

**EFFECT OF CO₂ ENRICHMENT ON GAS
EXCHANGE CHARACTERISTICS AND SEEDLING
GROWTH RATES IN A FEW TREE SPECIES**

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**DEPARTMENT OF CROP PHYSIOLOGY
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BANGALORE**

1990

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ವಿಶ್ವವಿದ್ಯಾಲಯ, ಸಿಕ್ಕಿಂ
ಸಿಕ್ಕಿಂ, ಹಿಮಾಲಯ (ಐ.)
28 MAR 1950
Th. 2441
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Thesis submitted to the
University of Agricultural Sciences, Bangalore
in partial fulfilment of the requirements
for the award of the Degree of

Master of Science (Agriculture)

IN

CROP PHYSIOLOGY

BANGALORE

FEBRUARY 1990

*Dedicated to
My Beloved Parents*

ACKNOWLEDGEMENT

I acknowledge with a deep sense of gratitude, the invaluable guidance of Professor M. Udaya Kumar, my Major Advisor, throughout the period of my M.Sc.(Agri.) programme. I thank him for the keen interest he showed in my work and for serving as a source of inspiration not just to me but to all the students in the Department.

I am thankful to Shri. Rama Rao, Dr. Shivashankar, Dr. Prasad, and Dr. Devendra, Members of my Advisory Committee for their constructive suggestions and critical reviewing of the manuscript.

My thanks are due to Dr. Uma Shaanker, Dr. Sashidhar and Mr. Ramaswamy who helped in many ways throughout my research work.

I am greatly indebted to all the students of the Department of Crop Physiology who helped me at every step of my work. A special word of thanks goes to Ravi, Shesha, Suresh and Uma.

My gratitudes to my parents, brothers and sisters for their constant encouragement and support throughout my course of study.

The Junior Fellowship awarded by Indian Council
of Agricultural Research, New Delhi, is thankfully
acknowledged.

Finally, I thank Mr.A. Ramesh for the neat
typing of the thesis.

Bangalore

February , 1990

(B.G. SUDHA)

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BANGALORE

CERTIFICATE

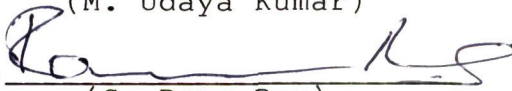
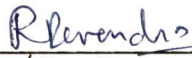
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Bangalore
February , 1990


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INTRODUCTION

I. INTRODUCTION

Afforestation programme is assuming considerable importance especially in tropical countries where there is a continuous deforestation of natural forests. In our country, agroforestry is also being popularised in order to meet the demand of fuel, wood products and other products. These two programmes are carried out by planting tree saplings in vast expanses of barren land, felled forest area, village outskirts and highways. For the expeditious and successful implementation of afforestation and agroforestry programmes, a continuous supply of healthy seedlings is required. To obtain transplantable size healthy seedlings in time is often a constraint because of their slow growth rates.

Generally trees are propagated by seeds or cuttings. They are germinated in seedbeds and subsequently grown in polythene bags. Seedlings of many tree species have poor growth rates. The rate of growth of seedlings in the earlier stages of germination is mainly determined by the stored seed constituents, leaf expansion rates and assimilation characteristics. Leaf expansion rates in woody plants is very low and so also the assimilation rates. To exemplify, net photosynthetic rate in Populees tremela and Acer platanoidis

12.2 and 8.9 mg CO₂ dm⁻²h⁻¹ whereas in Helianthus annuus it is as high as 35.7 mg CO₂ dm⁻²h⁻¹. Stomatal resistance in these tree species is high as 2.33 and 4.7 sec cm⁻¹ in Populus tremula and Acer platanoidis respectively where as it is as low as 0.38 sec cm⁻¹ in Helianthus annuus.

In addition to intrinsic factors, plant growth also depends on external factors. Environmental factors which greatly affects the plant growth are light, temperature, soil moisture and CO₂ levels. These factors can be varied to some extent to the plants' advantage to have a faster growth rate.

It is observed that in humid areas, the seedling growth rates are usually high. This is because of the prevailing high relative humidity (RH) and the resulting lower vapour pressure deficits (VPDs). High RH increases the leaf expansion rates and the low VPDs favour better gas exchange traits. As a result, net assimilation(A) per seedling increases and so also the total dry weight achieved by the seedling.

One more environmental factor which affects the assimilation and so the dry matter production is the atmospheric CO₂ concentration. Higher CO₂ levels in plant growing atmosphere maintained substantially above the normal favours growth. Results with intact plants

show that it is possible to increase, by as much as 50 to 100 per cent or more, the rate of photosynthesis under natural conditions by CO₂ fertilization. It has been unequivocally shown that increased ambient CO₂ levels increases the production. This technique of CO₂ fertilization is being profitably employed in the commercial production of glass house vegetables and fruits. The increased growth under elevated CO₂ is due to the enhanced apparent photosynthesis and a reduced photorespiration. Stomatal conductances are known to decrease at elevated CO₂ levels thus increasing the water use efficiency. Though the enhanced growth rate is observed in most of the species, the response of plants in terms of assimilation and conductances to enhanced CO₂ levels differ. So it is necessary to study the differential response of plants to elevated CO₂ levels in terms of their gas exchange characteristics and growth rates.

Keeping the above mentioned points in view, an experiment was conducted with the following objectives

- i) To enhance the seedling growth rates of forest species by altering the environmental conditions, mainly by elevating the ambient CO₂ levels.

- ii) To study the gas exchange characteristics of the seedlings at elevated CO_2 levels in order to understand the response of plants to elevated CO_2 levels.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Environmental factors regulate growth and development of the plants. Amongst all the environmental variables, temperature, RH and light intensity play an important role. Apart from these factors, ambient CO_2 concentration will also have a direct as well as indirect effect on plant growth and development. An attempt has been made in this investigation to study the effect of CO_2 on plant growth and development. Therefore, in this chapter the effect of CO_2 on plant growth and development has been reviewed.

CO_2 being one of the important inputs in photosynthesis, any change in the ambient CO_2 concentration will have a profound effect on the photosynthetic characteristics of the plants. Atmospheric CO_2 concentration has been increasing steadily in the recent years. Preindustrial CO_2 level might have been as low as 274 ppm as inferred by Stuiver (1978). During the early industrial period, prior to 1900, measurement made by Brown and Escombe (1905) indicated a CO_2 concentration of 290 ppm. The increase in atmospheric CO_2 concentration since the preindustrial era may have been 24 per cent and since

the turn of century about 17 per cent (Gates, 1983). For the last 85 years the annual increase in CO₂ concentration is about 1.5 ppm and it is predicted that the rate of increase may further increase.

Plants are always CO₂ limited for photosynthesis. They respond positively to increasing CO₂ concentrations and increases water use efficiency (WUE). There are also negative effects among some plant physiological responses, such as the reduction of stomatal conductance with increasing CO₂ concentration. It is possible that lower photosynthetic capacity will develop for plants grown at high CO₂ concentration, because of higher starch content, decreased chlorophyll concentration, decreased stomatal conductance and limited inorganic phosphate pool in the leaves. Diverse results has been obtained in terms of response of plants to elevated CO₂ concentration. A review of work done by various scientists in this area is given.

Effect on growth rates

There is a large body of information regarding the effect of CO₂ concentration on dry matter(DM) accumulation in plants. CO₂ enrichment increased DM production in cereal crops like wheat (Gifford, 1979), vegetable crops like cucumber and cabbage (Slack and Hand, 1985; Heij, et al., 1984) and fruit crops like

oranges and tomatoes (Downton et al., 1987; Calvert, 1975). Extensive work, which has been done on oilseeds like soybean also indicated the increased production of DM at elevated CO₂ concentration (Havelka, et al., 1984). Many studies on CO₂ enrichment showed an increase in root to shoot ratio. Such a change in the translocation pattern will increase the yield of root crops like cassava and potato (Imai, et al., 1984; Ku and Edwards 1977). It is not just the food crops or crops of commercial importance which would gain advantage out of enriched CO₂ concentrations, even weeds like water hyacinth and Echinochloa crusgalli have also been reported to accelerate their growth rates and produce greater DM (Poorter et al., 1988; Potvin and Strain, 1985). It can be concluded from several studies that increasing CO₂ concentration will increase the primary productivity of plant species to an extent of 40 per cent.

Increase in biomass could be due to increase in photosynthesis per unit leaf area or due to increase in canopy photosynthesis or due to a decrease in photorespiration and or respiration.

CO₂ enrichment effect on photosynthesis

In all short term experiments, where the leaves

were exposed to elevated CO₂ levels for not more than a period of 24 hours, increase in photosynthetic rate has been observed. Response of leaf photosynthetic rate to higher CO₂ levels was studied in soybean by Huber et al. (1984) and in many green house plants by Mortensen (1984). In both the studies, carbon exchange rate enhanced under higher CO₂ levels.

In long term experiments, where the plants were continuously exposed to enriched CO₂ levels, results have been contradictory. Increase in photosynthetic rate at higher CO₂ concentration throughout the period of exposure has been observed in soybean by Havelka et al. (1984a), Ackerson et al. (1984) and Campbell et al. (1988) and in Plantago major by poorter et al. (1988). In these studies, increase in photosynthetic rate is attributed to increase in substrate levels or increase in RuBISCO activity or due to substantial increase in specific leaf weight.

In contrast, a decrease in photosynthetic rate for plants grown at higher CO₂ concentration and measured at growth CO₂ concentration have been reported in water hyacinth (Spencer and Bowes, 1986) in cotton by Sasek et al. (1985) and in Desmodium paniculatum by Wulff and Strain (1981). The decrease in photosynthetic rate has been attributed to many reasons. Bruggunik

(1984) attributes the decrease in photosynthesis in carnation and tomato to the decreased carboxylation efficiency or increased mesophyll resistance but in cucumbers, the decrease is attributed to a decrease in stomatal conductance by Peet et al. (1986). End product inhibition of photosynthesis has been mentioned by Azcon Bieto^{and Øsmund} (1983). Similarly starch accumulation is stated to be the major inhibitor in a few studies (Sasek et al., 1985; Cave et al., 1981). Starch not only inhibits photosynthesis but is also supposed to reduce chlorophyll concentration and reduce grana formation (Wulff and Strain, 1982; Cave et al. 1981). Other inhibitory effects of starch are shading of light reaching the chloroplast^(Warren) Wilson, 1966) or increase in diffusive path length or interference of intercellular CO₂ transport due to the large starch grains (Nafziger and Koller, 1976). Even though many studies show starch accumulation as a consequence of CO₂ enrichment and a further decrease in photosynthesis as a consequence of starch accumulation, not all studies have reported such starch accumulation due to CO₂ enrichment. Riechers (1984) did not notice any starch accumulation at higher CO₂ concentration in C₄ grass land species.

RuBP carboxylase, the only enzyme which can fix CO₂ is also known to be affected by CO₂ enrichment. Vu et al. (1983) reported an increase in the level of RuBP

and a decrease in RuBP case activity. Overall activity of RuBP case has been reported to decline with long term CO₂ enrichment in a number of species (Hinkelton and Jolliffe, 1980; Pearcy and Bjorkman, 1983; Peet et al., 1986; Poorter and Gradzinski, 1984). Poorter and Gradzinski, (1984) reported not only a decline in Rubisco but also a decline in the activity of carbonic anhydrase in Phaseolus vulgaris grown for seven days in 1200 ul CO₂/l. In contrast, Koch, et al. (1986) noticed a higher RuBISCO activity in leaves of citrus seedlings grown at 660 ul l⁻¹ CO₂. But Campbell et al. (1988) observed no response in the activity of RuBISCO to short term and long term exposure of soybean plants to elevated CO₂ levels.

We can conclude that even though photosynthetic rate per unit area increases with CO₂ concentration in short term experiments, in long term experiments increase in photosynthesis rate per unit leaf area under higher CO₂ levels is not ubiquitous. Irrespective of the photosynthetic rates per unit leaf area, CO₂ enrichment has always resulted in an increase in dry matter. An explanation, for such a phenomenon is given by Raper and peedin (1978). They observe that absolute growth response to a CO₂ enriched atmosphere could be the result of a transitory plant response until the

steady state rate has been reestablished. During the exponential growth phase, a small initial difference in the absolute mass between plants can become exaggerated after a few weeks of steady state growth even though the relative growth rates of the plants are equal. In accordance to the above statement, leaf area has been found to increase as a result of CO₂ enrichment (Oberbauer et al., 1985; Koch et al., 1986). An increase in leaf area should result in an increase in canopy photosynthesis. In a study by Kridemann and Wong (1984), the greater biomass in cabbage and cucumber when grown at higher CO₂ concentration resulted partly due to a higher net Assimilation rate but more especially from faster leaf expansion during early exponential growth. Nijs et al. (1989), measured a stronger photosynthetic stimulation at the canopy level (103 per cent on average) than at leaf level (70 per cent in sunlight) due to accelerated foliage area development in white clover.

Effect on photorespiration

Higher concentrations of CO₂ not only increases photosynthesis but also competes with oxygen to bring down photorespiration. Ehleringer and Bjorkman (1977) have reported a decrease in oxygen inhibition of quantum yield in Eucalyptus californica from 72 per cent

at 88 μ bar internal CO_2 concentration (C_i) to 6 per cent at 1510 μ bar C_i . Valle, et al. (1985) noticed a significant decrease in CO_2 compensation point in soybean leaves adapted to high CO_2 levels indicating a decrease in photorespiration. Fock, et al. (1979) have reported a decrease in photorespiration to total photosynthesis ratio with increasing CO_2 concentrations. So it can be concluded that higher CO_2 levels reduce the extent of photorespiration.

Effect on stomatal conductances

It is an accepted fact that higher CO_2 levels decrease stomatal conductances (g_s) in most of the species. Such an observation has been made by Sasek et al. (1985) in cotton, in rice and maize by Morison and Gifford (1983). But not all plants respond in a similar way. Peet and Willits (1985) noticed an increase in stomatal resistances in CO_2 enriched plants of tomato but in cucumber, stomatal conductances was not affected by CO_2 concentrations. ' g_s ' of two C_4 species, Eleusine indica and Echinochloa crusgalli was unaffected when grown at higher CO_2 levels (Potvin and Strain, 1985). Akita and Moss (1972) generalised that C_3 species were less prone to closure than were stomata of C_4 species but Morison and Gifford (1983) found no evidence that stomata of C_4 species were more sensitive

and C_i/C_a ratio (i.e., internal to external CO_2 concentration ratio) was constant in C_3 and C_4 species at all concentration of Ca . In spite of decreasing ' g_s ', C_i has been found to increase with increasing Ca in rice and maize (Morison and Gifford, 1983) in sunflower (Whiteman and Koller, 1967) and in eucalyptus (Wong ~~et al.~~ 1978). Decrease in ' g_s ' was a temporary effect and it increases as the ambient CO_2 level reduces as reported by Sasek et al. (1985) but the decrease in ' g_s ' was permanent in water hyacinth and did not increase when the plants were shifted back to lower CO_2 levels as noticed by Spencer and Bowes (1986). Two things can be concluded that stomatal conductances decrease with increasing CO_2 concentration and C_i increases with Ca despite a decreasing ' g_s '.

Effect on Transpiration

Elevated CO_2 increases stomatal resistance and therefore reduces transpiration. Reduced transpiration was seen in soybean as reported by Huber et al. (1984). The decrease was from $140 \text{ mg H}_2\text{O m}^{-2}\text{s}^{-1}$ at ambient level to $80 \text{ mg H}_2\text{O m}^{-2}\text{s}^{-1}$ at 300 ppm of CO_2 above ambient level. Wong and Dunin (1987) measured a 30 per cent decrease in transpiration in eucalyptus at 680 μbar ambient partial pressure of CO_2 and at saturating light intensities. Jone, et al. (1985b) observed an

inverse relationship between CO₂ levels and transpiration rates in soybean plants with instantaneous response to changes in CO₂ concentration. On the contrary, Valle, et al. (1985b) failed to notice a significant decrease in transpiration rate of soybean plants grown at elevated CO₂ levels. Even though the extent of response varies, transpiration rate decreases at higher ambient CO₂ levels.

Effect on water use efficiency (WUE)

Elevated CO₂ affects both photosynthesis and transpiration. So it also affects the WUE of the plants. photosynthetic rates will generally increase at higher CO₂ concentration. As a consequence, WUE generally increases. Valle , et al (1985b) reported a doubling of WUE in soybean leaves at 660 ppm of CO₂, even though there was no significant decrease in transpiration rate. Same results has been obtained by Jones et al. (1985a) in soybean leaves. Tolley and Strain (1985) and Norby et al. (1986) have reported a higher WUE at elevated CO₂ levels in seedlings of sweetgum and Quercus alba. In Eucalyptus, a 80 per cent reduction in transpiration ratio resulted due to a 50 percent increase in photosynthetic rate and a 30 per cent reduction in transpiration (Wong and Dunin, 1987). The increase in WUE of plants at elevated CO₂ levels make

them more drought resistant.

The beneficial effect of elevated CO_2 under stress conditions has been demonstrated. Sionit and Patterson (1985) noticed that under stress conditions, net photosynthesis did not decline as rapidly at high CO_2 as at low CO_2 which could be due to the maintenance of turgor at high CO_2 concentrations. Sionit *et al.* (1981) suggested that increasing CO_2 concentration may induce wheat plants to osmoregulate more effectively and possibly enable them to adapt better to subsequent water stress. Stomata of the plants exposed to higher CO_2 would be still sensitive to stress 'gs' of plants exposed to high CO_2 concentration decreases as rapidly during a stress period as plants exposed to low CO_2 . Morison and Gifford (1983) noticed that the increase in transpiration efficiency at higher CO_2 level was relatively higher in C_3 plants than C_4 plants. So C_3 plants would be having a better drought resistance than C_4 plants. Marks and Strain (1989) worked on asters (C_3) and broomsedge (C_4) and concluded that the C_3 plants would be better competitors during well watered as well as water limited conditions under elevated CO_2 concentration.

Increased availability of substrates at elevated CO_2 concentrations should increase the respiration rates. Increase in respiration rate at elevated, CO_2 concentration has been reported by Potvin and Strain, 1985 in Eleusine indica and Echinochloa crusgalli. Once again the effect is different in different plant parts. Hrubec et al. (1985) found that dark respiration was enhanced in young leaves of soybean and not in mature ones. Shoot respiration was hardly affected by CO_2 enrichment in Plantago major where as root respiration was stimulated by CO_2 enrichment in young plants. CO_2 enrichment evokes differential response in different species of plants with regards to respiration. Gifford et al. (1985) studied the response of three species of plants and found that the root respiration of CO_2 enriched wheat plants was sometimes reduced, in sunflower respiration was enhanced, in mungbean no significant difference was found. The study of Kirschbaum and Faraguhar (1987) on eucalyptus has revealed that the respiration was virtually constant over the range of CO_2 concentrations from 50 to 1000 u bars. It can be concluded that the response of respiration rates in different plants to CO_2 enrichment is not uniform and even within a plant, different plant parts can respond in a different way.

Effect on ripening

CO₂ is known to be an ethylene action inhibitor and its effect has been extensively studied in fruits after harvest. Mature bartlett pear fruits when stored under a continuous flow of air with 10 per cent CO₂ for 4 days exhibited reduced respiration and ethylene evolution rates and remained greener and firmer than fruits stored in air (Kerbel et al. 1988). Quazi and Freebairn (1970) reported a delay in ripening of banana when the CO₂ concentration rate was increased in the absence of ethylene. This retarding effect of CO₂ was overcome by exogenously applied ethylene. So higher concentration of CO₂ delays ripening either by reducing respiration rate or by acting as a ethylene action inhibitor or by both the ways.

Effect on senescence

Varying reports of effect of high CO₂ on senescence has been given. Sionit et al. (1981) noticed that leaves of wheat plants grown in high CO₂ senesce 3 to 4 days earlier. It could be because they were initiated earlier. Same effect has been reported by Chang (1975). He noticed a decrease in chlorophyll content in cotton at 1000 u litres per litre CO₂. Even protein content decreased with no accompanying increase in its degraded products. On the contrary Hardy and

Havelka (1975) reported delayed senescence in soybeans probably because the high CO_2 enabled nodules to fix nitrogen faster delaying extraction of nitrogen from the leaves. In studies conducted by Havelka et al. (1984, a) and Havelka et al. (1984b), senescence was not affected in soybean and wheat grown at higher concentrations of CO_2 . Even though a few studies show that CO_2 affects senescence it appears that high CO_2 probably has little direct effect on senescence.

Interaction with vapour pressure deficit (VPD)

VPD has a direct effect on the stomatal conductances. The response of plants to elevated CO_2 greatly depends on the VPD to which the plants are exposed and the response of plants to VPD, in turn depends on other environmental factors. In Sesamum, Hall and Kaufmann (1975) found that with low intercellular CO_2 concentration, the humidity difference had less influence on leaf resistance compared to the influence it had with moderate intercellular CO_2 concentration. Similar results have been reported by Heath and Milthorpe (1950). They reported no effect of CO_2 concentration on leaf resistance of wheat in humid air and strong effect of CO_2 concentration on leaf resistance in dry air. Since higher VPD is going to reduce the stomatal

conductances, it should also alter the rates of C_i/C_a . Same effect has been observed by Morison and Gifford (1983) in two C_3 and C_4 species of plants. At small VPDs, the ratio of C_i/C_a was similar in all species (0.8-0.9) but at large VPD, C_i/C_a was 0.7 and 0.5 in C_3 and C_4 species respectively. In all the plants studied, response of conductance to VPD was maximum at low CO_2 and vice versa. However, they found that the relative response of 'gs' to either CO_2 or VPD was unaffected by the other variable.

Interaction with light

Photosynthesis enhancement by increased CO_2 concentration depends strongly on other environmental factors. Usually light intensities required to saturate photosynthesis increases with increasing ambient CO_2 concentration. Such an observation was made in Eucalyptus by Wong and Dunin (1987) and in soybean by Campbell (1988). Valle^{et al} (1985) noticed that soybean plants adopted to high CO_2 enrichment exhibited a capability to utilize radiation more effectively at elevated CO_2 throughout all light levels than leaflet grown at low CO_2 . In accordance with the above observation, Sharon and Mitchell (1988) found that leaf lettuce growth is most responsive to a combination of high light intensities and CO_2 enrichment. The maximum

absolute photosynthesis enhancement for C_3 species by CO_2 enrichment occurs at light intensities that are normally saturating for photosynthesis. However, the relative enhancement would be measured under light limiting conditions since the light compensation point decreases with an increase in CO_2 concentration (Pearcy and Bjorkman, 1983).

Interaction with temperature

The extent of plant's response to CO_2 enrichment depends greatly on the temperature. Sionit et al. (1988b) showed that plant height and number of branches increased slightly with CO_2 enrichment and more significantly with increasing temperature. Sionit et al. (1988a) observed that the reduction in dryweight of plant parts at lower temperature was ameliorated by increase in atmospheric CO_2 levels. Imai, et al. (1984) reported a greater enhancement of leaf area and whole plant dry weight in cassava at higher CO_2 concentration and higher temperature compared to higher CO_2 concentration and lower temperature. Sionit et al. (1988b) observed an increase in number of pods and seed in soybean at high temperature and high CO_2 concentration. In this study it was found that a combination of high temperature and high CO_2 levels not only enhanced the total growth of plants but also

hastened senescence of leaves. The enhancement of growth due to CO_2 enrichment is least at low temperature and increases continuously with increasing temperature. The probable cause is that the affinity of RuBP carboxylase for CO_2 declines with an increased temperature. As a result, the temperature optima for photosynthesis is generally higher at high CO_2 than at low CO_2 concentration (Pearcy and Bjorkman, 1983) and increase in temperature optima with increasing CO_2 concentration was noticed by Enoch and Hurd (1977) in spray carnations. All the above studies reveal that higher CO_2 concentration, no doubt is beneficial to plants at all temperature regimes, but the maximum benefit could be at a relatively higher temperature.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

Experiments were conducted to assess the effect of elevated CO₂ concentrations on the seedling growth rates in a few selected tree species. The experiments were carried out in the Department of Crop Physiology, University of Agricultural Sciences, Bangalore. The place is situated at a latitude of 12°58' North, longitude of 17°35' East and at an altitude of 930 m from the mean sea level. The average annual rainfall of the area is about 890.9 mm. The climatological data for the experimental period is provided in the Appendix.

Two important aspects were examined in the present investigation to understand the effect of CO₂ enrichment in a few tree species.

In the first aspect, the effect of enriched CO₂ on growth rates as measured by the increase in biomass and leaf area is determined in a few tree species. In the second aspect, the effect of elevated CO₂ on gas exchange characteristics was studied in the same tree species.

The following species were studied in the experiment:

1. Azadirichta indica

2. Dalbergia sissoo
3. Eucalyptus
4. Michelia champaka
5. Spathodia
6. Syzigium cumini
7. Atrocarpus heterophyllus

In the present investigation the major objective was to improve the seedling growth rates which is a major constraint in many forest species. Therefore in addition to the effect of elevated CO₂ concentrations, the effect of coirpith as a growth media and also the effect of soil volume were also investigated.

3.1 To study the first aspect, two experiments were conducted. In the first experiment, in addition to CO₂ enrichment treatment, the effect of coirpith, was also studied. In this experiment, the amount of soil or soil and coirpith used was 5 kgs of soil or 3 kgs of soil and coirpith mixture.

Procurement of the plant material: The seedlings were procured from the forest nursery, Karnataka Forest Department. The seedlings were six months old at the time of the procurement.

3.1.1 EXPERIMENT-I

Treatments

The main treatment consisted of the partial exposure of the seedlings to three different levels of CO_2 .

1. Control ambient level of CO_2
2. CO_2 - 1
3. CO_2 - 2

The sub-treatment consisting of growing the seedlings in two different growth media.

- (1) Soil alone - Seedlings were grown in polybags consisting of only red soil.
- (2) Soil and Coirpith mixture - Red soil and coirpith were mixed in a ratio of 4:1 weight by weight. Seedlings were grown in polybags consisting this mixture.

In each main treatment there were two sets of plants, one grown in soil and one in soil and coirpith mixture. In each species there were three plants for each sub-treatment. In total, for each species there were eighteen plants.

Preparation of the seedlings: The seedlings were

procured from the forest nursery and carefully transplanted into the new polybags with known quantity of soil or soil and coirpith mixture. The transplanted seedlings were kept under the shade of the big trees for a period of seventy days for acclimatisation. During this period they were watered thrice a day regularly.

Fertilizer application: Before transplanting the seedlings, soil and soil coirpith mixture was mixed with adequate quantity of farm yard manure. Fertilizers were applied immediately after transplantation and at every sixty days interval thereafter. The recommended quantity of nutrients given were nitrogen, phosphorus and potassium in the form of urea, superphosphate and muriate of potash. The fertiliser rate per seedling was calculated based on the volume of soil or soil and coirpith mixture

Imposition of the main treatment

Method adapted to enhance CO_2 concentration.

The main treatment consisted of exposing the plants to three different levels of CO_2 - control, CO_2 - 1 and CO_2 - 2.

Trenches were dug which measured 4 x 1 x 0.6 mts. Six such trenches were dug, two each for the three

different treatments of CO_2 . In two trenches which constituted CO_2 - 2 treatment, farm yard manure was added to facilitate additional build up of CO_2 over and above the levels of CO_2 built up only due to dark respiration of plants and soil. To obtain the desired concentration of CO_2 , the farm yard manure content added to the pit was altered. In CO_2 -1 treatment, the rate of build up of CO_2 in the covered trenches was much less compared to CO_2 treatment. Excepting the two trenches meant for control, the remaining four were covered with polythene sheets. A metallic frame measuring 12 feet length, 4 feet width and 2 feet depth was placed on each of the four trenches (Fig. 1). The frame provided support to polythene sheets for completely enclosing the trench. The trench was made air tight by burying the free end of the polythene sheets into the soil (Fig 2).

A new method to enhance the carbon dioxide concentration was adopted in this experiment. The CO_2 released during the dark respiration and soil respiration was trapped to enhance the ambient CO_2 levels. Seedlings in polybags were transferred to trenches which were covered with polythene sheets from 4.00 P.M. to 10.00 A.M. Soil respiration was increased by addition of decomposing organic matter. CO_2 released



Fig.1: The set up used for CO₂ enrichment studies.

by dark respiration and soil respiration was trapped during night for making it available to plants during morning hours upto 10.00 A.M.

Before initiation of the treatments, three plants from each sub-treatment (soil grown ones and coirpith grown ones) were taken for initial observations.

The following observations were taken at the end of the experiment.

