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Growth and carbon assimilation patterns in diverse multipurpose tree seedlings

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Abstract
Investigations were carried out in *Casuarina equisetifolia, Leucaena leucocephala, Azadirachta indica, Gmelina arborea* and *Eucalyptus tereticornis* seedlings to study the effect of ambient CO_2 in the atmosphere in carbon assimilation pattern at Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam. The observations on the biometric parameters revealed the carbon assimilation pattern in the selected multipurpose tree species. Studies on biometric attributes revealed that leaf area, specific leaf area, specific leaf weight and total dry matter accumulation were highly significant in *Eucalyptus tereticornis* and *Leucaena leucocephala*. The results showed that increasing carbon dioxide concentration of the atmosphere significantly improved the growth traits in the species studied. On analysing the biometric attributes *Eucalyptus tereticornis* and *Leucaena leucocephala* found superior among other multipurpose tree species. The results of the present study revealed that *Eucalyptus tereticornis* and *Leucaena leucocephala* were the best performers among the other multipurpose tree species. These species can be recommended for plantation forestry which could help to achieve a faster and efficient carbon assimilation and carbon gain through higher biomass and yield.

Keywords: Leaf area, dry matter production, carbon assimilation, plantation

Introduction
The issue of climate change is one of the most severe impacts on the environment humanity has ever confronted, and one that humans themselves have generated, placing all planetary life at risk. Reducing the emission of polluting gases that warm the atmosphere and damage the ozone layer through the use of clean technologies and removing many of these gases from circulation is part of the solution. Forest plantations play a significant role as global "cleaners," but the region's potential also lies in land where more trees can be planted or where forests can regenerate naturally, where efficient species can sequester more carbon to enhance their capacity to mitigate greenhouse gases. However, this requires the identification of ecosystems with high carbon sink capacity.

Brown *et al.* (1996) [4] estimated that, by 2050, plantations in tropical countries have the potential to capture as much as 16.4 Gt C whereas agroforestry has the potential to capture 6.3 Gt C. This privileged position is derived from the net potential of carbon from future plantations, implementation of agroforestry systems and induced regeneration of forests, making it possible to produce around 243 million tons of carbon (24.3 million tons a year) in the next decade. However, a methodology for evaluating the biological components of carbon flux remains a significant research issue. This concern may lead to international negotiations on carbon emissions, which will require in depth understanding of national-level carbon budgets. In addition to the scientific importance of understanding the C balance of trees, it is also of economic interest to known how trees will respond to further increases in atmospheric CO_2. Differences in % C among different tree species and among wood types within a single tree (Lamlom and Savidge, 2003) [13] indicated the need to estimate biomass and C content for each species and each tree component. Among various agricultural land-use and management strategies, bioenergy crops have the largest potential for C storage (Smith *et al.*, 2000) [23]. Fast growing multipurpose tree species such as *Eucalyptus tereticornis*, *Leucaena leucocephal*

Casuarina equisetifolia, Azadirachta indica, Gmelina arborea and Bambusa bambos are important components of tropical forest ecosystem and are especially well suited for plantation culture.

Environmental stresses such as drought can cause temporary change in shoot: root ratio that may have important effects on seedling quality and survival (Cannel *et al*., 1978) [5]. They concluded that allocation of biomass and morphology of the leaves has an important impact on plant's carbon economy and are major determinants of inter specific variation in RGR. Although a number of papers have been published on these multipurpose tree species, however, research regarding the effects of elevated CO_2 on its growth in seedling stage is rather few. The present study, therefore, were to determine the effects of the present CO_2 level upon growth and dry matter partitioning in the selected multipurpose tree species, *viz*., *Azadirachta indica, Bambusa bambos, Casuarina equisetifolia, Eucalyptus tereticornis, Gmelina arborea* and *Leucaena leucocephala* during the seedling stage and to investigate, through growth analysis, the relationship between source and sink, dry matter partitioning and its components under the present stress conditions. Such analytical tools as local Carbon budgets are needed to improve our preventive and mitigative strategies for dealing with global climate change. With this background, the current study is designed to assess the carbon assimilation pattern and estimate the carbon stock available in the selected multipurpose tree species in seedling stages.

