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A novel approach to obtain increased growth in nursery seedlings of *Hevea brasiliensis* using CO_2 fertilization

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Abstract

An indigenous technique developed at RRI India was used in the present study to expose nursery seedlings of *Hevea* to an elevated concentration of carbon dioxide (CO_2), relative humidity (RH) and warmer temperature daily from 3.30pm to 10.30am the following day for eight months from germination. *Hevea* seedlings were grown in nursery beds with and without the recommended fertilizer doses. CO_2, produced from the decomposition of farmyard manure, was retained in the vicinity of the seedlings using a UV- stabilized poly house which had a transparency of about 80% to sun light.

The CO_2 concentration inside the poly house increased from about 360ppm (normal ambient concentration) to about 700ppm within 45 minutes of closing the poly house at 3.30 pm. The level started to deplete during the morning of the next day after sunrise and reached around 370 ppm by 10.30am when the poly house was ventilated to prevent excessive build up of temperature inside. Inside the poly house during the night, when it remained closed, there was 28% more RH and 1.2° C higher temperature compared to the outside.

Our results show that the exposure of nursery plants to elevated CO_2, RH and warmer temperature as described above, led to a significant increase in total leaf area and total dry matter per plant. Elevated CO_2 induced an increase in leaf area per plant which was relatively more in plants that were supplied with fertilizers. However, relative stimulation of dry matter production due to elevated CO_2 was similar in plants grown with and without fertilizers. The mean stem girth showed a substantial increment in seedlings grown under elevated CO_2. This increase was more in plants grown with fertilizers than without fertilizers. The allocation of biomass into root was significantly higher in seedlings grown with elevated CO_2 and fertilizer. Also there was increased allocation of biomass to roots in seedlings grown under ambient air without fertilizer. Such a response is commonly noticed when roots are devoid of nutrients, especially nitrogen. The technique employed here may be useful in obtaining more robust seedlings for budding, whilst the increased allocation of biomass into roots in plants grown in elevated CO_2 levels could be of agronomic value for better harvesting of soil moisture in drought prone areas.

Introduction

Increased and faster rising levels of CO_2 in the atmosphere have lead to global warming and related changes in the environment. The major reasons for this increase in the atmospheric CO_2 concentration are deforestation, urbanization and fossil fuel burning. Currently CO_2 concentration in the atmosphere is increasing at the rate of 1.8 ppm/year[1] whilst the rate was only 0.05 to 0.1 ppm/year some two hundred years ago prior to the industrial revolution.

Atmospheric CO_2 concentration has a direct influence on the photosynthetic rates of plants[2,3]. Plants with a C_3 type of photosynthetic metabolism respond positively to elevated CO_2 concentrations[3,4] and produce more biomass[5] than plants with a C_4 type of photosynthetic metabolism[6]. Carboxylation is limiting in C_3 type of plants at the current ambient CO_2 concentration. Therefore, if a higher concentration of CO_2 is provided then carbon assimilation rates would increase in C_3 plants. *Hevea brasiliensis* is C_3 plant and, therefore, the exposure of *Hevea* seedlings to a higher CO_2 concentration can improve their growth.

Different methods can be followed to expose plants to elevated CO_2 levels, namely; controlled environment, open top chamber and Free Air CO_2 enrichment (FACE). All of these methods are effective and have their own merits and demerits. However, to obtain the higher concentration of CO_2 all of these methods require the addition to the ambient air of CO_2 from an external pure CO_2 source and sophisticated gadgets to control the micro-climate which make them rather expensive.

In this paper, an inexpensive technique is described to achieve the exposure of young nursery seedlings to higher concentrations of CO_2, relative humidity and warmer temperature and the use of this system is demonstrated to produce robust seedlings for budding.

Methodology

A poly house was erected, using UV-stabilized poly film of 150µ gauge, on a bamboo pole structure of dimensions 35ft long and 30ft wide. This poly house enclosed five nursery beds. Germinated seeds were planted in the nursery beds at 12" × 12" spacing. The poly house was erected on the nursery beds 10 days after planting of the germinated seeds. Decomposing organic matter was used to fill three quarters of the volume of 2 litre capacity plastic bowls which were placed inbetween the rows of seedlings. Eighty bowls were distributed uniformly inside the poly house. The poly house was maintained air tight from 3.30pm to 10.30am of the next day during which CO_2 released from the decomposing organic matter accumulated to reach 700 - 750 ppm inside the poly house thus exposing the seedlings to this elevated CO_2 concentration (Figure 1). The concentration of CO_2 increased to 750 (±15)ppm with in 40 - 45 minutes of closing the poly house (Figure 2). Using this system, in addition to the CO_2 level, the relative humidity and temperature also build up inside the chamber (Figure 3). The poly house was ventilated during the day time between 10.30am and 3.30pm in order to avoid excessive build up of temperature.