- | | |
|-----------------------|---|
| 1. Plant height | 7. Final drymatter |
| 2. Number of branches | 8. Total drymatter accumulated during the experimental period |
| 3. Number of leaves | 9. Root/shoot ratio |
| 4. Final leaf area | 10. Specific leaf weight |
| 5. Leaf biomass | 11. LA/DM ratio(LAR) |
| 6. Root weight | 12. LW/DM ratio(LWR) |

All these parameters were measured in the same plants.

1. Plant height:

Plant height was measured from the soil surface to the base of the youngest leaf on the main stem in all species except sissoo. In sissoo, the height was taken from the soil surface till the tip of the tallest

branch. Plant height is expressed as cm per plant.

The number of branches and leaves were counted in the seedlings before they were harvested for drymatter estimation and expressed as number per seedling.

2. Final leaf area

Leaf area was measured using LICOR leaf area meter (Model LI-3000) and expressed as cm^2 per seedling. Leaves were separated from the stem and then fed to the leaf area meter.

3. Dry matter estimation

Dry matter content of the seedlings was estimated at the beginning and at the end of the experiment. The plants were removed from the soil, along with the roots. The polythene covers were cut open and the soil mass adhering to roots was removed by washing with water. By this method, almost all the roots of plant could be removed. The seedlings were cut into root, stem and leaves and oven-dried at 80°C for 48 hours. After drying, the weights of root, stem and leaves of each seedling was taken separately. Total drymatter content, leaf biomass and root biomass is expressed as g drymatter per seedling. The total dry matter accumulated during the experimental period (ΔDM)

was computed as the difference between the initial and final drymatter of seedlings.

Root to shoot ratio (R/S ratio)

This was computed using the data on root drymatter and shoot drymatter, obtained during the harvest. This ratio was used to know if there was any change in the pattern of partitioning of metabolites due to the treatments imposed.

Specific leaf weight

The ratio of the average leaf biomass per seedling to the average leaf area per seedling is the specific leaf weight and expressed as grams per dm^2 .

LA/DM and LW/DM ratio

This ratio is an indication of the assimilation capacity per unit leaf area and per unit leaf weight respectively. These ratios were computed using the data on final drymatter, leaf biomass, leaf area measured at the end of the experiment.

$$\frac{\text{LA/DM in } \text{dm}^2/\text{g}}{=} = \frac{\text{Leaf area in cm}^2}{\text{Total drymatter (g)}} \times \frac{1}{100}$$

$$\text{Leaf wt/DM} = \frac{\text{Leaf biomass (g)}}{\text{Total drymatter (g)}}$$

3.1.2 EXPERIMENT-II

The objective of this experiment was very similar to that of the first experiment. The treatments imposed, preparation of plant material and the method of the imposition of the treatment was same as the first experiment. However the amount of soil or soil and coirpith mixture used was 2.5 and 1.5 kg respectively. Yet another variable in the present experiment was the duration of the experiment. The duration of the first experiment was from April through June and the duration of the second one was from April through August.

Both Experiment-I and Experiment-II were initiated at the same time and the plants belonging to both the experiments were located in the same trench. However, after terminating the first experiment, the plant material of the second experiment was continued in the same trench for a period of another two months. The initial and final observations were recorded as in the first experiment.

3.2 Gas Exchange Characteristics

The main approach in the present investigation was to enhance the seedling growth rates by altering the CO_2 , RH and temperature. The two important

variables are CO_2 concentration and RH which will have profound effect on CO_2 exchange rate, stomatal conductance (gs) and transpiration rate. Though the growth rates as measured by the increase in biomass and leaf area is a reflection of the response of the species to the altered environmental conditions, it is necessary to study the effect of these variables on Assimilation (A) and stomatal conductance. Keeping this in view, an attempt was made in this investigation to study the instantaneous change in gas exchange characteristics as influenced by the environmental conditions.

The following parameters were studied:

1. Differences in gas exchange traits amongst the seven species.
2. Light response curves of the species.
3. Gas exchange parameters at elevated CO_2 concentrations and CO_2 response curves.
4. Gas exchange parameters at different vapour pressure deficits (VPD) and VPD response curves.

The different gas exchange characters as influenced by the different environmental conditions were studied only in control plants which were not exposed to elevated CO_2 levels. In addition to gas

exchange characters, leaf chlorophyll content and stomatal frequency was also determined.

All gas exchange characters were determined using portable photosynthesis system, IRGA, ADC, LCA-2, (Fig. 2).

Portable ADC photosynthesis system (ADC-IRGA) is principally designed to measure photosynthesis rate (A), transpiration rate (T), stomatal conductance (gs) and internal CO₂ concentration (Ci). This instrument can also be used to measure the ambient CO₂ concentration (Fig. 2).

The main components of the photosynthesis system are:

1. Battery operated portable infra-red CO₂ analyser (LCA-2).
2. Parkinson's leaf chamber (plc-B)
3. Air supplying unit (ASUM 2)
4. Data processor/logger (DL-2)

Primary parameters measured by the instrument are:

1. Cuvette air temperature (ta) °C
2. Cuvette relative humidity (he) %

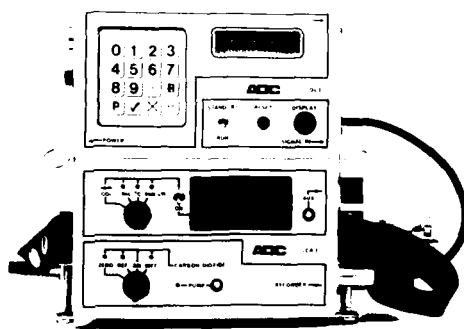


Fig.2: Photosynthesis system ADC - LCA-2.

3. Photon flux density (Q) $\mu \text{ mol m}^{-2} \text{ s}^{-1}$
4. CO_2 partial pressure in air both before entering the leaf chamber (C_a) and after leaving the leaf chamber (C_o) in $\mu \text{ bar}$.

System operation

This portable system works only as an open air system. In this system there is a continuous flow of air drawn from the atmosphere through a leaf chamber. An IRGA is used to measure CO_2 concentration of the air that is entering the chamber and also the air that is moving out of the chamber. For a continuous air flow through the leaf chamber a flow regulating air supply unit is provided.

The leaf chamber (Parkinson's leaf chamber) is designed in such a manner that it measures the cuvette temperature, the RH (due to the transpiration of the leaf) and the light intensity. The primary data on RH, cuvette temperature, leaf area, pressure and mass air flow were used to compute the transpiration rate of the leaf. Leaf temperature is computed based on light intensity, boundary layer resistance, cuvette temperature and other physical constants.

Leaf temperature, cuvette temperature, RH flow

rate and boundary layer resistance are used to compute the stomatal conductances.

IRGA in this system is sensitive to water vapour also. Hence, while calculating the photosynthesis rate based on CO_2 differences (CO_2 concentration of air entering and leaving the chamber) correction for water vapour has to be given. The flow rate and the area of leaf inserted into the leaf cuvette is also used for computing photosynthesis rate.

Internal CO_2 concentration is computed by using stomatal conductance, boundary layer resistance (T), assimilation rate and CO_2 concentration leaving the leaf chamber.

The calculated values are given in the following units:

1. Photosynthetic Rate (A) $\mu\text{mol}/\text{m}^2/\text{s}$
2. Transpiration (E) $\text{m mol m}^{-2}\text{s}^{-1}$
3. Stomatal conductance (gs) $\text{m mol m}^{-2}\text{s}^{-1}$
4. Intercellular CO_2 concentration (C_i) ppm
5. Vapour pressure deficit (VPD) millibars

The primary values were logged using data logger. These primary values were used to compute the derived parameters by software programme provided by

ADC Company using PC XT.

Bypass correction: One of the characteristic features in ADC is that dry air is pumped into the leaf chamber from ASU at a given flow rate. Therefore input RH is zero, increase in RH depends on transpiration rate of the leaf and often in turgid leaf the buildup of RH of many species is about 40 to 60 per cent. But in slow transpiring species or stressed leaves or when CO₂ concentration was enhanced, the RH build up in the cuvette would be low. This results in high VPD. VPD is the difference between leaf saturated vapour pressure and vapour pressure maintained in the chamber. The vapour pressure in the leaf chamber depends on the air temperature and the RH maintained in leaf chamber. Thus VPD depends on the air temperature, RH in the chamber and leaf saturated vapour pressure. Therefore to maintain constant vapour pressure it is necessary to maintain constant RH inside the leaf chamber and also the air temperature.

VPD has a direct effect on stomatal conductances. Therefore to compare the gas exchange characteristics of different species and between stressed and non-stressed plants, maintenance of same VPD is very essential. This can be achieved to some extent by using the bypass valve.

Approximately similar VPD levels were achieved by altering the leaf RH flowing into the leaf chamber by bypass valve. Bypass valve allows a small portion of air to pass directly into the leaf chamber without passing through the drying columns. Depending on the number of rounds of opening of the bypass valve and the flow rate, RH of the air supplied (RH IN) into the leaf chamber can be varied. Though RH IN can be altered by the bypass valves and the flowrate, it also depends on the ambient atmosphere. Therefore, ultimately depending on the rate of transpiration of leaves and ambient RH, the bypass valve and the flow rates were suitably altered to maintain cuvette RH which is very close to ambient RH. For all the species and for different treatments, by using these manipulations, the same RH and therefore the same VPD was maintained.

3.2.1 Differences in gas exchange characteristics amongst the seven species.

Gas exchange traits of all the seven species were measured under ambient conditions in order to understand the intrinsic gas exchange characteristics of the plants. Normally grown control plants were used in this study. VPD maintained during this study was quite high and was between 30 to 35 millibars. ADC LCA-2 was used in this study. Leaf was clamped in the

Parkinson's leaf chamber and once 'A' and 'gs' values were stabilised, the data were logged.

3.2.2 Light response curves

The response of assimilation 'A' to photon flux density was studied in a few forest species. These light response curves have three important applications.

- a) To assess light saturation assimilation rate
- b) To quantify the quantum yield based on initial slope of the light response.
- c) To identify PFD level for ' $A_{\max}$ ' measurement.

One of the important prerequisite to develop light saturation curve is to alter the light intensities received by the assimilating leaf, kept in the leaf chamber. This was done by using light filters which uniformly decreased the spectrum to a desired level. Differentially exposed x-ray film filters were used as light filters. Leaf was covered with these filters at a distance of 2 to 3 cms above the chamber to reduce the light intensity.

Light saturation curves were developed on a

bright sunny day when the PFD was around 2000-2200 $\mu\text{mol m}^{-2}\text{s}^{-1}$. ADC-LCA-2 was used to measure the gas exchange traits. Leaf was clamped into broad leaf Parkinson's leaf chamber after connecting the four components, ADC, IRGA, ASU and leaf chamber suitably. The leaf assimilation rate was measured under direct sunlight without using any filters. Later a single filter which decreased light intensity by only 20 per cent was kept over the leaf and other assimilation and 'gs' was recorded. Light intensity was gradually decreased by increasing the number of filters and the gas exchange traits were measured.

Care was taken to maintain the RH of the leaf chamber close to the ambient RH. Since light has a profound effect on 'gs', the RH within the chamber decreased with low light and vice-versa. By manipulating flow rate and the by-pass valve, required RH was maintained in the leaf chamber.

Assimilation rate was plotted against PFD using the principle of non-linear regression programme such as maximum likelihood programme (Ross, 1980). Using the same data of light effect on gas exchange traits, 'gs' response curves to light, assimilation response curves to conductance, 'A/gs' response to light were also plotted.

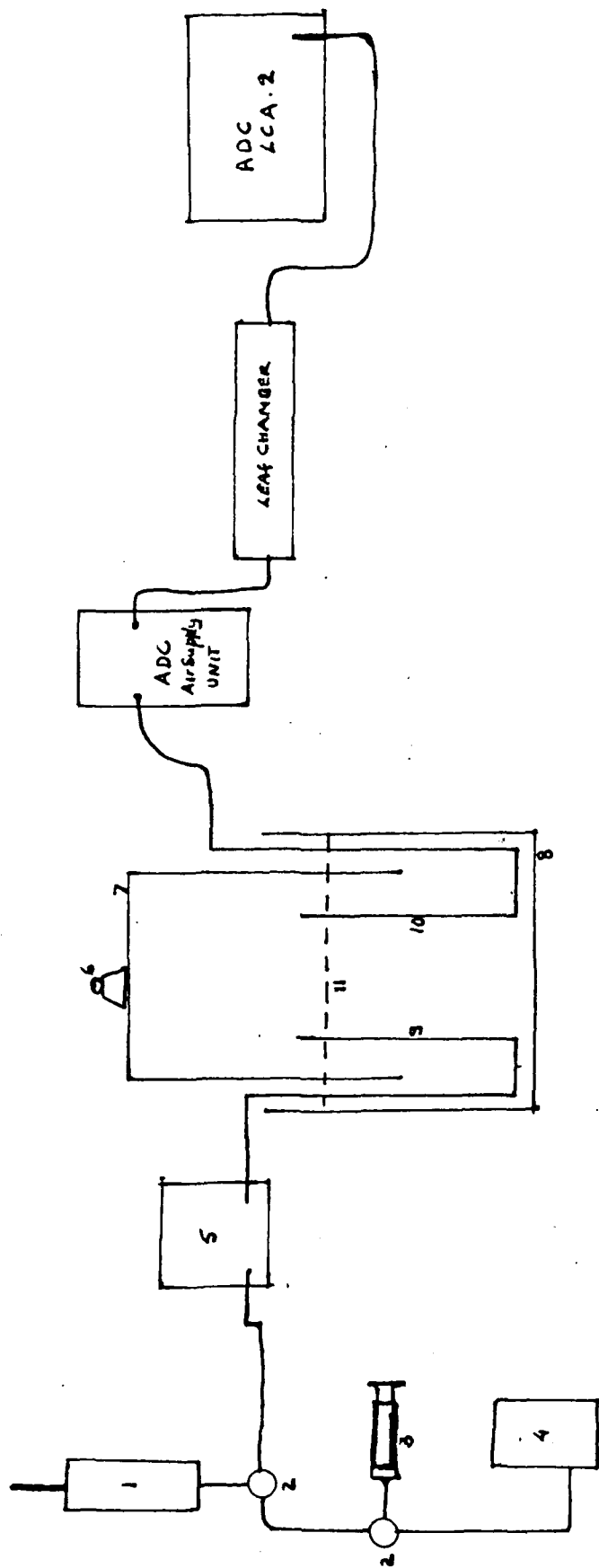
3.2.3 The A/Ci response curve

A/Ci response curves are developed by plotting assimilation 'A' against CO_2 concentration at mesophyll cell surface (C_i). The plot indicates the response of 'A' to CO_2 concentration in the absence of stomatal limitations. Such A/Ci response curves have two important applications (1) as a method to quantify and separate the stomatal and mesophyll limitations (2) to quantify carboxylation efficiency (VRuBISCO).

One of the approaches to develop A/Ci curve is by measuring gas exchange traits in plants or in leaves exposed to different ambient concentrations of CO_2 . One of the prerequisites for developing A/Ci curve is to possess sources of different CO_2 concentrations.

Development of different sources of CO_2

A simple device was developed to achieve different concentrations of CO_2 in aluminium drums of 25 litres capacity. As shown in Fig. 3, the aluminium drums were placed inverted over water in a plastic bucket of 50 litre capacity. When filled with air the drums float over water in the inverted position and when air was removed slowly the drums would slide down gradually, thus proportionately decreasing the volume of air in the drum. An iron frame was used to position the aluminium drum over the water and also to facili-



F19-3 Setup to obtain different CO₂ concentrations for A/ Ci curves using ADC LCA-2.

1-CO₂ Scrubber 2-3-Way valve 3-Syringe 4-CO₂ Source 5-Pump
 6-Weight 7-Inverted drum 8-Plastic waterbath 9-Inlet pipe
 10-Outlet pipe 11-Water level (6-7-8-9-10-11) Aspirator.

tate easy up and down sliding. An air pump was used to pump air into the drum. The connections into the drum were given in such a manner that air with known CO_2 concentration could be pumped into it and when once it was full, the air would be easily drawn out without any alteration in the composition of air maintained in the drum.

In all, five different sources of CO_2 were used to develop 'A/Ci' curves. The different concentrations of CO_2 used were two concentration levels below and two concentrations above the ambient CO_2 level. To obtain lower CO_2 concentrations the ambient air was pumped through a KOH-Soda lime trap and to increase CO_2 concentration above ambient CO_2 levels a known amount of enriched CO_2 ($\text{CaCO}_3 + \text{HCl}$) was pumped into the drums as shown in Fig. 3.

Measurement of gas exchange traits at different Ca

The gas exchange characteristics (A , g_s , C_i , R_H , T , PAR) were measured using a broad-leaved Parkinson's chamber suitably connected to ADC, IRGA and ASU. After clamping the leaf into the chamber the assimilation traits were determined by drawing air from a specific aluminium drum with a known CO_2 concentration. The leaf was acclimatized for a minimum period of one and half to two minutes until a steady state R_H and

'gs' was achieved. The RH, 'gs' and 'A' were continuously monitored using a data logger and only after reaching the steady state the data were logged for further computation.

Maintenance of RH in the leaf chamber

Since the stomata respond swiftly to the changes in CO_2 concentration, the RH in the leaf chamber gets altered. A change in RH will alter the VPD and hence has an effect on gas exchange characteristics. Therefore, it was necessary to maintain similar RH close to the ambient irrespective of ambient CO_2 concentration used. For this flow rates were suitably adjusted. Alternatively, in some cases bypass valve was used to maintain the required RH.

After generating the gas exchange data, the A/C_i curves were developed by adopting the following procedure.

The intercellular CO_2 concentration (C_i) was calculated from the formula

$$C_i = \frac{C_a (1/D_2 - E/2) - A}{(1/D_2 + E/2)} \quad \begin{array}{l} D_2 = 1.585 r_s + 1.37 r_b \\ r_b - \text{Boundary layer} \\ \text{resistance} \end{array}$$

where C_a is the ambient CO_2 level (ml l^{-1}) and 'A' is the photosynthetic rate ($\mu\text{mol m}^{-2}\text{s}^{-1}$).

Then A/C_i curves were plotted based on the principle of least squares with the help of a personal computer.

Utilising the same gas exchange data at different CO_2 concentration, ' C_i/C_a ' and ' g_s/C_i ' curves were plotted.

3.2.3.2 Estimation of stomatal and mesophyll limitations of photosynthesis in a few species

The stomatal and mesophyll limitations were studied in a few forest species.

The method proposed by Faraquhar and Sharkey (1982) has been used here to quantify the stomatal limitations of photosynthesis. Their method has been further modified to estimate mesophyll limitations of photosynthesis also (Fig. 4).

One of the prerequisites to estimate mesophyll and stomatal limitations is to develop A/C_i response curves. A/C_i curves were developed as described earlier. On the A/C_i curves the following points of the stomatal and mesophyll limitation were computed (refer Fig. 4).

1. A - observed photosynthetic rate at any given time.

2. A_0 - Potential photosynthetic rate when stomatal factors are not limiting and mesophyll factors are limiting.
3. A_g - Potential photosynthetic rate when mesophyll factors are not limiting and stomatal factors are limiting.
4. A_T - Potential maximum photosynthetic rate when mesophyll factors are not limiting and stomatal factors are not limiting.

Faraquhar and Sharkey (1982) gave the following formula to estimate the relative stomatal limitations (ls) for observed photosynthesis (A).

$$ls = \frac{A_0 - A}{A_0} \times 100$$

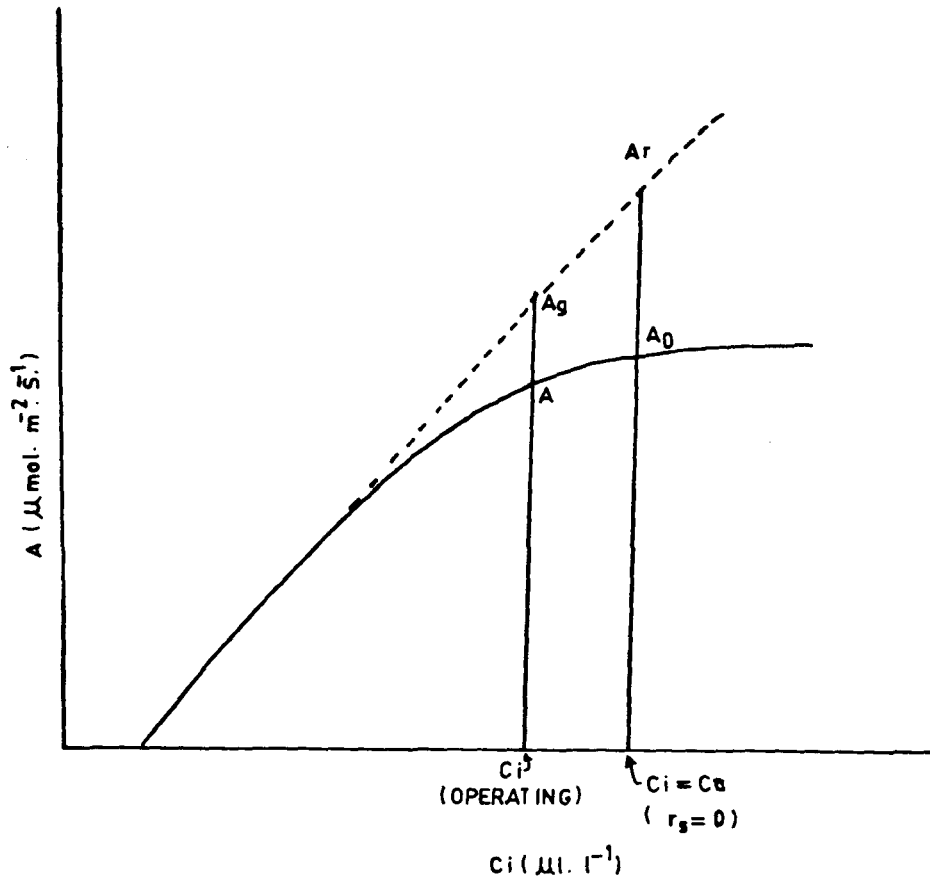
Mesophyll limitations (lm) of observed photosynthetic (lm) is defined as:

$$lm = \frac{A_g - A}{A_g} \times 100$$

Mesophyll limitation (ul) to the potential photosynthesis (AT) when there is no stomatal limitation is defined as

$$ML = \frac{AT - A_0}{AT} \times 100$$

Fig.4 ESTIMATION OF STOMATAL AND MESOPHYLL LIMITATIONS BY LINEAR RESISTANCE ANALYSIS.



- (i) A - OBSERVED Ph RATE AT ANY GIVEN TIME
- (ii) A_0 - POTENTIAL Ph RATE WHEN St. FACTORS ARE NOT LIMITING AND MESO. FACTORS ARE LIMITING.
- (iii) A_g - POTENTIAL Ph RATE WHEN MESO FACTORS ARE NOT LIMITING AND STOMATAL FACTORS ARE LIMITING.
- (iv) A_T - POTENTIAL MAXIMUM Ph RATE WHEN NEITHER MESO NOR St FACTORS ARE LIMITING.

$$I_s = \frac{A_0 - A}{A_0} \times 100$$

$$I_m = \frac{A_g - A}{A_g} \times 100$$

$$ML = \frac{A_T - A_0}{A_T} \times 100$$

(FARQUHAR AND SHARKEY 1982)

NOTE : I_s AND I_m REFER TO STOMATAL AND MESOPHYLL LIMITATIONS FOR OBSERVED PHOTOSYNTHESIS (A) AND ML REFERS TO THE MESOPHYLL LIMITATIONS TO A THEORETICALLY MAXIMUM PHOTOSYNTHESIS AT WHEN NO STOMATAL LIMITATIONS OPERATE.

These limitations were estimated in a few tree species which were grown under normal conditions.

3.2.3.3 Effect of CO₂ concentration on 'A' and 'gs'

In all the seven species, gas exchange traits were measured at two CO₂ concentrations, one-ambient and one at 650 to 750 ppm of CO₂. Ambient air was used for measuring at 340 ppm of CO₂. Air containing 650 to 750 ppm of CO₂ was obtained following the same method as in A/Ci curves.

The instantaneous response of plants to two CO₂ concentrations was measured. A single leaf was clamped in Parkinson's leaf chamber which was connected to ASU IRGA and data logger. Gas exchange traits were first measured at ambient CO₂ concentration and later at higher CO₂ concentration. Sufficient time was given for the stabilisation of 'A' and 'gs' values.

3.4.5 Effect of VPD on Assimilation and Conductances

When the plants in the trenches are covered by polythene sheets, the micro-environment around the plants change. The polythene cover not only traps CO₂ but also traps water vapour released by plants and soil surface. So RH increases in the evening and VPD decreases. But in the morning hours, temperature inside

the enclosed polythene sheet increases thereby there would be an increase in VPD irrespective of the high RH. However the VPD will be much less in the covered trench compared to controlled trenches which were not covered by polythene sheets. VPD has a direct effect on the stomata and stomata tend to close at high VPD (in dry air) restricting not only the water loss but also assimilation.

In view of the importance of this, it is necessary to study the gas exchange traits of plants at different VPDs. VPD response curves were developed using ADC, IRGA, LCA-2.

VPD depends on RH and temperature. The effect of VPD on 'A' and 'gs' was measured by instantaneously altering the VPD by altering the RH in the cuvette. In ADC, there is a provision to change the RH in the cuvette. RH can be altered by manipulating the by-pass throttle valve and the flow rates. When the by-pass throttle valve is opened, part of the air moves into the leaf chamber through the valve without passing through the dessicant. The extent of undried air passing through the valve could be reduced by reducing the extent of opening of the by-pass valve and can be fully stopped when the valve is closed. By using ASU, the inflow of air into the leaf chamber can be

monitored. The working range is 200 to 600 ml of air per minute. When the flow rates are small, the rate of dry air entering the leaf chamber would be lower. So RH in the leaf chamber would be at a higher level, depending on the extent of transpiration of the clamped leaf. When the flow rates are high, the air inside the leaf chamber is rapidly flushed out along with the water vapour transpired by the leaf. So when the flow rates are high, the RH in the leaf chamber would be low. By suitably manipulating the by-pass valve opening and flow rates, the required RH and thereby the VPD could be maintained in the chamber.

A fully expanded young leaf was clamped in the leaf chamber. The by-pass valve was fully opened and the flow rate was kept at minimum. This gave the maximum RH and minimum VPD. Observations were recorded at this VPD. Later the by-pass valve was closed and the flowrate was increased to get minimum RH and the maximum VPD. Observations were recorded at this VPD also. The range of VPD at which observations were recorded is 15 to 35 millibars.

A/VPD response curves. In two species, gas exchange traits were measured at six or seven levels of VPDs. By suitably manipulating bypass and flow rates, the desired VPD could be achieved. After achieving the

different VPD levels in the chamber, gas exchange traits were measured. The VPD levels were between 10 to 35 millibars. Using this data A-VPD response curve, 'gs-VPD' response curve, T-VPD response curve were plotted using PC XT.

In addition to the gas exchange traits, observations were recorded on the stomatal number in leaves. The chlorophyll content in the leaf was also measured. Normally grown control plants were used for the above study.

Stomatal frequency

Stomatal frequency in the leaves was measured by impression technique. A solution of thermocole and xylene was made of known consistency and smeared over the adaxial and abaxial surfaces of the leaf and the peelings were taken out after drying which has stomatal impressions and these peelings were observed under microscope.

Stomatal frequency was calculated by counting the number of stomata per microscopic field under high power (40x) objective and 10x eyepiece of the microscope and it was expressed as number of stomata per cm² using the formula:

$$\frac{1 \times A}{0.0086}$$

where 'A' is the number of stomata per microscopic field, 0.0086 is the area of the microscopic field under 40x magnification.

Estimation of chlorophyll content in leaves

Chlorophyll content in leaves at the end of the experiment was measured by DMSO technique (Hiscox and Iorolestan, 1979). Leaf discs weighing 0.1 g is incubated overnight in 7 ml of DMSO (Dimethyl Sulphoxide). After the period of incubation, the extract was decanted and the volume was made up to 10 ml. The O.D. of the extractant was taken at 645, 652 and 663 nm in a double beam spectrophotometer. Total chlorophyll, chlorophyll 'a' and chlorophyll 'b' were calculated using the following formulae.

$$\text{mg chlorophyll a/g tissue} = \frac{12.7(D_{663}) - 2.69(D_{645}) \times V}{a \times 1000 \times W}$$

$$\text{mg chlorophyll b/g tissue} = \frac{22.9(D_{645}) - 4.68(D_{663}) \times V}{a \times 1000 \times W}$$

$$\text{Total chlorophyll mg/g fresh weight} = \frac{20.2(D_{645}) + 8.02(D_{663}) \times V}{a \times 1000 \times w}$$

where, D = Optical density of DMSO

V = Final volume of the extract

W = Fresh weight of leaf discs

a = Length of pathlength in the photocell (1 cm)

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The objective of the present study was to enhance the seedling rates by altering environmental variables and growth media. The major variable attempted to study in this experiment was CO_2 concentrations. Soil water holding capacity and soil volume also affect growth rates of seedlings. Therefore the effect of growth media like coirpith and the volume of soil per seedling was also investigated simultaneously.

