Chapter 4

Chapter 4

Field Experiment: Plant Water Relations of Shrubs on Slopes

4.1 Introduction

Plants are always subjected to harsh environmental conditions on slopes. Water stress would be one of the stress factors that slope plants might very often be subjected to. Due to this stress, studies have found that, *inter alia*, increased stomatal closure concomitant with growth reduction (Davies and Zhang, 1991). In order to improve the growth of the species, it is thought that the species have to be pre-stressed before transferring them to the slopes.

Pre-stressed treatment by the previous studies can be described as applying water stress on plants, either by withholding or suppressing water, for a short period of time (e.g. Eamus, 1987; Bertin and Staudt, 1996). Earlier studies described the classical pre-treatment that was given to tropical grasses and legumes, had caused low stomatal sensitivity of these species to water stress (Ludlow *et al.*, 1985). Thomas *et al.* (1976) also found that pre-treatment induced stomatal conductance to increase when the plant were subjected to drought. Previous studies also found the positive effect of pre-treatment on net photosynthesis (e.g. Ludlow and Ng, 1976).

In this experiment, the three species of shrubs selected will be pre-stressed before growing along with legumes and vetiver on slopes. The objective is mainly to investigate the effectiveness of pre-treatment on these species, whether they would be acclimatised in several physiological aspects, including water relations and other gross parameters.

4.2 Materials and Methods

4.2.1 Plant Materials

Three species of shrubs (*L.camara*, *J.betonica* and *T.erecta*) in 60 replications were grown in polybags by stem cutting at Hulu Langat, Selangor. Prevailing conditions (PAR 800 – 1700 $\mu E\ m^{-2}\ s^{-1}$, temperature 28-35°C, daytime humidity 55 – 90%) were maintained by applying water every morning and evening. After 3½ months, all the plants were transferred to the glasshouse, Department of Botany, Universiti of Malaya. Thirty replicates for controlled shrubs were watered twice a day, the other thirty replicates were subjected to water-stress cycles for 10 days (16 October - 25 October 1996). Both treatments were placed in the glasshouse with relative humidity of 70-80%. The physiological measurements were taken during the pre-stressed period (see Table 4.1). The plants were transferred to the slopes on 31st October 1996. All shrubs of both treatments were grown in natural conditions on slopes until December 1997.

4.2.2 Experimental Design

Six plots (@ 6m x 4m) had been designed in such a way to compare the performance of the pre-treated shrubs and that non-pre-treated of control. In all plots, the species were grown along with legumes and vetiver (see Table 4.2).

4.2.3 Measurements

Photosynthesis, transpiration rates, stomatal conductance, WUE, leaf area and biomass were measured as mentioned earlier (2.2.2a, 2.2.2d, 2.2.2e and 3.2.3c, respectively.)

Table 4.1: The physiological parameters studied during the pre-stressed treatment (n=10)

Parameters	***L.camara***	***J.betonica***	***T.erecta***
Stomatal conductance (cm s^{-1})	0.06-0.19	0.013-0.07	0.14-0.25
Water potential (Bar)	-0.94 to –0.70	-0.58 to –0.48	-1.4 to –0.66
Relative Water Content (%)	52.0-63.2	71.2-77.3	54.0-60.4

Table 4.2 : Description of the experimental plot

PLOT	SPECIES	TREATMENT	SPACING
PLOT 1	*Lantana camara*	pre-treated	40 cm
PLOT 2	*Justicia betonica*	pre-treated	40 cm
PLOT 3	*Thunbergia erecta*	pre-treated	40 cm
PLOT 4	*Lantana camara*	control	40 cm
PLOT 5	*Justicia betonica*	control	40 cm
PLOT 6	*Thunbergia erecta*	control	40 cm

4.3 Results and Discussion

4.3.1 Water Relations of the Shrubs

(a) Photosynthesis Rate

Numerous studies have claimed the effect of water deficits on photosynthesis due to stomatal closure in order to conserve water in plant (e.g. Bradford and Hsiao, 1982; Wise *et al.*, 1990). However, this was not the case in all the pre-treated shrubs studied. All the pre-treated shrubs showed significantly high photosynthesis rate compared to the control. Photosynthesis rate of pre-treated *L.camara* was more than twice that of control (Table 4.3). This is similar to the findings of Irigoyen *et al.* (1996) that the pre-drought treatment had increased the assimilation rate of pre-droughted maize during the recovery. It was assumed that photosynthesis was affected by the short water-stress in the glass house (before transferring to the slopes) due to very low stomatal conductance. On the experimental slope, the pre-stressed plants showed positive stomatal behaviour which is one of the adaptive mechanisms (Fig. 4.1). As a result, the pre-treated shrubs performed better than the control.

