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WATER RELATIONS, CARBON FIXATION AND GROWTH RATES OF TWO LEAF SUCCULENT SPECIES UNDER DIFFERENT WATER REGIMES

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ABSTRACT

The leaf succulent species R. carolii and R. multiflora are common in the winter rainfall Karoo. Despite their similar growth form, their distribution in Worcester Veld Reserve appeared to differ. R. multiflora occupied a more exposed, arid North - facing slope, while R. carolii dominated a protected South - facing slope. Possible reasons for this distribution were investigated.

Plants of the two species were established in a growth chamber and subjected to different water regimes. The diurnal patterns of water loss and carbon gain were investigated under optimal and drought conditions after 30, 50 and 60 days of treatment.

Gas exchange data of both species showed that C3 photosynthesis was adopted when the plants were irrigated regularly, but also tended to accumulate malate, which indicated the activity of RuBPc and PEPc. R. carolii had higher photosynthetic and growth rates than R. multiflora under optimal conditions. This explained the dominance of R. carolii on the South - facing, protected slope. Under moderate water stress (after 30 days), the species behaved similarly. There was a tendency towards CAM activity, though results were inconclusive. During periods of extreme water shortage, R. carolii exhibited limited stomatal response and thus appeared to be dying, while R. multiflora adopted day time stomatal opening and CO_2 uptake. This strategy and in general the high tolerance to water stress shown by R. multiflora allowed it to outcompete R. carolii on the arid, exposed, North - facing slope.

INTRODUCTION

Ruschia carolii and Ruschia multiflora are two leaf succulent species common in the Winter rainfall Karoo. These species both occurred in the Worcester Veld Reserve (Cape Province, South Africa), but their observed distribution differed.

Ruschia multiflora appeared to occupy a more exposed North - facing slope in the reserve while Ruschia carolii though present on this slope, tended to favour the more sheltered South - facing slope where it dominated.

The aim of this study was to investigate what determined the success of Ruschia multiflora on the North slope and not Ruschia carolii. The reasons for dominance of Ruschia carolii on the protected South slope were also investigated.

The genus Ruschia has approximately 350 species and belongs to the family Mesembryanthemaceae (Herre 1971). This genus occurs in SWA/Namibia, the Cape Province, Orange Free State, parts of the Transvaal and Lesotho.

From ecophysiological studies undertaken by von Willert (1978, 1984, 1985) in the Namib Desert, it was shown that many of the species in this family are Crassulacean Acid Metabolism (CAM) plants. CAM is a term applied to the unique sequence of carbon uptake and acid metabolism in some succulents. It is characterised by diurnal fluctuations in tissue acidity and nocturnal stomatal opening (Nobel and Hartsock 1976, Kluge and Ting 1978). Many succulents in arid and semiarid regions are CAM plants. Since these plants have to cope with extreme heat and desiccation, yet sustain a net carbon dioxide (CO_2) uptake the

desiccation, yet sustain a net carbon dioxide (CO2) uptake the advantage of nocturnal stomatal opening becomes evident. The key to CAM is the ' succulence ' of the cells, brought about by a large central vacuole (Kluge and Ting 1978). Nearly all CO2 uptake by these plants occurs at night. The CO2 is incorporated into organic acids, such as malate which is stored in the large vacuoles of the chlorenchyma cells. During the daytime, the organic acids released from the vacuoles are decarboxylated resulting in a decrease in tissue acidity. The CO2 released in the chlorenchyma during the daytime is prevented from leaving the plant by the closing of the stomata and it is then fixed into carbohydrates and other photosynthetic products by the conventional C3 pathway (Neales et al. 1968, Kluge and Ting 1978, Nobel 1985). Stomatal opening during the cool nights minimizes transpirational water loss, since the tissue temperatures are lower and hence, the water vapour concentration gradients from the leaves to the air are considerably less than daytime values (Kluge and Ting 1978).

A number of papers with facultative CAM species have reported on the induction of CAM photosynthesis from C3 photosynthesis during water stress (Ting and Hanscom 1977, Hartsock 1976, Guralnick and Ting 1987). Other researchers have studied some CAM species to ascertain the effects of water stress on the CAM pathway during the shift from CAM to CAM - idling (Szarek et al. 1973, von Willert et al. 1985). CAM - idling is a response to drought in which stomata close, restricting water loss and exogenous CO2 uptake. By recycling organic acids, the plants maintain metabolic

activity and consequently respond rapidly to rainfall (Ting 1985).

The photosynthetic mode of Ruschia carolii and Ruschia multiflora is unknown, though often assumed to be CAM. In this study, I have examined the physiology of these two species under optimal and drought conditions, in the hope of elucidating what photosynthetic mode these plants adopt. From this investigation I hope to explain the distribution differences of these two Ruschia species in the Worcester Veld Reserve.

MATERIALS AND METHODS

Experimental Design

Distribution of Ruschia species:

Fifteen 5m x 2m quadrats were laid out on the North and South facing slopes. In each quadrat, the number of Ruschia carolii and Ruschia multiflora plants were counted. A two-way Chi-square statistical test was used to determine the significance of the distribution.

Collection and establishment of plants:

Twenty plants of each species were collected in the field and planted in 18cm pots containing soil from the same site. Plants were left to stabilise for 6 weeks in a greenhouse and received water once a day. The plants were then transferred to a High Light Intensity Phytotron Unit (Furcold,CT,SA), where they were left to stabilise under new conditions for a further two weeks (Plate I).

In the phytotron unit the plants received 14 hours of ` daylight ´ from 09:30 hours to 23:30 hours at a photon flux density of 1000µmol/m2/s. The bulbs in this chamber consisted of 14 metal halide (400W), 21 sodium H.P. (400W) and 24 incandescent bulbs (150W, 230V).

The temperature was set at 25°C during the ` day ´ and 20° C at ` night ´ and relative humidity at 60%.

Water stress treatment and control:

Ten plants of each species were given 100 ml of water once every third day, these served as the controls (RC+ and RM+). No water was added to the other twenty plants (10 of each species), these comprised the water stress treatment (RC- and RM-) (Plate II).

To ensure that the treatment plants were stressed by the time the first diurnal investigations were undertaken (30 days) a pre - dawn water potential investigation was carried out. The xylem potential of three shoots of each species and each treatment was determined using a (0-90bar) pressure chamber.

Diurnal Investigations after 30, 50 and 60 days of treatment

CAM investigation: Total titratable acidity

Leaf samples were taken every 3 hours from three individuals of each species and treatment, starting at 07:00 hours until 07:00 hours the next morning.

Total titratable acidity was determined using two methods. One procedure followed that of Nalborczyk, La Croix and Hill (1975) (a) and the second, similar to the method used by Smith, Schulte and Nobel 1987) (b).

a) Fresh leaf samples were weighed, ground up, added to 20ml of distilled water and boiled for 5 minutes. The supernatant was decanted and the residue was extracted four more times by blending with 10ml of hot distilled water and filtering through a glass wool filter. Extracts were combined and free

titratable acidity was determined by titrating the extract to pH 8.0 with 0.02N NaOH, using a pH meter 29 (Radiometer, Copenhagen). This procedure extracted in excess of 96% of the total acid in the plant material.

b) Fresh leaf samples were weighed and then frozen in liquid nitrogen. Leaves were ground up fine and 30ml of distilled water was added. This macerate was then titrated against 0.02N NaOH until pH 8.0.

In both methods, acidity was calculated as micro equivalents of acid per gram fresh weight (μeq/g/f.wt).