The response of different plant species to elevated CO_2 concentrations was examined by :

1. Assessing their growth rates when exposed to elevated CO_2 concentrations for prolonged periods and
2. by studying the gas exchange traits at higher CO_2 concentrations.

Growth rates due to elevated CO_2 and RH predominantly depend on the CO_2 assimilation characters of leaf. To assess the CO_2 assimilation characteristics, experiments were conducted to study the response of plants in terms of their assimilation to different CO_2 concentrations, light levels and vapour pressure

deficits.

To study the seedling growth rates at an altered environmental condition two experiments were conducted. The methodology in both experiments remained the same. The two variations introduced were with respect to soil volume and duration. In experiment-1, seedlings were grown in big polybags, duration being April through June. In the experiment-2, seedlings were grown in small polybags from April through August.

Technique to enrich CO₂ concentrations under the field conditions

A technique was developed to elevate the CO₂ levels using the CO₂ released during dark respiration of plants and soil respiration. The technique has been described in detail in material and methods. To study the efficacy of the method to enhance CO₂ concentrations, CO₂ levels were monitored at regular intervals after closing the pits with the polythene structure. The trenches were covered at 4.00 PM and the CO₂ build up was measured at every one hour interval till 9.00 PM. The data presented in the Fig. 5, indicates that there was a considerable increase in CO₂ levels inside the enclosed trench. The rate of increase and CO₂ level maintained in CO₂-2 treatment where decomposing farm

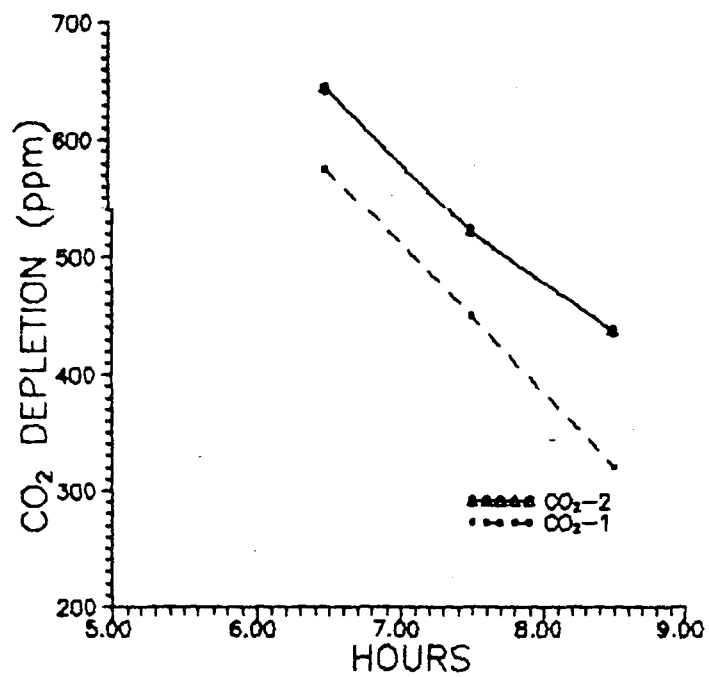
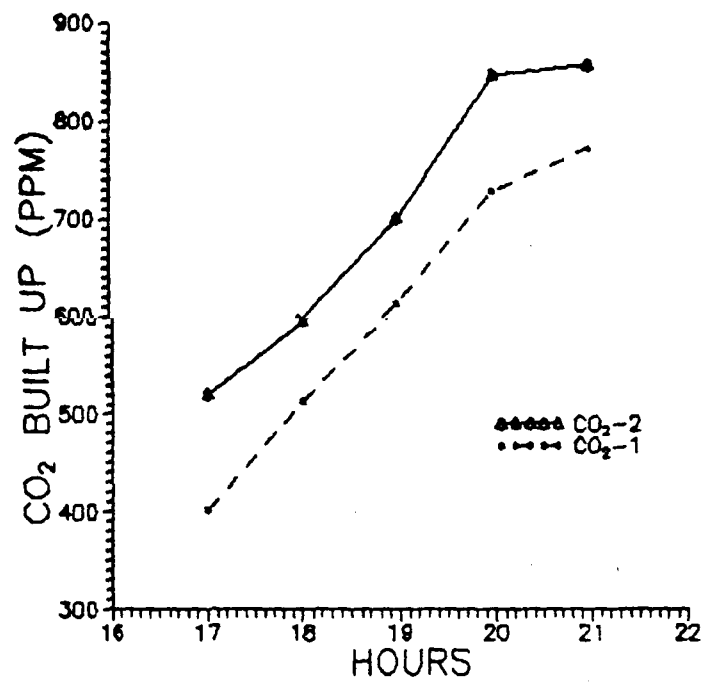


Fig.5: The change in the CO₂ concentration in trenches covered with polythene structure in CO₂ - 1 and CO₂ - 2 treatments.

yard manure was added was markedly more than CO₂-1 treatment.

The depletion of the CO₂ accumulated in the trench was also measured from morning 6.00 AM to 9.00 AM. With time, there was a significant decrease in CO₂ levels from 6.00 AM to 9.00 AM. The rate of decrease was same in both the CO₂ levels. In CO₂-1 treatment, within three hours, the CO₂ level came down to the ambient level (Fig. 5). The depletion was because of the CO₂ assimilation by plants. However, the loss of CO₂ due to diffusion cannot be ruled out. In the present experiment, it was not possible to account for the diffusion of the CO₂ from the enclosed trenches into the ambient atmosphere.

Covering the trenches with the polythene cover not only increased the CO₂ levels but also increased the relative humidity considerably. However in this experiment, RH inside could not be measured. But by visual observations, it could be said that the relative humidity was nearly 100 per cent. Any change in the seedling growth rates could not be ascribed only to the enriched CO₂ levels but may also be due to the increased RH.

One more environmental variable which was

changed due to the treatment was the air temperature inside the trench when covered by polythene sheet. Such a change was more pronounced only during morning hours. Air temperature was found to be higher inside the enclosed trench compared to outside between 7 to 10 AM. It was found to be 29°C inside the enclosed trench when the ambient temperature outside the trench was only 25°C. Increased temperature would also have had its impact on the growth rates of the seedlings.

4.1 Effect of CO₂ levels on the growth rates of forest species

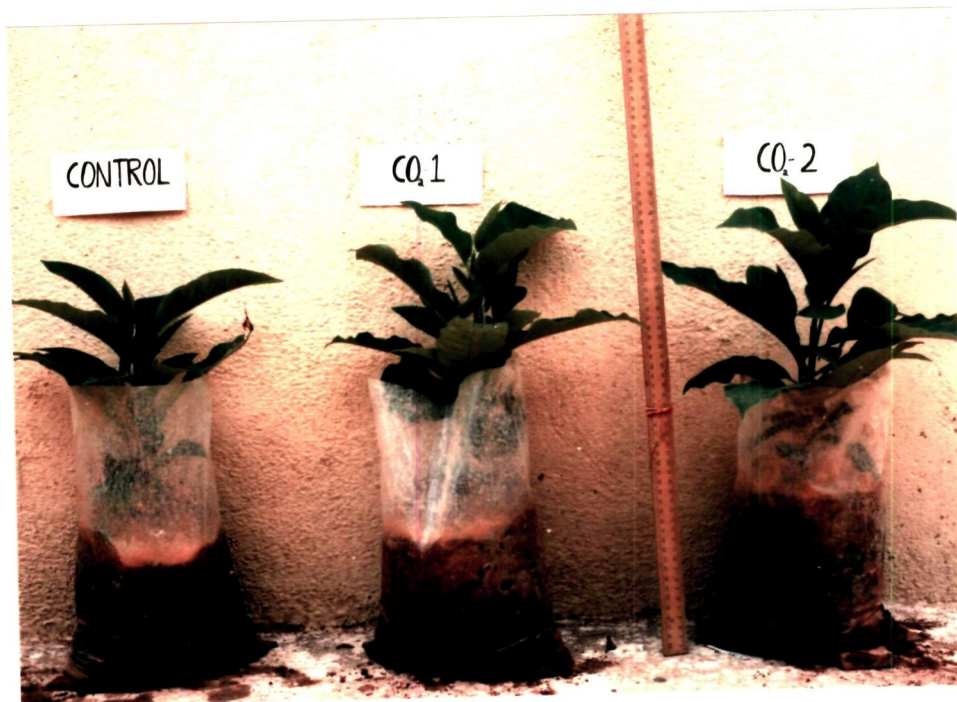
Any change in the seedling growth rates should be reflected in terms of increase or decrease in the drymatter, leaf area, leaf biomass and a host of other growth characteristics. In order to assess the effect of elevated CO₂ on growth rates, observations were recorded on drymatter, Δ DM i.e. drymatter accumulated during the experimental periods, root weight, leaf area, leaf biomass and other growth parameters.

4.1.1 EXPERIMENT-1

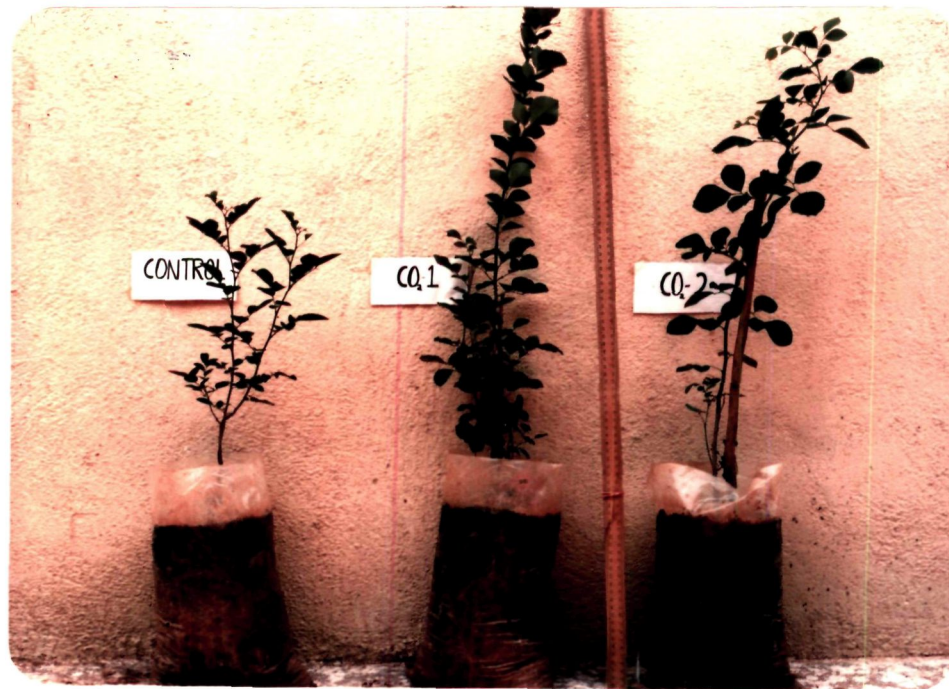
The effect of CO₂ enrichment on growth of plants was evident as early as one month after the initiation of the experiment (Fig. 5). Due to inadequate plant materials destructive sampling was not done.



Syzigium



Champaka



Sissoo

Fig.6: Response of (a) Syzigium, (b) Champaka and (c) Sissoo to CO_2 enrichment, one month after the initiation of the experiment.

Plant height, number of branches and leaf number

The plant height, number of branches, and number of leaves of the plants grown in control, CO₂-1 and CO₂-2 treatments are given in the Table Nos. 1a and 1b. The increase in plant height was about 25 and 33 per cent respectively in CO₂-1 and CO₂-2 treatments.

The highest per cent of increase in plant height was seen in neem, sissoo and jack, where in the CO₂-2 treatments, neem plants showed an increase of 100 per cent, sissoo and jack showed an increase by about 65 per cent. There was a marginal increase in syzigium, champaka and spathodia, whereas eucalyptus did not respond to the CO₂ enrichment in terms of plant height.

Branching was markedly enhanced in syzigium, and also in champaka, jack, sissoo and eucalyptus. In spathodia and neem, branching was not affected by CO₂ enrichment.

Leaf number per plant, across all species has increased markedly in CO₂-1 and CO₂-2 treatments, the per cent of increase being 71.3 and 60.6 respectively. Sissoo and Jack had the highest per cent increase in leaf number in CO₂-1 and CO₂-2 treatments. Spathodia showed a marginal increase in leaf number due to CO₂

Table 1 (a) : Plant height, number of branches and leaf number as affected by CO₂ concentrations

Treatments	Plant height (cm)	Number of branches	Leaf Number
Control	53.75 a	3.07 a	33.46 a
C1	67.00 b	5.29 b	57.32 b
C2	71.70 c	5.89 b	53.75 b
CD at 5%	4.01	1.43	6.073
CD at 1%	5.36	1.91	8.123
Soil	64.00	4.98	47.57
Coirpith	64.30	4.52	48.79
CD at 5%	NS	NS	NS

Table 1 (b) : Response of different species to CO₂ enrichments with regards to plant height, number of branches and leaf number

Species	Plant height (cm)		Number of branches			Leaf Number			
	Control	C1	C2	Control	C1	C2	Control	C1	C2
Syzigium	64.88	78.5	70.2	2.75	6.25	8.25	47.5	69.50	76.75
Champaka	33.13	42.63	40.88	3.50	6.50	6.25	28.5	39.00	41.50
Spathodia	31.75	38.00	41.00	0.00	0.00	0.00	13.75	17.25	16.25
Neem	39.00	61.13	78.00	1.50	1.75	1.25	34.50	50.25	37.75
Sissoo	63.00	90.63	104.63	5.00	9.25	11.50	45.25	107.00	82.25
Jack	36.13	54.25	59.13	3.50	4.75	6.00	20.30	36.50	42.25
Eucalyptus	108.38	103.88	108.00	5.25	8.50	8.00	44.25	81.75	79.50
CD at 5%	10.61		NS			16.07			
CD at 1%	36.82		NS			21.49			

enrichment.

Leaf area, leaf biomass and specific leaf weight

Leaf area of CO₂-1 and CO₂-2 plants across all species increased respectively by 47 and 42 per cent over control. The increase in leaf biomass was 33.9 and 24.7 per cent in CO₂-1 and CO₂-2 plants resulting in a decrease in specific leaf weight (Table 2a).

Leaf area has been doubled in case of jack, in both CO₂-1 and CO₂-2 treatments, CO₂-1 treatment could produce higher leaf area in species like neem, syzigium and champaka (Table 2b). Spathodia, sissoo and eucalyptus have also enhanced their leaf area in CO₂-1 and CO₂-2 treatments even though the per cent increase was lesser than the other species (Fig. 7).

Leaf biomass was enhanced in sissoo and neem. The extent of increase was almost same in champaka, spathodia and jack. Marginal increase was seen in syzigium but in eucalyptus no increase was seen.

Specific leaf weight has decreased in syzigium, champaka, jack and eucalyptus (Table 2b). The highest per cent of decrease was seen in jack by about 45 per cent and in rest of the three species the per cent decrease was more or less by the same extent. Spathodia

Table 2 a: Effect of elevated CO₂ concentration on leaf area, leaf biomass and specific leaf weight

Treatments	Leaf Area (cm ²)	Leaf weight (g)	Specific leaf weight (g/cm ² x 100)
Control	1334.62a	10.28a	0.854a
C1	1957.93b	13.76b	0.738a
C2	1896.73b	12.82b	0.700b
CD at 5%	181.87	1.40	0.134
CD at 1%	243.25	1.87	NS
Soil	1773.67	12.40	0.709
Coirpith	1685.84	12.18	0.819
CD at 5%	NS	NS	0.109
CD at 1%	NS	NS	0.146

Table 2b: Differential response of species with regards to leaf area, leaf biomass and specific leaf weight to CO₂ enrichment

Species	Leaf ₂ area (Cm ²)		Leaf biomass (g)		Specific leaf weight (g/cm ² x 100)				
	Control	C1	C2	Control	C1	C2	Control	C1	C2
Syzigium	1964	2600	2598	13.58	17.98	15.05	0.709	0.684	0.630
Champak	1621	2776	2479	8.78	13.20	12.80	0.539	0.476	0.518
Spathodia	1616	1932	2143	8.33	10.10	11.88	0.546	0.515	0.555
Neem	810	1444	1142	5.15	9.58	7.03	0.624	0.693	0.610
Sissoo	1021	1409	1478	6.70	9.78	10.45	0.651	0.711	0.701
Jack	707	1435	1522	12.95	17.23	16.35	1.889	1.183	1.035
Eucalyptus	1600	2046	1923	16.48	18.50	16.18	1.022	0.901	0.850
CD at 5%	NS				NS		NS		
CD at 1%	NS				NS		NS		

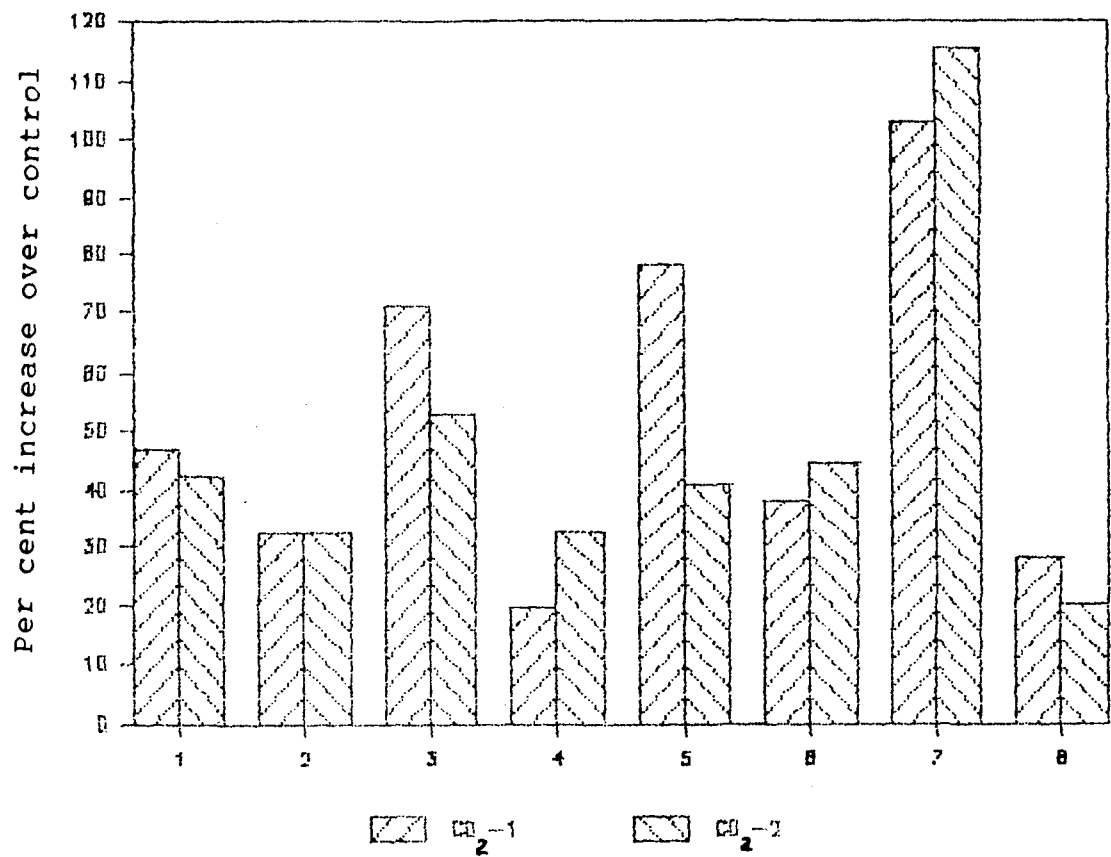


Fig.7: Effect of CO₂ enrichment on leaf area of different species.

1. Mean across all the species
2. Syzigium
3. Chamapaka
4. Spathodia
5. Neem
6. Sissoo
7. Jack
8. Eucalyptus

and neem did not show any particular trend but only in sissoo, specific leaf weight was increased marginally by 8 per cent.

Biomass, Final drymatter, Δ DM and Root biomass

Table 3a, gives the final drymatter (DM), drymatter accumulated during the experimental period (Δ DM) and the root biomass as affected by the CO_2 levels (Fig. 9) across all the species and as affected by soil and coirpith across all species. The final drymatter of the plants grown at the two higher levels of CO_2 was found to be significantly higher than the control plants. The increase was as high as 40 per cent compared to the control plants. However there was no significant difference in the final drymatter between plants grown at two elevated levels of CO_2 (Fig. 8). The Δ DM in CO_2 -1 and CO_2 -2 plants was 1.4 times greater than the Δ DM in the control plants.

The root biomass of the plants was 29 per cent and 34 per cent higher in CO_2 -1 and CO_2 -2 levels respectively, than the control plants.

The soil grown plants had a slightly higher final drymatter, Δ DM than the coirpith grown ones.

From this table, it can be concluded that the

Table 3a: Influence of CO₂ enrichment on Final DM,
 Δ DM and Root biomass

Treatments	Final DM (g)	Δ DM (g)	Root biomass (g)
Control	27.24a	22.1a	9.0a
C1	38.9b	33.8a	11.4b
C2	39.5b	34.4b	11.3b
CD at 5%	3.12	2.77	1.85
CD at 1%	3.83	3.76	2.48
Soil	35.8	30.2	10.27
Coirpith	34.6	29.9	10.81
CD at 5%	NS	NS	NS

Table 3 (b): Final DM, Δ DM and Root biomass of individual species as influenced by elevated CO₂ levels.

Species	Final DM (g)			Δ DM (g)			Root biomass (g)		
	Control	Cl	C2	Control	Cl	C2	Control	Cl	C2
Syzigium	43.1	51.5	51.5	35.4	43.6	43.6	10.1	12.6	12.0
Champak	20.2	31.0	27.7	17.1	28.0	24.7	7.2	9.9	7.5
Spathodia	31.1	36.9	43.1	24.5	30.3	36.5	14.5	14.4	15.6
Neem	9.8	27.3	21.9	8.0	25.9	20.5	3.3	6.6	5.7
Sissoo	25.9	35.5	44.8	22.1	32.9	42.2	9.8	11.6	14.7
Jack	13.2	30.0	29.4	5.5	23.1	22.4	4.0	8.9	7.2
Eucalyptus	47.4	59.9	58.0	41.1	52.8	50.8	14.3	15.7	15.8
CD at 5%	7.60			7.31			NS		
CD at 1%	10.16			9.78			NS		

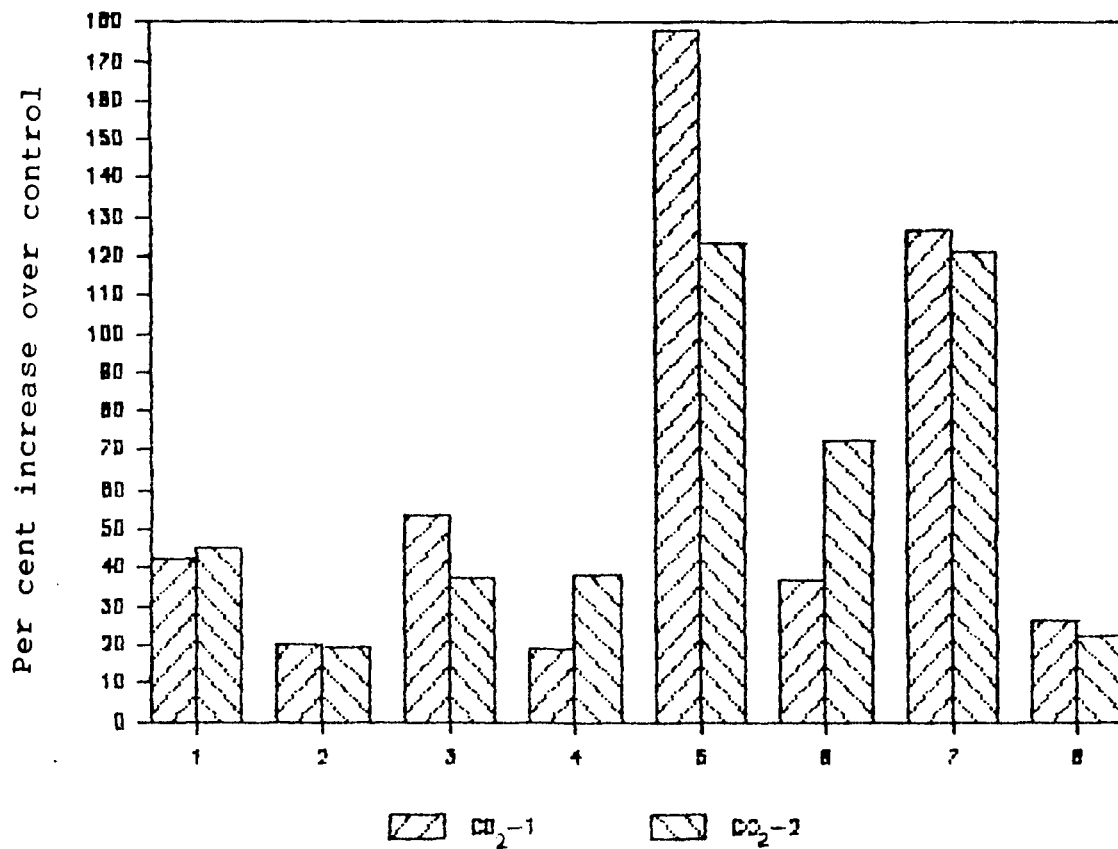


Fig.8: Effect of CO₂ enrichment on biomass of different species.

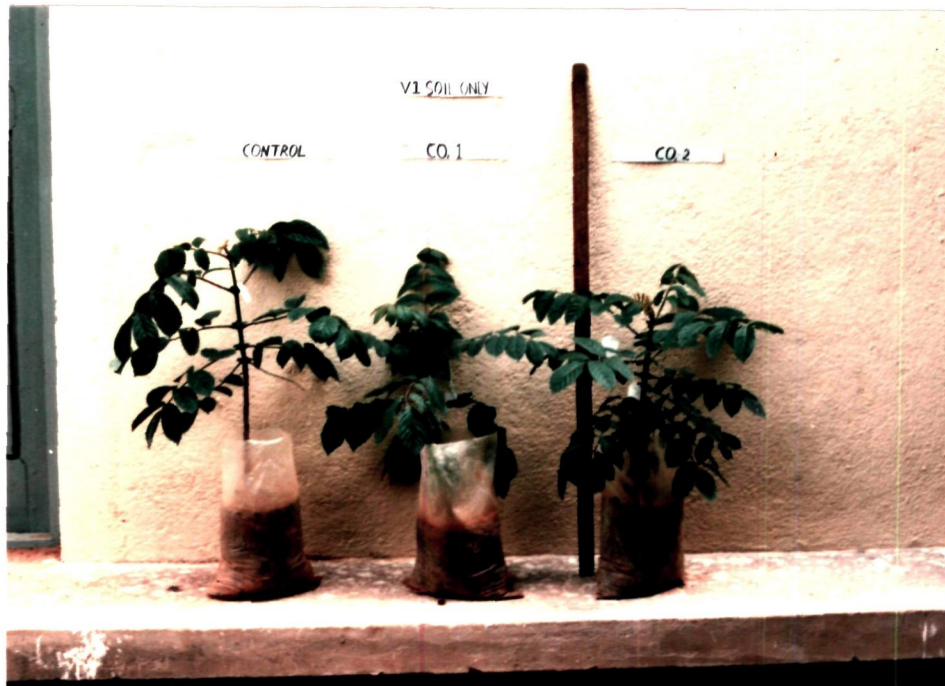
1. Mean across all the species
2. Syzigium
3. Chamapaka
4. Spathodia
5. Neem
6. Sissoo
7. Jack
8. Eucalyptus



Syzigium



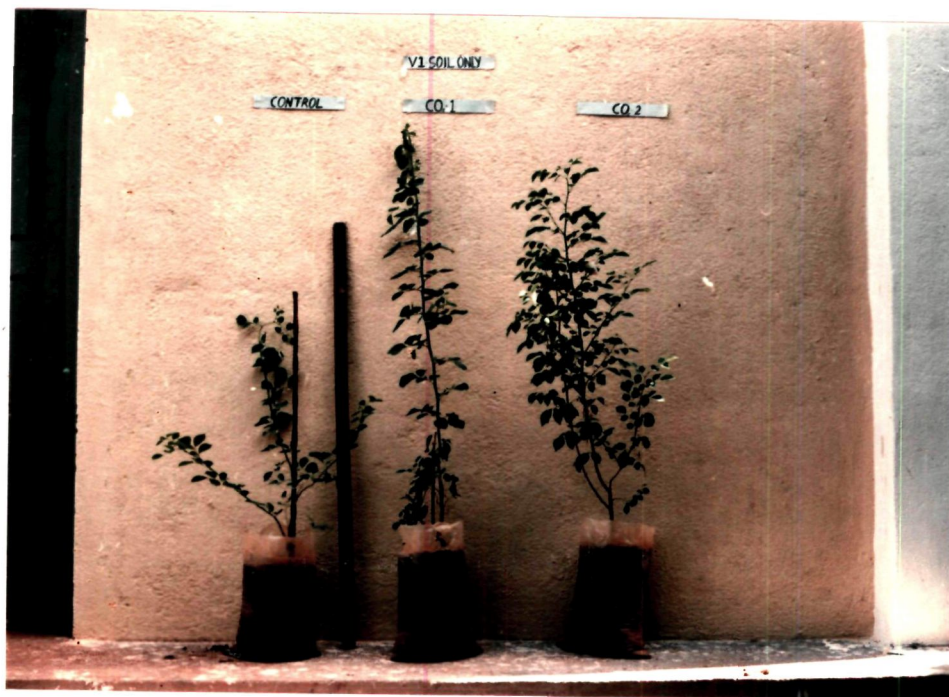
Champaka



Spathodia



Neem



Sissoo



Jack



Eucalyptus

Fig.9: Response of different tree species to CO_2 enrichment three months after the initiation of the Experiment-I.

higher levels of CO_2 had a significant effect on plants in terms of the above three characteristics whereas soil and coirpith did not make much of a difference to plants in terms of the above mentioned characteristics.

