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Chlorophyll *a* Fluorescence and Photosynthetic and Growth Responses of *Pinus radiata* to Phosphorus Deficiency, Drought Stress, and High CO_2[1]

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ABSTRACT

Needles from phosphorus deficient seedlings of *Pinus radiata* D. Don grown for 8 weeks at either 330 or 660 microliters CO_2 per liter displayed chlorophyll *a* fluorescence induction kinetics characteristic of structural changes within the thylakoid chloroplast membrane, *i.e.* constant yield fluorescence (F_O) was increased and induced fluorescence ($[F_P-F_I]/F_O$) was reduced. The effect was greatest in the undroughted plants grown at 660 μl CO_2 L^{-1}. By week 22 at 330 μl CO_2 L^{-1} acclimation to P deficiency had occurred as shown by the similarity in the fluorescence characteristics and maximum rates of photosynthesis of the needles from the two P treatments. However, acclimation did not occur in the plants grown at 660 μl CO_2 L^{-1}. The light saturated rate of photosynthesis of needles with adequate P was higher at 660 μl CO_2 L^{-1} than at 330 μl CO_2 L^{-1}, whereas photosynthesis of P deficient plants showed no increase when grown at the higher CO_2 concentration. The average growth increase due to CO_2 enrichment was 14% in P deficient plants and 32% when P was adequate. In drought stressed plants grown at 330 μl CO_2 L^{-1}, there was a reduction in the maximal rate of quenching of fluorescence (R_Q) after the major peak. Constant yield fluorescence was unaffected but induced fluorescence was lower. These results indicate that electron flow subsequent to photosystem II was affected by drought stress. At 660 μl CO_2 L^{-1} this response was eliminated showing that CO_2 enrichment improved the ability of the seedlings to acclimate to drought stress. The average growth increase with CO_2 enrichment was 37% in drought stressed plants and 19% in unstressed plants.

While the influence of the increasing levels of atmospheric CO_2 on plant growth has been recognized, the response of forest species under conditions of nutrient and drought stress is not well documented. P deficiency and periodic drought are commonly encountered by *Pinus radiata* growing in plantations in Australia and knowledge of the modifying effects of these stresses on the CO_2 response could enable more realistic prediction of long term growth.

The majority of CO_2 enrichment studies have been short term. These have shown that leaf photosynthesis in C_3 plants is limited by the present atmospheric concentration of CO_2 (340 μl L^{-1}) because RuBP[3]-carboxylase catalyzes both the oxygenation and carboxylation of RuBP (13). Consequently the rate of photosynthesis can be increased by 30 to 50% by raising the CO_2 concentration to up to 1000 μl L^{-1} or lowering the O_2 to 2% (16). This increase occurs only if electron transport capacity is large enough to regenerate RuBP and if the Pi concentration in the chloroplast is maintained at a concentration which is favorable for both photophosphorylation and the synthesis of starch and sucrose (17). While the effect of electron transport dysfunction is not known, feeding of hexoses which sequester Pi eliminates the low O_2 response (7). Witholding of water for 7 d (17) also produces this effect possibly because of a reduction in photophosphorylation capacity (19).

These short-term studies may be poor predictors of the long-term response because metabolic imbalances are likely when plants are grown under one set of conditions then transferred to another for the measurement of photosynthesis. In contrast plants are likely to be continuously exposed to 660 μl CO_2 L^{-1} by the end of the 21st century (6). Then even where water and P are adequate, increased photosynthetic rates will not be maintained if insufficient energy is available to process the products of the reductive cycle. Drought and P deficiency may also inhibit the response.

Under the present atmospheric conditions, acclimation occurs when plants are exposed to repeated episodes of drought or to low levels of nutrient availability (9, 14). This process involves metabolic changes which reduce perturbations in cellular functions such as photosynthesis (14). Continuous growth at 660 μl CO_2 L^{-1} may alter the levels of water and P necessary for acclimation. In particular the P requirement may be increased because each carboxylation causes the net esterification of ⅓ Pi, whereas each oxygenation causes the net release of ⅙ Pi (18).

This study was conducted to examine the response of *P. radiata* to P deficiency and drought stress where CO_2 was supplied at 330 and 660 μl L^{-1}. Photosynthetic electron transport function was assessed by measurement of Chl *a* fluorescence. Leaf photosynthesis and dry matter production were also deter-

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[3] Abbreviations: RuBP, ribulose-1,5-bisphosphate; F_O, constant yield Chl fluorescence; F_I, Chl fluorescence plateau after F_O; F_P, maximum Chl fluorescence (constant plus variable yield fluorescence); R_Q, maximal rate of Chl fluorescence quenching; NAR, net assimilation rate.

mined. Plants were exposed to the treatments for at least 22 weeks, long enough for acclimation to have occurred.

