants (Figures 2.6 & 2.7). Seven-millimetre holes were drilled in five of the lids to insert glass thermometers to monitor the soil temperature directly.

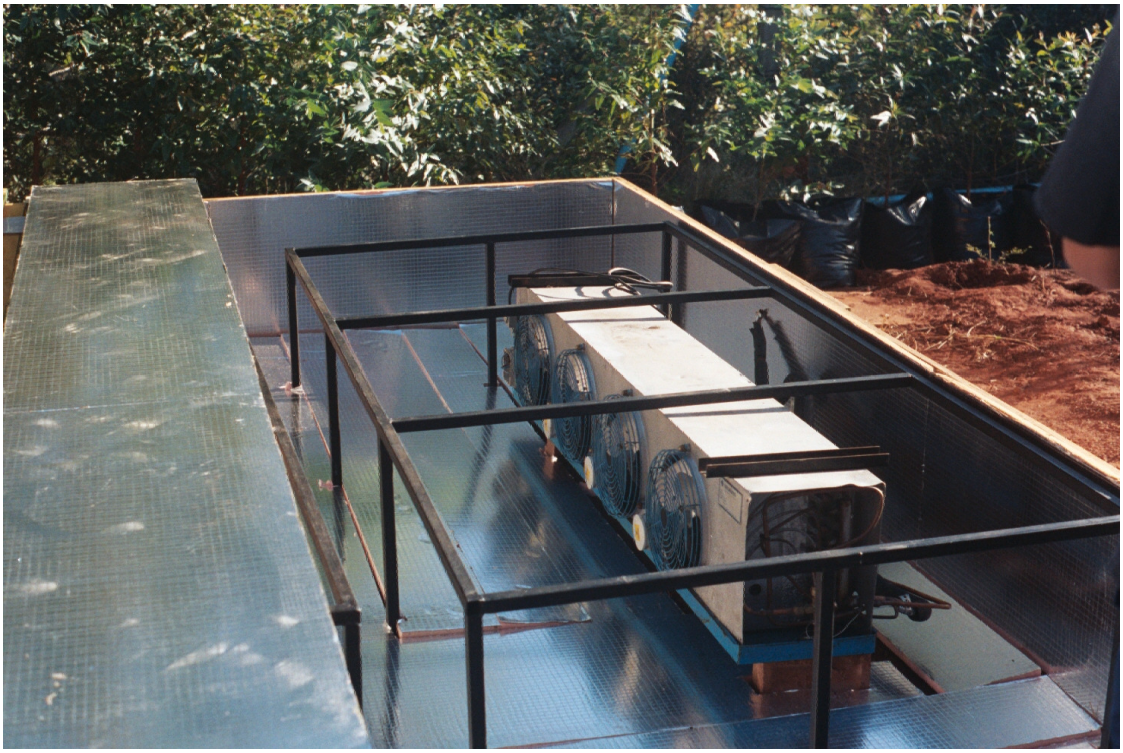

Figure 2.2: The blower and the square-tubing framework. The inner walls and floor (bare soil) were covered with 18mm thick polyeurathane; the polyeurathane was glued to the shutterboard using a commercially available contact adhesive.

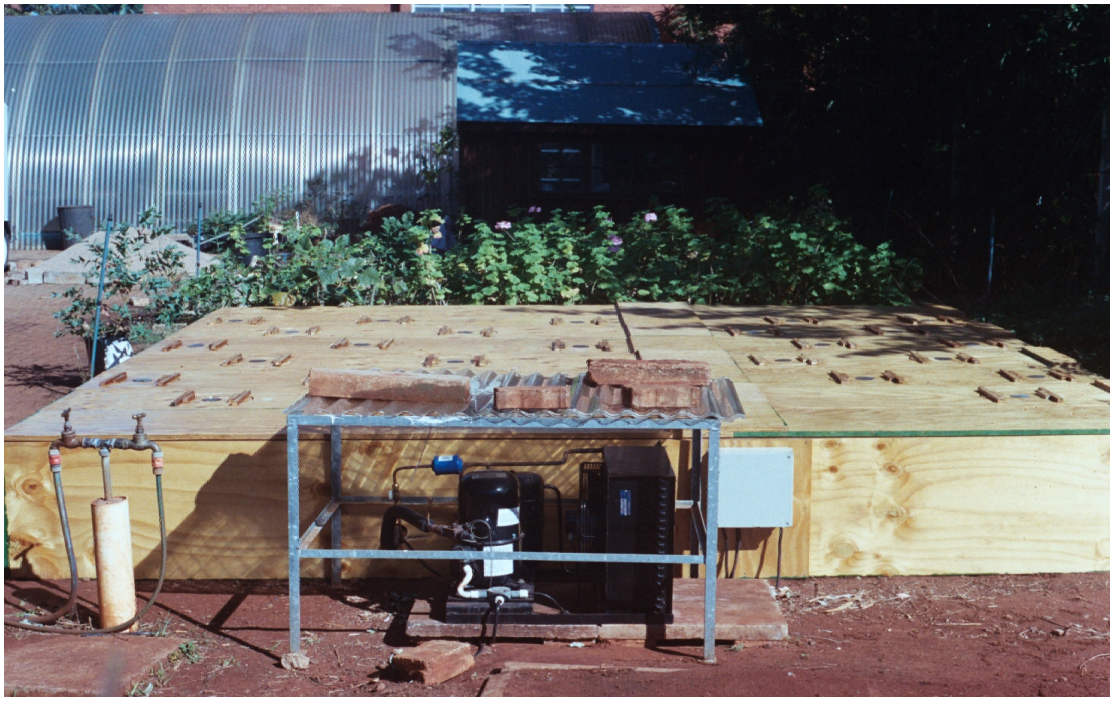

Figure 2.3: The completed box (4 x 3.8 x 0.5m) with the compressor and thermostat (square box on the right) attached.

Figure 2.4: Twenty five 300 mm holes were cut in the lid to insert the 25 plants.

Eight *E. grandis* and *E. grandis x nitens* plants and nine *E. nitens* plants were selected using the abovementioned random number table and placed inside the box. Their placement within the box was also determined using the random number table (see Figure 2.5). Each plant was supported by a seventeen-litre plastic bag that was filled with soil until the surface of the treatment-plant's soil was flush with the lid. This created a reasonable seal allowing the minimum of cold air to escape at the soil surface-lid interface under the slight positive pressure created by the blower. Each seventeen-litre bag was covered with plastic to prevent treatment plant roots from growing into the soil they contained. Name tags were nailed to the box close to each plant to keep track of data collected from each. The end result was a box containing 25 plants as shown by Figure 2.8. The thermostat was adjusted until a temperature of 10-13 °C was maintained in the soil inside the bags. Soil temperature was monitored every third or fourth day.

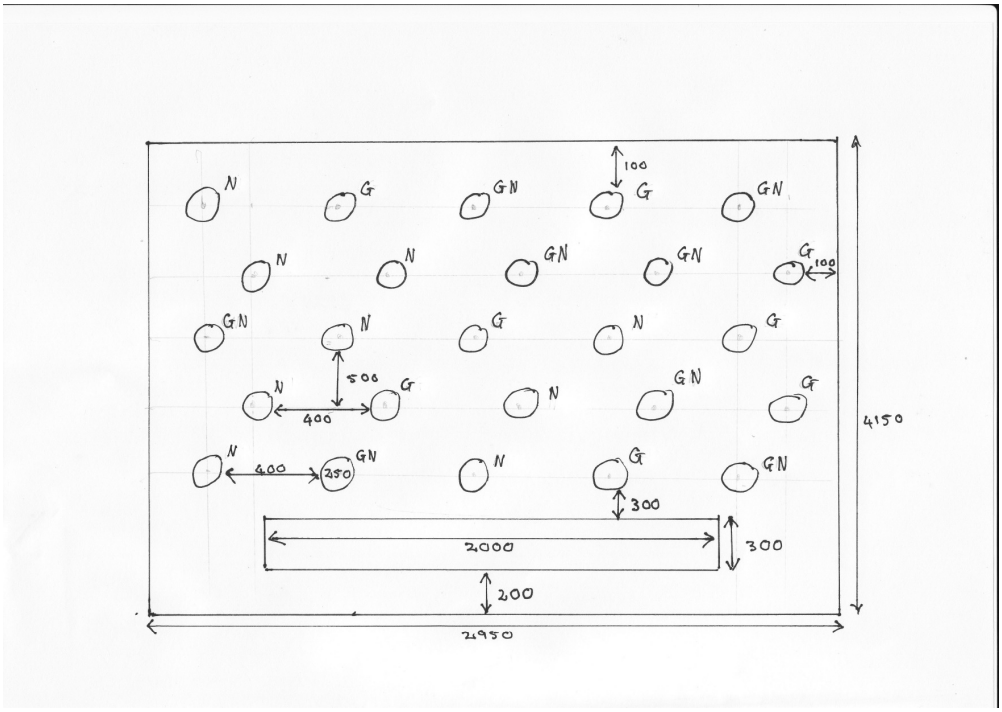

Figure 2.5: Sketch of the layout of the 25 plants within the box. G = *E. grandis*, N = *E. nitens* and GN = *E. grandis* x *nitens*. All the measurements are in millimetres. The sketch is not to scale.

Figure 2.6: An *E. grandis* x *nitens* plantlet inside the box with the polyeurathane discs in place. It is seated on a 17 litre bag filled with soil until the plant was at the required height.

Figure 2.7: An *E. grandis* x *nitens* plantlet with the polyeurathane discs and wooden discs flush with the lid.

Figure 2.8: The complete “phytotron” containing 25 two-month old *Eucalyptus* plants

Figure 2.9: Two-month old control plants wrapped in silver tape and with their soil covered by 18 mm thick polyeurathane discs. They are placed on polyeurathane discs to keep the transfer of heat from the soil to a minimum.

A proper control would have been an identical second box in which ambient air was circulated. Unfortunately there was too little room in the outside growing area for a second box; instead the control plants were grown next to the box as shown in Figure 2.9.

To minimise the substantial temperature fluctuations of the soil of the control plants, polyeurathane discs were cut to cover the exposed soil of control plants and the black exterior of the bags was covered with silver duct tape. The plants were also placed on polyeurathane discs to break contact with the warm soil (see Figure 2.9).

With the onset of summer in October soil temperatures in the box started to increase to 15 ºC during the warmest part of the day. To ameliorate this problem all gaps in the box were sealed with a one-component polyeurathane foam that hardens within hours (Figures 2.10 & 2.11); the plants were also watered with water at 10 ºC. This brought the midday temperatures back to between 10 and 13 ºC.

Figure 2.10: An *E. nitens* plant sealed in with the polyeurathane foam.

Figure 2.11: Top view of the box with one-component polyeurathane foam applied where the individual shutterboard sheets met.

2.3 Growth measurements

Stem diameter and height were measured every seven to twelve days on treatment and control plants to monitor their growth. Stem diameter was measured to the nearest 50 µm using plastic vernier callipers. Height was measured to the nearest centimetre using a tape measure.

2.4 Photosynthesis measurements

Light response curves of net photosynthetic CO_2 assimilation were measured with a Li-Cor 6400 Photosynthesis System (LiCor Inc., Lincoln, Nebraska) using an LED light source (Figures 2.12 & 2.14) when the plants were 7 to 8 months old. Measurements were taken between 8:00 am and 1:00 pm. The leaf in the sensor head chamber was acclimatised to high light by keeping the light intensity at 1500 $\mu mol.m^{-2}.s^{-1}$ for about ten minutes, after which the light intensity was decreased in a stepwise manner every two to three minutes to zero. The data were logged before each decrease and the IRGA was matched before every data point. This method is termed a "rapid" response curve as opposed to the "slow" response curve method where the one works from dark to light, waiting 15-20 minutes at each light level. From the initial slope of a light response curve the quantum efficiency can be calculated which is the efficiency with which absorbed light energy is being used to drive the biochemical reaction of photosynthesis. The light response curves obtained with the LiCor 6400 in this study were based on incident radiation and not that which is actually absorbed. The quantum effeciency calculated in this study is therefore termed the "apparent quantum efficiency".

Initial experiments indicated that there were no significant differences between light curves constructed by the two methods. The CO_2 concentration in the leaf

chamber was held at a constant 400 µmol.mol^{-1} using the CO_2 mixer that was connected to a cylinder of pure CO_2 (Figure 2.13). The CO_2 mixer was then screwed on to the side of the LiCor 6400 console. The pressure regulator on the CO_2 cylinder was set to give an outlet pressure of 13 kPa. The LiCor 6400 was powered by a truck battery (Figure 2.14).

Figure 2.12: The LI-COR 6400 sensor head with the "Red-Blue" Light Emitting Diode light source attached.

CO_2 response curves were measured alternately on a treatment and control plant from 8:00 am until 12:00 pm on sunny days. An autoprogramme that controlled all the parameters and logged data automatically was initiated. The PAR was held constant at 1000 µmol.m^{-2}.s^{-1} and the CO_2 concentration (in µmol.mol^{-1}) varied using the LiCor 6400's software with the "mixer" option in the following order: 400, 300, 200, 100, 50, 100, 200, 300, 400, 400, 600, 800, the two consequetive 400s was entered to allow the leaf some time to recover from the very low CO_2

levels. The CO_2 source was from the aforementioned CO_2 mixer-gas cylinder combination (Figure 2.13) with the regulator set to 1300 kPa. The LiCor 6400 was programmed to match whenever the difference in CO_2 concentration was greater than 15 ppm.

Figure 2.13: The cylinder containing pure CO_2 with the CO_2 mixer (in hand) attached.

Figure 2.14: The LiCor 6400 Photosynthesis System

2.5 Soil-plant hydraulic resistance

The hydraulic resistance of the soil-to-leaf pathway was assessed by measuring the relationship between the transpiration rate and the leaf water potential. This gives an indication of the soil-to-leaf water potential difference required to maintain a particular transpiration rate. Transpiration was measured using the LiCor 6400 and water potential using a pressure bomb. The LED light source, on the sensor head, was replaced with the "sun and sky" chamber and transpiration rates measured on a leaf from one control and treatment plant of each taxon (amounting to six measurements each of the control and treatment group) at 6:00, 7:00, 8:00, 9:00, 10:00 and 11:00 on four consequetive sunny mornings. Immediately afterward the twig was covered with a plastic bag, cut off with a pair of secateurs and placed in the pressure bomb. It was assumed that the water potential of the twig would be very similar to the water potential of the leaf it was carrying; this relationship has been found to be true for canola plants (Bernardi

2000). This methodology was unavoidable because *E. nitens* has sessile leaves and the other taxa had not developed petioles as yet and so water potential could not be measured on individual leaves. The six measurements took approximately 40 minutes to complete. Each day's measurements were done on a single individual, giving a picture of the changes in leaf water potential in one plant as the transpiration rate increased during the morning, in response to increasing vapour pressure deficit. Earlier measurements showed that beyond 11:00 am transpiration started to decline rapidly. Leaves that were wet from dew deposition were dried with a cloth before measurements were made. Data was logged when the "totalCV%" parameter was less than 1%. The LiCor 6400 was matched at every second leaf.

With the pressure bomb the point at which the stem changed colour as sap reached the surface was taken as the balance pressure. Leaf area and the leaf and stem dry weights were then determined in the laboratory after which the leaf material was discarded. The dry weight was determined by placing the plant material inside brown paper bags in a drying oven at 80 ºC for 24 hours.

2.6 Hydraulic conductance

2.6.1 General description: the HPFM

The High-Pressure Flow Meter was designed by Dr. M. Tyree and is described in Tyree *et al.* (1995) and Tyree *et al.* (1998). The HPFM measures the maximum conductance of a shoot or root system. When measuring shoot conductance water is forced up the excised shoot under high pressure (minimum of 0.4 MPa) until a "quasi steady-state" is reached, i.e. the volume of water passing between the two pressure transducers of the HPFM per unit time is constant. When measuring root conductance the HPFM actually measures the flow rate throught the root system under increasing pressure and conductance is then calculated from the slope of a

linear regression of the flow rate. Due to the nature of the setup water has to be forced down the root system opposite to the normal direction of flow (Figure 2.15). Reverse osmosis often causes a significant increase in root resistance; measurements of root hydraulic conductance are therefore done as fast as sound research practices allow and are called “transients”. The data-logging software is initiated and pressure valve opened simultaneously and the pressure in the Captive Air Tank (CAT) allowed to increase rapidly (3 to 5 $psi.s^{-1}$ or 20.5 to 34.5 kPa) to its maximum of just above 80 psi (551.3 kPa). The HPFM is therefore designed to force water into the base of a root system “while rapidly changing the delivery pressure and simultaneously measuring flow” (Tyree *et al.* 1995).

The two transducers are mounted on the two Omnifit manifolds – one is the inlet and the other the outlet. Each has eight outlets that are, in turn, associated with a capillary tube of different internal diameter and hence hydraulic resistance. The volume flow rate therefore depends upon the resistance of the capillary tube, the resistance of the plant material and the pressure applied to the CAT. The pressure applied to the CAT can be varied but ideally should be kept constant (otherwise quasi-steady state will never be reached) and high (to minimise the time necessary to complete a quasi-steady state measurement). The HPFM software gives a visual display of decreasing resistance with time as the shoot fills up with water; the steady state condition is indicated by a horizontal line.

Five parameters were continuously measured: pressure applied to the stem (MPa), the flow rate ($kg.s^{-1}$), the time (s), resistance ($MPa.s.kg^{-1}$) and conductance ($kg.s^{-1}.MPa^{-1}$). The CAT has a volume of approximately 3.2 litres and was filled with filtered, distilled water; 25 ml of concate HCL was added to 25 litres of the distilled water to make a 0.01 molar HCL solution. This was to

prevent microbial growth in the reservoir and in the xylem when doing long-term hydraulic measurements such as the quasi-steady state measurements (Sperry *et al.* 1988). The distilled, de-ionised water was filtered using a Millipore filtering system with a 0.22 μm final filter.

2.6.2 Hydraulic measurements

The hydraulic conductance of the root system and shoot of each plant was measured with a High-Pressure Flowmeter (HPFM) (Figure 2.16). The main stem was cut at an appropriate height and the cut end of the shoot placed in a bucket of water. About five cm of bark was then removed from the stump using a razor blade and a rubber bung of appropriate size forced over the stump. A few millimetres of the stump's upper surface was then removed with a clean razor blade to remove any fragments of wood or rubber that might obstruct the movement of water.The top half of the compression fitting was then screwed on and the fitting was then filled with a 0.05% safranin solution to make any leaks easily visible. A minimum of three transients were then performed on a root system and resistance plotted against pressure.

Figure 2.15: A compression fitting connected to an *E. nitens* root system. The shoot was cut off approximately ten centimetres from the soil surface with a handsaw. The bark was then peeled from the stump and the top three millimetres carefully removed with a clean razor blade to remove any splinters or particles that might block the xylem vessels.

Figure 2.16: The High Pressure Flow-Meter (HPFM). Changes in the hydraulic resistance of the plant material are displayed graphically on the laptop sitting on top of the HPFM.

Quasi steady-state measurements were performed on the shoots by attaching the compression fitting to the cut base (in the same manner as for the root stump), opening all the valves on the outlet Omnifit and forcing water up the stem to the leaves (Figures 2.18 & 2.19). This was done under high pressure (60-80 psi or 413.5 to 551.3 kPa) and it took between 15 and 40 minutes for the shoot to become filled with water depending upon its size. That quasi-steady state has been reached can also be seen on the shoot when water droplets appear on the leaf laminae as the water is forced out of the stomata under high pressure (Figure 2.17). At this point all valves on the outlet were closed except one and the quasi-steady state measurement initiated using the HPFM. Quasi steady-state test runs

on expendable *Eucalyptus* individuals determined which capillary tube had a suitable hydraulic resistance.

Figure 2.17: Water droplets emerging from the stomata after forcing water under high pressure up the shoot for 15 to 40 minutes depending upon the size of the specimen. This is an indication that quasi-steady state has been reached.

To determine the contribution of each component to the overall resistance, each component was removed and the parameters noted when the new quasi-steady state was reached. The different components measured were the minor veins, leaves, tertiary, secondary and primary twigs. To measure the resistance of the minor veins two parallel cuts were made with a razor blade along the major vein of each leaf. The leaves were then removed by hand (Figure 2.18) and the twigs with secateurs. In this way the hydraulic resistance of each component could be assessed.

Figure 2.18: A *E. grandis* x *nitens* plant after all its leaves had been removed during a quasi-steady state measurement.

Figure 2.19: The HPFM connected to a control plant during a quasi-steady state measurement.

2.7 Leaf and root surface areas and biomass allocation patterns

Leaf area was measured with a portable CI-202 leaf area meter (CID, Inc. Nebraska, USA). For root measurements the plastic bags were removed and the entire soil-root component soaked in water overnight. The water was then discarded and the soil removed with the aid of a gentle stream of water. Initially an aqueous 2.7 gramme/litre anhydrous sodium pyrophosphate ($Na_4P_2O_7$) solution was used as proposed by Schuurman and Goedewagen (1971). This did not significantly improve the removal of soil from the roots and was therefore discontinued. Schuurman and Goedewagen (1971) used it specifically to improve the dispersal of clay and the ineffectiveness of this technique could be because the soil had a low clay content.

Root surface area was estimated by laying the plant roots out on a white wooden slab, 570mm long x 500mm wide. A photograph was then taken of the slab with a Nikon Coolpix digital camera, using the flash for each picture. A 460mm long ruler was also placed on the slab. The pictures were transferred to a computer and analysed using ImagePro Plus AMG. Using a macro, that had been written to discriminate between a dark object and a white background, the software calculated the surface area of the roots; the ruler was used to calibrate each image. The calculation assumes that the roots are two dimensional and to improve on this it was assumed that the roots were circular in cross-section. The surface areas, calculated by the macro for each plant, were exported to Microsoft Excell and multiplied by π to give a more accurate measure of root surface area.

To detect any differences in biomass allocation patterns the leaves, twigs and roots were placed in separate brown paper bags and dried for 24 hours at 80 ºC, before measuring dry mass. The main stem was cut to a manageable size before

placing it into the oven. It was previously determined that 24 hours at 80 ºC was sufficient to dry the plant material to a constant mass.

2.8 Statistical analyses

Every statistical test has a number of assumptions associated with it. An independent-samples t-test was used to detect significant differences between control and treatment (chilled) groups. This test assumes equaity of variance and a normal distribution of the data. A one-sample Kolmogorov-Smirnoff test was performed on all data sets to determine if the data had a normal distribution. In all cases the data had a normal distribution making any transformation procedures unnecessary. The SPSS independent-samples t-test procedure automatically tests for equality of variance through the Levene's test. Where the assumption of equality of variance was invalid (indicated by a Levene's test statistic of less than 0.05) a non-parametric Mann-Whitney U test was performed.

One out of every 20 t-tests done is likely to give a significant P value purely buy chance. A Bonferroni correction was therefore applied to the stem diameter and height growth data due to the large number (8) t-test done in each case. This consisted of dividing the P value of 0.05 by the number of t-tests done and the quotient, in this case 0.00625, is taken as the new level of significance.

The light and CO_2 response curve data were fitted to the non-linear regression algorithm formulated by Causton and Dale (1990). The equation for the non-linear regression of light and CO_2 response curves is

$$y = a(1-e^{b-cx})$$

For a light response curve y represents the photosynthetic rate and x the light intensity (PPFD). a, b and c are constants. The ratio b/c is the light compensation

point. The dark respiration rate is given by $a(1-e^b)$ and the photochemical efficiency is given by ace^b.

For an A:Ci curve y represents the photosynthetic rate and x the CO_2 concentration in the internal cellular air spaces (ci). The ratio b/c is the CO_2 compensation point. The rate of photorespiration is the value of A, extrapolated to the point where the internal CO_2 concentration (x) is zero. This is therefore represented by $a(1-e^b)$. The carboxylation coefficient is represented by the term $a*c*e^b$.

SPSS 15.0 for Windows and Microsoft Exell 2000 was used for statistical tests.

3. Results

3.1 Growth data

3.1.1 Stem diameter

The reaction of stem diameter to root chilling seems to have been gradual in *E. grandis* x *nitens* but becoming increasingly pronounced (Fig. 3.4) until retardation of diameter growth became significant by day 150 (P = 0.003, Fig. 3.3; Table A1.1). In contrast *E. grandis*, showed an initial sharp reduction in diameter growth rate upon root chilling (P = 0.002, Fig. 3.1; Table A1.3), detected on day 29. However, by day 58 the difference was no longer significant. In *E. nitens* plants, differences in stem diameter remained non-significant for the duration of the experiment. In both *E. grandis* and *E. nitens* stem diameter of chilled plants then surpassed that of their unchilled counterparts from day 119 (Fig. 3.1 & 3.2). Although the chilled *E. grandis* and *E. nitens* plants maintained a heigher mean height from this point forward, it never became significant (Tables A1.2 & A1.3).

Stem diameter was thus significantly decreased in *E. grandis* x *nitens* by root chilling although the inhibitory effect took five months to take effect. *E. grandis,* in contrast, acclimatised within a two-month period to the root chilling treatment. Stem diameter in *E. nitens* was unaffected by the chilling treatment.

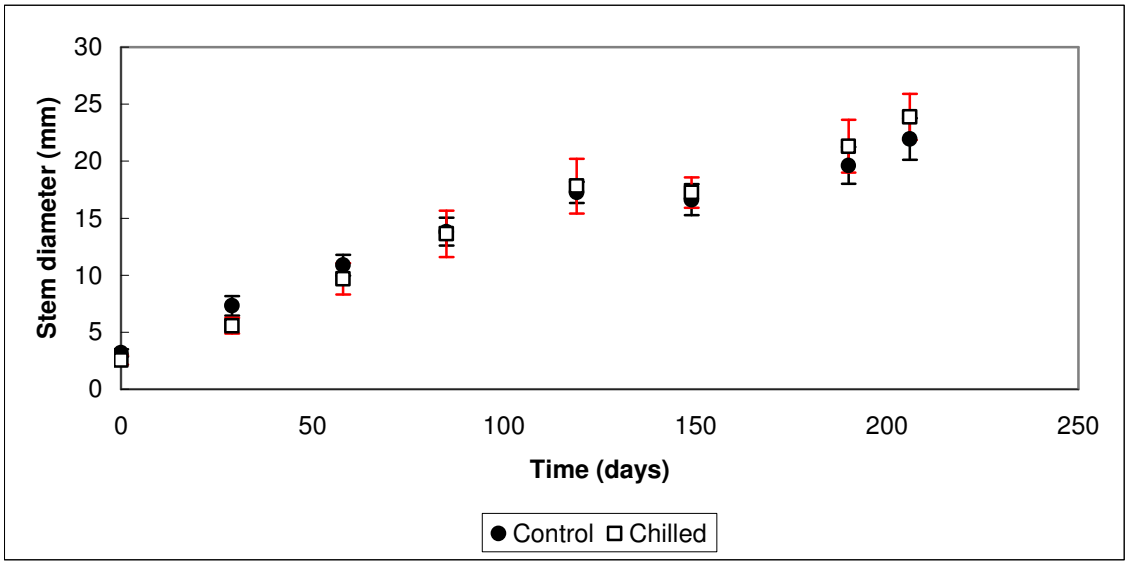

Figure 3.1: **S**tem diameter of *E. grandis* over the eight month period. Each point is reperesents the mean of 8 measurements. The vertical lines indicate 95% confidence limits (black – control; red – chilled).

