Tropical Plant Research
An International Journal
© Society for Tropical Plant Research

ISSN (E): 2349 – 1183
ISSN (P): 2349 – 9265
3(3): 551–557, 2016
DOI: 10.22271/tpr.2016.v3.i3.072

Research article

Intra-specific variation in response of Neem (*Azadirachta indica* A. Juss) to elevated CO_2 levels and biochemical characterization of differently responding plants

C. Buvaneswaran*, K. Arivoli, T. Sivaranjani, E. Menason, K. Vinothkumar, S. Padmini and S. Senthilkumar

Institute of Forest Genetics and Tree Breeding, (ICFRE), R.S.Puram, Coimbatore, Tamil Nadu, India
*****Corresponding Author: buvanesc@icfre.org [Accepted: 23 October 2016]

Abstract: Global climate change the looming environmental threat is mainly due to the increase in atmospheric CO_2 levels, which was increasing earlier by about 1.55 ppm per year and currently by 2.76 ppm per year. Thus, CO_2 concentration has reached 400.16 ppm in 2015. To understand the response of various tropical tree species to such an elevated CO_2, experiments were conducted in Automated Open Top Chambers (AOTC) facility at Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore (India). The results of initial studies indicated the scope for exploring intra-specific variation in response of tropical trees to elevated CO_2. Subsequently, experiments were carried out to assess intra-specific variation in Neem (*Azadirachta indica*). The selected phenotypes of Neem (varieties or clones) were exposed to four treatments *viz.*, i) CO_2 of 600 ppm, ii) CO_2 of 900 ppm, iii) chamber control- without any CO_2 enrichment and iv) ambient conditions. The parameters studied were shoot length, root length, dry matter accumulation in shoot and root. The results of the study showed that there existed significant variation among different treatments of CO_2 as well as among various phenotypes or clones in terms of growth characteristics. This intra-specific variation in biomass accumulation under elevated CO_2 levels could be exploited for future breeding programmes in developing climate ready genotypes having greater potential to sequester more carbon and produce greater biomass under forecasted elevated levels of atmospheric CO_2. Another objective in this study was to analyze intra-specific variation in selected biometrical and biochemical characteristics of leaf samples of neem trees in relation to their differential response to elevated CO_2. Among parameters of leaf, Fumaric acid, Malic acid and Oxalic acid, leaf Nitrogen and Specific Leaf Weight may be considered as a biochemical and biometrical marker to categorize the plants adapted to elevated CO_2 environments.
Keywords: Response to elevated CO_2 - Neem (*Azadirachta indica*) - Open Top Chambers - Within species variation in growth.

[Cite as: Buvaneswaran C, Arivoli K, Sivaranjani T, Menason E, Vinothkumar K, Padmini S & Senthilkumar S (2016) Intra-specific variation in response of Neem (*Azadirachta indica* A. Juss) to elevated CO_2 levels and biochemical characterization of differently responding plants. *Tropical Plant Research* 3(3): 551–557]

INTRODUCTION

Even the most environment friendly emission scenarios lead to an increase in atmospheric CO_2 concentration over the next 100 years, to about double the pre-industrial levels up to 550 ppm (IPCC 2007). Currently, CO_2 concentration has reached 400.16 ppm in 2015 Mike McGee (2015). This increasing concentration of carbon dioxide (CO_2) in the atmosphere may have a direct effect on the physiology of plants: higher CO_2 tends to suppress plant transpiration through reduced stomatal conductance (Field *et al*. 1995). In tree species, short term experiments with *Pinus ponderosa*, *Quercus coccinea*, *Pinus radiata* and *Populus deltoides* have shown a definite increase in photosynthesis rate up to 40–80% under 600 ppm levels of CO_2 (Couteaux *et al.* 1992). Studies conducted at Institute of Forest Genetics and Tree Breeding (IFGTB) showed the existence of greater inter- and intra-specific variation in tropical tree species in response to elevated CO_2 and temperature (Buvaneswaran *et al*. 2010). With reference to intra-specific clonal variation in tree species, Johanna *et al.* (2003) reported that two clones of silver birch (*Betula pendula*) responded differently to elevated CO_2 levels. In one clone, total biomass increased by 40% under elevated CO_2, but in another clone no response was found.

