# GROWTH AND HYDRAULIC CHARACTERISTICS OF TWO TROPICAL DECIDUOUS SEEDLINGS SUBJECTED TO DIFFERENT WATER SUPPLY.

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## INTRODUCTION

Plant water deficit occurs when the rate of transpiration (E) exceeds the water uptake from soil (Bray, 1997). This is caused by several different external stresses, including drought, salinity and temperature difference (Bray, 1997) as well as the design of hydraulic architecture (Zimmermann, 1983; Tyree and Ewers, 1991). The ability of the whole plant to respond and survive cellular water deficit depends on whole-plant mechanisms (Bray, 1997) and the responses depend on the species and genotype (Tyree $et\ al.$, 2003), the length and severity of water loss (Lovisolo and Schubert, 1998), the age and stage of development, the organ and cell type and the sub cellular compartment (Bray, 1997).

Higher plants respond to water stress within variety of physiological and molecular mechanisms (Lovisolo and Schubert, 1998). A common response is decrease of conductivity to water flow along the soil-plant-atmosphere water pathway (Moreshet $et\ al.$, 1990; Sperry $et\ al.$, 2002). This is achieved through an increase of the resistance to water flow of the components of water pathway such as roots and shoots (Nardini and Tyree, 1999), leaves (Nardini, 2001) and stomata (Meinzer $et\ al.$, 1995). Further, hydraulic conductivity decreases as a result of disruption of water column in xylem by cavitation (Tyree and Sperry, 1989; Tognetti $et\ al.$, 1996), when xylem tension exceeds the cavitation threshold value of xylem pressure potential ($\phi_p$). Xylem cavitation is generally a catastrophic dysfunction of the water-conducting system (Tyree and Sperry, 1989; Tardieu and Davis, 1993; Hacke and Sauter, 1995). However, cavitation-induced xylem embolism may act as a rapid hydraulic signal for initiate the stomatal response (Tyree and Ewers, 1991; Cochard $et\ al.$, 1996) and prevent runaway of embolism (Tyree and Sperry, 1988; Salleo $et\ al.$, 1996) under water stress condition.

The reduced hydraulic conductance by its hydraulic architecture (Sperry $et\ al.$, 1993; Saliendra $et\ al.$, 1995; Meinzer and Grantz, 1990; Kavanagh $et\ al.$, 1999) and the increased xylem tension by transpiration energy (Tyree and Sperry, 1988; Jones and Sutherland, 1991) influence stomatal behavior, which reduces photosynthesis by reducing stomatal conductance ($g_s$) and closure of stomata (Makela $et\ al.$, 1996; Tyree $et\ al.$, 2002; Xu and Baldocchi, 2003; Munne-Bosch $et\ al.$, 2003; Rieger $et\ al.$, 2003). Such stomatal responses occur due to either hydraulic (Tyree and Sperry, 1988; Jones and Sutherland, 1991) or chemical signals generated from root (Khalil and Grace, 1993; Tardieu and Davis, 1993; Davis and Zhang, 1991) as a consequence of water stress. So, reduction in $CO_2$ assimilation by stomatal movement reduces total biomass (Li $et\ al.$, 2000; Vander Willigen and Pammenter, 1998; Osorio $et\ al.$, 1998) and ultimately productivity of plants (Tyree and Dixon, 1986).

Stomata responses to changes in hydraulic conductance of the flow path from soil to leaf (Meinzer and Grantz, 1990). Ryan and Yoder (1997) hypothesized that, as trees grow taller and age, $g_s$ and photosynthesis decrease due to increasing axial hydraulic resistance, because of increasing path length and aging (Hydraulic limitation hypothesis). There were evidences that both in support (Meinzer and Grantz, 1990; Sperry and Pockman, 1993; Saliendra *et al.*, 1995; Ryan *et al.*, 2000; Hubbard *et al.*, 1999 & 2001) and against (Hubbard *et al.*, 2002; Phillips *et al.*, 2002; Barnard and Ryan, 2003; McDowell *et al.*, 2003) the hydraulic limitation hypothesis.

In this study, two tropical trees, *Berrya cordifolia* and *Terminalia arjuna* were subjected to high and low watering treatment to measure any impact of watering treatment on *B. cordifolia* and *T. arjuna* and relate that with the growth characteristics and the leaf physiology of $\phi_{Leaf}$, to study the effect of watering treatment and genus on hydraulic conductivities (above ground main branch only) and the percentage loss in conductivity and to assess the relationship among hydraulic characteristics and growth.

## MATERIALS AND METHODS

### PLANT MATERIALS AND STUDY SITE :

Seedlings of *Berrya cordifolia* (Savandala) and *Terminalia arjuna* (Marudai) were obtained from District Forest office, Batticaloa. Soils of loamy: compost: gravel (3:1:1) v/v were evenly mixed with hand and mammoty for the pots. Then soil mixture was compactly filled in 6 l white polythene bags (pots), which have four drainage holes at the bottom, by adding water soil layer by layer.  The pots were randomized for each genus with particular watering treatment (*T. arjuna* /High, *T. arjuna*/Low, *B. cordifolia*/ High and *B. cordifolia*/Low) and randomly lined up in 5 lines and 16 rows in a flat, cleared, open area (8mx18m) at the Agroforestry, Eastern University, Sri Lanka. According to the randomization and labeling, all *T. arjuna* and *B. cordifolia* seedlings (total of 40 in each genus) were transplanted into pots on 28th April, 2003 and vertically supported. The spacing between each line and row was 1.5 m x 1 m respectively to avoid competition. Under each pot a double layer of polythene was placed in order to prevent root penetration into the soil from the pots. During this experimental period, rainfall was excluded by means of covering clear polythene sheets over the potted soil surface of each seedling somewhat like a 'top skirt'. Also existence of anaerobic condition was avoided keeping interspaces between the pots and the soil covering sheets.

### IMPOSING WATERING TREATMENTS :

Two watering treatments were implemented, high and low respectively, for 10 months from 1st May, 2003 to 12th February, 2004. Eleven years of mean monthly rainfall (1991-2001) of Batticaloa region, Sri Lanka was taken and simulated for the watering treatments. Water per pot was calculated using the average rainfall per month and the surface area of the pots. This calculated volume was further simulated to 10 fold for high watering treatment and 3 fold for low watering treatment to optimize the treatments. Plants were watered twice a week on Mondays (3 days of water) and Thursdays (4 days of water).

### Leaf water potential ($_{Leaf}$)

Leaf water potential ($\phi_{leaf}$) was measured destructively using a pressure chamber (Soil Moisture Equipment Crop, Santa Barbara, CA USA) on potted plants across watering treatments and

genus. Soil water potential ($\emptyset_{Soil}$) was assessed by measuring predawn $\emptyset_{leaf}$ at 5.45 h and midday ($\emptyset_{Midday}$) water potential was measured at 12.00 h.