Gas exchange investigation:

Leaf transpiration and diffusive resistance were measured using a L1 - 1600 steady state porometer. Resistance values were corrected, as a number of assumptions are involved in the direct resistance value of the porometry reading. The correction involved using the formula $R = \frac{Patm - Pleaf}{\text{transpiration rate}} - 0.85$

R =resistance (s/cm) Patm =Psat x RH Psat =water vapour density

RH =relative humidity

Pleaf =water vapour density at the leaf temperature

0.85 =boundary layer resistance

Daily CO2 exchange was only measured after 60 days of treatment. An Infra-Red-Gas-Analyzer (IRGA) LCA-2 was used for these measurements.

A copper-constantan thermocouple was inserted into one of the leaves to measure leaf temperature.

Whole plant water loss per day

Three water stressed and three control plants of both species were used for this investigation. To these 12 potted plants a layer (1cm) of polyurethane beads were laid on the soil surface, this served to reduce water loss from the soil.

Pots were weighed at the same time every day. A decrease in weight was assumed to be the grams of water lost from the plant. A control pot containing only soil and a layer of beads was also watered and weighed every day, to see how much water was lost from the soil alone. By subtracting the average water loss per day from this pot, it was possible to arrive at a more accurate total water loss value for each plant per day.

Growth Analysis

Growth estimate in mm/week:

One shoot on 20 plants was marked where new growth was detected, at the beginning of the experiment. Every second week over a period of six weeks, the length of new shoots and new leaves was measured. After the six week period all initial new growth measurements (day 1) were deducted from the total new growth. This gave a rough estimate of how fast a plant spread in vegetative cover.

Relative Growth Rates:

Five watered plants of each species were monitored for new growth over a period of 10 weeks. At the end of the ten week period all new growth was harvested. Old plant

material was also harvested. The harvested material was then oven dried for 7 days at 80 C. Dry weights were recorded and Relative Growth Rates calculated using the formula:- $RGR=\frac{\log W2-\log W1}{T2-T1}$

W2=total weight (g)
W1=old growth (g)
T2-T1=time period (weeks)

(Hunt 1982).

Plate I : Plants established and monitored in the high light intensity growth chamber (Phytotron Unit)

Plate II : The two species, Ruschia carolii (RC) and Ruschia multiflora (RM).
- = water stressed individuals
+ = watered (control) individuals

RESULTS

The distribution of the two species Ruschia carolii and Ruschia multiflora on the North and South - facing slopes in Worcester Veld Reserve was significantly different (Chi-square $p < 0.01$) (Table 1)

Table 1: Distribution of Ruschia species

SPECIES	MEAN NO. on N-slope + SE	MEAN NO. on S-slope + SE	EXPECTED f
R.carolii	4 + 0.65	24.19 + 2.06	14.09
R.multiflora	15.9 + 1.48	0	7.94

Chi-square value at 0.5% significance level = 7.8794 (99.5% confidence).

Chi-square calculated = 30.35

7.8794 < 30.35 therefore distribution of Ruschia species differs significantly.

Species comparison at 30 days

The xylem potential values of the control and treatment plants differed significantly (t-test, $p<0.01$ for R.carolii and $p<0.001$ for R.multiflora)

Table II: Xylem potentials of the control and treatment plants.

SPECIES	TREATMENT	XYLEM POTENTIAL (bars)
R.carolii	control	-14.6
R.carolii	control	-12.6 mean=13.26+.67
R.carolii	control	-12.6
R.carolii	stressed	-28.4
R.carolii	stressed	-19.6 mean=20.22+3.3
R.carolii	stressed	-23.4
R.multiflora	control	-11.0
R.multiflora	control	-13.2 mean=18.4+3.6
R.multiflora	control	-12.4
R.multiflora	stressed	-27.4 mean=21.3+3.1
R.multiflora	stressed	-23.0

Total Titratable Acidity:

The control plants of both species tend to accumulate acid overnight (Figure 2), though acidity fluctuated more and reached higher levels in R.carolii. The stressed plants of this species showed no typical trend in diurnal acidity pattern, thus no night time accumulation of acid was evidenced (Figure 2) in R.carolii, but was in R.multiflora. Examining the day/night levels, the

R. carolii controls had significantly more acid during the night ($p<0.05$) and while R. multiflora control plants tended to accumulate acid, the difference between day and night levels was not significant (Figure 3). There was clearly no difference in day/night acidities of the stressed R. carolii individuals, while there was in the stressed R. multiflora plants ($p<0.05$).

Stomatal Conductance:

Control plants exhibited greater stomatal conductances than the stressed plants (Figure 4). The stomatal conductance was greater in R. carolii under irrigated conditions. In both species and treatments day time stomatal opening was apparent, in addition though, R. multiflora showed some evidence for nocturnal stomatal opening, (07:00 hours at the start of the diurnal monitoring period and again at the end of this period, from 04:00 hours until 07:00 hours the next morning.

Transpiration:

As for the conductance, transpiration in R. carolii controls were higher than R. multiflora and this was during the day. Stressed plants of both species transpired at much lower rates than the controls (Figure 5) and while R. carolii transpired at these low rates during the day, R. multiflora transpired for a brief period at night. After 10:00 hours there was a decline in transpiration and no further transpiration was recorded for the rest of the day, until 07:00 hours the next morning.