Received: 15 June 2016 Published online: 30 November 2016
https://doi.org/10.22271/tpr.2016.v3.i3.072

Similar such intra-specific clonal variations have been reported in Sitka spruce (Murray *et al.* 1994), Poplar (Ceulemans *et al.* 1995), *Hevea brasiliensis* (Devakumar *et al.* 1998), *Populus tremuloides* (Isebrands *et al.* 2003). However, not much study has been conducted on intra-specific variation in response of tree species to elevated CO_2 levels, particularly the plantation forestry species of India. Hence, the present study was conducted to generate knowledge on intra-specific variation in important tropical plantation species besides helping to screen assembled productive clones of Neem (*Azadirachta indica* A. Juss) for higher carbon sequestration potential under elevated CO_2. This selection of clones adapted to elevated CO_2 levels may also be used for developing 'climate ready genotypes' in future through biotechnological interventions. The information generated could also help in identification of biometrical and biochemical markers, if any, for higher carbon sequestration potential of the selected species under elevated CO_2.

MATERIALS AND METHODS

The present research experiment was conducted using Automated Open Top Chambers (AOTC) facility at Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, India. This study site is located on 11°59'0.69" N, 76°57'2.32" E and 437 m MSL. The facility of AOTCs has chambers which are cubical type structures of 3 × 3 × 3 m dimension, fabricated with galvanized iron (GI) pipe frames and covered with polyvinyl chloride (PVC) sheet of 120 micron gauge to have more than 90% transmittance of light. The upper portion of the chamber is kept open to maintain near-natural conditions. A software facility called Supervisory Control and Data Acquisition (SCADA) was used to continuously control record and display the actual and desired CO_2 level, relative humidity and temperature in each OTC by feedback control loop passing through Programmable Logical Controllers (PLC).

The species selected for the present study was Neem (*Azadirachta indica* A. Juss) in which 12 clones (varieties) were selected and subjected to various treatments. The initial shoot length was recorded for all the plants. Then, these plants were exposed to four treatments with the different levels of CO_2 *viz.*, i) chamber with 600 ppm of CO_2, ii) chamber with 900 ppm concentration of CO_2, iii) chamber without any elevation of CO_2 and iv) ambient control- in open area. CO_2 enrichment was done by using CO_2 cylinders. The design of the experiment adopted is Completely Randomized Block Design (CRBD).

At end of exposure period under various treatments, plants were sampled and growth parameters were measured like shoot length, root length, and fresh weight of leaf, shoot and root. Then, the plant samples were kept in hot air oven at 60ºC till the constant dry weight is obtained. Then dry weight was recorded for in root, shoot and leaves. The data on growth and dry matter production were subjected to analysis of variance for completely randomized design with replications.

To analyze intra-specific variation in few biometrical and biochemical characteristics of Neem trees in relation to their differential response to elevated CO_2, Four different clones (varieties) of Neem *viz.* IFGTB-AI-9, IFGTB-AI-11, IFGTB-AI-3 and IFGTB-AI-5 were used to assess intra-specific variation in biochemical characteristics in neem trees in relation to their differential response to elevated CO_2. Among these four clones selected for the present study, two clones IFGTB-AI-9 and IFGTB-AI-11 were responding positively under elevated CO_2 environments in the earlier experiments conducted under Open Top Chambers at Institute of Forest Genetics and Tree Breeding, Coimbatore. On the other hand, clones IFGTB-AI-3 and IFGTB-AI-5 were responding poorly to the elevated CO_2 conditions by recording lesser dry matter accumulation in biomass of various plant parts.

The mother plants of these four selected clones of neem, belonging to two categories with respect to their response to elevated CO_2 environments - were studied for variation in chlorophyll content (chlorophyll - a, chlorophyll - b and total chlorophyll), Organic acids (Fumaric acid, Malic acid, Citric acid and Oxalic acid), Nutrient elements (Nitrogen, Phosphorus, Potassium, Calcium and Magnesium) and also for leaf weight, leaf area and Specific Leaf Weight. All these parameters were assessed using leaf samples collected from the respective clones available in the germplasm assemblage of neem in IFGTB campus, Coimbatore.