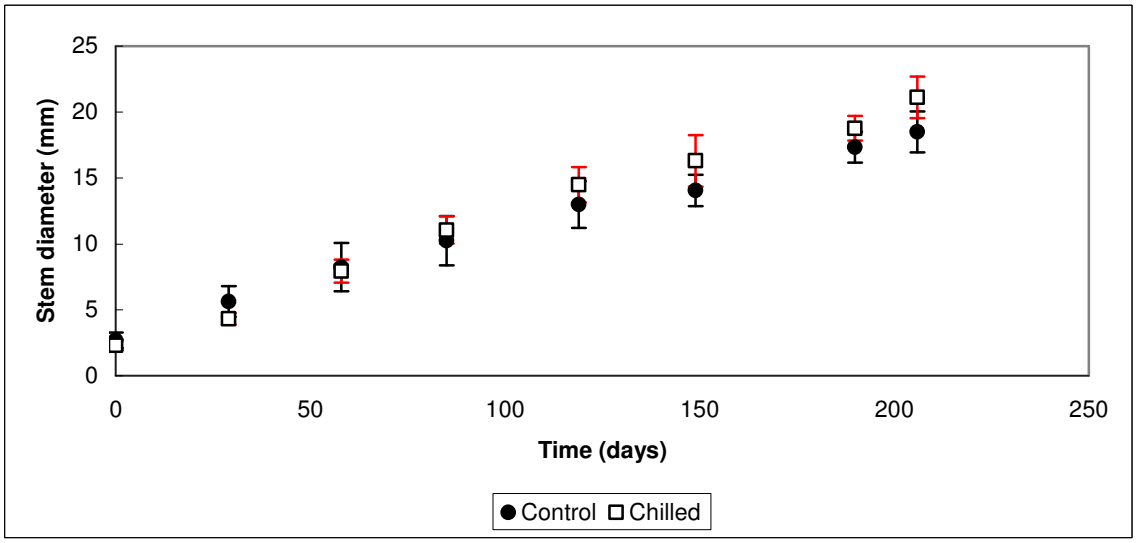

Figure 3.2: **S**tem diameter of *E. nitens* over the eight month period. Each point is reperesents the mean of 8 measurements. The vertical lines indicate 95% confidence limits (black – control; red – chilled).

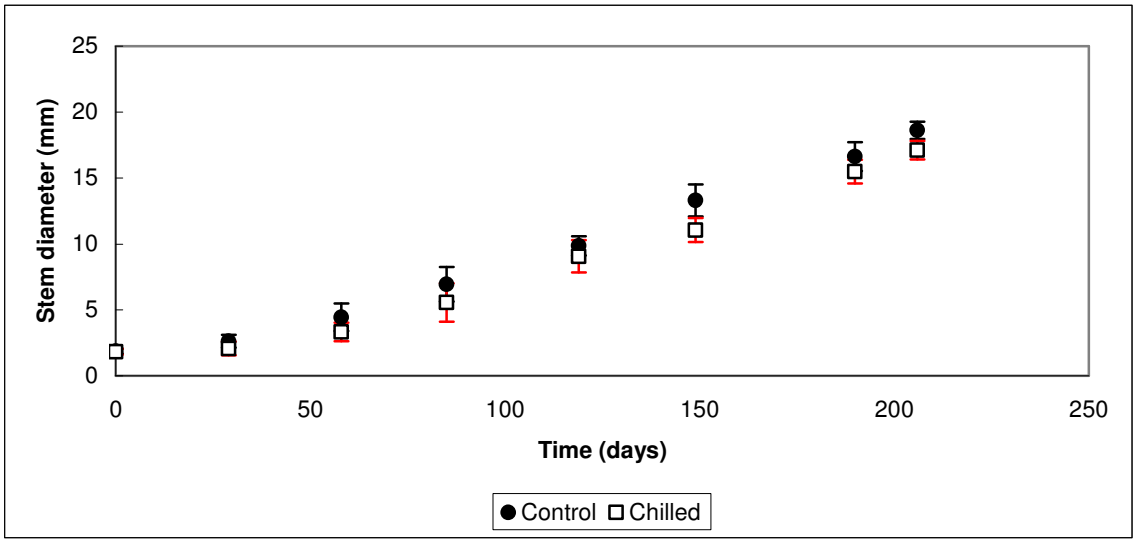

Figure 3.3: Stem diameter of *E. grandis* x *nitens* over the eight month period. Each point is reperesents the mean of 8 measurements. The vertical lines indicate 95% confidence limits (black – control; red – chilled)

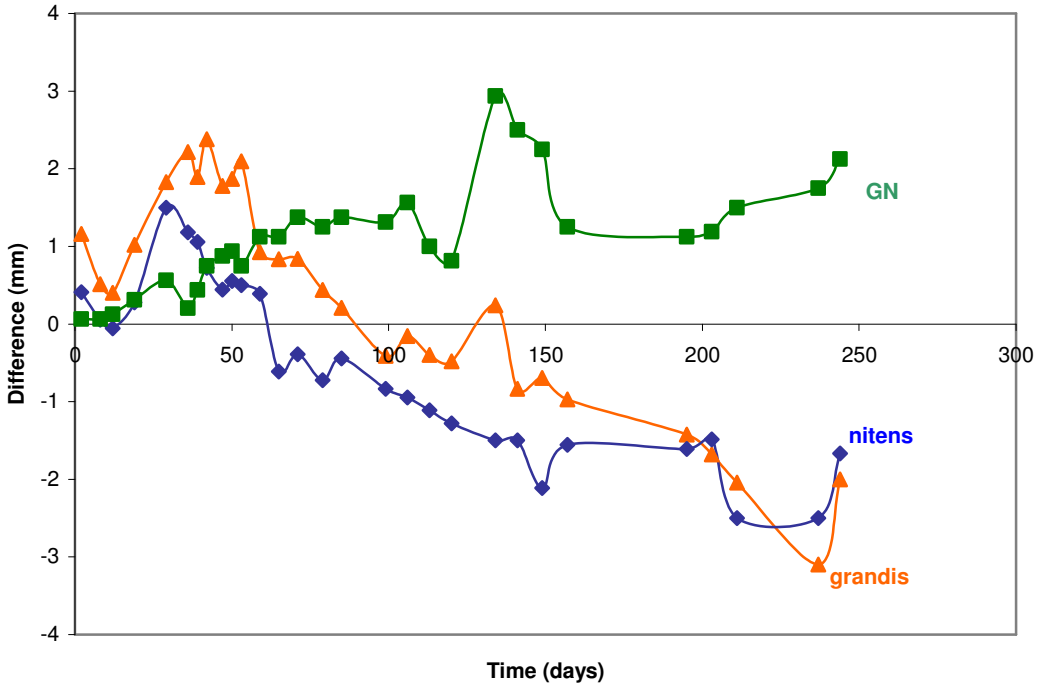

Figure 3.4: Difference in stem diameter between chilled and control plants (control diameter-chilled diameter).

3.1.2 Plant height

Root chilling had no significant effect on height growth in the *E. grandis* plants (Figs. 3.5 & 3.8; P = 0.753; Table A2.3). By day 100 height in chilled plants surpassed that of the unchilled plants but the difference never became significant (Fig. 3.5). Height of the chilled *E. nitens* (Fig. 3.6) and *E. grandis* x *nitens* (Fig. 3.7) plants surpassed that of their unchilled counterparts at about day 150 and 200 respectively (Fig. 3.8). However, during the entire experimental period none of the differences in height for *E. nitens* or *E. grandis* x *nitens* were significant (Tables A2.2 & 2.1, respectively).

Plant height was thus unaffected by the root chilling treatment in all three taxa. *E. grandis* and *E. grandis x nitens* showed an opposite trend in their stem diameter growth. *E. grandis* showed a siginificant initial decrease followed by acclimatisation within a period of two months. In *E. grandis* x *nitens* root chilling caused a gradual inhibition of stem diameter which became significant at about four months; its height was unaffected by the chilling treatment (Fig. 3.7; P = 0.003). In *E. nitens* both stem diameter and height were unaffected by root chilling . It should be kept in mind that *E. nitens* is the renowned for its cold-tolerance.

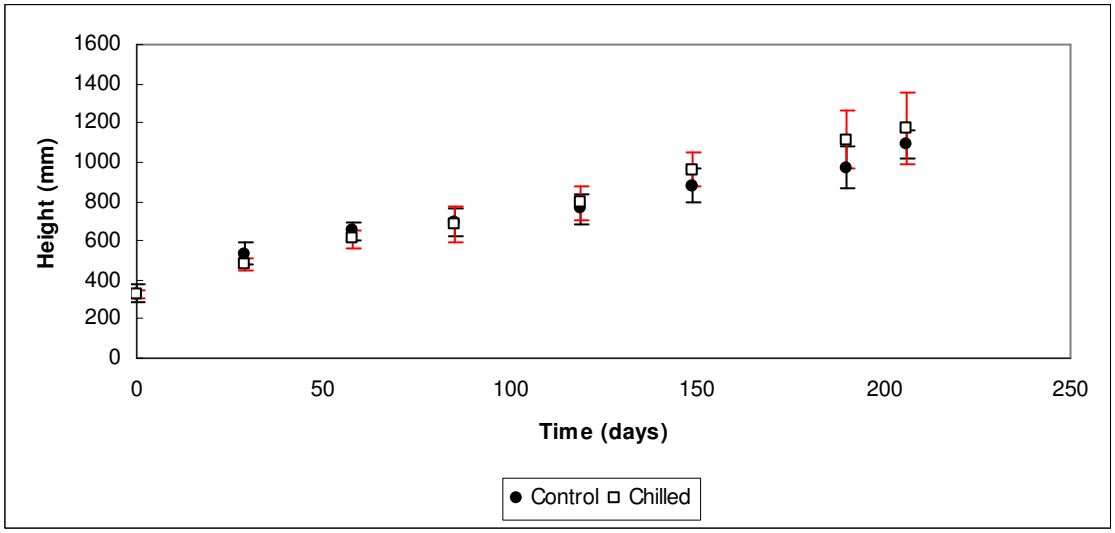

Figure 3.5: Height of *E. grandis* over the eight month period. Each point is reperesents the mean of 8 measurements. The vertical lines indicate 95% confidence limits (black – control; red – chilled).

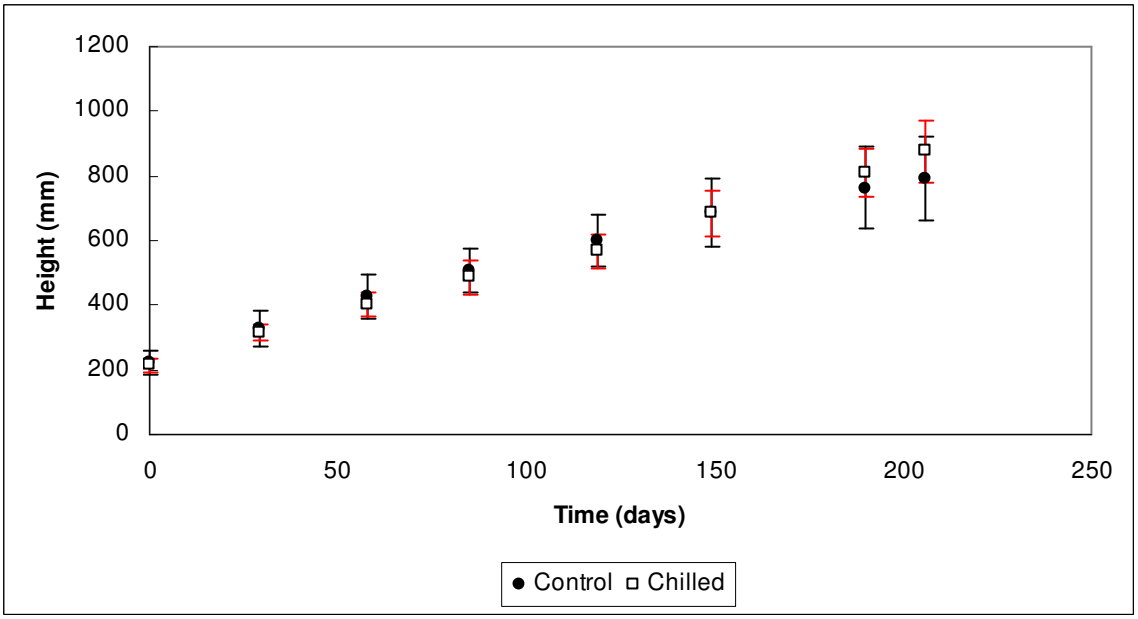

Figure 3.6: Height of *E. nitens* plants during the experimental period. Each point represents the mean of 8 measurements. The vertical lines indicate 95% confidence limits (black – control; red – chilled).

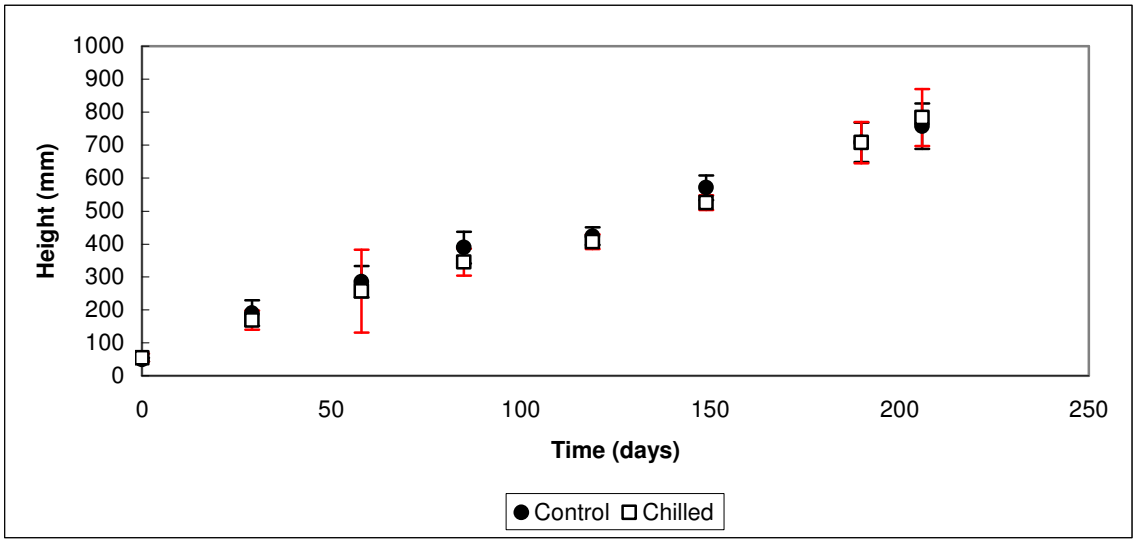

Figure 3.7: Height of *E. grandis* x *nitens* plants during the experimental period. Each point represents the mean of 8 measurements. The vertical lines indicate 95% confidence limits (black – control; red – chilled).

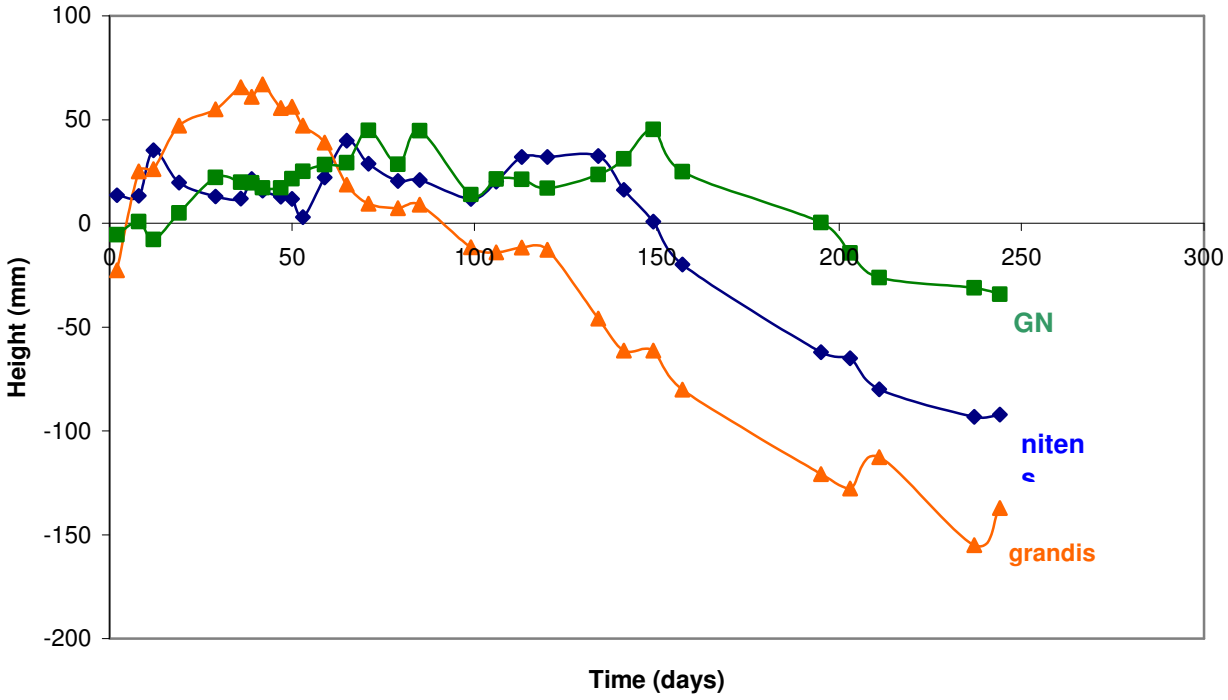

Figure 3.8: Differences in height (control height – chilled height) between the chilled and control groups for the duration of the chilling period.

3.1.3 Final biomass

Figure 3.9 shows a reduction in total plant dry weight, when harvested after 260 days, in plants subjected to root chilling across all genotypes. This reduction was significant for *E. grandis* x *nitens* (P = 0.007; Table A3.3) and *E. grandis* (P = 0.02; Table A3.1). The reduction was not significant in *E. nitens* (P = 0.279; Table A3.2).

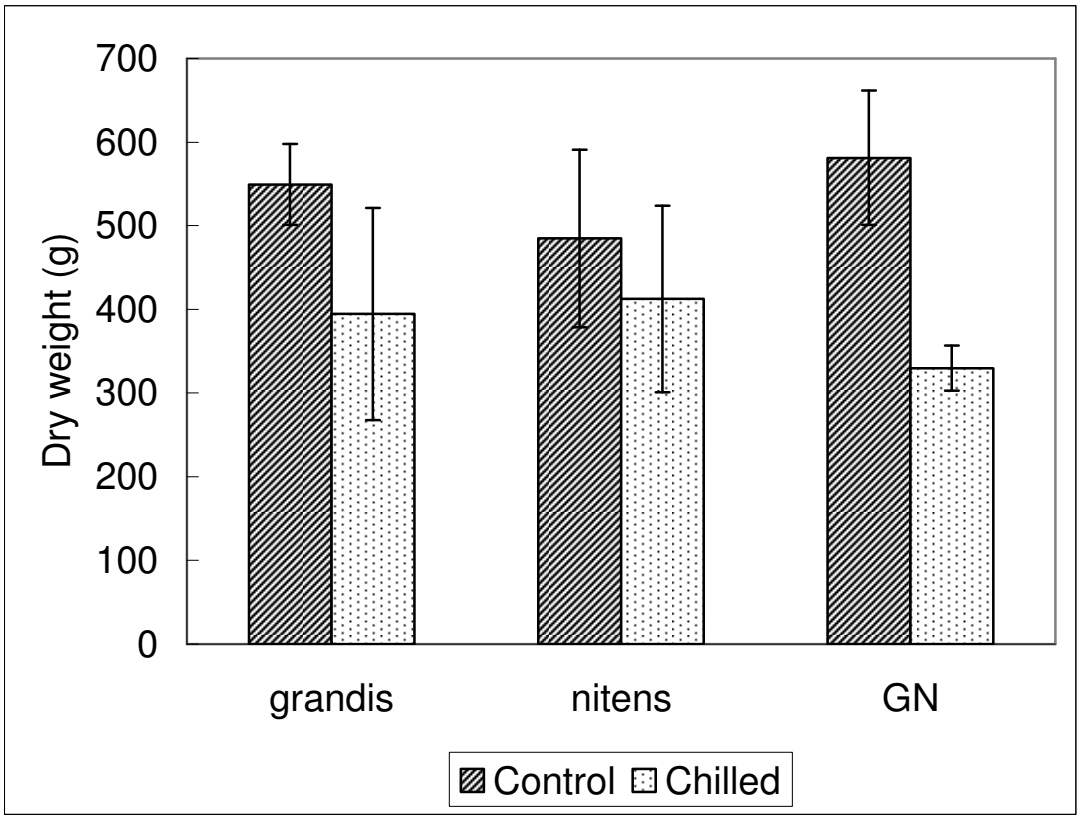

Figure 3.9: Total dry weight of the control and chilled plants of all three genotypes. The vertical lines above the bars indicate the standard deviation about the mean (n = 5).

3.1.4 Biomass partitioning

Figure 3.10 shows the variation in biomass partitioning between chilled and unchilled *E. grandis* plants. The decrease in root dry weight in the chilled plants was not significant (P = 0.880; Table A3.1). Instead the significant decreases in dry weight took place aboveground in the leaves (P = 0.018) and stem (P = 0.047), the stem dry weight calculated by subtracting the foliage mass from the aboveground biomass. It therefore seems that, in *E. grandis*, the alterations made to the biomass partitioning pattern to maintain the balance between water uptake and loss, have occurred aboveground in the leaves and stem dry weight.

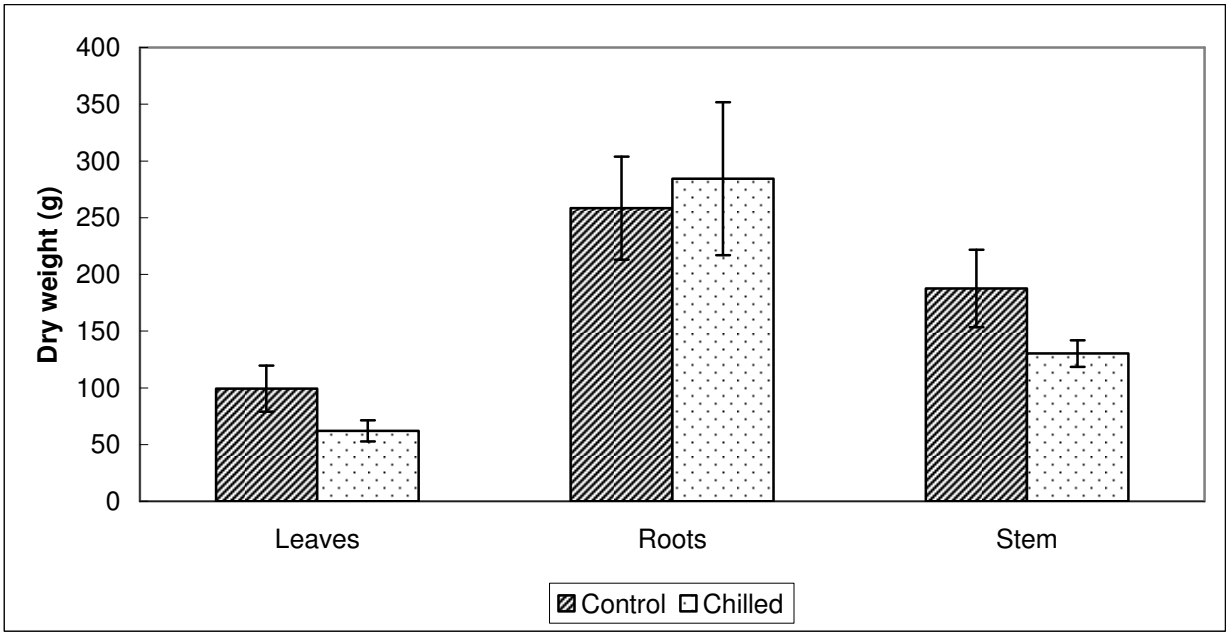

Figure 3.10: Leaf, stem and root dry weight in control and chilled *E. grandis* plants. The vertical lines above the bars indicate the standard deviation about the mean (n = 4).

E. nitens is the most cold-tolerant genotype and in accordance with this, Fig 3.11 shows that the root chilling treatment had little effect on biomass partitioning. The difference in the leaves (P = 0.713; Table A3.2), roots (P = 0.295) and stem (P = 0.236) dry weight was not significant. Interestingly the root dry weight of chilled plants were slightly greater than the unchilled plants.