MATERIALS AND METHODS

Plant Culture. *Pinus radiata* D. Don seedlings were grown from seed in 950 g of soil, which had a low P availability. Each 1 L pot contained three seedlings. At planting a basal dressing of $NaH_2PO_4 \cdot 2H_2O$ was added to provide 0.25 mg P per pot. Throughout the experiment nutrients other than P were supplied at regular intervals. After 8 weeks growth in a glasshouse, the seedlings were transferred either to a growth chamber with ambient CO_2 (330 μl CO_2 L^{-1}) or to one with high CO_2 (660 μl CO_2 L^{-1}). The high CO_2 level was maintained by continuously injecting CO_2 into one of the chambers. The levels in both chambers were continuously monitored by an IR gas analyzer (Uras 2, Hartmann and Braun, Frankfurt, FRG). Both chambers were maintained at 25 ± 0.5°C for a 16 h light period and 18 ± 0.5°C for an 8 h dark period. The vapor pressure deficit was 18 ±1 Pa kPa^{-1} and 9 ±1 Pa kPa^{-1} for the corresponding periods. The photosynthetic photon flux density at the top of the plants was 450 μmol m^{-2} s^{-1}.

P was applied to the soil at two levels, 4.4 and 40.0 mg per pot as $NaH_2PO_4 \cdot 2H_2O$, 3 weeks before and 9 weeks after commencing the CO_2 treatments. The P concentration in the needles was 0.07 to 0.08% dry weight for the 4.4 mg per pot treatment and 0.10 to 0.15% dry weight for the 40 mg per pot treatment. The latter concentration is adequate to sustain the potential maximum rate of growth while the former is not (25).

Two levels of water availability were established by restoring the gravimetric water content of the pots to field capacity (−0.03 MPa) at intervals of 1 d (undroughted), or 7 d (drought stressed). At the end of the 7th d the soil water content reached wilting point (−1.5 MPa).

Chl *a* Fluorescence. On the 6th d of the last 7 d watering cycle prior to each harvest, three of the youngest fully expanded needles were removed from every seedling. Needles from each treatment were pooled and cut into 1.5 cm lengths. Eight samples were prepared by laying the needles parallel and adjoining one another on eight plastic discs. The needles were held in place by double-sided adhesive tape, with their curved surfaces exposed. Samples were dark-adapted for at least 1 h. During this period moisture loss was minimized by placing samples on damp filter paper in a Petri dish. Chl *a* fluorescence kinetics of each sample was measured once at room temperature, during irradiation with red light of photon flux density 15 μmol m^{-2} s^{-1}, using a fluorometer (model SF-10, Richard Brancker Research, Ottawa, Canada) and a DASAR data acquisition, storage and retrieval system (American Instrument Co., Silver Spring, MD) connected to an X-Y plotter. Chl fluorescence values were measured directly from the DASAR utilizing the oscilloscope trace of the fluorescence signal and digital readout to obtain F_O and F_I. Data points were stored every one ms until F_I was reached and at 20 ms intervals thereafter.

Gas Exchange. Measurements were made 22 weeks after commencement of CO_2 enrichment. For each measurement approximately 60 needles attached to a single shoot were arranged in the cuvette in a planar array to avoid self shading. Plants were not subjected to drought stress for the 7 d prior to these measurements. Gas exchange was measured using a differential IR gas analyzer system. The temperature and vapor pressure deficits in the chamber were maintained at 22.5°C and 10 to 15 Pa kPa^{-1}. Needle temperature was measured using a thermocouple attached to the lower side of a needle. The CO_2 concentration in the air flowing into the leaf chamber (1.5–2.0 L min^{-1}), was maintained at either 330 or 660 μl CO_2 L^{-1}. PAR was determined at the needle level and was varied with neutral density filters. Net photosynthesis and leaf conductance rates were calculated according to Küppers (11). The rates reported here must be multiplied by 2.4 for comparison with those calculated on a projected area basis (2).