Materials and Methods

The laboratory and the nursery studies were carried out in order to study the carbon assimilation pattern and carbon gain in the selected multipurpose tree species in the seedling stage at Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam. The species under study includes *Casuarina equisetifolia* (T_1), *Leucaena leucocephala* (T_2), *Bambusa bambos* (T3), *Azadirachta indica* (T_4), *Gmelina arborea* (T_5) and *Eucalyptus tereticornis* (T_6). The Institute is located in the western zone of Tamil Nadu. Geographically it is located at 11º 19' N latitude and 77º 56' E longitude and an altitude of 300 m above msl. The soil used was non-calcareous, red loam (Typic Ustropept), having pH 7.24 and Organic Carbon 0.426%. The status of available nitrogen, phosphorus and potassium of the experimental site were 187.6, 51.4 and 64 kg ha^{-1}. Fresh seeds of all the selected multipurpose tree species were collected from single identified trees at Forest College and Research Institute, Mettupalayam. The same seeds were used for raising the seedlings in the nursery under study. The experiment was laid out in a Complete Randomized Design (CRD) with six treatments, each replicated four times. The observations on biometric parameters were recorded at 30, 60, 90 and 120 days after transplanting (DAT) @ four seedlings per replication. The leaf area was estimated by graphic method as suggested by Ashley *et al.* (1963) [2]. To estimate the leaf area, the outline of the leaf is drawn on a graph paper and the number of full squares, half squares and quarter squares are counted and added. The leaf area was expressed as cm^2 $leaf^{-1}$. Estimation of carbon assimilatory efficiency of leaves or to estimate the leafiness of plants, Radford (1967) [19] suggested leaf area ratio as a measure of leaf area to the weight of the whole plant. It was expressed as cm^2 g^{-1}. SLA is the ratio of assimilating surface to its dry weight. Kvet *et al.* (1971) [12] suggested Specific leaf area as a measure of leaf area per plant to the leaf dry weight per plant. It was expressed as cm^2 g^{-1}. Specific leaf weight (SLW) is the ratio of leaf dry weight to its area of assimilating surface. The formula was suggested by Pearce *et al.* (1968) [17] and expressed as mg cm^{-2}. The shoot and root length is expressed in cm. Dry weight of shoot and root in g $seedling^{-1}$.

The estimation of biomass carbon stock is based on biomass and carbon content (%) in different components of tree species. The total biomass carbon was calculated by using below given formulae.

- AGB carbon (g C $plant^{-1}$) = Above ground biomass (g $plant^{-1}$) × Carbon content (%)
- BGB carbon (g C $plant^{-1}$) = Below ground biomass (g $plant^{-1}$) × Carbon content (%)
- Total biomass carbon stock (g C $plant^{-1}$) = AGB carbon + BGB carbon

The data were analyzed by standard statistical procedure described by Snedecor and Cochran (1967) [24]. The stage wise data were analyzed separately using AGRES software.

Results and Discussion

To achieve the objectives outlined in the introduction, experiments were conducted on seedlings of *Casuarina equisetifolia, Leucaena leucocephala, Bambusa bambos, Azadirachta indica, Gmelina arborea*, and *Eucalyptus tereticornis*. These experiments aimed to study the carbon gain among the selected multipurpose tree species. Observations on biometric attributes under ambient CO_2 conditions were recorded to reveal the carbon assimilation patterns of these species. The collected data were subjected to statistical analysis, and the results are comprehensively presented and discussed in this chapter.

1. Biometric attributes

a. Leaf area

The study revealed significant variations in leaf area among the selected tree species at the seedling stage. *Eucalyptus tereticornis* exhibited the largest leaf area, measuring 567 cm² per plant. This was followed by *Leucaena leucocephala*, which had a leaf area of 475 cm². In contrast, *Bambusa bambos* had the smallest leaf area, recorded at 94 cm² (Table 1). The leaf area is a crucial indicator of the plant's photosynthetic capacity, as it directly correlates with the plant's ability to assimilate carbon and produce dry matter. The growth analysis indicated that the leaf area in all species increased exponentially over time, highlighting the dynamic nature of leaf development during the seedling stage.

