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SEASONAL AND INTRASPECIFIC VARIABILITY OF CHLOROPHYLL FLUORESCENCE, PIGMENTATION AND GROWTH OF *PINUS PONDEROSA* SUBJECTED TO ELEVATED CO_2

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Abstract

Atmospheric CO_2 is expected to double in the next century, and these increases will have substantial impact on forest ecosystems. However, the database on the effects of elevated CO_2 on forests is limited, and the extent of intraspecific variability remains unknown. We are investigating the effects of elevated CO_2 on the intraspecific variability of quantum yield (as measured through chlorophyll fluorescence Fv/Fm ratio) and pigmentation, and how these are correlated to variability in growth.

Four-year-old *Pinus ponderosa* seedlings were obtained from nine different sources (either half-sibling or open-pollinated) across California. These seedlings were grown in standard outdoor exposure chambers for sixteen months at either ambient levels of CO_2, ambient+175ppm CO_2, or ambient+350ppm CO_2. The seedlings were periodically measured for growth, pigmentation, and chlorophyll fluorescence (Fv/Fm).

The results showed a variable growth response of the nine sources during all measurement periods. For example, stem diameter increases varied from 2.6% to 22.4% during the month of October 1993 (comparing ambient to ambient+350ppm).

Increasing CO_2 resulted in a decrease in Fv/Fm (compared to ambient) among sources ranging from -2.1% to -23.2% in February, and 3.1% to -12.5% in June. The source that had the best growth throughout the study, also had a minimal reduction in quantum yield (Fv/Fm) in the presence of elevated CO_2. For the seedlings of fastest growing sources, the correspondence between total growth and chlorophyll fluorescence was strongest during the February measurement period.

Our results also showed a significant reduction in pigmentation (chlorophyll a and carotenoids) due to increased CO_2. However, there were no significant effects due to family.

There are at least three explanations for the different responses during each measurement periods. First, the trees could be adapting favorably to increasing CO_2. Secondly, 1993 needles (which were used for chlorophyll fluorescence) could be under less physiological stress than the current year needles, which will be measured in August 1994. Third, there is a seasonal effect dependent upon temperature or light which is influencing the Fv/Fm ratio and pigmentation.

Introduction

Levels of atmospheric CO_2 have been gradually increasing in modern times. It has been estimated that prior to the industrial revolution, atmospheric CO_2 levels were between 260 and 290 $\mu l\ l^{-1}$. Measurements taken in 1980 show an almost 16% increase to 335 to 340 $\mu l\ l^{-1}$. It has been projected that CO_2 levels could increase to 600 $\mu l\ l^{-1}$ by the middle of the next century (Bacastow and Keeling 1973, Bolin et al. 1986).

Since CO_2 plays a major role in photosynthesis, these increasing levels of CO_2 may have a significant effect on plants. The majority of studies investigating the effect of elevated CO_2 have involved annular species, such as agricultural or floral crop plants. Of those few studies researching longer lived species, many were short-term. It is the goal of the present study to explore the effects of elevated CO_2 levels in a long term study of the forest species *Pinus ponderosa*.

Specifically, this report focuses on the effect of increased CO_2 on two aspects of the photosynthetic system. The first part of our study will utilize chlorophyll fluorescence to analyze the effects of CO_2 on photosynthesis. It has been shown that changes in chlorophyll fluorescence emission during the induction of photosynthesis are closely related to the rate of CO_2 assimilation (Krause and Weis 1991). It was even suggested as early as 1874 by N.J.C. Muller that there is an inverse relationship between chlorophyll fluorescence and CO_2 assimilation (Lichtenthaler 1992). Additionally, Lichtenthaler and Rinderle (1988) have written that the reciprocal relationship between *in vivo* chlorophyll fluorescence and photosynthetic activity can be used to detect stress effects on green plants and to study the potential photosynthetic activity of leaves. Other researchers such as Schmidt et. al. (1990) have also stated that chlorophyll fluorescence has been useful in studying the effects of environmental stresses such as temperature, air pollution and water stress. The fluorescence parameter which we will principally be examining is the ratio of the variable fluorescence to the maximal fluorescence (Fv/Fm), which indicates the relative photochemical efficiency of photosystem II (Krause and Weis 1991).

Since these electron transfers use pigment molecules, we will also assay the levels of different pigments found in the needles. Krause and Weis (1991) have claimed that fluorescence at Fo (non-variable fluorescence) is an emission by antenna chlorophyll *a* molecules. So then chlorophyll *a* concentrations might directly influence chlorophyll fluorescence. Fv/Fm would be influenced by pigment levels since Fv is a function of Fo. Changes in pigmentation alone have been used as an indication of air pollution induced stress (Houpis et al. 1988). We will look at changes in pigmentation levels as an indication of stress or possibly as an adaptive response to increased CO_2.