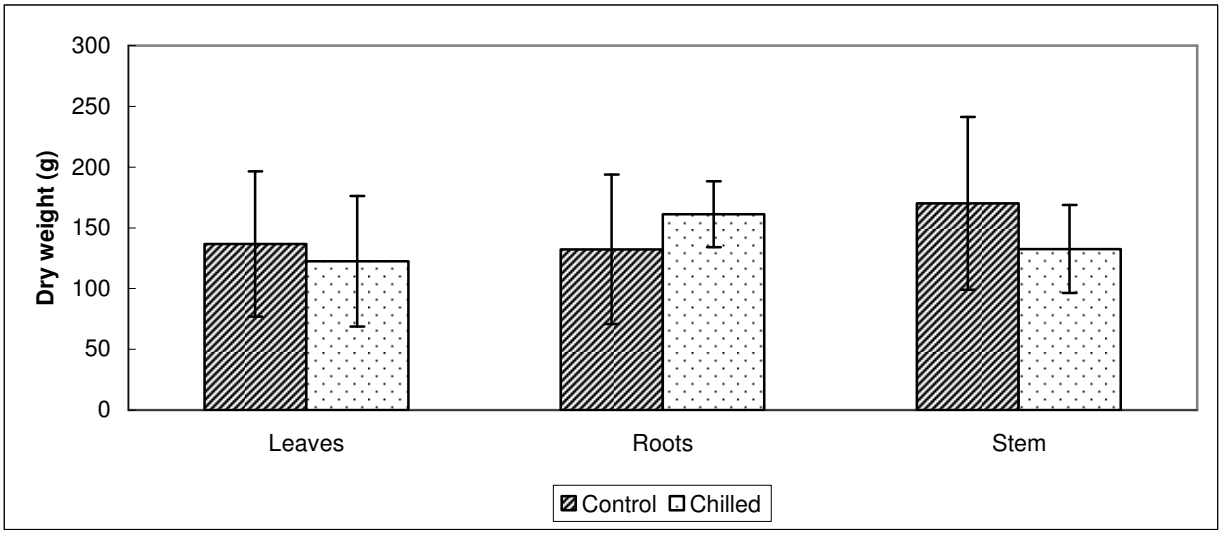

Figure 3.11: Leaf, stem and root dry weight in control and chilled *E. nitens* plants. The vertical lines above the bars indicate the standard deviation about the mean (n = 3).

E. grandis x *nitens* proved to be the most malleable with respect to biomass partitioning as shown by Fig. 3.12. The difference between treatments in dry weight of the leaves (P < 0.001; Table A3.3) and stem (P = 0.003) was significant, the chilled plants having much lower dry weights in each case. Root dry weight, however, did not differ significantly.

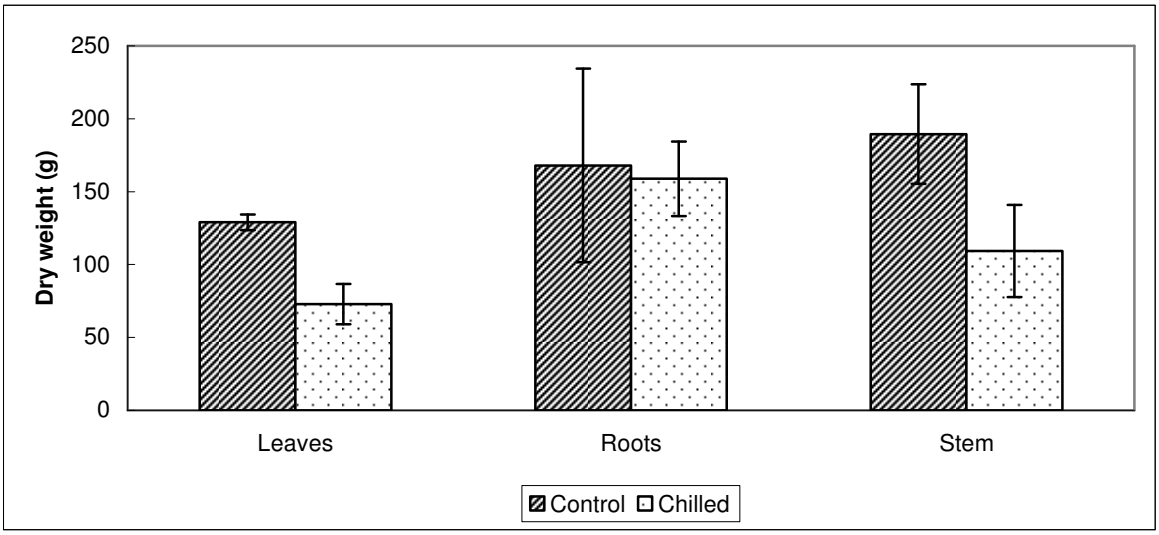

Figure 3.12: Leaf, root and stem dry weight between control and chilled *E. grandis* x *nitens* plants. The vertical lines above the bars indicate the standard deviation about the mean (n = 5).

3.1.5 Leaf surface area

Total leaf surface area was significantly reduced in the chilled plants compared with the unchilled plants. Figure 3.13 illustrates this for all three genotypes: *E. grandis* (P = 0.01; Table A3.1), *E. nitens* (P = 0.008; Table A3.2) and *E. grandis* x *nitens* (P < 0.001; Table A3.3).

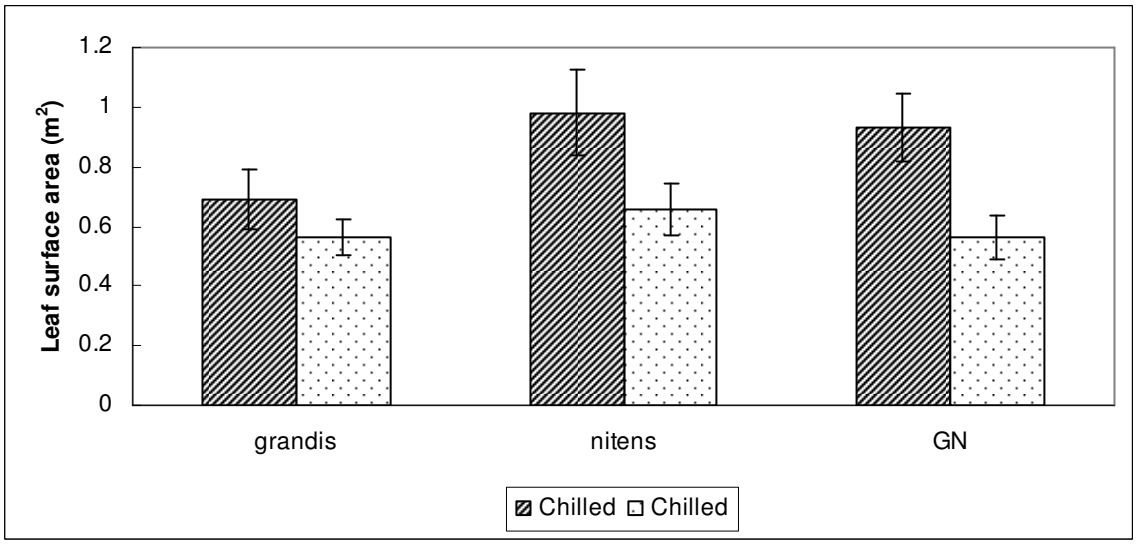

Figure 3.13: Total leaf surface area of control and chilled plants in each genotype. The vertical lines above the bars indicate the standard deviation about the mean (n = 5).

3.1.6 Root surface area

Figure 3.14 illustrates the variation brought about in root surface area by the root chilling in each genotype. The treatment plants generally had a higher root surface area than control plants but only in *E. nitens* was this increase significant (P = 0.028; Table A3.2).

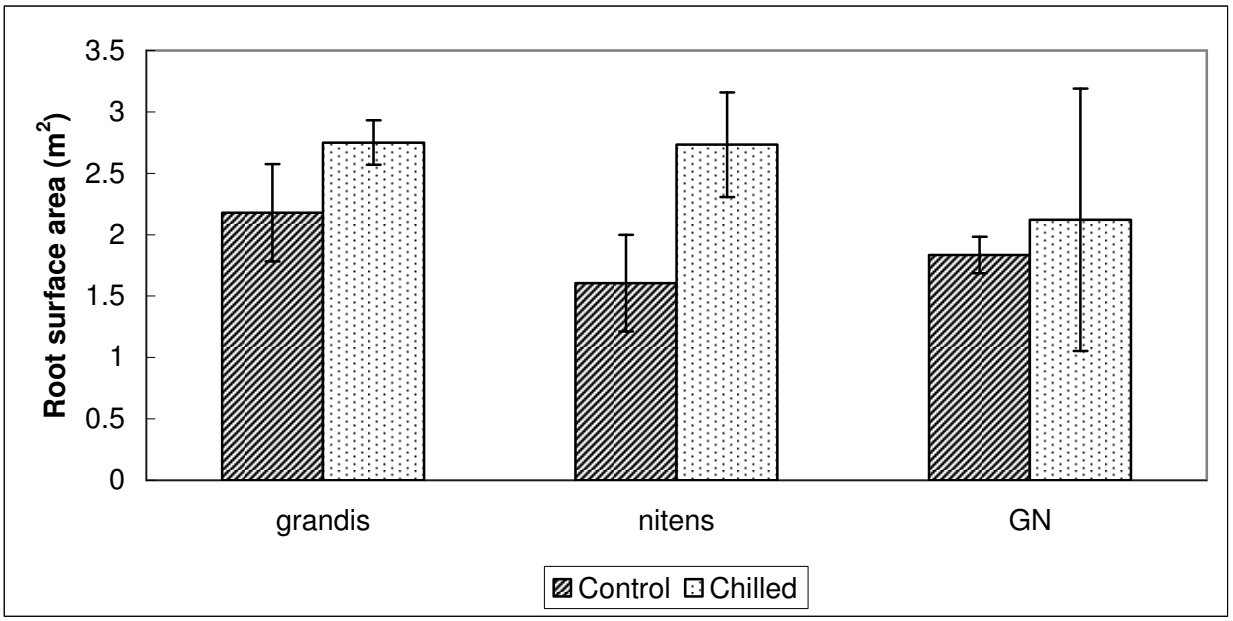

Figure 3.14: Root surface areas of control and chilled plants of each genotype. The vertical lines above the bars indicate the standard deviation about the mean (n = 3).

3.1.7 Below- to above-ground ratios

Figure 3.15 shows that the chilled plants had an increased root to shoot dry weight ratio. This increase was significant in *E. grandis* (P = 0.012; Table A3.1) but not in *E. grandis* x *nitens* (P = 0.088; Table A3.3) or *E. nitens* (P = 0.267; Table A3.2).

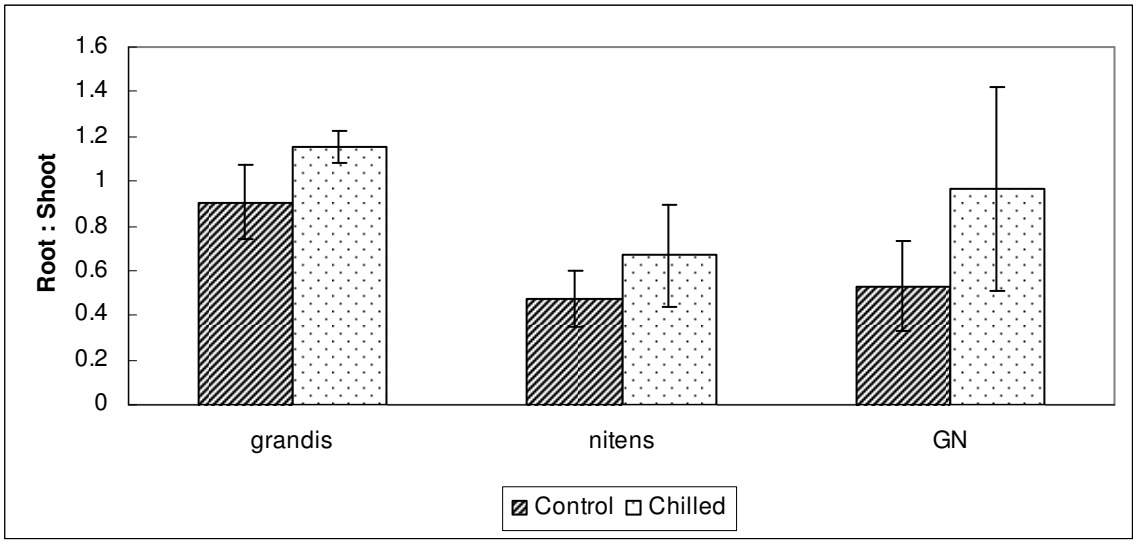

Figure 3.15: Root:shoot ratio of the control and chilled plants in each genotype. The vertical lines above the bars indicate the standard deviation about the mean (n = 5).

3.1.8 Root surface area : leaf surface area

Figure 3.16 illustrates the differences in the ratio of root surface area to leaf surface area due to root chilling. The ratio was significantly increased in all three genotypes: *E. grandis* (P = 0.035; Table A3.1), *E. nitens* plants (P = 0.01; Table A3.2) and in the hybrid (P = 0.05; Table A3.3).

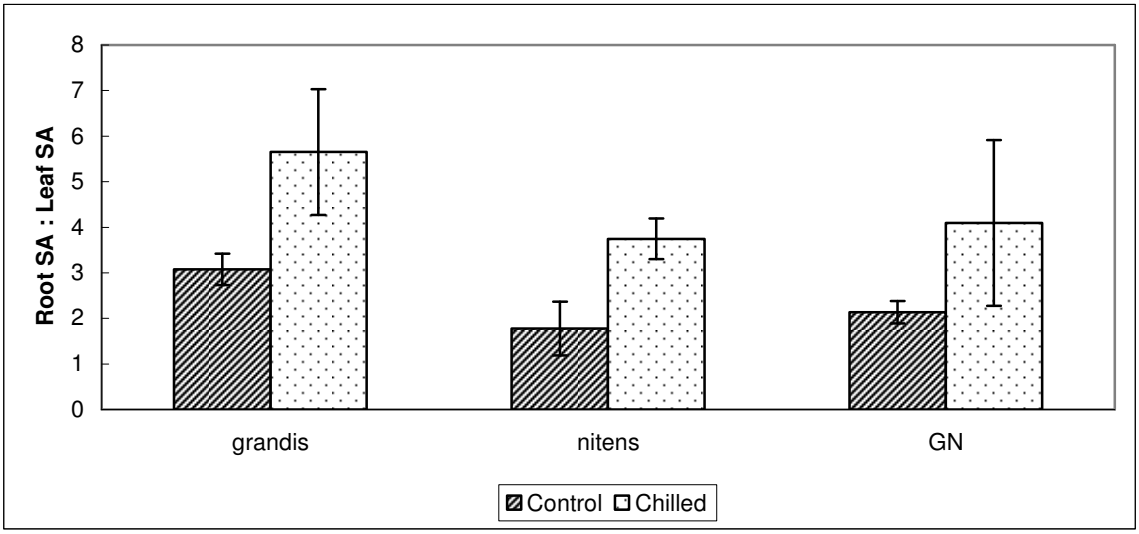

Figure 3.16: Root surface area to leaf surface area ratio between control and chilled plants for each genotype. The vertical lines above the bars indicate the standard deviation about the mean (n = 5).

The balance between absorptive and evaporative surface area seems to have been a critical factor that was maintained in different ways: *E. nitens* increased its root surface area, whereas the others increased their leaf surface area. It should be remembered that a large portion of the fine roots are lost when washing the soil from the roots. This is the part of a root system that is the most active in water absorption because it has the highest surface area to mass ratio. This should be taken into account when interpreting the data illustrated in Figure 3.16.

3.2 Physiological data

3.2.1 Photosynthetic characteristics

A light response curve illustrates the dynamics of the photosynthetic processes that are sensitive to light. Initially carbon assimilation increases linearly with increasing light intensity; this constitutes the light limited region (Fig. 3.17). The slope of this linear region is the maximum quantum yield (denoted by the Greek "alpha"), which is a measure of the efficiency with which absorbed quanta are used to fix CO_2. A point is then reached where assimilation levels off where photosynthesis is limited by other factors, especially CO_2 supply. The light response curves in this study are based on incident, rather than absorbed quanta and the quantum efficiency calculated is therefore the apparent quantum efficiency.

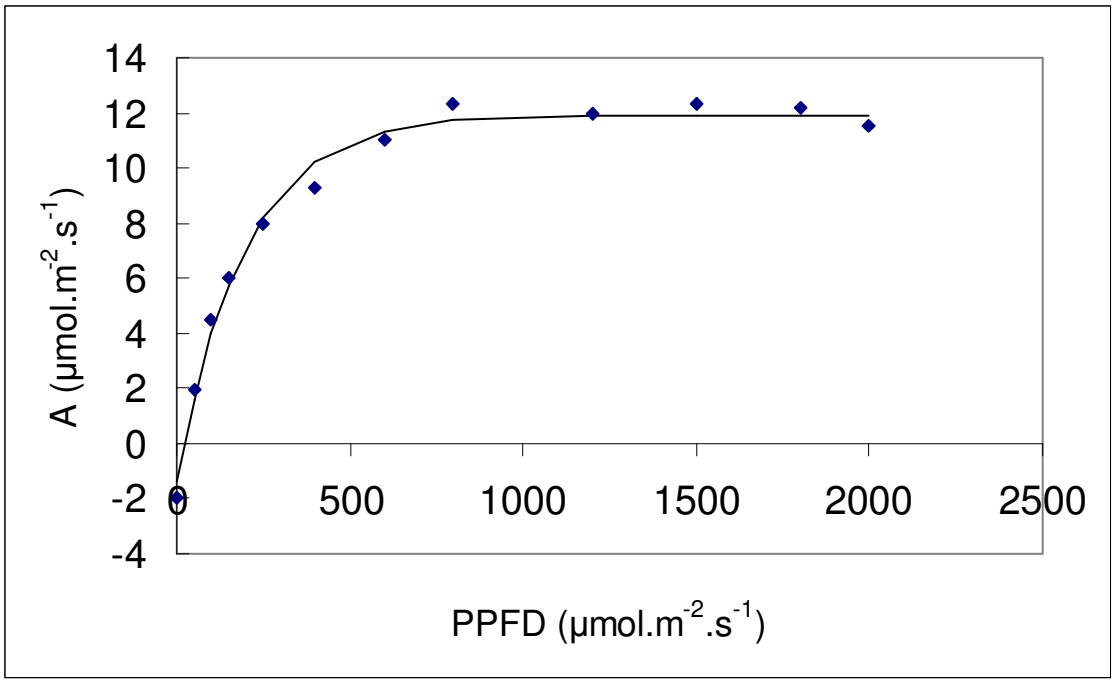

Figure 3.17: Light response curve of an *E. grandis* control plant.

Regression lines were fitted to the light curve using the monomolecular function as suggested by Causton and Dale (1990). This function provided an accurate fit

to the data points and takes the form A = a(1-exp(b-c*ppfd)). The constants were obtained by running a non-linear regression on the raw assimilation data in SPSS 13.0.

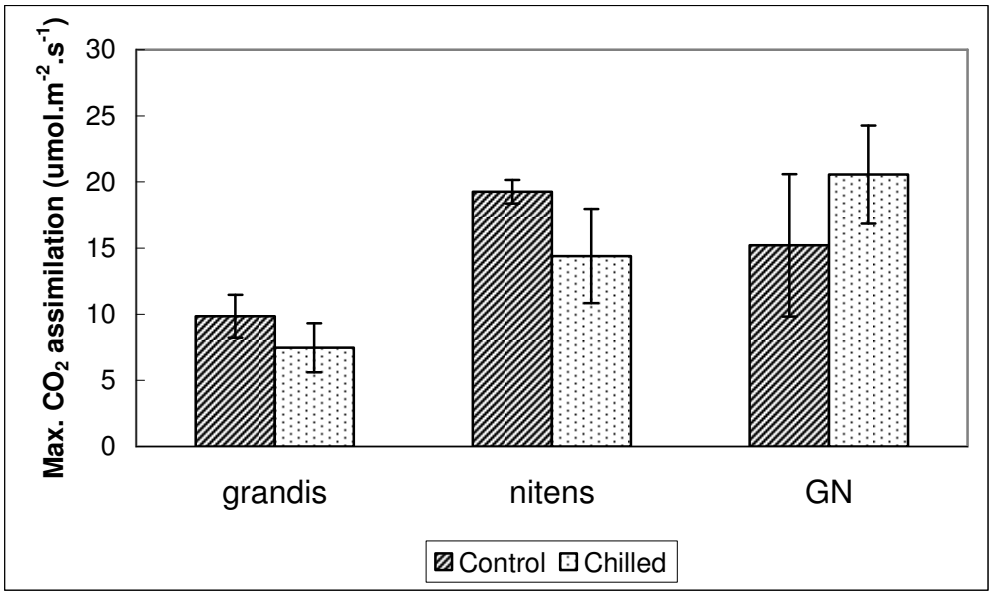

Figure 3.18: Mean maximum CO_2 assimilation values of the control and chilled plants. The vertical lines above the bars indicate the standard deviation about the mean (n = 5).

Figure 3.18 is an illustration of the maximum rates of carbon assimilation (A_{max}) in each genotype from both control and chilled groups. Root chilling did not reduce A_{max} significantly in *E. grandis* (P = 0.061; Table A4.1) or *E. grandis* x *nitens* (P = 0.277; Table A4.5). A_{max} was significantly reduced in *E. nitens* (P = 0.011; Table A4.3), an unexpected result because it is considered the most chilling tolerant of the three genotypes.

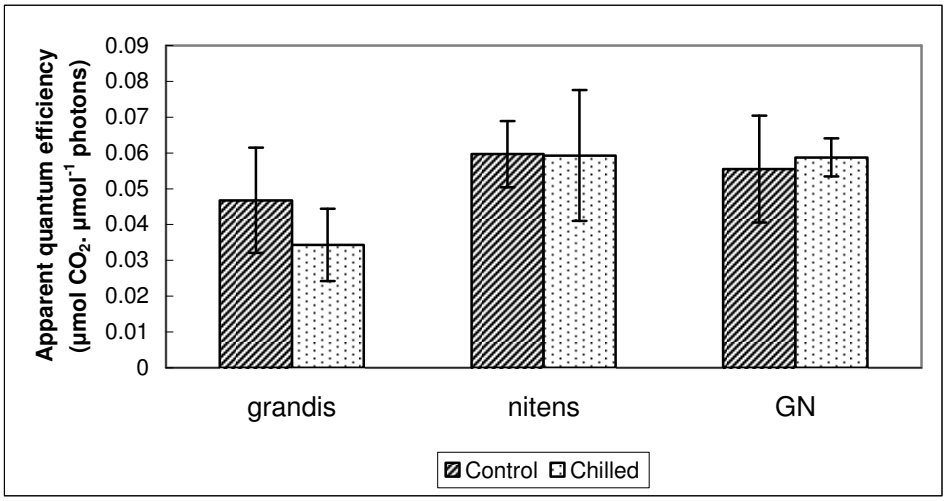

Figure 3.19: Mean apparent quantum efficiency values for control and chilled plants. The vertical lines above the bars indicate the standard deviation about the mean (n = 5).

Figure 3.19 is illustrates the mean apparent quantum efficiency values, based on incident radiation, of the control and treatment groups. None of the differences induced by the chilling treatment were significant (see Tables A4.1, A4.3 & A4.5) which indicates that it had no effect on the efficiency with the photosynthetic machinery was using light energy to drive biochemical reactions.

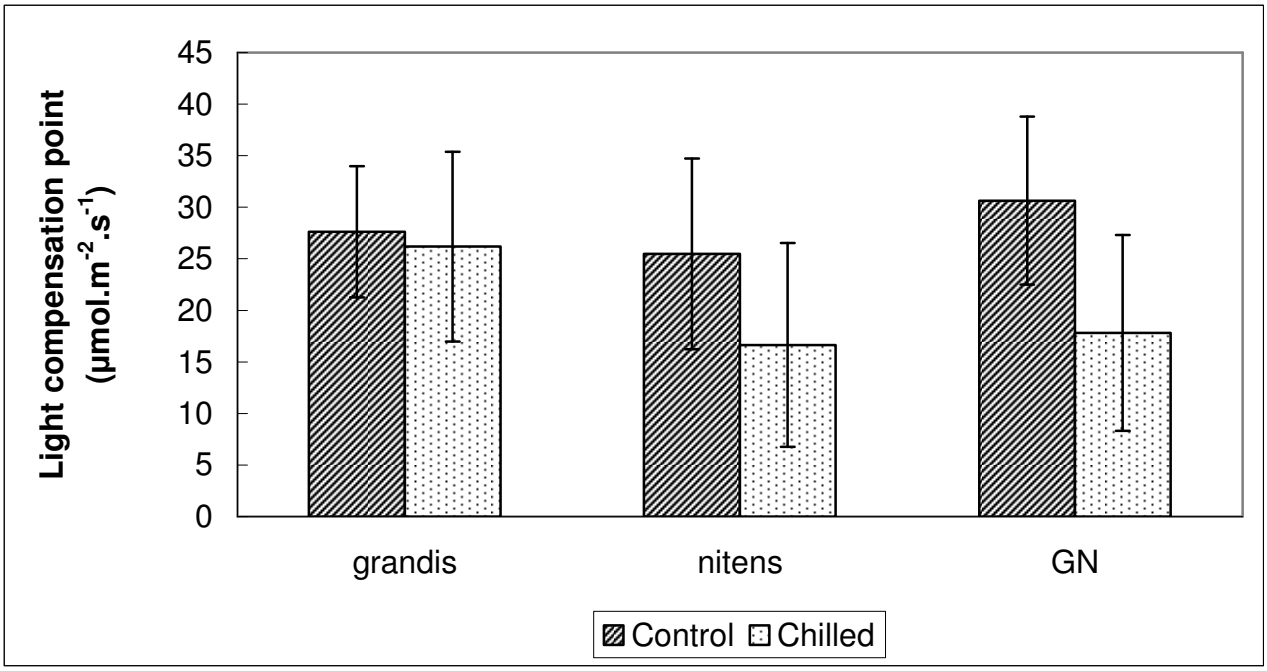

Figure 3.20: Mean light compensation points of the control and chilled plants. The vertical bars represent the standard deviation about the mean (n = 5).

Figure 3.20 shows the mean light compensation points for the six experimental groups. The light compensation point is the point where CO_2 fixed through photosynthesis balances that produced by respiration. A higher compensation point implies a higher level of light is needed to balance photosynthesis and respiration and therefore a high dark respiration rate is occurring. None of the differences between chilled and unchilled plants of *E. grandis* or *E. nitens* were significant (P = 0.296 and 0.131 respectively; see Tables A4.1 & A4.3). In *E. grandis* x *nitens* the control plants had significantly higher (P = 0.043; Table A4.5) light compensation points than chilled plants. This is most likely not a temperature effect because the control and chilled groups had similar leaf temperatures.

