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Interactive effects of elevated CO_2 and potassium deficiency on photosynthesis, growth, and biomass partitioning of cotton

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Abstract

In modern cotton production systems, potassium (K) deficiency is one of the major factors limiting lint yield and affecting fiber quality. Although influence of K deficiency on cotton plant physiology and growth and lint yield responses to K fertilizer applications have received intensive studies, it is not clear whether elevated atmospheric CO_2 concentration [CO_2] affects plant requirements and sensitivity to K. An experiment was conducted in sunlit controlled-environment chambers to determine the interaction effects of elevated [CO_2] and K deficiency during squaring and flowering on cotton plant growth, photosynthesis, and biomass accumulation and partitioning. The treatments included two levels of [CO_2] (360 and 720 $\mu L\ L^{-1}$) and five levels of K supply (optimum (control) and 40, 20, 5, and 0% of the control K) at each [CO_2] level. Elevated [CO_2] significantly increased photosynthesis, leaf area and biomass production of K sufficient plants, but did not affect leaf K concentration. Potassium deficiency not only reduced these growth variables but also changed biomass partitioning among plant tissues with the greatest decrease in fruit biomass. There were significant interactive effects of [CO_2] × K on leaf area, canopy photosynthesis, and biomass accumulation and partitioning. The stimulation of the physiological and growth parameters observed due to elevated [CO_2] was lost under severe K deficiency. Under ambient [CO_2], leaf critical K level depended on growth variables measured and was 17 g kg^{-1} for leaf area expansion and 12 g kg^{-1} for canopy photosynthesis, stem elongation and biomass accumulation. Plants grown under elevated [CO_2] were more sensitive to K deficiency with higher leaf critical K levels. The information from this study is useful for understanding the cotton K requirement in the present as well as in the future higher [CO_2] environment and for recommendations of K application.

Keywords: Cotton *(Gossypium hirsutum* L.); Elevated CO_2; Potassium deficiency; Leaf K concentration; Leaf area; Biomass; Photosynthesis; Leaf critical K levels

1. Introduction

Potassium (K) is one of the major mineral nutrients impacting cotton (*Gossypium hirsutum* L.) plant growth, development, lint yield, and fiber quality (Kerby and Adams, 1985; Cassman et al., 1990). In

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recent years, the incidence of K deficiency in cotton production has been increasing across the United States Cotton Belt (Oosterhuis, 1994). Introduction of faster fruiting, higher boll-load and high lint yield cotton cultivars with reduced root growth and ion uptake during reproductive growth may be the primary contributing factors for the observed in-season K deficiency (Oosterhuis, 1994). Compared to other field crops, cotton appears to be more susceptible to K availability and shows symptoms of K deficiency much earlier under limited soil K (Cope, 1981; Cassman et al., 1989). Many studies have been indicated that K deficiency negatively affects cotton plant photosynthesis (Bednarz et al., 1998; Zhao et al., 2001), leaf area (Zhao et al., 2001; Pettigrew, 2003), biomass production (Zhao et al., 2001), and finally resulting in a lower lint yield (Pettigrew, 2003) and poor fiber quality (Pettigrew et al., 1996). Soil or foliar applications of K have been reported to improve cotton yield and quality (Howard et al., 1998; Adeli and Varco, 2002).

Climate change and crop production are intimately connected. Atmospheric CO_2 concentration [CO_2] has increased by more than 28% since the beginning of the industrial revolution mainly because of increased utilization of fossil fuels and deforestation (IPCC, 2001). The [CO_2] in the atmosphere is projected to double from the current 370 $\mu L\ L^{-1}$ by the end of the current century (IPCC, 2001). Elevated atmospheric [CO_2] generally enhances leaf and canopy photosynthesis, especially in C_3 crops, because the present [CO_2] is not sufficient to saturate Rubisco and because high CO_2 inhibits the competing process of photorespiration (Lawlor and Mitchell, 2000). Numerous studies have demonstrated that crop growth and yield are favored by elevated [CO_2] (Kimball, 1983; Amthor, 2001; Kimball et al., 2002).