Neem and jack has shown a maximum increase in final drymatter (Table 3b). All the species except spathodia had a significantly higher drymatter at harvest in both the elevated CO_2 levels. In spathodia, only the plants grown in the highest CO_2 level had a significantly higher biomass when compared to controlled plants (Fig. 8).

The DM in the different species differed a lot between the control and CO_2 enriched plants. The Δ DM in the CO_2 enriched plants of neem and jack was about 3 to 4 times the control. In champaka and sissoo, Δ DM in CO_2 -1 and CO_2 -2 plants was 50 per cent greater than DM in control plants.

Root weight increased in jack, neem and sissoo. A very marginal increase was found in syzigium, champaka, spathodia and neem.

Root to shoot ratio, LAR and LWR

Though the root biomass increased in CO_2 enriched plants in both the treatments, R/S ratios decreased in the CO_2 -1 and CO_2 -2 plants.

Altering the growth media did not have any significant effect on root to shoot ratio but a slight increase was seen in coirpith grown plants (Table 4a).

Root/shoot ratios of all species has been affected differentially by CO₂ enrichment. Root/shoot ratio was found to have decreased in all the species except syzigium and jack. In these two species Root/Shoot ratio did not seem to be affected by CO₂ enrichment as they did not show any particular trend either a definite increase or decrease (Table 4b).

LA/DM(LAR) and LW/DM(LWR) reflects the assimilation rate of the leaves. These ratios give the amount of leaf area and leaf weight required to produce a unit of drymatter. A decrease in LAR and LWR speaks of the higher net photosynthetic rate per unit leaf area and leaf weight respectively. LAR and LWR in the CO₂ enriched plants has decreased across all species. The decrease in LWR in CO₂-2 plants was by 25 per cent and the decrease in LAR in CO₂-2 plants was a marginal 8 per cent.

The difference in LAR and LWR between the soil and coirpith grown plants was very marginal.

LAR of the individual species showed a differential response (Table 4b), to elevated CO₂ levels.

Table 4 (a): Root/Shoot ratio, LAR and LWR in control
CO₂-1 and CO₂-2 treatments

Treatments	R/S ratio	LA/DM	Leaf weight/DM
Control	0.538 a	0.561	0.445 a
C1	0.437 b	0.527	0.346 b
C2	0.405 b	0.515	0.344 b
CD at 5%	0.094	N.A.	0.078
CD at 1%	0.126	N.S.	N.S.
Soil	0.416	0.542	0.362
Coirpith	0.504	0.527	0.406
CD at 5%	NS	NS	NS

Table 4(b): Influence of CO₂ enrichment on Root/Shoot ratio, LAR and LWR of different species

Species	R/S ratio		LA/DM		L. Wt/DM	
	Control	C1	Control	C1	Control	C1
Syzigium	0.307	0.324	0.302	0.472	0.521	0.502
Champak	0.578	0.484	0.389	0.807	0.896	0.896
Spathodia	0.891	0.648	0.574	0.542	0.525	0.497
Neem	0.515	0.339	0.375	0.816	0.516	0.531
Sissoo	0.626	0.488	0.494	0.393	0.403	0.327
Jack	0.413	0.422	0.320	0.552	0.483	0.516
Eucalyptus	0.437	0.358	0.377	0.346	0.343	0.335
CD at 5%	NS	NS	NS	NS	NS	NS

While syzigium and champaka showed an increase, spathodia, neem and jack showed a decrease in LWR. Eucalyptus and sissoo showed a marginal decrease in the CO₂ enriched plants.

LWR decreased in the CO₂ elevated plants in spathodia, neem, jack and eucalyptus. Rest of the species did not respond in an unequivocal manner to the CO₂ enrichment.

Of all the above studied parameters, significant differences were seen in plant height, number of leaves, leaf area and DM. Number of leaves and leaf area have increased proportionately, keeping leaf area per leaf constant in the CO₂ enriched plants.

Effect of soil volume

Experiment-1 and Experiment-2 were initiated simultaneously. The effect of soil volume on plant growth was distinctly evident after three months of treatment (Fig. 11). The plants grown in the bigger volume had better growth rates than the plants grown in smaller volume of soil. Due to inadequate plant material, destructive sampling of experiment-2 plants was not done to give the difference in the growth characteristics.



CHAMPAKA



SISSOO

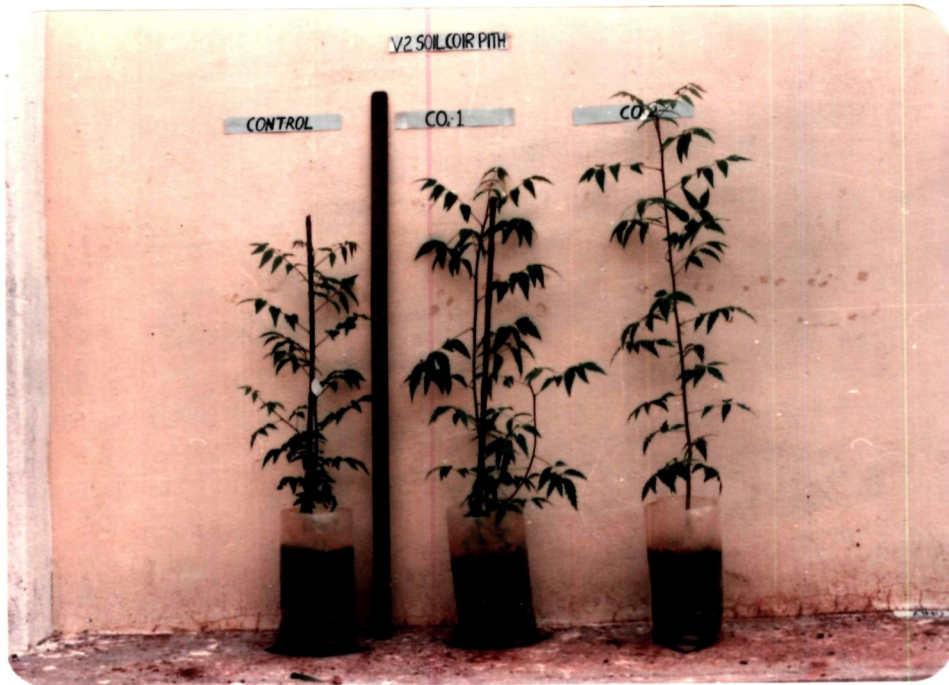
Fig.10: Effect of soil volume on growth rates of champaka and sissuo one month after transplanting.

4.1.2 EXPERIMENT - 2

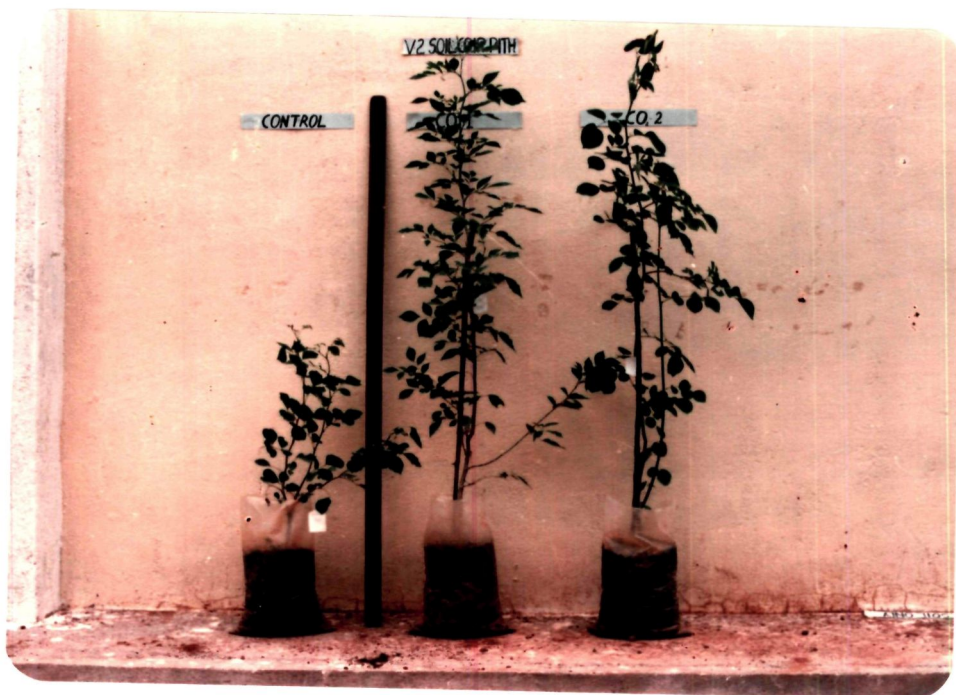
This experiment was similar to experiment-1 in all respects except for soil volume and duration. Even in this experiment, effect of CO₂ enrichment on plant growth was evident after three months of initiation of the experiment (Fig. 11). Destructive sampling was not done due to inadequate plant material. The duration of this experiment was extended to 5 months to see if the differences observed due to CO₂ enrichment are maintained for a longer time. The results obtained are given in the Tables 5 to 8.

Growth characteristics

Plant height, number of branches per plant, number of leaves per plant, leaf area and leaf biomass per plant, final drymatter and Δ DM of the plants grown at elevated CO₂ levels were significantly higher than the control plants. The differences between the two elevated levels of CO₂ was found to be non-significant. Leaf area of CO₂-1 and CO₂-2 plants was enhanced by 41 and 48 per cent respectively. The final drymatter of CO₂-1 plants and CO₂-2 plants had increased by 34 and 39 per cent respectively. Such a highly significant difference was also seen in Δ DM where CO₂-1 and CO₂-2 plants had accumulated respectively 38 and 43 per cent higher drymatter.



Neem



Sissoo



Jack

Fig.11 Response of neem, sissoo and jack to CO₂ enrichment, three months after the initiation of the experiment-II

Specific leaf weight and Root/Shoot ratios of control plants was higher than the CO₂ enriched plants. There was a slight increase in the root biomass and LAR ratio. However LWR of all the three treatments remained more or less the same. The differences in the above discussed parameters were found to be non-significant.

Soil and coirpith grown plants differed significantly with regard to only three parameters, leaf number, leaf area and leaf biomass per plant. Soil grown plants had higher number of leaves, leaf area per plant and leaf biomass per plant compared to the coirpith grown plants. The increase being 27, 16 and 17 per cent respectively. The same trend was seen in final drymatter, Δ DM and root biomass.

When the response of individual species to the CO₂ treatments was observed (Table 5b, 6b, 7b and 8b) plant height, number of branches and leaf number had increased in plants exposed to higher CO₂ levels. The increase was marginal in case of number of branches in all species but increase in plant height was good in all species except eucalyptus. Leaf number had increased substantially in all species.

In terms of response of leaf area to CO₂ enrichment, the magnitude of response was less than in

the experiment-1. Here sissoo and spathodia showed an increase in leaf area by 148 and 100 per cent respectively and champaka by 53 per cent. The increase in leaf area of jack was only 41 per cent. Leaf biomass per plant was found to be enhanced in all the species except eucalyptus and neem. The highest increase was seen in sissoo where the increase was by 200 per cent. Spathodia and champaka also had a marked increase in leaf area by 62 and 45 per cent respectively. Specific leaf weight of all the species except sissoo was found to have decreased due to CO₂ enrichment.

Species variation in response to CO₂ enrichment in terms of final drymatter and Δ DM was very considerable. Highest increase was shown by sissoo followed by champaka. Sissoo and champaka showed an increase in final drymatter by 145 and 81 per cent respectively.

Root weight of all species increased in higher CO₂ plants except eucalyptus. Sissoo and spathodia had higher root biomass in both CO₂-1 and CO₂-2 plants.

Root/Shoot ratio varied a lot in the species. A decrease in root/shoot ratio was seen in champaka, sissoo and eucalyptus whereas the response was not consistent in the other species.

Table 5 a: Plant height, number of branches and leafnumber as affected by CO₂ concentration

Treatments	Plant height (cm)	Number of branches	Leaf Number
Control	58.4a	3.96a	38.8a
C1	75.3b	5.57b	56.3b
C2	71.0b	6.21b	60.0b
CD at 5%	4.8	1.60	11.3
CD at 1%	6.5	2.14	15.1
Soil	68.5	5.64	57.8a
Coirpith	68.0	4.86	45.6b
CD at 5%	NS	NS	9.2
CD at 1%	NS	NS	12.4

Table 5 (b): Response of different species to CO₂ enrichment with regard to plant height number of branches and leaf number

Species	Plant height (cm)		Number of branches		Leaf Number				
	Control	C1	C2	Control	C1	C2	Control	C1	C2
Syzigium	56.0	74.0	71.5	6.00	6.75	6.50	77.8	101.3	89.0
Champak	31.8	54.0	46.3	6.00	7.25	8.50	31.3	52.3	49.5
Spathodia	38.8	36.5	47.3	0.00	0.50	0.50	7.3	16.3	13.5
Neem	59.0	81.8	79.8	2.50	2.75	4.50	28.0	36.5	45.0
Sissoo	69.0	102.3	90.0	5.00	9.50	10.00	29.0	62.0	76.0
Jack	55.0	68.3	63.5	3.00	5.75	5.75	32.0	51.3	52.8
Eucalyptus	99.0	110.3	99.0	5.25	6.50	7.75	66.5	74.8	94.3
CD at 5%	NS		NS		NS		NS		NS

Table 6 (a): Effect of elevated CO₂ concentration on leaf area, leaf² biomass and specific leaf weight

Treatments	Leaf Area (cm ²)	Leaf Weight (g)	Specific leaf Weight ₂ (g/cm ² x 100)
Control	1228 a	11.31 a	0.860
C1	1734 b	14.90 b	0.852
C2	1818 b	15.17 b	0.817
CD at 5%	262.0	2.13	NS
CD at 1%	350.4	2.85	NS
Soil	1722 a	14.9 a	0.839
Cairpith	1465 b	12.7 b	0.847
CD at 5%	213.9	1.7	NS
CD at 1%	286.1	2.3	NS

Table 6 (b): Differential response of species with regard to leaf area, leaf biomass and specific leaf weight CO₂ enrichment

Species	Leaf ₂ area (cm ²)		Leaf weight (g)		Specific ₂ leaf weight (g/cm ² x 100)	
	Control	C1	Control	C1	Control	C1
Syzigium	2000	3099	2863	25.6	37.8	35.0
Champak	1395	2670	2139	8.8	15.6	12.8
Spathodia	1070	1464	2135	7.1	8.8	11.5
Neem	911	983	1056	7.0	7.5	7.1
Sissoo	594	1068	1476	3.6	9.1	10.8
Jack	1189	1657	1683	10.7	13.6	14.3
Eucalyptus	1438	1200	1377	16.4	12.2	14.8
CD at 5%	NS		NS		NS	

Table 7(a): Influence of CO₂ enrichment on Final DM, Δ DM and Root biomass

Treatments	Final DM (g)	Δ DM (g)	Root weight (g)
Control	40.1a	36.7a	15.6
C1	53.9b	50.5b	20.2
C2	55.9b	52.5b	20.9
CD at 5%	6.0	6.0	NS
CD at 1%	8.0	8.0	NS
Soil	51.6	48.1	19.2
Coirpith	48.4	45.0	18.6
CD at 5%	NS	NS	NS

Table 7(b): Final DM, Δ DM and Root biomass of individual species as influenced by elevated CO₂ levels

Species	Final DM (g)		Δ DM (g)		Root weight (g)	
	Control	C1	C2	Control	C1	C2
Syzigium	58.7	87.8	83.5	52.7	81.8	77.5
Champak	24.6	37.0	44.5	22.6	35.0	42.5
Spathodia	43.6	57.4	57.6	40.7	54.5	54.7
Neem	28.0	31.6	32.0	27.2	30.8	31.2
Sissoo	24.2	52.6	59.5	22.4	50.8	57.6
Jack	41.8	55.0	58.9	37.5	50.7	54.6
Eucalyptus	60.1	56.2	55.6	53.8	49.8	49.3
CD at 5%	NS		NS		NS	

Table 8(a): Root/Shoot ratio, LAR and LWR in Control, CO₂-1 and CO₂-2 treatments

Treatments	R/S ratio	LA/DM	L.Wt/DM
Control	0.706	0.324	0.272
C1	0.648	0.340	0.272
C2	0.637	0.339	0.266
CD at 5%	NS	NS	NS
Soil	0.647	0.349	0.283
Coirpith	0.680	0.319	0.256
CD at 5%	NS	NS	NS

Table No.8(b): Influence of CO ₂ enrichment on R/S ratio, LAR and LWR of different species										
Species	R/S ratio		LA/DM				Leaf weight/DM			
	Control	C1	C2	Control	C1	C2	Control	C1	C2	C2
Syzigium	0.406	0.427	0.405	0.344	0.358	0.342	0.437	0.440	0.422	
Champaka	0.611	0.398	0.591	0.580	0.705	0.521	0.361	0.415	0.308	
Spathodia	1.055	1.099	0.723	0.254	0.291	0.385	0.167	0.173	0.214	
Neem	0.651	0.615	0.782	0.321	0.310	0.333	0.255	0.236	0.224	
Sissoo	0.920	0.850	0.785	0.242	0.206	0.250	0.146	0.175	0.182	
Jack	0.696	0.630	0.747	0.287	0.301	0.285	0.264	0.249	0.242	
Eucalyptus	0.605	0.516	0.428	0.238	0.209	0.258	0.271	0.215	0.273	
CD at 5%	NS		NS				NS			

LAR and LWR did not differ in the three treatments. However both LAR and LWR decreased at higher CO₂ levels in spathodia. LWR decreased in neem and jack but increased in sissoo.

Conclusion

The observed differences at the end of 3 months of exposure was not maintained when the duration of treatment was increased to 5 months (Fig. 12). The soil volume could be the major limitation. Growth rates in CO₂ enriched plants had decreased due to the soil volume limitation during this time, control plants which were still smaller grew up narrowing down the differences between enriched and non-enriched plants. So Experiment-1 seems to be more acceptable for studying CO₂ enrichment effects on seedling growth rates.

4.2 Gas exchange characteristics in different species

The observed increase in growth rates when exposed to elevated CO₂ concentrations was predominantly due to higher CO₂ levels and also due to higher RH. RH directly influences VPD, which affects 'gs' and thus the gas exchange characteristics. In order to understand the response of plants to altered environmental condition, in control plants which were grown in



Syzigium



Champaka



Spathodia



Neem



Sissoo



Jack



Eucalyptus

Fig.12 Response of different tree species to the CO₂ enrichment at the end of experiment-II

normal conditions, the difference in assimilation rate 'A', light response curves, difference in 'A' at higher CO₂ levels, the effect of VPD on gas exchange characteristics and VPD response curves were studied. All gas exchange characteristics were studied in attached leaves by using a portable photosynthesis system ADC, LCA-2.

4.2.1 Species variation in "Assimilation" rate

The P_{max} (Assimilation rate at saturated light intensities) of the different species was determined with an objective to assess the species variation in 'A' and 'gs' and also to arrive at 'A/gs' values which is a measure of intrinsic WUE at the leaf level. In this experiment, VPD at which 'A' rate was determined was quite high and ranged between 30 to 35 millibars except in eucalyptus where it was 39 millibars. Since 'A' was measured at similar VPD, the species differences in gas exchange traits are comparable.

There was a considerable difference among the species in terms of their 'A', 'gs' and 'A/gs' ratios (Table 9). Sissoo and eucalyptus had a higher 'A' followed by neem, jack and spathodia. Syzigium and champaka had lower assimilation rates.

Stomatal conductances of all the species except

Table No. 9: Gas exchange characteristics of different species

Species	Light intensity ($\mu\text{mols m}^{-2}\text{s}^{-1}$)	RH (%)	VPD m bars	Leaf tempera- ture $^{\circ}\text{C}$	Ci ppm	A u mols $\text{m}^{-2}\text{s}^{-1}$	gs $\mu\text{mol m}^{-2}\text{s}^{-1}$	A/gs
Syzigium	2200	42	35.48	33.10	232.7	6.20+0.26	111.04+2.33	0.056
Champak	2200	35	32.10	31.5	236.8	5.12+0.01	101.6+1.25	0.050
Spathodia	1825	30	30.5	30.2	243.3	7.35+0.04	161.0+0.05	0.046
Neem	2100	39	29.8	32.1	263.0	9.42+0.89	253.0+0.83	0.037
Sissoo	2100	34	31.6	32.1	218.3	16.17+0.04	277.0+2.70	0.058
Jack	1700	42	27.9	32.0	266.2	8.91+0.14	265.1+1.19	0.034
Eucalyptus	2500	40	39.3	33.6	271.2	12.16+0.04	409.0+1.1	0.029
CD at 5%						1.67	4.35	

eucalyptus were generally lower. Eucalyptus had a 'gs' of $400 \text{ m mols m}^{-2} \text{ s}^{-1}$. 'gs' of jack, sissoo and neem were comparable which were around $200 \text{ m mols m}^{-2} \text{ s}^{-1}$. Once again syzigium and champaka had a very low conductance values.

'A/gs' values also varied a lot among the species. Sissoo, syzigium and champaka had higher 'A/gs' values where as in eucalyptus, it was very low. Though sissoo had a relatively higher 'gs', its high 'A' had led to a high 'A /gs' value. In champaka and syzigium, both 'A' and 'gs' were low. So 'A' per unit conductance had increased. Eucalyptus though had a higher 'A', its 'A/gs' value was less because of a very high conductance.

In order to understand the difference in the conductances among these species, an attempt was made to study the stomatal frequencies in these species (Table 10). Sissoo and eucalyptus were found to be amphistomatous and rest all were hypostomatous. The amphistomatal nature of sissoo and eucalyptus explains its higher conductance. Among the hypostomatal species, jack and spathodia had a greater stomatal number averaging around 43,000 to 48,000 per cm^2 of leaf area. Neem and syzigium had lower stomatal number per unit leaf area, lowest being in syzigium. Though champaka

Table 10: Stomatal frequency (no./cm²) in different species

Species	Upper surface	Lower surface
Syzigium	0	26259
Champaka	0	35217
Spathodia	0	48137
Neem	0	29279
Sissoo	2229	24005
Jack	0	43274
Eucalyptus	14928	32165

showed lower 'gs' based on porometric observations (LCA-2), its stomatal number was higher than that of neem.

4.2.2 Light response curves

Light response curves were studied in different species with an objective of studying the 'A' response to PFD also to arrive at the PFD at which 'A' was saturating. Experiments were conducted with normally grown control plants acclimatized to high light intensities. The instantaneous response of the plants to the altering light intensities were recorded (Figs. 13 to 18).

In general 'A' response to light is a curve-linear, biphasic curve. Spathodia and champaka showed the well accepted biphasic response. In both the species plateauing was seen at $1500 \text{ u mols m}^{-2}\text{s}^{-1}$. Saturation was not reached in neem, sissoo and jack even upto $2200 \text{ u mols m}^{-2}\text{s}^{-1}$. In neem the initial slope of the curve reduced after 700 to 800 $\text{u mols m}^{-2}\text{s}^{-1}$ nevertheless 'A' went on increasing with light intensity even upto $2500 \text{ u mol m}^{-2}\text{s}^{-1}$. Jack and sisoo showed a linear response to light, at all levels of light till $2500 \text{ u mol m}^{-2}\text{s}^{-1}$.

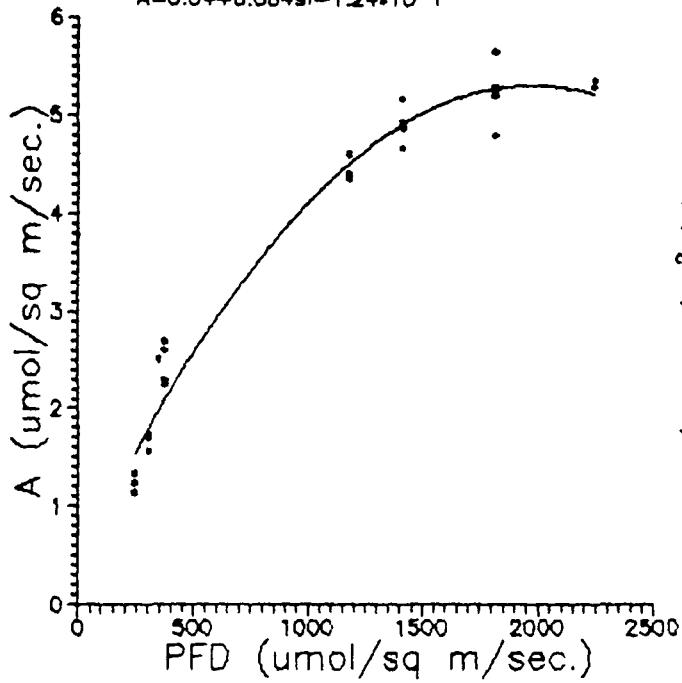
Light has a very direct effect on conductances.

So the 'gs' response to light was also studied. Differential responses were noticed among the species. In champaka, the response of 'gs' to PFD was parallel to the response of 'A' to PFD, both saturating at $1500 \text{ u mol m}^{-2}\text{s}^{-1}$. Spathodia showed a linear response till $1700 \text{ u mol m}^{-2}\text{s}^{-1}$ and later tended towards saturation. The 'gs' response to PFD was similar in jack, sissoo and eucalyptus, response increasing with increasing light intensities. 'gs' continued to increase even upto $2500 \text{ u mol m}^{-2}\text{s}^{-1}$.

Light affects both 'A' and 'gs' but the response of plants to light in terms of 'A' and 'gs' may not be similar. In this context, study of response of 'A' to 'gs' would become interesting. 'A' was plotted against 'gs', which was changing as a consequence of changing light intensities (Figs. 13 and 14). Champaka and spathodia showed a more or less linear response of 'A' to 'gs' at all levels of 'gs'. Here it could be recalled the response of 'gs' and 'A' to light was parallel. In eucalyptus, 'A' increased at a higher rate at lower light intensities whereas 'gs' increased at a higher rate at higher light intensities. As a consequence of this, 'A' seemed to plateau off at higher levels of 'gs'.