Needle Chl and Density. Chl and density were measured on a sample of needles from each pot. Chl was determined spectrophotometrically in an extract prepared by grinding the needles in 80% acetone and centrifuging the suspension. Density was calculated from the dry weight and volume, the latter being estimated from the difference in weight of needles suspended in

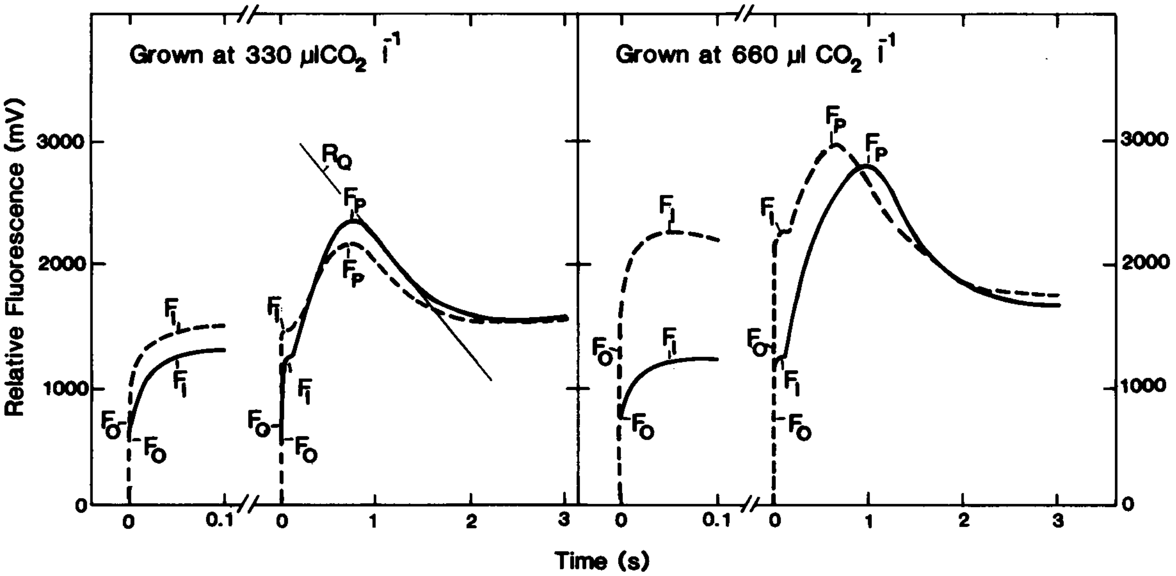

FIG. 1. Chl *a* fluorescence of *P. radiata* needles detached from undroughted seedlings. The concentration of P in the needles was either adequate (0.10–0.15% dry weight) (——) or deficient (0.07–0.08% dry weight) (25) (----). Seedlings were exposed to either 330 or 660 μl CO_2 L^{-1} for 21 weeks. Zero time indicates initiation of excitation and data acquisition. The fast transient on the left of each box was obtained by slowly replaying stored data. F_O, F_I, and F_P indicate constant fluorescence yield, the yield at the minor plateau and the maximum fluorescence yield, respectively. R_Q represents the maximum rate of quenching after F_P.

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air and water.

Seedling Dry Weight. The total seedling dry weight per pot was measured nine and 22 weeks after the commencement of CO_2 enrichment.

RESULTS

Chl *a* Fluorescence. The fluorescence kinetics of *P. radiata* needles are illustrated in Figure 1. F_O, F_I, and F_P (Fig. 1) refer to fluorescence transients occurring after dark-adapted photosynthetic tissue is irradiated. A brief explanation of their relevance to events occurring in the photosynthetic electron transport system follows. A more complete description is available elsewhere (15). After dark adaptation, the electron acceptors of PSII should be in the oxidized state. The rise in Chl fluorescence to F_O occurs within milliseconds. Its primary source is energy captured within the structure of the photon harvesting system that cannot be used in photochemistry. Within 0.1 s there is a further rise (F_I−F_O) to a minor plateau (F_I). This rise is thought to be related to the reduction of the primary electron acceptors at the PSII reaction centers. The subsequent rise to F_P is correlated with the flow of electrons through PSII. R_Q is the maximum rate of quenching after F_P and is probably associated with reoxidation of the electron acceptors of PSI.

Table I. *Chl a Fluorescence—Interactive Effects of CO_2 and Phosphorus*

Seedlings were continuously exposed to either 330 or 660 μl CO_2 L^{-1} for the stated periods. The concentration of P in the needles was either adequate (0.10–0.15% dry weight) or deficient (0.07–0.08% dry weight) (25). The interaction was significant for all variables at week 21 ($P < 0.01$).

Fluorescence Parameter	CO_2 Exposure		Fluorescence Value[a] Phosphorus	
	Time	Concentration	Deficient	Adequate
	weeks	*μl L^{-1}*		
F_O (mV)	8	330	807a	517b
		660	1210c	677ab
	21	330	680a	637a
		660	1116b	822c
$F_I - F_O$ (mV)	8	330	741a	633b
		660	963c	726a
	21	330	524a	523a
		660	772b	551a
$(F_P - F_I)/F_O$	8	330	2.04a	3.83b
		660	1.18c	3.49b
	21	330	1.81a	2.17a
		660	0.89b	1.95a

[a] Values are averaged across the water treatments. At each CO_2 exposure time, fluorescence variables followed by the same letter do not differ significantly ($P < 0.01$).