(b) Transpiration Rate

Transpiration rates of pre-treated *L.camara* and *T.erecta* was much higher than the control (Table 4.3). The results can be attributed to the maximal root system of the pre-treated species established during the pre-treatment. It can then be argued that the observed higher water absorption capacity might contribute to high transpiration rate. Moreover, in the case of pre-treated *T.erecta*, high leaf area may contribute to high transpiration rate per plant (Fig. 4.2), an important criterion for a slope plant.

Table 4.3 : Comparison of performance of the shrubs in controlled and pre-treated shrubs in December 1997. Each value represents the mean of 6-10 replications.

(a) *Lantana camara*

Parameter	Control	Pre-treated	F-value
Photosynthesis rate (μmol m^{-2} s^{-1})	6.76 ± 0.72	16.40 ± 0.62	729.6 ***
Transpiration rate (mmol m^{-2} s^{-1})	2.84 ± 0.34	4.47 ± 0.43	62.6 ***
Stomatal conductance (cm s^{-1})	4.17 ± 0.85	4.26 ± 0.72	N.S.
WUE	2.39	3.71	40.7 ***

(b) *Justicia betonica*

Parameter	Control	Pre-treated	F-value
Photosynthesis rate (μmol m^{-2} s^{-1})	8.95 ± 0.51	15.94 ± 1.02	189.5 ***
Transpiration rate (mmol m^{-2} s^{-1})	3.58 ± 0.46	4.06 ± 0.43	N.S.
Stomatal conductance (cm s^{-1})	2.35 ± 0.48	4.27 ± 0.75	18.95 **
WUE	2.54	3.99	18.8 **

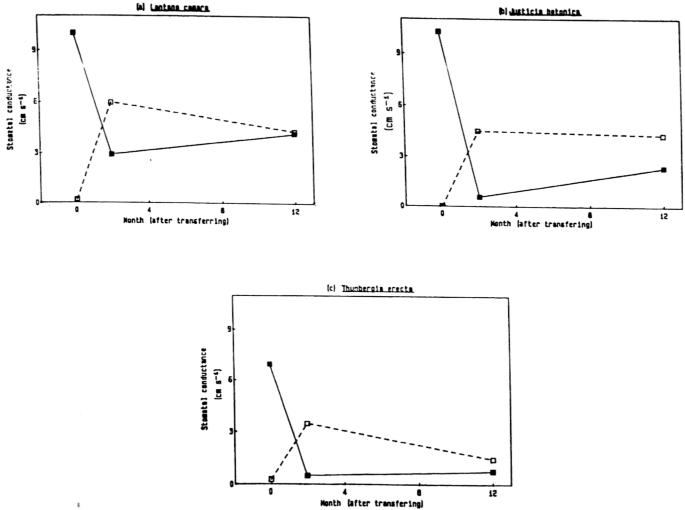
(c) *Thunbergia erecta*

Parameter	Control	Pre-treated	F-value
Photosynthesis rate (μmol m^{-2} s^{-1})	7.60 ± 0.74	9.60 ± 1.3	12.5 **
Transpiration rate (mmol m^{-2} s^{-1})	3.14 ± 0.45	3.64 ± 0.06	8.58 *
Stomatal conductance (cm s^{-1})	0.77 ± 0.13	1.44 ± 0.28	23.66 ***
WUE	2.46	2.64	N.S.

*** Significant at P = 0.001, ** Significant at P = 0.01, * Significant at P = 0.05, N.S. = Not Significant

Fig. 4.1 : Comparison of stomatal conductance between (■) controlled and (□) pre-treated shrubs. Each point represents the mean of 10 determinations. The value "zero month" was taken as the day of planting plants on the experimental slope.

Fig.4.1: Stomatal conductance of the shrubs studied



Despite the reduction of leaf area in pre-treated plant, *J.betonica* showed similar transpiration rates in both treatments. This implies that on per plant basis, transpiration rate is higher non pre-treated control. Factor of stomatal pores may attribute to these results in which water loss is proportional to the diameter of the pores rather than the leaf area (Baron, 1967).

(c) Stomatal Conductance

Amazingly, stomatal conductance of pre-treated *J.betonica* and *T.erecta* were significantly higher than the control (Table 4.3). This apparent contradiction with results in the preceeding paragraph may be attributed to different measurement techniques — steady state in the former and transient in the latter. Stomatal conductance of the pre-treated shrubs (after transferring to the slope) was positively increased compared to the controlled ones (Fig. 4.1). This would partly be due to the development of high leaf water potential in the pre-treated shrubs. Mansfield and Davies (1985), for instance, reported that stomatal preconditioning is an important component of drought resistance. In addition, Shultze *et al.* (1986) found that the shrubs which had previously been exposed to a period of water stress would modify the stomatal responses to leaf water potential.