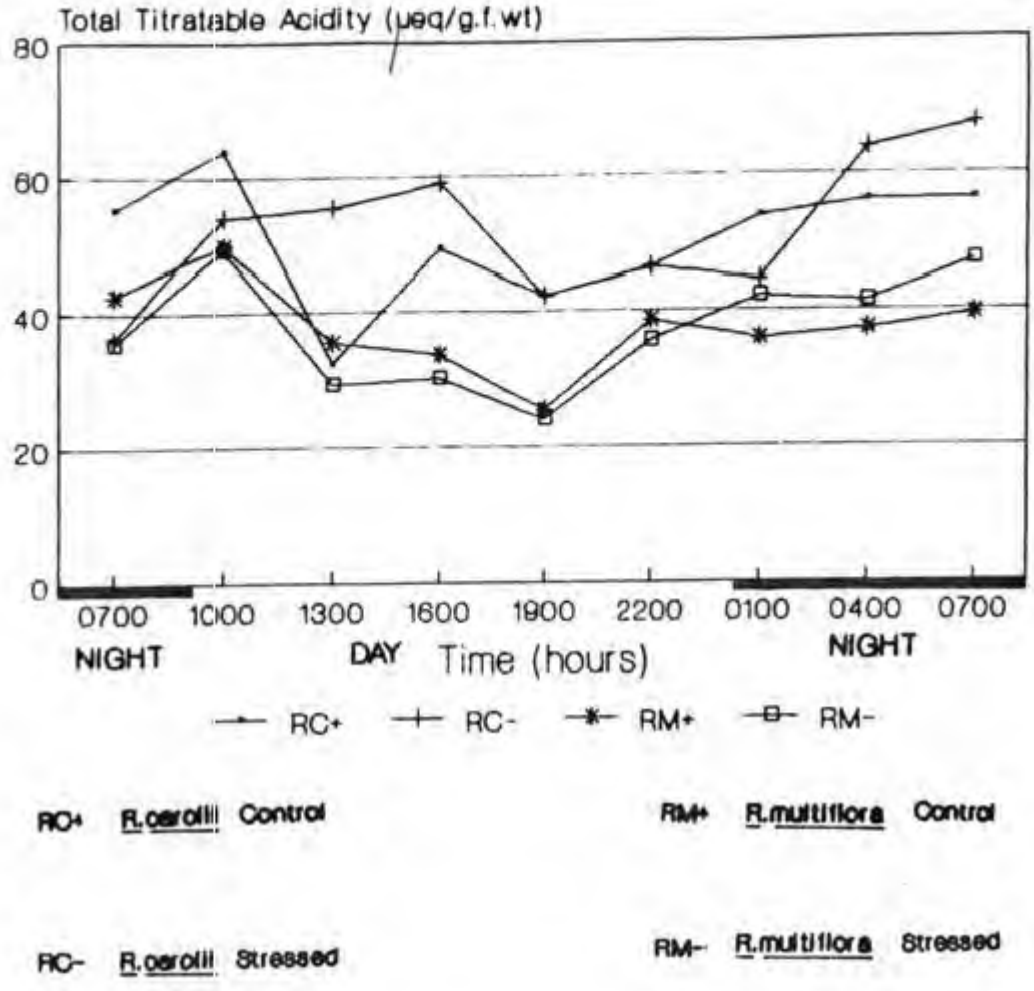

Figure 2: Diurnal Acidity Pattern after 30 Days Stressed and Control Plants

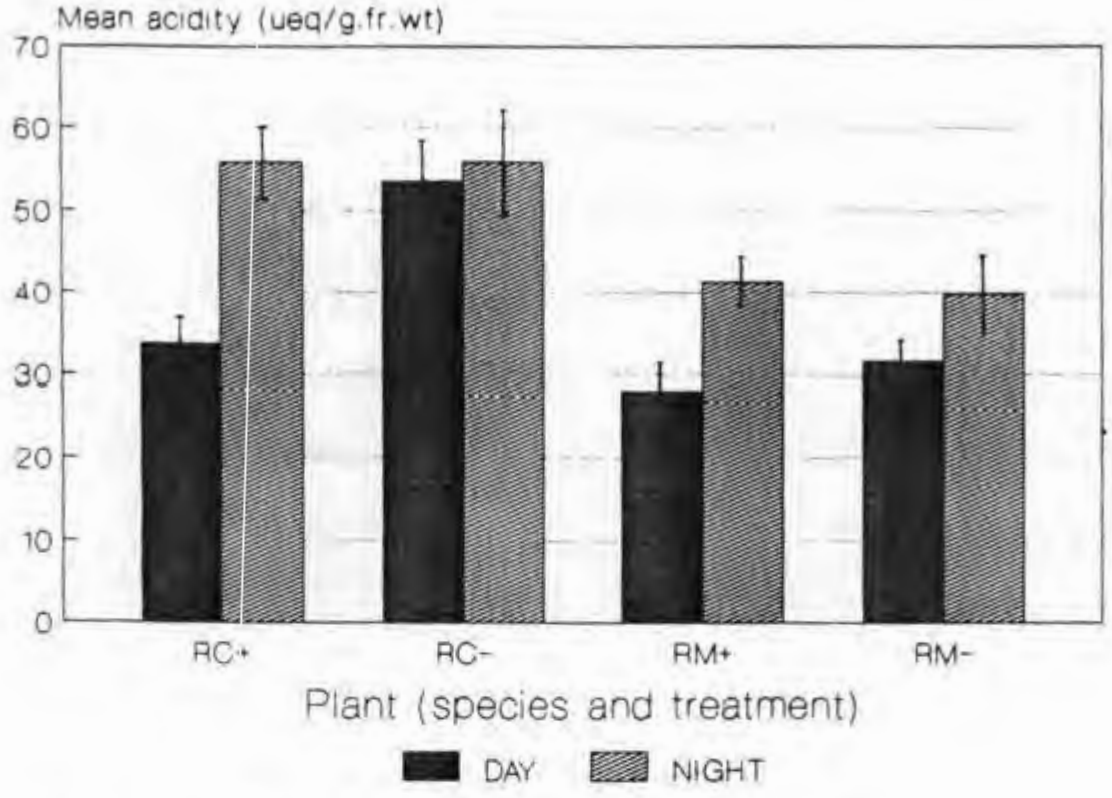

Figure 3: Mean Day/Night Acidity after 30 Days Stressed and Control Plants

Vertical bars represent standard errors. Significant differences found in R.carolii controls (RC+) and R.multiflora stressed (RM+), $p<0.05$ in both cases.