Methods

The study was performed at Lawrence Livermore National Laboratory (Livermore, CA) utilizing outdoor CO_2 exposure facilities. All plants were grown in 3m x 3m open-top chambers (Allen et al 1992). There were 18 chambers containing *P. ponderosa* seedlings, with three CO_2 treatments. Six chambers exposed seedlings to ambient levels of CO_2, six exposed seedlings to approximately 1.5 times the ambient concentration of CO_2 (+ 175 $\mu l\ l^{-1}$ CO_2) and the final chambers housed plants exposed to approximately twice the atmospheric level of CO_2 (+ 350 $\mu l\ l^{-1}$ CO_2). All chambers were arranged in a completely randomized experimental design. All trees were approximately four-years-old. The seedlings were placed in the exposure chambers in April 1993, and were continually exposed for 16 months.

To analyze intraspecific variability, we included seedling source (family) as an additional treatment, using a split-plot experimental design. We included nine different families in this experiment. There were four sets of half siblings, obtained from the El Dorado National Forest (Family 3087, 3088, 3354, and 3399). Five additional open-pollinated sources were obtained from different geographic regions: Mendocino California (OP5), Sierra (eastern) California (OP6), San Bernandino California (OP7), Santa Clara California (OP8) and El Dorado County California (OP9).

For each of the two assays, nine trees from each chamber and control plot were studied, for a total of 189 trees per sampling. Each of the nine trees sampled were of a different genotype. The fascicles sampled were taken from the 1993 age class of needles. Measurements were taken periodically from January to July 1994.

For the first assay we used a CF-1000 Chlorophyll Fluorescence Measurement System (Version 2.00 Morgan Scientific, Inc., Andover MA), which measures fluorescence at 690 nm. Cuvettes were used to dark adapt the needles, and were attached to one fascicle 45 minutes prior to measurement. Settings on the CF-1000 were : light level = 1000 μmole/m^2 s^1 and sample time = 50 s. The CF-1000 automatically calculates the photochemical efficiency of photosystem II (PSII: Fv/Fm) of the sample being studied.

The second assay was for needle pigmentation. Foliar samples were collected for analysis of chlorophyll *a* and *b* content, and carotenoids. One fascicle from each plant was collected and measured for leaf area. After removing the bundle sheath, the needles were cut into approximately 1 cm fragments, and then immersed in N,N-dimethyl formamide (DMF). The samples were kept in the dark at 4 °C during a 21 day pigment extraction period.. Following extraction, the absorbance of the extract was measured spectrophotometrically at wavelengths of 440 nm, 644 nm and 662 nm using an ultraviolet diode array spectrophotometer (Hewlett Packard HP8452A). The total content of chlorophyll *a*, chlorophyll *b* and carotenoids was calculated based on absorbance coefficients of Lichtenthaler and Wellburn (1983). Pigment concentrations were expressed on a leaf area basis.

Growth measurements consisted of the height and diameter of the main stem. Height measurements were taken to the nearest half centimeter using a tape measure. Diameter was measured at the cotyledon whorl with a vernier caliper to the nearest 1/10th of a millimeter.

Results

Growth

Family differences in stem diameter were statistically significant ($p \leq 0.05$) in all measurement periods (Figure 1). Additionally there was a trend of increased stem diameter growth with increased CO_2 treatment. Height measurements in January tended to show an increase relative to ambient trees at +175 ppm CO_2, while growth was minimal or negative at + 350 ppm CO_2 (Figure 2). Stem height changes in July were in most cases similar to those seen in January, but there was more of a trend of increased height relative to ambient trees of the same family (Figure 2).

Chlorophyll fluorescence

Our February chlorophyll fluorescence results showed a family specific response to the different CO_2 treatments, as indicated by the relative efficiencies of photochemical transfer in photosystem II (Fv/Fm; Figure 3a). April results showed a variable family specific response independent of CO_2 treatment, while the June measurements were similar to those seen in February, although not as dramatic (Figures 3b and 3c respectively). Certain trends were visible throughout, however, such as the obvious negative effect on family OP6 (a seedling source from the eastern Sierra Nevada). Increasing CO_2 resulted in a decrease in Fv/Fm in all sources of seedlings in February (Fv/Fm mean ratios of 0.74, 0.69 and 0.66 for ambient, ambient + 175 and ambient + 350 respectively) and eight of the nine families in June. The variability in percent change in Fv/Fm (comparing ambient to ambient + 350) ranged from -2.1% to -23.2% in February, to 3.1% to -12.5% in June. The source of *P. ponderosa* that had the best growth performance throughout the length of the study (Family 3399), also had a minimal reduction in quantum yield (maintained Fv/Fm) in the presence of elevated CO_2. Growth measurements of the better growth performers corresponded the best to chlorophyll fluorescence during the February measurement period.