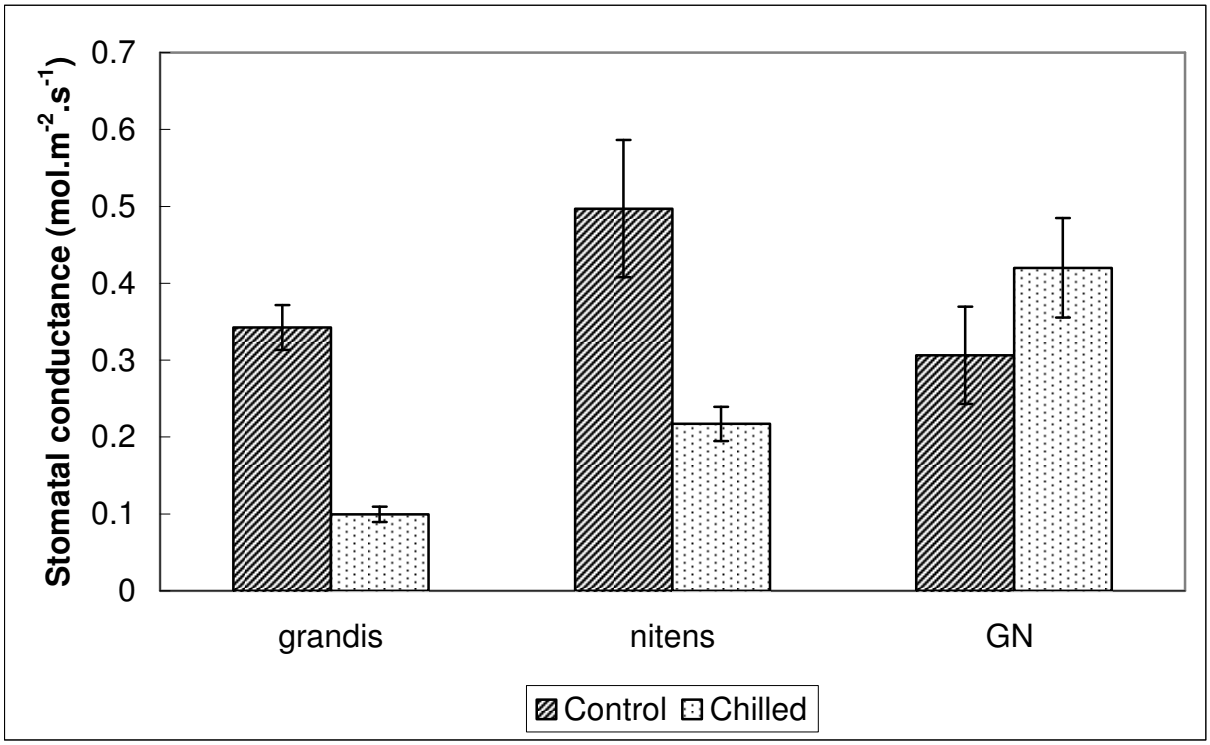

Figure 3.21: Mean stomatal conductance (to H_2O) values for the control and chilled plants. The vertical bars represent the standard deviation about the mean (n = 5).

Figure 3.21 illustrates the mean differences in stomatal conductance between control and chilled plants. The means were calculated using those conductance values from light response curves where the stomatal conductance is light saturated (from 800 to 2000 PPFD). Light response data were collected only on sunny days, between 08h00 and 12h00. Stomatal conductance was significantly reduced in chilled plants of *E. grandis* (P = 0.009; Table A4.2) and *E. nitens* (P = 0.009; Tabe A4.4). Chilled plants of *E. grandis* x *nitens*, however, had a significantly higher stomatal conductance (P = 0.023; Table A4.5) than control plants.

A:Ci curves (Fig. 3.22) are plots of assimilation versus the CO_2 concentration of the internal air spaces within the leaf, and therefore assess how efficiently the biochemical processes are functioning. The four variables assessed were the maximum rate of CO_2 assimilation, the carboxylation coefficient, the CO_2 compensation point and the rate of photorespiration.

In an A:Ci curve maximum assimilation rates are termed J_{max} and represent the maximum rate of electron transport and therefore the rate at which the photosynthetic carbon reduction cycle turns over. The carboxylation coefficient is measured as the initial slope of the curve and is a measure of how efficient Rubsico is at catalysing the reaction joining RuBP and CO_2. The CO_2 compensation point reflects the point where photosynthesis and photorespiration balance each other. Higher CO_2 compensation points would suggest a negative effect on the biochemistry of photosynthesis because it implies that Rubisco needs higher substrate concentrations to function optimally. The amount of photorespiration occurring was assessed by the value of A, extrapolated to the point where the internal CO_2 concentration is zero.

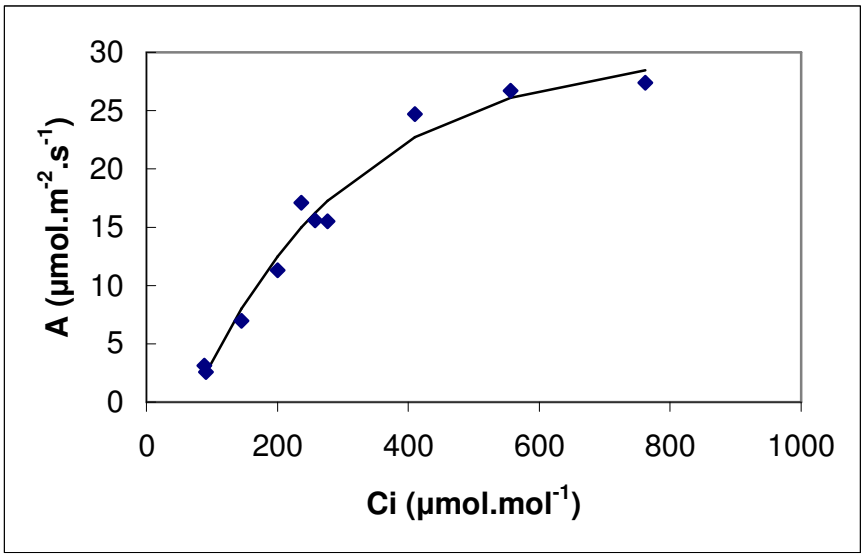

Figure 3.22: An A:Ci curve of an *E. nitens* control plant.

The same monomolecular function used to fit regression lines to the light curves was used for the A:Ci curves. In the IRGA's autoprogramme for A:Ci curves the CO_2 concentration in the photosynthesis chamber started at ambient, decreased to very low levels and increased finally to well above ambient.

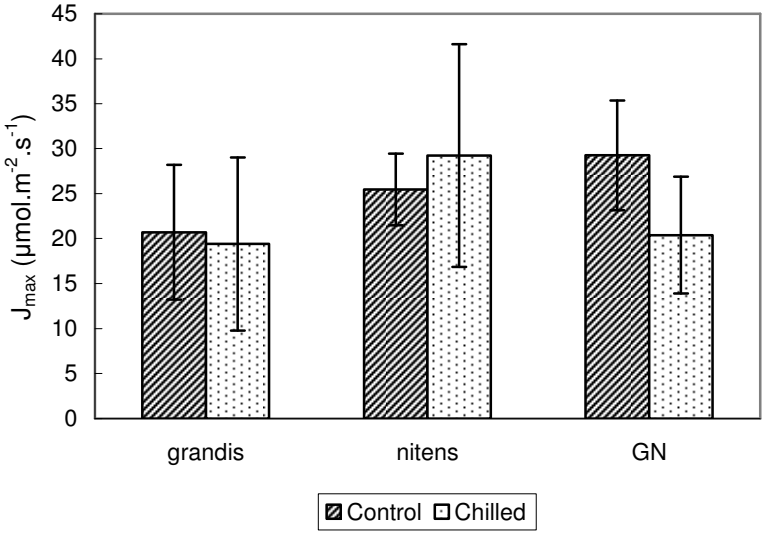

Figure 3.23: Mean J_{max} values for the control and chilled plants. The vertical lines above the bars indicate the standard deviation about the mean (n = 5).

Figure 3.23 shows the difference in the maximum rate of electron transport (J_{max}) for each genotype. Root chilling had no significant effect on the J_{max} values of any of the genotypes (Tables A4.6, A4.8 & A4.9).

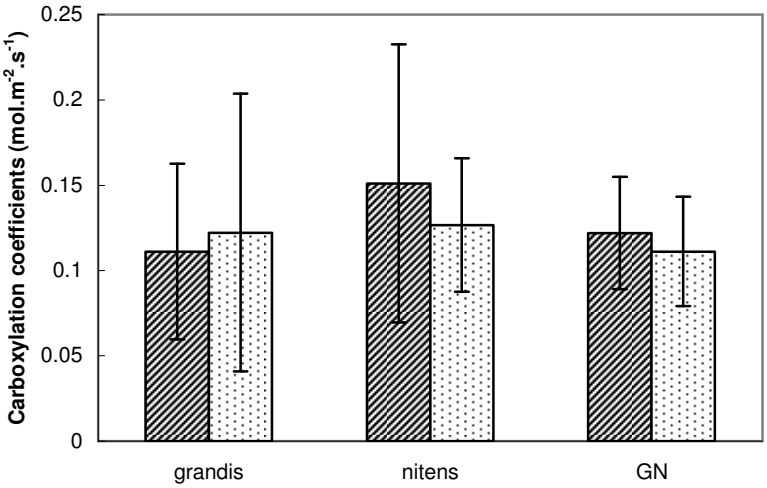

Figure 3.24: Mean carboxylation coefficients for the control and chilled plants. The vertical bars represent the standard deviation about the mean (n = 5).

Figure 3.24 illustrates differences in the carboxylation coefficients for each genotyp. Root chilling had no effect on the carboxylation coeffecients in any of the genotypes (Tables A4.6, A4.7 & A4.9).

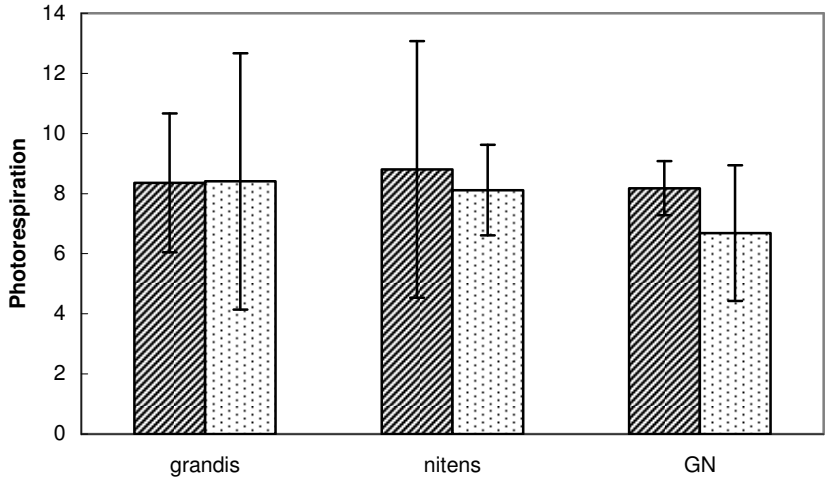

Figure 3.25: Mean photorespiration values for the control and chilled plants. The vertical lines represent the standard deviation about the mean (n = 5).

Figure 3.25 illustrates differences in the rates of photorespiration, induced by root chilling. Again, the treatment did not induce significant differnce in any genotype (Tables A4.6, A4.7 & A4.9).

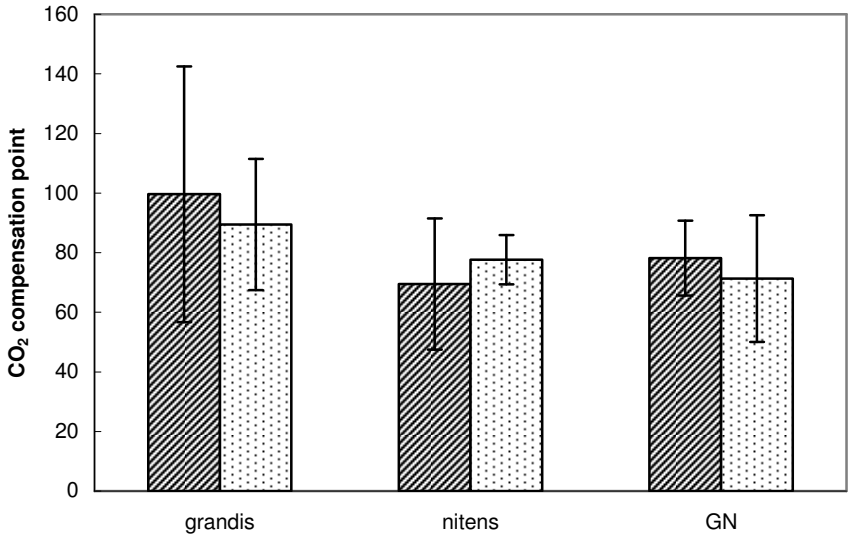

Figure 3.26: Mean CO_2 compensation points for control and chilled plants. The vertical bars represent the standard deviation about the mean (n = 5).

Similarly, differences in CO_2 compensation point, illustrated in figure 3.26, induced by the chilling treatment were not significant within any genotype (Tables A4.6, A4.7 & A4.9).

Treatment plants of *E. grandis* and *E. nitens* had a significantly reduced A_{max}. However, root chilling appears to have had no significant effect on the biochemical photosynthetic characteristics of the *Eucalyptus* taxa concerned, although there were some genotypic differences with respect to the light driven processes. It can therefore be concluded that the effect of root chilling on growth does not occur by alterations in the photosynthetic parameters.

3.2.2 Soil-to-leaf hydraulic resistance

The slope of leaf water potential versus transpiration rate is an indication of the hydraulic resistance of the soil-to-leaf pathway. In *E. grandis* and *E. grandis* x *nitens* there was no significant difference in the resistance of this pathway between the chilled and unchilled plants (Figures 3.27 & 3.29; P = 0.265 and 0.993, respectively; Table A5.1). In *E. nitens* (Fig. 3.28) the chilled plants had a significantly shallower slope, indicating a lower resistance than the unchilled plants (P = 0.002; Table A5.1). This suggests that the plants have acclimatised to the chilling treatment, counteracting the imbalance in the whole-plant hydraulic resistance induced by this treatment.

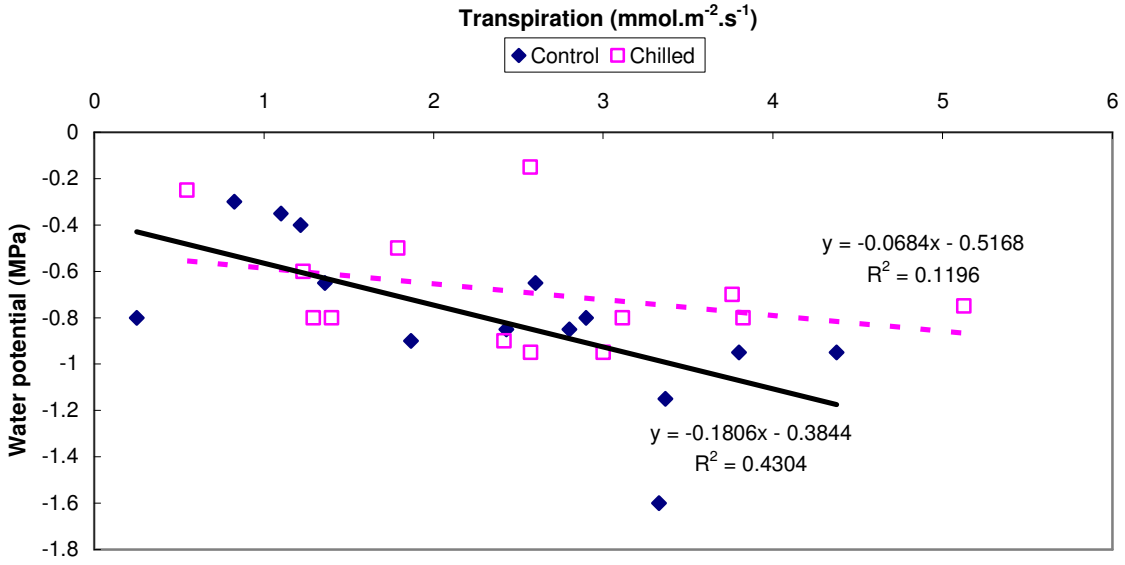

Figure 3.27: Leaf water potential as influenced by transpiration rates of control and chilled plants of *E. grandis*. The slopes of these plots are taken as an indication of the soil-to-leaf hydraulic resistance.

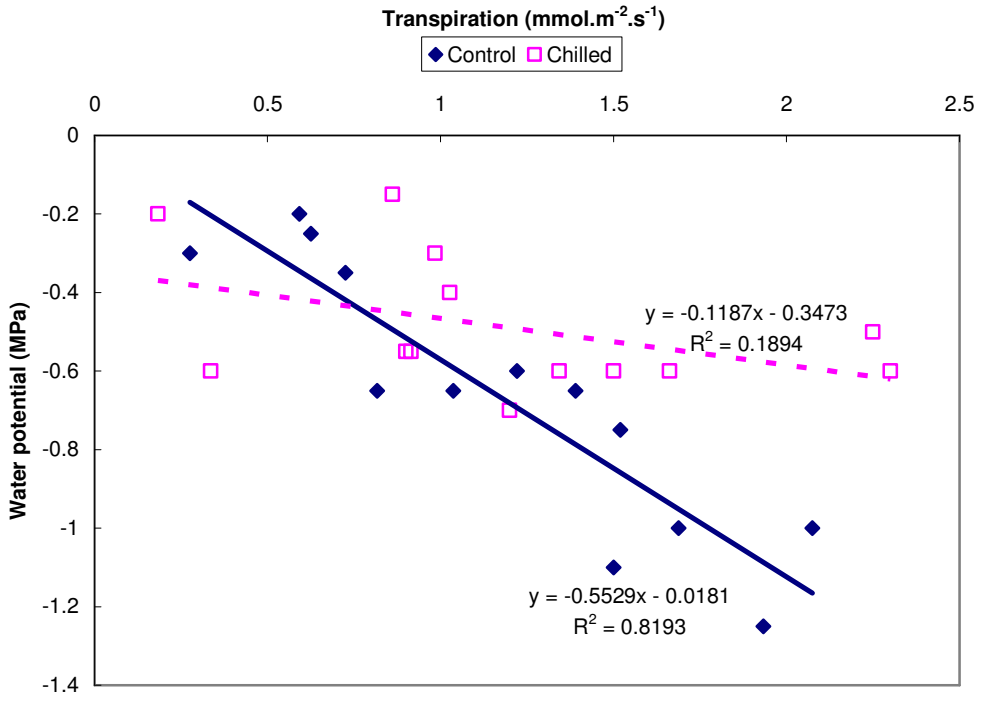

Figure 3.28: Leaf water potential as influenced by transpiration rates of control and chilled plants of *E. nitens*. The slopes of these plots are taken as an indication of the soil-to-leaf hydraulic resistance.

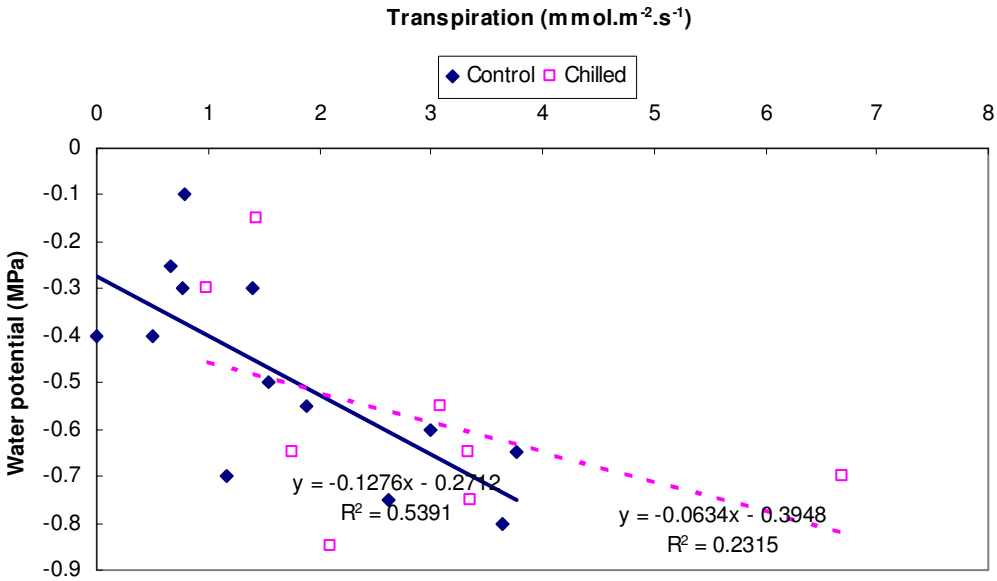

Figure 3.29: Leaf water potential as influenced by transpiration rates of control and chilled plants of *E. grandis* x *nitens*. The slopes of these plots are taken as an indication of the soil-to-leaf hydraulic resistance.

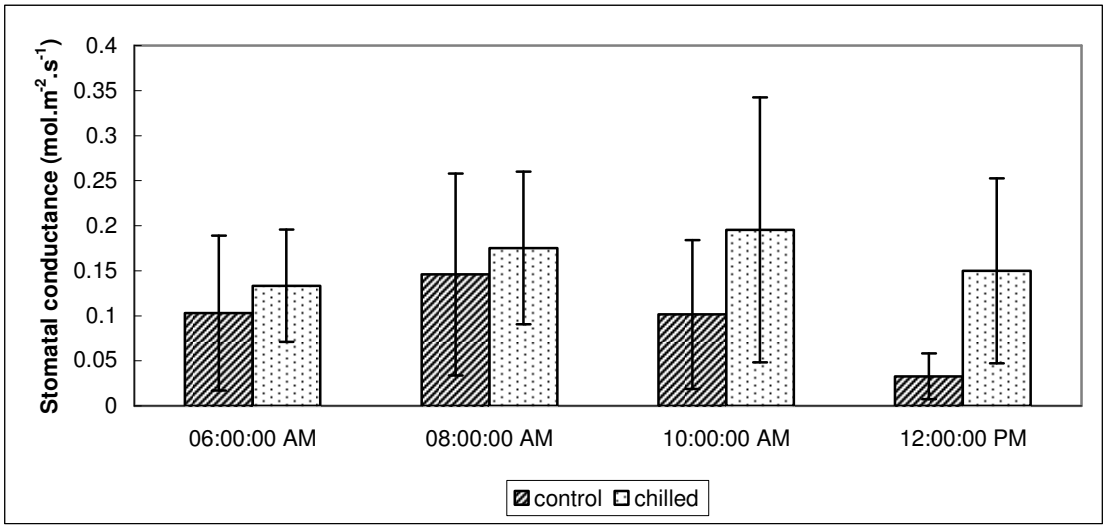

Figure 3.30: Stomatal conductance spot-measurements taken during the course of the morning on *E. grandis* plants. Each bar represents the mean of four measurements taken at each time on four sunny mornings. Vertical bars are the standard deviation about the mean.

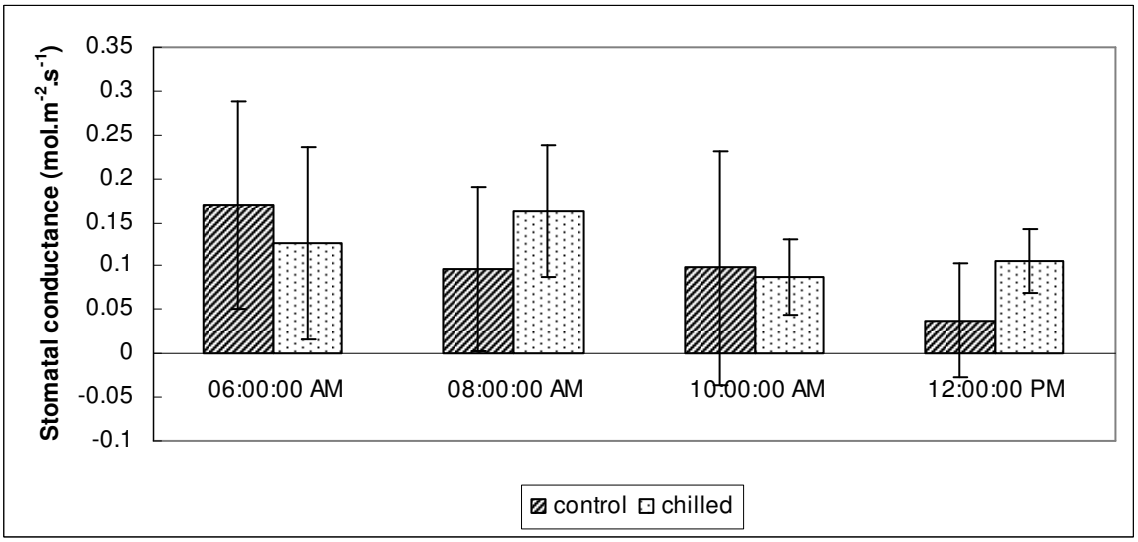

Figure 3.31: Stomatal conductance spot-measurements taken during the course of the morning on *E. nitens* plants. Each bar represents the mean of four measurements taken at each time on four sunny mornings. Vertical bars are the standard deviation about the mean.

Figures 3.30, 3.31 and 3.32 show the mean stomatal conductance as the morning progressed. Chilled *E. grandis* plants (Fig. 3.30) maintained a higher conductance, although differences were never significant at any point in time (Table A5.4). Similarly, in *E. nitens* (Fig. 3.31) conductance values did not differ

significantly and both control and chilled plants conductance decreased as the day progressed (Table A5.6).

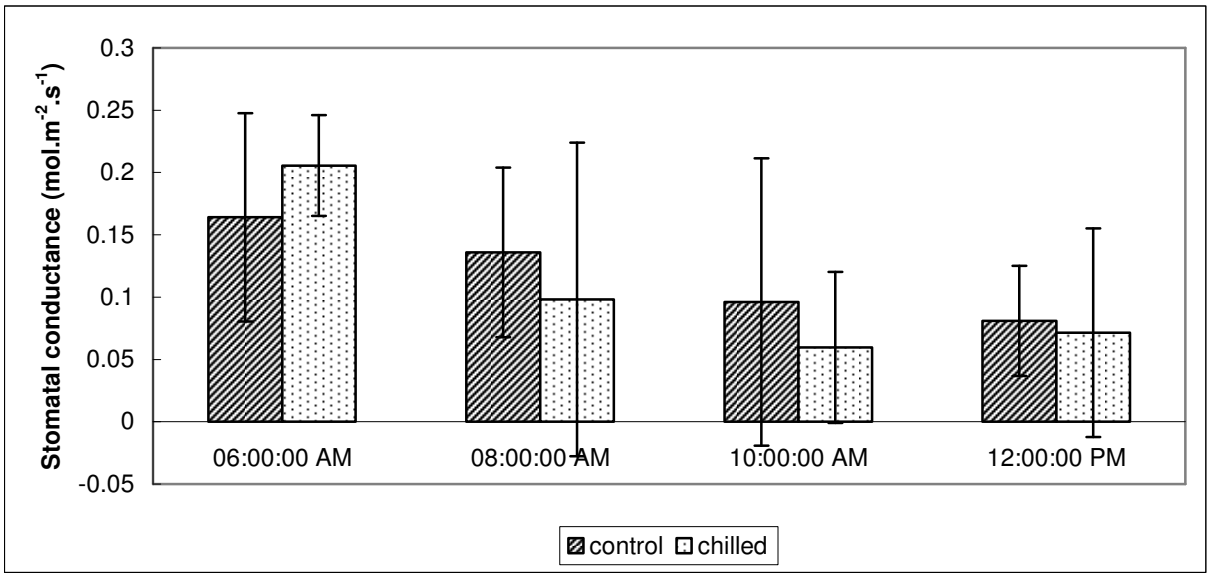

Figure 3.32: Stomatal conductance spot-measurements taken during the course of the morning on *E. grandis* x *nitens* plants. Each bar represents the mean of four measurements taken at each time on four sunny mornings. Vertical bars are the standard deviation about the mean.