The leaf area expansion was significant in the present study which could be significantly observed in *Eucalyptus tereticornis* which was in confirmative with the findings of Ilango (1997) [10] who reported a three fold increase in leaf area in *Albizia lebbeck* with increase in age of seedlings. The increase in the leaf area due to enhanced carbon dioxide levels can be assigned to the effective assimilatory pattern of C_3 leaf dry weight to its area of assimilating surface. The formula was suggested by Pearce *et al.* (1968) [17] and expressed as mg cm^{-2}. The shoot and root length is expressed in cm. Dry weight of shoot and root in g $seedling^{-1}$.

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- AGB carbon (g C $plant^{-1}$) = Above ground biomass (g $plant^{-1}$) × Carbon content (%)
- BGB carbon (g C $plant^{-1}$) = Below ground biomass (g $plant^{-1}$) × Carbon content (%)
- Total biomass carbon stock (g C $plant^{-1}$) = AGB carbon+

BGB carbon

The data were analyzed by standard statistical procedure described by Snedecor and Cochran (1967) [24]. The stage wise data were analyzed separately using AGRES software.

Towards realizing the objectives enumerated in introduction, experiments were carried out in *Casuarina equisetifolia, Leucaena leucocephala, Bambusa bambos, Azadirachta indica, Gmelina arborea* and *Eucalyptus tereticornis* seedlings, to study the carbon gain among the selected multipurpose tree species. The observations on the biometric attribute in the ambient CO_2 condition revealed the carbon assimilation pattern in the selected multipurpose tree species. The data obtained from these experiments were statistically analyzed and the results are presented and discussed thoroughly in this chapter.

1. Biometric attributes

1.1 Leaf area

In this study, *Eucalyptus tereticornis* exhibited the highest leaf area per plant (567 cm^2), followed by *Leucaena leucocephala* (475 cm^2), while *Bambusa bambos* had the lowest leaf area (94 cm^2) (Table 1). The leaf area represents the primary photosynthesizing apparatus responsible for carbon fixation and subsequent dry matter production in terrestrial plants. The growth analysis demonstrated that the leaf area of all species increased exponentially over time. Notably, *Eucalyptus tereticornis* showed a significant expansion in leaf area, aligning with Ilango's (1997) [10] findings of a threefold increase in *Albizia lebbeck* leaf area as seedlings aged. This increase can be attributed to the efficient carbon assimilation patterns characteristic of C3 plants, a category to which most tree species belong. Elkohen *et al*. (1993) [8] also supported these findings, reporting that elevated CO_2 levels significantly boosted net photosynthesis in beech seedlings, leading to a substantial increase in total leaf area per plant due to more growth flushes and larger leaves.

1.2 Leaf area ratio

The highest leaf area ratio (85.26 $cm^2\ g^{-1}$) was found in *Leucaena leucocephala*, followed by *Azadirachta indica* with a ratio of 66.58 $cm^2\ g^{-1}$. In contrast, *Bambusa bambos* exhibited the lowest leaf area ratio at 26.35 $cm^2\ g^{-1}$ (Table 1). Significant variations were noted at different growth stages. The leaf area ratio, indicating the proportion of leaf area to total plant weight, is influenced by the rate of leaf expansion, net assimilation rate, leaf area, and leaf weight. Among these, leaf area is the primary dependent variable. Enhanced carbon dioxide levels notably improved leaf area expansion during the seedling growth stage.

In the present study, the leaf area ratio (LAR) values showed a significant increase from 30 to 60 days after transplanting. Following this period, there was a marginal increase until 90 days, after which the LAR declined. This decline can be attributed to the senescence of older leaves and the redistribution of dry matter or carbon sources to the stem and branches, contributing to the overall plant biomass. These observations are consistent with findings by Reich *et al*. (1992) [21], who noted that a decrease in LAR with increasing leaf lifespan is due to the translocation of nitrogen and other resources from older leaves to other parts of the plant, leading to a reduction in leaf nitrogen content over time.