Photosynthetic pigments

Chlorophylls: February results showed a significant reduction in chlorophyll a due to elevated CO_2 (13.52, 12.44, and 11.89 mg • m^{-2} for ambient, ambient+175 ppm CO_2, and ambient+350 ppm CO_2 respectively). In April we observed a greater significant reduction in chlorophyll a due to elevated CO_2 (15.36, 13.45, and 12.51 mg • m^{-2} for ambient, ambient+175 ppm CO_2, and ambient+350 ppm CO_2, respectively). Additionally, there were no significant family effects or family x CO_2 interactions.

Chlorophyll *b* levels and the chlorophyll *a/b* ratio showed no significant differences between ambient and treatment groups at any of the three measuring periods.

Carotenoids: February carotenoid levels followed the pattern of the corresponding chlorophyll measurements with a statistically significant ($p \leq 0.05$) decrease in the treatment groups relative to ambient. Also, with increasing length of study, there was an increasing difference in carotenoid levels between the CO_2 treatment levels (for June, the carotenoid levels were 13.01, 12.06, and 11.02 mg • m^{-2} for ambient, ambient+175 ppm CO_2, and ambient+350 ppm CO_2 respectively).

Discussion

The Fv/Fm ratio showed the most intraspecific variability with regard to CO_2 treatment during the February measuring period. We also saw the most intraspecific CO_2 interaction in the January stem diameter measurements. In general, almost all of the treated groups (regardless of Fv/Fm) showed a consistent increase in growth compared to ambient. The results indicate a tendency for the treatment with the greater growth to correspond to less of a decrease in Fv/Fm compared to ambient.

The increased growth might be explained by the fact that more carbon is readily available to the plants, so more is assimilated and utilized for new tissue growth. However, among certain sources of seedlings, it is then possible that the poor growth performers are not as well adapted to utilize the increase in atmospheric carbon availability. These seedlings responded to elevated CO_2 with decreased photosynthetic efficiency, thereby emitting more fluorescence. Although, these seedlings still have a slight increase in growth compared to seedlings grown at ambient CO_2 concentration. It is interesting to note that one of the least adapted families is that of OP6 from the eastern Sierra Nevada, where air pollution (and presumably CO_2 levels) are lower than in other areas of California. This family may not have developed an adaptive carbon assimilation physiology or morphology that would make it better adapted to higher CO_2 levels. A recent study on cotton leaves (Betsche, 1994) has also found that the long-term response of leaves to atmospheric CO_2 enrichment was variable.

With regard to photosynthetic pigments, instead of an intraspecific reaction to the CO_2 treatments, we saw that the pigments of all trees were affected approximately equally by the treatments. This may lead us to conclude that it is not the decrease in pigments which is responsible for the intraspecific variability in the decrease in photosystem II efficiency (Fv/Fm). This is supported by the findings of Hagg et al. (1992), who observed a drop in chlorophyll and carotenoid content to be unrelated to chlorophyll fluorescence, in spruce needles.

One possible explanation for the observed trend in pigmentation is that the decrease in pigment levels might be that an adaptive alteration in their physiology has occurred, rather than a sign that the seedlings are stressed, (Houpis et al. 1988). This would mean that the plants need to harvest less light for photosynthesis, and are actually functioning more efficiently. Apparently there is a reduction in light harvesting pigments, but even with a reduction in pigments, there still may be a surplus of light energy as indicated by a reduction in quantum efficiency (as indicated by Fv/Fm) but an increase in growth. It is also possible that the seedlings are being stressed, as Houpis et al. (1988) also found that at ambient + 300 ppm CO_2 treatment there actually was a decrease in growth. Perhaps as the present study continues, we will see a decrease in growth in certain families at the highest CO_2 treatment. Houpis et. al. (1988) also found some indication of intraspecific variety relating to pigment concentration which we have not yet observed at present.

Betsche (1994) mentions several ways in which high concentrations of CO_2 could cause stress to a plant: Oversized starch granules (which have been observed in trees from the present study), formed in response to elevated CO_2 levels could hinder gas diffusion or cause physical membrane damage. High CO_2 concentrations may induce low inorganic phosphate concentrations which can limit chloroplast ATP synthase. Alternatively the treatments could induce feedback-inhibition and photosynthetic decline because of imbalance between CO_2 fixation and assimilate utilization.

Upon examination of the data, for both chlorophyll fluorescence and pigmentation, the CO_2 specific variation observed is less in the summer than it was in the winter. There are at least three possible explanations for this observation. One possibility is that the trees are adapting favorably to the increased CO_2. At first the trees were not able to cope with the increased CO_2, but as time went on perhaps some adaptive mechanism developed. A second possibility is that there is a seasonal effect relating to temperature, length of day or intensity of light. A third consideration is that 1993 needles (which were used for the present study) are under less physiological stress or are less active than current year needles.

By studying chlorophyll fluorescence and the photosynthetic pigment levels of *P. ponderosa* under treatment with elevated levels of atmospheric CO_2 we have gained some insight into the long-term effects of this gas (which is rapidly accumulating in our environment) upon a dominant forest species. Through further work of this nature at Lawrence Livermore National Laboratory and similar facilities we hope to be able to gain a greater understanding of plant physiology while learning how atmospheric conditions affect forests.