Table II. *Chl a Fluorescence—Interactive Effects of Water and Phosphorus*

Soil water was restored to field capacity every 7th d (drought stressed) or daily (undroughted). Measurements were made on the 6th d of the 7 d watering cycle. The P concentration in the needles was either adequate (0.10–0.15% dry weight) or deficient (0.07–0.08% dry weight) (25). The interaction was significant for all variables at week 21 only ($P < 0.01$).

Fluorescence Parameter	Water Exposure		Fluorescence Value[a] Phosphorus	
	Period	Treatment	Deficient	Adequate
	weeks			
F_O (mV)	8	Drought stressed	962a	623b
		Undroughted	1055a	572b
	21	Drought stressed	813a	794ac
		Undroughted	983b	664c
$(F_P - F_I)/F_O$	8	Drought stressed	1.74a	3.44b
		Undroughted	1.48a	3.89b
	21	Drought stressed	1.46ad	1.70a
		Undroughted	1.24bd	2.43c

[a] Values are averaged across the CO_2 treatments. At each time, values followed by the same letter do not differ significantly ($P < 0.01$).

Table III. *Chl a Fluorescence—Interactive Effects of CO_2 and Water*

Seedlings were continuously exposed to either 330 or 660 μl CO_2 L^{-1} for the stated periods. Soil water was restored to field capacity every 7th d (drought stressed) or daily (undroughted). Measurements were made on the 6th d of the 7 d watering cycle. The interaction was significant at week 21 only ($P < 0.01$).

Fluorescence Parameter	CO_2 Exposure		Fluorescence Value[a] Water	
	Time	Concentration	Drought stressed	Undroughted
$mV\ s^{-1}$	*weeks*	*μl L^{-1}*		
R_Q	8	330	1797ac	1973bc
		660	2115b	2054b
	21	330	1079a	1571b
		660	1460b	1486b

[a] Values are averaged across P treatments. At each exposure time, values followed by the same letter do not differ significantly ($P < 0.01$).

In this study $(F_P-F_I)/F_O$ was used as a measure of variable fluorescence rather than the commonly used F_V/F_O (*i.e.* $[F_P-F_O]/F_O$) because F_I-F_O in *P. radiata* is large in comparison with F_P-F_I and because the changes in the F_O to F_I and the F_I to F_P rises in response to P deficiency were quite different (Table I). Nevertheless, the changes in F_V/F_O and $(F_P-F_I)/F_O$ were in the same direction, the latter being larger in magnitude (data not shown).

P deficiency increased F_O and F_I-F_O and reduced $(F_P-F_I)/F_O$ (Table I). During the first 8 weeks needles from each of the CO_2 treatments displayed these characteristics. By week 21 they were exhibited only in the needles developed at 660 μl CO_2 L^{-1} (Table I and Fig. 1). Similarly, differences in F_O and $(F_P-F_I)/F_O$ due to P deficiency occurred in both drought stressed and undroughted plants at week 8 but only in the latter at week 21 (Table II). P deficiency had only a small effect on R_Q, reducing it by 10% at week 21 only (data not shown). In needles with adequate levels of P, CO_2 enrichment increased F_O without concomitantly decreasing $(F_P-F_I)/F_O$ (Table I).

Drought stress did not affect F_O, although in the needles with adequate P it reduced $(F_P-F_I)/F_O$ at both CO_2 levels (Table II), there being no significant interaction between the CO_2 and water treatments. The reduction was not as large as that caused by P deficiency at high CO_2 (Table I). After 21 weeks of growth at 330 μl CO_2 L^{-1}, R_Q was reduced by drought stress (Table III). This effect was not observed in the needles grown at the higher level of CO_2 or in those exposed to drought stress for 8 weeks at either CO_2 level.

Steady-State Leaf Photosynthesis. Stomatal response to the growth conditions was such that the intercellular CO_2 was higher when the ambient CO_2 concentration during measurement was 660 μl CO_2 L^{-1} (Table IV). The higher CO_2 concentration increased the light saturated rate of photosynthesis only when P was adequate (Table IV and Fig. 2, B and D). Needles with adequate P also had higher light saturated rates of photosynthesis than P deficient needles measured under the same conditions