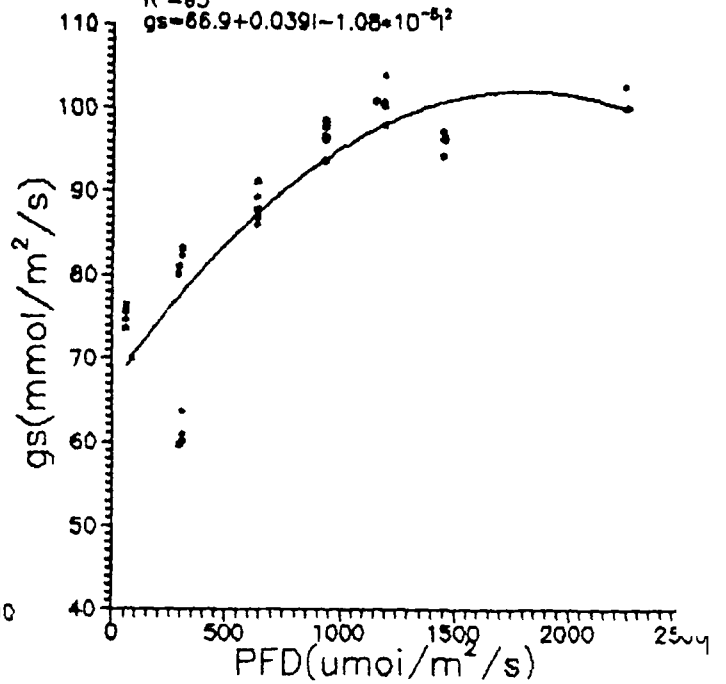
When 'A' was plotted against 'gs' across all

LIGHT RESPONSE CURVE OF CHAMPAKA
 $R^2=97$
 $A=0.04+0.0049I-1.24 \times 10^{-6}I^2$



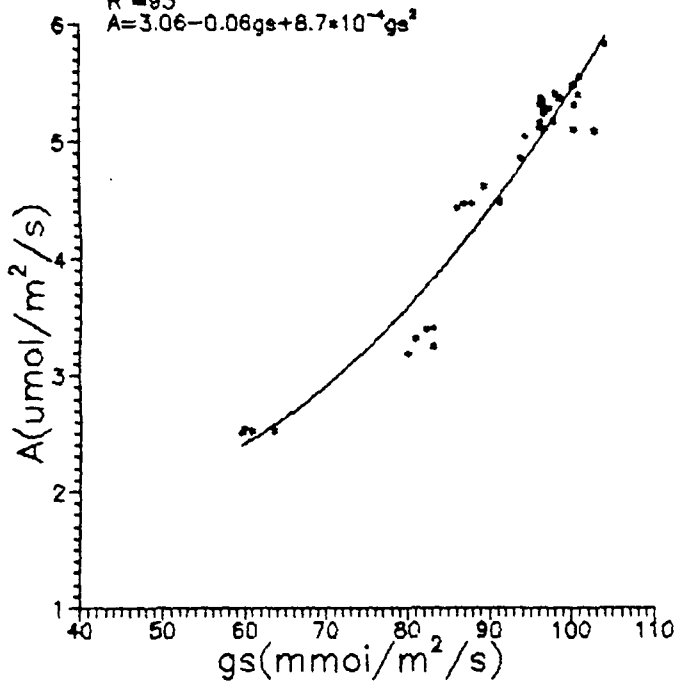
(a) A/PFD curve

g_s/PFD in champaka
 $R^2=95$
 $g_s=66.9+0.039I-1.08 \times 10^{-6}I^2$



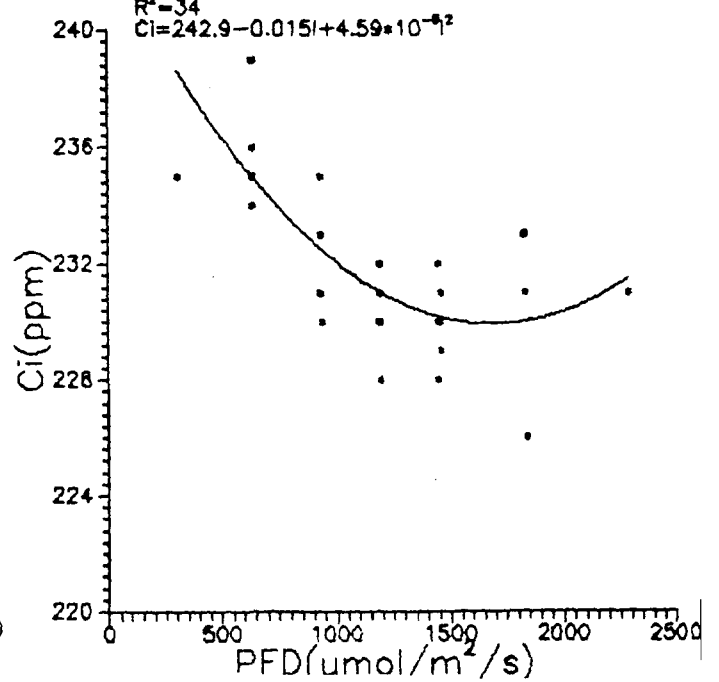
(b) g_s/PFD curve

A/g_s in Champaka
 $R^2=83$
 $A=3.06-0.06g_s+8.7 \times 10^{-4}g_s^2$

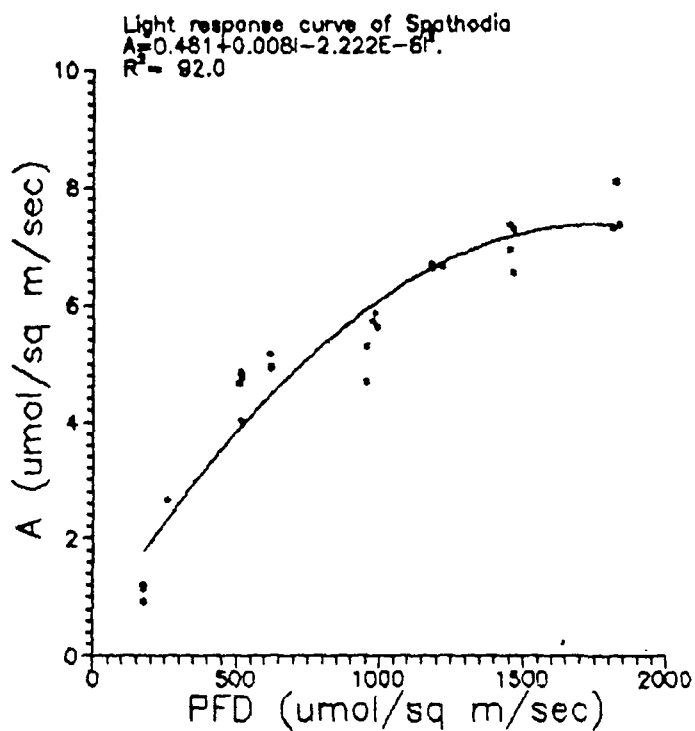


(c) A/ g_s curve

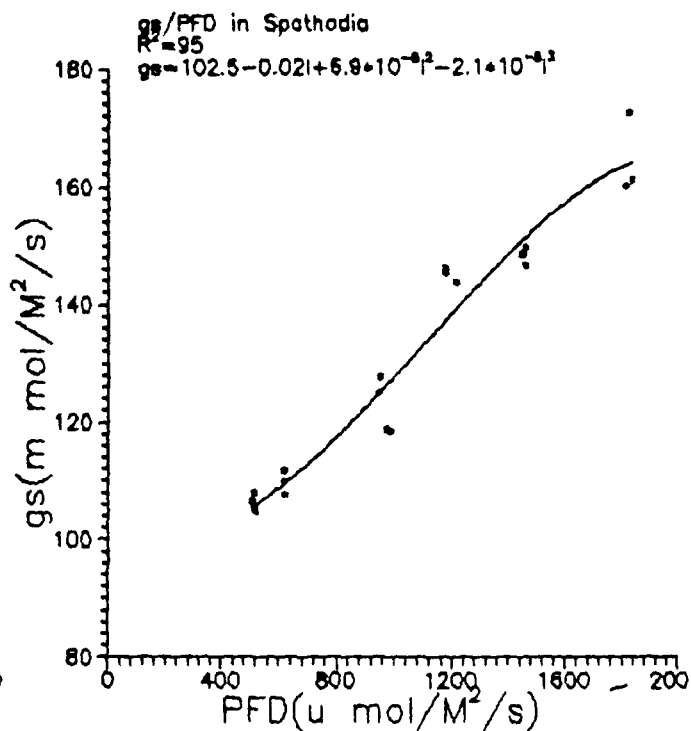
C_i/PFD in Champaka
 $R^2=34$
 $C_i=242.9-0.015I+4.59 \times 10^{-6}I^2$



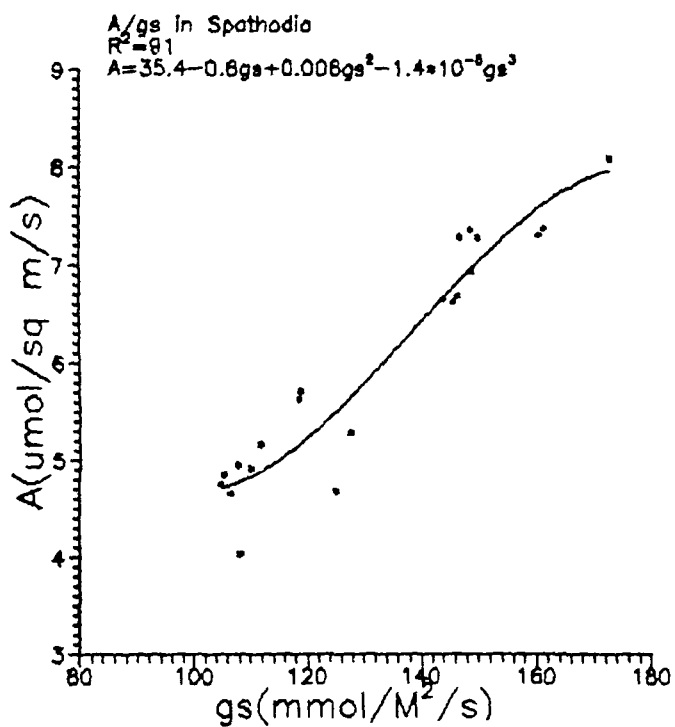
(d) C_i/PFD curve



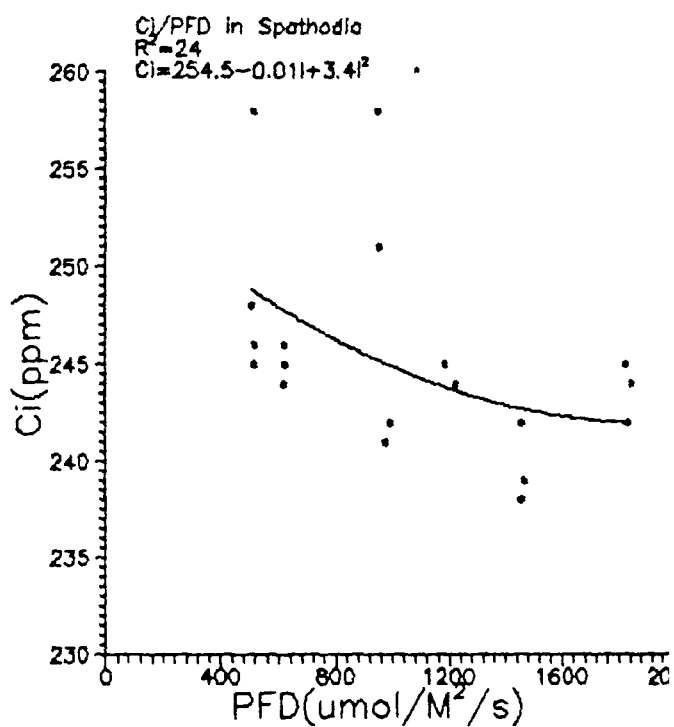
(a) A/PFD curve



(b) g_s /PFD curve

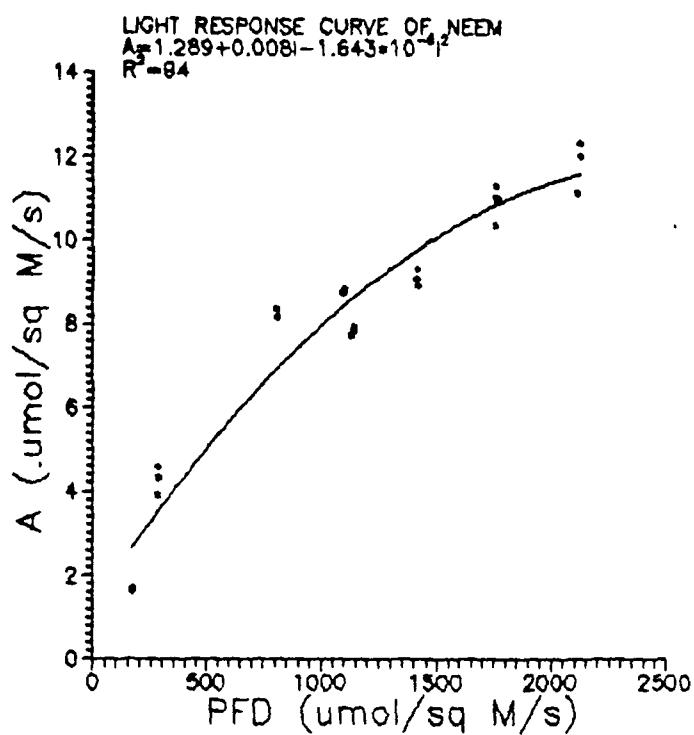


(c) A/ g_s curve

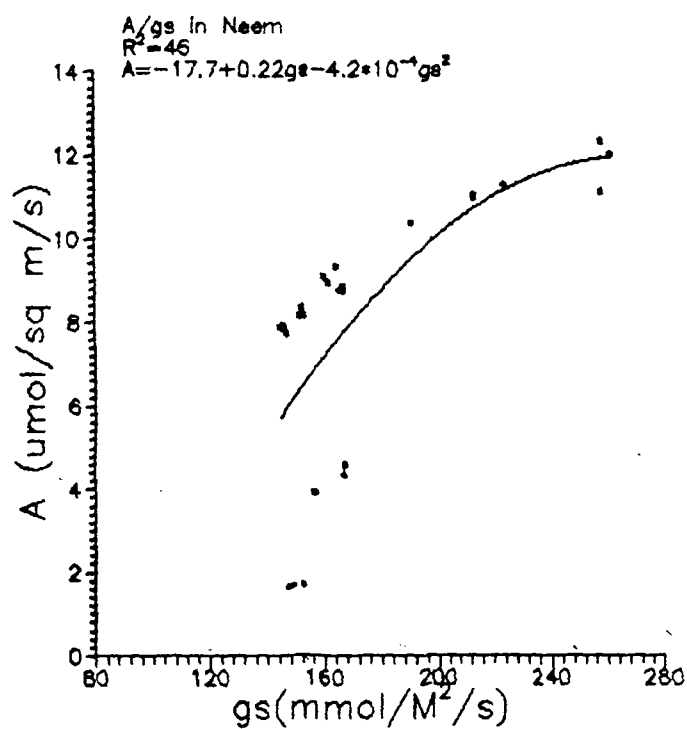


(d) C_i /PFD curve

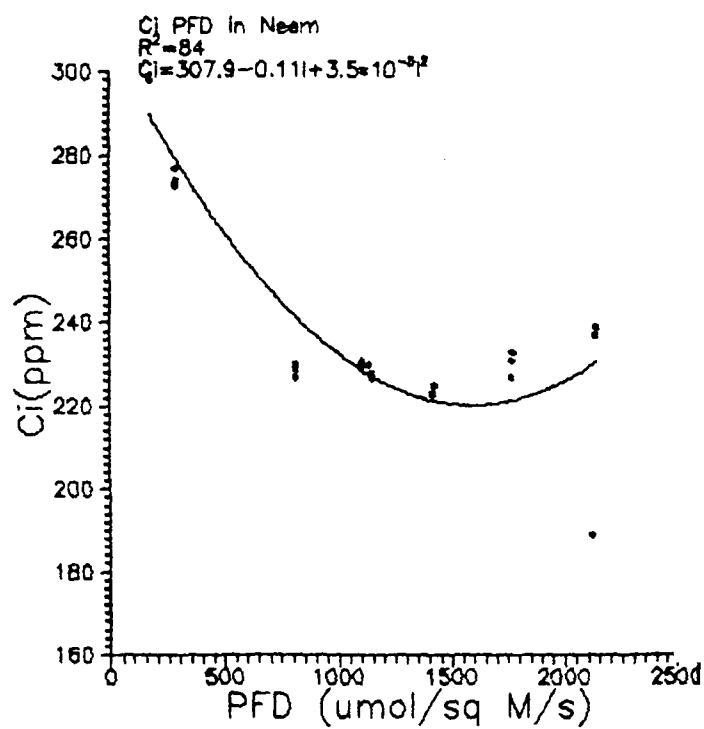
Fig.14: Light response curves in *Spathodia*.



(a) A/PFD curve

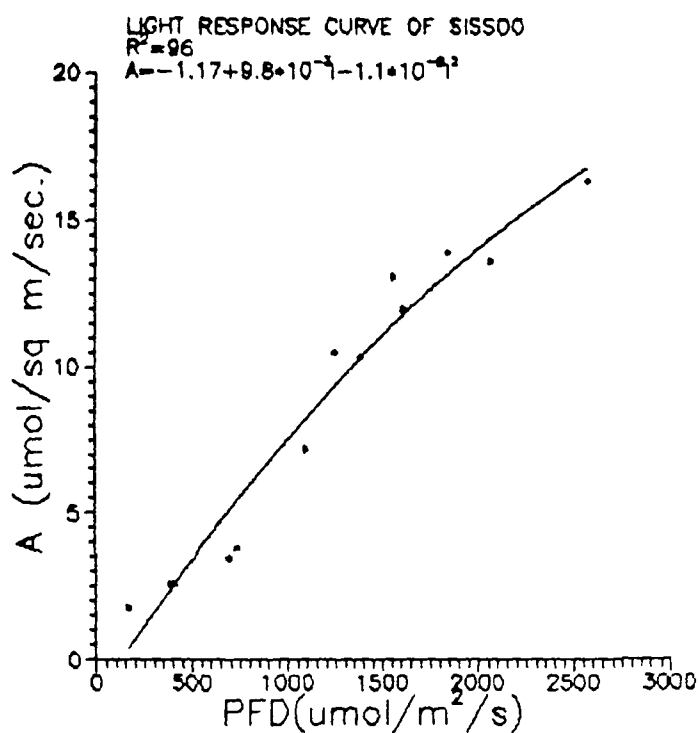


(b) A/gs curve

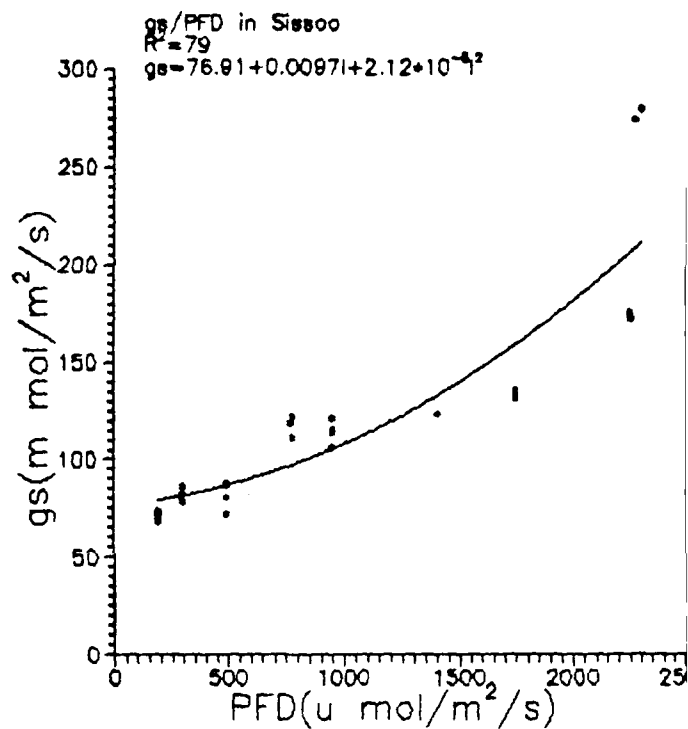


(c) C_i /PFD curve

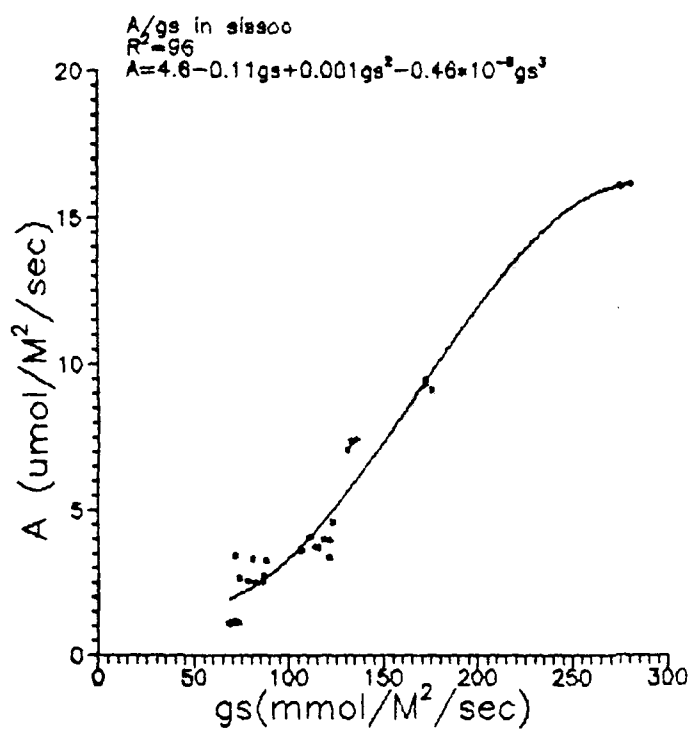
Fig.15: Light response curves in Neem.



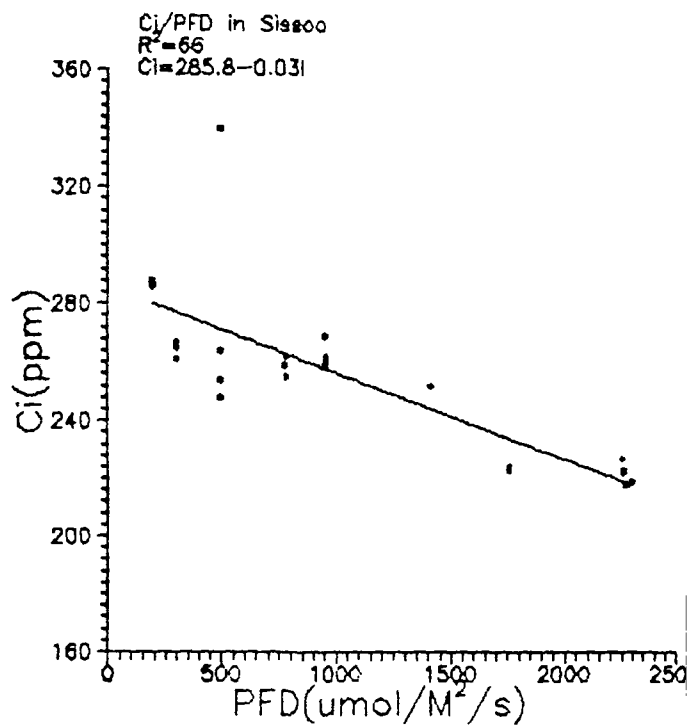
(a) A/PFD curve



(b) gs/PFD curve

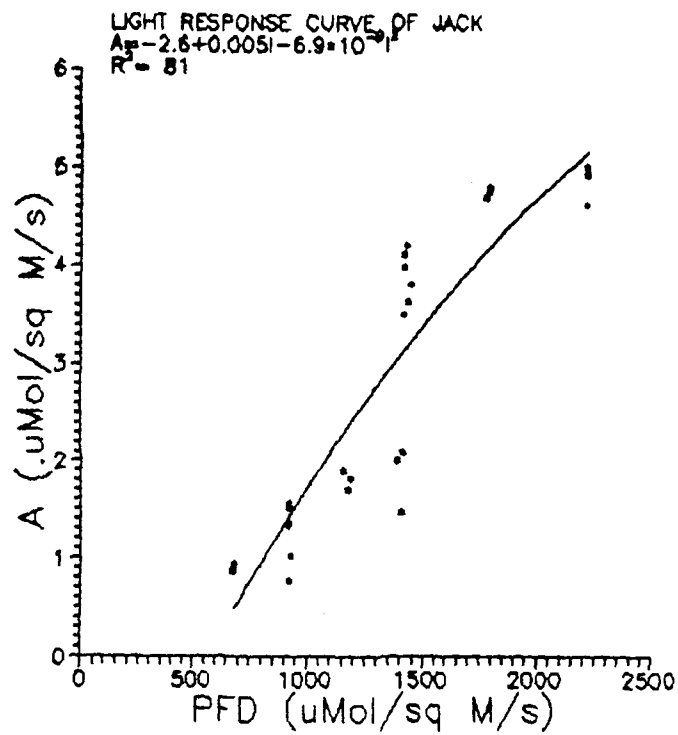


(c) A/gs curve

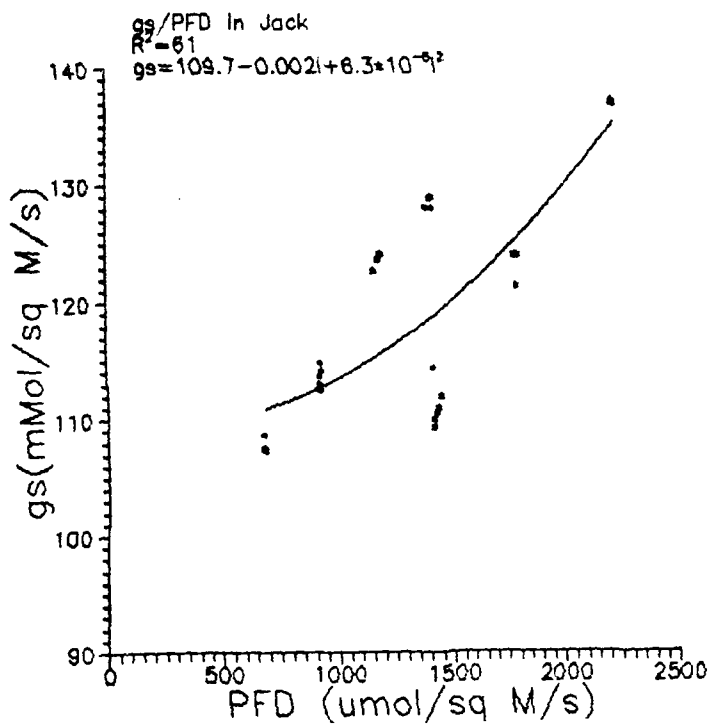


(d) Ci/PFD curve

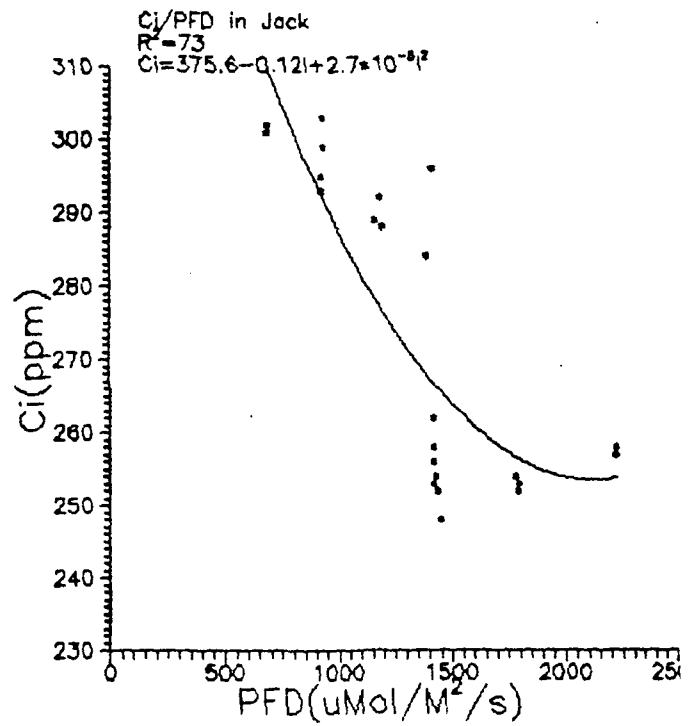
Fig.16:Light response curves in Sisso.