Acknowledgments

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References

Allen, L.H. Jr, Drake, B.G., Rogers, H.H., and Shinn, J.H. . 1992. Field techniques for exposure of plants and ecosystems to elevated CO_2 and other trace gases. In FACE: Free-air CO_2 enrichment for plant research in the field. CRC Press, Boca Raton, Fl. Pp. 85-119.

Bacastow, R. and Keeling, C.D. 1973. Atmospheric carbon dioxide and radiocarbon in the natural carbon cycle. II. Changes from AD 1700 to 2070 as deduced from a geochemical model. In Carbon and the Biosphere. USAEC, Washington, D.C. Pp. 86-134.

Bolin, B. 1986. How much CO_2 will remain in the atmosphere? The carbon cycle and projections for the future. In The greenhouse effect, climate change, and ecosystems. John Wiley and Sons, Chinchester, UK. Pp. 98-99, 144.

Betsche, T. 1994. Atmospheric CO_2 enrichment: kinetics of chlorophyll a fluorescence and photosynthetic CO_2 uptake in individuals, attached cotton leaves. Environ. Exp. Botany 34:75-86.

Hagg, C., Stober, F., and Lichtenthaler, H.K. 1992. Pigment content, chlorophyll fluorescence and photosynthetic activity of spruce clones under normal and limited mineral nutrition. Photosynthetica 27:401-411.

Houpis, J.L.J., Surano, K.A., Cowles, S., and Shinn, J.H. 1988. Chlorophyll and carotenoid concentrations in two varieties of *Pinus ponderosa* subjected to long-term elevated carbon dioxide. Tree Physiol. 4:187-193.

Krause, G.H., Weis, E. 1991. Chlorophyll fluorescence: the basics. Annual Review of Plant Physiol. 42:313-349.

Lichtenthaler, H.K. 1992. The Kautsky effect: 60 years of chlorophyll fluorescence induction kinetics. Photosynthetica 27:45-54.

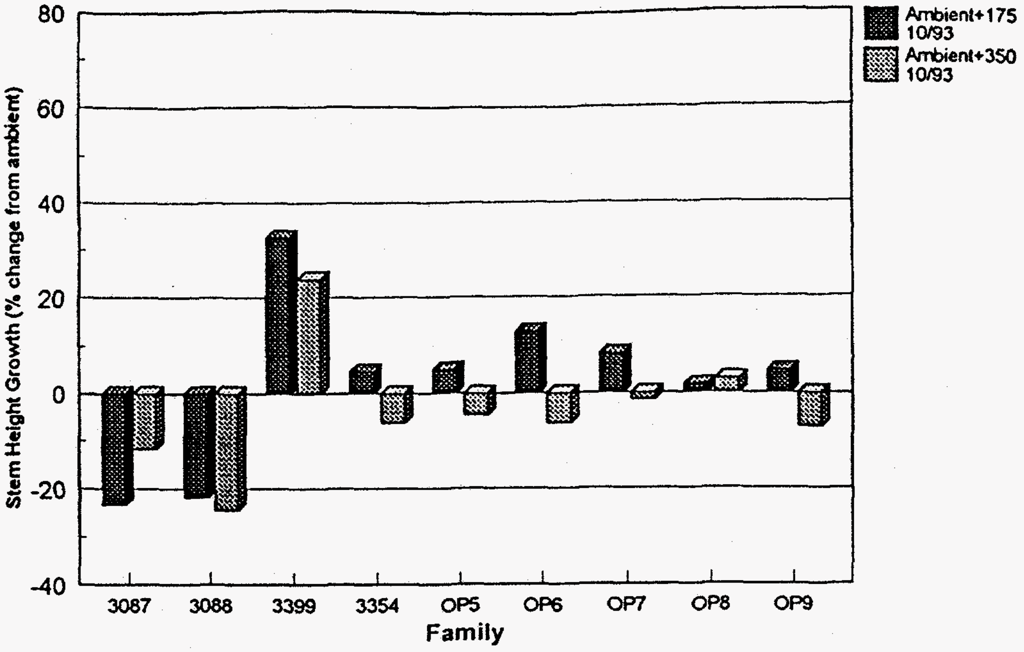
Lichtenthaler, H.K., and Wellburn, A.R. 1983. Determinations of total cartenoids and chlorophylls a and b of leaf extracts in different solvents. In Abstracts of the 6th International Congress on Photosynthesis. Brussels, Belgium. Pp. 415.

Lichtenthaler, H.K., and Rinderle, U. 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. CRC Critical Reviews in Anal. Chem. 19:S29-S85.