RESULTS AND DISCUSSIONS

A) *Response of Azadirachta indica to elevated CO_2 in Open Top Chambers*

A set of 12 phenotypes (clones) of Neem (*Azadirachta indica* A. Juss) were used in the experiments conducted in Automated Open Top Chambers (AOTC) facility at IFGTB, Coimbatore. The results of the study showed that there existed significant variation among different treatments of CO_2 as well as among various clones in terms of growth characteristics. Overall mean shoot length was 59.73 cm for plants exposed to 600 ppm of CO_2 and it was only 28.25 cm under ambient conditions, irrespective of clones (Table 1). Root length was greater (39.33 cm) under 900 ppm CO_2 level than that recorded in ambient conditions (30.07 cm). Dry matter accumulation in shoots was 78% more under 600 ppm CO_2 level than that of in ambient conditions. Similarly, 58% more dry matter accumulation was recorded in roots of plants grown under 600 ppm of CO_2 levels when compared to the plants grown in ambient conditions (Table 1).

Among 12 clones studied, clone IFGTB-AI-12 recorded 86% more total dry matter accumulation in biomass of plants grown under 600 ppm of CO_2 as compared to that observed for the same clone under ambient conditions. Clone IFGTB-AI-9 accumulated greater amount of total dry matter in biomass under 900 ppm CO_2 levels that is 89% over and above the total dry matter accumulated in the same clone under ambient environments (Fig 1). In Neem, only one clone that is IFGTB-AI-5 showed negative response in term of total dry matter accumulation under 900 ppm CO_2 level by registering 3% less dry matter accumulation in biomass when compared to that of ambient grown plants of the same clone. Followed by clone IFGTB-AI-3 which recorded lesser accumulation of total dry weight when compared to all other positively responding clones.

Table 1. Shoot and root characteristics of *Azadirachta indica* A. Juss plants exposed to various levels of CO_2 in open top chamber experiments conducted in IFGTB, Coimbatore, India.

	600 ppm	900 ppm	Chamber control	Ambient
Mean shoot length (cm)	59.73 ± 3.48	54.69 ± 2.12	62.05 ± 2.56	28.25 ± 1.59
Mean root length (cm)	36.10 ± 3.11	39.33 ± 2.15	33.20 ± 2.79	30.07 ± 1.01
Leaf dry weight (g per plant)	6.22 ± 0.26	5.52 ± 0.48	6.85 ± 0.40	4.53 ± 0.29
Shoot dry weight (g per plant)	12.24 ± 0.80	10.72 ± 0.61	11.50 ± 0.55	6.86 ± 0.54
Root dry weight (g per plant)	9.12 ± 0.90	8.29 ± 0.61	7.54 ± 0.57	5.78 ± 0.49

Note: *Values shown are Mean ± Standard Error*

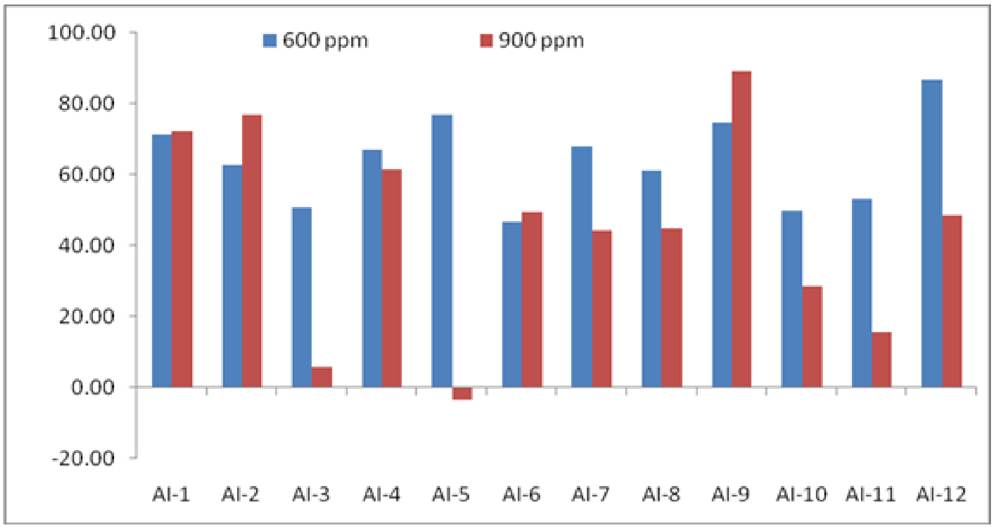

Figure 1. Increase or decrease in total dry weight (%) under different levels of CO_2 as percentage over that of ambient grown respective clones of *Azadirachta indica* A. Juss.