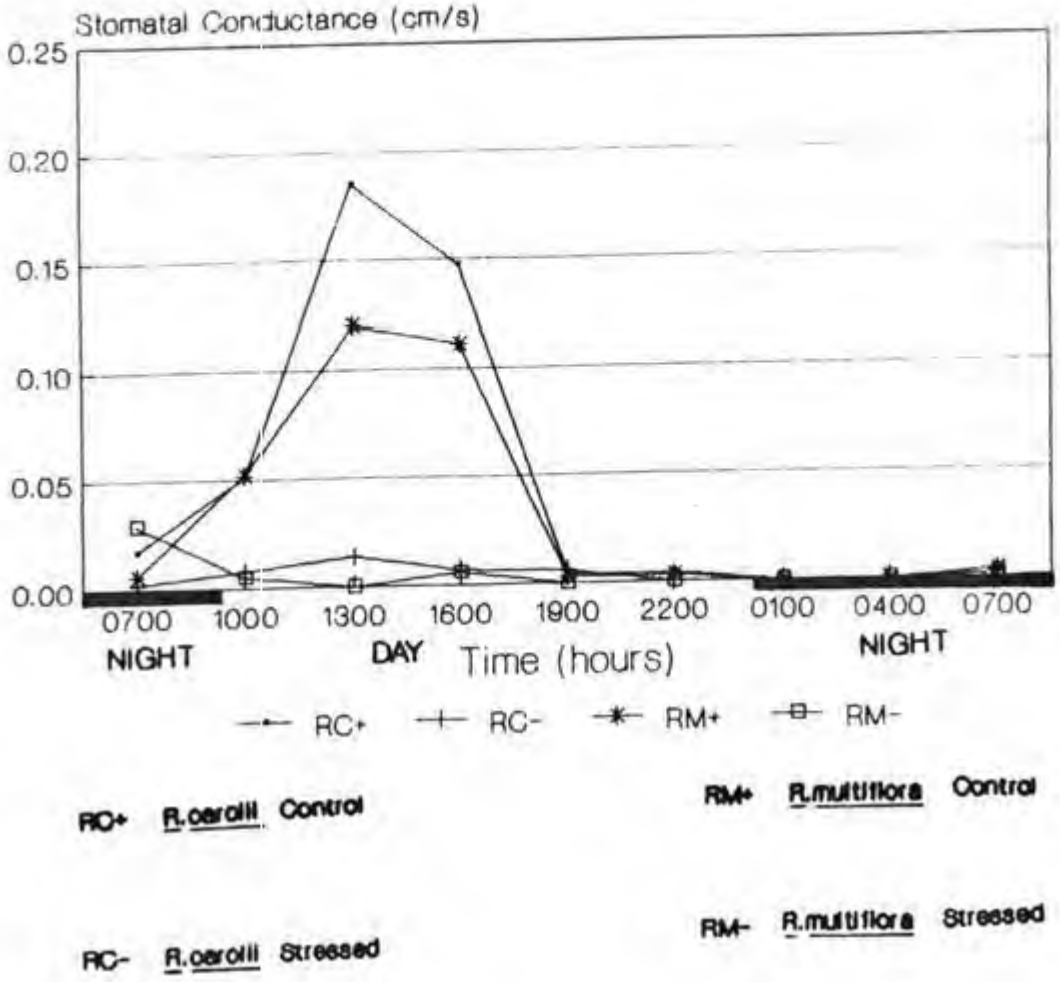

Figure 4: Diurnal Stomatal Conductance after 30 Days Stressed and Control Plants

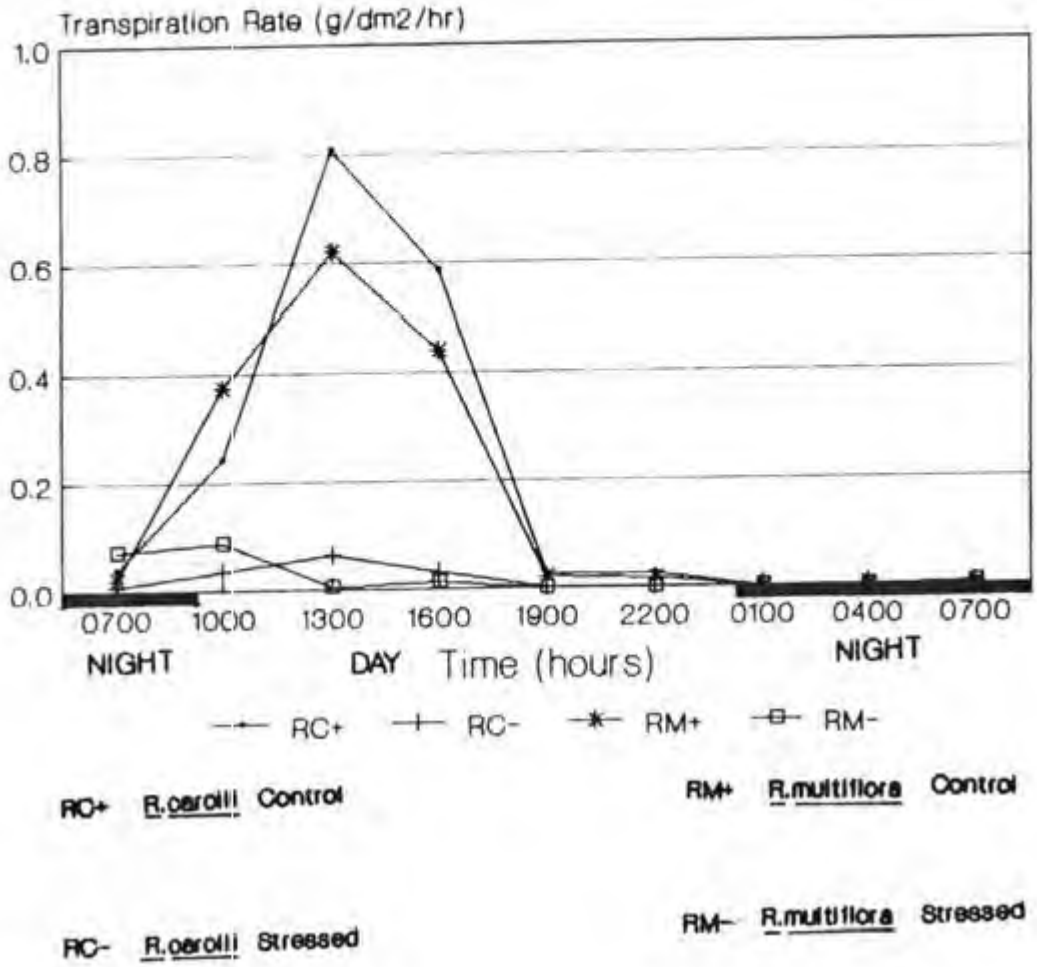

Figure 5: Diurnal Transpiration after 30 Days Stressed and Control Plants

Species comparison at 50 days

Total Titratable Acidity:

No diurnal acidity patterns were evidenced in either species in and in either treatment (Figure 6). Only in the control R.carolii was there a tendency for acids to accumulate at night (Figure 7), though acid levels during the day and night were statistically non-significant (students t-test, $p>0.05$)

Stomatal Conductance:

Both species under irrigation opened their stomata during the day (Figure 8). Conductance values were much lower in the stressed plants than the controls. Stressed R.multiflora individuals had greater stomatal conductances than stressed R.carolii plants and while the latter tended to adopt a strategy of nocturnal stomatal opening, R.multiflora clearly opened it's stomata during the day (Figure 8).

Transpiration:

In comparison to the 30 day results where control R.carolii plants transpired more than R.multiflora controls, at 50 days R.multiflora transpired more (Figure 9). Since conditions remained the same at 30 and 50 days this result is probably due to careless application of water. Stressed R.carolii plants transpired during the night unlike R.carolii controls which transpired at high rates during the day. Nocturnal transpiration in R.carolii stressed individuals was slower than the stressed R.multiflora plants which evidently transpired during the day (Figure 9).