Schmidt, W., Neubauer, C., Kolbowski, J., Schreiber, U., and Urbach, W. 1990. Comparison of effects of air pollutants (SO_2, O_3, NO_2) on intact leaves by measurements of chlorophyll fluorescence and P_{700} absorbance changes. Photosynthesis Research 25:241-248.

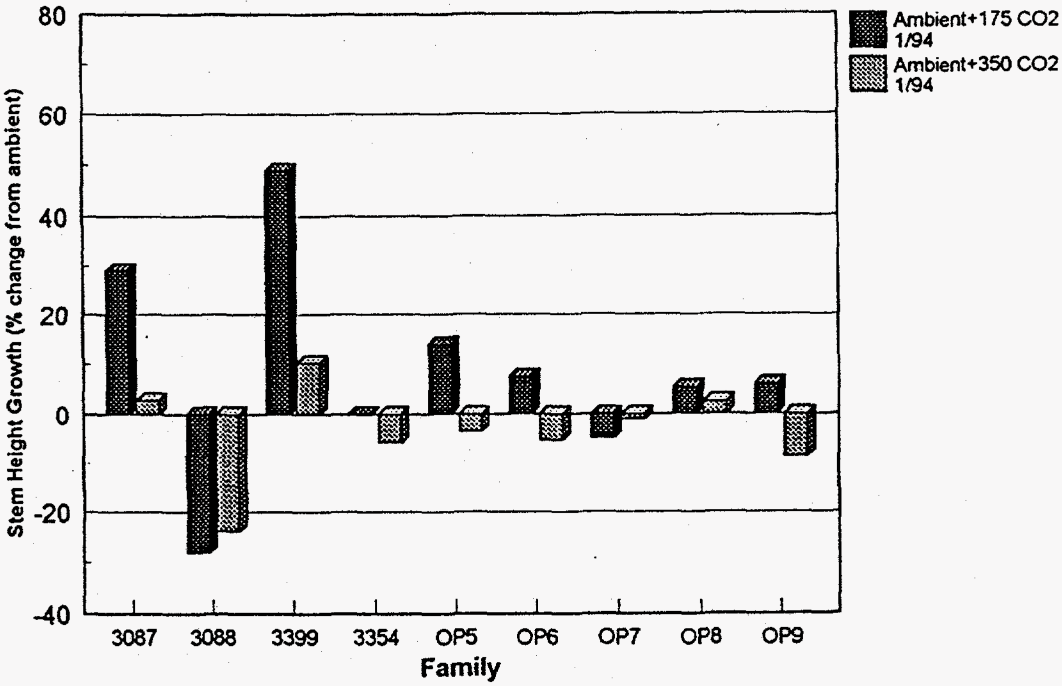
List of Figures

a)



Ambient+175 10/93
Ambient+350 10/93
Stem Height Growth (% change from ambient)
80
60
40
20
0
-20
-40
3087
3088
3399
3354
OP5
OP6
OP7
OP8
OP9
Family

b)



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Ambient+350 CO2 1/94
Stem Height Growth (% change from ambient)
80
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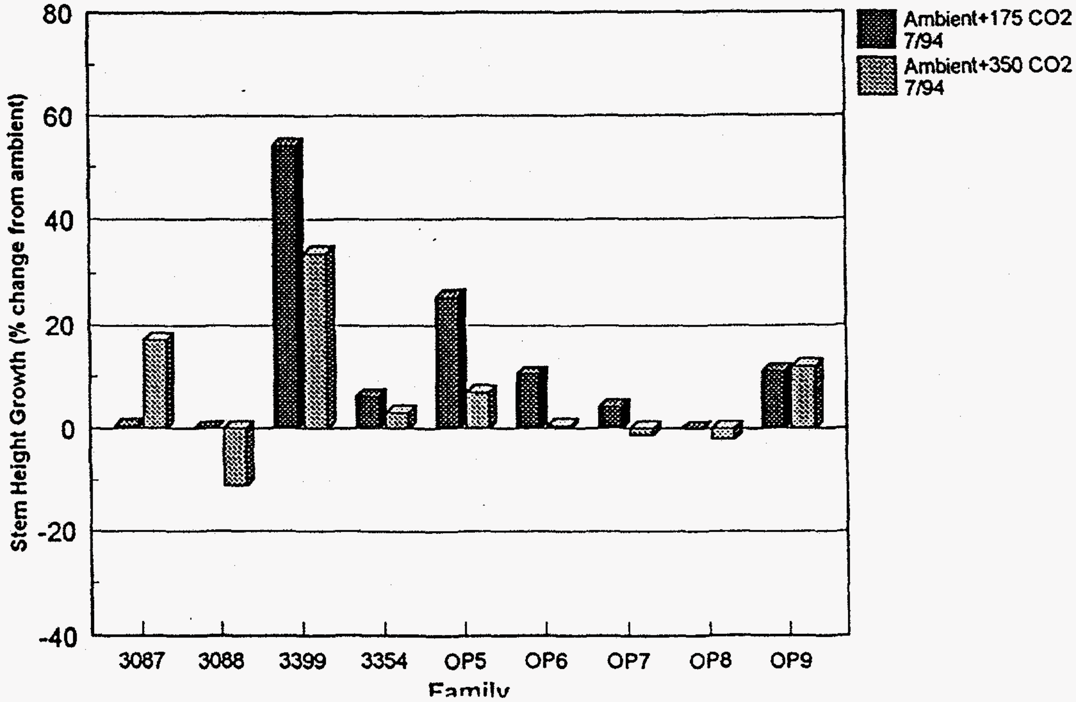
3399
3354
OP5
OP6
OP7
OP8
OP9
Family

c)