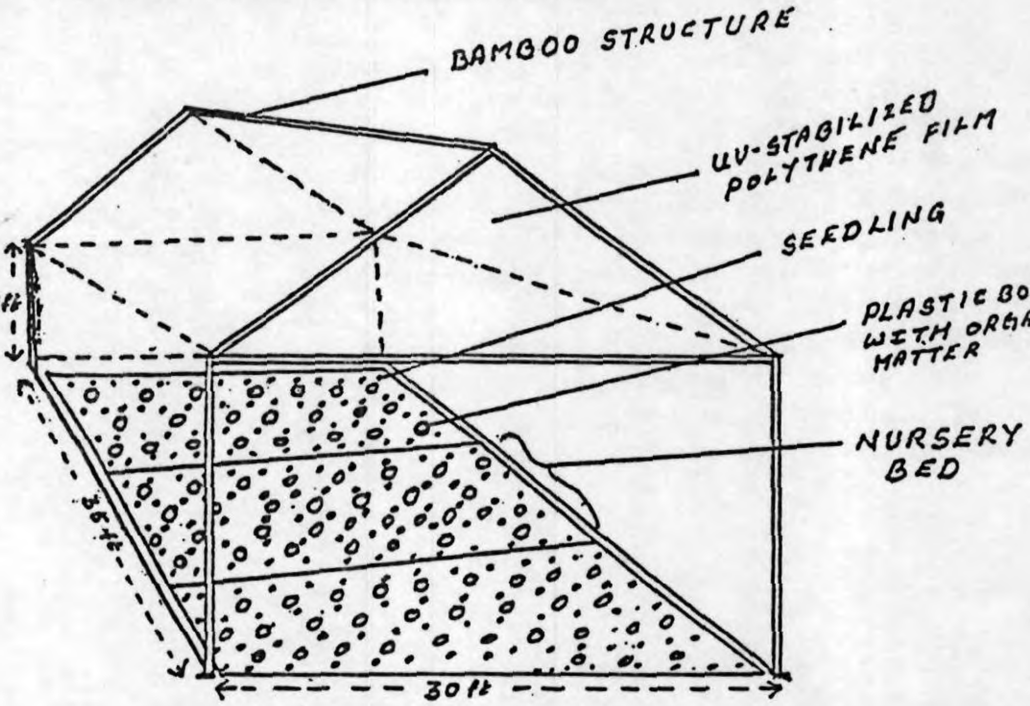

Figure 1 ***Description of poly house and setup of the structure used to obtain higher CO_2 concentration, RH and temperature.***

One set of seedlings were maintained with zero fertilizer whilst another set received the recommended dose of fertilizers in order to find out the effect of elevated CO_2 under these two fertilizer levels. A further set of plants were maintained under ambient air as a control. Mulching was provided to seedlings in the nursery beds during peak summer to conserve moisture. Irrigation was provided to seedlings inside the poly house whenever there was rain since, otherwise, control seedlings in the open air would have the advantage of rainwater while seedlings exposed to elevated CO_2 would be deprived of this inside the poly house.