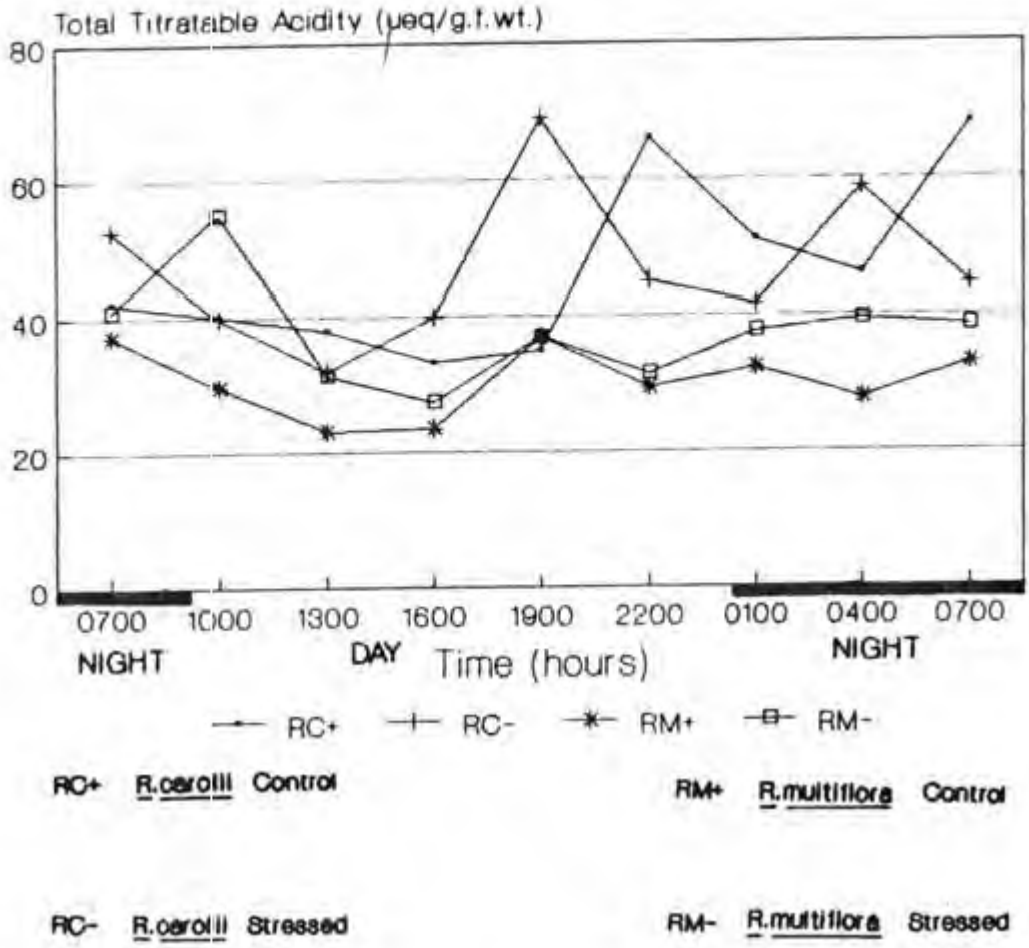

Figure 6: Diurnal Acidity Pattern after 50 Days Stressed and Control Plants

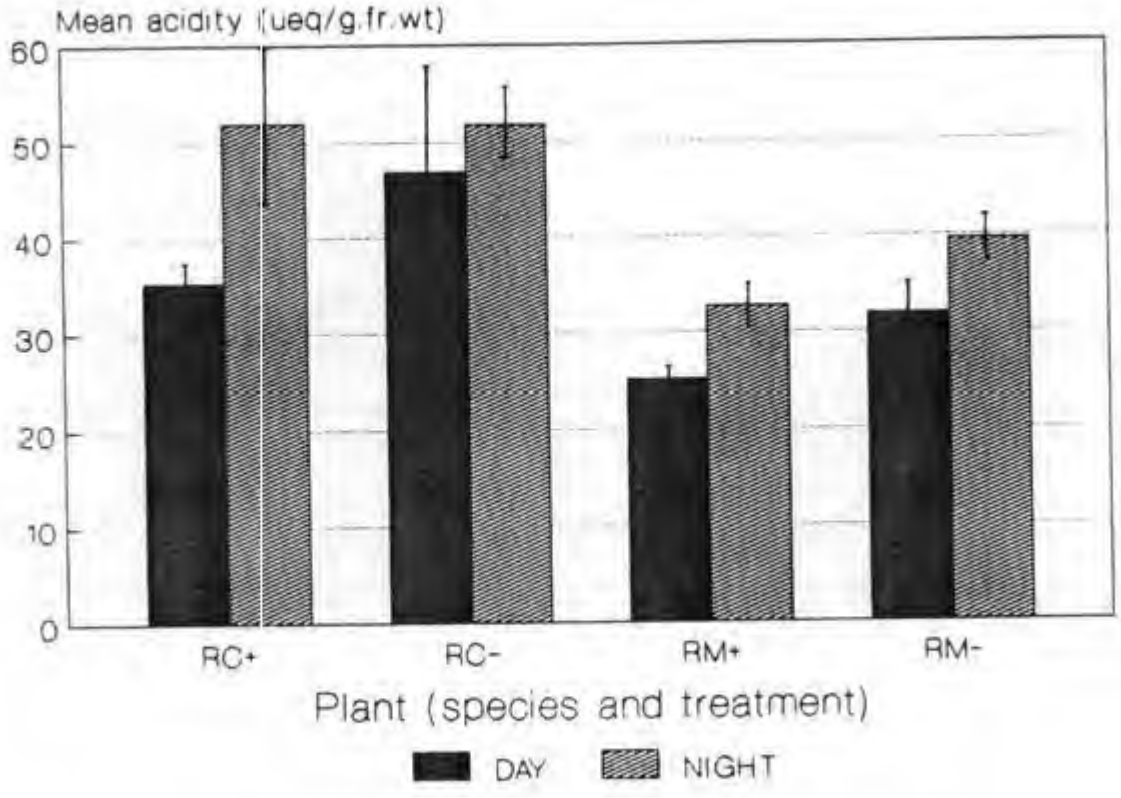

Figure 7: Mean Day/Night Acidity after 50 Days Stressed and Control Plants

Vertical bars represent standard errors. There were no significant differences in mean day/night acidities for either treatment and either species.