In *E. grandis* x *nitens* both control and chilled plants showed decreasing conductance (Fig. 3.32) as the morning progressed, with no significant differences between the groups at any point in time (Tables A5.8 & A5.9). It therefore appears, from Figures 3.30 to 3.32, that stomatal conductance was unaffected by the root chilling treatment.

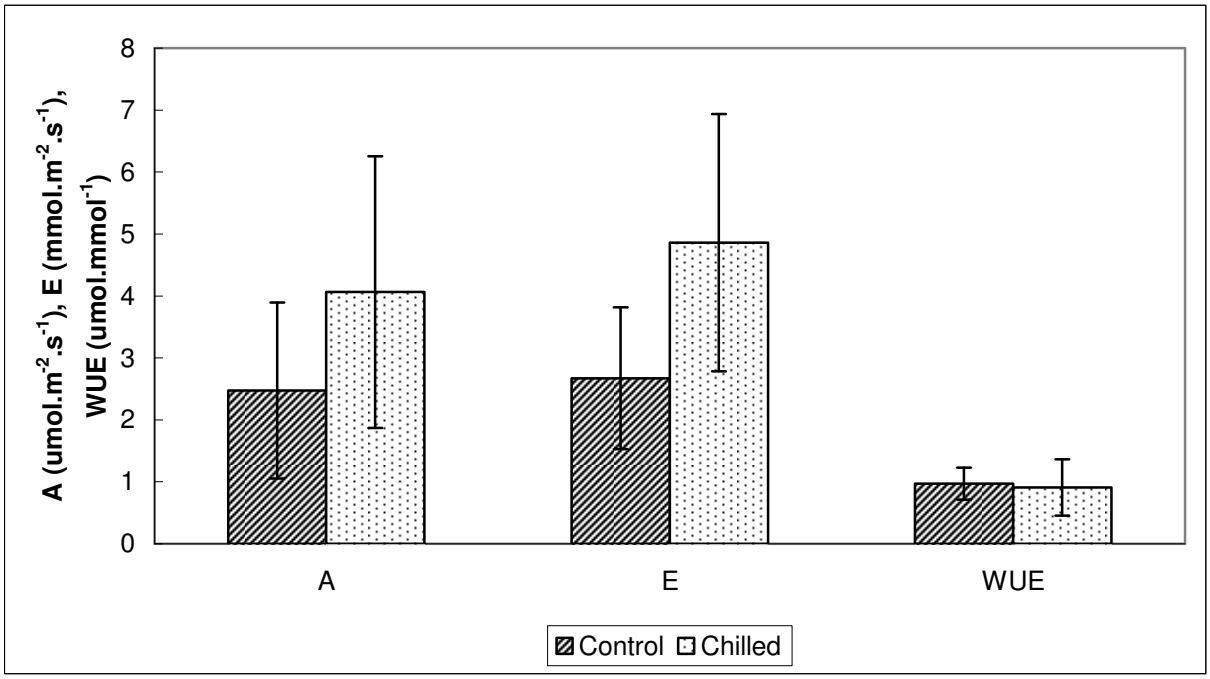

Figure 3.33: Spot measurements of assimilation, transpiration and water use efficiency (A/E) of *E. grandis* plants. Vertical bars are standard deviation about the mean

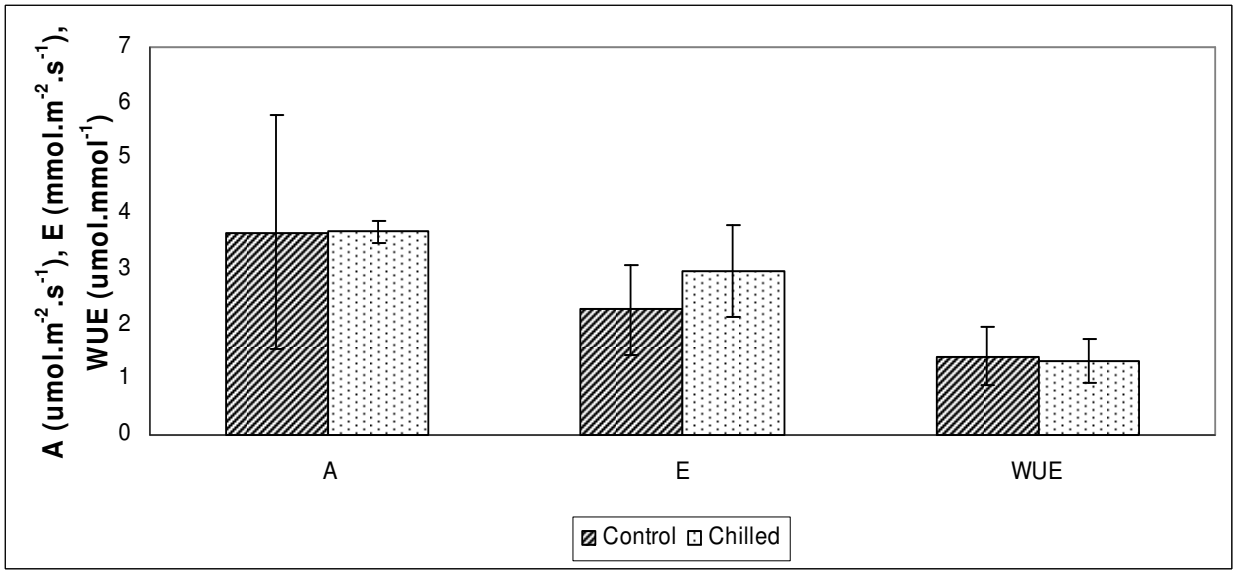

Figure 3.34: Spot measurements of assimilation, transpiration and water use efficiency (A/E) of *E. nitens* plants. Vertical bars are standard deviation about the mean

Figures 3.33 and 3.34 are bar graphs of spot measurements of carbon assimilation (A), transpiration (E) and water use efficiency (WUE) taken with a sun-sky

chamber. Each bar represents the mean of data from six plants measured through the course of a morning. Contrary to the expected trend, chilled *E. grandis* showed substantially higher carbon assimilation and transpiration rates (Table A5.13). The difference in mean transpiration rate was significant (P = 0.013). In *E. nitens* differences in the three parameters were negligible, indicating that *E. nitens* was not perturbed by the root chilling treatment (Table A5.14).

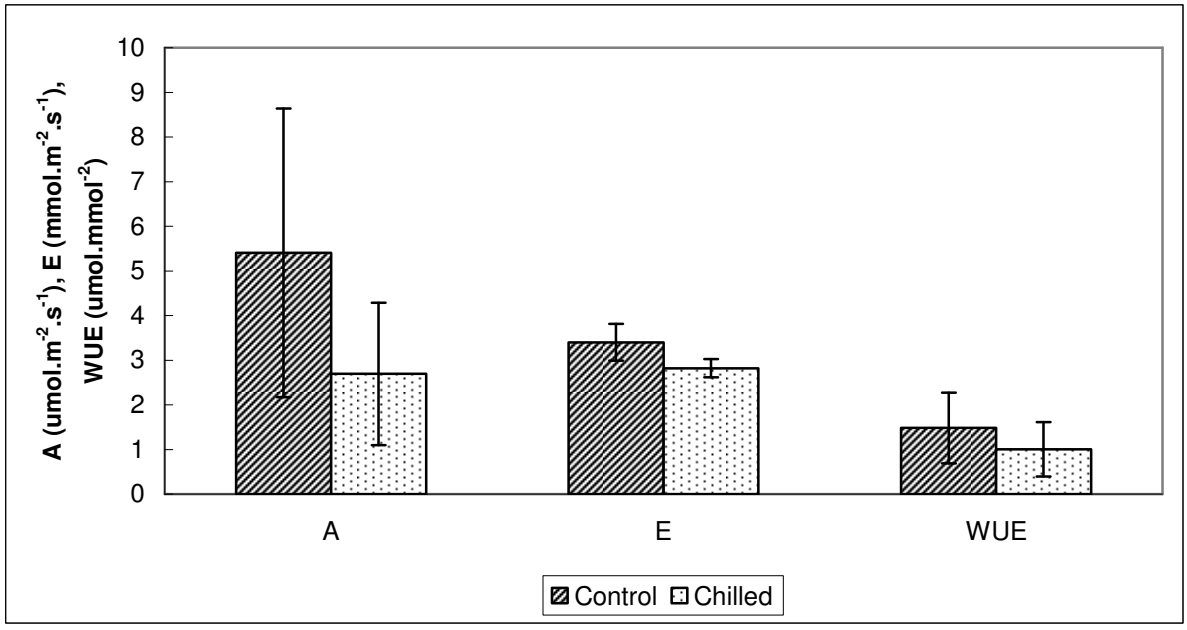

Figure 3.35: Spot measurements of assimilation, transpiration and water use efficiency (A/E) of *E. grandis* x *nitens* plants. Vertical bars are standard deviation about the mean

Figure 3.35 shows that chilled *E. grandis x nitens* plants had a substantially, although not significantly, lower carbon assimilation rate (P = 0.096). The differences in transpiration and water use efficiency were not significant. The substantially decreased assimilation rate is reflected by the significantly lower stomatal conductance and marked alterations in biomass allocation in the hybrid.

3.2.3 Hydraulic characteristics

The plants subjected to root chilling exhibited higher absolute hydraulic resistances compared to unchilled plants (Fig. 3.36). In *E. grandis* and *E. nitens* this difference was not significant (P = 0.265 and 0.0521 respectively; Table A5.16). In *E. grandis* x *nitens* the chilled plants had a significantly higher whole-plant resistance (P = 0.00671).

When the effect of plant size was removed by normalising by total dry weight (Fig. 3.37) *E. grandis* control plants had a slightly higher whole-plant resistance but the difference remained non-significant (P = 0.727; Table A5.17). In *E. nitens* the chilled plants still had a higher resistance but not significantly so (P = 0.192). In *E. grandis* x *nitens* the chilled plants still had a significantly higher whole-plant resistance (P = 0.039).

When the effect of differences in leaf surface area was removed by normalising by leaf area (Fig. 3.38) *E. grandis* control plants still had a higher resistance than chilled plants (P = 0.582; Table A5.18) but the difference between the *E. nitens* groups was almost zero. *E. grandis* x *nitens* chilled plants still had a higher resistance but not significantly so (P = 0.0750). The chilling treatment caused a significant reduction in leaf surface area in all genotypes (Fig.3.13). Although the chilling treatment had no significant effect on total plant resistance in *E. grandis* or *E. nitens*, Figure 3.38 does indicate that the leaf area reduction did ameliorate the higher resistance somewhat. This reduction was important in *E. grandis* x *nitens* because the significant difference in whole-plant resistance of Figure 3.36 is no longer significant after the effect of leaf area had been removed.

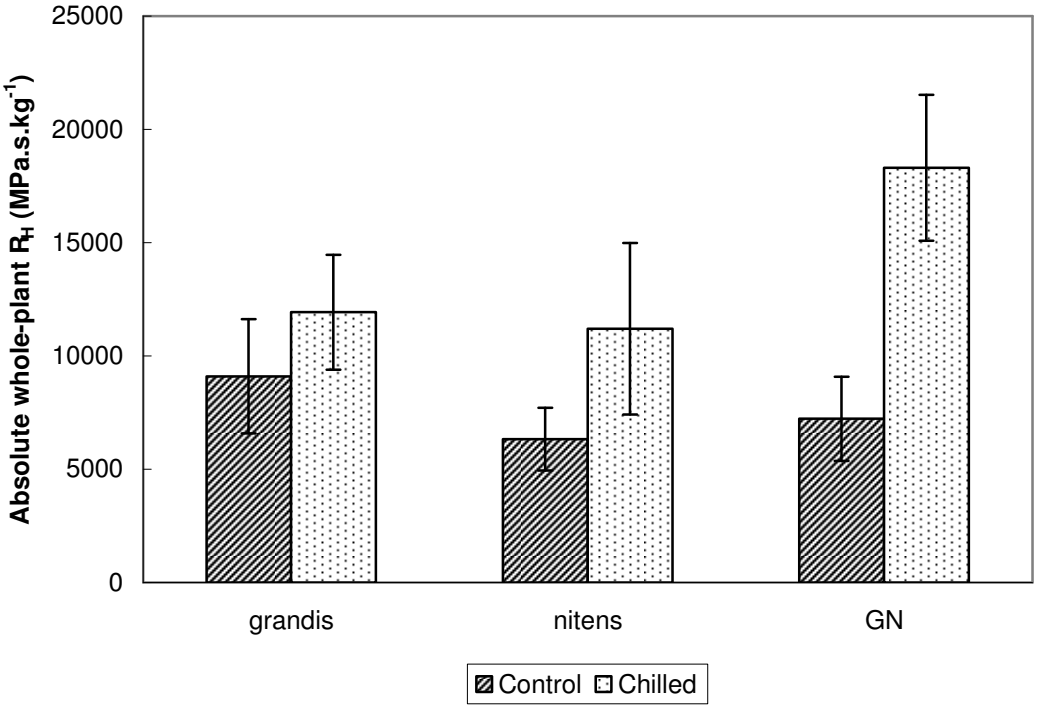

Figure 3.36: Absolute values of total whole plant hydraulic resistance (R_H) of all genotypes. The vertical lines above the bars indicate the standard deviation about the mean (n = 4).

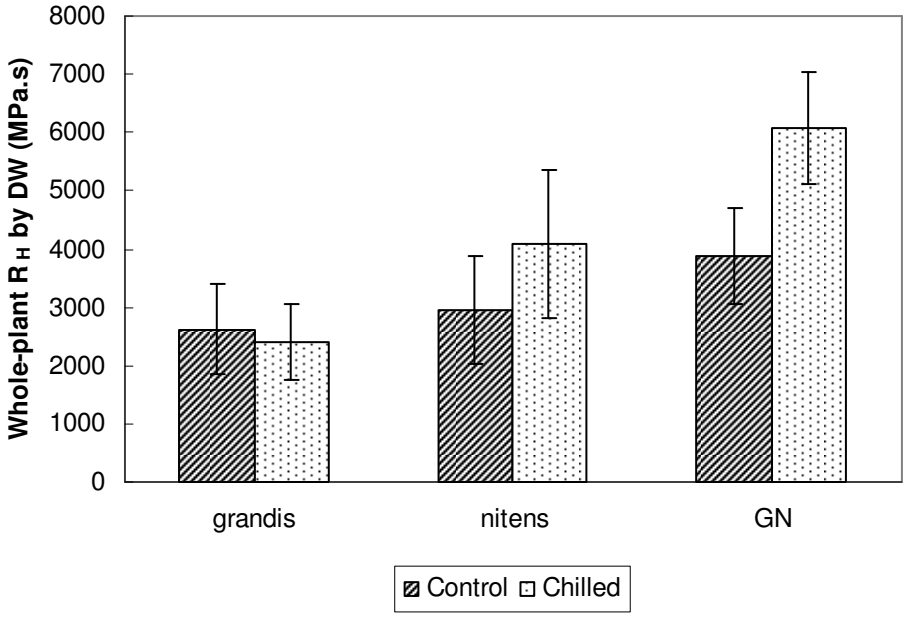

Figure 3.37: Total whole plant hydraulic resistance (R_H), normalised by dry weight, for all genotypes. The vertical lines above the bars indicate the standard deviation about the mean (n = 4).

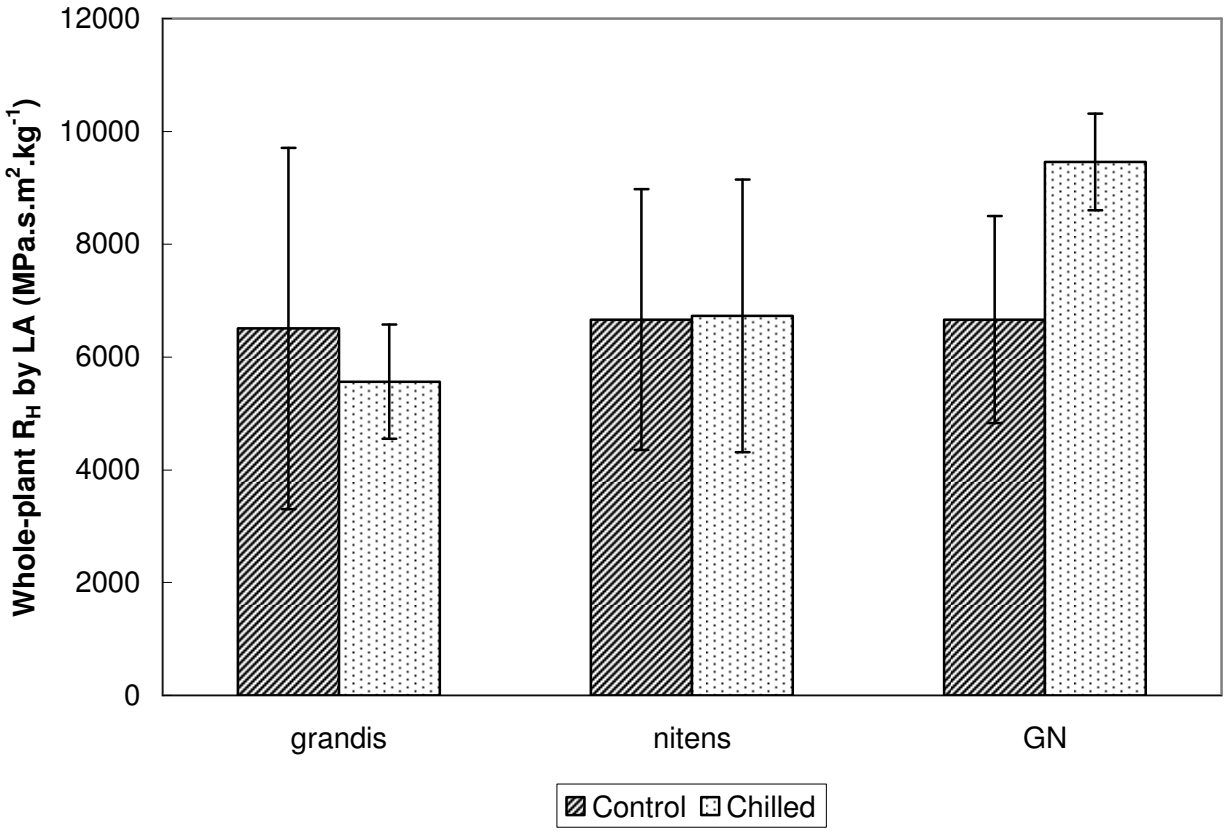

Figure 3.38: Total whole plant hydraulic resistance (R_H), normalised by leaf area, for all genotypes. The vertical lines above the bars indicate the standard deviation about the mean (n = 4).

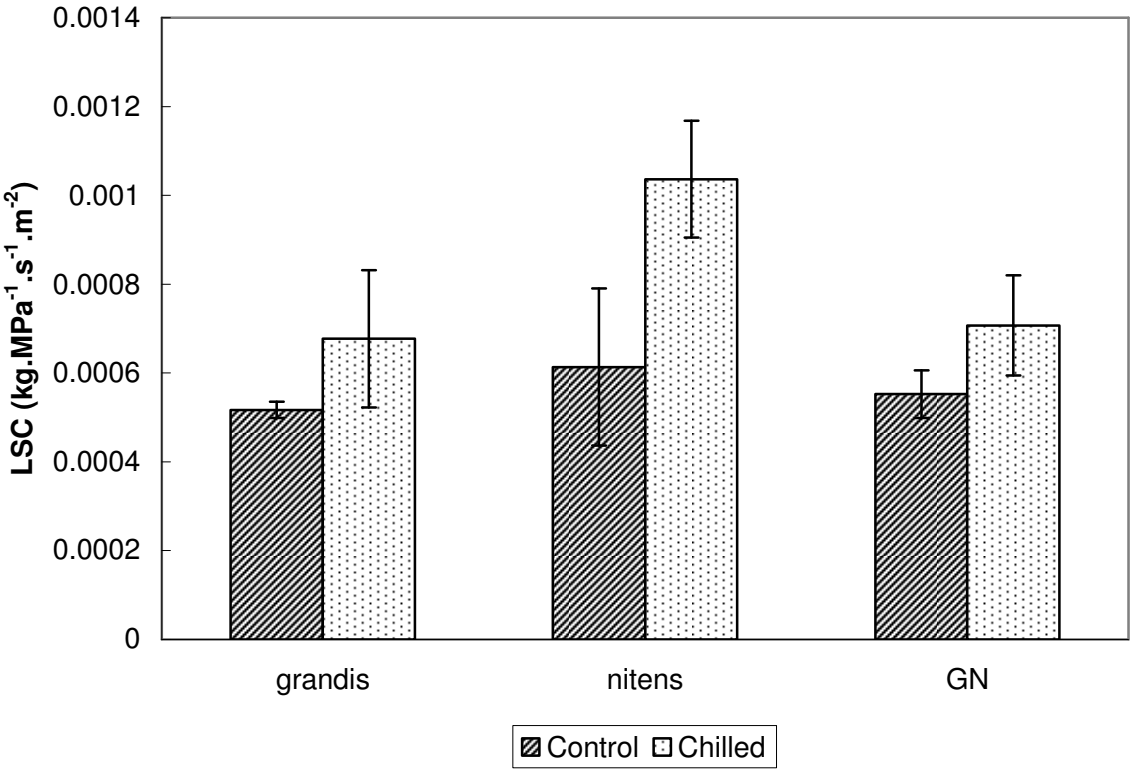

Figure 3.39: Aboveground conductance normalised by leaf area for the aboveground biomass for each genotype. The vertical lines above the bars indicate the standard deviation about the mean (n = 4).

The leaf specific conductance (LSC) values illustrated in Figure 3.39 were calculated by dividing the shoot conductance (mainstem and branches) by the leaf area for each plant. In each genotype the chilled plants exhibited higher LSCs than the unchilled plants. Only in *E. nitens*, however, was this difference significant (P = 0.009; Table A5.19).

Figure 3.40 shows that the distribution of resistances (absolute values) throughout the plant was altered significantly by the chilling treatment with respect to the roots and leaves in *E. grandis*. Root resistance, as a proportion of the total resistance, was significantly higher (P = 0.0117; Table A5.20) in the treatment plants where it accounted for 42% of the entire plant resistance, as opposed to 15% in the control plants. In contrast the leaf resistance was

significantly higher in the control plants (P = 0.00753) and made up 53% of the total plant resistance, as opposed to the 30% contribution of leaves in the chilled plants.

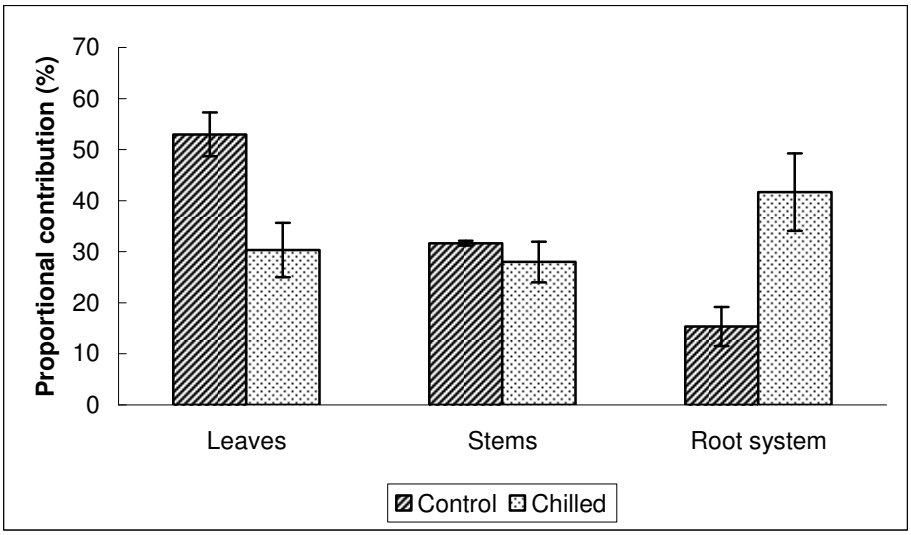

Figure 3.40: The proportional contribution of each of the three main plant components to the whole plant resistance in *E. grandis* plants. The vertical lines above the bars indicate the standard devaition about the mean (n = 4).

When the effect of dry weight was removed for the entire aboveground part of the plant, the stem (aboveground minus the leaves) and the root system, only the difference in the root component remained significant (Fig. 3.41; P = 0.0482; Table A5.21). Similarly, when the effect of leaf area was removed (Fig. 3.42) the root component in chilled plants had a significantly higher resistance than control plants (P = 0.0203; Table A5.22). The significantly higher absolute leaf resistance was not significant when normalised by dry weight (Fig. 3.41; P = 0.0749) or leaf area (Fig. 3.42; P = 0.116). Although not significant, leaf resistance was greatly reduced in the chilled plants, indicating that it is an important factor in compensating for increased whole-plant resistance.