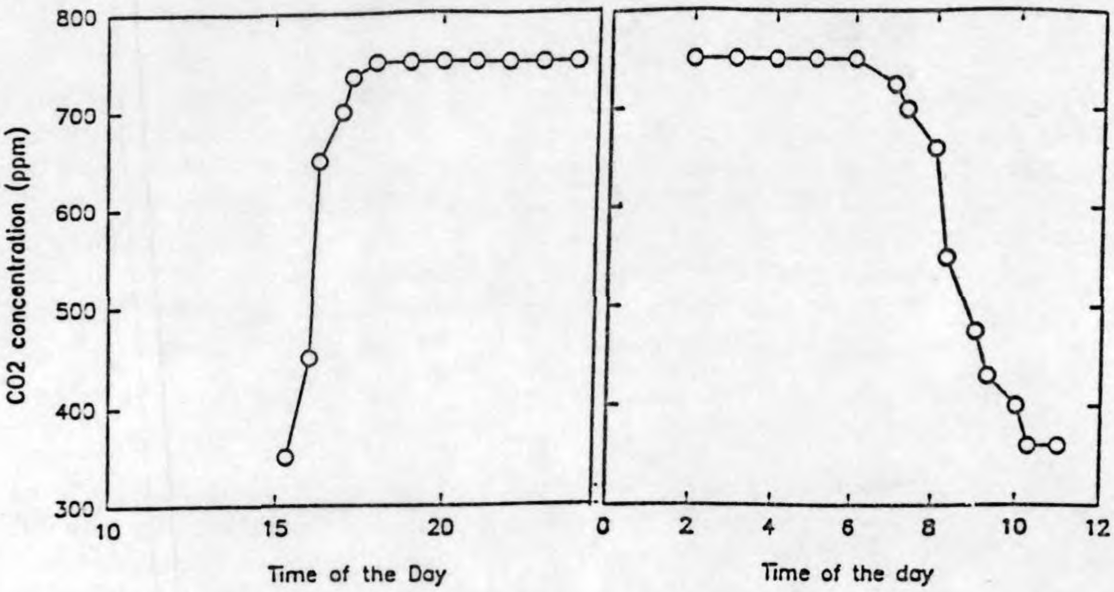

Figure 2 *Changes in the carbon dioxide concentration inside the poly house with time after closing the structure between 3.30pm and 10.30am the following day.*

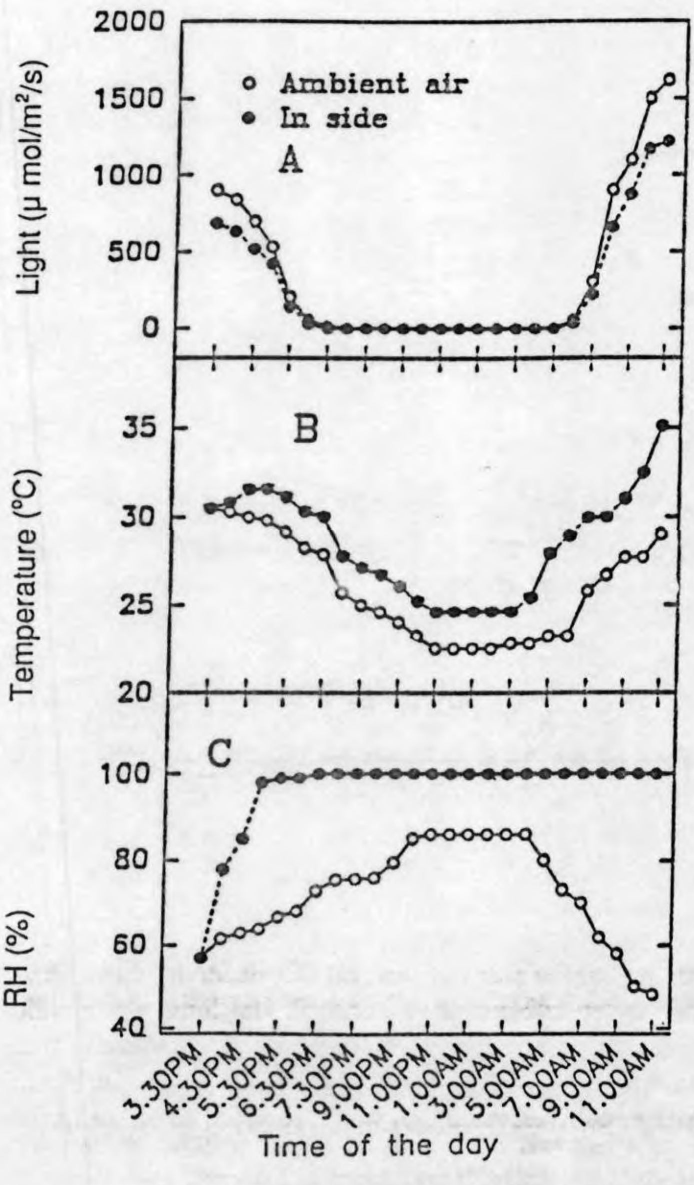

Figure 3 *Pattern of light intensity, relative humidity and temperature inside the poly house and in the outside.*