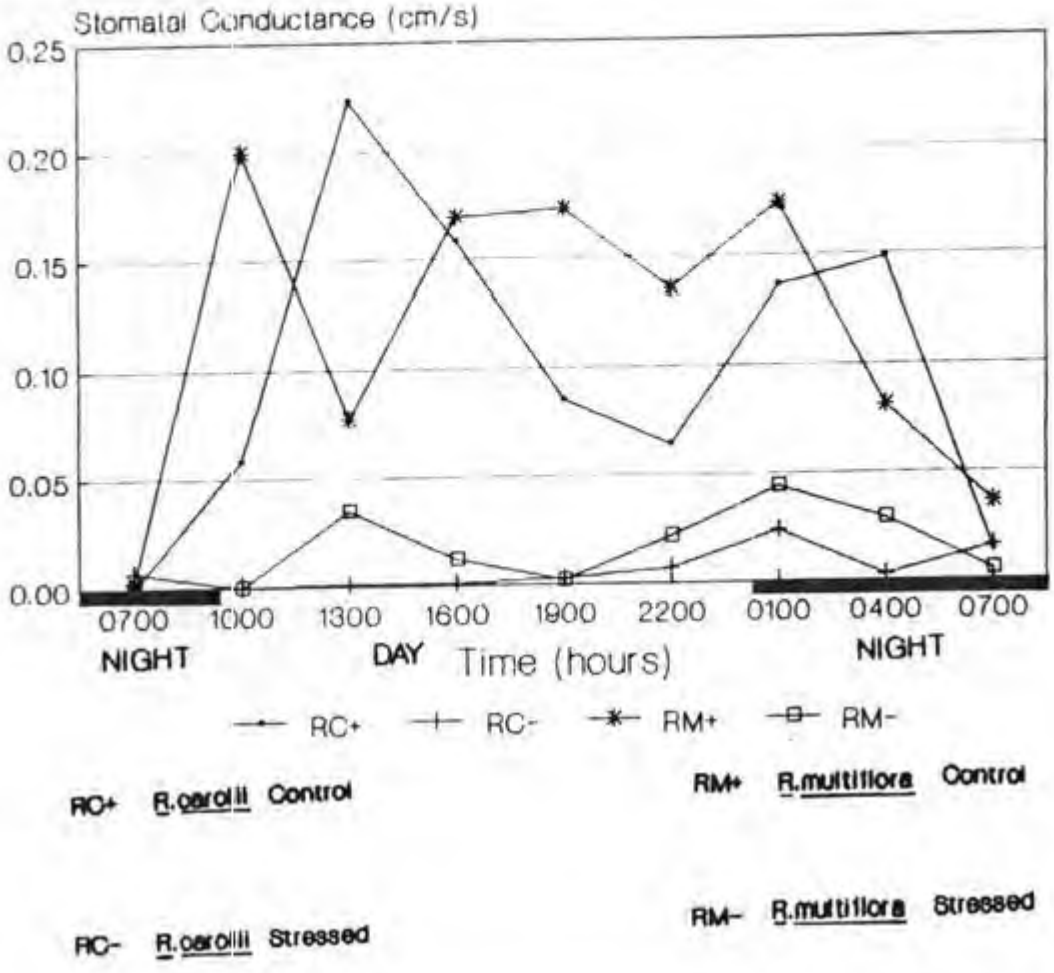

Figure 8: Diurnal Stomatal Conductance after 50 Days Stressed and Control Plants

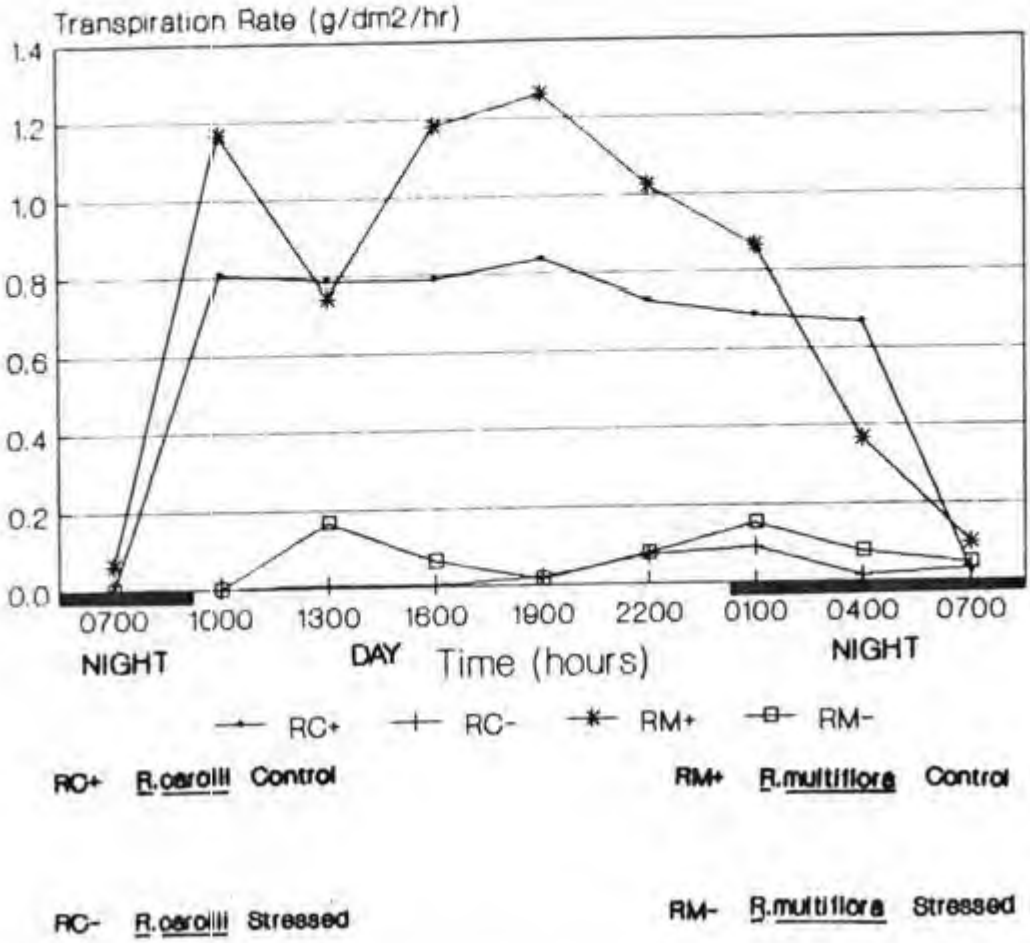

Figure 9: Diurnal Transpiration after 50 Days Stressed and Control Plants

Species comparison at 60 days

Generally, stomatal conductance and transpiration values were higher in R. carolii controls than the R. multiflora controls. The stressed plants of the former were closed for the entire monitoring period, thus no stomatal conductance, transpiration or CO2 uptake was evidenced . The stressed R. multiflora individuals clearly opened their stomata and transpired during the day and also succeeded at taking up CO2 during the day (Fig. 10, 11, 12)

Total Plant Water loss per day

Ruschia multiflora lost significantly more water than Ruschia carolii for both control and stressed individuals ($p<0.05$). Water loss from the watered control pot lost 8.55mg of water per day, so this value was deducted from the water loss value of the control plants. It was assumed that weight decreases in the stressed plants were due to plant water loss only (Table III).

Table III: Water loss/day by the two species

SPECIES + TREATMENT	MEAN H20 LOSS+SE (mg/day)	CORRECTED H20 LOSS(mg/day
R.carolii +	134.7+11.62	126.15
R.carolii −	5.5+2.14	
R.multiflora +	178.96+8.05	170.35
R.multiflora −	7.8+2.67	

Growth Analysis

mm/week:

R. carolii grew faster than R. multiflora ($p<.05$) (Table III).

Table III: Growth rates of the 2 species

SPECIES	MEAN GROWTH RATE mm/week (+SE)	p	RGR g/week (+SE)	p
R.carolii	12.74+2.08	0.01	1.056+.009	0.05
R.multiflora	5.82+0.85		1.022+.042	

RGR = relative growth rate

p = significance level SE = standard error

Relative Growth Rate:

Under optimal conditions, R.carolli had a significantly faster relative growth rate than R.multiflora (student t-test $p<0.05$)

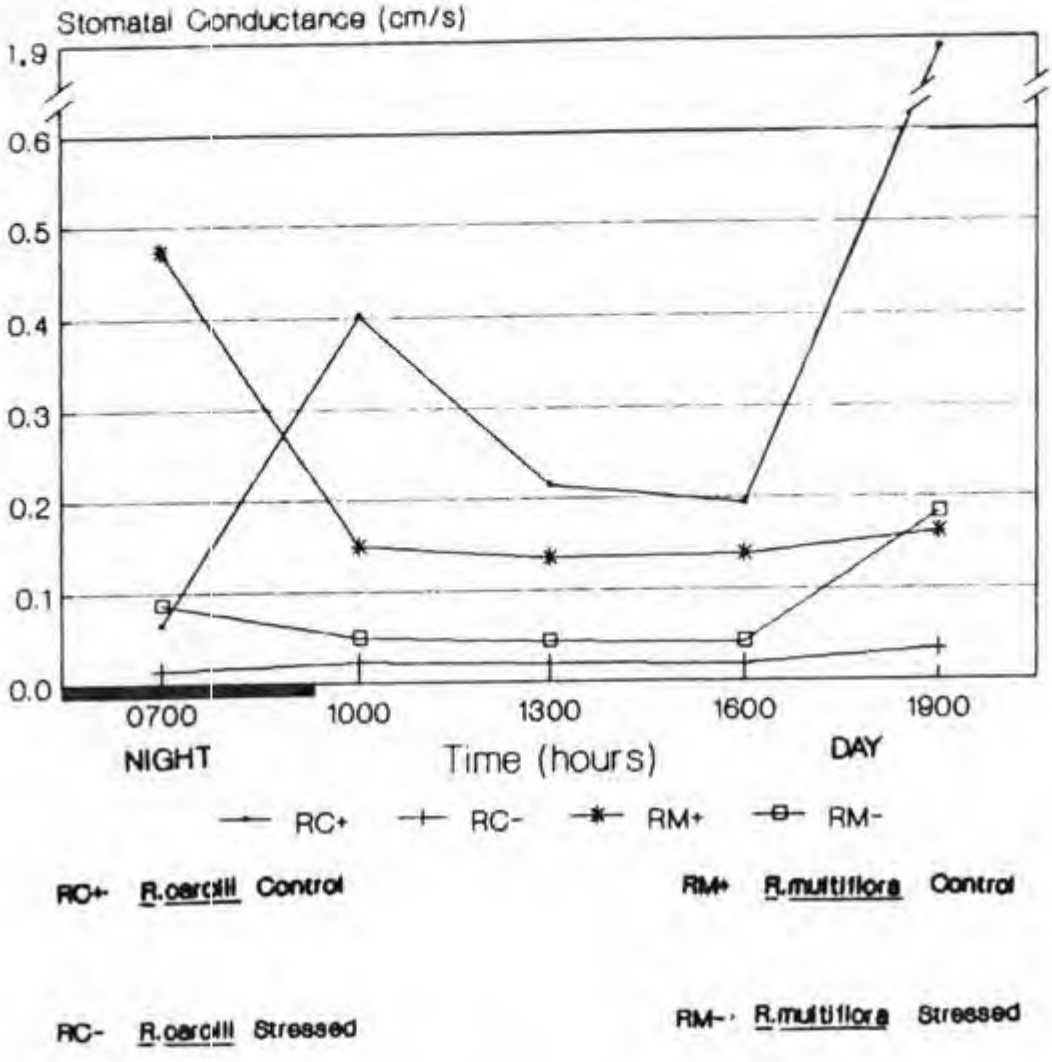

Figure 10: Diurnal Stomatal Conductance after 60 Days Stressed and Control Plants

