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Accelerated Early Growth of Rice at Elevated CO_2

Is It Related to Developmental Changes in the Shoot Apex?[1]

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The influence of elevated CO_2 on the development of the shoot apex and on subsequent vegetative growth and grain yield was investigated using rice (*Oryza sativa* L. cv Jarrah) grown in flooded soil at either 350 or 700 μL CO_2 L^{-1}. At 8 d after planting (DAP), elevated CO_2 increased the height and diameter of the apical dome and lengths of leaf primordia and tiller buds but had no effect on their numbers. By 16 DAP, there were five tiller buds in the apex at 700 μL CO_2 L^{-1} compared with only three tiller buds at 350 μL CO_2 L^{-1}. These changes in development of the shoot apex at high CO_2 were forerunners to faster development of the vegetative shoot at elevated CO_2 between 11 and 26 DAP as evidenced by increases in the relative growth rates of the shoot and tillers. Accelerated development at high CO_2 was responsible for the 42% increase in tiller number at the maximum tillering stage and the 57% enhancement of grain yield at the final harvest. The link between high CO_2 effects on development during the first 15 DAP and final tiller number and grain yield was demonstrated by delaying exposure of plants to high CO_2 for 15 d. The delay totally inhibited the tillering response to high CO_2, and the increase in grain yield of 20% arose from a greater number of grains per panicle. Consequently, it can be concluded that accelerated development in the shoot apex early in development is crucial for obtaining maximum increases in grain yield at elevated atmospheric CO_2 concentrations.

Strong evidence that indicates that increases in atmospheric CO_2 concentration are likely to continue has challenged physiologists to predict the responses of plants to these increases. Early experiments clearly showed that raising the concentration of CO_2 above ambient levels increased the rate of photosynthesis of expanded (source) leaves. Further studies indicated that predicting the magnitude of the growth response to high CO_2 could not simply be based on measuring photosynthesis after these step changes in CO_2 because long-term exposure to high CO_2 resulted in the down-regulation of photosynthesis (Webber et al., 1994; Xu et al., 1994). Nevertheless, a study of 156 species showed that long-term growth at double the ambient CO_2 concentration increased productivity by about 37% (Poorter, 1993). Although the largest growth response to high CO_2 occurred in C_3 species, C_4 plants also responded because of small increases in leaf photosynthesis (Ghannoum et al., 1997). It is important that the CO_2 response of both C_3 and C_4 species can be eliminated if generation of sinks for photosynthate are inhibited by depriving plants of nitrogen or, for some species, by growing them under artificial light (Rogers et al., 1996; Ghannoum et al., 1997). These studies indicate that, although an increase in photosynthesis is the most important primary response to high CO_2, the magnitude of the long-term growth enhancement is dependent on generation of sinks for carbohydrates.

For C_3 grain crops such as wheat and rice, sink generation is particularly important in determining the magnitude of yield response to high CO_2. These grass plants have the capacity to generate a large number of carbohydrate sinks by increasing the numbers of tillers that originate as buds at the base of the leaf of the main plant and subsequent tillers. Under optimal growth conditions, a single plant can produce as many as 50 tillers and each tiller can produce grain. Consequently, grain yield of wheat was enhanced by 79% by increasing the CO_2 concentration from 350 to 900 μL CO_2 L^{-1}, primarily because tiller production was 64% greater at the elevated CO_2 concentration (Conroy et al., 1995; Rogers et al., 1996). There was little response to high CO_2 at low nitrogen supply because the capacity for tillering was inhibited. An increase in grain yield of 58% has been also demonstrated for rice and, as with wheat, was associated with a large increase in tiller number (Seneweera et al., 1994).

The maximum number of tiller buds that a plant can produce is genetically determined, and elongation of buds is synchronized with the initiation and elongation of leaves on the main plant or tillers from which they emerge (Skinner and Nelson, 1995). The tiller buds and leaf primordia are initiated in the shoot apex, which is enclosed in the older leaves at the base of the grass plant. At any one time,

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Abbreviations: DAP, days after planting; RGR, relative growth rate.

the apex has a succession of leaf primordia and tillers at various stages of development. The growth of new tillers generally commences when elongation of the associated leaf ceases. The initiation of growth of these tiller buds, however, is strongly dependent on environmental factors such as nitrogen supply and PPFD, and it is therefore possible that a new leaf can emerge while the tiller bud will remain suppressed indefinitely (Skinner and Nelson, 1995). Since growth of secondary tillers is dependent on the emergence of primary tillers, favorable growth conditions early in development are essential for the plant to reach its maximum growth potential. Consequently, following the growth of the tiller buds and the leaf primordia in the apex in the very early stages of vegetative growth should provide valuable information about the influence of elevated atmospheric CO_2 on the growth potential and ultimate grain yield of grasses.

We report on the changes in the shoot apex of rice (*Oryza sativa* L. cv Jarrah) after 8 d of growth at either 350 or 700 μL CO_2 L^{-1} on subsequent growth rates of the main shoot and primary and secondary tillers, and on grain yield. The importance of early exposure to high CO_2 in determining ultimate grain yield was investigated by delaying the commencement of CO_2 enrichment for 15 DAP.