Photosynthetic photon flux (PPF) over a wave length of 400 - 700nm, CO_2 concentration and relative humidity were measured using the LI-6200 Portable Photosynthesis system (LI-Cor Inc, USA). PPF measurement inside the poly house was made 15cm from the roof of the polytene structure. The temperature inside the poly house and ambient air were measured simultaneously. Leaf area was measured using a portable leaf area meter (LI-3000, LI-Cor, INC, Nebraska, USA). Dry matter was measured after oven drying of the plant material at 70°C for 72 hours until three subsequent dry weights were constant. All the seedlings were irrigated to saturation at the time of final harvest in order to uproot the seedlings with most of the roots still intact thus causing least damage to the roots. All other cultural operations as recommended for nursery seedlings were followed as per the cultivation practices.

Results and discussion

Carbon dioxide concentration inside the poly house started to build up after closing the poly house and reached 700 - 750ppm in less than 45 minutes. It remained high all through the night and started to fall after sunrise in the morning (Figure 2). Next day after sunrise, photosynthetically active radiation started gradually to increase (Figure 3A). This increase is in accordance with a decrease in the CO_2 concentration inside the poly house (Figure 2B). This suggests utilization of the higher concentration of CO_2 by the plants in the presence of sunlight for photosynthesis. CO_2 concentration never fell below the ambient level which means that there was replenishment of CO_2 inside the poly house from the organic matter while plants are photosynthesizing in the presence of sunlight.

The temperature inside the poly house started increasing after sunrise (Figure 3B). An average of 1.21°C rise was seen inside the chamber compared to ambient air up to 8.00am from the time of keeping poly house airtight (3.30pm previous day) whereas the difference from 8.00am to 11.00am was 4.69°C. Because of this temperature increase inside the poly house, it was not possible to keep the poly house closed throughout the day. Along with CO_2 and temperature, a rise in the relative humidity (RH) was also taking place inside the trench (Figure 3C). Relative humidity built up inside the chamber within thirty minutes of closing the poly house and reached the saturation level. When the poly house was ventilated, the relative humidity decreased gradually to reach the ambient levels.

Using this system, seedlings planted in the nursery beds were exposed to elevated CO_2, high relative humidity and warmer temperature. Seedlings were subjected to this treatment for eight months and their growth responses were assessed under two nutrient levels. Growth rates were assessed three months after exposure to elevated CO_2, RH and temperature and finally after eight months. After three months there was a significant difference in leaf area produced per plant. There was also a significant difference in leaf area between the two fertilizer levels. Under elevated CO_2 concentrations seedlings with a normal dose of fertilizer had 20.9% more leaf area than the seedlings grown without any fertilizer and 30.6% more leaf area was seen in seedlings provided with a normal dose of fertilizers and grown under elevated CO_2 than plants grown under ambient air with a normal dose of fertilizers (Table 1). Dry matter produced per plant also showed a significant increase. A 13.13% increase in dry matter was seen in seedlings grown under elevated CO_2 and fertilizers compared with seedlings provided with elevated CO_2 but no fertilizers. A significant 30.7% more dry matter per plant was seen in plants grown with elevated CO_2 concentration and fertilizer when compared to ambient air grown plants with fertilizer (Table 1). Seedlings grown under elevated CO_2, irrespective of the fertilizer levels had more growth than plants grown under ambient CO_2 levels (control) with or without fertilizer. A similar trend in leaf area and biomass production was seen after exposing seedlings to elevated CO_2 for eight months (Table 2). Girth, which is a reflection of the overall growth, measured after 8 months of the treatment, showed a significant increase in seedlings grown under elevated CO_2 concentration in relation to ambient air grown seedlings irrespective of the fertilizer levels (Table 2). It is evident that growth rates were best under elevated CO_2 concentration when provided with normal levels of nutrients when compared to zero fertilizer.

Allocation of dry matter to root growth was significantly more in seedlings grown under elevated CO_2 concentration especially when seedlings were provided with normal doses of fertilizers (25.55%). Similar reports have been already made in other crops[7,8]. In plants grown in ambient air, the biomass allocated to roots was more in plants grown with deficient as opposed to sufficient nutrients. Plants have a tendency to divert more biomass to root growth when they experience stress such as nutrient deficiency. This data along with the stem girth suggests that plants grown under elevated CO_2 concentrations may be more drought tolerant[9].