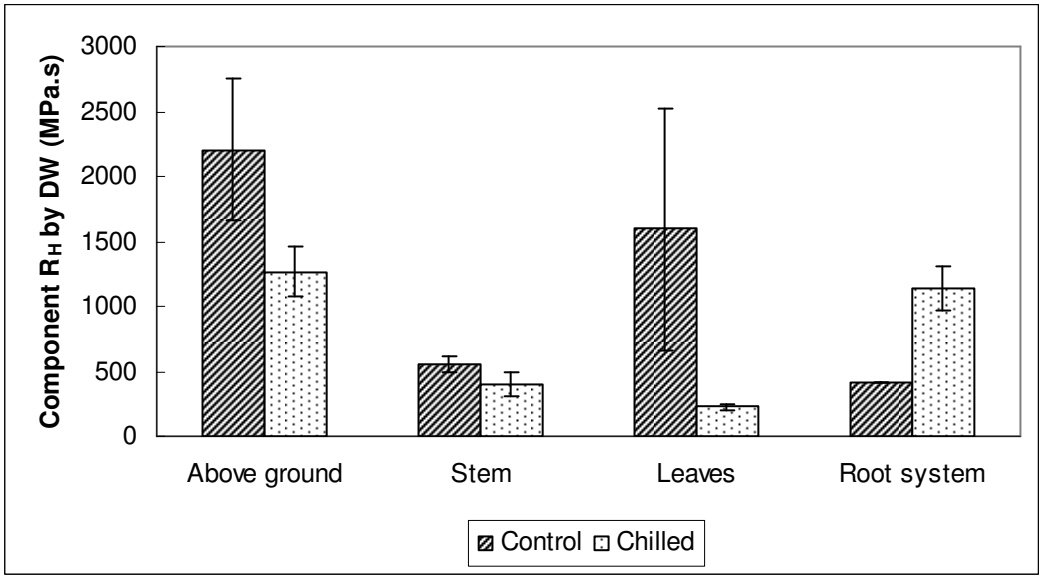

Figure 3.41: Hydraulic resistance of the different components normalised by component dry weight for *E. grandis.* The vertical lines above the bars indicate the standard deviation about the mean (n = 4).

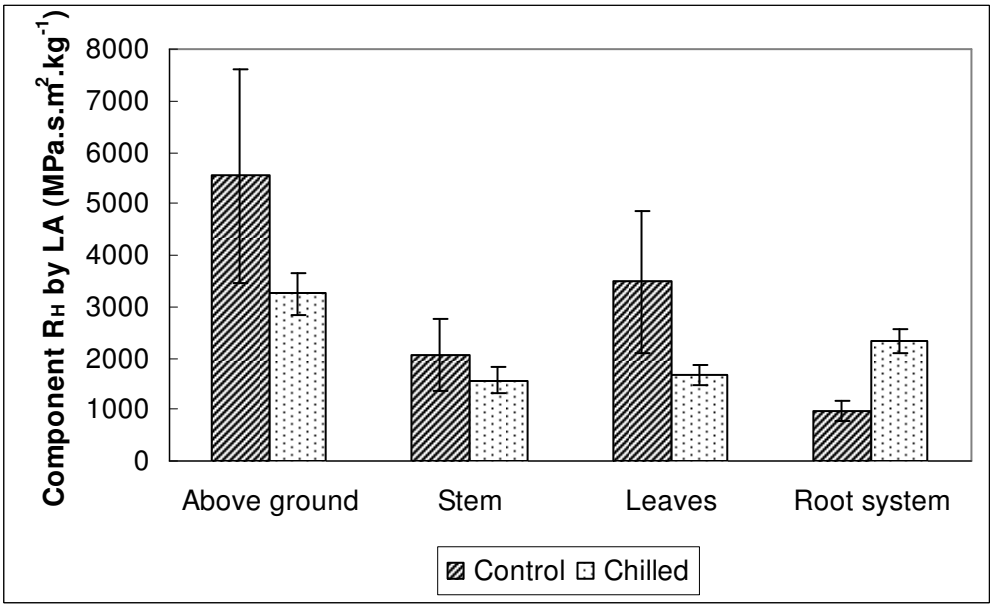

Figure 3.42: Hydraulic resistance of the different components normalised by leaf area for *E. grandis*. The vertical lines above the bars indicate the standard deviation about the mean (n = 4).

Figure 3.43 illustrates the proportion of the total hydraulic resistance (absolute values) of the leaves, stem and root system in *E. nitens.* Control plants had a significantly higher (P = 0.0119; Table A5.20) stem hydraulic resistance than the

stems of chilled plants. Although the root hydraulic resistance was much increased in chilled plants the absolute values were not significantly different (P = 0.118).

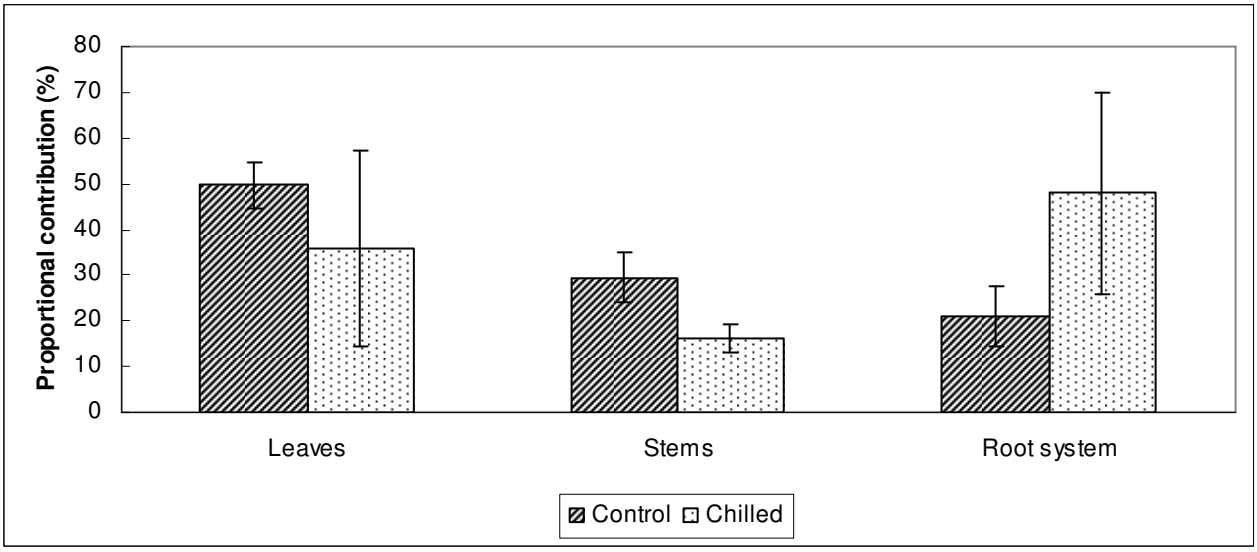

Figure 3.43: The proportional contribution of the three main components to whole plant resistance in *E. nitens* plants. The vertical lines above the bars indicate the standard deviation about the mean (n = 4).

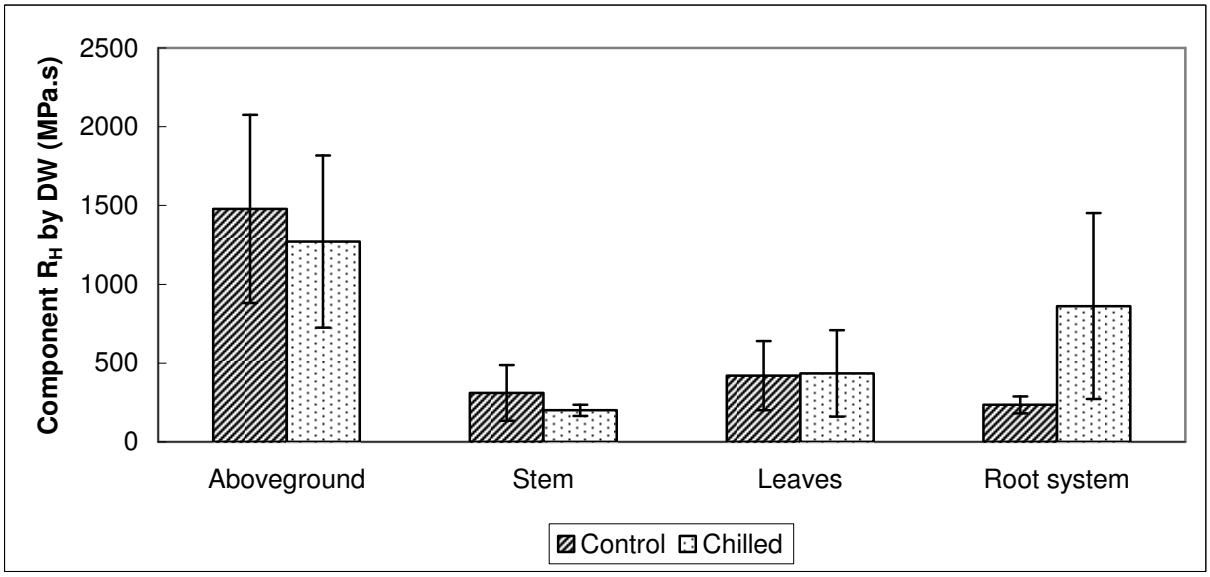

Figure 3.44: Hydraulic resistance of the different components normalised by component dry weight for *E. nitens*. The vertical lines above the bars indicate the standard deviation about the mean (n = 4).

Figure 3.44 illustrates the differences in resistance values of each component, in *E. nitens*, when normalised by the dry weight of each component. All differences were non-significant. Although not significant the considerable increase in root resistance in chilled plants approached the significance level with a P value of 0.079 (Table A5.21).

Figure 3.45 illustrates the differences in resistance values of the different *E. nitens* components when normalised by total plant leaf area. None of the differences were significant although the lower stem resistance of chilled plants had the borderline P value of 0.0532 (Table A5.22).

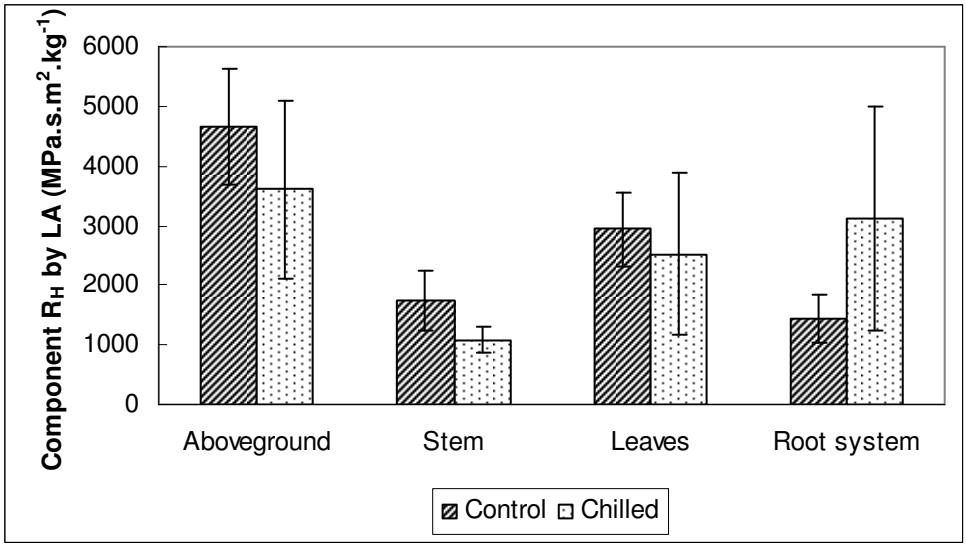

Figure 3.45: Hydraulic resistance of the different components normalised by leaf area in *E. nitens*. The vertical lines above the bars indicate the standard deviation about the mean (n = 4).

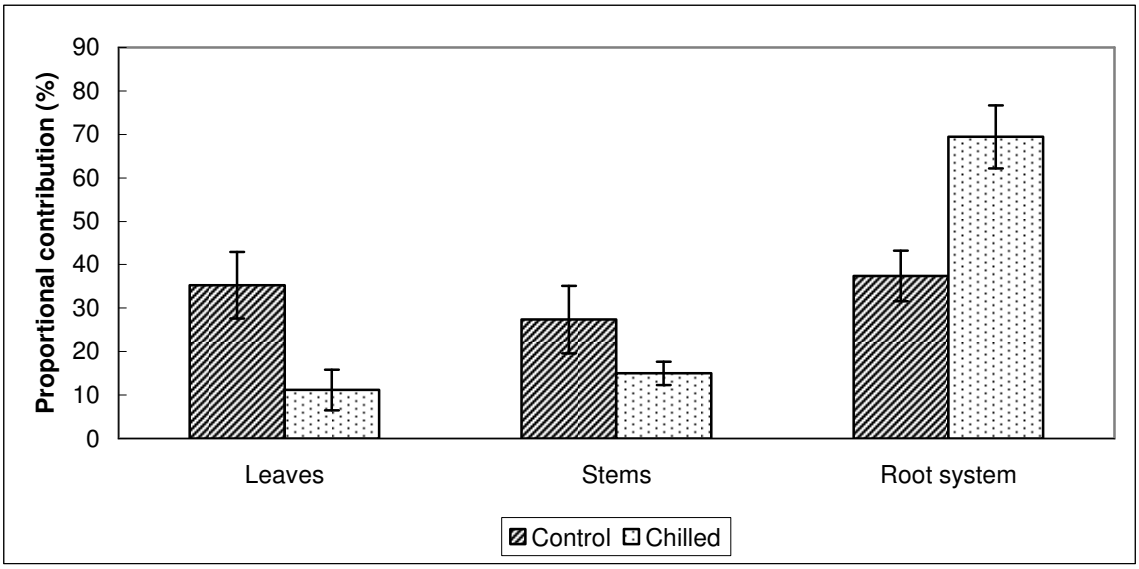

Figure 3.46: Proportional contribution of each of the main components of *E. grandis* x *nitens* plants. The vertical lines above the bars indicate the standard deviation about the mean (n = 3)

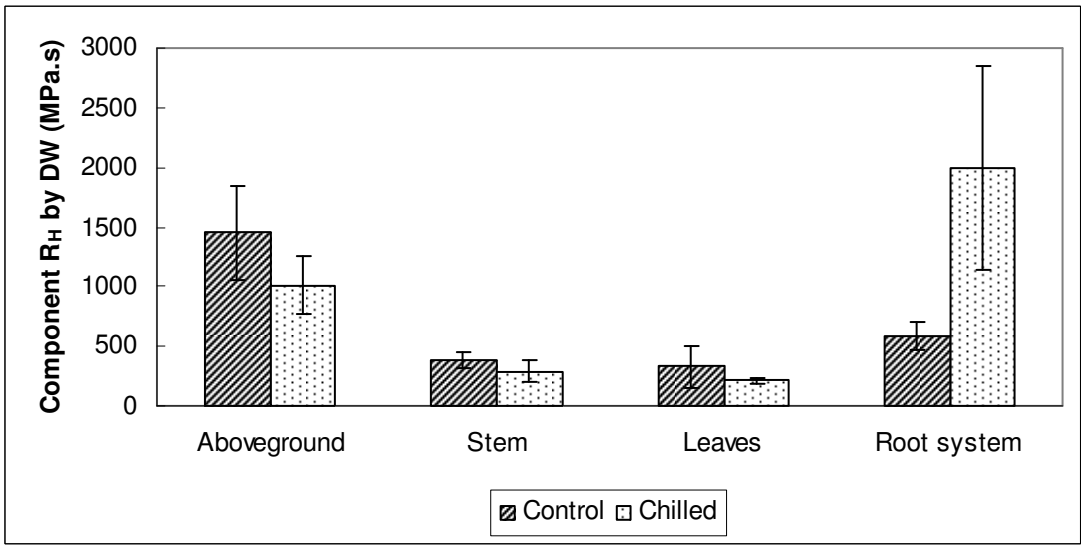

Figure 3.47: Hydraulic resistance of the different components normalised by component dry weight in *E. grandis* x *nitens*. The vertical lines above the bars indicate the standard deviation about the mean (n = 3)

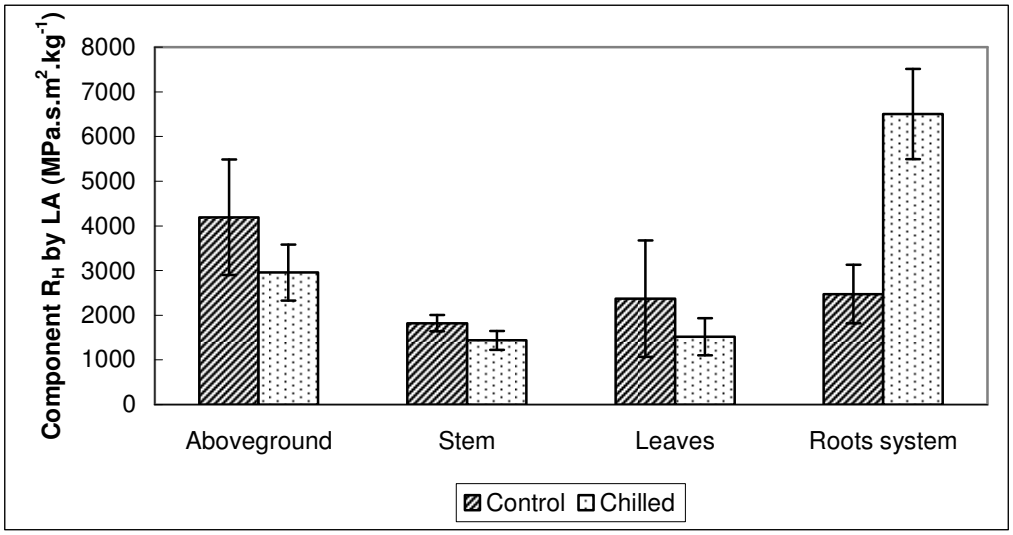

Figure 3.48: Hydraulic resistance of the different components normalised by leaf area in *E. grandis* x *nitens*. The vertical lines above the bars indicate the standard deviation about the mean (n = 3).

Figure 3.46 illustrates the proportion of the total hydraulic resistance (absolute values) of the leaves, stem and root system in *E. grandis* x *nitens* control plants. The hydraulic resistance of the leaves and stems of the chilled plants was significantly lower than that of the control plants (P = 0.0418 and 0.0476, respectively; Table A5.20). The root resistance however, was significantly higher (P = 0.00435) in the chilled plants as opposed to the control plants. When normalised by each component's dry weight (Fig. 3.47) or total leaf area (Fig. 3.48) only the increase in the root system resistance remained significant (P = 0.0468 and 0.00416, respectively).

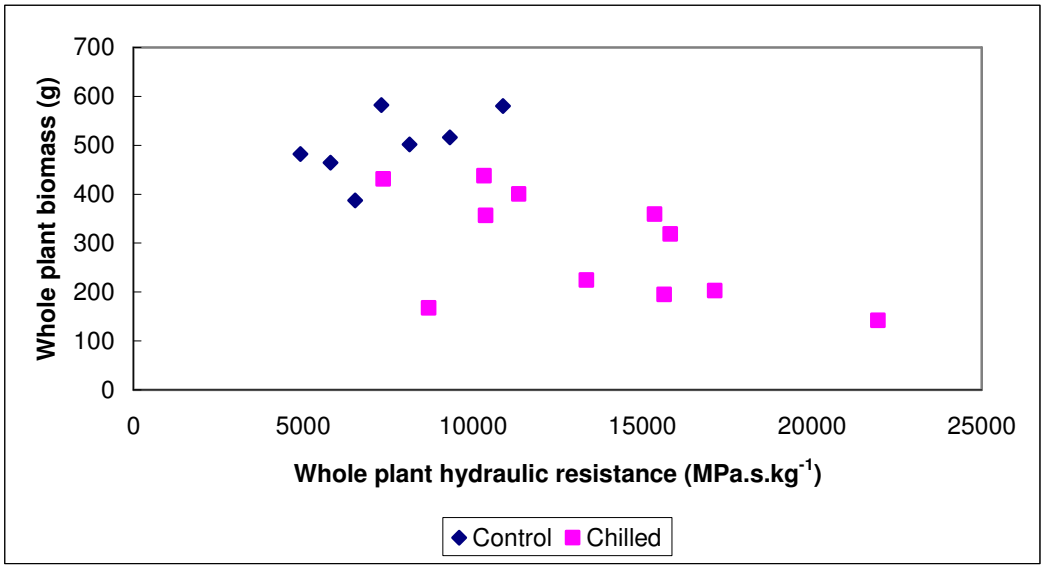

Figure 3.49: Whole plant biomass as a function of absolute whole plant resistance. Each point represents a single plant

Each point in Figure 3.49 represents a single plant and relates the growth of each plant to its hydraulic resistance. The plot shows that high hydraulic resistance and low growth clearly go hand-in-hand and that high growth is correlated with low hydraulic resistance. A pearson correlation analsis indicated that the negative correlation was significant
(P = 0.002; Table A5.24).

4. Discussion

According to the Hydraulic Limitation Hypothesis (HLH) an increased hydraulic resistance should result in lower leaf water potentials, lower stomatal conductance or earlier stomatal closure, either in combination or on their own. This study aimed to clarify whether this occurs after a relatively long period (eight months) in plants subjected to root chilling. Growth, leaf gas exchange and hydraulic characteristics were measured to assess whether growth is significantly reduced, through which mechanism, and whether the plants compensated for increased hydraulic resistances in any way. Figure 3.49 showed that growth was indeed negatively related to higher whole-plant hydraulic resistances.

4.1 Growth

Height growth of all three genotypes was unaffected by the root chilling treatment. Stem diameter growth was more sensitive to root chilling. Stem diameter was significantly reduced upon root chilling in the cold-sensitive *E. grandis*. Within a period of about two months *E. grandis* had acclimatised because the significant difference at day 29 was not significant by day 58. Neither height growth nor stem diameter were affected by root chilling in the cold-tolerant *E. nitens* plants.

E. grandis x *nitens* stem diameter growth did not respond immediately to root chilling. The inhibition of stem diameter growth in the hybrid increased with time and became significant five months after the start of the experiment. Manoharan (unpublished Ph.D. thesis, 2002) chilled two *Eucalyptus* clones to 15 ºC for a period of three months and did not find significant differences in height or over-bark stem diameter. Chesterfield *et al.* (1991), however, found a similar period of

root chilling to cause significant reductions in plant height. In their study *E. nitens* grew significantly taller than *E. delegatensis* and *E. fastigata* at soil temperatures of 5 to 10 ºC. The genotypes showed significant growth effects due to the root chilling treatment.

E. grandis showed significant decreases in total plant dry weight, stem and leaf dry weight as well as leaf surface area. This decrease in the aboveground component caused a significant increase in the root:shoot ratio. *E. grandis* x *nitens* showed significant decreases in the same parameters as *E. grandis* except the root:shoot ratio. The significant decrease in both the above- and belowground components in the hybrid, caused there to be no differences in the root:shoot ratios of chilled and unchilled plants. In *E. nitens* the root chilling did not cause a significant decrease in total plant dry weight. It did, however, display a significant decrease in leaf surface area and increase in root surface area. This resulted in a significantly higher root surface area:leaf surface area ratio in root-chilled *E. nitens*, a ratio that did not differ significantly in the other genotypes.

Trees generally have a developmental pattern that operates to maintain a balanced relationship between the absorbing and transpiring surfaces (Ledig *et al.* 1970). All the genotypes decreased their transpiring surface areas and in *E. nitens* the absorbing surface area was increased as well. The most influential environmental factors determining the root:shoot ratio are mineral nutrients, light availability and temperature (Russel 1977) and metabolically the internal balance of labile nitrogen and carbon is thought to determine dry matter partitioning between the root and shoot system (Ericsson 1995). Root temperature, in turn, influences partitioning of the internal nitrogen pool, with the amount allocated to the roots increasing as soil temperature decreases (Rufty *et al.* 1981). The ability

to maintain an adequate carbohydrate supply to the root tips is also a likely limiting factor to root growth under low soil temperatures (Crawford and Huxter 1977).

An increased root:shoot ratio is a trait commonly acquired in response to water stress (Chen and Reynolds 1997; Jacobs and Schloeder 2003) although an overall reduction in growth, instead of resource-balance driven biomass partioning, has been reported (Cortina *et al.* 2007). Low soil temperatures also generally cause an increase in the root:shoot ratio (Davidson 1969; Russel 1977; Kleier *et al.* 1998; Equiza *et al.* 2001). This was indeed obeserved in all genotypes, although it was only significantly increased in *E. grandis.* This, together with the significantly decreased leaf surface area, which was observed in all genotypes, suggests that they experienced a reduced water absorbing ability.

At the age of eight months the maximum heights for *E. grandis*, *E. nitens* and *E. grandis* x *nitens* were 1.22, 0.93 and 0.84 metres respectively. These heights are far below the height at which gravitational or hydraulic limitations are typically expected to become significant (Ryan and Yoder 1997). Manoharan (unpublished Ph.D. thesis, 2002) found root chilling to have no significant effect on height and states that it "… is perhaps not surprising as biomass … is related to volume, not linear dimensions of tissue". It is still a striking feature of the *Eucalyptus* genotypes used in this study, that they showed significant reductions in growth and biomass allocation, leaving height unaffected. Perhaps, being forest pioneer species, the genetic programming dictating maximal height growth, to intercept as much light as possible, was an overriding factor.

Direct temperature effects on plant metabolism and growth cannot be ruled out. Low soil temperatures are known to reduce ion and nutrient uptake and translocation, thereby decreasing nutrient transport to the shoot and carbohydrate transport to the roots (Nielsen and Humphries 1966; McNaughton *et al.* 1974; Levitt 1980; Cruz *et al.* 2003), and cause overall reductions in plant productivity (McMichael and Burke 2002). The reduced uptake of nitrogen could result in low foliar nitrogen concentrations, thereby reducing photosynthesis (DeLucia *et al.* 1992). As part of this, however, a decrease in the foliar concentration of these two elements could be expected to decrease the carboxylation coefficients and J_{max}, the maximum rate of turnover in the PCR cycle, but this was not observed. Hormonal production or communication can also be altered directly by low root temperatures (Skene and Kerridge 1967; Cruz *et al.* 2003; Veselova *et al.* 2005) causing reductions in growth.

4.2 Photosynthesis and stomatal conductance

The biochemistry of the Photosynthetic Carbon Reduction (PCR) cycle was unaltered by the root chilling treatment; the only significant differences were intergenotypic differences in the light response curves. Photosynthesis and water absorption are thermodynamic processes (Davidson 1969) and plants have an optimum temperature for both (Davidson 1969; Wand *et al.* 2001; Yamori *et al.* 2005). Air temperature is more variable than soil temperature and can reach much lower levels than the latter (Crawford 1989). Low air temperatures are known to inhibit photosynthesis. This could be due to a reduction in photochemistry of Photosystem II, reducing the rate of energy dissipation via the electron transport chain, leading to photo-inhibitory damage (Baker *et al.* 1989; Adams and Demmig-Adams 1994; Close *et al.* 2001). Alternatively the low-temperature inhibition could act on the chlorophyll content or the enzymes

involved in the photosynthetic carbon reduction cycle (Hurry and Huner 1991; Stamp 1980). However, leaves of the control and chilled groups experienced the same air temperatre and therefore these effects are not likely.