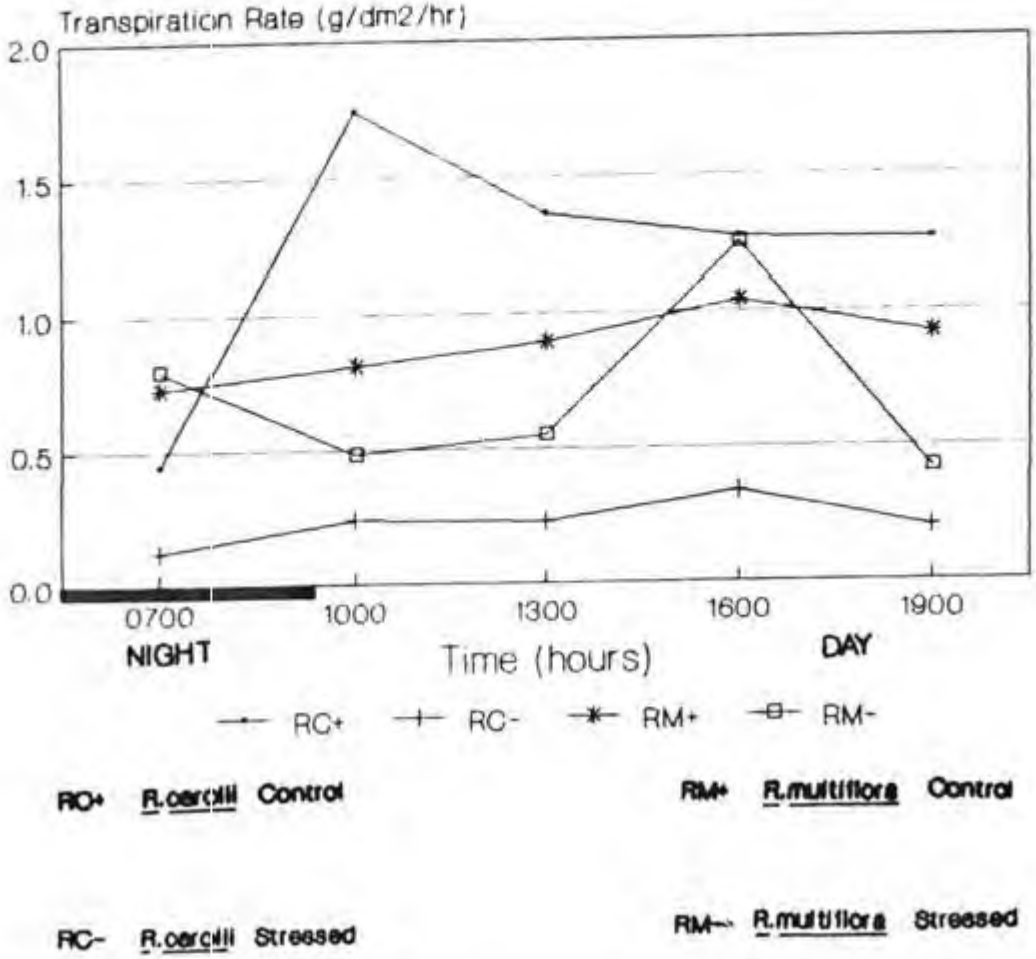

Figure 11: Diurnal Transpiration after 60 Days Stressed and Control Plants

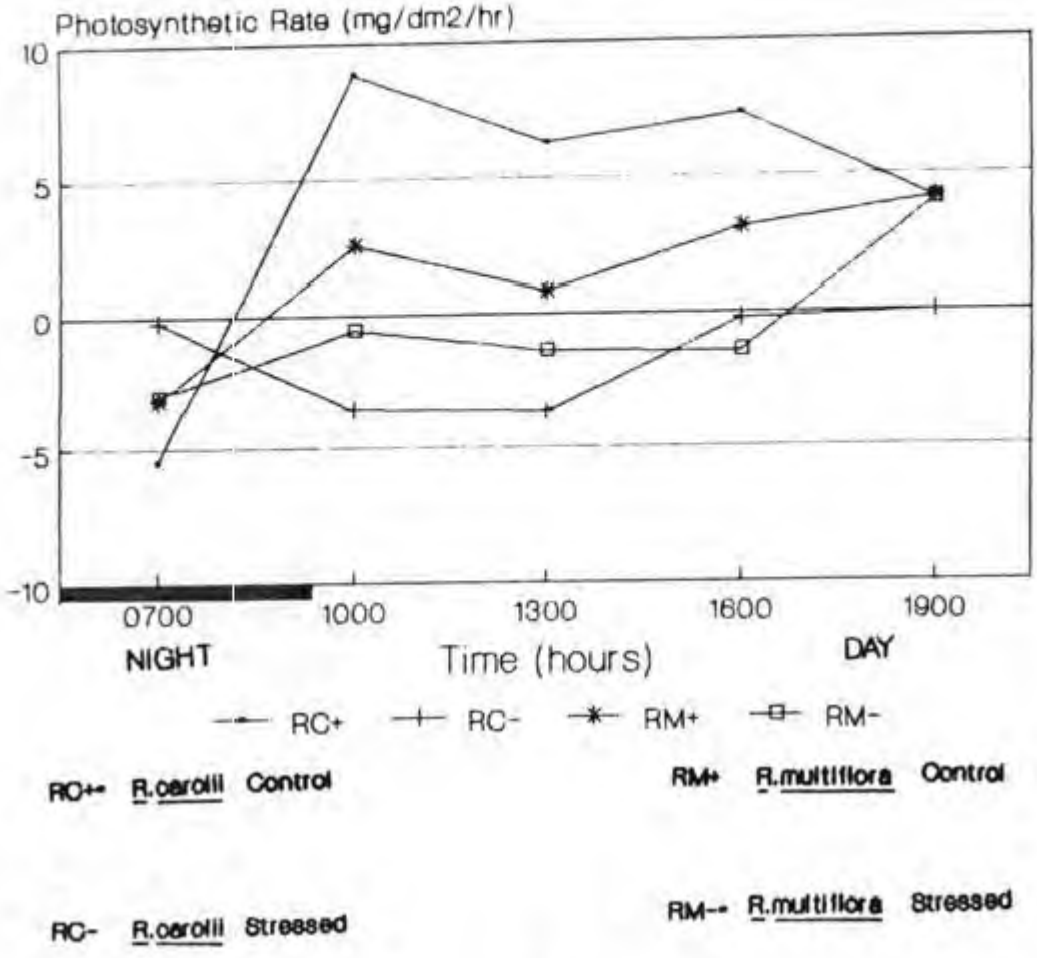

Figure 12: Diurnal Carbon Dioxide Uptake after 60 Days Stressed and Control Plants

DISCUSSION

The gas exchange data of both species showed that C3 photosynthesis was the metabolic pathway followed when the plants were irrigated regularly. Though control R.carolii plants exhibited acid accumulation at night, there was no evidence of night time stomatal opening. The diurnal fluctuations in acidity and higher nocturnal acid levels could possibly have reflected the ability of this species to store organic acids, such as malate. Clearly, CO2 uptake occurred during the day when the stomata were open, however, exogenous CO2 taken up was either utilized immediately in C3 photosynthesis or converted to organic acids and temporarily stored. Inferring from the fluctuation in acidity i.e. not following a particular diurnal trend, these acids were utilized during the day and night, though perhaps more so in the day. This irregularity was probably influenced by the rate of CO2 uptake at any particular time. I suggest therefore that excess CO2 not immediately entering the C3 cycle and being converted to Phosphoglyceric acid was possibly shunted off to the vacuole after malate synthesis had occurred. The rapid and high rates of CO2 uptake evidenced by this species under irrigation lends some support to this suggestion. Some C3 plants in the Richtersveld have also been found to accumulate organic acids (von Willert, Brinckmann, Eller and Scheitler 1984). The acidity levels also fluctuated considerably during the day in their study. Though von Willert et.al (1984) could not draw any conclusions from this study, since the role of malate in C3 plants was unknown, it was suggested that photosynthetic CO2

fixation of those C3 plants was not restricted to the action of RuBPc (ribulose bisphosphate carboxylase) but also occurred via PEPc (phosphoenol pyruvate carboxylase). Total carbon fixation by R.carolii under irrigation is high and though no conclusive statements can be made, it is apparent from this study that water has a role to play in this acid (malate) accumulation. With moderate water stress (30 days) stomata still opened during the day but conductance values were low and this must have caused CO2 uptake to decline as well. Malate synthesis did occur and levels were unexpectedly similar to those of the irrigated plants. From this one can infer that most CO2 taken up was synthesized into organic acids and not fed into the C3 cycle indicating greater PEPc activity. With such a reduced stomatal conductance and therefore CO2 uptake during stress, the similarity in acid levels between control and stressed plants cannot otherwise be explained.