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Ambient+350 CO2 7/94
Stem Height Growth (% change from ambient)
80
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OP5
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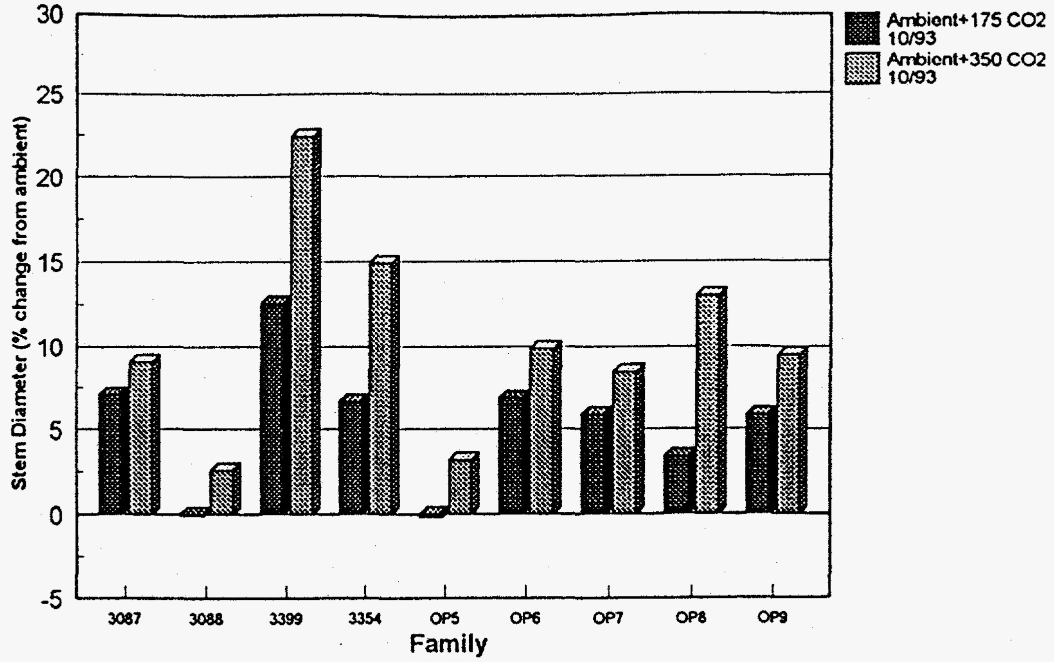
OP8
OP9
Family

a)


Ambient+175 CO2 10/93
Ambient+350 CO2 10/93
Stem Diameter (% change from ambient)
30
25
20
15
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-5
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OP5
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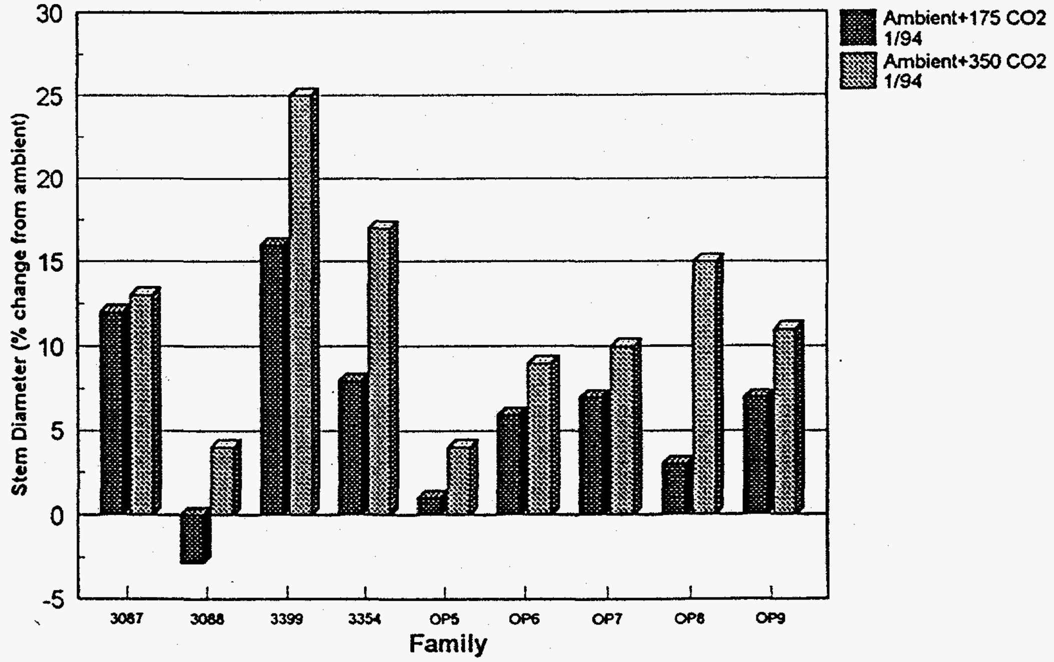
OP9
Family