In the present study, shoot length was increased under elevated CO_2 conditions when compared to ambient conditions. As observed in the present study, Pokorny *et al.* (2012) observed that the shoot length was increased frequently under elevated CO_2 conditions. With regard to increased root length under elevated CO_2 environment, it is reported that the responses of roots to CO_2 are dependent on experimental conditions (Ceulemans & Mousseau 1994). When the plants exposed to increased CO_2, root have observed to become more numerous, longer, thicker and faster growing in crops (Chandhuri *et al.* 1990) with increased root length in many plant species (Norby 1994, Pritchard & Rogers 2000, Bernecchi *et al.* 2000). Lengths and volumes of tap root and fine roots were higher for CO_2 enhanced cotton plants (Rogers *et al.* 1993). 110% increase in root length of soybean was observed as CO_2 concentration increased (Rogers *et al.* 1992). Increased root length and number under elevated CO_2 environments has also been reported in sweet potato (Bhattacharya *et al.* 1990) and *Phaseolus acutifolius* and *P. vulgaris* (Salsman *et al.* 1999).

In the present study, dry matter accumulation both in shoot and root biomass was greater in plants grown under elevated CO_2 environments as compared to that in ambient grown plants. Similar findings were reported by Cao *et al.* (2008) in *Gossypium hirsutum* and the above ground biomass was increased under 600 ppm of elevated CO_2 level. Lukac *et al*. (2003) revealed that the CO_2 enrichment increases the belowground biomass allocation in three *populus* species were investigated and the standing root biomass enhanced by 47–76%. Moreover the growth of Minjiang fir showed significantly positive responses of elevated CO_2 with greater increases of total biomass than the control conditions (Hou *et al.* 2011). Ghasemzadeh & Jaafar (2011) reported in two varieties of *Zingiher officinale* were exposed to different CO_2 concentration (400 and 800 ppm) resulted in increasing total plant biomass over the ambient conditions. Mohamed (2013) reported that there is no significant intra specific difference in dry matter production in the tropical dry land forest species of Neem. But when the plants were exposed on increased atmospheric CO_2 the final plant biomass, above ground biomass and below ground biomass was significantly increased in tree species (Madhu & Hatfield 2013). Similarly, the shoot biomass was approximately 35% greater for creeping bentgrass plants grown under elevated CO_2 compared to plants maintained under ambient CO_2, while the root biomass increased by 37% due to elevated CO_2 (Burgess & Huang 2014). Wang *et al.* (2015) studied the responses of rice production under elevated CO_2, there was significant stimulation in above ground biomass 28 per cent and below ground biomass of rice was 42 per cent increases. Similar findings of root biomass was higher under elevated CO_2 levels has been reported by several authors and it was increased of 55 per cent in *Pinus sylvestris* (Jach *et al.*, 2000), 32 per cent in *Pinus taeda* by Jackson *et al.* (2009).

With reference to intra-specific clonal variation in tree species, it is observed in the present study that huge variation existed among the clones in terms of total dry matter accumulation in Neem. Similarly, Johanna *et al.* (2003) also reported that two clones of silver birch (*Betula pendula*) responded differently to elevated CO_2 levels. In one clone, total biomass increased by 40% under elevated CO_2, but in another clone no response was found. Similar such intra-specific clonal variations have been reported in Sitka spruce (Murray *et al.*1994), Poplar (Ceulemans *et al.* 1995), *Hevea brasiliensis* (Devakumar *et al.* 1998), Populus tremuloides (Isebrands *et al.* 2003).

B) *Foliar analysis of differently responding clones for biometrical and biochemical traits*

In the present study, the existing variation in chlorophyll contents among the clones studied were not corresponding to the growth response observed for the respective clones under the elevated CO_2 environments (Table 2). Similarly, Nicolas *et al.* (2007) also reported that there is no link of chlorophyll with tree growth irrespectively of site and family in hybrids of poplar. However, Kumar & Paramathama (2005) reported that all the traits studied, via., plant height, collar diameter. Number of branches, survival percent, chlorophyll content and suitability index were strongly associated with volume index in 44 clones of Casuarina equisetifolia assembled from Tamilnadu, Andhra Pradesh, Orissa and Pondicherry. Reddy *et al.* (2003) also reported that chlorophyll a, b and total chlorophyll showed significant positive correlation with leaf area and yield in different genotype of Mulberry.