## MEASUREMENTS OF HYDRAULIC PROPERTIES

Hydraulic conductivity of whole plant main shoot stem was measured by Low Pressure Flow Meter (LPFM) (Sperry *et al.*, 1988). The apparatus was fabricated with superficial modifications. Hydraulic measurements were carried out on 7 plants per treatment per genus when the plants were 9 months old. The potted whole plant shoots were cut at 5 cm above from the soil surface at 12.00 h. The cut proximal end was immediately sealed with Para film and brought into laboratory in black polythene bags. The whole plant shoot was left on the bench top for an hour in order to equilibrate the shoot water potential. The proximal end of the equilibrated whole plant shoot was re-cut perpendicularly under water to remove any embolized vessel from the initial cut. Then cut end was cleanly shaved with a sharp razor blade and connected to the Reservoir 1 of the conductivity apparatus via PVC tubes (Fig.1). Likewise, the distal end of the shoot was cut under water and connected to the drain reservoir of the apparatus.
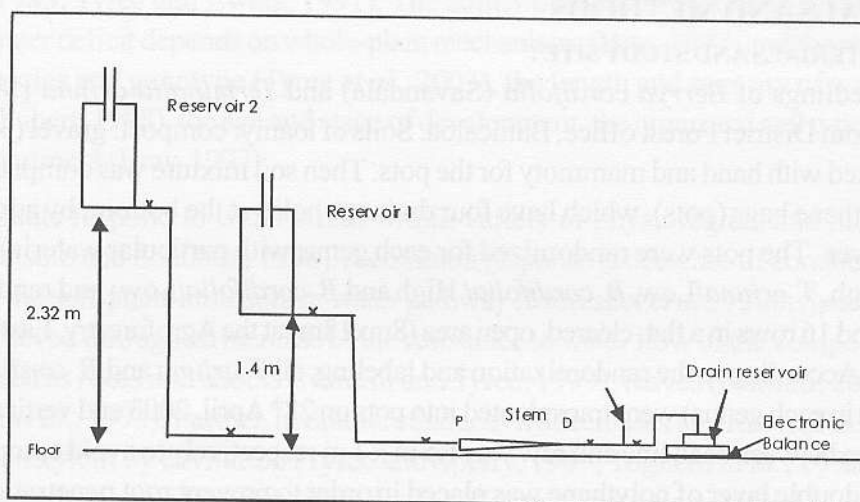


**Fig.1** Schematic diagram of modified Low Pressure Flow Meter (LPFM) from the version of Sperry *et al.* (1988). P- Proximal end of the shoot, D- Distal end of the shoot, X-Taps to control delivery supplies.

Fig. 1 illustrates the hydraulic apparatus designed to measure the conductivity in the main shoot stem using flow with pressure difference of 0.014 MPa (Head pressure). The solution used was distilled, degassed, filtered (0.47ìm cellulose micro fiber membrane filter) 0.01M HCl, and stored in supply tank (Reservoir 1) at 1.4m height from ground floor in order to supply the solution at a pressure difference of 0.014 MPa. Filtering prevented clogging of dust particles in the vessels and HCl was used to prevent short term microbial growth (Sperry *et al.*, 1988). The solution was allowed to flow through PVC tubes from the Reservoir 1 through the stem and into a drain reservoir via distal PVC connection on an electronic balance (Mettler PJ300, Switzerland, resolution 0.001g). The second supply tank (Reservoir 2) at 2.32m height was kept to dissolve embolism in the stem at higher pressure of 0.023 MPa immediately after an initial conductivity measurement by flushing the whole shoot stem for over night.

The hydraulic conductivity ($k_h$) was determined by measuring the mass flow (F, kgs$^{-1}$) of solution passing through a stem segment of length (l, m) connected to a constant pressure reservoir (P, MPa).

$$k_h = \frac{\text{Flow (F) x Twig length (l)}}{\text{Head pressure (P)}}$$

The degree of embolism in a main shoot stem was estimated by its initial conductivity, as a percentage of maximum obtained after removal of emboli. i.e Percentage Loss in Conductivity (PLC)

$$PLC = \frac{(k_{max} - k_i) \times 100}{k_{max}}$$

At the end, hydraulic conductivity was normalized to the leaf area at the distal end, sapwood area and total dry mass of whole plant shoot.

# GROWTH PARAMETERS

## LEAF AREA

Projected leaf area and total leaf area per plant were calculated from leaf area vs. biomass relationship (Ryan et al., 2000; Hubbard et al., 2002) manually. For that, fresh leaf piece of B. cordifolia (4 x 4 cm²) and of T. arjuna (2 x 2 cm²) were taken from each plant, oven dried at 60⁰C for 48 hours and weighed using an electronic balance for the dry mass.

## SAPWOOD AREA AND VESSEL DIAMETER

Sapwood area and vessel diameter at the distal end of stems that were subjected to hydraulic measurement was measured with ocular lens and stage micrometer using light microscope (Olympus HSC, Tokyo, Japan)

## HEIGHT AND STEM OVER BARK DIAMETER (OBD)

Height (m) and stem over bark diameter (OBD) (mm) of all potted seedlings were measured at 15 days interval from 1ˢᵗ May, 2003 until final harvest. Height was measured from the soil surface to apical bud using measuring tape. OBD was measured with a hand vernier caliper at 10 cm height for T. arjuna where as for B. cordifolia at 5 cm height, since the genus had crowded-lower canopy.

## DRY MASS

After hydraulic measurements, the shoots were segmented into leaves and stem and oven dried at 60⁰ C for 48 h and weighed using electronic balance for dry mass determination. Roots were carefully excavated and washed with splash of water in a bucket and all broken rootlets were separated using a sieve. Then the roots were oven dried as stated above for dry mass determination.

## STATISTICAL ANALYSIS

Two-way ANOVA for genus and watering treatments were performed using Statistical Package for Social Sciences (SPSS, 10.0 for windows. SPSS Inc., 1989-1999). Kolmogorov-Smirnov test (K-S test) was performed to test normality of the variables. One-way ANOVA was performed across genus and watering treatment separately since the numbers of variables were low enough for post-hoc tests. The relationship between maximum hydraulic conductivity ($k_{max}$,), xylem specific conductivity ($k_s$) and vessel diameter and the relationship between leaf specific hydraulic conductivity ($k_l$) and total shoot, total root, whole plant dry mass were explored by using linear regression analysis of the GraphPad Prism 3.02 (GraphPad Software Inc., 2000).

# RESULTS

## GROWTH PARAMETERS

### HEIGHT AND STEM OVER BARK DIAMETER (OBD)

The growth response in terms of height in *Terminalia arjuna* and *Berrya cordifolia* subjected to high and low water treatments is shown in Table 1. There was no significant effect of watering treatments on plant height at the time of harvest (F=0.52, P=0.478), but there was a clear genus effect (F=205.85, P=0.000), where *T. arjuna* grew taller than *B. cordifolia*. The interaction between genus and watering treatments was also significant (F=5.16, P=0.032). Surprisingly, *T. arjuna* seedlings subjected to low water treatment were taller than high water treatment (P<0.05). In *B. cordifolia*, the high watered plants were taller than that of low watered plants but the difference was insignificant (P>0.05).