MATERIALS AND METHODS

Plant Culture

We conducted three separate experiments under identical conditions. In each experiment rice (*Oryza sativa* L. cv Jarrah) was grown from seed in large pots containing flooded soil, which was supplied continuously with nutrients at concentrations shown to promote the maximum response to elevated CO_2 (Seneweera et al., 1994). In two of the three experiments, plants were exposed to either 350 or 700 μL CO_2 L^{-1} immediately after the seeds had germinated. In the third experiment plants were grown for 15 DAP at 350 μL CO_2 L^{-1} before exposure to elevated CO_2. The CO_2 levels were maintained at either 350 or 700 μL CO_2 L^{-1} by injecting compressed FOGG grade CO_2 (BOC, Australia) into two matched growth chambers. The CO_2 was passed through a column (Purafil International, Doraville, GA) prior to injection to remove possible ethylene contamination. The temperature was maintained at 28°C and 21°C for 12-h light and dark periods, respectively. The PPFD at the leaf surface was 1000 μmol photons m^{-2} s^{-1}.

Morphology and Anatomy during the Early Vegetative Phase: Experiment 1

Whole Shoot Growth and Morphology

Five plants per CO_2 treatment were harvested at 11, 16, 21, and 26 DAP. At each harvest, the numbers of emerged leaves on the main plant and on each tiller were counted. The lengths of the main plant and of each tiller were measured from the base of the shoot to the tip of the longest leaf blade. The latter data were then used to calculate the RGR over the intervals 11 to 16 d, 16 to 21 d, and 21 to 26 d, according to the method of Masle et al. (1993). The leaf sheaths were then removed from the main plant and the primary tillers. The numbers of emerged and emerging tillers were counted, the latter using a dissecting microscope. The significance of the difference between the CO_2 treatments was estimated using SE.

Development of the Shoot Apex

At 8 and 16 DAP in the same experiment, three plants per CO_2 treatment were sampled as described for morphological measurements. The roots were removed and a section (approximately 1 cm long) was cut from the base of the main plant of each plant and fixed in 5% glutaraldehyde in 0.1 M potassium phosphate buffer (pH 7.4) for 12 h. These sections contained the shoot apex. The shoot sections were then washed in 0.1 M phosphate buffer (pH 7.4), dehydrated in an alcohol series ranging from 30% (v/v) ethanol in water to absolute alcohol, and cleared in xylene. The sections were then embedded in paraplast. Serial, transverse sections (7 μm thick) were cut beginning at the base of the shoot apex and finishing at the tip of the apex. They were stained with 1% safranin and each of them was examined under a light microscope. These serial sections were then used to determine the sizes of the apical dome, leaf primordia, stem internodes, tiller buds, and leaf midribs and to observe the pattern of differentiation of leaf primordia and other developmental features.

For the apices sampled at 8 DAP, the numbers of 7-μm transverse sections were counted between specific points on the apex to make the following measurements: from the base of the fifth leaf primordia to the tip of the apical dome (the height of the apical dome); between the base and tip of leaf primordia 4 and 5 (lengths of leaf primordia 4 and 5); between the base and the tip of primary tiller buds 1 and 2 (lengths of primary tiller buds 1 and 2); and between the bases of leaf primordia 1 and 2, 2 and 3, 3 and 4, and 4 and 5 (lengths of internodes 1–4). The maximum diameter of the apical dome was measured using an ocular graticule after examining all of the transverse sections of each apical dome. The widths of leaf primordia 1 to 5 at the midrib were measured similarly. There were five replicates for each measurement. For apices sampled at 16 DAP, typical transverse sections from near the base of the shoot apex were photographed to show tiller development.

Tiller Number and Grain Yield of the Mature Plant: Experiments 2 and 3

In experiment 2, plants were grown from germination at either 350 or 700 μL CO_2 L^{-1}. In experiment 3, plants were grown for 15 DAP at 350 μL CO_2 L^{-1}, after which one-half of the plants was left at 350 μL CO_2 L^{-1} and the other half was transferred to 700 μL CO_2 L^{-1}. Both experiments were continued until the grain had reached maturity (135 and 146 DAP for the 700 and 350 μL CO_2 L^{-1} treatments, respectively). In each experiment, five plants from each treatment were sampled at maximum tillering stage (approximately 60 DAP) and again at grain maturity. The numbers of tillers were counted at the first harvest. At the second harvest, the total grain weight per plant, and grain numbers per panicle and per plant were measured. The 100 grain weight was estimated from the total grain weight and grain numbers.

RESULTS

Morphology and Anatomy during the Early Vegetative Phase: Experiment 1

Whole Shoot Growth and Morphology

The stimulation of shoot growth by CO_2 enrichment was demonstrated by the increases in length of the main shoot and each individual tiller at 11, 16, 21, and 26 DAP (Figs. 1, 2, and 3). The most important feature of the growth response to high CO_2 was that the difference between the CO_2 treatments was greatest during the early phases of growth. For example, the increase in shoot length due to CO_2 enrichment was 26% at 11 DAP, fell to 12% at 16 DAP, and was only 4% at 26 DAP (Fig. 1). Similar trends were observed for the enhancement of tiller lengths by elevated CO_2 concentration (Figs. 2 and 3). For example, there was a more than a 3-fold increase in the length of primary tiller 1 at 16 DAP, but by 26 DAP the difference in length between the CO_2 treatments was only 11% (Fig. 2).

The acceleration of shoot and tiller elongation during their early growth phase was reflected in their RGR, which showed that development was faster at high CO_2 (Table I). During the 11- to 16-DAP growth period, the main shoot and primary tillers 1 and 2 had presumably passed their most rapid phase of growth and had lower RGR at elevated CO_2 (Table I). Only tiller 3, which had recently emerged, had a faster RGR at elevated CO_2. However, by the 16- to 21-DAP growth period the effect of high CO_2 on the RGR of tiller 3 was no longer evident, and only tiller 4, which had recently appeared, had a faster RGR at high CO_2 (Table I). Similarly, only tiller 5 had a greater RGR during the 21- to 26-DAP period.