Table 2. Intra-specific Variation in Chlorophyll contents (mg chlorophyll per g leaf tissue) in selected Neem clones.

Clones	Chlorophyll-a	Chlorophyll-b	Total Chlorophyll
IFGTB-AI-9	0.0135 ± 0.0016	0.0115 ± 0.0037	0.0359 ± 0.0072
IFGTB-AI-11	0.0074 ± 0.0025	0.0155 ± 0.0046	0.0298 ± 0.0049
IFGTB-AI-3	0.0049 ± 0.0023	0.0048 ± 0.0025	0.0245 ± 0.0079
IFGTB-AI-5	0.0140 ± 0.0014	0.0196 ± 0.0028	0.0490 ± 0.002

Note: *Values shown are Mean ± Standard Error*.

Among various organic acids, Fumaric and Malic acid was in greater quantity in clones positive to elevated CO_2 when compared to other two clones which are responding poorly to elevated CO_2. On the contrary, Oxalic acid content was observed to be lesser in quantity in clones adaptive to elevated CO2 than in clones less adaptive to elevated CO_2. However, there is not much difference among clones in respect of Citric acid content (Table 3). Similar variation in organic acid contents under exposure to stress environments has been reported by Silva *et al.* (2004) who reported that Gas chromatography/mass spectrometry and ion chromatography analyses indicated that root exposure to AI led to a greater than 200% increase in Malic acid concentration in the root tips of all eucalypt species. The increase in Malate concentration in response to AI treatment is correlated with the tree species. A small increase in citric acid `concentration was also observed in all species, but there were no consistent changes in the concentration of other organic acids in response to AI treatment. In all eucalypt species, AI treatment induced the secretion of citric acid and Malic acid in root exudates. But no trend with respect to AI tolerance was observed. Thus, although malate and citrate exudation by roots may partially account for the overall high AI tolerance of these eucalypt species, it appears that tolerance is mainly derived from the internal detoxtification of AI by complexation with malic acid.

Table 3. Intra-specific Variation in organic acids (mg per g leaf tissue) in selected Neem clones.

Clones	Fumaric Acid	Malic acid	Citric acid	Oxalic acid
IFGTB-AI-9	11.56 ± 0.022	11.70 ± 0.322	1.80 ± 0.027	0.049 ± 0.001
IFGTB-AI-11	10.36 ± 0.093	11.34 ± 0.305	2.04 ± 0.002	0.2697 ± 0.012
IFGTB-AI-3	9.40 ± 0.066	10.40 ± 0.907	1.79 ± 0.032	0.4767 ± 0.065
IFGTB-AI-5	8.32 ± 0.057	10.54 ± 0.636	2.42 ± 0.099	0.3967 ± 0.085

Note: *Values shown are Mean ± Standard Error*

Significant observation in the present study was that among various nutrient elements studied, Nitrogen content was greater in amount in clones which are categorized as adaptive to elevated CO_2 (0.112 to 0.14%) when compared to that in clones which are not adaptive to elevated CO_2 environments (0.028 to 0.056%) (Table 4). It is reported that the largest single pool of nitrogen in leaves is RuBisCO and hence it can be inferred that the greater amount of Nitrogen in the leaves of adaptive clones may indicate greater amount of RuBisCO availability. This higher RuBisCO availability is related to higher biomass production, as photosynthesis is catalysed by this RuBisCO, which fixes carbon to form carbohydrates. Moore *et al.* (1998) also reported that short term exposure of elevated CO_2 for plants generally leads to increased rates of leaf-level photosynthesis due to enhanced activity of RuBisCO. On the other hand, photosynthesis down regulation is characterized at the biochemical and leaf levels by reduced chlorophyll content, reduced RuBisCO content and activity and decreased leaf nitrogen concentration on a leaf mass basis (Sage 1994, Tissue *et al.* 1995). It warrants further research to understand the reason for higher leaf N in some species and lower N levels in leaf in other species when plants are exposed to elevated CO_2.