High watered plants had greater OBD than low watered plants (F=38.57, P=0.000). The reduction in OBD by low water treatment was by 36% in *T. arjuna*, and 22% in *B. cordifolia* (see Table 3.1). *T. arjuna* had greater OBD than that of *B. cordifolia* (F=87.78, P=0.000), although OBD measured at two different heights. Further, water treatment x genus interaction was significant (F=10.59, P= 0.003) on stem OBD at the time of harvest.

### LEAF AREA

High watered plants produced higher leaf area than low watered plants (F=5.79, P=0.024). The percentage differences between the watering treatments was 22% higher in *T. arjuna* (P>0.05) and 52% in *B. cordifolia* (P<0.05). Of the genus, *B. cordifolia* had higher total leaf area than *T. arjuna*, though the difference was insignificant (F=0.04, P=0.84). As like, the interaction between genus and watering treatment was also insignificant (F=1.38, P=0.251).

**Table 1** Growth parameters of *T. arjuna* and *B. cordifolia* subjected to high and low watering treatments. Means ± SEM (n=7). Within columns, different capital letters (High water) and small letters (Low water) indicate significant differences at P<0.05 (One-way ANOVA) separately for watering treatments.

| Species | Water | Height (m) | OBD (mm) | Total leaf Area (m²) | Leaf Dry mass (g) | Total shoot Dry mass (g) | Root Dry mass (g) | Root: shoot ratio | Whole plant Dry mass (g) |
|---|---|---|---|---|---|---|---|---|---|
| T. arjuna | High | 0.92±0.05ᴬ | 19.49±0.86ᴬ | 0.28±0.03ᴬ | 35.54±3.22ᴬ | 121.63±4.32ᴬ | 214.48±14.19ᴬ | 1.78±0.14ᴬ | 336.12±13.41ᴬ |
| | Low | 1.04±0.05ª | 12.49±0.28ª | 0.21±0.02ª | 24.39±3.15ª | 76.63±5.58ª | 68.23±6.39ª | 0.89±0.08ª | 144.86±10.31ª |
| B. cordifolia | High | 0.44±0.05ᴮ | 10.16±1.11ᴮ | 0.34±0.09ᴬ | 11.60±2.75ᴮ | 22.50±5.95ᴮ | 13.86±3.95ᴮ | 0.60±0.05ᴮ | 36.36±9.85ᴮ |
| | Low | 0.37±0.02ᵇ | 7.97±0.71ᵇ | 0.16±0.03ª | 7.23±1.26ᵇ | 13.40±2.27ᵇ | 8.09±1.27ᵇ | 0.63±0.05ᵇ | 21.49±3.44ª |

### SAPWOOD AREA

There was no significant difference between the genus (F=0.29, P=0.599) and watering treatments (F=0.000, P= 1) in production of sapwood area at the distal end. It was $2.99\pm0.36\times10^{-7}m^2$, $2.45\pm0.28\times10^{-7}m^2$ in high and low watered *T. arjuna* and $2.85\pm0.35\times10^{-7}m^2$ and $3.09\pm0.86\times10^{-7}m^2$ in high and low water *B. cordifolia*, respectively.

## DRY MASS ALLOCATION

Table 1 shows the allocation of dry mass in whole plant and its plant components. The low watering treatment reduced the dry mass of whole plant and its components in both genus (F=125.44, P=0.000). Of the genus effect, *T. arjuna* had significantly higher total plant dry mass than *B. cordifolia* (F=528.60, P=0.000). Total allocation was 66% and 41% higher in high watered plants of *T. arjuna* (P<0.05) and *B. cordifolia* (P>0.05) respectively than that of the low watered plants. The effect of watering treatment (F=24.72, P=0.000), genus (F=70.89, P=0.000) and the genus x watering treatment interaction (F=27.81, P=0.000) on root: shoot ratio was highly significant. High watered *T. arjuna* invested more carbon in roots than shoot (R:S = 1.78:1), although low watered plants invested marginally opposite (R:S = 0.89:1). *B. cordifolia* invested 62% of dry mass in shoot and 38% in root, regardless of watering treatments (r: s =0.6: 1).

## PLANT WATER STATUS

### LEAF WATER POTENTIAL: PREDAWN AND MIDDAY

The response of $\emptyset_{Leaf}$ to the high and low watering treatment is shown in Table 2. Both $\emptyset_{Predawn}$ and $\emptyset_{Midday}$ were significantly higher in *B. cordifolia* than that of *T. arjuna* (F=10.16, P=0.003; F=4.49, P=0.045). There was no significant effect of watering treatments on $\emptyset_{Predawn}$ (F=0.91, P=0.348) and $\emptyset_{Midday}$ (F=1.69, P=0.206). In *B. cordifolia*, $\emptyset_{Predawn}$ and $\emptyset_{Midday}$ was equal in both water treatments. Both $\emptyset_{Predawn}$ and $\emptyset_{Midday}$ were lower in low watered *T. arjuna* than that of high watered plants (P>0.05).

**Table 2** Predawn and midday leaf water potential of *T. arjuna* and *B. cordifolia* subjected to high and low watering treatments. Means ± SEM (For predawn n=10 and midday n=7). Within columns, different capital letters (High water) and small letters (Low water) indicate significant differences at P<0.05 (One-way ANOVA) separately for watering treatments.

| Species | Watering treatment | $\Psi_{Predawn}$ (MPa) | $\Psi_{Midday}$ (MPa) |
|---|---|---|---|
| *T. arjuna* | High | -0.61±0.26[A] | -1.14±0.09[A] |
| | Low | -0.95±0.32[a] | -1.47±0.09[a] |
| *B. cordifolia* | High | -0.21±0.04[A] | -1.06±0.19[A] |
| | Low | -0.21±0.01[b] | -1.05±0.10[b] |

## Preliminary investigation of stem xylem architecture: vessel diameter

Table 3 shows the xylem conduit diameter of the distal end of the shoot stem of *T. arjuna* and *B. cordifolia* subjected to high and low watering treatments. High watered plants had significantly wider conduits than that of low watered plants (F=27.87, P=0.000). It was 30% and 16% higher in high watered plants in *T. arjuna* and *B. cordifolia*, respectively. But the difference between the watering treatments was not significant in *B. cordifolia*. Of the genus, *T. arjuna* had significantly wider xylem conduit diameter than that of *B. cordifolia* (F=82.22, P=0.000). The interaction between the genus and treatment also showed a significant difference (F=7.94, P=0.010).