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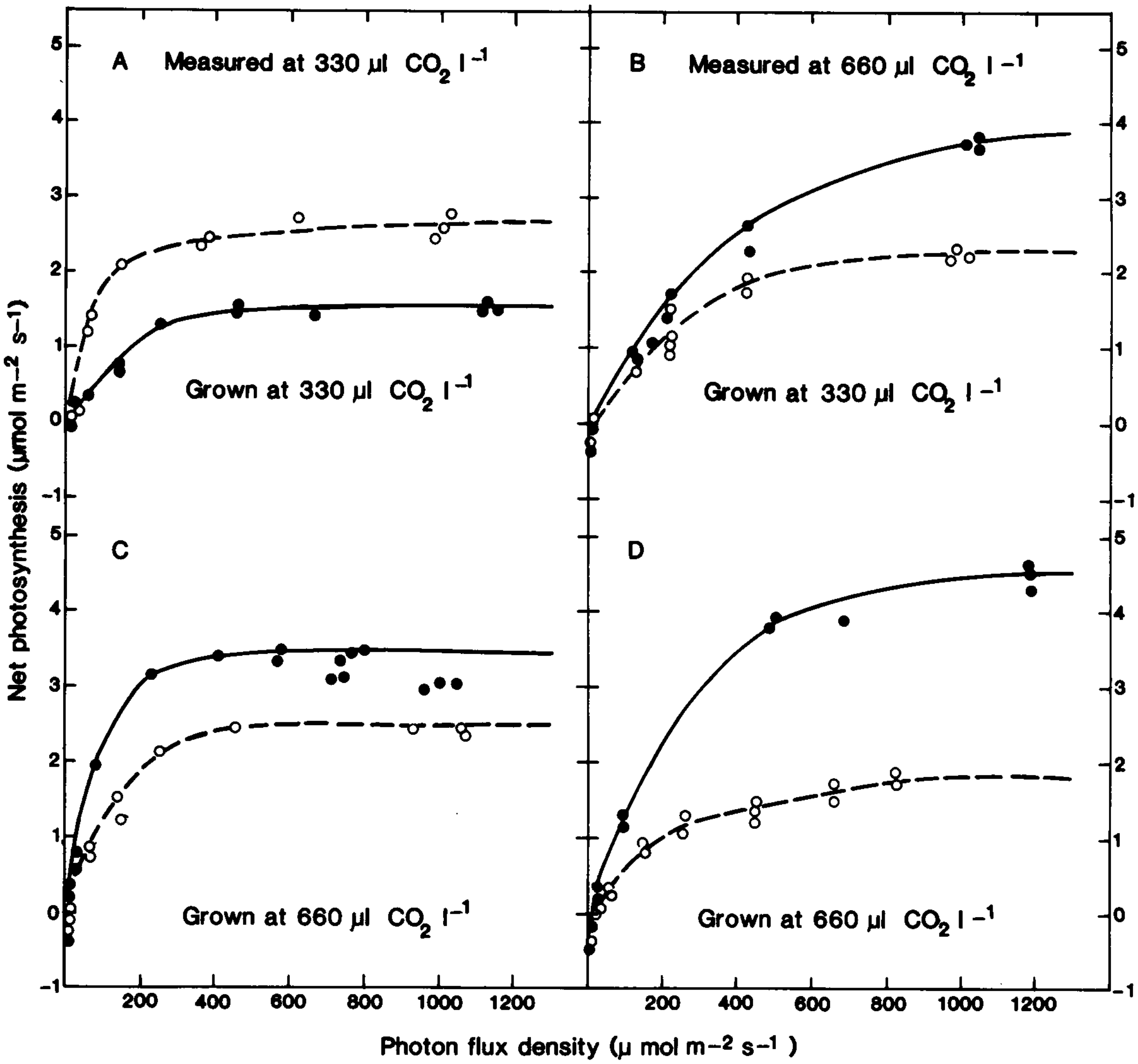

FIG. 2. Effect of photosynthetic photon flux density on the net photosynthesis of undroughted *P. radiata* needles with either adequate (0.10–0.15% dry weight) (●——●) or deficient (0.07–0.08% dry weight) (25) (○----○) concentrations of P, 22 weeks after the commencement of CO_2 enrichment. Needles were A, grown and measured at 330 µl CO_2 L^{-1}; B, grown at 330 µl CO_2 L^{-1} and measured at 660 µl CO_2 L^{-1}; C, grown at 660 µl CO_2 L^{-1} and measured at 330 µl CO_2 L^{-1}; D, grown and measured at 660 µl CO_2 L^{-1}.

(Table IV and Fig. 2 B–D), except when the seedlings were grown and measured at 330 µl CO_2 L^{-1} (Table IV and Fig. 2A). Drought stress had no effect (Table IV).

Needle Chl and Density. P deficiency increased the ratio of Chl per unit surface area and the Chl *a:b* ratio (Table V). Needle density was not affected by P deficiency but was increased by CO_2 enrichment and by drought stress (Table V).