Table 4. Intra-specific Variation in nutrient elements (% in leaf tissue) in selected Neem clones.

Clones	N %	P %	K%	Ca	Mg %
IFGTB-AI-9	0.140	0.196	2.00	0.56	0.08
IFGTB-AI-11	0.112	0.136	2.87	0.28	0.21
IFGTB-AI-3	0.056	0.143	1.97	1.24	1.87
IFGTB-AI-5	0.028	0.135	2.22	1.16	1.74

Another important observation made in the present study was that the actual dry weight and leaf area of clones were not differentiated in terms of clonal variation in respect of their response to elevated CO_2. However, when Specific Leaf Weight (SLW) was worked out, this SLW parameter clearly got differentiated in clones. Greater SLW was recorded in clones which were responding positively to the elevated CO_2 enforcing. Hence, clones IFGTB-AI-9 and IFGTB-AI-11 recorded greater SLW values of 13.06 and 10.75 mg per cm^2 respectively (Table 5). On the other hand, clones IFGTB-AI-3 and IFGTB-AI-5 recorded lesser SLW, which were the clones responded poorly to the elevated CO_2 environments in the earlier experiments. Sicher *et al.* (1994) who studied the photosynthetic acclimation to elevated CO_2 occurs in transformed Tobacco and reported that the dry weight gain was due to increased specific leaf weight. Specific leaf weight was shown to be a valuable index for comparing photosynthesis various parts of a tree canopy over a season or throughout an entire year. Mean annual photosynthetic rate in five separate portions of a spruce canopy was directly proportional to observed differences in specific leaf weight (Ram 1984).

In brief, among various parameters of leaf, organic acids particularly Fumaric acid, Malic acid and Oxalic acid, leaf Nitrogen content and Specific Leaf Weight may be considered to act as a biochemical and biometrical marker to categorize the plants adapted to elevated CO_2 environments, specifically to neem trees.

Table 5. Intra-specific variation in leaf dry weight, leaf area and Specific Leaf Weight in clones of Neem.

Clones	Dry weight	Leaf area (cm^2)	Specific Leaf Weight (mg per cm^2)
IFGTB-AI-9	389.6 ± 0.129	31.67 ± 5.89	13.06 ± 2.16
IFGTB-AI-11	451.7 ± 0.075	43.00 ± 7.00	10.75 ± 2.31
IFGTB-AI-3	519.7 ± 0.087	56.67 ± 6.64	9.36 ± 0.84
IFGTB-AI-5	221.2 ± 0.037	28.00 ± 3.05	7.99 ± 0.68

Note: *Values shown are Mean ± Standard Error*

CONCLUSION

The present study evidently proves that there exists significant intra-specific variation in *Azadirachta indica* in response to elevated CO_2. Greater variation existed in dry matter accumulation in biomass among different clones under elevated CO_2 conditions. This variation could be explored in all other commercially important tropical tree species and superior tree varities can be identified for higher productivity and carbon sequestration potential under forecasted elvated CO_2 levels of the future envirnment.

When four different clones (varieties) of Neem (*Azadirachta indica*) *viz.* IFGTB-AI-9, IFGTB-AI-11, IFGTB-AI-3 and IFGTB-AI-5 were used to assess intra-specific variation in biochemical characteristics in neem trees in relation to their differential response to elevated CO_2, it is observed that among various parameters of leaf, organic acids particularly Fumaric acid, Malic acid and Oxalic acid, leaf Nitrogen content and Specific Leaf Weight may be considered to act as a biochemical and biometrical marker to categorize the plants adapted to elevated CO_2 environments, specifically to neem trees. Further research is suggested to confirm these observations in other tropical tree species which will aid in large scale screening of various tree species for their adaptation potential to ever increasing atmospheric CO_2.

ACKNOWLEDGMENTS

The authors very much thankful to the Director, Institute of Forest Genetics and Tree Breeding, Coimbatore and Director General, Indian Council of Forestry Research and Education, Dehra Dun for providing the opportunity to conduct the experiments in Automated Open Top Chambers.