**Table 3** Xylem vessel diameter at the distal end of whole shoot stem of *T. arjuna* and *B. cordifolia* subjected to high and low watering treatments. Means ± SEM (n=7). Within columns, different capital letters (High water) and small letters (Low water) indicate significant differences at P<0.05 (One-way ANOVA) separately for watering treatments.

| Species | Water treatment | Xylem vessel diameter ($\mu$m) |
|---------|-----------------|-------------------------------|
| T. arjuna | High | $147.67 \pm 4.71^A$ |
| | Low | $104.04 \pm 6.03^a$ |
| B. cordifolia | High | $83.63 \pm 7.94^B$ |
| | Low | $70.37 \pm 3.75^b$ |

# HYDRAULIC PROPERTIES

## MAXIMUM HYDRAULIC CONDUCTIVITY ($K_{MAX}$)

T. arjuna and had higher $k_{max}$ than that of B. cordifolia (F=20.33, P=0.000). Of the watering treatments, $k_{max}$ of high watered plants was two fold greater than that of low water treatment in T. arjuna (F=10.79, P=0.003) (Fig. 2.A). $k_{max}$ in high watered T. arjuna was $9.86 \pm 1.51 \times 10^{-6}$ kg s$^{-1}$ m$^{-1}$ MPa$^{-1}$. Difference in reduction in $k_{max}$ was by 47% in low watered plants (P<0.05). In B. cordifolia, $k_{max}$ of high water treatment was $4.10 \pm 1.04 \times 10^{-6}$ kg s$^{-1}$ m$^{-1}$ MPa$^{-1}$ and there was a 38% reduction in low water treatment but the reduction was insignificant (P>0.05).
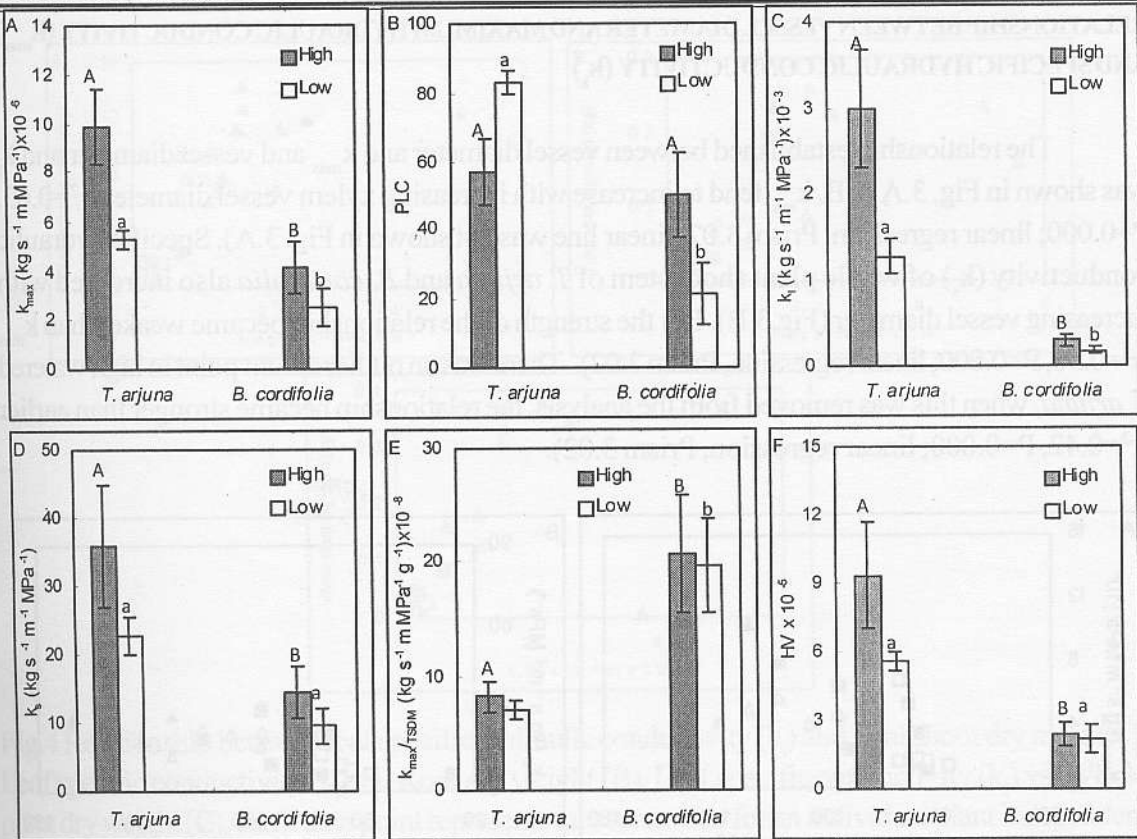
## PERCENTAGE LOSS IN HYDRAULIC CONDUCTIVITY (PLC)

Higher PLC occurred in T. arjuna than B. cordifolia (F=17.45, P=0.000). But the watering treatment was insignificant (F=0.02, P=0.883). In high watered T. arjuna, PLC was $56.79 \pm 9.65$ and significantly increased to $83.27 \pm 3.73$ in low water treatment (P<0.05). In contrast, high PLC was quantified in B. cordifolia ($50 \pm 12.49$) subjected to high watering treatment than that of low watered plants ($21.07 \pm 8.78$). But the differences between them were insignificant (P>0.05; Fig.2.B).

## LEAF SPECIFIC HYDRAULIC CONDUCTIVITY ($K_L$) AND SPECIFIC HYDRAULIC CONDUCTIVITY ($K_S$)

$k_l$ was higher in T. arjuna than that of B. cordifolia (F=31.99, P=0.000). Of the watering treatments, high watered plants had higher $k_l$ and it was decreased in low watering treatment (F=7.39, P=0.012). The interaction between the genus and watering treatment was significant (F=5.57, P=0.027). In T. arjuna, $k_l$ was $2.99 \pm 0.06 \times 10^{-3}$ kg s$^{-1}$ m$^{-1}$ MPa$^{-1}$ in high watering treatment and reduced by 58% in low watering treatment (P<0.05). Likewise, $k_l$ was $0.29 \pm 0.07 \times 10^{-3}$ kg s$^{-1}$ m$^{-1}$ MPa$^{-1}$ in B. cordifolia subjected to high water and reduced by 40% in low water treatment. But the reduction was insignificant (P>0.05).

igher $k_s$ was observed in T. arjuna than that of B. cordifolia (F=12.79, P=0.002). Though the plants subjected to high water treatment showed higher $k_s$ than that of low watering treatment, the effect of watering treatments were insignificant at 95% confidence interval (F=3.56, P=0.072). An interaction between the genus and watering treatment were insignificant (F=0.74, P=0.400).

**Fig.2** Hydraulic properties of *T. arjuna* and *B. cordifolia* subjected to high and low watering treatments. (A) Maximum hydraulic conductivity of whole shoot stem ($k_{max}$), (B) Percentage loss in hydraulic conductivity (PLC), (C) Leaf specific hydraulic conductivity ($k_l$), (D) Specific hydraulic conductivity ($k_s$), (E) $k_{max}$ normalized by total shoot dry mass ($k_{max}/_{TSDM}$), (F) Huber value (HV). Means± SEM, n =7. Different capital letters (High water) and small letters (Low water) indicate significant differences at P<0.05 (One-way ANOVA) separately for watering treatments.