Stomatal conductance of the pre-treated shrubs had positive relationship with leaf water potential (before transferring) (Table 4.3). Hence, leaf water potential was not measured, assuming the increase in stomatal conductance was associated with an increase in leaf water potential. Due to this, the pre-treated plants might maintain turgor potential to a substantial level through osmotic adjustment. Thus, total closure of stomata or uneven closure, patchiness, had not been observed and plant water status had not reached to the levels that substantially would damage the plants tissue. (Bradford and Hsiao, 1982; Sharkey and Seeman, 1989; Chaves, 1991). Cornish and Zeevaart (1985) suggested that this lack of reopening of stomata in the shrubs, following having normal condition on slopes is mediated through increased foliar abscisic acid (ABA) levels or increased partitioning of ABA into apoplast. It can be concluded that, due to the pre-treatment, both species adapted to the harsh condition on slopes that it would exhibit non-conservative behaviour of stomatal closure (Tardieu and Davies, 1993), a positive effect of pre-treatment.

In the case of *L.camara*, there is no positive effect of pre-treatment on stomatal conductance (Table 4.3).

(d) Water Use Efficiency (WUE)

Similar to Irigoyen's finding on pre-treated maize (1996), WUE of pre-treated *L.camara* and *J.betonica* was significantly higher than the control. This is probably because of the drought-induced stomatal closure which caused a greater reduction in transpiration but higher photosynthesis rates. This is one of the drought-tolerance mechanisms observed in the pre-treated plants. The short water-stressed duration prior to the transferring process to the slope may attribute to the adaptation of the plants to the normal conditions on slopes, with low maintenance of water supply and possibly large shift of soil moisture.

However, WUE of *T.erecta* did not show any difference and the results indicate no positive effect of pre-treatment on this species (Table 4.1).

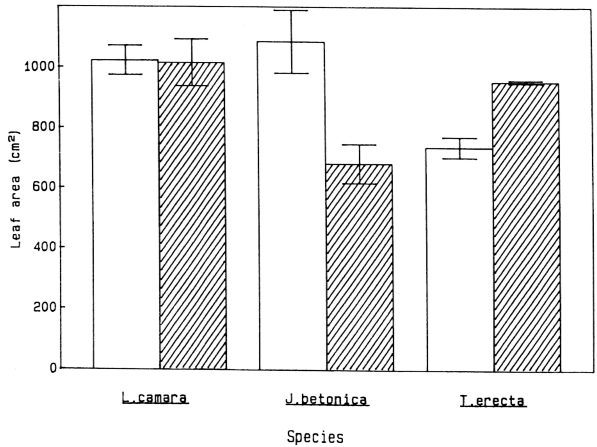
4.3.2 Gross Measurement of the Shrubs

(a) Leaf Area per Plant

Leaf area of pre-treated *J.betonica* was significantly lower compared to that of the control (Fig. 4.2). The results may due to the drought-tolerance mechanism of the species in order to conserve water by reducing leaf area (e.g. Jones, 1992). However,

Fig. 4.2 : Comparison of leaf area per plant of the shrubs studied between (□) controlled and (▨) pre-treated shrubs. Each point represents the mean of 10 determinants. Vertical line on bar represents value of standard deviation.

Fig.4.2: Leaf area per plant of the shrubs studied in both treatments



T.erecta showed the positive effect of the pre-treatment when the pre-treated plants had more leaves than the control. Moreover, results showed that stomatal conductance of its treated plants was significantly higher compared to the control (Table 4.3). Studies have found that stomatal aperture is generally proportional to the turgor pressure inside the guard cells and it has been described as a turgor-operate valve. Subsequently, it affects the leaf area development as do turgor pressure on leaf area expansion (Feng *et al.*, 1994). Whereas *L.camara* showed no response to the pre-treatment. This would probably be due to the stressed gene developed during the pre-treatment did not get switched on during the recovery period on slopes. Thus, some physiological activities were not effected and exhibited no response.

(b) Dry Weight Partitioning and Above Ground Biomass

L.camara and *J.betonica* showed lower stem dry weight in its pre-treated plant (Table 4.4). Same response was observed in the leaf weight of the pre-treated *J.betonica*. The results also show that the biomass of all three shrub species was much influenced by the pre-treatment, with control non pre-treated plants having as much as twice that of the pre-treated plants in *L.camara* and *J.betonica* (Fig. 4.3). Studies have shown that total biomass decreased under dry condition (e.g. Kramer, 1983). The results obtained in the current experiment may attribute to the adaptive feature of high root to shoot ratios of the pre-treated shrubs presumably to maximise the water uptake in order to survive on slopes (Jones and Zur, 1984). In addition, due to the pre-stressed condition, the carbon partitioning to the roots of the plant might have been favoured as a function of drought-tolerance mechanism (Finn and Brun, 1980).