REFERENCES

Bernacchi CJ, Coleman JS, Bazzaz FA & McConnaughay KDM (2000) Biomass allocation in old-field annual species grown in elevated CO_2 environments: No evidence for optimal partitioning. *Global Change Biology* 6: 855–863.

Bhattacharya NC, Hileman DR, Ghosh PP, Musser RL, Bhattacharya S & Biswas PK (1990) Interaction of enriched CO_2 and water stress on the physiology and production in sweet potato grown in open-top chambers. *Plant, Cell & Environment* 13: 933–940.

Burgess P & Huang B (2014) Growth and physiological responses of creeping bentgrass (*Agrostis stolonifera*) to elevated carbon dioxide concentrations. *Horticulture Research* 1: 1–8.

Buvaneswaran C, Edwin Raj E, Warrier RR & Jayaraj RSC (2010) Scope and opportunities of research on elevated CO_2 and plant response in tropical tree species. *ENVIS Forestry Bulletin* 10(2): 10–16.

Cao L, Eby M, Ridgwell A, Caldeira K, Archer D, Ishida A, Joos F, Matsumoto K, Mikolajewicz U, Mouchet A, Orr JC, Plattner GK, Schlitzer R, Tokos K, Totterdell I, Tschumi T, Yamanaka Y & Yoo A (2008) The importance of ocean transport in the fate of anthropogenic CO_2. *Biogeosciences Discussions* 5: 4521–4557.

Ceulemans R & Mousseau M (1994) Effects of elevated atmospheric CO_2 on woody plants. *New Phytologist* 127: 425–446.

Ceulemans R, Jiang XN & Shao BY (1995) Growth and physiology of one-year old poplar (*Populus*) under elevated atmospheric CO_2 levels. *Annals of Botany* 75(6): 609–617.

Chaudhuri UN, Kirkham MB & Kanemasu ET (1990) Root growth of winter wheat under elevated carbon dioxide and drought. *Crop Science* 30: 853–857.

Couteaux MM, Bottner P, Rouhier H & Billes G (1992) Atmospheric CO2 Increase and Plant Material Quality: Production, Nitrogen Allocation and Litter Decomposition of Sweet Chestnut. In: Teller A, Mathy P & Jeffers JNR (eds) *Responses of Forest Ecosystems to Environmental Changes*. Springer, pp. 429–436.

Devakumar AS, Shesha Shayee MS, Udayakumar M & Prasad TG (1998) Effect of elevated CO2 concentration on seedling growth rate and photosynthesis in *Hevea brasillensis*. *Journal of Bioscience* 23(1): 33–36.

Field C, Jackson R & Mooney H (1995) Stomatal responses to increased CO_2: implications from the plant to the global scale. *Plant, Cell & Environment* 18: 1214–1255.

Ghasemzadeh A & Jaafar HZE (2011) Effect of CO_2 Enrichment on Synthesis of some primary and Secondary Metabolites in Ginger (*Zingiber officinale* Roscoe). *International Journal of Molecular Science* 12: 1101–1114.

Hou Y, Qu J, Luo Z, Zhang C & Wang K (2011) Morphological mechanism of growth response in tree line species Minjiang fir to elevated CO_2 and temperature. *Silva Fennica* 45(2): 181–195.

IPCC (2007) Climate change 2007: The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge Univ. Press, Cambridge, UK.

Isebrands JG, McDonald EP, Kruger E, Hendrey GR, Percy KE, Pregitzer KS, Sober J & Karnosky DF (2003) Growth responses of aspen clones to elevated carbon dioxide and ozone. *Developments in Environmental sciences* 3: 411–435.

Jach ME, Laureysens I & Ceulemans R (2000) Above and below ground production of young Scots pine (*Pinus sylvestris* L.) trees after three years of growth in the field under elevated CO_2. *Annals of Botany* 85: 789–798.

Jackson RB, Cook CW, Pippen JS & Palmer SM (2009) Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. *Ecology* 90: 3352–3366.

Johanna R, Lindsberg MM, Holopainen T, Oksanen E, Lappi J, Peltonen P & Vapaavuori E (2003) Silver birch and climate change: variable growth and carbon allocation responses to elevated concentrations of carbon dioxide and ozone. *Tree Physiology* 24(11): 1227–1237.

Kumar A & Paramathama M (2005) Correlation and path coefficient studies in *Casuarina equisetifolia* L. Johnson. *Indian Forester* 131(1): 47–55.