MAXIMUM HYDRAULIC CONDUCTIVITY NORMALIZED BY TOTAL SHOOT DRY MASS ($k_{max}/_{TSDM}$)

When $k_{max}$ was normalized to total shoot dry mass (to show carbon investment in the hydraulic system), *B. cordifolia* had significantly higher value than that of *T. arjuna* (F=15.70, P=0.001) (Fig.2.E). The high watering treatment showed a marginal increase in $k_{max}/_{TSDW}$ than that of low watering treatment (F=0.13, P=0.719) also the genus x watering treatment interaction was insignificant (F=0.00, P=1). $k_{max}/_{TSDM}$ was 20.68±5.24 x$10^{-8}$ kg s$^{-1}$ m MPa$^{-1}$g$^{-1}$ in high watered *B. cordifolia* and thus reduced to 19.54±4.08 x$10^{-8}$ kg s$^{-1}$ m MPa$^{-1}$g$^{-1}$ in low water treatment. In *T. arjuna*, for high and low water treatments the value was 8.21±1.28 and 7.06±0.74 x $10^{-8}$ kg s$^{-1}$ m MPa$^{-1}$g$^{-1}$ respectively.

HUBER VALUE (HV)

The sapwood area per unit leaf area (HV), the measure of investment of stem tissue per unit leaf area, was significantly higher in *T. arjuna* than that of *B. cordifolia* (F=20.14, P=0.000) (Fig. 2.F). The watering treatments had not any significant effect on HV (F=2.96, P=0.098). The genus x water treatment interaction was also insignificant (F=2.49, P=0.127).

# RELATIONSHIP BETWEEN VESSEL DIAMETER AND MAXIMUM HYDRAULIC CONDUCTIVITY ($k_{max}$) AND SPECIFIC HYDRAULIC CONDUCTIVITY ($k_s$)

The relationship established between vessel diameter and $k_{max}$ and vessel diameter and $k_s$ was shown in Fig. 3.A & B. $k_{max}$ tend to increase with increasing xylem vessel diameter ($r^2=0.63$, P=0.000; linear regression, Prism 3.02, linear line was not shown in Fig. 3.A). Specific hydraulic conductivity ($k_s$) of whole plant shoot stem of *T. arjuna* and *B. cordifolia* also increased with increasing vessel diameter (Fig.3.B). But the strength of the relationship became weaker than $k_{max}$ ($r^2=0.38$, P=0.000; linear regression, Prism 3.02). There was an outlier datum point in high watered *T. arjuna*, when this was removed from the analysis, the relationship became stronger than earlier ($r^2=0.42$, P=0.000; linear regression, Prism 3.02).
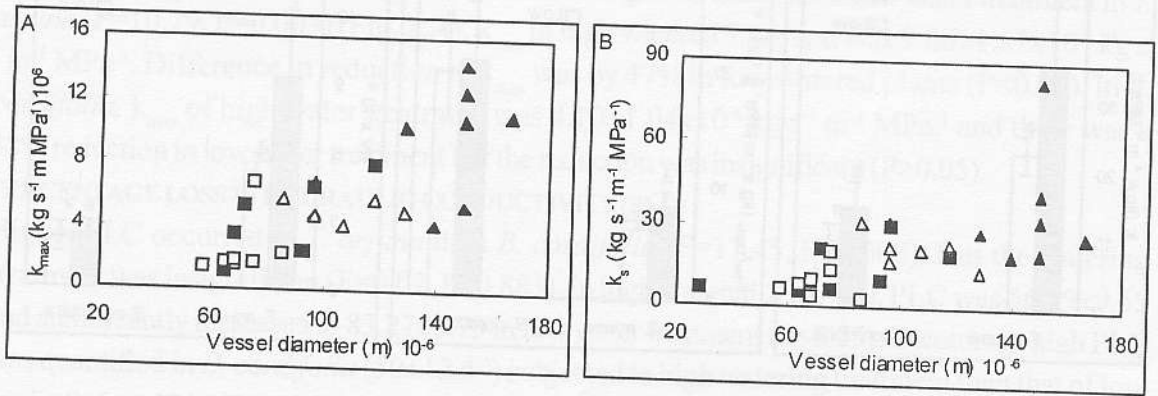


**Fig.3** The relationship between $k_{max}$ (A) and $k_s$ (B) and vessel diameter Each data point represents measurements for an individual plant shoot independent of both genus and watering treatments (n=28). Symbols: ²% High watered *T. arjuna*,   Low watered *T. arjuna*, % High watered *B. cordifolia*, ¡% Low watered *B. cordifolia*.

## RELATIONSHIP BETWEEN LEAF SPECIFIC HYDRAULIC CONDUCTIVITY ($k_l$) AND DRY MASS

The relationship between leaf specific conductivity and plant dry mass is shown in Fig 4. As $k_l$ increased, the total shoot dry mass (Fig. 4.A, $r^2= 0.58$, P=0.000; linear regression, Prism 3.02), total root dry mass (Fig. 4.B, $r^2=0.56$, P= 0.000; linear regression, Prism 3.02) and the whole plant dry mass (Fig.4.C, $r^2=0.59$, P=0.000; linear regression, Prism 3.02) increased. The relationship between $k_l$ and total shoot dry mass and total root dry mass showed $r^2=0.77$, P=0.000 and for the whole plant dry mass $r^2=0.79$, P=0.000. This suggests that dry mass allocation increased concurrently with increasing $k_l$ in all cases. But $k_l$ vs. total shoot dry mass relationship is realistic and physiologically meaningful than other cases.
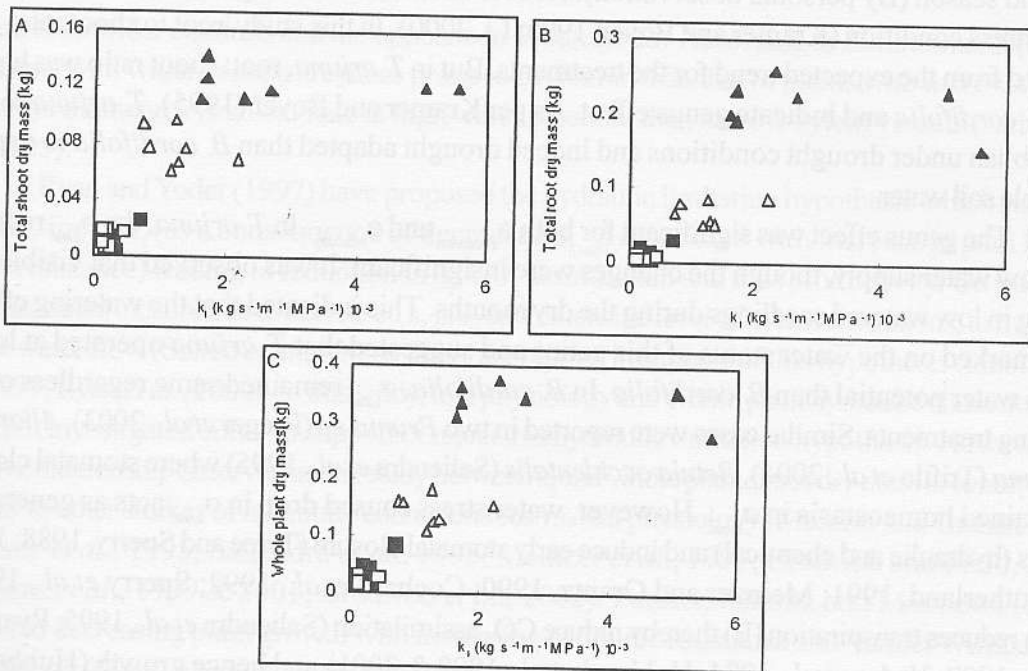
**Fig.4** Relationship between leaf specific hydraulic conductivity ($k_l$) and total shoot dry mass (A), Leaf specific conductivity ($k_l$) vs. Root dry weight (B), Leaf specific conductivity ($k_l$) vs. Whole plant dry weight (C). Each data point represents measurements for an individual plant independent of both genus and watering treatments (n=28). Symbols: [20]% High watered *T. arjuna*, " Low watered *T. arjuna*, % High water *B. cordifolia*, ¡% Low watered *B. cordifolia*.