Differences in the carboxylation coefficients and apparent quantum efficiency were not significant between control and chilled plants. The apparent quantum efficiency values varied from 0.029 to 0.04 µmol of photons absorbed per µmol CO_2 fixed which is within the range for healthy plants, which is from 0.01 to 0.05 (Taiz and Zeiger 1998). In addition, root chilling did not induce significant differences in the light and CO_2 compensation points or the dark respiration values. Light compensation points were higher for the control plants indicating that they recquired higher light intensities to balance photosynthesis and respiration. Respiration produces the energy for maintenance, transport and anabolic processes (Lambers *et al.* 2002) and higher temperatures are known to induce higher respiration rates in plants (Cruz *et al.* 2003; Kleier *et al.* 1998) and has been found to reduce water use efficiency (Calder *et al.* 1993). The lack of significant differences in photosynthetic performance suggest that the reduction in growth did not occur via the biochemistry of the PCR cycle or the electron transport processes.

The effect of soil temperature on roots may be direct, but on the leaves and photosynthesis it will be indirect. Low soil temperatures can decrease carbon assimilation through reducing water absorption and thereby leaf water potential and stomatal conductance (Starr *et al.* 2004) as well as through non-stomatal factors such as carbohydrate feedback limitations (DeLucia *et al.* 1992). Leaves experience a more variable environment than the roots and have evolved a circadian growth pattern with growth mechanisms that compensate for short-term

fluctuations (Walter and Shurr 2005). Roots, in contrast, do not show such inherent variations in diurnal growth (Walter *et al.* 2002) but respond immediately to changes in environmental parameters to optimise resource use efficiency (Walter and Shurr 2005).

DeLucia *et al.* (1992) found that soil temperatures in the range of 5 to 15 ºC had no effect on leaf water potential in the prairie grass *Andropogon gerardii* Vitman but that soil temperatures of 30 ºC and above did lower leaf water potential, possibly due to high transpiration rates. They also found that low soil temperatures significantly lowered assimilation and stomatal conductance, which fits well into the theory of stomatal regulation to maintain a threshold leaf turgor.

When interpreting light and carbon dioxide response curves it should be kept in mind that the leaf was responding to controlled conditions within the leaf chamber. They give very useful information about what the leaves are capable of under particular conditions. Stomatal conductance values, obtained from rapid light response curves (as in Fig. 3.17), are unequilibrated in that they have not had enough time to adjust to the change in light intensity and tend to be more open at low light intensities than they normally would be (LiCor 6400 Manual). When the time interval between logging different data points is increased to eight to ten minutes significantly different conductance values have been obtained (Xu and Baldocchi 2003).

The spot-measurements, however, give a more realistic picture of what the leaves are doing under natural conditions and are therefore inherently more noisy. From these measurements it appears as if chilled *E. grandis* plants had a considerably higher rate of carbon assimilation than control plants. In addition their water use

efficiency was very similar. Similarly, *E. grandis* x *nitens* showed a substantially higher, although non-significant, assimilation rate with neglible differences in transpiration and water use efficiency. In *E. nitens* no difference was detected between carbon assimilation and both transpiration and water use efficiency were very similar.

The finding of a considerably lower rate of carbon assimilation in chilled *E. grandis* x *nitens* plants fits well with the fact that it had a significantly lower leaf, stem, root and total plant biomass. Similarly, the fact that *E. nitens* showed no difference in assimilation rate upon chilling agrees with the finding that its biomass was not significantly reduced at the whole-plant level or that of any plant component. It should also be remembered that *E. nitens* is renowned for its cold-tolerance (Chesterfield *et al* 1991; Stewart 1993; Swain and Gardner 2004).

E. grandis, however, showed a significantly lower leaf, stem and total plant biomass upon chilling and yet those very plants exhibited a higher, albeit not significant, carbon assimilation rate. A statistically significant difference is not always necessary to cause a separation between two groups with respect to a particular factor. A portion of the photosynthate is re-invested to produce more leaves driving plant growth exponentially (Pearcy *et al.* 1987). Small differences in the rates of carbon assimilation can therefore have a significant effect on foliage mass and whole plant growth over time (Sheriff 1992). Instantaneous measures of leaf photosynthesis are relatively accurate assessments of *in situ* leaf function (Kruger and Volin 2006), however, whole-plant growth also depends upon the total amount of foliage (Sheriff 1992). Although the assimilation per unit foliage may have been no different (Fig. 3.18) or higher (Fig. 3.33) in chilled

E. grandis plants, leaf photosynthesis *per se* has been found inadequate to explain whole plant growth (Heichel 1971) which may explain why they exhibited a significantly lower total biomass despite having a higher assimilation rate at the leaf-level. Structural properties, especially the ability to produce new assimilating leaf area, often co-determine whole-plant photosynthesis (Monsi 1968; Körner 1991)

4.3 Hydraulic characteristics

The slope of a plot of transpiration versus leaf water potential is an effective measure of the resistance of the soil-to-leaf pathway or whole-plant resistance (Jarvis 1976; Saliendra *et al.* 1995; Kolb and Sperry 1999; Vander Willigen *et al.* 1999). It was anticipated that the root chilling treatment would increase the whole-plant resistance. However, contrary to the expected pattern the slope of control and chilled plants of *E. grandis* and *E. grandis* x *nitens* did not differ significantly. Surprisingly, chilled *E. nitens* plants had a significantly lower soil-to-leaf resistance than their unchilled counterparts. Stomatal conductance was measured simultaneously and it also showed no difference at the leaf level between the two groups. This suggests that the chilled plants, in every genotype, had compensated in some way for the much higher root hydraulic resistance to keep the shoot water status comparable to that of the unchilled plants.

Kleier *et al.* (1998) defined compensation as the growth response or physiological change in an unaffected part to offset the decreased function in that same plant component. Leaf dry weight and surface area were significantly reduced in chilled plants of all three genotypes, indicating that the plants compensated by reducing their transpiration surfaces. As a result the chilled plants had higher leaf specific conductivities but only in *E. nitens* was this

difference significant. This could partly explain why *E. nitens* could maintain a total plant biomass similar to unchilled plants, and why *E. grandis* and *E. grandis* x *nitens* did not.

Although stomatal conductance did not differ significantly, total plant biomass was reduced in chilled *E. nitens* plants and significantly so in *E. grandis* and *E. grandis* x *nitens*. It may be that the relationship between leaf conductance and canopy conductance is not linear. The compensations allowed stomatal conductance to be similar to the control group at the leaf-level, but this might not have translated to a similar whole-plant gas exchange which could have significantly reduced carbon assimilation. Differences in leaf physiology are difficult to relate to canopy conductance and assimilation because of the varying distribution of leaf area, photosynthetic capacity and light within the canopy (Ryan *et al.* 2006). In addition, changes in photosynthetic capacity can cause changes in assimilation unrelated to hydraulic limitations (Ryan *et al.* 2006).

Root chilling increased the whole plant absolute resistance in all genotypes and to a significant degree in *E. grandis* x *nitens*. This significant difference still remained, even after removing the effect of plant dry weight. When the absolute resistances were normalised by leaf area the difference was no longer significant, indicating that this was the more important factor in ameliorating the decreased water transporting ability. The graphs of normalising by dry weight and leaf area (Figures 3.37 & 3.38) indicate that altering leaf area had a greater effect in ameliorating decreases in water transport in
E. grandis and *E. nitens*.

The hydraulic resistances of the different plant components showed significant changes upon root chilling. In chilled *E. grandis* and *E. grandis* x *nitens* plants, the absolute resistance values for the root systems were significantly higher and the leaf resistance significantly lower. In addition, *E. grandis* x *nitens* also had significantly lower stem resistance. In both genotypes only the increased root resistance remained significant when normalised by component dry weight or plant leaf area.

In *E. nitens* only the absolute resistance of the stem was significantly reduced in chilled plants. None of the differences were significant when normalised to dry weight or leaf area although the stem resistance was still much reduced in chilled plants.

E. grandis and *E. grandis* x *nitens* therefore experienced significantly higher root hydraulic resistances and both genotypes showed significant reductions in dry weight of the entire plant and its different components, as well as leaf surface area reductions. The cold-tolerant *E. nitens* responded to this increased root resistance by reducing the leaf surface area to root surface area ratio.

It appears as though growth was reduced through root chilling but not via a reduced stomatal conductance or leaf water potential, as predicted by the HLH. Medhurst and Beadle (2002) found that the amount of leaf area that can be supported in *E. nitens* increases disproportionately with an increase in sapwood area. The increasing stem diameter of chilled plants in *E. grandis* and *E. nitens* could have compensated to increase the water supply to the foliage of chilled plants. The limitation to growth still seems to have had a hydraulic basis because the balance between the absorptive and transpirational surfaces was among the

factors significantly altered. After reviewing 51 studies that measured one or more factors integral to the HLH Ryan *et al.* (2006) concluded that the hydraulic limitation of gas eschange is common but not universal. They suggest that future investigations of this phenomenon should test whether trees are carbon limited and if so, whether such a limitation influences growth.

4.4 Conclusion

It is concluded from this study that root chilling and the consequent increased root hydraulic resistance necessitated significant compensations in the long term. The response common to all genotypes, regardless of cold-tolerance or root architecture, was a significant reduction in leaf area and leaf dry weight, suggesting that the balance between the evaporative and absorptive surfaces is an important factor in optimising plant water relations. It also supports the idea that plant water-use cannot exceed the water supply from the soil-to-leaf pipeline because a negative correlation existed between plant dry weight and hydraulic resistance. Despite substaintially increased hydraulic resistances, stomatal conductance and carbon assimilation were not significantly reduced in *E. grandis* or *E. nitens*; however, *E. grandis* x *nitens* did show the expected trend of decreased assimilation upon chilling. The prediction of the HLH of reduced assimilation via reduced stomatal conductance is therefore not supported by the findings of this long-term study. The significant reduction in dry weight in *E. grandis*, despite having a higher assimilation rate at the leaf-level, is possibly due to its reduced foliage biomass and area and the fact that leaf-level assimilation does not scale linearly to canopy assimilation.

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Table A1.1: Independent-samples t-test of the effect of chilling on stem diameter for *E. grandis x nitens* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Day 1	Equal variances assumed	1.000	.334	.509	14	.619	.06250	.12275	-.20077	.32577
	Equal variances not assumed			.509	13.829	.619	.06250	.12275	-.20107	.32607
Day 29	Equal variances assumed	.085	.775	1.865	14	.083	.56250	.30160	-.08436	1.20936
	Equal variances not assumed			1.865	13.937	.083	.56250	.30160	-.08463	1.20963
Day 58	Equal variances assumed	.718	.411	2.126	14	.052	1.12500	.52928	-.01019	2.26019
	Equal variances not assumed			2.126	12.342	.054	1.12500	.52928	-.02466	2.27466
Day 85	Equal variances assumed	.028	.870	1.660	14	.119	1.37500	.82848	-.40192	3.15192
	Equal variances not assumed			1.660	13.850	.119	1.37500	.82848	-.40373	3.15373
Day 119	Equal variances assumed	3.545	.081	1.340	14	.202	.81250	.60642	-.48814	2.11314
	Equal variances not assumed			1.340	11.396	.206	.81250	.60642	-.51658	2.14158
Day 149	Equal variances assumed	.132	.722	3.535	14	.003	2.25000	.63650	.88484	3.61516
	Equal variances not assumed			3.535	13.012	.004	2.25000	.63650	.87506	3.62494
Day 190	Equal variances assumed	.966	.342	1.888	14	.080	1.12500	.59574	-.15274	2.40274
	Equal variances not assumed			1.888	13.487	.081	1.12500	.59574	-.15732	2.40732
Day 206	Equal variances assumed	.000	1.000	3.691	14	.002	1.50000	.40642	.62831	2.37169
	Equal variances not assumed			3.691	13.959	.002	1.50000	.40642	.62807	2.37193

Table A1.2: Independent-samples t-test of the effect of chilling on stem diameter for *E. nitens* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Day 1	Equal variances assumed	.759	.398	1.263	14	.227	.37500	.29693	-.26186	1.01186
	Equal variances not assumed			1.263	12.027	.231	.37500	.29693	-.27180	1.02180
Day 29	Equal variances assumed	3.722	.074	2.507	14	.025	1.31250	.52345	.18982	2.43518
	Equal variances not assumed			2.507	9.017	.033	1.31250	.52345	.12872	2.49628
Day 58	Equal variances assumed	3.075	.101	.362	14	.723	.31250	.86312	-1.53871	2.16371
	Equal variances not assumed			.362	10.023	.725	.31250	.86312	-1.61007	2.23507
Day 85	Equal variances assumed	2.574	.131	-.894	14	.386	-.81250	.90848	-2.76099	1.13599
	Equal variances not assumed			-.894	10.874	.390	-.81250	.90848	-2.81488	1.18988
Day 119	Equal variances assumed	1.471	.245	-1.595	14	.133	-1.50000	.94017	-3.51647	.51647
	Equal variances not assumed			-1.595	13.031	.135	-1.50000	.94017	-3.53064	.53064
Day 149	Equal variances assumed	2.586	.130	-2.331	14	.035	-2.25000	.96536	-4.32049	-.17951
	Equal variances not assumed			-2.331	11.598	.039	-2.25000	.96536	-4.36145	-.13855
Day 190	Equal variances assumed	1.260	.281	-1.688	14	.114	-1.12500	.66648	-2.55446	.30446
	Equal variances not assumed			-1.688	13.200	.115	-1.12500	.66648	-2.56263	.31263
Day 206	Equal variances assumed	.003	.957	-2.810	14	.014	-2.62500	.93422	-4.62870	-.62130
	Equal variances not assumed			-2.810	13.996	.014	-2.62500	.93422	-4.62876	-.62124

Table A1.3: Independent-samples t-test of the effect of chilling on stem diameter for *E. grandis* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Day 1	Equal variances assumed	2.612	.128	2.853	14	.013	.62500	.21907	.15514	1.09486
	Equal variances not assumed			2.853	12.988	.014	.62500	.21907	.15169	1.09831
Day 29	Equal variances assumed	.280	.605	3.751	14	.002	1.75000	.46651	.74943	2.75057
	Equal variances not assumed			3.751	13.322	.002	1.75000	.46651	.74463	2.75537
Day 58	Equal variances assumed	1.119	.308	1.699	14	.111	1.18750	.69877	-.31122	2.68622
	Equal variances not assumed			1.699	12.173	.115	1.18750	.69877	-.33260	2.70760
Day 85	Equal variances assumed	.538	.475	.187	14	.854	.18750	1.00306	-1.96386	2.33886
	Equal variances not assumed			.187	11.479	.855	.18750	1.00306	-2.00905	2.38405
Day 119	Equal variances assumed	3.559	.080	-.516	14	.614	-.56250	1.08947	-2.89918	1.77418
	Equal variances not assumed			-.516	9.010	.618	-.56250	1.08947	-3.02665	1.90165
Day 149	Equal variances assumed	.000	1.000	-.775	14	.451	-.62500	.80595	-2.35359	1.10359
	Equal variances not assumed			-.775	13.999	.451	-.62500	.80595	-2.35361	1.10361
Day 190	Equal variances assumed	.889	.362	-1.418	14	.178	-1.68750	1.19032	-4.24047	.86547
	Equal variances not assumed			-1.418	12.489	.181	-1.68750	1.19032	-4.26975	.89475
Day 206	Equal variances assumed	.047	.832	-1.684	14	.114	-1.93750	1.15026	-4.40457	.52957
	Equal variances not assumed			-1.684	13.852	.114	-1.93750	1.15026	-4.40704	.53204

Table A2.1: Independent-samples t-test of the effect of chilling on plant height for *E. grandis x nitens* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Day 1	Equal variances assumed	.611	.447	-.973	14	.347	-5.50000	5.65528	-17.62936	6.62936
	Equal variances not assumed			-.973	9.742	.354	-5.50000	5.65528	-18.14606	7.14606
Day 29	Equal variances assumed	.796	.387	1.080	14	.298	22.12500	20.48164	-21.80376	66.05376
	Equal variances not assumed			1.080	12.987	.300	22.12500	20.48164	-22.12732	66.37732
Day 58	Equal variances assumed	.179	.679	1.058	14	.308	28.37500	26.82029	-29.14881	85.89881
	Equal variances not assumed			1.058	13.829	.308	28.37500	26.82029	-29.21565	85.96565
Day 85	Equal variances assumed	.174	.683	1.668	14	.117	44.62500	26.75013	-12.74831	101.99831
	Equal variances not assumed			1.668	13.675	.118	44.62500	26.75013	-12.87659	102.12659
Day 119	Equal variances assumed	1.035	.326	1.162	14	.265	17.00000	14.62935	-14.37683	48.37683
	Equal variances not assumed			1.162	13.613	.265	17.00000	14.62935	-14.46083	48.46083
Day 149	Equal variances assumed	1.949	.184	2.474	14	.027	45.37500	18.33803	6.04383	84.70617
	Equal variances not assumed			2.474	11.406	.030	45.37500	18.33803	5.18797	85.56203
Day 190	Equal variances assumed	.190	.670	.007	14	.995	.25000	36.56251	-78.16878	78.66878
	Equal variances not assumed			.007	13.970	.995	.25000	36.56251	-78.18464	78.68464
Day 206	Equal variances assumed	.355	.561	-.559	14	.585	-26.12500	46.69953	-126.28553	74.03553
	Equal variances not assumed			-.559	13.374	.585	-26.12500	46.69953	-126.72706	74.47706

Table A2.2: Independent-samples t-test of the effect of chilling on plant height for *E. nitens* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Day 85	Equal variances assumed	.173	.684	.335	14	.743	12.00000	35.84117	-64.87166	88.87166
	Equal variances not assumed			.335	13.775	.743	12.00000	35.84117	-64.98942	88.98942
Day 119	Equal variances assumed	1.139	.304	.584	14	.568	23.25000	39.80348	-62.11997	108.61997
	Equal variances not assumed			.584	12.482	.570	23.25000	39.80348	-63.10421	109.60421
Day 149	Equal variances assumed	1.527	.237	-.127	14	.901	-6.50000	51.15815	-116.22332	103.22332
	Equal variances not assumed			-.127	12.290	.901	-6.50000	51.15815	-117.67303	104.67303

Table A2.3: Non-parametric Mann-Whitney U test for differences in height between control and chilled *E. nitens* plants on day 1

	Day1
Mann-Whitney U	28.000
Wilcoxon W	64.000
Z	-.420
Asymp. Sig. (2-tailed)	.674
Exact Sig. [2*(1-tailed Sig.)]	.721(a)

a Not corrected for ties.
b Grouping Variable: Grouping

Table A2.4: Non-parametric Mann-Whitney U test for differences in height between control and chilled *E. nitens* plants on day 29

	Day29
Mann-Whitney U	31.500
Wilcoxon W	67.500
Z	-.053
Asymp. Sig. (2-tailed)	.958
Exact Sig. [2*(1-tailed Sig.)]	.959(a)

a Not corrected for ties.
b Grouping Variable: Grouping

Table A2.5: Non-parametric Mann-Whitney U test for differences in height between control and chilled *E. nitens* plants on day 58

	Day58
Mann-Whitney U	28.000
Wilcoxon W	64.000
Z	-.420
Asymp. Sig. (2-tailed)	.674
Exact Sig. [2*(1-tailed Sig.)]	.721(a)

a Not corrected for ties.
b Grouping Variable: Grouping

Table A2.6: Non-parametric Mann-Whitney U test for differences in height between control and chilled *E. nitens* plants on day 190

	Day190
Mann-Whitney U	27.000
Wilcoxon W	63.000
Z	-.525
Asymp. Sig. (2-tailed)	.600
Exact Sig. [2*(1-tailed Sig.)]	.645(a)

a Not corrected for ties.
b Grouping Variable: Grouping

Table A2.7: Non-parametric Mann-Whitney U test for differences in height between control and chilled *E. nitens* plants on day 206

	Day206C
Mann-Whitney U	23.000
Wilcoxon W	59.000
Z	-.946
Asymp. Sig. (2-tailed)	.344
Exact Sig. [2*(1-tailed Sig.)]	.382(a)

a Not corrected for ties.
b Grouping Variable: Grouping

Table A2.8: Non-parametric Mann-Whitney U test of differences in height between control and chilled *E. grandis* plants on day 29

	Day29
Mann-Whitney U	17.000
Wilcoxon W	53.000
Z	-1.575
Asymp. Sig. (2-tailed)	.115
Exact Sig. [2*(1-tailed Sig.)]	.130(a)

a Not corrected for ties.
b Grouping Variable: Grouping

Table A2.9: Independent-samples t-test of differences in height of control and chilled *E. grandis* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Day1	Equal variances assumed	2.065	.173	.045	14	.965	1.00000	22.07617	-46.34867	48.34867
	Equal variances not assumed			.045	10.387	.965	1.00000	22.07617	-47.94143	49.94143
Day 58	Equal variances assumed	.000	.984	1.543	14	.145	43.87500	28.43661	-17.11546	104.86546
	Equal variances not assumed			1.543	13.970	.145	43.87500	28.43661	-17.12781	104.87781
Day 85	Equal variances assumed	.533	.478	.270	14	.791	13.00000	48.22043	-90.42254	116.42254
	Equal variances not assumed			.270	12.925	.792	13.00000	48.22043	-91.23510	117.23510
Day 119	Equal variances assumed	.257	.620	-.605	14	.555	-30.25000	50.01620	-137.52409	77.02409
	Equal variances not assumed			-.605	13.772	.555	-30.25000	50.01620	-137.69131	77.19131
Day 149	Equal variances assumed	.091	.767	-1.578	14	.137	-81.87500	51.89143	-193.17106	29.42106
	Equal variances not assumed			-1.578	13.997	.137	-81.87500	51.89143	-193.17338	29.42338
Day 190	Equal variances assumed	.681	.423	-1.926	14	.075	-144.75000	75.17402	-305.98225	16.48225
	Equal variances not assumed			-1.926	12.885	.077	-144.75000	75.17402	-307.30170	17.80170
Day206	Equal variances assumed	4.193	.060	-.959	14	.354	-78.50000	81.88428	-254.12432	97.12432
	Equal variances not assumed			-.959	8.967	.363	-78.50000	81.88428	-263.83821	106.83821

Table A3.1: Independent-samples t-test of differences in biomass allocation between control and chilled *E. grandis* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
TotBiomass	Equal variances assumed	1.008	.349	2.999	7	.020	175.75182	58.60480	37.17348	314.33015
	Equal variances not assumed			3.221	6.245	.017	175.75182	54.55934	43.50960	307.99403
Leaf DW	Equal variances assumed	.844	.389	3.073	7	.018	33.17910	10.79868	7.64428	58.71393
	Equal variances not assumed			2.889	4.645	.037	33.17910	11.48462	2.96618	63.39203
Above-ground DW	Equal variances assumed	.002	.966	5.196	8	.001	90.16170	17.35063	50.15109	130.17231
	Equal variances not assumed			5.244	6.780	.001	90.16170	17.19319	49.23712	131.08628
Stem DW	Equal variances assumed	.209	.662	2.400	7	.047	69.02852	28.76575	1.00833	137.04870
	Equal variances not assumed			2.504	6.943	.041	69.02852	27.57272	3.72033	134.33670
Root DW	Equal variances assumed	.627	.454	-.157	7	.880	-6.56915	41.90162	-105.65074	92.51245
	Equal variances not assumed			-.166	6.724	.873	-6.56915	39.64815	-101.10898	87.97069
Root:Shoot	Equal variances assumed	2.384	.161	-3.256	8	.012	-.27739	.08520	-.47386	-.08091
	Equal variances not assumed			-2.970	4.717	.033	-.27739	.09339	-.52186	-.03291
Leaf SA	Equal variances assumed	1.147	.315	3.325	8	.010	.18444	.05546	.05654	.31234
	Equal variances not assumed			3.102	5.116	.026	.18444	.05946	.03264	.33624
Root SA	Equal variances assumed	3.275	.145	-2.270	4	.086	-.57139	.25173	-1.27030	.12753
	Equal variances not assumed			-2.270	2.804	.114	-.57139	.25173	-1.40509	.26232
RootSA:Leaf SA	Equal variances assumed	8.083	.047	-3.132	4	.035	-2.57452	.82202	-4.85681	-.29222
	Equal variances not assumed			-3.132	2.246	.076	-2.57452	.82202	-5.76499	.61596

Table A3.2: Independent-samples t-test of differences in biomass allocation between control and chilled *E. nitens* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
TotBiomass	Equal variances assumed	.235	.653	1.252	4	.279	140.31787	112.11403	-170.96059	451.59633
	Equal variances not assumed			1.252	3.894	.281	140.31787	112.11403	-174.33624	454.97198
Leaf DW	Equal variances assumed	9.043	.040	-.396	4	.713	-13.83217	34.97036	-110.92544	83.26110
	Equal variances not assumed			-.396	2.098	.729	-13.83217	34.97036	-157.78612	130.12178
Above-ground DW	Equal variances assumed	5.194	.085	.290	4	.786	16.54597	57.05570	-141.86606	174.95799
	Equal variances not assumed			.290	2.394	.795	16.54597	57.05570	-194.01090	227.10284
Stem DW	Equal variances assumed	2.678	.153	1.316	6	.236	47.98600	36.46055	-41.22975	137.20175
	Equal variances not assumed			1.316	3.326	.272	47.98600	36.46055	-61.87317	157.84517
Root DW	Equal variances assumed	3.178	.149	-1.205	4	.295	-43.53850	36.12807	-143.84612	56.76912
	Equal variances not assumed			-1.205	2.137	.345	-43.53850	36.12807	-189.79942	102.72242
Root:Shoot	Equal variances assumed	1.535	.283	-1.288	4	.267	-.19373	.15040	-.61130	.22384
	Equal variances not assumed			-1.288	3.059	.286	-.19373	.15040	-.66717	.27971
Leaf SA	Equal variances assumed	.875	.386	3.863	6	.008	.32183	.08332	.11796	.52570
	Equal variances not assumed			3.863	4.866	.012	.32183	.08332	.10586	.53780
Root SA	Equal variances assumed	.091	.778	-3.367	4	.028	-1.12814	.33507	-2.05845	-.19783
	Equal variances not assumed			-3.367	3.974	.028	-1.12814	.33507	-2.06084	-.19544
RootSA:Leaf SA	Equal variances assumed	.306	.610	-4.627	4	.010	-1.96947	.42566	-3.15129	-.78765
	Equal variances not assumed			-4.627	3.717	.012	-1.96947	.42566	-3.18763	-.75131