Lukac M, Calfapietra C & Godbold D (2003) Production, turnover and mycorrhizal colonisation of root systems of three *Populus* species grown under elevated [CO_2] (POPFACE). *Global Change Biology* 9: 838–848.

Madhu M & Hatfield JL (2013) Dynamics of Plant Root Growth under Increased Atmospheric Carbon Dioxide. *Agronomy Journal* 105(3): 657–669.

Mike McGee (2015) Available from: https://www.co2.earth (accessed: 10 Mar. 2016)

Mohamed EA (2013) Growth Performance and Physiological Characteristics of Seedlings of Six Tropical Dry land Forest Tree Species in the Sudan. *Journal of natural resources and environmental studies* 1(2): 25–33.

Moore BD, Cheng SH & Seeman JR (1998) Effects of short and long term ellevated CO2 on the expression of Ribulose 1-5-bisphosphate carboxylase/oxygenase genes and carbohydrate accumulation in leaves of *Arabidopsis thaliana* L. Heynh. *Plant Physiology* 116: 715–723.

Murray MB, Smith RI, Leith ID, Fowler D, Lee HSJ, Friend AD & Jarvis PG (1994) Effects of elevated CO2, nutrition and climatic warming on bud phenology in Sitka spruce (*Picea sitchensis*) and their impact on the risk of frost damage. *Tree Physiology* 14: 69l–706.

Nicolas M, Sophie YD & Reinhart C (2007) Evaluation of leaf traits for indirect selection of high yielding poplar hybrids. *Environmental and Experimental Botany* 61: 103–116.

Norby RJ (1994) Issues and perspectives for investigating responses to elevated atmospheric carbon dioxide. *Plant Soil* 165: 9–20.

Pokorný R, Tomášková I, Drápelová I, Kulhavý J & Marek MV (2012) Long-term effects of CO_2 enrichment on bud phenology and shoot growth patterns of Norway spruce juvenile trees. *Journal of Forestry Science* 56: 251–257.

Pritchard SG & Rogers HH (2000) Spatial and temporal deployment of crop roots in CO_2-enriched environments. *New Phytology* 147: 55–71.

Ram O (1984) *Leaf area index and specific leaf weight: keys to interpreting canopy photosynthesis and stand growth*. Thesis of College of Forestry, Oregon State University, USA.

Reddy BK, Rao DMR, Reddy MP, Jayaram RH, Suryanarayana N (2003) Variation of cholorophyll content and its relationship with leaf area and leaf yield in Mulberry genotypes (*Morus* spp.). *Advances in plant sciences* 16(1): 277–280.

Rogers HH, Peterson CM, McCrimmon JM & Cure JD (1992) Response of soybean roots to elevated atmospheric carbon dioxide. *Plant, Cell & Environment* 15: 749–752.

Rogers HH, Prior SA & O'Neill EG (1993) Cotton root and rhizosphere responses to free-air CO_2 enrichment. In: *Free-air CO_2 enrichment for plant research in the field*. CRC Press, Boca Raton, FL.

Sage RF (1994) Acclimation of photosyntheiss to increasing atmospheric CO_2: the gas exchange perspective. *Photosynthesis Research* 39: 590–596.

Salsman KJ, Jordan DN, Smith SD & Neuman DS (1999) Effect of atmospheric enrichment on root growth and carbohydrate allocation of *Phaseolus* spp. *International Journal of Plant Sciences* 160: 1075–1081.

Silva IR, Novais RF, Jham GN, Barros NF, Gebrim FO, Nunes FN, Neves JC & Leite FP (2004) Responses of eucalypt species to aluminum: the possible involvement of low molecular weight organic acids in the Al tolerance mechanism. *Tree Physiology* 24(11): 1267–1277.

Tissue DT, Griffin KL, Thomas RB & Strain BR (1995) Effects of low and elevated CO_2 on C3 and C4 annuals. II. Photosynthesis and leaf biochemistry. *Oecologia* 101: 21–28.

Wang J, Wnag C, Chen N, Xiong Z, Wolfe D & Zou J (2015) Response of rice production to elevated [CO_2] and its interaction with rising temperature or nitrogen supply: a meta-analysis. *Climatic Change* 130: 529–543.