**DISCUSSION :**

As expected, biomass accumulation and growth of *T. arjuna* and *B. cordifolia* seedlings were affected by low water supply. The greatest watering effect was observed in *T. arjuna* than *B. cordifolia* (see Table 1). *T. arjuna* seedlings grew taller than *B. cordifolia* seedlings and this shows probability of genus competition effect to acquire more photosynthetic active radiation (PAR). Whole plant dry mass and plant component dry mass such as leaves, stem and petioles and root dry mass were significantly reduced by low watering treatment (Table 1). Similar to this findings, drought-induced reduction in plant dry mass was reported in two *Prunus* sp (Rust and Roloff, 2002), *E. microtheca* (Li *et al.*, 2000), *E. globulus* (Osorio *et al.*, 1998) and *E. grandis* clones (Vander Willigen and Pammenter, 1998). It is presumed that the drought induced reduction in dry mass production probably due to stomatal closure and thus limits $CO_2$ assimilation (Rust and Roloff, 2002; Li, 2000; Jones and Sutherland, 1991).

The pattern of biomass allocation was genotypically different in this study. *B. cordifolia* preferentially allocated dry mater to leaves, and stem and petioles (shoot system) while *T. arjuna* invested less on the shoot system. Though relative investment and root growth were lower in *B. cordifolia*, the higher leaf area/ root dry mass ratio suggested that this genus had higher efficiency to transport water to the leaves than *T. arjuna* (Data not shown). However, *T. arjuna* had extensive root system with higher root investment and higer root: shoot ratio. This greater allocation to roots may exploit more soil water and supply to the shoot (Larcher, 1995 *loc. cite* Li *et al.*, 2000). The shallow root system of *B. cordifolia* also evident as this genus easily uprooted during

the wind season (By personal observation). Root to shoot ratio was reported to increase under water stress condition (Kramer and Boyer, 1995; Li, 2000). In this study, root to shoot ratio was reversed from the expected trend for the treatments. But in *T. arjuna*, root: shoot ratio was higher than *B. cordifolia* and indicate genus effect. As per Kramer and Boyer (1995), *T. arjuna* seems to establish under drought conditions and indeed drought adapted than *B. cordifolia* to exploit available soil water.

The genus effect was significant for both $\emptyset_{Predawn}$ and $\emptyset_{Midday}$. In *T. arjuna*, the $\emptyset_{Leaf}$ reduced with low water supply, though the changes were insignificant. It was observed that visible leaf wilting in low watered seedlings during the dry months. This indicated that the watering effects were marked on the water status of this genus and suggested that *T. arjuna* operated at lower xylem water potential than *B. cordifolia*. In *B. cordifolia*, $\emptyset_{Leaf}$ remained same regardless of the watering treatments. Similar cases were reported in two *Prunus* sp (Rieger *et al.*, 2003), *Alianthus altissima* (Trifilo *et al.*, 2004), *Betula occidentalis* (Saliendra *et al.*, 1995) where stomatal closure maintained homeostasis in $\emptyset_{Leaf}$. However, water stress caused drop in $\emptyset_{Leaf}$ acts as generating signals (hydraulic and chemical) and induce early stomatal closure (Tyree and Sperry, 1988; Jones and Sutherland, 1991; Meinzer and Grantz, 1990; Cochard *et al.*, 1992; Sperry *et al.*, 1993), which reduces transpiration (E) thereby reduce $CO_2$ assimilation (Saliendra *et al.*, 1995; Ryan and Yoder, 1997; Yoder *et al.*, 1994; Hubbard *et al.*, 1999 & 2001) and hence growth (Hubbard *et al.*, 1999). This was indirectly reflected on the weighed dry mass in this study, where *B. cordifolia* resulted with lower dry mass. Surprisingly, though *T. arjuna* functioned at lower water potential, produced higher whole plant, above ground and root dry mass and physiological reasons for this is unknown.

In this study, water supply affected the above ground plant hydraulic characteristics. The seedlings that were subjected to high watering treatment showed higher $k_{max}$, $k_l$ and $k_s$ of the whole plant shoot stem than low watered plants. Yet, the watering effect was more marked on *T. arjuna* than *B. cordifolia*. In accordance with this data, water effect reported to change the hydraulic conductivities both in potted and field grown plant species. In *Vitis vinifera*, $k_h$, $k_l$ and $k_s$ of shoot reduced by water stress (Lovisolo and Schubert, 1998) and the branches of *Eucalyptus grandis* clones (Vander Willigen and Pammenter, 1998). Further, whole plant hydraulic conductance ($K_{Plant}$) was reduced by drought in *Fraxinus excelsior* saplings (Stohr and Losch, 2004) and both root hydraulic conductance ($K_{Root}$) and shoot hydraulic conductance ($K_{Shoot}$) were reduced during desiccation in *Licania platypus* seedlings (Tyree *et al.*, 2002).

Higher hydraulic conductivities are desirable for growth performances in plants (Tyree, 2002; Nardini and Tyree, 1999; Nardini and Salleo, 2000). Because at a particular E, higher $k_l$ maintains lower xylem pressure gradient along the soil-plant-atmosphere pathway (Tyree and Ewers, 1991). This prevents excessive water stress in the xylem (xylem tension), thus prevents xylem dysfunction (Tyree *et al.*, 1991) and maintains low water stress at growing meristem, which leads to higher growth (Nardini and Tyree, 1999). On the other hand, higher $k_l$ permits higher E rates; therefore maximizing $g_s$ and photosynthesis (Tyree *et al.*, 1991; Hubbard *et al.*, 1999 & 2001). $k_s$ is a measure of porosity of xylem conduit (Tyree *et al.*, 1991). At a particular HV, higher $k_s$ maintain higher $k_l$ ($k_l$ = HV x $k_s$) (Tyree and Ewers, 1991 & 1996).