b)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Ambient+350 CO2 1/94
Stem Diameter (% change from ambient)
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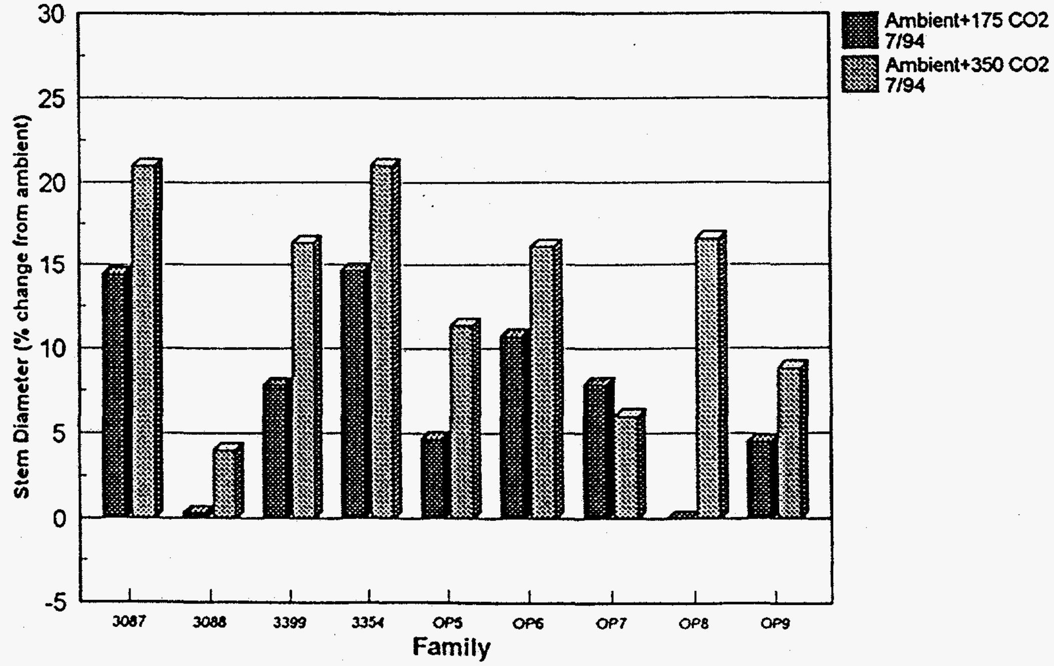
Ambient+175 CO2 7/94
Ambient+350 CO2 7/94
Stem Diameter (% change from ambient)
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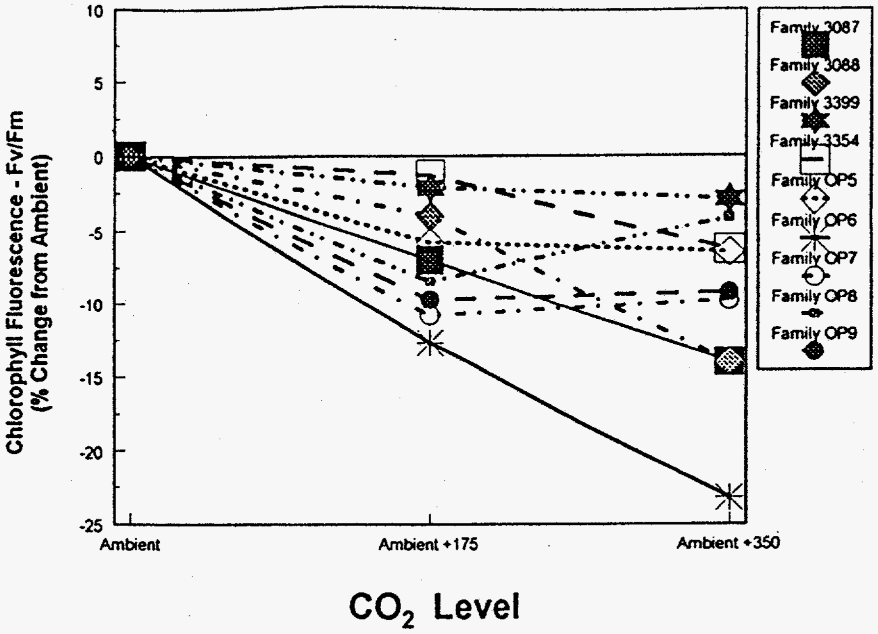
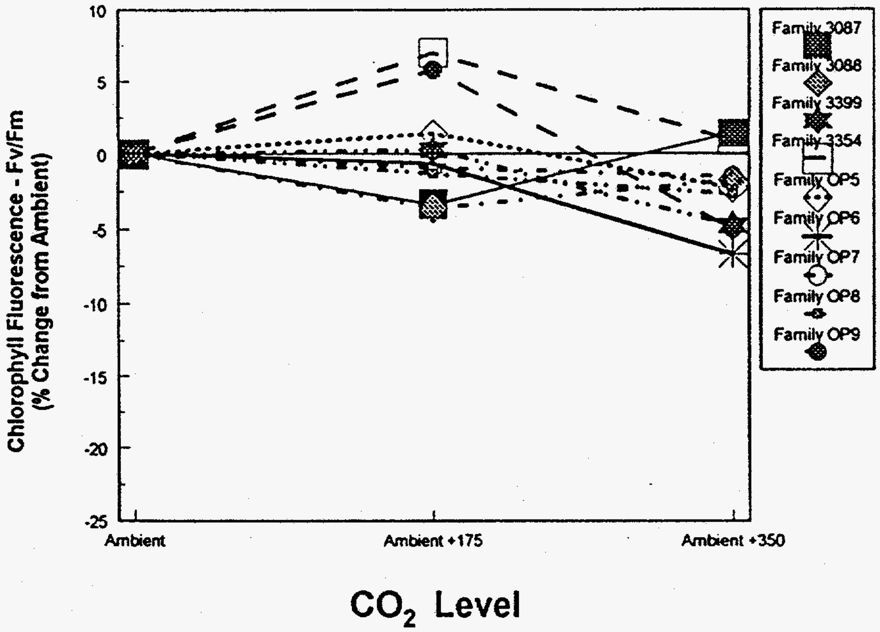
Family