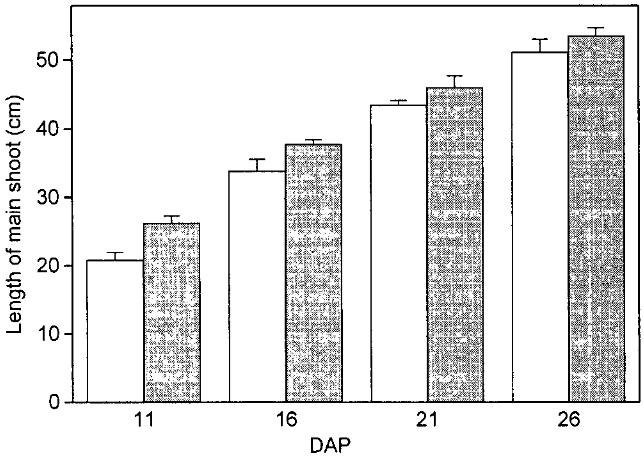


Figure 1. Influence of CO_2 enrichment on the length of the main shoot at 11 to 26 DAP. Plants were grown at either 350 (open bars) or 700 (shaded bars) μL CO_2 L^{-1}. Measurements were made from the base of the shoot to the tip of the longest leaf blade. Values are the means of five replicates and bars represent one SE.

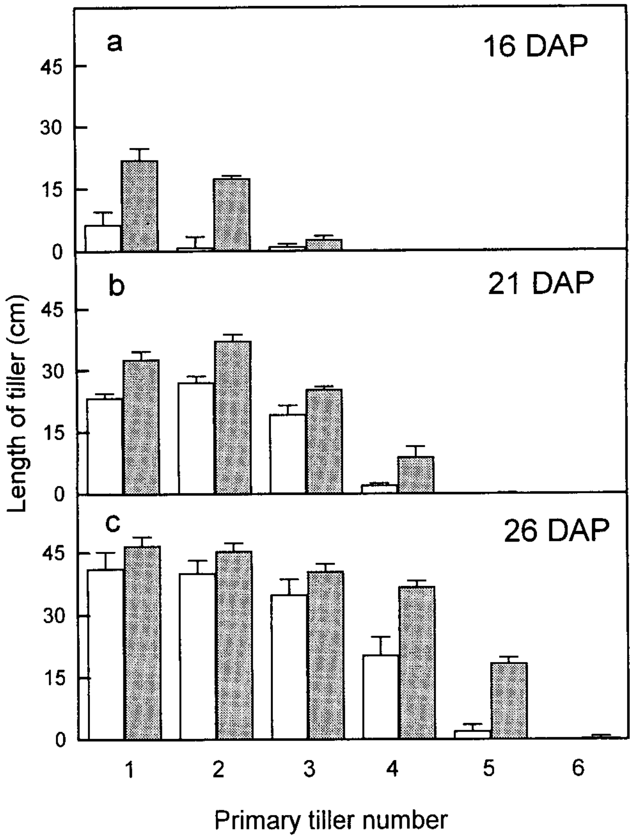


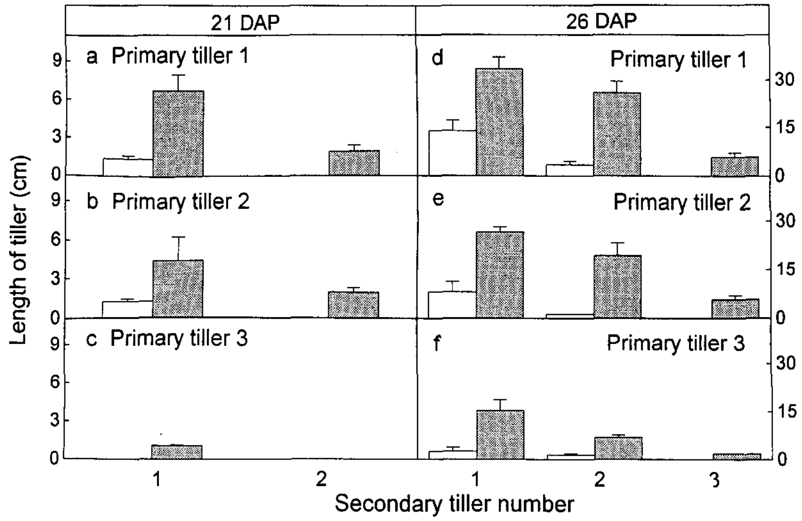
Figure 2. Length of primary tillers of rice grown at either 350 (open bars) or 700 (shaded bars) μL CO_2 L^{-1}. Measurements were made at 16 (a), 21 (b), and 26 (c) DAP from the base of the tiller to the tip of the longest leaf blade. Values are the means of five replicates and bars represent one SE.

Leaf number per main plant and per tiller was another developmental index influenced by the CO_2 treatments (Table II). As with RGR, the effect of high CO_2 on leaf number was most pronounced on the most recently emerged tillers. For example, at 26 DAP tiller 4 had more than double the number of leaves at elevated CO_2 (Table II). In contrast, there was an average of 7.6 emergent leaves on the main shoot at the ambient CO_2 treatment compared with an average of 8 at high CO_2 (Table II).