1.3 Specific leaf area

The specific leaf area indirectly measures the leaf expansion rate in relation to leaf weight. Significant variation in specific leaf area was found at different stages of growth in the study. Specific Leaf Area was found to be higher (1.03 $cm^2\ mg^{-1}$) in *Leucaena leucocephala*, followed by *Casuarina equisetifolia*, which was on par with *Azadirachta indica* recording 0.25 $cm^2\ mg^{-1}$. *Eucalyptus tereticornis* recorded the lowest specific leaf area of 0.16 $cm^2\ mg^{-1}$ (Table 1). The reduction in specific leaf area was noted from 30 to 60 DAT and showed a marginal increase till 120 DAT. *Eucalyptus tereticornis* recorded lower specific leaf area, which is an indirect measure of carbon gain or dry matter accumulation. This finding is highly supported by the findings of Ostman and Weaver (1982) [15] who reported that the decrease in specific leaf area at the initial stage followed by an increase near the end presumably indicates retranslocation of organic matter in and out of leaves during senescence. The results were also in conformity with the findings of Paramaguru and Thamburaj (1991) [16] in Cassava who found the decreased specific leaf area with ageing of plants.

Reich *et al.* (1992) [21] also confirmed the specific leaf area of seedlings decreased with increase in leaf life span. Peneulas and Matamala (1990) [18] indicated that parallel to the increase in atmospheric carbon dioxide from 278 µ mol mol^{-1} in 1750 AD to the current ambient 348 µ mol mol^{-1}, there have been overall decrease in specific leaf area from 184% to 100% today.

Table 1: Biometric attributes (LA, LAR, SLA & SLW) in the selected multipurpose tree species at seedling stage

Species	LA (cm^2)				LAR ($cm^2\ g^{-1}$)				SLA ($cm^2\ mg^{-2}$)				SLW ($mg\ cm^{-2}$)			
	30 DAT	60 DAT	90 DAT	120 DAT	30 DAT	60 DAT	90 DAT	120 DAT	30 DAT	60 DAT	90 DAT	120 DAT	30 DAT	60 DAT	90 DAT	120 DAT
Casuarina equisetifolia	23	40	68	100	30.59	23.25	25.69	28.78	0.31	0.26	0.21	0.20	3.34	4.02	4.89	5.21
Leucaena leucocephala	56	155	286	475	61.49	93.99	90.90	85.26	0.43	0.66	0.45	1.03	4.49	5.44	7.35	5.09
Bambusa bambos	20	42	59	94	23.89	22.52	21.54	26.35	0.24	0.21	0.19	0.18	4.31	5.09	5.34	5.82
Azadirachta indica	12	48	131	206	33.41	71.36	74.39	66.58	0.26	0.21	0.24	0.27	4.02	5.02	4.29	3.73
Gmelina arborea	23	79	215	448	16.67	25.98	44.06	65.46	0.14	0.17	0.20	0.17	7.35	5.91	5.03	6.09
Eucalyptus tereticornis	32	87	256	567	30.03	45.62	79.44	64.03	0.13	0.11	0.15	0.16	8.19	9.11	7.12	6.39
SEd	27	75	169	315	32.68	47.12	56.00	56.08	0.25	0.27	0.24	0.34	5.28	5.76	5.67	5.39
CD (P = 0.05)	2.00	3.07	2.27	5.82	1.52	1.06	1.37	0.96	0.005	0.005	0.003	0.003	0.004	0.003	0.003	0.014

1.4 Specific leaf weight

The specific leaf weight of trees indirectly reveals the thickness of the leaf. The ratio of assimilating surface to its dry weight is represented as specific leaf weight. Measure of specific leaf weight also gives the assimilatory efficiency of the leaf which decreased towards crop maturity, since proteins and metabolites are translocated from the leaf to the developing sink (Pearce *et al*., 1968) [17]. *Eucalyptus tereticornis* has recorded the highest specific leaf weight of 6.39 mg cm^{-2}, followed by *Gmelina arborea* which recorded 6.09 mg cm^{-2}. The lowest specific leaf weight of 3.73 mg cm^{-2} was recorded in *Azadirachta indica* (Table 1).