(a) A/PFD curve

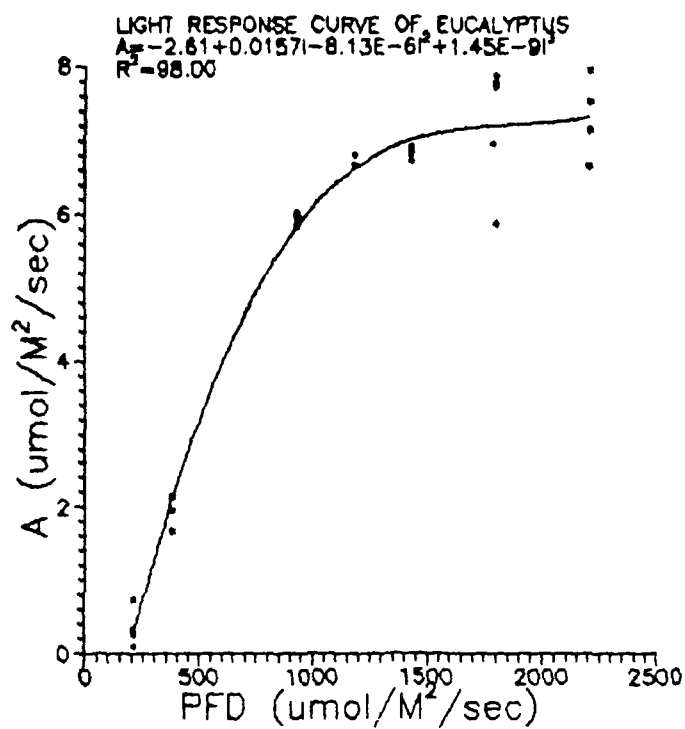


(b) g_s/PFD curve

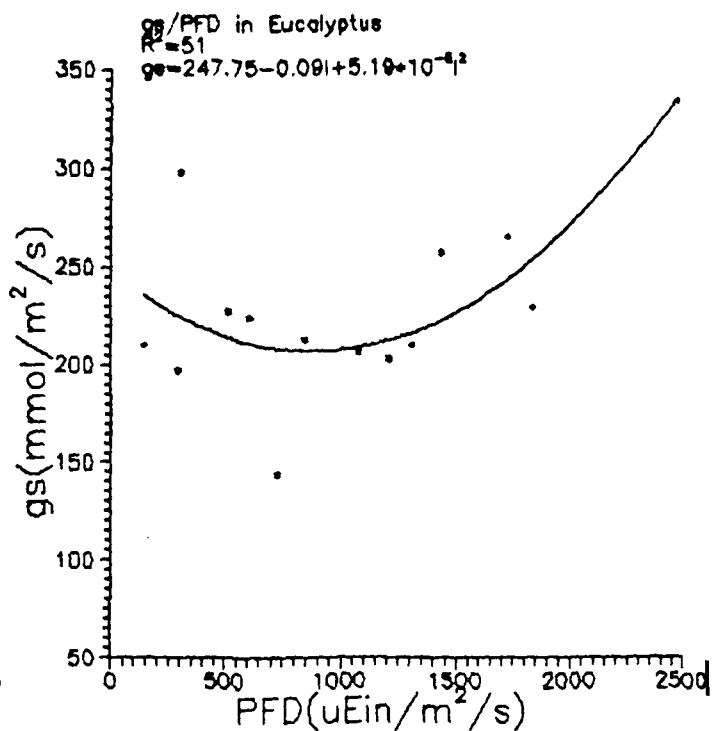


(c) C_i/PFD curve

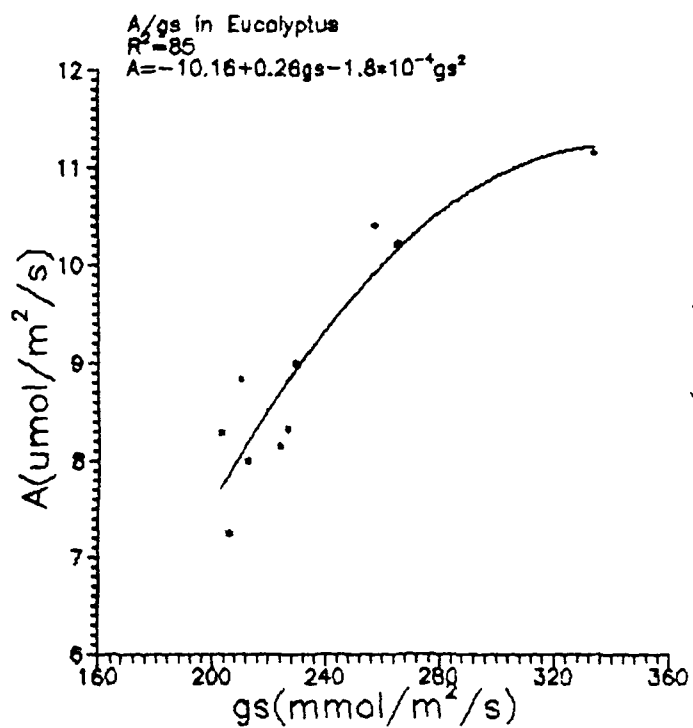
Fig.17: Light response curves in Jack.



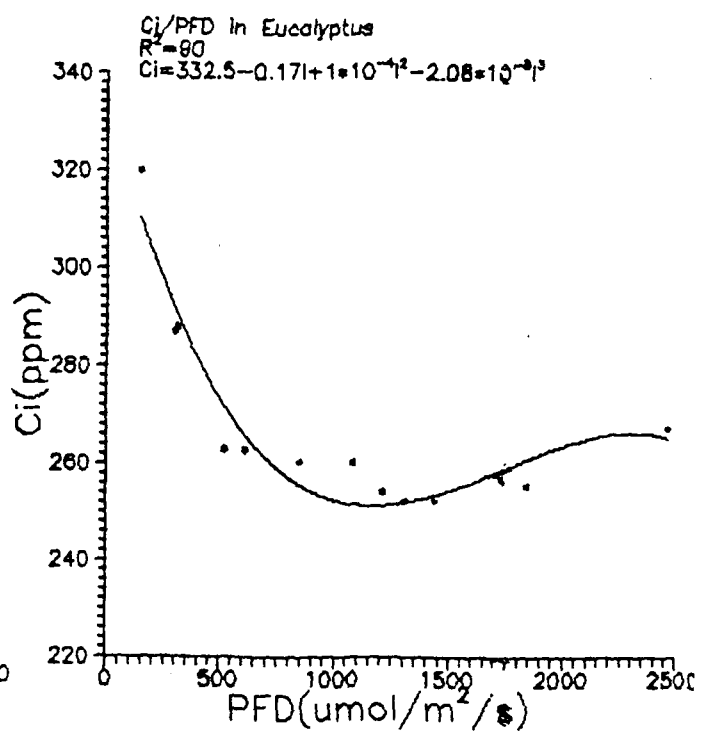
(a) A/PFD curve



(b) gs/PFD curve



(c) A/gs curve



(d) Ci/PFD curve

Fig.18: Light response curves in Eucalyptus.

species, 'A' was found to linearly increase with 'gs' (Fig. 19).

'A/gs' values at different light intensities are given in the table 11. The light intensity at which 'A/gs' is maximum could be considered optimum for the plants for maximum WUE. These values give the intrinsic WUE of the plants at the leaf level at different light intensities. 'A/gs' values were maximum at a light intensity of 1400 to 1800 $\mu\text{mol m}^{-2}\text{s}^{-1}$ in neem, sissoo, jack and eucalyptus (Fig. 20). In champaka, maximum 'A/gs' values were seen at 100 PFD. 'A/gs' values reached its maximum very early in syzigium and spathodia, at 900 and 700 $\mu\text{mol m}^{-2}\text{s}^{-1}$ respectively. But in syzigium, the value decreased at higher light intensities but in spathodia it varied a little till 1800 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

Light levels can alter C_i in two ways by increasing 'A' and by increasing 'gs'. When C_i was plotted against light, two types of response could be seen (Figs. 13 to 18). In eucalyptus, and neem, champaka and jack, there was a dramatic decrease in C_i levels till 1300 to 1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and later plateaued off. In spathodia and sissoo, the decrease in C_i was linear.

Table 11 : Assimilation to conductance ratios (A/g_s) at different light intensities

Light intensity u mols m ⁻² s ⁻¹	Syzigium	Champaka	Spathodia	Neem	Sissoo	Jack	Eucalyptus
300	0.0290	0.0253	N.A.	0.0260	0.0080	0.0083	0.0290
500	NA	NA	0.0460	0.0430	N.A.	0.0098	NA
900	0.0470	NA	0.0440	0.0540	0.0132	0.0098	0.0470
1100	0.0450	0.0420	0.0460	0.0550	0.0241	0.0150	0.0450
1400	0.0460	0.0330	0.0470	0.0570	0.0310	0.0360	0.0460
1800	0.0420	0.0357	0.046	0.0510	0.0390	0.0390	0.0420
2200	0.0400	0.0317	NA	0.0460	0.0370	0.0360	0.0400

NA - Not Available

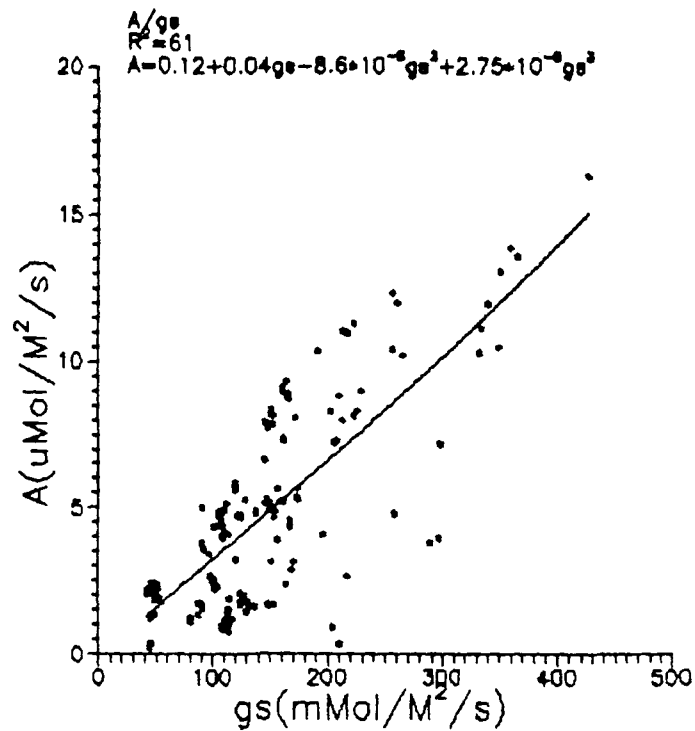


Fig.19: Inter-relationship between 'A' and 'g_s' in different tree species.

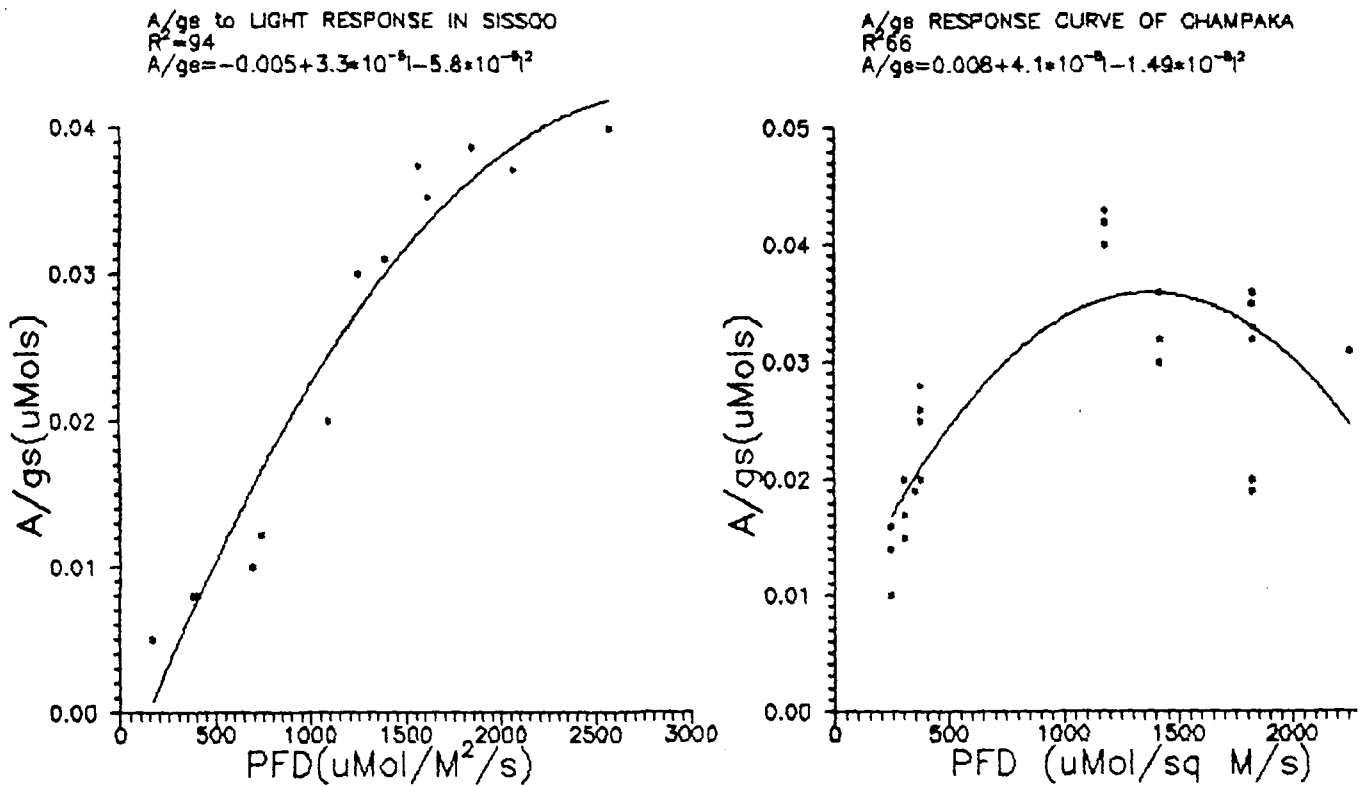


Fig.20: Relationship between A/g and PFD in Sissoo and Champaka.

4.2.3.1 Response of 'A', 'gs' to CO₂ levels

The response of 'A' and 'gs' to the CO₂ levels was studied. To determine this, gas exchange characters were studied in control plants at two levels of CO₂ ambient and 750 ppm of CO₂. Same leaf was used to study gas exchange characters at both the levels of CO₂. The response determined in this study was instantaneous. A single intact leaf of the seedling was clamped in the Parkinson's leaf chamber and air containing two levels of CO₂ was supplied to the same leaf, first ambient and then air containing higher CO₂ concentration. ADC-LCA-2 was used in this study. Data was logged only after the stabilisation of 'A' and 'gs' values.

The response of 'A' and 'gs' is given in Table 12. All the species showed a very large increase in 'A' when the CO₂ concentration was increased. Maximum response was seen in spathodia, champaka and sissoo where the 'A' doubled at high CO₂. Rest of the species increased its 'A' by about 65 to 85 per cent (Fig. 21).

'gs' values in general reduced at high CO₂ concentrations. Syzigium and champaka were found to be very sensitive to elevated CO₂. They responded by a large reduction in their conductances. Eucalyptus, spathodia and jack were relatively less responsive

Table 12: Effect of higher CO₂ concentration (Ca) of 'A' and 'gs'

Species	CO ₂ concn. ppm	VPD mbars	ci ppm	A u molsm ⁻² s ⁻¹	gs mmolsm ⁻² s ⁻¹	A/gs
Syzigium	335.3* 775.4	24.17 26.87	285.47 665.17	3.97+0.71 7.29+0.80	186.27+6.97 150.26+6.96	0.021 0.049
Champaka	337.2 739.6	30.25 32.23	282.75 563.52	1.96+0.41 4.06+0.11	89.8+4.65 57.8+1.08	0.020 0.080
Spathodia	340.8* 764.2	26.94 28.88	250.06 542.38	8.61+0.35 20.45+0.23	195.28+3.00 180.48+0.69	0.044 0.114
Neem	328.0* 737.0	17.80 20.03	279.20 738.60	8.96+0.56 15.58+0.00	393.27+1.37 296.00+0.00	0.023 0.053
Sissoo	346.0* 773.2	27.76 31.90	227.21 552.60	9.95+0.27 21.31+0.27	165.88+2.95 124.22+0.52	0.060 0.172
Jack	338.5* 697.5	27.89 29.88	266.21 552.59	8.91+0.14 15.82+0.14	265.13+1.19 234.24+1.46	0.034 0.068
Eucalyptus	338.4* 649.6	19.15 20.50	277.55 541.13	12.97+0.24 21.36+0.59	477.98+2.11 436.78+4.72	0.027 0.056

*ambient CO₂ concentration

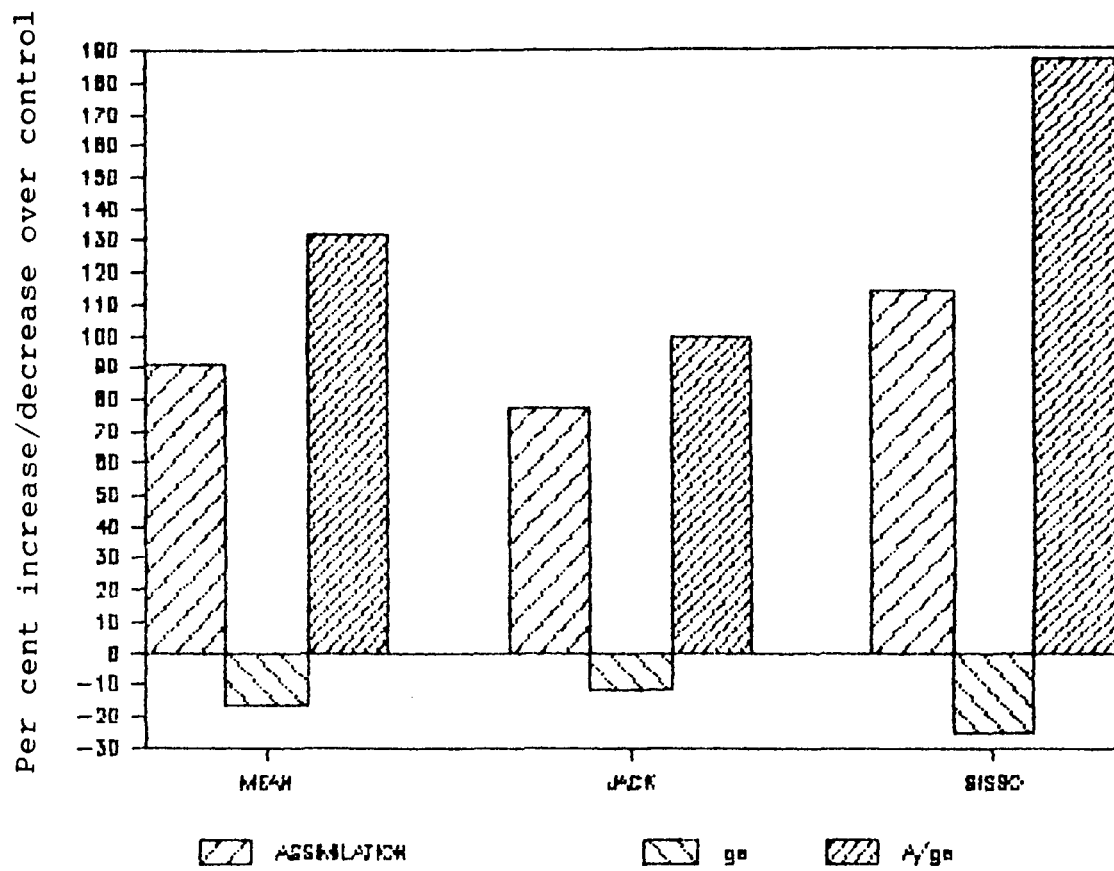


Fig.21: Effect of CO_2 on gas exchange characteristics.

where the 'gs' reduced only by 10 per cent. Neem and sissoo were found to be moderately responsive to elevated CO_2 in terms of their 'gs' values.

'A/gs' values increased at higher CO_2 level. The increase was more than 100 per cent in sissoo and champaka. In other species, the 'A/gs' values nearly doubled at elevated CO_2 levels. This clearly indicates that the plants will have a higher intrinsic WUE at high CO_2 levels which could be achieved in two ways - increase in 'A' and decrease in 'gs'.

4.2.3.2 Assimilation response curves to CO_2 (A/Ci curves)

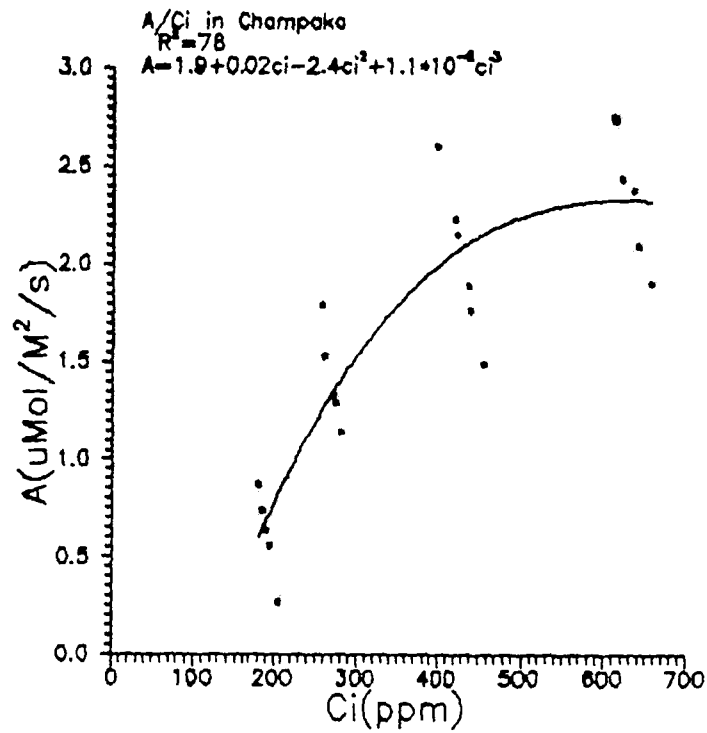
Assimilation response curves to CO_2 (A/Ci) are developed by plotting 'A' against internal CO_2 concentration (Ci). The plot indicates the response of 'A' to the CO_2 concentration when stomatal limitations are absent. This increase in A/Ci curve is also used to measure and separate the relative stomatal and mesophyll limitation.

Gas exchange characteristics were studied at five or six levels of CO_2 and were plotted as a function of intercellular CO_2 concentration. Assimilation, 'gs' and intercellular CO_2 concentrations were determined at various levels of ambient CO_2 .

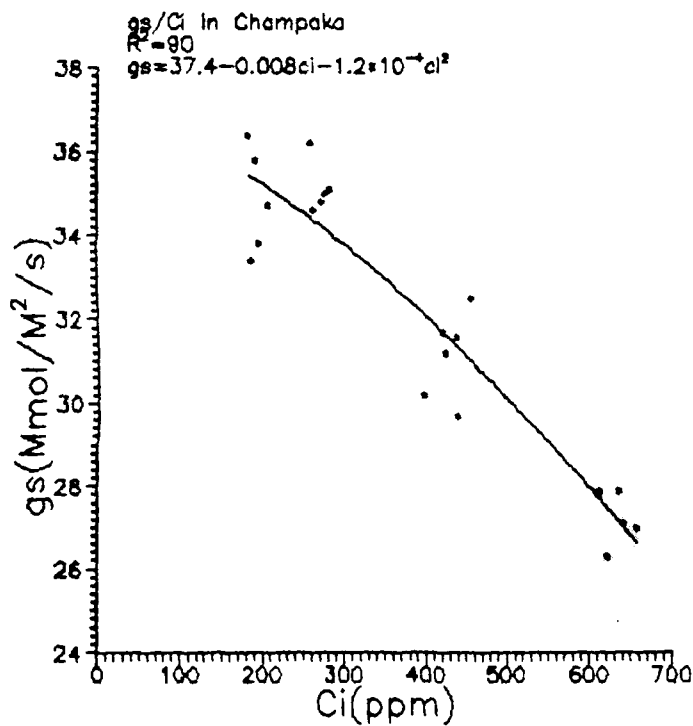
A/Ci curves : A/Ci curves were developed only in four species - jack, eucalyptus, spathodia and champaka (Figs. 22 to 25). As seen from the data on the dry-matter accumulation, the plants based on their response to CO₂ enrichment could be grouped into highly responsive, moderately responsive and marginally responsive. Jack belongs to the first group, champaka and eucalyptus to the second group and spathodia to the third group.

A/Ci curves are biphasic with an initial linear response followed by a reduced response at higher levels of Ci (Figs. 22 to 25), spathodia, champaka and eucalyptus showed a linear increase in 'A' till 400 to 480 ppm Ci and later plateaued off but the change in 'A' for a unit change in Ci was greater in eucalyptus till 400 ppm Ci, as compared to champaka and spathodia. In jack, 'A/Ci' curve was slightly different. 'A' increased linearly with Ci and continued to be so even at 600 ppm Ci.

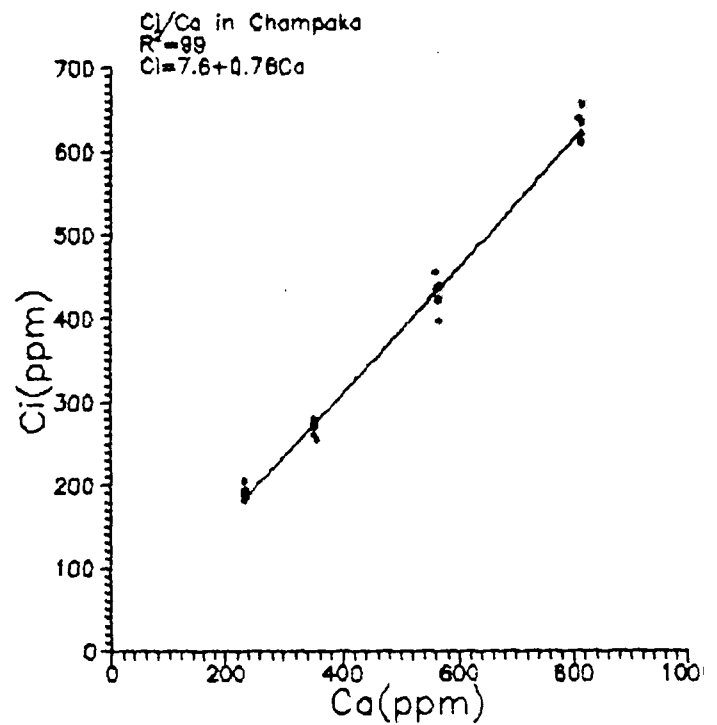
The response of 'gs' to Ci was not uniform in all the species (Figs. 22 to 25). In three out of the four species, 'gs' decreased with increasing Ci. In eucalyptus, 'gs' decreased markedly till 250 ppm Ci and later stabilised. In spathodia, stomata was almost insensitive till 250 ppm of Ci and on further increase



(a) A/Ci curve

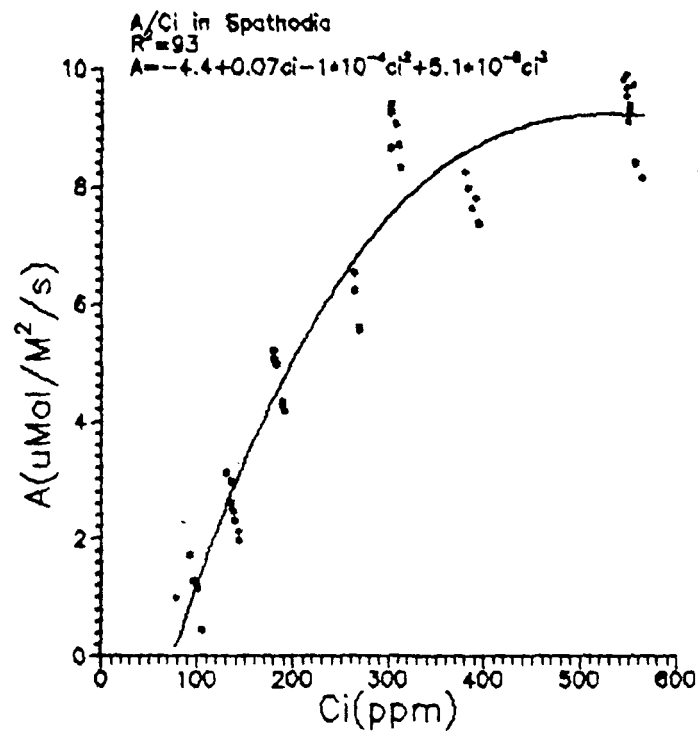


(b) Ci/gs curve

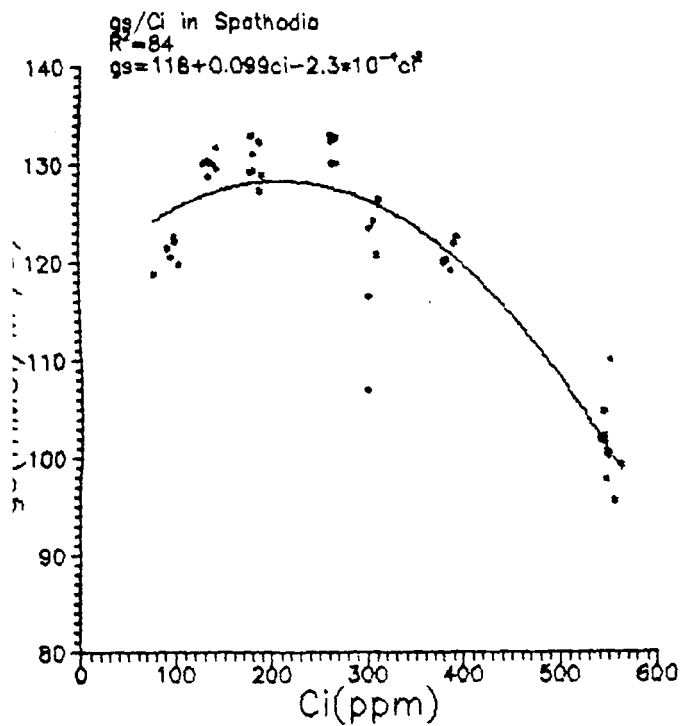


(c) Ca/Ci curve

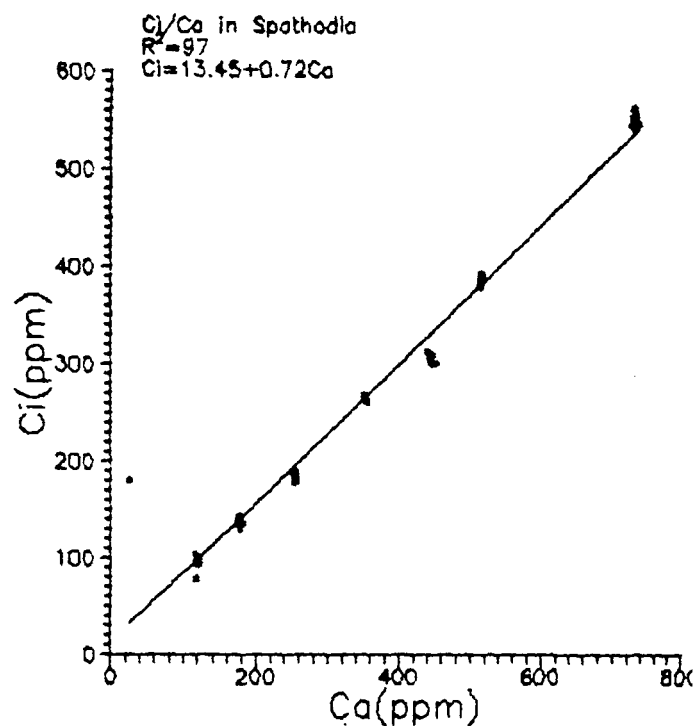
Fig.22: Internal CO₂ concentration response curves
 (A/Ci) in Champaka.