Development of the Shoot Apex

Figure 4 is a schematic drawing of the shoot apex at 8 DAP, which shows the height and diameter of the apical dome (*DH, DD*), leaf primordia 1 to 5 (*L1* and *L5*), primary tillers 1 and 2 (*T1, T2*). The lengths of internodes 1 to 4 (*IN1–IN4*) were also measured. Figure 5, a and b are photographs of transverse sections cut through the base of the fifth leaf primordia at 8 DAP showing the coleoptile leaf, leaf primordia 1 to 5, the mid rib of the leaf primordia, and the diameter of the apex. It is clear from the photograph that the diameter of the apical dome and the midrib thickness of the leaf primordia are larger at 700 than at 350 μL CO_2 L^{-1} (Fig. 5, a and b). Quantification of the sizes of

Figure 3. Influence of elevated CO_2 on length of secondary tillers of rice emerging from primary tillers 1 (a and d), 2 (b and e), and 3 (c and f). Plants were grown at either 350 (open bars) or 700 (shaded bars) µL CO_2 L^{-1}. Measurements were made at 21 (a–c) and 26 DAP (d–f) from the base of the tiller to the tip of the longest leaf blade. Values are the means of five replicates and bars represent one SE.



various parts of the apex showed that elevated CO_2 had a dramatic effect on apex development (Table III). For example, leaf primordia 4 and 5 were almost twice as long at high CO_2. Elevated CO_2 had a much greater influence on the thickness of the midrib of the oldest leaf primordia (leaf 1) than on those recently formed (Fig. 6).

Although high CO_2 influenced the size of the shoot apex at 8 DAP, it had no effect on the numbers of leaf primordia or tiller buds (Fig. 5, a and b). In contrast, transverse sections through the base of the shoot apex at 16 DAP clearly showed that there were five tiller buds present at elevated CO_2 compared with only three in the 350 µL CO_2 L^{-1} treatment (Fig. 5, c and d).

Tiller Number and Grain Yield of the Mature Plant: Experiments 2 and 3

The most important consequence of early exposure of plants to elevated CO_2 was that the tiller number was increased by 42% at the maximum tillering stage (Fig. 7a). Each tiller produced grain and the enhancement of grain yield per plant due to CO_2 enrichment was 57% (Fig. 7b). An increase in grain number per panicle from 57 at ambient CO_2 to 68 at elevated CO_2 and in 100 grain weight from 2.4 to 2.9 also contributed to the greater grain yield. In contrast, when 15 d had elapsed before the plants were exposed to high CO_2, the tiller number was unaffected by the CO_2 treatments. In that experiment the increase in grain yield per plant was 20% (Fig. 7b) because of an increase in grain number per panicle from 62 to 82 and a change in 100 grain weight from 2.4 to 2.6 due to elevated CO_2.

Table I. *Influence of elevated CO_2 on the RGR of main plant and primary tillers*

Rice plants were grown in flooded soil at a CO_2 concentration of 350 (ambient) or 700 µL L^{-1}. The RGR was determined from the lengths of the main plant and tillers measured at 11, 16, 21, and 26 DAP. The data represent the mean values ± SE (n = 5).

Plant Part	CO_2 concentration	RGR		
		11–16 DAP	16–21 DAP	21–26 DAP
	µL L^{-1}		*mm mm^{-1} d^{-1} × 10^{-3}*	
Main plant	350	42 ± 4	22 ± 4	14 ± 4
	700	32 ± 2	17 ± 6	13 ± 3
Primary tiller 1	350	154 ± 61	149 ± 54	49 ± 8
	700	107 ± 9	36 ± 9	32 ± 6
Primary tiller 2	350	309 ± 36	98 ± 25	35 ± 6
	700	293 ± 8	51 ± 4	31 ± 4
Primary tiller 3	350	240 ± 50	283 ± 45	53 ± 7
	700	348 ± 18	212 ± 25	41 ± 2
Primary tiller 4	350		311 ± 23	199 ± 20
	700		415 ± 17	135 ± 24
Primary tiller 5	350			222 ± 43
	700			389 ± 21

DISCUSSION

Influence of High CO_2 on Development of Shoot Apex

Elevated atmospheric CO_2 concentrations caused dramatic changes in the shoot apex of rice plants that were evident as early as 8 DAP and became more pronounced by 16 DAP (Table III; Fig. 5). These changes in the apex at elevated CO_2 were consistent with accelerated development as demonstrated by increases in the diameter and

Table II. *Influence of elevated CO_2 on the average number of leaves emerged on the main plant and primary tillers at different stages of ontogeny*

Rice plants were grown in flooded soils at either 350 or 700 µL CO_2 L^{-1}. The numbers of leaves visible above the leaf sheath were counted at 11, 16, 21, and 26 DAP. Values are the means of five replicates.