In this study, an increase in specific leaf weight was observed across all species, likely due to elevated atmospheric CO_2 concentrations. This trend aligns with previous research by Rao *et al*. (1999) [20], who found that seedlings exposed to higher CO_2 levels exhibited greater specific leaf weight. Among the species studied, *Eucalyptus tereticornis* demonstrated the highest specific leaf weight. The increase was particularly notable between 30 to 60 days after transplanting (DAT), followed by a decline towards the end of the growth stage. This decrease can be attributed to the translocation of proteins and metabolites from the leaves to developing parts of the plant, a phenomenon also noted by Pearce *et al.* (1968) [17].

1.5 Shoot length and Root length

Growth is one of the most fundamental and conspicuous characteristics of living organism. The growth in length of the plant is due to the meristematic activity of the apical meristems that takes place in the root and shoots apices. Among the tree species, *Eucalyptus tereticornis* recorded the maximum shoot length (65.6 cm), followed by *Leucaena leucocephala* which measured 55.1 cm. The lowest shoot length of 21.8 cm was recorded in *Azadirachta indica* (Table 2). The shoot length varied significantly at all the stages of growth. Bertomeu and Sungkit (1998) [3] also reported an increasing growth rate in Eucalyptus deglupta Blume. in the seedling stage. Similarly the root length also varied significantly at all the stages of the growth. The maximum root length of (55.5 cm) was recorded in *Casuarina equisetifolia*, followed by *Leucaena leucocephala* which measured 47.7 cm. The lowest root length of 30.0 cm was recorded in *Azadirachta indica* (Table 2). The increase in the root length was noted in all the species with increase in age of the seedlings which was in conformity with the findings of Saravanan (2000) [22] who reported an increasing root length trend with increase in age of the seedlings. *Casuarina equisetifolia* showed maximum root length. This is in conformity with the findings of Adalarasan (2002) [1] who reported a similar trend of root length values in *Casuarina equisetifolia* when grown as control. The reason could be the roots of Casuarina seedlings possess nodules capable of fixing atmospheric nitrogen which enhances the root growth.

1.6 Shoot and root dry weight

The increase in the shoot dry weight was found significant in all the species. Among the tree species, *Eucalyptus tereticornis* recorded the maximum shoot dry weight (6.44 g seedling^{-1}), followed by *Leucaena leucocephala* which measured 4.81 g seedling^{-1} (Table 2). This could be because of faster growth rate, longer growth period and better photosynthetic efficiency of the leaves which supply abundant energy for biomass production. This finding is supported by the findings of Jarvis and Jarvis (1964) [11] who revealed that long growth period had a significant effect on annual production of dry matter by the trees which coincide with high yielding species of Eucalyptus and Populus have long growth period. In contrast, *Casuarina equisetifolia* recorded the maximum root dry weight (3.28 g seedling^{-1}), followed by *Leucaena leucocephala* which recorded 3.12 g seedling^{-1}. Casuarina seedlings had maximum root dry weight among all the treatments. The reason that could be attributed to greater 'N' transfer rate which is in conformity with the findings of Srivastava and Ambasht (1995) [25] who concluded that in Casuarina stands, root nodules contribute significantly to below ground production.