The high watered seedlings significantly developed wider vessels than low watered seedlings (see Table 3). Of the genus, *T. arjuna* had wider conduits than *B. cordifolia*. The level of embolism can also be related to the differences in the vessel size. i.e. higher PLC quantified in *T. arjuna* was

related to its wider conduits and the opposite in *B. cordifolia*. According to Zimmermann (1983), the plants with wider vessels are more prone to cavitation than that of plants with narrow vessels i.e. larger diameter xylem cavitate at high water potential than narrow xylem vessels (Jarbeau *et al.*, 1995).

Ryan and Yoder (1997) have proposed the hydraulic limitation hypothesis which suggests that decline of $k_l$ (as a consequence of decreased $\varnothing_{Leaf}$) associated with increase in path length (axial resistance) leading to reduction in $g_s$, $CO_2$ assimilation and thus productivity. On the other hand, stomatal closure due to decreased $k_l$ prevents extensive level of xylem cavitations, to maintain stable water flow (Salleo *et al.*, 2000). There are reports in support of this hypothesis (Hubbard *et al.*, 1999; Ryan *et al.*, 2000). In this study, leaf physiology and whole plant hydraulic characteristics were not investigated either to support or reject this hydraulic limitation hypothesis. However, the positive relationship observed in this study between $k_l$ and whole plant shoot dry mass is conceptually similar to other studies of hydraulic characteristics vs. leaf physiology (Meinzer and Grantz, 1990; Meinzer *et al.*, 1995; Saliendra *et al.*, 1995; Meinzer *et al.*, 1999; Brodribb and Field, 2000; Hubbard *et al.*, 1999 & 2001; Brodribb *et al.*, 2002). Further, Schultz and Matthews (1988) observed decreasing plant growth with increasing hydraulic resistance and Vander Willigen and Pammenter (1998) observed increasing growth efficiency (wood growth) with increasing $k_s$ in support of hydraulic limitation hypothesis. So, this result is possibly another documentary proof to support hydraulic limitation hypothesis.

In conclusion, growth and higher dry mass production was observed in high watered seedlings than that of the seedlings subjected to low watering treatment. The genus effect also evident on plant growth that *T. arjuna* achieved higher growth than *B. cordifolia* in both watering treatments. The differences in plant growth were closely related to the hydraulic characteristics of both genus and the effect of watering treatments. Low water supply decreased $k_l$, $k_s$, HV and increase PLC in *T. arjuna*. However, the watering effect on the same hydraulic parameters was not remarkable on *B. cordifolia*. The watering effect also seemed to have altered the xylem vessel diameter and in turn influences the hydraulic conductivities. There were positive relationships between vessel diameter and $k_{max}$ and $k_s$ and between $k_l$ and plant dry masses that possibly support the hydraulic limitation hypothesis.

## REFERENCES

Barnard, H. R. and Ryan, M. G. (2003) 'A test of the hydraulic limitation hypothesis in fast-growing *Eucalyptus saligna*', *Plant, Cell and Environment* 26: 1235-1245.

Bray, E. A. (1997) 'Plant responses to water deficit', *Trends in Plant Science Review* 2: 48-54.

Brodribb, T. J. and Field, T. S. (2000) 'Stem hydraulic supply is linked to leaf photosynthetic capacity: evidence from New Caledonian and Tasmanian rainforests', *Plant Cell and Environment* 23: 1381-1388.

Brodribb, T. J., Holbrook, N. M. and Gutierrez, M. V. (2002) 'Hydraulic and photosynthetic co-ordination in seasonally dry tropical forest trees', *Plant, Cell and Environment* 25: 1435-1444.

Cochard, G., Cruiziat, P. and Tyree, M. T. (1992) 'Use of positive pressures to establish vulnerability curves: Further support for the air-seeding hypothesis and possible problems for pressure-volume analysis', *Plant Physiology* 100: 205-209.

**Cochard, H., Breda, N. and Granier, A.** (1996) 'Whole tree hydraulic conductance and water loss regulation in *Quercus* during drought: evidence for stomatal control of embolism?', *Annales Des Sciences Forestieres* 25: 197-206.

**Davis, W. J and Zhang, J.** (1991) 'Root signals and the regulation of growth and development of plants in drying soil', *Annual Review of Plant Physiology and Molecular Biology* 42: 55-76.

**Hacke, U. and Sauter, J.** (1995) 'Vulnerability of xylem to embolism in relation to leaf water potential and stomatal conductance in *Fagus sylvatica, F. purpurea* and *Populus balsamifera*", Journal *of Experimental Botany* 46: 1177-1183.

**Hubbard, R. M., Bond, B. J. and Ryan, M. G.** (1999) 'Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees', *Tree Physiology* 19: 165-172.

**Hubbard, R. M., Ryan, M. G., Stiller, V. and Sperry, J. S.** (2001) 'Stomatal conductance vary linearly with plant hydraulic conductance in ponderosa pine', *Plant, Cell and Environment* 24: 113-121.

**Hubbard, R. M., Bond, B. J., Senock R. S. and Ryan, M. G.** (2002) 'Effects of branch height on leaf gas exchange, branch hydraulic conductance and branch sap flux in open-grown ponderosa pine', *Tree Physiology* 22: 575-581.

**Jarbeau, J., Ewers, F. W. and Davis, S. D.** (1995) 'The mechanism of water stress-induced embolism in two chaparral shrubs', *Plant, Cell and Environment* 18: 189-196.

**Jones, H. G. and Sutherland, R. A.** (1991) 'Stomatal control of xylem embolism', *Plant, Cell and Environment* 14: 607-612.

**Kavanagh, K. L., Bond, B. J., Aitken, S. N., Gartner, B. and Knowe, S.** (1999) 'Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings', *Tree Physiology* 19: 31-37.

**Khalil, A. A. M. and Grace, J.** (1993) 'Does xylem sap ABA control the stomatal behavior of water-stressed Sycamore (*Acer pseudoplatanus* L.) seedlings', *Journal of Experimental Botany* 44: 1127-1134.

**Kramer, P. J. and Boyer, J. S.** (1995) *Water relations of plants and soils.* San Diego, CA: Academic Press, 20, 139-140, 216 & 234.

**Li, C., Berninger, F., Koskela, J. and Sonninen, E.** (2000) 'Drought responses of *Eucalyptus microtheca* provencances depend on seasonality of rainfall in their place of origin', *Australian Journal of Plant Physiology* 27: 231-238.

**Lovisolo, C. and Schubert, A.** (1998) 'Effects of water stress on vessel size and xylem hydraulic conductivity in *Vitis vinifera* L.', *Journal of Experimental Botany* 49: 693-700.**Makela, A., Berninger, F. and Hari, P.** (1996) 'Optimal control of gas exchange during drought: theoretical analysis', Annals *of Botany* 77: 461-467.

**McDowell, N.G., Phillips, N., Lunch, C., Bond, B. J. and Ryan, M. G.** (2002) 'An investigation of hydraulic limitation and compensation in large, old Douglas-fir trees', *Tree Physiology* 22: 763-774.

**Meinzer, F. C. and Grantz, D. A.** (1990) 'Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustment to water transport capacity', *Plant, Cell and Environment* 13: 383-388.