a)



10
5
0
-5
-10
-15
-20
-25
Chlorophyll Fluorescence - Fv/Fm
(% Change from Ambient)
Ambient
Ambient +175
Ambient +350
CO2 Level
Family 3087
Family 3088
Family 3399
Family 3354
Family OP5
Family OP6
Family OP7
Family OP8
Family OP9

b)



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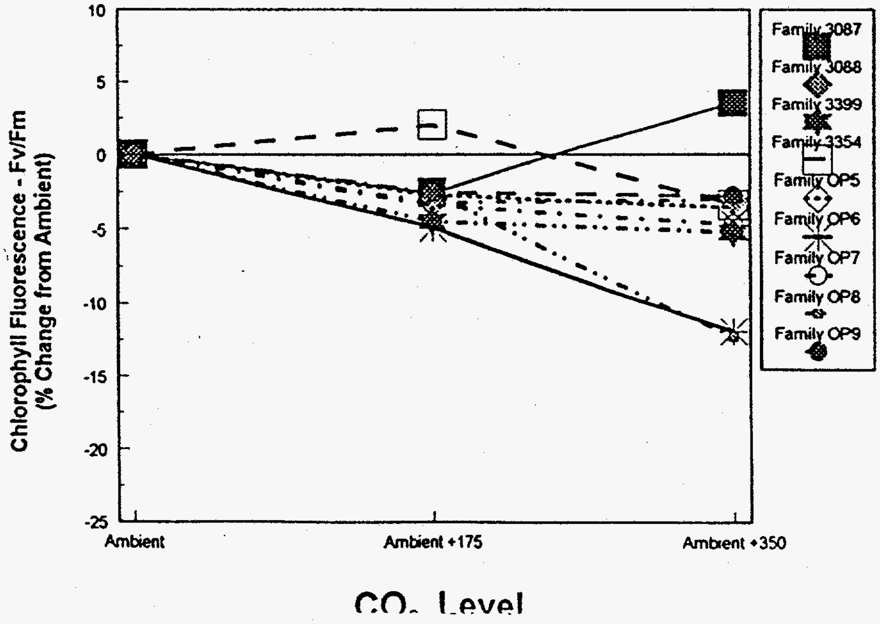
-20
-25
Chlorophyll Fluorescence - Fv/Fm
(% Change from Ambient)
Ambient
Ambient +175
Ambient +350
CO2 Level
Family 3087
Family 3088
Family 3399
Family 3354
Family OP5
Family OP6
Family OP7
Family OP8
Family OP9

c)



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-5
-10
-15
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-25
Chlorophyll Fluorescence - Fv/Fm
(% Change from Ambient)
Ambient
Ambient +175
Ambient +350
CO2 Level
Family 3087
Family 3088
Family 3399
Family 3354
Family OP5
Family OP6
Family OP7
Family OP8
Family OP9