Seedling Dry Matter Production. The greatest dry matter production occurred in the undroughted seedlings grown at 660 µl CO_2 L^{-1} with adequate P (Fig. 3). Although drought stressed plants produced less dry matter, their growth increase due to CO_2 enrichment was significant at both P treatment levels (Fig. 3). In well watered plants, P deficiency completely inhibited the growth response to high CO_2.

DISCUSSION

Response to CO_2 at Adequate Water and P Levels. Neither electron flow through PSII, inferred from the magnitude of (F_P–F_I/F_O, Chl *a:b*, nor Chl per unit surface area were increased by CO_2 enrichment (Tables I and V). Nevertheless, the leaf photosynthetic capacity was greater in plants grown at high CO_2 as evidenced by their higher light saturated rates of photosynthesis (Table IV and Fig. 2, C and D). In some species CO_2 enrichment during growth leads to the formation of a third layer of mesophyll cells (22) and this could partially account for increases in leaf photosynthetic capacity. However, we have previously shown that in *P. radiata* the number of mesophyll cells per unit cross-sectional area and the ratio of mesophyll to total needle cross-sectional area were unaffected by the CO_2 treatments (5). We also found that long-term photosynthesis measured as NAR was higher at high CO_2 over both the 0 to 9 week and 9 to 22 week growth intervals (5). These results and the observed increase in dry weight indicate that there was no substantial long-term feedback inhibition of photosynthesis.

Response to CO_2 at Deficient P Levels. The Chl *a* fluorescence kinetics recorded from P deficient *P. radiata* needles indicate that structural changes occurred within the chloroplast thylakoid membranes. These affected the ability of the photon harvesting assemblages to trap photons and pass the converted energy to the electron acceptors of the PSII electron transport system (Table I). It is known that loss of PSII reaction centers increases the amount of energy emitted as F_O fluorescence, while decreasing that emitted as induced Chl fluorescence (10, 12, 21).

Of special interest is the large size of the F_O to F_I rise in *P.*

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Table IV. *Gas Exchange Characteristics—Effect of CO_2 Enrichment, P Deficiency, and Periodic Drought Stress During Growth*

Seedlings were exposed to either 330 or 660 μl CO_2 L^{-1} for 22 weeks before measurement. Soil water was restored to field capacity every 7th d (drought stressed) or daily (undroughted). Plants were not subjected to drought stress during the 7 d prior to the measurements. The concentration of P in the needles was either adequate (0.10–0.15% dry weight) or deficient (0.07–0.08% dry weight) (25). Gas exchange was measured at the same ambient CO_2 concentration as that provided during growth. The net photosynthesis value for each treatment is the maximum value estimated from a single light response curve measured up to 1200 μmol m^{-2} s^{-1}. The corresponding conductance and intercellular CO_2 concentrations are the values obtained at the maximum photosynthetic rate.

Growth Conditions			Net Photosynthesis	Conductance	Intercellular CO_2 Concentration
Water	P	CO_2			
		μl L^{-1}	*μmol m^{-2} s^{-1}*	*mmol m^{-2} s^{-1}*	*μl L^{-1}*
Drought stressed	Deficient	330	2.6	26	210
		660	2.6	20	380
	Adequate	330	1.7	20	210
		660	4.1	20	330
Undroughted	Deficient	330	2.6	22	180
		660	1.8	12	390
	Adequate	330	1.8	20	210
		660	4.5	20	310

Table V. *Effect of CO_2, P Deficiency and Drought Stress on Needle Density and Chl Level, 22 Weeks after Commencement of CO_2 Treatments*

Seedlings were exposed to either 330 or 660 μl CO_2 L^{-1} for 22 weeks. Soil water was restored to field capacity every 7th d (drought stressed) or daily (undroughted). The concentration of P in the needles was either adequate (0.10–0.15% dry weight) or deficient (0.07–0.08% dry weight) (25). For CO_2, the values are averaged over the P and water treatments; for P, the values are averaged over the water and CO_2 treatments; for water, the values are averaged over the CO_2 and P treatments. There were no significant interactions between the treatments. Values followed by the same letter do not differ significantly ($P < 0.01$).

Treatment	Level	Density	Chl *a:b*	Chl per Unit Surface Area
		kg m^{-3}	*ratio*	*mg m^{-2}*
CO_2 (μl L^{-1})	330	270a	2.43a	157a
	660	300b	2.48a	155a
P	Deficient	290a	2.52a	167a
	Adequate	280a	2.39b	144b
Water	Drought stressed	310a	2.46a	157a
	Undroughted	270b	2.44a	153a

radiata and the fact that like F_O its magnitude was increased by P deficiency (Fig. 1 and Table I) even though the F_I to F_P rise was decreased (Fig. 1). The F_o to F_I rise is generally thought to indicate charge separation taking place at the PSII reaction center sites (15). It is suggested instead that the F_O to F_I rise measured here may be related to the F_O emission.