DAP	CO_2	Main Plant	Tiller 1	Tiller 2	Tiller 3	Tiller 4
	µL L^{-1}			*No. of leaves*		
11	350	4.0				
	700	4.0				
16	350	4.8				
	700	5.0				
21	350	6.0				
	700	6.4				
26	350	7.6	3.8	3.0	2.2	1.0
	700	8.0	4.8	4.4	3.6	2.6

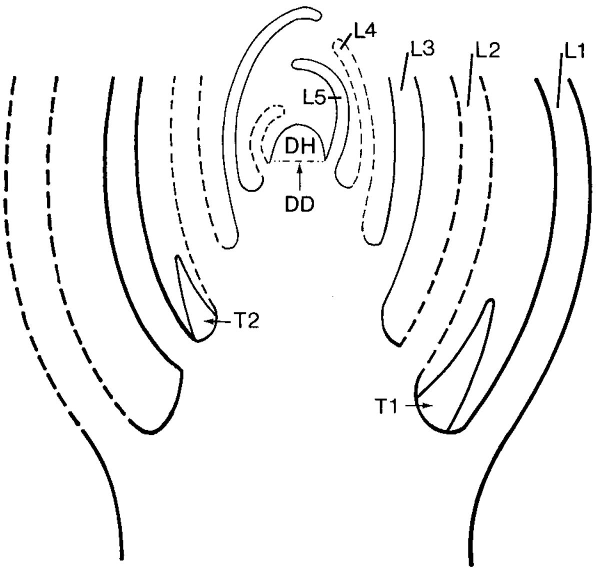


Figure 4. Schematic diagram of the shoot apex of rice at 8 DAP. Serial transverse sections (7 μm) were cut through the apex, commencing at its base. The numbers of sections between specific points were counted to quantify changes in apex development due to CO_2 enrichment. DH and DD, the height and diameter of the apical dome; L1–L5, leaf primordia 1 to 5; T1 and T2, primary tiller buds 1 and 2.

height of the apical dome, and in the lengths of the leaf primordia, tiller buds, and internodes (Table III). High CO_2 also had a greater effect on the thickness of midrib of leaf primordia 1 (the oldest leaf) than on that of leaf primordia 5 (the youngest leaf) (Fig. 6). By 16 DAP, the effect of elevated CO_2 on development had become more obvious because the number of tiller buds had increased to five at 700 μL CO_2 L^{-1}, whereas there were still only three present in the apices of plants grown at 350 μL CO_2 L^{-1} (Fig. 5, c and d).

The importance of high CO_2 in stimulating early leaf growth in grasses was also demonstrated in 7-d-old wheat plants (Robertson and Leech, 1995; Robertson et al., 1995). In those studies, CO_2 enrichment greatly enhanced mitochondrial biogenesis in the cells of the growing zone at the base of the first leaf, the effect being evident as early as 12 h postmitosis (Robertson et al., 1995). Mesophyll cell and chloroplast volumes increased by 10 and 25%, respectively, at 650 μL CO_2 L^{-1} compared with 350 μL CO_2 L^{-1}, indicating that this early enhancement of activity at the cellular level was translated into changes in the mature leaf (Robertson and Leech, 1995).

Higher metabolic activity may also have occurred in the shoot apex of our rice plants at elevated CO_2 and could have resulted in accelerated rates of cell division. It has previously been demonstrated that in the grass tall fescue, nitrogen supply affects rates of cell division, and this plays an important role in the regulation of tiller and leaf development (Skinner and Nelson, 1994). Farrar (1996) suggests that the supply of Suc to dividing cells in growing tissue may be the key to determining rates of cell division and that control of cell division could be the critical link between the production of photosynthate by the source leaves and the generation of sink capacity in the growing tissue. We have shown that there are higher concentrations of soluble carbohydrates in the fully expanded source leaves of rice grown at high CO_2 (Seneweera et al., 1995). It is possible that the flux of carbohydrates to the shoot apex may also be higher and may account for the faster rates of development at high CO_2.

Relationship between Early Changes in Apex and Plant Morphology Later in Ontogeny

The early effects of CO_2 enrichment on development of the shoot apex of rice at 8 DAP, when only the first and second leaves had emerged, were the forerunners to accelerated growth later in ontogeny, and consequently they determined the ultimate growth potential and grain yield of the plant. At 11 DAP there were still only four leaves visible above the sheath on the main plant and no tillers were visible at either 350 or 700 μL CO_2 L^{-1} (Table II). However, by 16 and 21 DAP there were increases in average leaf number on the main plant due to CO_2 enrichment (Table II). The effect of high CO_2 was even more pronounced as leaves emerged on the primary tillers, and at 26 DAP only 1 leaf had emerged on primary tiller 4 at 350 μL CO_2 L^{-1} compared with an average of 2.6 leaves at 700 μL CO_2 L^{-1} (Table II). These observations are also consistent with acceleration of development.

The finding that high CO_2 had a larger influence on the development of tillers than on the main plant was also demonstrated by measuring the length of the main shoot and primary and secondary tillers (Figs. 1–3). These measurements represent changes in the length of the leaf (sheath plus blade) because the stem does not elongate until flowering occurs (Seneweera et al., 1994). At 11 DAP, length of the main shoot was greater at elevated CO_2, but by 26 DAP the plants grown at 350 μL CO_2 L^{-1} were catching up with those grown at 700 μL CO_2 L^{-1} (Fig. 1). High CO_2 also had a dramatic effect on the length of both the primary and secondary tillers (Figs. 2 and 3). Nevertheless, as with the main shoot, the effect was more pronounced at the early stages of tiller growth (Fig. 2). Calculation of RGR, based on shoot and tiller lengths, revealed that growth rates were only stimulated by high CO_2 during the early growth phase and that the RGR slowed down sooner at elevated CO_2 (Table I). This suggests that the rate of growth of leaves on the tillers and main shoot were faster at high CO_2 but that the duration of elongation was shorter. This would explain why the size of the main shoot was similar at both CO_2 concentrations by 26 DAP. We have previously shown that elongation rates of rice leaves measured over a single day were faster at elevated CO_2. The leaf growth rates were correlated with the Suc phosphate synthase activity at ambient and elevated CO_2, suggesting that supply of Suc from the source leaf may be important in regulating leaf growth (Seneweera et al., 1995). The acceleration of leaf growth is important in terms of rate of tiller development because the generation of new tillers commences when the associated leaf ceases to grow