(a) A/Ci curve

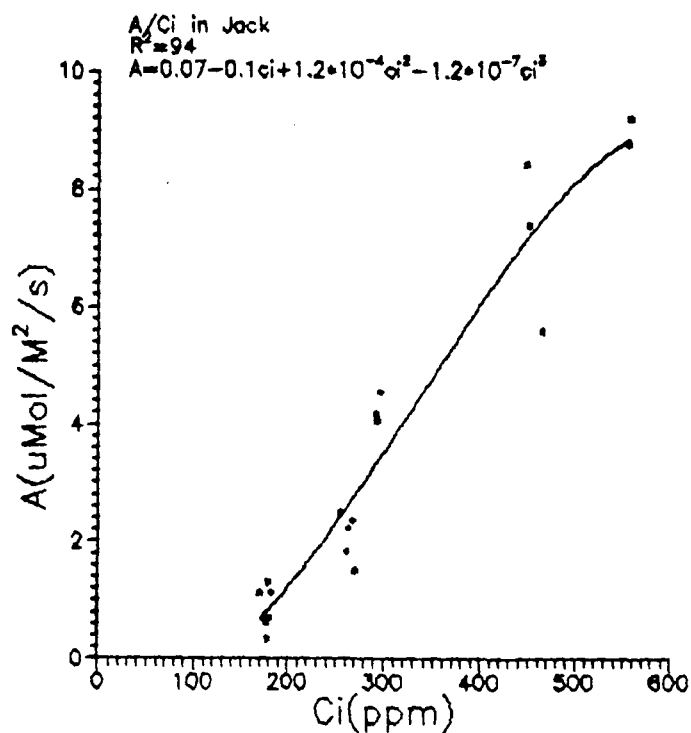


(b) Ci/gs curve

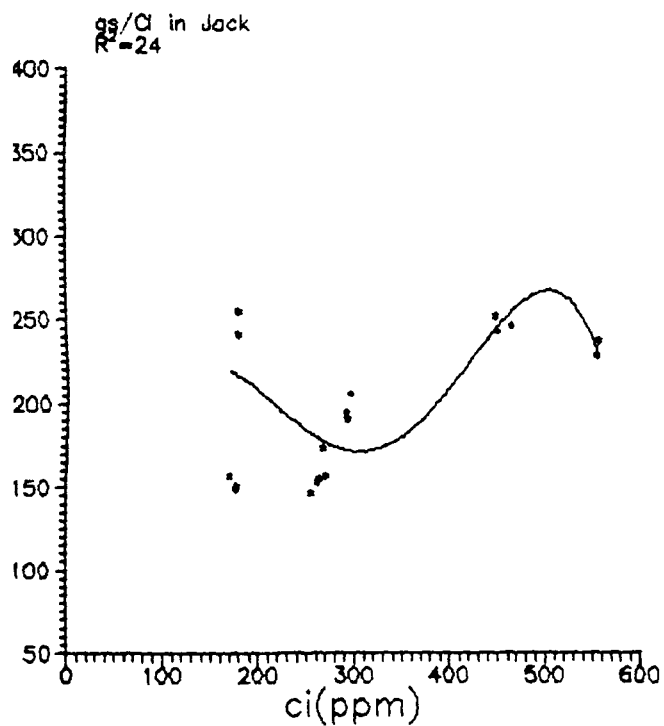


(c) Ca/Ci curve

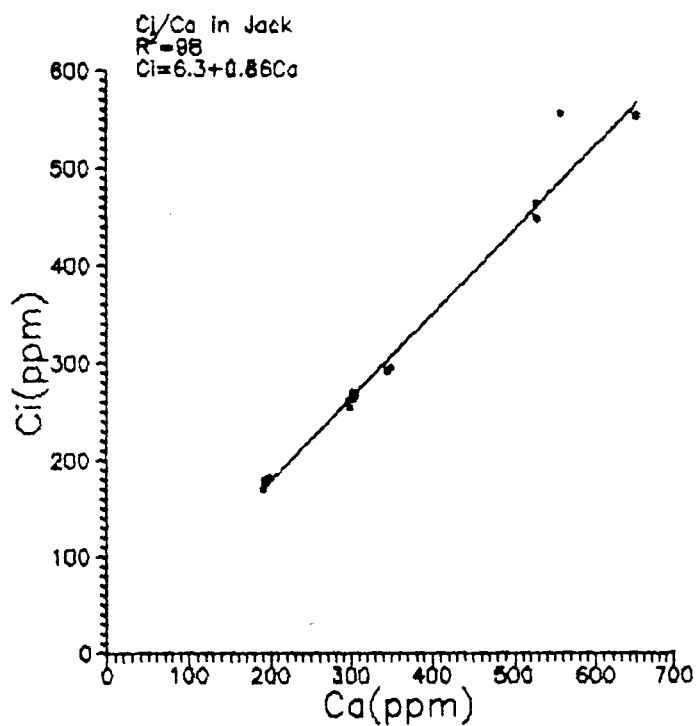
Fig.23: Internal CO₂ concentration response curves
 (A/Ci) in Spathodia.



(a) A/Ci curve

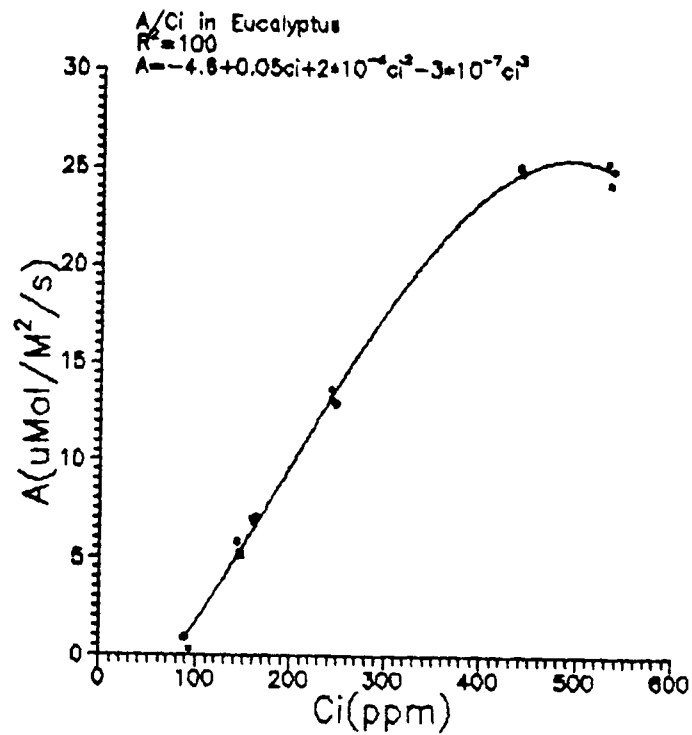


(b) Ci/gS curve

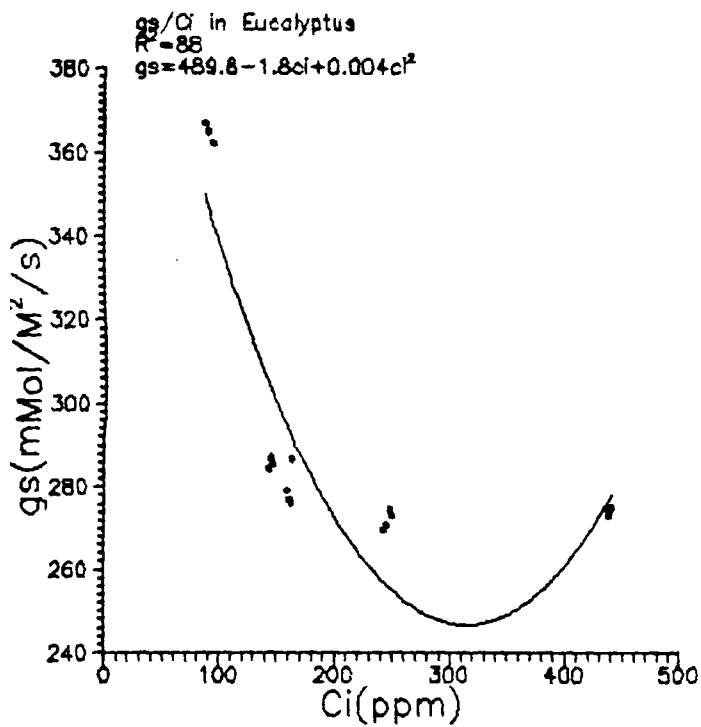


(c) Ca/Ci curve

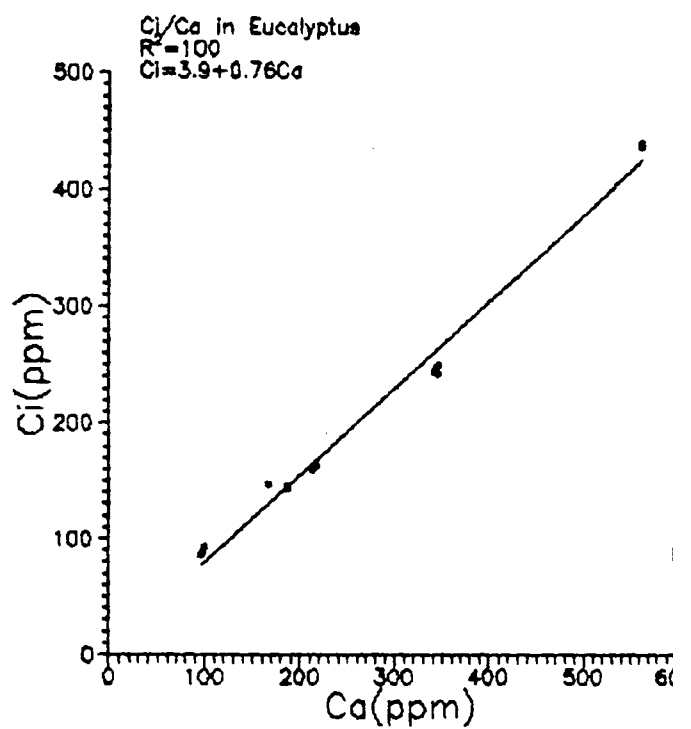
Fig.24: Internal CO₂ concentration response curves
 (A/Ci) in Jack.



(a) A/Ci curve



(b) Ci/gs curve



(c) Ca/Ci curve

Fig.25: Internal CO₂ concentration response curves
 (A/Ci) in Eucalyptus

in C_i , ' g_s ' reduced linearly. In champaka, ' g_s ' showed an uniform reduction with C_i . No such trend was seen in jack. Here ' g_s ' was not affected by C_i .

The C_i levels and its variation with C_a was studied as ' A ' and ' g_s ' depends mainly on C_i and not on C_a (Figs. 22 to 25). Even though higher CO_2 levels reduce ' g_s ' and increase ' A ', C_i increased linearly with C_a at all concentrations of C_a upto 700 ppm in all species. However C_i/C_a ratio varied among species. *Spathodia*, champaka and eucalyptus had a C_i/C_a ratio of 0.76 whereas jack had a ratio of 0.85 at all concentrations of C_a . In all the species, ' A ' and ' g_s ' have varied in such a way so as to maintain a constant C_i/C_a at all levels of C_a .

4.2.3.3 Relative stomatal and mesophyll limitation to Assimilation in a few species

Relative stomatal and mesophyll limitations to Assimilation was estimated in a few species (Table 13). There was no mesophyll limitations in champaka, jack and eucalyptus and the limitation to assimilation was only due to stomata. In *spathodia*, mesophyll limitation was higher than stomatal limitation. In all these species, there is a possibility of increasing ' A ' by increasing stomatal conductances.

Table 13 : Relationship between stomatal and mesophyll limitation to Assimilation in a few species

Species	A _O	A	A _g	ls	lm	ls as % of total limitation	lm as % of total limitation	(dA/dci) x 10
Champaka	1.75	1.25	1.25	28.6	0	100	0	0.07
Spathodia	7.90	6.60	8.40	16.5	21.4	43.95	56.05	0.23
Jack	4.30	3.20	3.20	25.6	0.0	100.0	0	0.25
Ecualyptus	20.0	14.50	14.50	27.50	0.0	100.0	0	0.93
A _O = u mol m ⁻² s ⁻¹	A = u mol m ⁻² s ⁻¹			dA/dci = u mol m ⁻² s ⁻¹ ul l ⁻¹				
ls = $\frac{A_O - A}{A_O} \times 100$	lm = $\frac{A_g - A}{A_g} \times 100$							

4.2.4 Response of 'A' and 'gs' to VPD

VPD had a direct effect on stomata and can alter the 'gs'. So VPD indirectly affects 'A' of the plant. So the response of gas exchange characteristics as affected by different levels of VPD was studied in all the species. The response of plants to an instantaneous change in the VPD, due to a change in RH was determined using ADC-LCA-2. Sufficient time was given for the stabilisation of 'A' and 'gs'.

Refer table 14, 'gs' decreased with an increase in VPD in all the species. However, the magnitude of decrease was different. *Spathodia* and *jack* decreased its 'gs' by 64 and 58 per cent respectively whereas the decrease in *champaka* and *sisoo* was 21 per cent. The decrease in 'A' in the remaining three species was about 29 per cent (Fig. 26).

Assimilation decreased at a higher VPD in all species. The decrease was within 20 per cent in all species except *champaka* and *syzigium*. *Champaka* showed an incredible decrease of 77 per cent to be followed by *syzigium* which decreased response of 'A' and 'gs' to VPD by 29 per cent. In *eucalyptus*, 'A' was least affected and the decrease was very low (Fig. 26).

Table 14: Effect of VPD on A and gs in different species

Species	VPD millibars	RH %	Leaf Temp. °C	A $\mu\text{mol m}^{-2}\text{s}^{-1}$	gs $\text{mmol m}^{-2}\text{s}^{-1}$	Ci ppm	A/gs
Syzigium	23.76	49	30.56	8.74+0.21	157.52+0.50	241.78	0.056
	35.48	42	33.10	6.20+0.26	111.04+2.33	232.72	0.056
Champaka	19.86	56	28.80	5.87+0.29	46.96+1.52	130.58	0.125
	32.27	32	31.18	1.32+0.24	36.70+0.27	270.48	0.036
Spathodia	17.90	54	28.00	5.22+0.02	304.0+5.40	288.20	0.017
	29.10	30	29.13	4.47+0.18	110.7+0.84	246.57	0.041
Neem	17.39	62	30.19	11.53+0.49	345.9+7.98	279.37	0.034
	29.80	39	32.10	9.42+0.89	253.0+0.83	263.10	0.037
Sissoo	15.17	63	29.87	14.87+0.20	454.7+11.23	279.35	0.035
	26.27	46	32.63	12.53+0.52	357.5+0.72	267.40	0.035
Jack	17.05	60	28.83	6.64+0.35	271.63+0.87	291.68	0.025
	34.33	31	32.00	5.34+0.31	113.48+0.41	247.15	0.052
Eucalyptus	14.90	63	28.0	12.75+0.05	357.40+32.8	275.0	0.036
	25.90	35	28.5	12.15+0.12	268.62+1.95	253.5	0.045

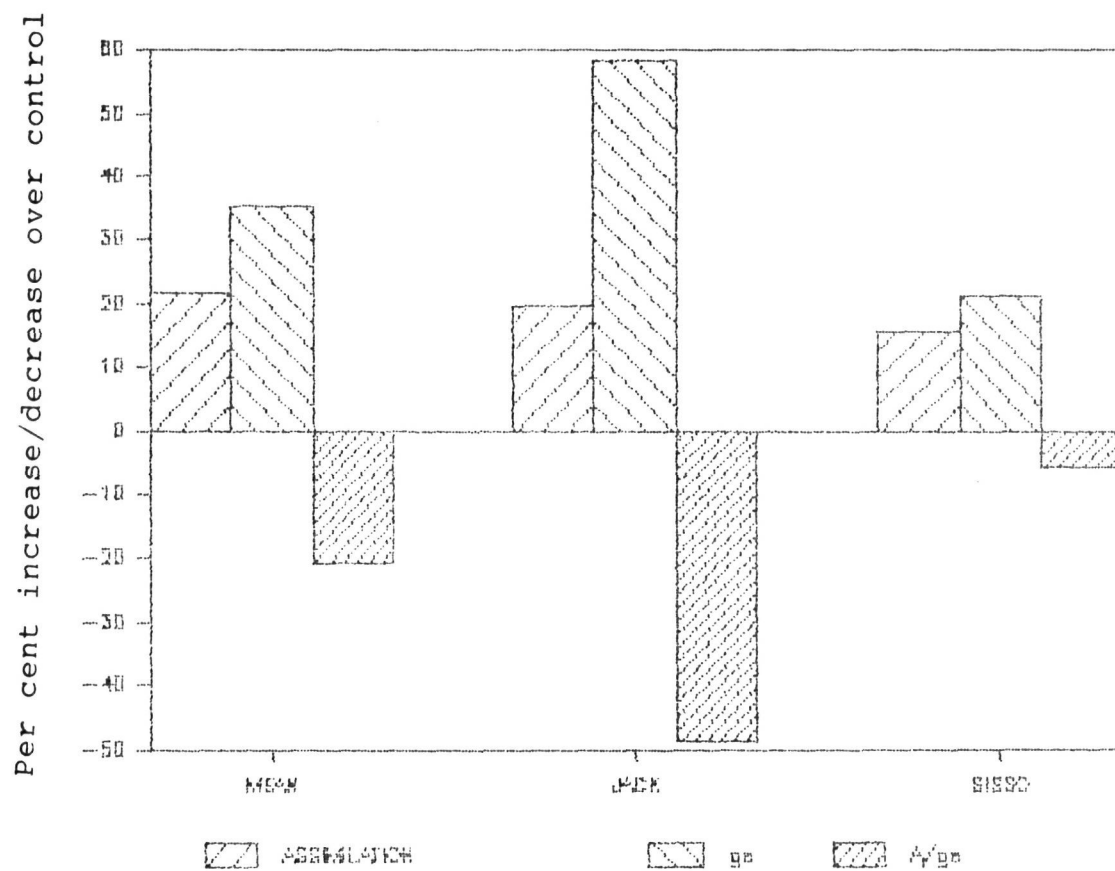


Fig.26: Effect of VPD on gas exchange characteristics.

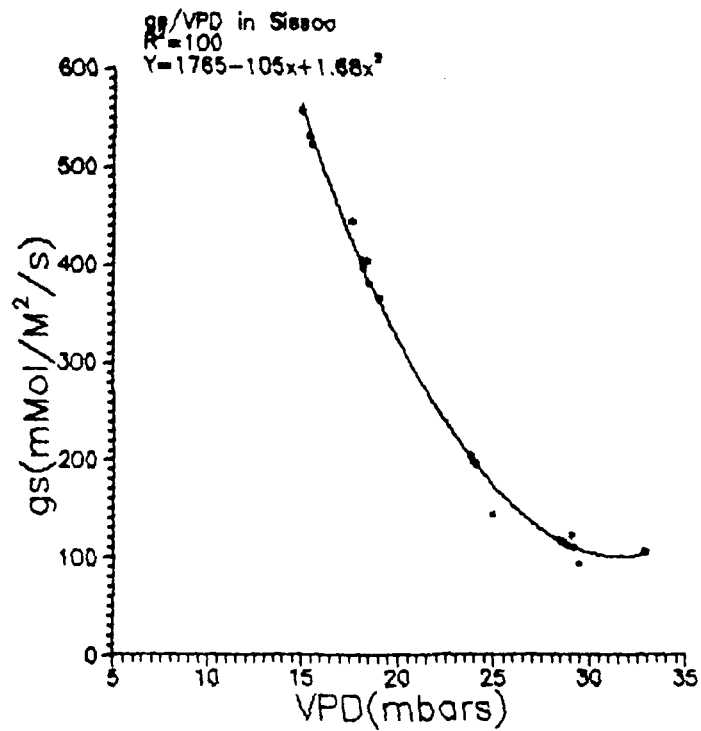
A/gs values were derived for the different species at two different levels of VPD. A/gs value decreased only in champaka because of a very high reduction in 'A' compared to 'gs'. In eucalyptus and jack, owing to a very large decrease in gs alone and not A, A/gs has increased. A/gs remained almost the same in the other species.

VPD response curve

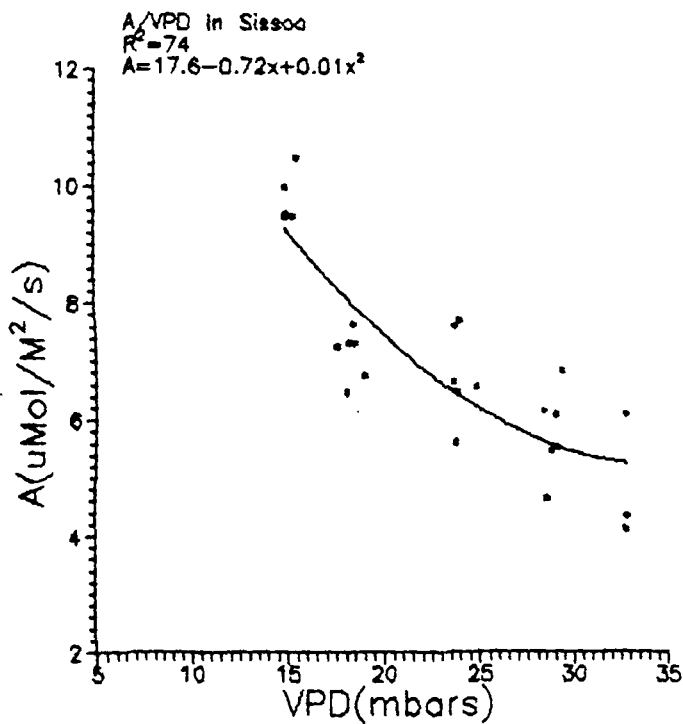
Gas exchange characteristics were studied at a range of VPD in eucalyptus and sissoo. The instantaneous response of the species to the changes in VPD was studied. VPD level was altered by altering RH in the cuvette.

As shown from the figs. 27 and 28, 'gs' decreased markedly with VPD in both the species. In eucalyptus when VPD was increased from 10 to 26 millibars, 'gs' decreased by 66 per cent. In sissoo the decrease was 82 per cent when VPD increased from 15 to 35 millibars.

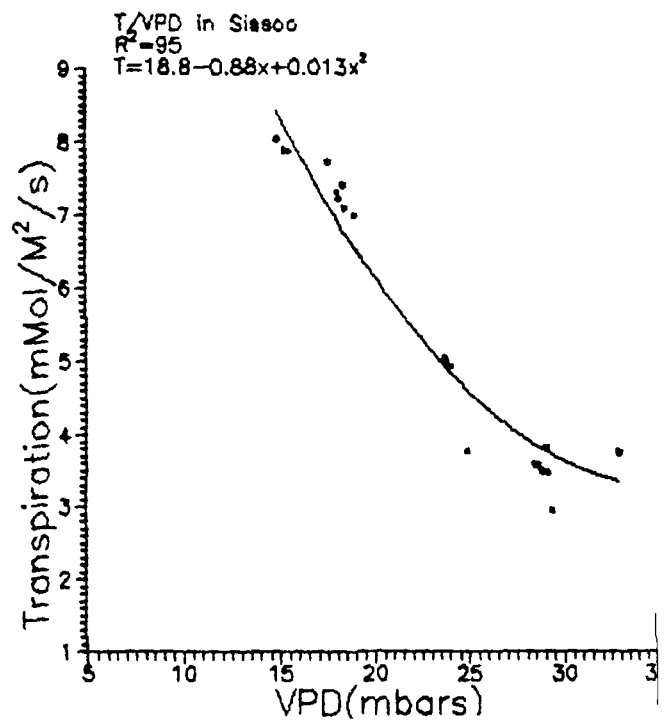
Assimilation showed a uniform linear decrease with increasing VPD in both the species (figs. 27 and 28). The decrease was just 18 per cent in eucalyptus but in sissoo it was as high as 49 per cent.



(a) gs/VPD response curve

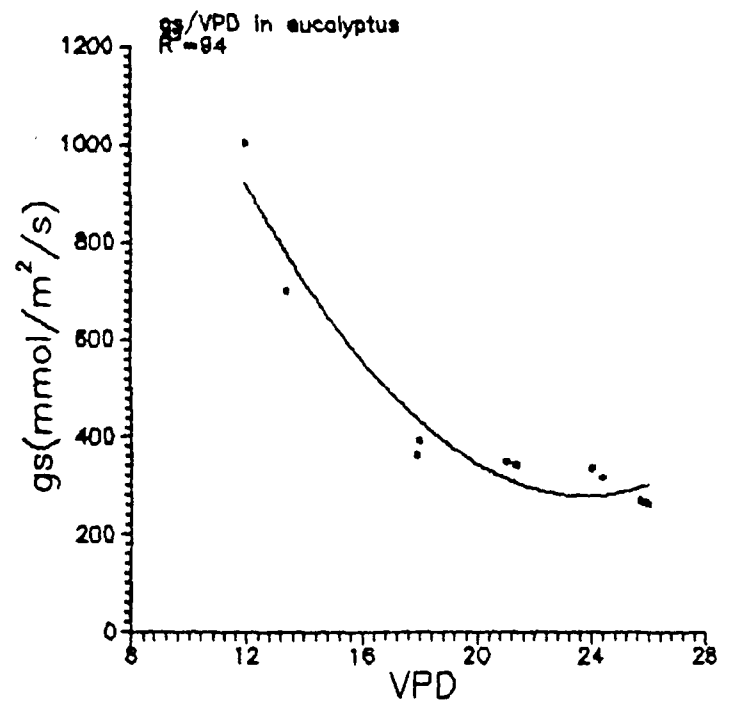


(b) A/VPD response curve

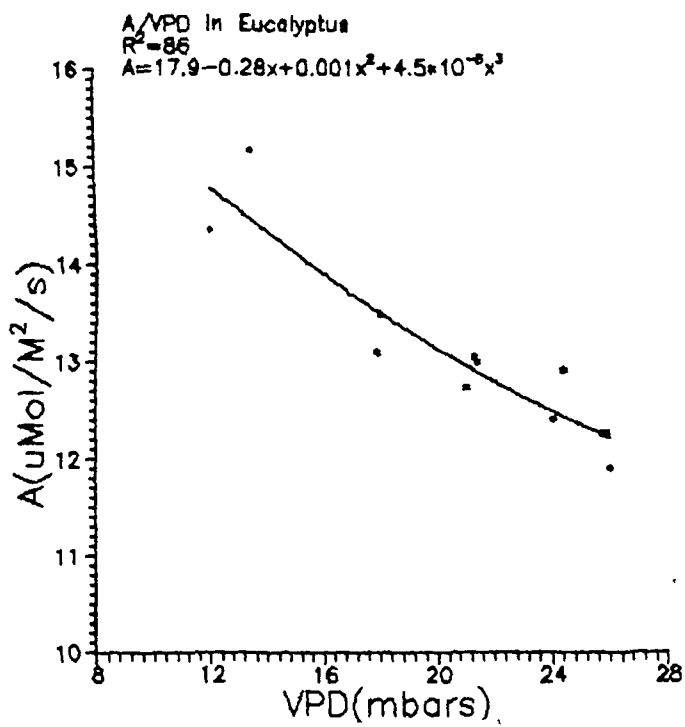


(c) T/VPD response curve

Fig.27: VPD effect on gas exchange characteristics of sissoo.