Table 1 ***Leaf area production per plant and total dry matter production of seedlings after three months exposure to elevated CO_2, RH and temperature.***

	Elevated CO_2 grown plants		Ambient air grown plants	
	+N	-N	+N	-N
Leaf area (cm^2/plant)	3348[ab]	2646[ab]	2338[a]	1992
Total dry matter (g/plant)	48.65[ab]	42.17[ab]	33.65[b]	28.55

a - indicates significant p value within the treatment; b - indicates significant p value between the treatments.

Table 2 ***Leaf area production per plant and total dry matter production of seedlings after eight months exposure to elevated CO_2, RH and temperature.***

	Elevated CO_2 grown plants		Ambient air grown plants	
	+N	-N	+N	-N
Leaf area (cm^2/plant)	3651[ab]	2011[b]	1976[b]	1237
Total dry matter (g/plant)	93[ab]	70.64[ab]	63.13[b]	48.01
Stem girth (mm)	10.12[ab]	8.74[ab]	7.9[NS]	7.85
% biomass diverted for root growth	26	21	21	23

a - indicates significant p value within the treatment; b - indicates significant p value between the treatments; NS - not significant

In the above described method, plants are make use of higher concentrations of CO_2 for carbon assimilation in the presence of light in the morning hours between 7.00 and 11.00am. Evidence from a large body of literature show that it is possible to increase biomass production by growing plants under elevated CO_2[5,6,10]. In the present method of CO_2 fertilization, plants were also experiencing relatively higher temperatures compared to ambient air grown plants. Imai *et al*[11] noticed enhancement of leaf area and whole plant dry weight at higher CO_2 levels when associated with higher temperature. A proportionate increase in carbon assimilation at elevated CO_2 will increase with temperature[4]. This reference also found that elevated CO_2 will alter the magnitude of the response of the leaf canopy carbon gain to rising temperature and relative humidity which apparently enhanced growth. Therefore, higher temperature and humidity associated with higher concentrations of CO_2 helped in higher carbon assimilation and leaf expansion rates respectively. Since seedlings had higher growth rates when exposed to elevated CO_2 concentrations, they may also need more nutrients to support the additional growth. This is evident from the growth differences noticed between seedlings grown with and without fertilizers.

Another important plant character that showed a remarkable increase under elevated CO_2 was leaf area per plant. A significant increase in leaf area was noticed under elevated CO_2 when compared to ambient air grown plants. Studies conducted under controlled conditions have reported an increase in leaf area in plants grown under high concentrations of CO_2[12,13,14]. When a higher temperature is associated with elevated CO_2 concentrations, leaf area is further enhanced[15] which is due to higher leaf initiation rate, leaf expansion and individual leaf area. Saman *et al*[16] have shown that leaf elongation rates were more under elevated CO_2

concentrations and were associated with higher sucrose phosphate synthase activity during the early vegetative stage when growing blades were strong carbohydrate sinks. Leaf elongation rate is one of the ways by which leaf area development can be stimulated. Relative humidity is another important weather parameter that determines leaf growth. Relative humidity can reduce the vapour pressure deficits and thus help in maintaining high turgidity of the cells which provides congenial conditions for cell division, elongation and expansion.

Plant height and stem diameter were significantly higher in elevated CO_2 grown plants in both of the clones which is a reflection of higher growth rates under elevated CO_2 concentration (Table 1). Another possible reason for higher growth seen under this system could be lower dark respiration rates at elevated CO_2. According to Tanaka[17] the efficiency of respiration increases as a result of whole plant CO_2 enrichment, but the respiration rates decrease at higher concentrations of CO_2[18,19,20].

Conclusions

In the present system of exposure of plants to elevated CO_2, the main advantages are that there is no need for a pure source of CO_2 and the whole system is inexpensive. Construction of a poly house is simple with minimal technicalities involved. This system can be effectively used to enhance the initial growth rates of the seedlings in the nurseries to obtain robust seedlings for budding and, in turn, help in improving budding success. A normal dose of fertilizer appears to be helpful in obtaining maximum response to elevated CO_2. Higher biomass allocation to root growth in plants exposed to elevated CO_2 would prove to be useful under a drought situation.

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