Dysfunction of PSII was accompanied by elimination of the leaf photosynthetic response to high CO_2 (Table IV and Fig. 2, B and D). A diminished response was also evident at the whole plant level (5) (Fig. 3).

Response to CO_2 under Drought Stress. Chl *a* fluorescence kinetics demonstrated that the sites of perturbation in electron transport function were different from those affected by P deficiency (Tables I–III). The reduction in the rate of quenching (R_Q) after the major peak indicated that electron flow subsequent to PSII was most affected by drought (Table III). Drought stress has been shown to reduce *in vitro* electron transport rates in *Helianthus annuus* (14) while no effect on uncoupled electron transport was found in *Picea sitchensis* (1), *Phaseolus vulgaris* (3) and in isolated mesophyll cells of *Xanthium strumarium* (19). A gradual decline in R_Q relative to the initial rate of rise to F_P occurred with dehydration of *Borya nitida* (8), suggesting a preferential slowing of electron flow subsequent to PSII. This is in agreement with the view that photophosphorylation is the photosynthetic function most sensitive to drought stress (19). Our results indicate that measurement of R_Q may be a useful method for following the development of drought stress in photosynthetic tissues.

Drought stress did not affect the leaf photosynthetic response to high CO_2 (Table IV); however, the periodically droughted plants had not been droughted during the 7 d prior to gas exchange measurements. During this period recovery of photosynthesis may have occurred. Complete recovery has been reported for *H. annuus* (14), while only partial recovery occurred in *P. vulgaris* (3). These may be real interspecific differences or artifacts due to experimental differences in the rates of induction of drought stress.

Interaction between CO_2, Drought Stress, and P Deficiency. Electron transport dysfunction was greatest in the needles from P deficient undroughted plants (Table II). This was not due to differences in the P concentration between the droughted and undroughted needles (0.07% dry weight). A possible explanation is that the demand for cytosolic Pi for functions other than photosynthesis was greater where growth was increased by additional water availability (Fig. 3). PSII capacity was greatest in undroughted plants with adequate P (Table II). Thus at the whole plant level, the capacity to respond to high CO_2 was least in the undroughted P deficient seedlings and was greatest in the undroughted seedlings with adequate P (Fig. 3).

Acclimation to P Deficiency. At 330 μl CO_2 L^{-1} the differences between the Chl *a* fluorescence kinetics of the needles with deficient and adequate P levels had disappeared by week 21, suggesting that acclimation to the low P conditions had occurred (Table I and Fig. 1). Further evidence for acclimation is that the foliage was not showing yellowing symptoms typical of P deficiency even though the P concentration was deficient at week 9 (0.08% dry weight) and if anything, continued to decline until the end of the experiment (0.07–0.08% dry weight). By that time the light saturated rate of photosynthesis in P deficient needles was higher than that in needles with adequate P (Table IV and Fig. 2A). In the latter, reductions in Chl per unit surface area

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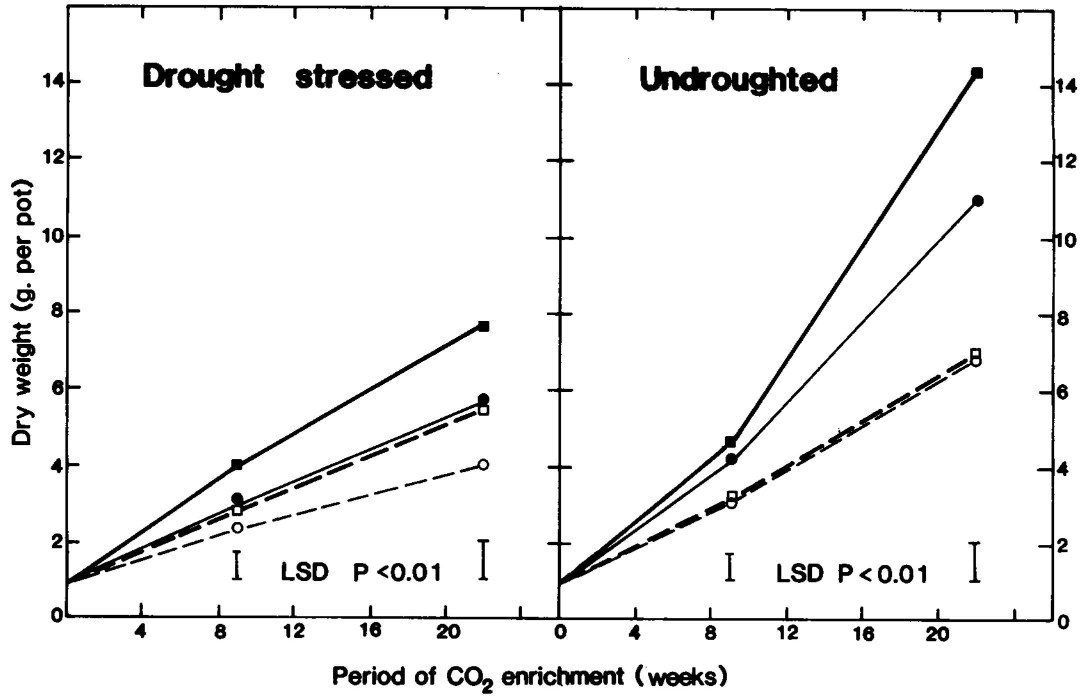