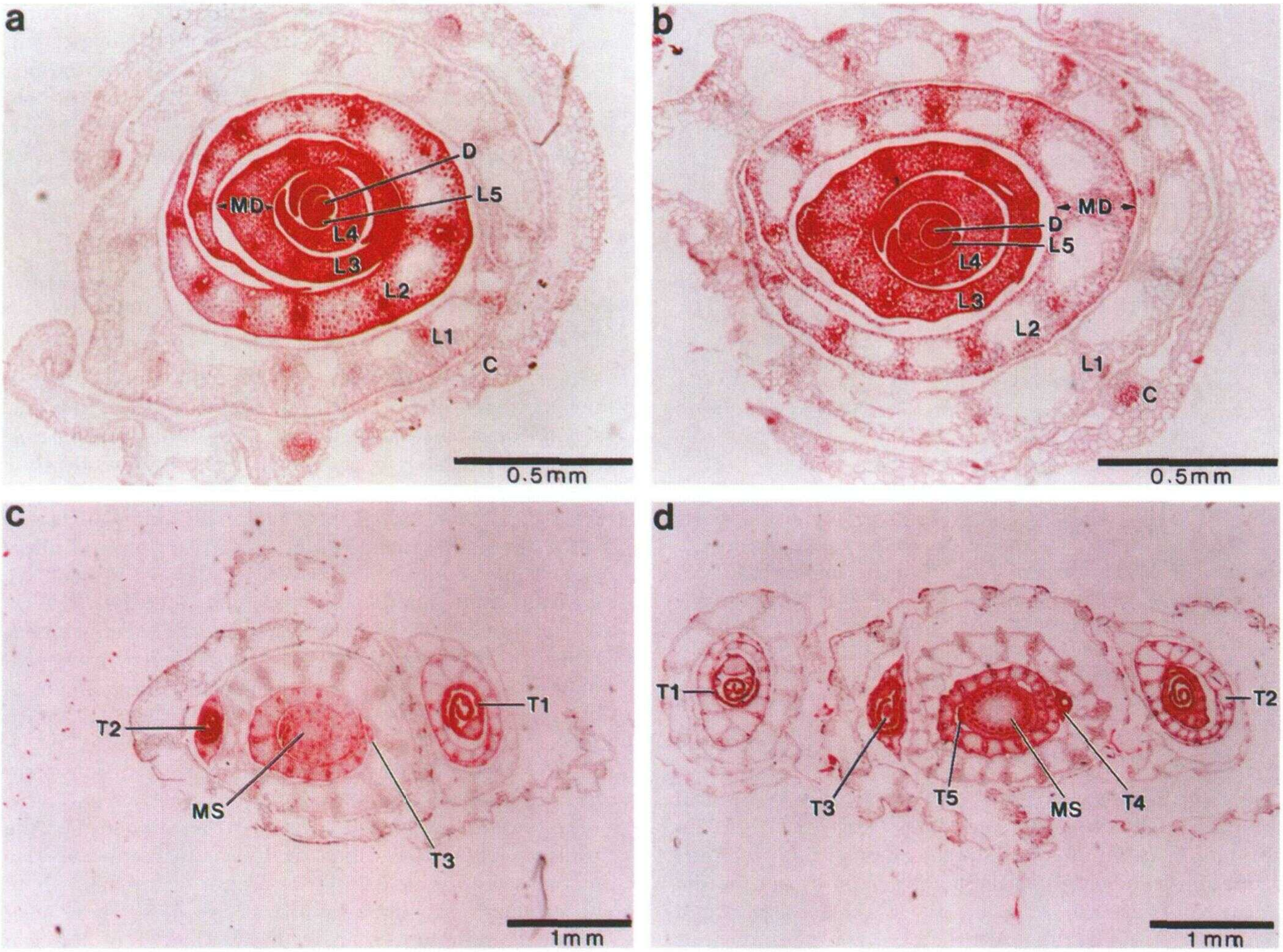


Figure 5. Photomicrographs of transverse sections of shoot apices of rice plants grown at either 350 μL CO_2 L^{-1} (a and c) or 700 μL CO_2 L^{-1} (b and d). Sections were cut through the base of the fifth leaf primordia at 8 DAP (a and b) and near the base of the apex at 16 DAP (c and d). D, the apical dome; L1–L5, leaf primordia 1 to 5; C, the coleoptile leaf; MD, the midrib; T1–T5, tiller buds 1 to 5; MS, the main shoot.

(Skinner and Nelson, 1995). Thus, the faster a leaf reaches its final size, the faster the tillers will appear.

Masle et al. (1993) showed that the RGR (calculated on a whole plant dry weight basis) of *Nicotiana tabacum* was increased when the photosynthetic rates of the source leaves were increased either by genetic manipulation of Rubisco or by increasing the CO_2 concentration. However, the increase in RGR lasted only for a few days during early development, after which the RGR was similar for all of the treatments. This long-term insensitivity of RGR to increases in rates of photosynthesis of the source leaves was not directly caused by changes in carbon partitioning between the roots and the shoots nor by feedback inhibition of photosynthesis. The factors controlling the early enhancement of RGR and the subsequent attainment of a steady state still remain enigmatic.