In contrast, *T.erecta* had higher dry weight in its pre-treated shoot (leaf and stem) and biomass of pre-treated *T.erecta* was twice that of the control. Unlike other species, *T.erecta* was not sensitive towards low plant water in which it possibly developed lower root to shoot ratios due to the pre-stressed conditions. The pre-treated condition

Table 4.4 : Dry weight partitioning of the shrubs studied in the two treatments.

Species	Leaf (g)		Stem (g)		Flower (g)		Total biomass (g)	
	Control	Pre-treated	Control	Pre-treated	Control	Pre-treated	Control	Pre-treated
L.camara	7.80 ± 1.5	6.00 ± 0.38	16.83 ± 2.14	5.97 ± 0.65	0.31 ± 0.03	0.62 ± 0.04	24.93 ± 0.97	12.59 ± 0.96
	(N.S.)		(7.3 **)		(0 ***)		(4.43 ***)	
J.betonica	4.73 ± 0.17	2.45 ± 0.34	11.24 ± 1.4	5.62 ± 0.81	0.27 ± 0.05	0.81 ± 0.0	16.15 ± 1.3	8.35 ± 0.45
	(1.19 **)		(3.16 **)		(2.43 ***)		(4.48 **)	
T.erecta	2.43 ± 0.16	3.84 ± 0.14	2.45 ± 0.16	4.93 ± 1.1	-		4.88 ± 0.11	8.77 ± 1.1
	(0.65 ***)		(2.15 *)				(2.17 **)	

(F-value)

*** Significant at p=0.001, ** Significant at p=0.01, * Significant at p=0.05, N.S. = Not Significant

Fig.4.3: Biomass of the shrubs studied in both treatments

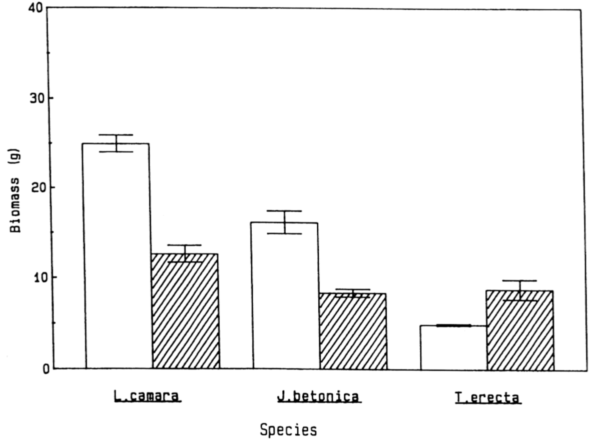


Fig. 4.3 : Comparison of biomass of the shrubs studied between (□) controlled and (▨) pre-treated shrubs. Each point represents the mean of 10 determinations. Vertical line on bar represents standard deviation.

possibly gave positive effects on carbon partitioning in which the carbon reserves were remobilised out of shoot and their roots appeared to be accumulating most of them. Soil water and temperature stress may affect turgor potential and osmotic adjustment in plant growth (Feng *et al.*, 1990)

The flower weight of the pre-treated *L.camara* and *J.betonica* were significantly higher than that in the control. No flower was observed in both treatments of *T.erecta,* arguably because this species is being shaded by other plants.

4.4 General Discussion

Overall results show that there are diverse responses of pre-treatment among the species and the parameters studied (Table 4.5). The observations are probably due to several reasons:

(a) Since these species were grown with other plants on the experimental slopes, diverse responses and competition in terms of whole plant water use and fluctuation in soil water status in the mixed plant communities may occur.

(b) The positive or negative responses of pre-treatment are possibly caused by the stressed gene that developed during the pre-treatment getting switched on. The so-called "chemical" signalling mediated through phytohormone may influence several physiological characteristics discussed earlier. Conversely, the stressed gene presumably do not get switched on during the pre-treatment and this resulted in no response of the species to the pre-treatment.

Apart from some impairments of physiological activities due to the pre-treatment, in terms of plant growth, it can be concluded that *L.camara*, *J.betonica* and *T.erecta* improved even though the mechanisms behind this is not clear.

Table 4.5 : Overall results of plant water relations and gross parameters studies of the pre-treated shrubs as compared to the controlled shrubs. (↑ = significantly higher than the control, ↓ = significantly lower than the control and N.S. = not significant)

Parameters	Species		
	L.camara	***J.betonica***	***T.erecta***
Photosynthesis rate	↑	↑	↑
Transpiration rate	↑	N.S.	↑
Stomatal conductance	N.S.	↑	↑
WUE	↑	↑	N.S.
Leaf area	N.S.	↓	↑
Leaf Dry weight	N.S.	↓	↑
Stem dry weight	↓	↓	↑
Flower dry weight	↑	-	N.S.
Biomass	↓	↓	↑