Meinzer, F. C., Goldstein, G., Jackson, P., Holbrook, N. M., Gutierrez, M. V. and Cavelier, J. (1995) 'Environment and physiological regulation of transpiration in tropical forest gap species: the influence of boundary layer and hydraulic properties', *Oecologia* 101: 514-522.Meinzer, F. C., Goldstein, G., Franco, C., Bustamante, M., Igler, E., Jackson, P., Caldas, L. and Rundel, W. (1999) 'Atmospheric and hydraulic limitations on transpiration in Brazilian cerrodo woody species', *Functional Ecology* 13: 273-282.

Moreshet, S., Cohen, Y., Green, G. C. and Fuchs, M. (1990) 'The partitioning of hydraulic conductances within mature orange trees', *Journal of Experimental Botany* 41: 833-839.

Munne-Bosch, S., Jubany-Mari, T. and Alegre, L. (2003) 'Enhanced photo- and antioxidative protection, and hydrogen peroxide accumulation in drought-stressed *Cistus clusii* and *Cistus albidus* plants', *Tree Physiology* 23: 1-12.

Nardini, A. and Tyree, M. T. (1999) 'Root and shoot hydraulic conductance of seven *Quercus* species', *Annals of Forest Science* 56: 371-377.

Nardini, A. and Salleo, S. (2000) 'Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation?', *Trees* 15: 14-24.

Nardini, A. (2001) 'Are Sclerophylls and Malacophylls hydraulically different?', *Biologia Plantarum* 44: 239-245.

Osorio, J., Osorio, M. L., Chaves, M. M. and Pereira, J. S. (1998) 'Water deficits are more important than in changing patterns of carbon allocation in *Eucalyptus globulus*', *Tree Physiology* 18: 363-373.

Phillips, N., Bond, B. J., McDowell, N. G. and Michael, G. (2002) 'Canopy and hydraulic conductance in young, mature and old Douglas-fir tree', *Tree Physiology* 22: 205-211.

Rieger, M., Lo Bianco, R. and Okie, W. R. (2003) 'Response of *Prunus ferganensis*, *Prunus persica* and two interspecific hybrids to moderate drought stress', *Tree Physiology* 23: 51-58.

Rust, S. and Roloff, A. (2002) 'Reduced photosynthesis in old oak (*Quercus robur*): the impact of crown and hydraulic architecture', *Tree Physiology* 22: 597-601.

Ryan, M. G. and Yoder, B. J. (1997) 'Hydraulic limits to tree height and tree growth', *BioScience* 47: 235-242.

Ryan, M. G., Bond, B. J., Law, B. E., Hubbard, R. M., Woodruff, D., Cinenciala, E. and Kucera, J. (2000) 'Transpiration and whole-tree conductance in ponderosa pine trees of different heights', *Oecologia* 124: 553-560.

Salleo, S., Lo Gullo, M. A., De Paoli, D. and Zippo, M. (1996) 'Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism', *New Phytologist* 132: 47-56.

Salleo, S., Nardini, A., Pitt, F. and Lo Gullo, M. A. (2000) 'Xylem cavitation and hydraulic control of stomatal conductance in Laurel (*Laurus nobilis* L.)', *Plant, Cell and Environment* 23: 71-79.

Saliendra, N. Z., Sperry, J. S. and Comstock, J. P. (1995) 'Influence of leaf water status on stomatal response to humidity, hydraulic conductance and soil draught in *Betula occidentalis*', *Planta* 196: 357-366.

Schultz, H. R. and Matthews, M. A. (1988) 'Resistance to water transport in shoots of *Vitis vinifera* L.', *Plant Physiology* 88: 718-724.

Sperry, J. S., Donnelly, J.R. and Tyree, M. T. (1988) 'A method for measuring hydraulic conductivity and embolism in xylem', *Plant, Cell and Environment* 11: 35-40. Sperry, J. S., Alder, N. N. and Eastlack, S. E. (1993) 'The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation', *Journal of Experimental Botany* 44: 1075-1082.

Sperry, J. S. and Pockman, W. T. (1993) 'Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*', *Plant, Cell and Environment* 16: 279 – 287.

Sperry, J. S., Hacke, U. G., Oren, R. and Comstock, J. P. (2002) 'Water deficits and hydraulic limits to leaf water supply', *Plant, Cell and Environment* 25: 251-267.

Stohr, A. and Losch, R. (2004) 'Xylem sap flow and drought stress of *Fraxinus excelsior* spalings', *Tree Physiology* 24: 169-180.

Tardieu, F. and Davies, W. J. (1993) 'Integration of hydraulic and chemical signaling in the control of stomatal conductance and water status of droughted plants', *Plant, Cell and Environment* 16: 341-349.

Tognetti, R., Raschi, A., Beres, C., Fenyves, A. and Ridder, H. (1996) 'Comparison of sap flow, cavitation and water status of *Quercus petraea* and *Quercus cerris* trees with special reference to computer tomography', *Plant, Cell and Environment* 19: 928-938.

Trifilo, P., Raimonds, F., Nardini, A., Lo Gullo, M. A. and Salleo, S. (2004) 'Drought resistance of *Ailanthus altissima*: root hydraulics and water relations', *Tree Physiology* 24: 107-114.

Tyree, M. T., Fiscus, E. L., Wullschleger, S. D. and Dixon, M. A. (1986) 'Detection of xylem cavitation in corn under field conditions', *Plant Physiology* 82: 597-599.

Tyree, M.T. and Sperry, J. S. (1988) 'Do plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress?', *Plant Physiology* 88: 574-580.

Tyree, M. T. and Sperry, J. S. (1989) 'Vulnerability of xylem to cavitation and embolism', *Annual Review of Plant Physiology and Plant Molecular Biology* 40: 19-38.

Tyree, M. T. and Ewers, F. W. (1991) 'The hydraulic architecture of trees and other woody plants', *New Phytologist* 119: 345-360.

Tyree, M. T., Vargas, G., Engelbrecht, B. M. J. and Kursar, T. A. (2002) 'Drought until death do us part: a case study of the desiccation-tolerance of a tropical moist forest seedling-tree, *Licania platypus* (Hemsl.) Fritsch', *Journal of Experimental Botany* 53: 2239-2247.

Tyree, M. T., Engelbrecht, B. M. J., Vargas, G. and Kursar, T. A. (2003) 'Desiccation tolerance of five tropical seedlings in Panama. Relationship to a field assessment of drought performance', *Plant Physiology* 132: 1439-1447.

Vander Willigen, C. and Pammenter, N. W. (1998) 'Relationship between growth and xylem hydraulic characteristics of clones of *Eucalyptus* spp'. at contrasting sites', *Tree Physiology* 18: 595-600.

Xu, L. and Baladocchi, D. D. (2003) 'Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature', *Tree Physiology* 23: 865-877.

Yoder, B. J., Ryan, M. G., Waring, R. H., Schoettle, A. W. and Kaufmann, M. R. (1994) 'Evidence of reduced photosynthetic rates in old trees' *Forest Science* 40: 513-527.

Zimmermann, M.H. (1983) *Xylem Structure and the Ascent of Sap*. Springer-Verlag, Berlin.