A possible explanation is that higher rates of carbohydrate production may alter hormone concentrations, which in turn may control the longer-term growth and development of the plant so that the carbohydrate supplied by photosynthesis matches the generation of growing sinks. This balance between source and sink activity would account for maintenance of a constant RGR, but since both rates of photosynthesis and sink generation would be higher at elevated CO_2 concentrations, the ultimate size of the plant would be greater at high CO_2. A possible candidate for regulation of source/sink balance at elevated CO_2 is the hormone ethylene because its release from expanded leaves is increased at high CO_2 in rice (Conroy et al., 1994) and tomato plants (Woodrow and Grodzinski, 1993).

Long-Term Effects of Early Changes in the Shoot Apex at High CO_2

Clear evidence that early enhancement of development by elevated CO_2 has a dramatic influence on ultimate grain yield was demonstrated by the finding that the 42% increase in tiller number at the maximum tillering stage due to elevated CO_2 was associated with a 57% increase in

Table III. *Influence of elevated CO_2 concentrations on dimensions of apical dome and lengths of leaf primordia, internodes, and tiller buds in apices of 8-d-old rice*

Rice plants were grown in flooded soil at either 350 or 700 μL CO_2 L^{-1}. The height (DH) and diameter (DD) of the apical dome and leaf primordia 4, 5 (L4, L5), and tiller buds 1, 2 (T1, T2) are illustrated in Fig. 4. IN1 to IN4 are the internodes between corresponding leaves. The data are the means of five replicates with SE in parentheses.

Parameter	Position	CO_2	Size
		$\mu L\ L^{-1}$	*μm*
Height of apical dome		350	33 (2)
		700	54 (2)
Diameter of apex		350	62 (2)
		700	84 (3)
Length of leaf primordia	L4	350	574 (15)
		700	1760 (18)
	L5	350	61 (6)
		700	124 (8)
Length of internodes	IN1	350	240 (6)
		700	303 (6)
	IN2	350	149 (2)
		700	165 (5)
	IN3	350	61 (2)
		700	71 (3)
	IN4	350	36 (1)
		700	64 (4)
Length of tiller buds	T1	350	124 (6)
		700	194 (8)
	T2	350	60 (5)
		700	114 (10)

grain yield. An increase of approximately 20% in the grain number per panicle and the average grain weight also contributed to the enhanced grain yield at elevated CO_2, indicating that the generation of sinks in the floral apex and the endosperm of the grain also plays a role in determining grain yield.

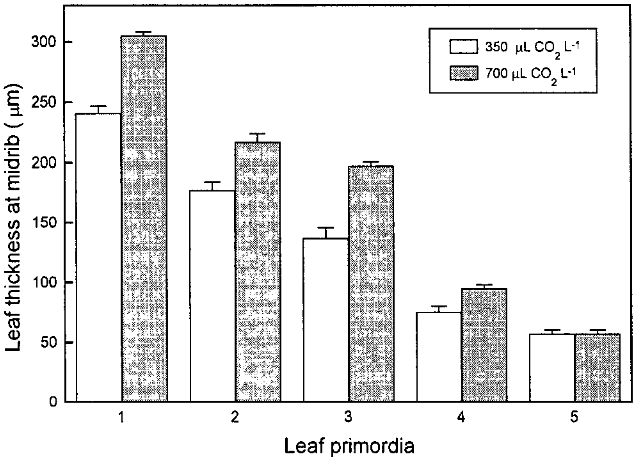


Figure 6. Influence of elevated CO_2 on midrib thickness of leaf primordia 1 to 5. Plants were grown at either 350 (open bars) or 700 (shaded bars) μL CO_2 L^{-1} for 8 DAP. Measurements were made on transverse sections of apices at the point (MD) indicated in Figure 5a. Values are the means of five replicates and bars represent one SE.

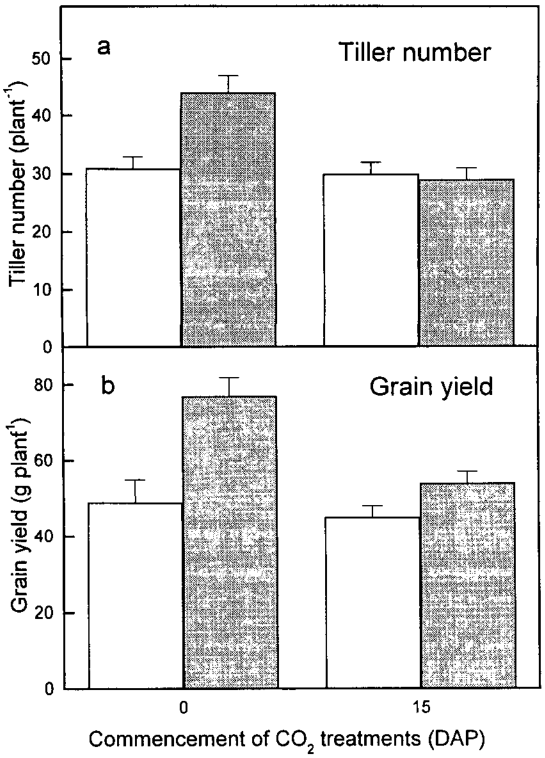


Figure 7. Influence of elevated CO_2 concentration on tiller numbers (a) and grain yield (b). Plants were exposed to either 350 or 700 μL CO_2 L^{-1} from 0 or 15 DAP. In the latter, plants were grown at 350 μL CO_2 L^{-1} for 15 d before being transferred to the CO_2 treatments. There were five replicates per treatment. Bars represent one SE.