(a) gs/VPD response curve



(b) A/VPD response curve

Chlorophyll content in leaves

Leaf chlorophyll content in the control and CO₂ enriched plants (CO₂-1) was estimated to see if enriching CO₂ had any effect on the chlorophyll content of leaves (Table No. 15). Total chlorophyll content had increased in spathodia, jack and eucalyptus but had reduced in champaka. Chlorophyll a also followed the same trend. Chlorophyll b had increased in all species and the maximum increase was seen in spathodia, jack and eucalyptus. Chlorophyll a/b ratios had reduced in all species except spathodia and sissoo. Marked decrease was seen in syzigium, champaka, eucalyptus, jack and neem.

Table 15: Leaf chlorophyll content (mg/g of leaf fresh weight) in plants grown in ambient (control) and CO₂ enriched (CO₂-1) conditions.

Species	Total chlorophyll		Chlorophyll a		Chlorophyll b		Chlorophyll a/b	
	Control	CO ₂ enriched	Control	CO ₂ enriched	Control	CO ₂ enriched	Control	CO ₂ enriched
Syzigium	2.78±0.00	2.92±0.10	1.97±0.00	2.11±0.08	0.52±0.01	0.81±0.00	3.79	2.60
Champak	2.20±0.11	1.76±0.10	1.80±0.12	1.40±0.07	0.35±0.02	0.46±0.01	5.14	3.04
Spathodia	1.95±0.16	2.77±0.07	1.57±0.12	2.25±0.06	0.38±0.04	0.52±0.01	4.13	4.33
Neem	1.89±0.04	1.86±0.05	1.56±0.02	1.51±0.03	0.33±0.02	0.35±0.02	4.73	3.29
Sissoo	3.02±0.16	3.22±0.14	2.21±0.12	2.36±0.09	0.83±0.07	0.86±0.06	2.66	2.74
Jack	2.76±0.23	3.32±0.51	2.28±0.17	2.48±0.38	0.47±0.04	0.84±0.13	4.85	2.95
Eucalyptus	2.80±0.04	3.57±0.03	2.22±0.01	2.49±0.01	0.58±0.02	1.08±0.03	3.83	2.13

DISCUSSION

V. DISCUSSION

In this study, an attempt was made to develop a technique wherein growth rates of the generally slow growing forest species seedlings could be enhanced. Though growth rates are species specific, they are still amenable to environmental variables like RH, temperature, CO_2 and nutrients. The effect of CO_2 on plant growth is well documented. CO_2 is known to increase the total DM and economic yield of plants. CO_2 fertilization is being extensively resorted to, to increase the productivity of vegetable and fruit crops grown under glass house conditions. RH has both direct and indirect effect on plant growth. It affects the stomatal behaviour and water relations of plants. Temperature affects plant growth in many ways, by affecting stomatal behaviour, water relations and enzyme activity. Keeping this in view, the effect of CO_2 and RH levels were studied in this experiment.

1. Method to enhance CO_2 levels

A novel method of enhancing CO_2 levels was used here. CO_2 given out during respiration of plants and soil was trapped to enhance its levels. This technique is very simple and can be conveniently used to increase CO_2 and RH. A substantial increase was noticed within a few hours after closing with polythene structure. It

was also possible to alter the relative accumulation of CO_2 by addition of organic matter in the trenches. In this technique, plants were covered from evening 4 to morning 10. The objective here was to see that higher CO_2 concentration was utilized in the morning hours. Yet another advantage of this technique is that the plants were not continuously exposed to high CO_2 and RH as it is generally done in controlled growth chamber studies. Plants were exposed to ambient conditions from 10 A.M. To 4 P.M. everyday. Therefore, at the end of experimental period, there was no need for specifically acclamatising the plants to ambient conditions. Even though, the main objective of using such a structure was to build up CO_2 , this method invariably builds up the RH level.

In this experiment, in addition to CO_2 and RH, an attempt was also made to find out if coirpith as a growth media had any favourable effect on the growth of plants. But nothing to this effect was detected in this study. There was no much difference between soil and coirpith grown plants. The main effect on plant growth was only due to CO_2 enrichment. So further discussion is only on the CO_2 enrichment effect on plant growth and physiology.

2. Response of plants to enriched CO₂ levels

The response of plants to elevated CO₂ levels was studied in two ways by assessing its effect on growth rates of seedlings and by studying the gas exchange characteristics at different levels of CO₂.

Response of the selected forest species to elevated CO₂ based on growth characteristics

Two experiments were conducted to study the elevated CO₂ response by determining the biomass increase for a known growth period. The approach in the two experiments was exactly the same but in the II experiment the plants were exposed to elevated CO₂ levels for 5 months and the soil volume was less compared to I experiment.

Much emphasis is being laid on the experiment-1 with regard to understanding the response to CO₂ concentration because here the seedlings were grown for a period of 3 months and there was no limitation in terms of soil volume. In experiment-2, soil volume was very less. They were grown for a period of five months. Seedlings in the later stages had experienced a severe limitation of soil volume leading to a poor vigour, unhealthy look and a faster senescence of the older leaves. Therefore the results obtained from the first experiment is a better reflection of species response

to CO₂.

All the seven species have responded to CO₂ enrichment in terms of dry matter accumulation. Jack and neem showed the maximum response. Sissoo and Champaka were moderate in their response. Eucalyptus, syzigium and Spathodia responded to a lesser degree nevertheless their response was also significant. Increase in DM due to exposure to elevated levels of CO₂ has been reported in many species like cassava (Imai, et al 1984), Sweet potato (Bhattacharya, et al 1985), citrus (Koch, et al 1986) and lactua sativa (Sharon, and Mitchell, 1988).

CO₂ enrichment could result in an increased biomass by either increasing the photosynthesis per unit leaf area or unit leaf weight or by decreasing photorespiration or by both. In this study photorespiration was not studied but a decrease in photorespiration was expected. Most of the tree species are C₃ in nature. It is known that in C₃ plants, increasing the CO₂ to O₂ concentration ratio will decrease PR. Ehleringer and Bjorkman (1977) have reported a decrease in oxygen inhibition of the quantum yield in Eucalyptus californica from 72 per cent at 88 u bar Ci to 6 per cent at 1510 u bar Ci.

A rapid decline in the level of accumulated CO_2 in the trenches was observed in the morning time. This depletion could be because of utilisation of CO_2 by plants and partly due to diffusion. Diffusion can occur all through after build up of CO_2 in the trenches which was noticed within 2-3 hours after closing the trenches. In spite of the diffusion, CO_2 levels were maintained as there would be a constant release of CO_2 into the trenches by plants and soil. In morning hours, diffusion and Assimilation depletes CO_2 levels rapidly. So we can infer that elevated CO_2 concentrations are being utilised by Assimilating plants.

LA/DM on leaf area ratio (LAR) was computed in this study, as a reflection of net assimilation rate of the leaves. Plant with higher photosynthetic rate should have a lower ratio and vice versa. When LAR and LWR of enriched and non enriched plants were compared across species, LAR was found to have decreased by 6.0 and 8.0 per cent in CO_2 -1 and CO_2 -2 treatments respectively. LWR was decreased by 18.2 and 22.7 per cent respectively. This certainly reflects the increased rate of 'A' in the enriched plants. LAR and LWR has decreased in three species neem, jack and spathodia. The decrease in eucalyptus was very marginal. In rest of the species, these ratios were not affected. Based on this ratio, the increase in biomass of neem and jack

seems to be understandable. In champaka, there was actually an increase in LWR by 11 per cent.

Gas exchange characteristics at elevated CO₂ levels

The gas exchange characteristics at elevated CO₂ levels is yet another approach to understand the response of species to CO₂ levels. For this, it is necessary to study the intrinsic gas exchange characteristics of the plants at different CO₂ concentrations. Instantaneous response to elevated CO₂ levels at a single leaf level was studied by using an open system - IRGA in intact leaves. Initially the instantaneous gas exchange traits at ambient levels were studied.

There was a considerable difference among the species in terms of 'A' 'gs' and 'A/gs' ratios. Sissoo and eucalyptus had a higher 'A' followed by neem, jack and spathodia. 'gs' of all the species except eucalyptus was generally lower. The magnitude of response to environmental factors like CO₂, RH of any parameter would be directly related to the magnitude of that parameter. So the response should be higher in the species like sissoo, eucalyptus, jack and neem.

The polythene structure used to cover the trenches was not fully transparent and it reduced the light intensity inside the trenches. Light response

curves were studied in these species to see if the reduced light intensity could have had any effect on the 'A' of the plants. All the species showed a linear increase in 'A' with PFD upto $2000 \mu\text{mol m}^{-2}\text{s}^{-1}$. Only champaka and Spathodia reached Pmax at $1600 \mu\text{mol m}^{-2}\text{s}^{-1}$. In many studies Pmax was achieved at comparatively low light intensities (Nijs et al 1989,b). In most of the species, gs was found to increase linearly with light intensity. So a combined effect of low light and high CO_2 prevailing within the enclosed trench would have decreased 'gs' considerably. Further, Wong, and Dunin, (1987) say that light intensity required to saturate photosynthesis increased with increasing ambient partial pressure of CO_2 in eucalyptus.

'A/gs' values were calculated at different light intensities. A/gs ratios were higher at high light intensities. SO A/gs values of the plants in the enclosed trench would be generally low owing to the reduced light intensities inside the trench.

When Assimilation rates were studied at elevated CO_2 levels, all the species responded very well. Maximum response was seen in spathodia and champaka. Such response has been reported in many species (Campbell et al, 1988, Morison and Gifford 1983, Mortensen, 1984).

Even though a good response was seen in all the species, A/c_i curves were developed in a few species to assess the relative mesophyll and stomatal limitation. As could be inferred from A/c_i curves, the mesophyll limitation to fix CO_2 was almost nil in three out of the four species. This contradicts with earlier reports where a considerable mesophyll limitation was also noticed (Jacob, 1988).

Faraquhen and Sharkey (1982) argue that stomata usually impose only a little limitation on net assimilation. dA/dC_i values reflect the mesophyll conductance of the species. A higher value shows that the mesophyll can increase the assimilation rate to a greater extent for a given change in C_i . dA/dC_i values are higher in eucalyptus and jack indicating a greater g_m but is lower in champaka indicating a lower g_m . Therefore the stomata offers the major limitation for Assimilation under ambient conditions under elevated CO_2 levels also, stomata might pose a limitation to obtain the required response.

Increased CO_2 levels is known to decrease ' g_s ', in general. The extent of reduction determines the extent of response of ' A ' to elevated CO_2 levels. So one more parameter which needs to be studied is ' g_s '. The plants in which the reduction in ' g_s ' is going to

be less would be more benefitted. Eucalyptus, jack and spathodia had the least decrease in 'gs' due to CO_2 enrichment. Maximum decrease was seen in champaka 'gs/ci' response curves were plotted to see the general response of 'gs' to increasing C_i . 'gs' reduced with C_i in spathodia, eucalyptus and champaka, but the extent of reduction was different in different species. Maximum decrease was seen in champaka and minimum decrease in spathodia. Jack did not show any particular response of 'gs' to C_i . But in spite of a decreased 'gs', plants could increase its C_i with C_a at a constant rate and could maintain a constant ratio of c_i/c_a at all concentrations of C_a . Similar results have been reported in eucalyptus by Wong, et al (1978) where c_i/c_a was almost constant at all concentrations of CO_2 till 800 ppm.

If CO_2 is going to decrease 'gs' to a greater extent than 'A', 'A/gs' ratio increases resulting in an increase in WUE. Therefore 'A/gs' values were calculated at the levels of CO_2 to arrive at intrinsic WUE of the leaf. 'A/gs' increased by more than 100 per cent in all species. Highest increase was seen in champaka. Since 'gs' decreases at high CO_2 , an increase in 'A/gs' should be able to compensate the decrease in 'gs'. In soybean, it was found that WUE in units of CO_2 fixed per unit water transpired was twice as high with high

than low CO_2 (Jones, et al, 1985).

The Response of species to elevated CO_2 in terms of growth rates and gas exchange characteristics - A comparison

Considering both 'A' and 'gs' at elevated CO_2 concentrations, Spathodia should have had the maximum benefit in terms of increase in DM. The other species which could have derived benefit would be jack and neem, which as we have seen has had the desired effect. The extent of response expected in neem should be lesser than jack, as the 'gs' has reduced by a larger extent in neem than jack. Sissoo despite a reduction in 'gs' by 25 per cent should have been able to get the maximum benefit as the increase in 'A' at higher CO_2 levels was more than 100 per cent. In champaka, the decrease in 'gs' was by 36 per cent. In spite of the unlimited mesophyll conductance to fix CO_2 , champaka did not respond to expected level to an enhanced CO_2 . In eucalyptus, as seen from 'A/ci' curves, mesophyll limitation was absent and the reduction in 'gs' was also very less. But the desired response in terms of increase in DM was not seen.

If the increased biomass was only due to the beneficial effect of CO_2 , then the trend in biomass accumulation and Assimilation at higher CO_2 should have

had a similar trend. But it was not so. In Desmodium paniculatum, increase in DM production was not associated with an increase in photosynthetic capacity per unit leaf area (Wulff and Strain, 1982). In Pentaclethra macroloba seedlings the instantaneous response in terms of PE was $6.0 \text{ u mols of CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ but on acclimatisation, PE was found to have reduced to $4.7 \text{ u mols CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Oberbauer, et al 1985). Such an observation has also been made by Raper Jr and Peedin (1978). They observe that net carbon exchange rate in short term experiments in response to environmental conditions are not representative of long term behaviour. There are two possible reasons. One is that instantaneous response of 'A' to high CO_2 levels as measured in this study may not be a true reflection of CO_2 effect under long term conditions. The other possibility is in addition to CO_2 , a change in other environmental factors might have attributed to the observed differences in growth.

For the plants to get acclimated, they have to be continuously exposed to elevated CO_2 . In this study out of the 16 hours of exposure, only 4-5 hours were the light period. A big lacuna in this study is the absence of any data on the A levels of plants exposed to elevated CO_2 levels taken at different time intervals on a day after exposure and on different days

after the actual start of the experiment. In a similar study on green house carnation where plants were exposed to high CO_2 (600-800 ppm) at midnight, CO_2 concentrations fell between 0600 to 0800 hrs. The photosynthetic rate of carnation increased between 200 and 1100 hrs and decreased between 1300 and 1800 hrs (Miura, 1983). In this study it was difficult to measure 'A' at growth CO_2 in the trenches (fig. 6) and the plants were not completely exposed to higher CO_2 concentrations throughout the day. But the negative adaptation due to acclimatisation seems to be unlikely. Mortensen (1984) observed that intermittent CO_2 application on green house plants could reduce the negative adaptation of plants to prolonged exposure to CO_2 enriched air.

One more striking aspect in this study was that there was not much difference between CO_2 -1 and CO_2 -2 treatment. Even though the levels of CO_2 was not strictly maintained in both the treatments, in general CO_2 -2 treatment always had 900 ppm of CO_2 which was at least 200 ppm higher than the CO_2 -1 level. An increase in CO_2 concentration by 200 ppm is quite sufficient to enhance the response to that extent of the enhanced CO_2 level. So CO_2 -2 was expected to have a more beneficial effect on the plants. Since the differential response

was not seen, the observed response in growth rates could be also due to some other factor present in the CO_2 -1 and CO_2 -2 treatments and not in control. RH or VPD was one such factor.

VPD as an environmental variable affecting plant growth

RH sufficiently increased in the CO_2 -1 and CO_2 -2 trenches. Though the temperature in the morning times was higher, it could not have had a very deleterious effect on plants by increasing VPD because RH was nearly at saturation level in CO_2 -1 and CO_2 -2 treatments. At that time outside VPD was about 26-28 mbars and by the enclosure of polythene structure VPD could have reduced sufficiently.

VPD has a direct effect on stomatal and water relation and thus affects both 'gs' and 'A'. It is well documented that high RH and low VPD has a beneficial effect in increasing the plant growth. To assess this, once again instantaneous response of plants to altering VPD was measured. Studying the effect of VPD on plant growth by studying its response in a small area of the leaf which alone is exposed to altered environmental condition may not give the true picture. In soybean, sunflower, wheat, barley and sorghum photosynthesis and stomatal resistance were not affected by changing the VPD from 8 to 27 mbars, when the response was studied

on a single leaf but when the whole plant response was studied both photosynthesis & 'gs' decreased with increasing VPD (Rawson, et al 1977). Rawson, (1977) opines that in studies where there has been no stomatal response to VPD, only a small part of the plant has been exposed to the measuring conditions, while the remainder of plant remained under condition of low evaporative demand. He also argues that when small leaf area (relative to root volume) is exposed to increasing VPD, the plant can more readily meet the increased evaporative demand and stomata do not close.

In our study, when VPD was altered in the leaf chamber, there was a rapid response reflected by change in 'A' and 'gs' in all the species. The increase was more than 100 per cent. The per cent increase in 'gs' in all the other species was less than 40 per cent.

'A' also increases along with 'gs' when exposed to low VPD. Increase in 'A' in plants grown at high RH could be due to higher 'gs'. Increase in 'A' was about 345 percent in champaka which should certainly increase its biomass at a lower VPD in spite of a lower increase in 'gs'. Jack and syzigium were also found to have increased 'A' by 25 and 41 per cent respectively. If going only by 'A', champaka, syzigium and jack should have had a higher response to this technique. Jack

showed a good response, champaka moderate and syzigium poor response. So it was not VPD alone which evoked the observed response in growth.

Cowpea experiment to understand VPD effect

A small experiment was conducted to delineate the effects of VPD and CO_2 on plant growth (data not given in the results). Pot grown 20 day old cowpea plants were subjected to three different treatments, control, t1 and t2.

t1 - cowpea plants were placed in the trench and the trench was covered with polythene structure between 4 P.M. and 6 A.M.

t2 - The trench with cowpea plants was covered with polythene structure between 4 P.M. and 11 A.M.

Here the comparison was mainly between t1 and t2 treatments. In t1 the response would be only due to increased RH and reduced VPD. In t2, the response would be due to both VPD and CO_2 effect. In this study, the response in t1 and t2 treatments in terms of leaf area and biomass were similar.

The conclusion from the above experiment to some extent can be applied to growth rates of the seedlings in trenches. But the beneficial effect of the CO_2 cannot be completely ruled out. A decrease in LAR and

LWR can be explained only as a CO₂ effect and a decrease in specific leaf weight is due RH effect. LAR has been found to have decreased in wheat plants. Lactuaia sativa when exposed to higher CO₂ concentration (Neales and Nicholls, 1978, Sharon and Mitchell, 1988). In many of the experiments with CO₂ enrichment, specific leaf weight is known to have increased. In soybean, CO₂ concentrations of 990 ul/l had increased specific leaf weight from 21.4 to 30.5 g dry wt m⁻² (Campbell et al 1988).

Possible effect of temperature

This technique as a means to enhance the seedling growth rates is quite effective. All the species have responded positively to the treatments. But the response varied among species. The species which responded to the maximum extent were jack and neem. As seen from the gas exchange characteristics, Jack could effectively use the altered environmental conditions to its benefit. As could be inferred from the study on gas exchange, champaka and spathodia also should have responded very well, but the expected response was not seen. May be there were other factors which have affected these seedlings and have gone unnoticed like high temperatures.

Many species have responded well to the decreased VPD and also to the enhanced CO₂ level when given separately. But plants were exposed to decreased VPD and enhanced CO₂ at the same time. So plants should have had a very high 'A' which should have resulted in a very high increase in DM. Though the plants have enhanced their DM production significantly, the expected increase was much higher than the observed increase. This diminished increase in DM could be because of changed respiration patterns in the plants subjected to the enhanced CO₂ levels during and after the period of enrichment. In Echinochloa crusgalli, respiration rates were increased by an average of 49 per cent when they were exposed to high CO₂ levels (Potvin, and Strain, 1985). In plantago major respiration levels were enhanced in the beginning of the exposure (Poorten, et al 1988). Gifford et al (1985) described some results in a few species. In wheat, root respiration of enriched plant was sometimes reduced, in sunflower respiration was enhanced and in mungbean no significant difference was found. In this study as we do not have any data on the respiration rate of plants, drawing any conclusion would be difficult.

From this investigation, we conclude -

1. The technique used in this study is effective

in enhancing the seedling growth rates of the slow growing forest species.

2. Assimilation in all the species increased instantaneously when exposed to higher CO_2 concentrations. Though species differed in their response of 'gs' to higher CO_2 concentration in general 'gs' decreased at higher CO_2 levels. As a result A/g_s increased at higher CO_2 levels.
3. All the species responded to instantaneous change in VPD in terms of 'A' and 'gs' indicating that low VPDs increases 'A' and 'gs'.
4. The observed response in terms of growth rates could not be attributed to CO_2 or RH alone. It could be due to combined effect of CO_2 and RH and also due to some other factors like temperature which were not investigated in the present experiments.

SUMMARY

SUMMARY

The main objective of the present investigation was to enhance the seedling growth rates by CO₂ enrichment and altering RH. The response of seven different tree species to elevated CO₂ and RH was studied by adopting a new technique, in which CO₂ released by plants and soil during respiration was trapped by covering the trenches with polythene structure. Plants kept in the trenches were covered with polythene structures from 4 P.M. to 10 A.M. A substantial increase in CO₂ concentration and RH was noticed within a few hours after closing the trenches. Two experiments were conducted to study the response of plants in terms of growth rates in altered environmental conditions. To understand the observed plant growth responses to altered environmental conditions, gas exchange traits of different species were studied at different CO₂ levels and VPDs. The effect of coirpith as a growth media and the effect of volume of growth media on plant growth rates was also studied. Response was seen only to CO₂ enrichment, soil volume and not to coirpith. Plants grown in bigger volume had better growth rates than compared to the plants grown in smaller volume of soil in control as well as CO₂ enriched plants.

control in CO₂-1 and CO₂-2 treatments respectively. In all the species the difference between CO₂-1 and CO₂-2 treatments with regard to growth parameters was not significant.

4. To understand growth responses to CO₂ and RH, gas exchange traits were studied.
 - a) Intrinsic gas exchange characteristics were studied at ambient conditions. Considerable difference among species in terms of 'A', 'gs' and 'A/gs' was noticed. Sissoo and eucalyptus were the species which had higher 'A' and 'gs'. Intrinsic WUE at leaf level, calculated by ratio of Assimilation to stomatal conductance was found to be higher in sissoo and syzigium.
 - b) Light response curves: Spathodia, eucalyptus and champaka showed a biphasic response to light intensities. Pmax was reached at 1500 U mols m⁻²s⁻¹. In neem, jack, 'A' never seemed to reach maximum even at 2000 u mols m⁻²s⁻¹, even though the rate of increase in 'A' had reduced at higher light intensities. In all the species, 'gs' increased with increasing light intensities as a result 'A' and 'gs' were linearly related. Intrinsic WUE at leaf level,

Based on the results, the salient findings of the present investigation are:

1. In the method tried in this study CO_2 concentrations were found to have increased within a few hours of closing the trenches. RH also increased in the enclosed trenches and used to reach saturation levels in the morning hours.
2. Even though response of plants to elevated CO_2 in terms of increase in DM was observed in both the experiments, the data from the first experiment was given more importance with regard to understanding the plants response to CO_2 enrichment. In experiment-1, the duration was three months and the soil volume was large enough to accommodate the whole root system. In experiment-2, the duration was longer, the volume of soil was too less. The plant growth was found to be severely inhibited in the later stages of the experiment.
3. All the species responded to CO_2 enrichment in terms of increasing plant height, leaf number, leaf area and DM accumulation. The increase in leaf area in CO_2 -1 and CO_2 -2 over control was 47 and 42 per cent respectively and the increase in DM was 43 and 45 per cent over

calculated as ratio of 'A' to 'gs', was generally higher at high light intensities.

- c) Response to elevated CO_2 : All the species showed an instantaneous response to elevated CO_2 . Maximum response was seen in spathodia and champaka. However, in all species, increase in 'A' was by more than 50 per cent. The effect of CO_2 on 'gs' was lesser compared to its effect on 'A'. In syzigium and champaka, reduction in 'gs' was maximum. In all the other species reduction in 'gs' was very less. Intrinsic WUE at elevated CO_2 increased by at least 100 per cent as could be inferred from 'A'/'gs' values, which doubled at higher CO_2 concentrations.

In a few species A/Ci curves were developed. 'A' and 'gs' were plotted against Ci to see the response of 'A' and 'gs' to Ci and to quantify the relative stomatal and mesophyll limitations to photosynthesis. 'A' increased in all the species and almost linearly in jack and eucalyptus. Eucalyptus, champaka and spathodia reached saturation at 500 ppm Ci, 'gs' reduced with increasing Ci in all the three species, eucalyptus, champaka and spathodia, but in jack, 'gs' seemed to be unaffected by Ci. In

jack, eucalyptus and champaka, stomatal limitation seemed to be the major limitation, whereas mesophyll limitation to Assimilation was nil. In spathodia, both the limitations to Assimilation was observed.

- d) Response to VPD. Effect of VPD on 'A', 'gs' and 'A/gs' was studied in all the species. 'A' and 'gs' was maximum at lower VPDs and reduced when VPD was increased, the magnitude of decrease being greater in 'gs' than 'A'. Decrease in 'gs' was maximum in spathodia and jack and decrease in 'A' was maximum in champaka and syzigium. 'A/gs' values was reduced in champaka at higher VPD and increased in eucalyptus and jack. In rest of the species, it was unaltered.

5. The observed response in growth rates to elevated CO_2 was not similar to the observed response of gas exchange traits to increased CO_2 . Therefore the increase in growth rates of the seedlings could be due to a combined effect of elevated CO_2 and RH and also may be due to some other factors along with CO_2 and RH. Temperature could have had a role in determining the response.

6. Since the response of all the plant species to the present method of enriching CO₂ levels was very good, this method could be considered very effective in enhancing seedling growth rates.

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APPENDIX

Appendix-I: Meteorological data for 1989

Weeks	Mean temperature		Mean RH (%)	Rain- fall (mm)	Sunshine hours
	Min. °C	Max. °C			
2-8 April	19.1	34.7	56.4	0.4	10.0
9-15	20.1	34.3	55.0	0.0	10.4
16-22	20.7	32.1	60.0	0.0	8.0
23-29	21.4	33.4	60.0	1.2	7.9
30 Apr- 6 May	21.3	34.6	57.0	16.5	10.3
7-13	20.3	34.7	61.0	18.5	8.5
14-20	20.2	34.0	58.0	6.6	8.9
21-27	19.3	31.5	61.0	10.6	9.3
28 May- 3 June	19.2	32.7	58.0	0.0	9.7
4-10	19.0	29.4	70.0	4.8	5.8
11-17	18.8	29.4	68.0	1.3	5.4
18-24	18.5	30.5	56.0	0.0	8.3
25 June- 1 July	18.7	29.9	71.0	8.4	5.9
2-8	19.1	30.0	72.0	51.1	6.4
9-15	19.5	28.1	71.0	26.2	7.0
16-22	17.9	25.0	83.0	57.7	4.2
23-28	18.0	25.2	70.0	15.3	2.2
30 July- 5 August	18.3	28.4	66.0	1.2	8.6
6-12	18.0	27.7	72.0	22.7	7.0
13-19	18.0	26.6	76.0	4.8	4.2
20-26	18.0	27.0	71.0	12.6	4.9