Water stress seems to have reduced night time acid accmulation (day/night levels similar) despite the brief periods of nocturnal stomatal opening which would allow CO2 uptake. Von Willert et al (1985) also yielded a similar result to this and suggested that the decline in malate was related to the water storage or leaf water content of the plants. The irrigated and moderately stressed plants seemed to follow the same metabolic pathway but stressed plants (i.e.at 50 days) tended to adopt some form of CAM where nocturnal stomatal opening occurred, when most CO2 is taken up and converted to malate. A decreasing day time acidity pattern was not evident in these plants however because stomata

opened briefly during the day as well . Decarboxylation of malate that was synthesized at night must have occurred during the day but was not detected since malate was also synthesized in the day. The effectiveness of PEPc in trapping CO2 is thus evidenced. These results after 50 days of stress further indicate that PEPc is more active than RuBPc. Though no diurnal acidity investigations were carried out after 60 days of stress, one can infer from the infra-red gas analyser results as to what possibly occurred. Stomatal conductance was near zero and no CO2 uptake was evidenced throughout the monitoring period neither in the day nor night. CO2 was clearly lost through respiration. The ability to trap any CO2 that might escape in respiration was obviously lost. Malate synthesis probably stopped since there was no substrate (i.e.CO2) for it's formation. As leaf water storage was further impaired after 60 days one can assume that malate synthesis was also hindered because of this. PEPc activity was presumably also affected.

Ruschia carolii under water stress conditions exhibited one of the characteristics of CAM (nocturnal stomatal opening), yet the acidity pattern typical of CAM was not evidenced. I suggest that further ecophysiological investigations are carried out since I believe a potential for CAM exists in this species.

This study was undertaken in a controlled chamber thus the artificiality of this must be emphasised. In the field temperature, relative humidity and irradiance changes gradually as the day progresses but this was abrupt in the chamber. Night time temperatures are much lower than 20°C in the field, relative humidity constantly changes and reaches very low levels in the

day but the chambers' relative humidity was kept at 60%. It is likely that these artificial conditions affected the normal diurnal plant behaviour. The high night time temperature (20 ^{0} C) must surely have affected CAM activity. Von Willert et.al (1985) mentioned the importance of temperature, humidity and thus the vapour pressure deficit in permitting CAM activty. With a high VPD during the night, stomata may be prevented or restricted in opening. This would limit nocturnal CO2 uptake and thus malate synthesis.

If CAM activity does occur, it is probably induced by water stress, since the irrigated plants clearly exhibit day time CO2 uptake. This ability is restricted with drought conditions (60 day data) as there was very little stomatal response and no CO2 uptake during the day or night. The plants were either dying or in a state of ' suspended life ', which upon watering might have recovered. The fact that this species appeared to deteriorate after 60 days of stress however is significant when the behaviour of the other species, R. multiflora is discussed.

Ruschia multiflora controls behaved similarly to R. carolii controls, though in general stomatal conductance, transpiration and CO2 uptake was lower in R. multiflora. The C3 mode of photosynthesis is also adopted by this species under conditions of irrigation and also accumulates acid at night. The role of PEPc as well as RuBPc is again evident. Fewer fluctuations and lower organic acid levels may be explained by the lower rates of CO2 uptake during the day. Thus less excess CO2 was available so most carbon taken up entered the C3 pathway directly with very

little being used for malate synthesis. The slightly higher levels of acid at night was probably a result of refixation of respiratory CO2.

A difference in the behaviour of the two species was detected however once stress was induced. R. multiflora accumulated significantly higher levels of acid at night, recall this did not occur in the other species, neither was there any indication of nocturnal stomatal opening which was evident for R. multiflora. Though these periods were brief, the fact that the stomata did open at night is significant. Thus with moderate stress (30days) this species adopts a CAM type of carbon fixation. Again the artificiality of the phytotron conditions might have limited or masked this activity. Interesting results were evidenced after 50 and 60 days of stress. There was a more or less constant level of organic acids throughout the day, and stomata were definitely open for most of the day, which was not the case at 30 days. After 60 days of stress there was evidence of CO2 uptake during the day in R. multiflora. Loss of respiratory CO2 was less than in R. carolii. The former species was also able to take up CO2. With increased stress, R. multiflora was able to revert back to C3 despite the extreme shortage of water, R. carolii at this stage appeared to be dying, clearly intolerant of such stress. Since activity came to a halt in the latter species, the former could still photosynthesise and grow. Stress tolerance was greater in R. multiflora. The fact that significantly more water was lost by R. multiflora suggests that the metabolism and physiology of this species can withstand greater water loss.

The reasons underlying the two species' distribution can now be explained. R. carolii does not thrive on the exposed North-facing slope since it's carbon fixation ability is drastically impaired under intense water stress conditions. In Summer, such conditions prevail on this slope. R. multiflora has an advantage over R. carolii therefore because it can still fix carbon efficiently during stress. Though a proliferate user of water, R. multiflora stressed plants have the ability to outcompete R. carolii because of the carbon fixation strategy adopted and that they are generally more tolerant to water stress. Further studies should be undertaken to investigate the importance of the smaller leaf size of R. multiflora. Desert plants tend to have smaller leaves compared with closely related species in more mesic environments (Nobel 1976). This acts to reduce transpiration since the smaller leaves lose heat more easily, they can therefore maintain lower leaf temperatures and so reduce the driving force for transpiration (Gates et al. 1968). From this study, instantaneous water loss was lower for the smaller leafed species only under moderate stress. Total water loss estimates were greater in this species however. Perhaps field studies would yield more accurate results.

Under optimal conditions of irrigation R. carolii photosynthesises at greater rates than R. multiflora. This carbon can therefore be channelled into rapid growth. The optimal, sheltered conditions on the South - facing slope are thus ideally suited to R. carolii which has the capacity to grow faster than R. multiflora thus leaving little chance for the latter species to successfully

establish itself. So, under more optimal conditions R.carolii outcompetes R.multiflora.

It is evident from this study that the ecophysiological behaviour of the two species differ. Much speculation has gone into this discussion which is a direct result of the lack of published work on Karoo species in the field. The limitations of ecophysiological work being carried out under controlled conditions have already been mentioned.

I have suggested that the two Ruschia species are C3 plants under irrigation, but the role of malate in C3 plants urgently needs to be investigated. Moderate stress in these plants (30 days) induces changes in their behaviour, but whether one considers this to be restricted C3 or CAM - idling is unclear. More conclusive are the results after 50 and 60 days of stress whereby the hardiness of R.multiflora allows it to outcompete R.carolii on the North slope. The greater carbon fixation and faster growth rates of R.carolii under optimal conditions allows it's rapid establishment and spread, clearly reducing the probability of successful establishment by R.multiflora on the South slope.

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