FIG. 3. Dry weight of *P. radiata* seedlings after 22 weeks of exposure to either 330 µl CO_2 L^{-1} (○---○), ●—●) or 660 µl CO_2 L^{-1} (□---□, ■—■). The solid lines were used where the P concentration is adequate (0.10–0.15% dry weight) and the dashed lines where it was deficient (0.07–0.08% dry weight) (25). Soil water was restored to field capacity every 7th d (droughted) or daily (undroughted).

and Chl *a:b* could account for the lower light saturated photosynthetic rate (Table V). We have also shown that at the whole plant level, NAR was reduced by P deficiency during the first 9 weeks but was unaffected during the subsequent 13 weeks (5). Thus it appears that there was a lag period prior to acclimation. The occurrence of a lag phase has been reported for birch seedlings after the supply of nitrogen had been reduced from adequate to deficient (9). Deficiency symptoms were visible during the lag phase but subsequently, growth was reduced to match nutrient availability, resulting in stable physiological conditions and the disappearance of deficiency symptoms.

In seedlings grown at 660 µl CO_2 L^{-1}, the differences in fluorescence between the deficient and adequate P needles persisted up to week 21, implying that acclimation had not occurred (Table I). The P concentration in the needles of P deficient plants was the same at both CO_2 levels (0.07–0.08% dry weight) yet at 660 µl CO_2 L^{-1} typical P deficiency symptoms were evident. In addition at 660 µl CO_2 L^{-1} the light saturated photosynthetic rates (Table IV and Fig. 2D) and NAR (5) of the P deficient plants were lower than those with adequate P grown under the same conditions. Plants grown at 660 µl CO_2 L^{-1} may be unable to acclimate to the lower P level provided in this experiment because the greater demand for Pi caused by the favoring of the reductive cycle (18, 24) may reduce P available for such purposes as chloroplast membrane synthesis and could account for the structural differences in the chloroplasts of P deficient needles (Table I).

Acclimation to Drought Stress. Acclimation was improved by supplying CO_2 at 660 µl L^{-1}. This eliminated the effect of stress on R_Q and caused a relatively large increase in dry matter production (Fig. 3) and NAR in the stressed plants (5). It is unlikely that the leaf water potential was affected because water use and therefore soil water potential was the same in both CO_2 treatments (data not shown). A more likely explanation is that the higher rates of photosynthesis at 660 µl CO_2 L^{-1} (Table IV) resulted in a larger accumulation of carbohydrate. This would also account for the greater density of the needles grown at 660 µl CO_2 L^{-1} (Table V). Large accumulations of carbohydrate have been reported for other plants grown at high CO_2 (4). Under drought stress this could facilitate osmotic adjustment and turgor maintenance and could explain the elimination of the effect of drought stress on R_Q (Table III). Exposure of drought stressed wheat to 1000 µl CO_2 L^{-1} rather than 350 µl CO_2 L^{-1} decreased osmotic potential and increased dry matter production (20). CO_2 enrichment has also been shown to delay the onset of drought stress effects on photosynthesis in *Liquidambar straciflua*, but not in *Pinus taeda* (23) indicating that there may be interspecific differences in osmoregulation at high CO_2.

Concluding Remarks. If the atmospheric CO_2 concentration rises to 660 µl CO_2 L^{-1}, leaf photosynthesis of *P. radiata* will be maintained at a higher level and growth will be increased, providing the supply of P is adequate. P deficiency will decrease the magnitude of the response, particularly in situations where soil water availability is high. The levels of P which are sufficient for acclimation at 330 µl CO_2 L^{-1} will no longer be sufficient under the new atmospheric conditions. Persistance of photosynthetic dysfunction could ultimately lead to the cessation of growth. In contrast, the deleterious effects of drought stress on photosynthesis are likely to be ameliorated and growth could be improved in situations where water currently limits growth.

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