1.7 Total dry matter production

The study's results illustrate significant variations in carbon assimilation and biomass production among the evaluated tree species. *Eucalyptus tereticornis* emerged as the most effective in terms of both total dry matter production (8.86 g) and biomass carbon (3.22 g C plant^{-1}), indicating its superior ability to convert resources into biomass compared to other species like *Leucaena leucocephala*, which had lower values of 7.93 g and 2.64 g C plant^{-1}, respectively. *Azadirachta indica* showed the least biomass accumulation, with only 3.09 g of total dry matter. These findings are consistent with Monteith's (1977) principle that photosynthetic efficiency and light interception—both influenced by leaf area and tree architecture—are critical determinants of plant productivity. The enhanced growth and biomass in Eucalyptus seedlings can be attributed to their larger leaf area, which facilitates better light capture and photosynthesis, aligning with Hideaki's (2004) observation that increased leaf area contributes to higher biomass production. The study also confirms that elevated CO_2 levels significantly impact biomass accumulation, supported by Damesin *et al.* (1996) and others, who found increased shoot and root biomass under elevated CO_2 conditions. This research highlights *Eucalyptus tereticornis and Leucaena leucocephala* as promising species for carbon sequestration, thanks to their efficient carbon assimilation and higher biomass yield. These species are thus recommended for use in plantation forestry to optimize carbon capture and storage, contributing to effective climate change mitigation strategies.

Table 2: Biometric attributes (SL, RL, SDW & RDW) in the selected multipurpose tree species at seedling stage

Species	SL (cm)				RL(cm)				SDW (g seedling^{-1})				RDW (g seedling^{-1})			
	30 DAT	60 DAT	90 DAT	120 DAT	30 DAT	60 DAT	90 DAT	120 DAT	30 DAT	60 DAT	90 DAT	120 DAT	30 DAT	60 DAT	90 DAT	120 DAT
Casuarina equisetifolia	16.5	22.8	38.0	48.1	15.4	28.1	39.9	55.5	0.35	0.66	1.21	3.21	0.41	0.98	1.23	3.28
Leucaena leucocephala	18.1	25.3	41.0	55.1	16.3	21.0	31.3	47.7	0.51	0.94	1.91	4.81	0.40	0.72	1.21	3.12
Bambusa bambos	8.2	14.3	21.1	25.9	13.5	19.4	25.0	38.0	0.47	0.88	1.45	1.98	0.41	0.85	1.41	1.81
Azadirachta indica	6.9	10.8	18.5	21.8	9.8	14.9	20.5	30.0	0.18	0.33	0.86	1.80	0.20	0.35	0.87	1.29
Gmelina arborea	15.2	22.3	32.1	40.7	12.4	17.3	27.4	35.9	0.62	0.78	1.63	4.27	0.23	0.48	1.10	2.63
Eucalyptus tereticornis	19.2	29.5	48.3	65.6	12.4	17.3	21.5	31.0	0.82	1.46	2.25	6.44	0.28	0.45	0.96	2.42
SEd	14.0	20.8	33.2	42.9	13.3	19.7	27.6	39.7	0.49	0.84	1.55	3.75	0.32	0.64	1.13	2.43
CD(P = 0.05)	0.33	0.48	0.78	0.92	0.21	0.40	0.76	0.77	0.01	0.01	0.02	0.03	0.01	0.01	0.02	0.05

Table 3: Total dry matter production (g seedling^{-1}) and biomass carbon stock (g C plant^{-1}) in the selected multipurpose tree species at seedling stages.

Species	120 Days after transplanting			
	TDP	AGB carbon	BGB carbon	Total biomass carbon
Casuarina equisetifolia	6.49	1.01	1.09	2.10
Leucaena leucocephala	7.93	1.57	1.07	2.64
Bambusa bambos	3.79	0.54	0.51	1.05
Azadirachta indica	3.09	0.42	0.33	0.75
Gmelina arborea	6.89	1.24	0.81	2.05
Eucalyptus tereticornis	8.86	2.29	0.93	3.22
Mean	6.18	1.18	0.79	1.97
SEd	0.06	0.028	0.031	0.05
CD (P = 0.05)	0.11	0.06	0.06	0.11

Conclusion

In this study, the total biomass carbon stock, which represents the amount of carbon stored in a particular reservoir or system, showed a notable increase in Eucalyptus seedlings. This can be attributed to the relatively larger photosynthetic area of Eucalyptus, which enhances its ability to assimilate carbon and consequently supports a faster growth rate compared to the other species examined. Additionally, the elevated atmospheric carbon dioxide levels likely contributed to improved net carbon assimilation, further accelerating growth in Eucalyptus seedlings.

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