Table A3.3: Independent-samples t-test of differences in biomass allocation between control and chilled *E. grandis* x *nitens* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
TotBiomass	Equal variances assumed	.003	.956	3.561	8	.007	191.80213	53.85835	67.60454	315.99971
	Equal variances not assumed			3.561	7.919	.008	191.80213	53.85835	67.38380	316.22046
Leaf DW	Equal variances assumed	1.371	.275	9.567	8	.000	60.60211	6.33477	45.99411	75.21010
	Equal variances not assumed			9.567	5.306	.000	60.60211	6.33477	44.59666	76.60755
Above-ground DW	Equal variances assumed	.062	.810	6.012	8	.000	150.87015	25.09456	93.00198	208.73831
	Equal variances not assumed			6.012	7.981	.000	150.87015	25.09456	92.97838	208.76191
Stem DW	Equal variances assumed	.002	.963	4.303	8	.003	90.06840	20.93190	41.79936	138.33744
	Equal variances not assumed			4.303	7.970	.003	90.06840	20.93190	41.76802	138.36878
Root DW	Equal variances assumed	5.564	.046	.112	8	.914	3.61468	32.26724	-70.79372	78.02308
	Equal variances not assumed			.112	5.389	.915	3.61468	32.26724	-77.56006	84.78942
Root:Shoot	Equal variances assumed	1.297	.288	-1.939	8	.088	-.43345	.22354	-.94894	.08203
	Equal variances not assumed			-1.939	5.524	.105	-.43345	.22354	-.99207	.12516
Leaf SA	Equal variances assumed	1.233	.299	6.303	8	.000	.38097	.06044	.24159	.52035
	Equal variances not assumed			6.303	6.959	.000	.38097	.06044	.23787	.52407
Root SA	Equal variances assumed									
	Equal variances not assumed									
RootSA:Leaf SA	Equal variances assumed									
	Equal variances not assumed									

Table A3.4: Non-prametric Mann-Whitney U test of root surface area between control and chilled *E. grandis* x *nitens* plants

	RootSA
Mann-Whitney U	3.000
Wilcoxon W	9.000
Z	-.655
Asymp. Sig. (2-tailed)	.513
Exact Sig. [2*(1-tailed Sig.)]	.700(a)

a Not corrected for ties.
b Grouping Variable: Group

Table A3.5: Non-prametric Mann-Whitney U test of the ratio between root surface area to leaf surface area between control and chilled *E. grandis* x *nitens* plants

	RsaLsa
Mann-Whitney U	.000
Wilcoxon W	6.000
Z	-1.964
Asymp. Sig. (2-tailed)	.050
Exact Sig. [2*(1-tailed Sig.)]	.100(a)

a Not corrected for ties.
b Grouping Variable: Group

Table A4.1: Independent-samples t-test of light curve parameters of control and chilled *E. grandis* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Amax	Equal variances assumed	.242	.636	2.175	8	.061	2.38783	1.09800	-.14417	4.91983
	Equal variances not assumed			2.175	7.875	.062	2.38783	1.09800	-.15117	4.92683
Alpha	Equal variances assumed	.349	.571	1.562	8	.157	.01245	.00797	-.00593	.03083
	Equal variances not assumed			1.562	7.082	.162	.01245	.00797	-.00636	.03125
Light compensation point	Equal variances assumed	3.174	.113	-1.117	8	.296	-22.81261	20.41762	-69.89572	24.27051
	Equal variances not assumed			-1.117	4.621	.319	-22.81261	20.41762	-76.61955	30.99434

Table A4.2: Non-parametric Mann-Whitney U test for significant differences between control and chilled stomatal conductance for *E.grandis* plants

	GsEG
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008(a)

a Not corrected for ties.
b Grouping Variable: Grouping

Table A4.3: Independent-samples t-test of light curve parameters of control and chilled *E. nitens* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Amax	Equal variances assumed	5.084	.051	3.180	9	.011	4.73305	1.48860	1.36560	8.10050
	Equal variances not assumed			3.465	5.903	.014	4.73305	1.36586	1.37749	8.08862
Alpha	Equal variances assumed	2.466	.151	-.077	9	.940	-.00065	.00836	-.01955	.01825
	Equal variances not assumed			-.082	8.035	.937	-.00065	.00793	-.01891	.01762
Light compensation point	Equal variances assumed	.003	.956	1.712	7	.131	11.02562	6.43844	-4.19886	26.25010
	Equal variances not assumed			1.701	6.395	.137	11.02562	6.48071	-4.59788	26.64913

Table A4.4: Non-parametric Mann-Whitney U test for significant differences in stomatal conductance between control and chilled *E. nitens* plants

	GsEN
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008(a)

a Not corrected for ties.
b Grouping Variable: Grouping

Table A4.5: Independent-samples t-test of light curve parameters of control and chilled *E. grandis* x *nitens* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Amax	Equal variances assumed	.105	.755	-1.178	7	.277	-3.89803	3.30874	-11.72195	3.92589
	Equal variances not assumed			-1.154	5.966	.293	-3.89803	3.37806	-12.17514	4.37908
Alpha	Equal variances assumed	.523	.493	.116	7	.911	.00098	.00845	-.01901	.02097
	Equal variances not assumed			.111	5.237	.916	.00098	.00883	-.02140	.02336
Light compensation point	Equal variances assumed	.827	.393	2.472	7	.043	15.50482	6.27305	.67141	30.33823
	Equal variances not assumed			2.540	6.991	.039	15.50482	6.10461	1.06576	29.94388
Stomatal conductance	Equal variances assumed	.013	.913	-2.807	8	.023	-.11372	.04052	-.20715	-.02029
	Equal variances not assumed			-2.807	7.996	.023	-.11372	.04052	-.20715	-.02028

Table A4.6: Independent-samples t-test of A:ci curve parameters between control and chilled *E. grandis* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
J_{max}	Equal variances assumed	1.886	.219	.215	6	.837	1.30952	6.09857	-13.61313	16.23217
	Equal variances not assumed			.215	5.658	.838	1.30952	6.09857	-13.83475	16.45379
Carboxylation coefficient	Equal variances assumed	.482	.514	-.230	6	.825	-.01110	.04819	-.12902	.10681
	Equal variances not assumed			-.230	5.067	.827	-.01110	.04819	-.13449	.11228
Photorespiration	Equal variances assumed	2.668	.154	.022	6	.983	.05268	2.42561	-5.88258	5.98795
	Equal variances not assumed			.022	4.617	.984	.05268	2.42561	-6.34123	6.44660
CO_2 compensation points		1.338	.291	.421	6	.688	10.16306	24.11824	-48.85214	69.17826
				.421	4.480	.693	10.16306	24.11824	-54.06205	74.38817

Table A4.7: Independent-samples t-test of A:ci curve parameters between control and chilled *E. nitens* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Carboxylation coefficient	Equal variances assumed	.181	.688	.898	5	.410	.02437	.02714	-.04540	.09414
	Equal variances not assumed			.872	3.943	.433	.02437	.02796	-.05370	.10244
Photorespiration	Equal variances assumed	1.539	.270	-.766	5	.478	-.69077	.90121	-3.00741	1.62587
	Equal variances not assumed			-.706	3.070	.530	-.69077	.97860	-3.76537	2.38384
CO_2 compensation points	Equal variances assumed	.288	.620	-.813	4	.462	-8.18768	10.06538	-36.13365	19.75830
	Equal variances not assumed			-.954	3.201	.406	-8.18768	8.58192	-34.55440	18.17905

Table A4.8: Non-parametric Mann-Whitney U t-test of J_{max} between control and chilled *E.nitens* plants

	JmaxEN
Mann-Whitney U	6.000
Wilcoxon W	12.000
Z	.000
Asymp. Sig. (2-tailed)	1.000
Exact Sig. [2*(1-tailed Sig.)]	1.000(a)

a Not corrected for ties.
b Grouping Variable: Grouping

Table A4.9: Independent-samples t-test of A:ci curve parameters between control and chilled *E. grandis x nitens* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
J_{max}	Equal variances assumed	.061	.814	1.859	5	.122	8.88892	4.78273	-3.40548	21.18331
	Equal variances not assumed			1.840	4.288	.135	8.88892	4.83070	-4.17555	21.95339
Carboxylation coefficient	Equal variances assumed	.757	.424	.325	5	.758	.01084	.03336	-.07491	.09659
	Equal variances not assumed			.349	4.959	.742	.01084	.03109	-.06928	.09097
Photorespiration	Equal variances assumed	.011	.921	-.874	5	.422	-1.49137	1.70708	-5.87957	2.89682
	Equal variances not assumed			-.871	4.413	.428	-1.49137	1.71141	-6.07260	3.08985
CO_2 compensation points		3.602	.116	.609	5	.569	6.83860	11.22462	-22.01521	35.69241
				.532	2.388	.640	6.83860	12.86406	-40.72558	54.40279

Table A5.1: Levels of significance of the difference between the slopes of control and treatment groups for each taxon.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Grandis	0.00	1	0.00	1.68	0.265
Nitens	0.32	1	0.32	48.56	0.002
Hybrid	0.00	1	0.00	0.00	0.993

Table A5.2: Significance values of differences leaf water potential and transpiration between control and chilled plants of each genotype.

Source	Dependent Variable	Mean	SD	Sig.
Grandis	Leaf WP control	-0.80	0.34	
	Leaf WP chilled	-0.69	0.25	0.34
	Leaf transp control	2.30	1.23	
	Leaf transp chilled	2.51	1.27	0.67
Nitens	Leaf WP control	-0.67	0.34	
	Leaf WP chilled	-0.49	0.17	0.09
	Leaf transp control	1.18	0.56	
	Leaf transp chilled	1.19	0.63	0.98
Hybrid	Leaf WP control	-0.48	0.21	
	Leaf WP chilled	-0.89	0.47	0.01
	Leaf transp control	1.67	1.23	
	Leaf transp chilled	2.07	1.73	0.50

Table A5.3: Descriptive statistics of changes in stomatal conductance throughout the day in *E. grandis* plants

	Grouping	N	Mean	Std. Deviation	Std. Error Mean
GsEG6am	1.00	4	.1031	.08588	.04294
	2.00	4	.1334	.06245	.03122
GsEG8am	1.00	4	.1459	.11219	.05610
	2.00	4	.1753	.08483	.04241
GsEG10am	1.00	4	.1014	.08252	.04126
	2.00	4	.1954	.14693	.07347
GsEG12pm	1.00	4	.0328	.02545	.01273
	2.00	4	.1499	.10275	.05137

Table A5.4: Independent-samples t-test of changes in stomatal conductance throughout the day in *E. grandis* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
GsEG6am	Equal variances assumed	.088	.776	-.571	6	.589	-.03032	.05309	-.16024	.09959
	Equal variances not assumed			-.571	5.479	.591	-.03032	.05309	-.16329	.10265
GsEG8am	Equal variances assumed	2.339	.177	-.418	6	.690	-.02941	.07033	-.20149	.14267
	Equal variances not assumed			-.418	5.585	.691	-.02941	.07033	-.20464	.14582
GsEG10am	Equal variances assumed	1.645	.247	-1.115	6	.307	-.09397	.08426	-.30015	.11221
	Equal variances not assumed			-1.115	4.721	.318	-.09397	.08426	-.31447	.12653
GsEG12pm	Equal variances assumed	2.099	.198	-2.212	6	.069	-.11705	.05293	-.24656	.01245
	Equal variances not assumed			-2.212	3.367	.104	-.11705	.05293	-.27557	.04146

Table A5.5: Descriptive statistics of changes in stomatal conductance throughout the day in *E. nitens* plants

	Grouping	N	Mean	Std. Deviation	Std. Error Mean
GsEN6am	1.00	4	.1695	.08362	.04181
	2.00	4	.1259	.04044	.02022
GsEN8am	1.00	4	.0964	.06806	.03403
	2.00	4	.1622	.12581	.06291
GsEN10am	1.00	4	.0980	.11521	.05761
	2.00	4	.0872	.06066	.03033
GsEN12pm	1.00	4	.0451	.04084	.02042
	2.00	4	.1071	.08267	.04134

Table A5.6: Independent-samples t-test of changes in stomatal conductance throughout the day in *E. nitens* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
GsEN6am	Equal variances assumed	1.340	.291	.938	6	.384	.04357	.04644	-.07007	.15721
	Equal variances not assumed			.938	4.330	.398	.04357	.04644	-.08159	.16872
GsEN8am	Equal variances assumed	4.522	.078	-.920	6	.393	-.06581	.07152	-.24081	.10920
	Equal variances not assumed			-.920	4.617	.403	-.06581	.07152	-.25433	.12272
GsEN10am	Equal variances assumed	1.548	.260	.167	6	.873	.01086	.06510	-.14844	.17016
	Equal variances not assumed			.167	4.545	.875	.01086	.06510	-.16166	.18338
GsEN12pm	Equal variances assumed	1.206	.314	-1.344	6	.228	-.06195	.04610	-.17476	.05086
	Equal variances not assumed			-1.344	4.382	.244	-.06195	.04610	-.18567	.06177

Table A5.7: Descriptive statistics of changes in stomatal conductance throughout the day in *E. grandis* x *nitens* plants

	Grouping	N	Mean	Std. Deviation	Std. Error Mean
GsGN6am	1.00	4	.1641	.11814	.05907
	2.00	4	.2055	.10958	.05479
GsGN8am	1.00	4	.1360	.09472	.04736
	2.00	4	.0983	.07543	.03772
GsGN10am	1.00	4	.0961	.13309	.06654
	2.00	4	.0596	.04432	.02216
GsGN12pm	1.00	4	.0696	.07467	.03733
	2.00	4	.0715	.03687	.01843

Table A5.8: Independent-samples t-test of changes in stomatal conductance throughout the day in *E. grandis* x *nitens* plants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
GsGN6am	Equal variances assumed	.113	.748	-.514	6	.626	-.04141	.08057	-.23855	.15573
	Equal variances not assumed			-.514	5.966	.626	-.04141	.08057	-.23882	.15599
GsGN8am	Equal variances assumed	.319	.593	.623	6	.556	.03775	.06054	-.11040	.18589
	Equal variances not assumed			.623	5.714	.557	.03775	.06054	-.11222	.18771
GsGN10am	Equal variances assumed	2.721	.150	.520	6	.622	.03649	.07014	-.13513	.20811
	Equal variances not assumed			.520	3.657	.633	.03649	.07014	-.16565	.23863
GsGN12pm	Equal variances assumed	12.621	.012	-.045	6	.966	-.00185	.04164	-.10374	.10003
	Equal variances not assumed			-.045	4.381	.966	-.00185	.04164	-.11360	.10989

Table A5.9: Non-parametric Mann-Whitney U test o differences in stomatal conductance between control and chilled *E. grandis* x *nitens* plants at midday

	GsGN12pm
Mann-Whitney U	8.000
Wilcoxon W	18.000
Z	.000
Asymp. Sig. (2-tailed)	1.000
Exact Sig. [2*(1-tailed Sig.)]	1.000(a)

a Not corrected for ties.
b Grouping Variable: Grouping

Table A5.10: Raw data of changes in stomatal conductance in *E. grandis* throughout the day. Each data point represents a different day.

6am control	6am chilled	8am control	8am chilled	10am control	10am chilled	12pm control	12pm chilled
0.008	0.059	0.043	0.062	0.001	0.002	0.005	0.014
0.215	0.199	0.250	0.258	0.106	0.337	0.041	0.168
0.109	0.169	0.236	0.163	0.203	0.276	0.021	0.156
0.080	0.107	0.055	0.218	0.096	0.167	0.064	0.263

Table A5.11: Raw data of changes in stomatal conductance in *E. nitens* throughout the day. Each data point represents a different day.

6am control	6am chilled	8am control	8am chilled	10am control	10am chilled	12pm control	12pm chilled
0.050	0.072	0.049	0.015	0.013	0.013	0.016	0.029
0.240	0.144	0.171	0.103	0.268	0.092	0.018	0.093
0.211	0.121	0.029	0.287	0.068	0.161	0.103	0.224
0.177	0.167	0.136	0.244	0.044	0.083	0.012	0.074

Table A5.12: Raw data of changes in stomatal conductance in *E. grandis* x *nitens* throughout the day. Each data point represents a different day.

6am		8am		10am		12pm	
0.014	0.045	0.006	0.001	0.006	0.006	0.001	0.025
0.237	0.285	0.205	0.184	0.289	0.046	0.136	0.114
0.128	0.266	0.126	0.114	0.081	0.111	0.132	0.066
0.278	0.226	0.207	0.094	0.009	0.076	0.054	0.081

Table A5.13: Average values of carbon assimilation (A), transpiration (E) and water use efficiency between *E. grandis* control and chilled plants. Each point is the average of four different days.

	grandis C			grandis T		
Time	A	E	WUE	A	E	WUE
06:00:00 AM	1.157	1.750	0.677	1.035	2.078	0.462
08:00:00 AM	2.427	3.486	0.838	6.274	4.474	1.542
10:00:00 AM	4.459	3.822	1.244	4.524	6.602	0.872
12:00:00 PM	1.860	1.632	1.117	4.416	6.278	0.763
	A	E	WUE			
t-test	0.270	0.115	0.829			
Average	2.476	2.673	0.969	4.062	4.858	0.910
SD	1.421	1.143	0.258	2.190	2.076	0.456

Table A5.14: Average values of carbon assimilation (A), transpiration (E) and water use efficiency between *E. nitens* control and chilled plants. Each point is the average of four different days.

	nitens C			nitens T		
Time	A	E	WUE	A	E	WUE
06:00:00 AM	2.868	2.660	1.068	3.870	2.115	1.671
08:00:00 AM	3.319	2.039	1.567	3.433	2.364	1.654
10:00:00 AM	6.693	3.097	2.084	3.774	3.508	1.053
12:00:00 PM	1.760	1.226	0.949	3.583	3.838	0.911
	A	E	WUE			
t-test	0.996	0.277	0.782			
Average	3.660	2.256	1.417	3.665	2.956	1.322
SD	2.126	0.812	0.519	0.196	0.845	0.397

Table A5.15: Average values of carbon assimilation (A), transpiration (E) and water use efficiency between *E. grandis* x *nitens* control and chilled plants. Each point is the average of four different days.

	hybrid C			hybrid T		
Time	A	E	WUE	A	E	WUE
06:00:00 AM	2.432	2.945	0.949	0.777	3.119	0.264
08:00:00 AM	10.005	3.435	2.622	4.587	2.690	1.679
10:00:00 AM	4.393	3.937	0.945	2.254	2.776	0.795
12:00:00 PM	4.790	3.279	1.406	3.152	2.693	1.279
	A	E	WUE			
t-test	0.183	0.488	0.378			
Average	5.405	3.399	1.481	2.692	2.820	1.004
SD	3.235	0.413	0.791	1.598	0.204	0.612

Table A5.16: Descriptive statistics and significance values for the absolute whole plant hydraulic resistance values.

	N	Minimum	Maximum	Mean		Std. Deviation	
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	t-test
egc	2	7309.95	10891.34	9100.64	1790.70	2532.43	
egt	4	10337.09	15641.75	11929.09	1259.69	2519.37	0.265
enc	4	4926.89	8134.33	6329.18	690.54	1381.07	
ent	4	7361.64	15368.24	11196.86	1892.38	3784.75	0.052
gnc	4	5820.00	9330.00	7229.59	756.87	1513.74	
gnt	4	15800.00	22000.00	18301.21	1334.79	2669.58	0.007

Table A5.17: Descriptive statistics and significance values for the absolute whole plant hydraulic resistance values, normalised to total plant dry weight.

	N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	t-test
	Statistic	Statistic	Statistic	Statistic		Statistic	
Egc	2	2073.85	3165.84	2619.84	546.00	772.16	
Egt	4	1823.53	3314.71	2401.28	319.87	639.74	0.728
Enc	4	2024.39	4081.56	2940.04	463.25	926.50	
Ent	4	2883.71	5528.00	4096.25	636.31	1272.63	0.192
Gnc	3	3315.78	4817.63	3883.51	470.66	815.21	
Gnt	3	5041.17	6923.98	6074.32	551.21	954.72	0.039

Table A5.18: Descriptive statistics and significance values for the absolute whole plant hydraulic resistance values, normalised to total plant leaf area.

	N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	t-test
	Statistic	Statistic	Statistic	Statistic		Statistic	
Egc	2	4244.87	8773.05	6508.96	2264.09	3201.90	
Egt	4	4591.27	6951.46	5566.06	506.21	1012.42	0.583
Enc	4	4889.65	9964.64	6665.60	1155.42	2310.83	
ent	4	4479.96	9292.20	6732.07	1207.20	2414.39	0.970
gnc	3	5421.86	8771.52	6664.18	1059.28	1834.73	
gnt	3	8649.63	10357.88	9459.75	495.09	857.52	0.075

Table A5.19: Descriptive statistics and significance values for the leaf specific conductivity of chilled and control plants in each genotype.

	N	Range	Minimum	Maximum	Mean		Std. Deviation	
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	t-test
EGC	2	2.61E-05	0.000504	0.00053	0.000517	1.3E-05	1.84279E-05	
EGT	4	0.000345	0.000461	0.000807	0.000677	7.73E-05	0.000154589	0.239447
ENC	4	0.000425	0.000418	0.000843	0.000613	8.85E-05	0.000177004	
ENT	4	0.000316	0.000861	0.001177	0.001036	6.59E-05	0.000131714	0.008613
GNC	3	0.000103	0.000492	0.000595	0.000552	3.1E-05	5.37246E-05	
GNT	3	0.000197	0.00064	0.000837	0.000707	6.5E-05	0.000112601	0.097807

Table A5.20: Mean values, standard deviation and levels of significance of absolute resistance for the three main components in all three genotypes

		Leaves	SD	Stems	SD	Roots	SD
grandis	control (2)	52.983	4.276	31.658	0.472	15.359	3.804
	chilled (4)	30.338	5.349	27.991	3.970	41.671	7.607
	t-test	0.008		0.246		0.012	
Nitens	control (4)	47.855	4.900	28.207	5.546	23.938	6.383
	chilled (4)	38.256	21.387	16.899	3.099	44.845	22.049
	t-test	0.415		0.012		0.118	
GN	control (3)	33.934	9.340	28.672	7.776	37.394	5.803
	chilled (3)	16.128	4.659	15.265	2.665	68.606	7.263
	t-test	0.042		0.048		0.004	

Table A5.21: Mean values, standard deviation and levels of significance of absolute resistance normalised by component dry weight for all three genotypes

		Above ground	SD	Leaves	SD	Stems	SD	Roots	SD
grandis	control (2)	2205.83	768.57	1593.80	1316.38	554.61	90.58	414.02	3.59
	chilled (4)	1268.01	395.94	226.15	54.29	405.40	188.07	1133.27	341.13
	t-test	0.103		0.075		0.366		0.048	
nitens	control (4)	1478.65	597.51	421.12	219.41	310.57	176.02	235.07	53.95
	chilled (4)	1270.80	546.26	434.71	275.03	200.31	35.17	861.99	590.70
	t-test	0.626		0.941		0.265		0.079	
GN	control (3)	1451.90	397.49	327.48	175.31	383.35	73.00	587.74	119.19
	chilled (3)	1012.01	236.61	210.60	23.36	288.72	91.21	2001.11	853.13
	t-test	0.175		0.316		0.233		0.047	

Table A5.22: Mean values, standard deviation and levels of significance of absolute resistance normalised by leaf area for all three genotypes. Sample size indicated in brackets

		Above ground	SD	Leaves	SD	Stems	SD	Roots	SD
grandis	control (2)	5535.68	2940.75	3478.34	1955.71	2057.34	985.04	973.28	261.16
	chilled (4)	3248.65	837.00	1683.71	388.88	1564.94	484.90	2317.41	456.24
	t-test	0.182		0.116		0.429		0.020	
nitens	control (4)	4670.32	977.06	2934.25	625.51	1736.08	501.85	1443.17	396.10
	chilled (4)	3611.87	1498.18	2528.81	1358.07	1083.05	209.46	3120.21	1868.64
	t-test	0.281		0.607		0.053		0.130	
GN	control (3)	4192.33	1294.52	2370.43	1303.86	1821.90	184.51	2471.85	655.38
	chilled (3)	2955.37	626.41	1518.96	417.41	1436.42	209.15	6504.38	1012.12
	t-test	0.211		0.342		0.075		0.004	

Table A5.23: Mean values and standard deviation of the total plant dry weight and absolute hydraulic resistance.

	Plant mass (g)		Total R (MPa.s.kg^{-1})	
	Control	Chilled	Control	Chillled
	582.30	437.72	7309.95	10337.09
	580.58	400.57	10891.34	11356.03
	387.48	356.86	6539.90	10381.49
	482.12	194.73	4926.89	15641.75
	501.77	224.02	8134.33	13353.35
	516.04	431.41	9328.65	7361.64
	464.54	359.70	5816.53	15368.24
		167.34		8704.21
		318.46		15829.79
		202.32		17133.28
		141.90		21951.47
Sample size	7	11		
Average	502.12	294.09	7563.94	13401.67
SD	67.99	110.50	2066.87	4278.47

Table A5.24: Pearson correlation between total biomass and total hydraulic resistance

Correlations

		Biomass	Resistance
Biomass	Pearson Correlation	1	-.684**
	Sig. (2-tailed)		.002
	N	18	18
Resistance	Pearson Correlation	-.684**	1
	Sig. (2-tailed)	.002	
	N	18	18

**. Correlation is significant at the 0.01 level (2-tailed).

Table A6.1: Elemental composition of the fertilisers administered

	N	P	K	S	Mg	Cl	Fe	Mn	Zn	Mo	Cu	B	Ca
	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	g/kg
Nutrifert natgro	61.7	34.1	262.4	100	35.8	29	2.302	0.508	646	0.062	0.084	0.663	0
Plant calcium	140	0	16	0	26	0	0	0	0	50	0	200	129
Mondi blue	187 (57% as NO_3)	75	162	0	0	0	0.69	0.3	0.27	0.09	0.14	0.44	0
Mondi orange	129 (34% as NO_3)	176	111	0	0	0	0	0	0	0	0	0	0
Trelmix	0	0	0	0	0.3	0	22.6	2.9	2.4	0.3	3.2	0	0