Delaying exposure of plants to high CO_2 until 15 DAP severely reduced the impact of high CO_2 on grain yield because the tillering response to high CO_2 was inhibited (Fig. 7). This fits in with the observations that tiller initiation in the shoot apex was greatly enhanced by high CO_2 at 16 DAP (Fig. 5, c and d) and tiller growth was subsequently greater at high CO_2 (Figs. 2 and 3). Nevertheless, there was a 20% increase in grain yield due to CO_2 enrichment (Fig. 7) because there was still potential to increase the number of grain per panicle and grain size, in this case by 32% and 10%, respectively. It is possible that faster rates of cell division at high CO_2 are not only important during vegetative shoot initiation in the apex but also during grain initiation in the panicle and endosperm development in the grain. The importance of timing of exposure to elevated CO_2 in obtaining a yield increase at high CO_2 was also demonstrated by Havelka et al. (1984) using wheat. When plants were exposed to high CO_2 during grain initiation, there was an increase in yield; however, there was no response to high CO_2 when plants were exposed to high CO_2 during grain filling only. The results of that study indicated that the potential to alter sink size was past at the grain-filling stage.

In conclusion, the generation of sinks in the shoot apex appears to be a major factor in determining the long-term productivity of grain crops under rising atmospheric CO_2 concentrations. We suggest that at high CO_2 there is a greater flux of carbohydrates from the source leaves to the shoot apex, which results in increased rates of sink development. During the vegetative stage, a greater supply of carbohydrates also accelerates the rate of development of the leaves on the main shoot and on each tiller, stimulating the appearance of new tillers. If exposure to high CO_2 is delayed, some of the tiller buds remain permanently suppressed. Since most of tiller can produce grain, the maximum increases in grain yield at elevated atmospheric CO_2 concentrations can only be realized by exposing plants to high CO_2 immediately after germination.

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LITERATURE CITED

Conroy JP, Rogers GS, Seneweera S, Basra AS, Jones B, Ghannoum O (1995) Influence of global change on crop productivity and quality. 2nd Asian Agronomy Conference. Fukai, Japan

Conroy JP, Seneweera S, Basra AS, Rogers G, Nissen-Wooller B (1994) Influence of rising atmospheric CO_2 concentrations and temperature on growth, yield and grain quality of cereal crops. Aust J Plant Physiol **21:** 741–758

Farrar J (1996) Regulation of shoot-root ratio is mediated by sucrose. Plant and Soil **185:** 13–19

Ghannoum O, von Caemmerer S, Barlow EWR, Conroy JP (1997) The effect of CO_2 enrichment and irradiance on the growth, morphology and gas exchange of a C_3 (*Panicum laxum*) and a C_4 (*Panicum antidotale*) grass. Aust J Plant Physiol **24:** 227–237

Havelka UD, Wittenbach VA, Boyle MG (1984) CO_2 enrichment effects on wheat yield and physiology. Crop Sci **24:** 1163–1168

Masle J, Hudson GS, Badger MR (1993) Effects of ambient CO_2 concentration on growth and nitrogen use in tobacco (*Nicotiana tabacum*) plants transformed with an antisense gene to the small subunit of ribulose-1,5-bisphosphate carboxylase oxygenase. Plant Physiol **103:** 1075–1088

Poorter H (1993) Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. Vegetatio **104/105:** 77–97

Robertson EJ, Leech RM (1995) Significant changes in cell and chloroplast development in young wheat leaves (*Triticum aestivum* cv. Hereward) grown in elevated CO_2. Plant Physiol **107:** 63–71

Robertson EJ, Williams M, Harwood JL, Lindsay JG, Leaver CJ, Leech RM (1995) Mitochondria increase three-fold and mitochondrial proteins and lipid change dramatically in postmeristematic cells in young wheat leaves grown in elevated CO_2. Plant Physiol **108:** 469–474

Rogers GS, Milham PJ, Gillings M, Conroy J (1996) Sink strength may be the key to growth and nitrogen responses in N-deficient wheat at elevated CO_2. Aust J Plant Physiol **23:** 253–264

Seneweera S, Milham P, Conroy J (1994) Influence of elevated CO_2 and phosphorus nutrition on the growth and yield of a short-duration rice (*Oryza sativa* L cv. Jarrah). Aust J Plant Physiol **21:** 281–292

Seneweera SP, Basra AS, Barlow EW, Conroy JP (1995) Diurnal regulation of leaf blade elongation in rice by CO_2. Is it related to sucrose-phosphate synthase activity? Plant Physiol **108:** 1471–1477

Skinner RH, Nelson CJ (1994) Epidermal cell division and the coordination of leaf and tiller development. Ann Bot **74:** 9–15

Skinner RH, Nelson CJ (1995) Elongation of the grass leaf and its relationship to the phyllocron. Crop Sci **35:** 4–10

Webber AN, Nie GY, Long SP (1994) Acclimation of photosynthetic proteins to rising atmospheric CO_2. Photosynth Res **39:** 413–425

Woodrow L, Grodzinski B (1993) Ethylene exchange in *Lycopersicon esculentum* Mill: leaves during short-term and long-term exposures to CO_2. J Exp Bot **44:** 471–480

Xu DQ, Gifford RM, Chow WS (1994) Photosynthetic acclimation in pea and soybean to high atmospheric CO_2 partial pressure. Plant Physiol **106:** 661–671