Similar to other field crops, cotton growth, physiology and yield respond well to elevated atmospheric [CO_2]. Kimball and Mauney (1993) found that cotton grown under 550 $\mu L\ CO_2\ L^{-1}$ had a 35% higher biomass, 40% higher fruit weight, and 60% higher lint yield than plants grown under 350 $\mu L\ CO_2\ L^{-1}$ under free-air CO_2 enrichment conditions. However, plant growth and physiological responses to elevated [CO_2] are closely dependent on other environmental factors. For instance, cotton canopy photosynthesis increased by about 80% when [CO_2] was increased from 360 to 720 $\mu L\ L^{-1}$ under optimum temperature (26–28 °C) conditions, but the elevated CO_2 only resulted in a 40% increase in canopy photosynthesis when plants were grown under low (20 °C) or high (32 °C) temperature conditions (Reddy et al., 2000). Khader and Ravichandran (2002) reported that plants grown under elevated [CO_2] were more tolerant to water deficit stress compared to plants grown under ambient CO_2 conditions. In contrast, several studies have documented that elevated [CO_2] did not ameliorate the adverse effects of high temperature (Reddy et al., 1995a), water deficient stress (Samarakoon and Gifford, 1996) and high UV-B radiation (Zhao et al., 2003).

Although crop growth, physiology and yield responses to a single factor of K deficiency or CO_2 enhancement have received intensive studies, to date, there are no reports on the interactive effects of K nutritional supply and elevated [CO_2] on cotton. We hypothesize that elevated atmospheric [CO_2] may increase the sensitivity of cotton plants to K deficiency or the CO_2 enhancement effect may be reduced by limitation of K supply. The objectives of this study were to determine the interactive effects of elevated [CO_2] and K supply on cotton growth, development, and photosynthetic characteristics under controlled-environmental conditions. Information related to whole plant processes will contribute to filling gaps in our knowledge and to understanding of relationships between these two major nutrients affecting growth, and development of cotton.

2. Materials and methods

2.1. Experimental facility and plant culture

The experiment was conducted at the Mississippi Agricultural and Forestry Experiment Station, Mississippi State, Mississippi (33°28′N, 88°47′W), USA using ten sunlit Soil-Plant-Atmosphere-Research (SPAR) units. The SPAR facility has the capability to precisely control temperature, [CO_2], water, and nutrients at predetermined set points for plant growth studies in near natural solar radiation regimes. Details of the SPAR operation and controls have been described by Reddy et al. (2001). Briefly, each SPAR unit consists of a steel soil bin (1 m × 2 m× 0.5 m),

and a Plexiglas chamber (2.5 m × 2 m × 1.5 m) to accommodate above-ground plant parts, a heating and cooling system, and an environment monitoring and control system. Each SPAR unit can provide a 1 m^2 area for plant growth. The Plexiglas of the chambers transmits 97% of incoming photosynthetically active radiation (PAR, 400–700 nm). Air temperature and [CO_2] in each SPAR chamber were monitored and adjusted every 10 s throughout the experimental period.

Thirty 12 L white polyvinyl chloride (PVC) pots were inserted in each SPAR soil bin and filled with fine sand. The cylinder-shaped pots were 0.65 m in height and 0.15 m in diameter with a small hole at bottom to drain excess water. Seeds of cotton cv. NUCOTN 33B, a mid-season Upland Bt cultivar, were sown on 2 May 1996 with five seeds each pot. Emergence was observed five days after sowing. Seedlings were thinned to one per pot after plant emergence. All SPAR chambers were maintained at 30/22 °C (day/night) temperatures during the experiment. Plants were irrigated three times a day with defined nutrient solutions, based on K treatments, delivered at 08:00, 12:00, and 17:00 h to ensure favorable water conditions for plant growth and development. Irrigation was provided through an automated and computer-controlled drip system. Variable-density black shade cloths (Hummert Seed Co., St. Louis, Missouri, USA) were placed around the edges of plants and adjusted regularly to simulate natural shading by other plants.

2.2. Treatments

Ten treatments included two levels of [CO_2]: 360 μL L^{-1} (ambient) and 720 μL L^{-1} (elevated), and five levels of K supply. The [CO_2] treatments were imposed from emergence through final harvest, 85 DAE. The five K treatments were initiated around first square stage (23 DAE) and included: (1) a full K supply (Control, 100% K) irrigated with half-strength Hoagland's nutrient solution containing 0.234 g K L^{-1} throughout the experiment; (2) K reduction to 40% of the control level (40% K); (3) 20% K of the control (20% K); (4) 5% K of the control (5% K); and (5) 0% K of the control (0% K), until final harvest (85 DAE). All plants in the reduced (the 40, 20, and 5% K) and withheld K (the 0% K) treatments received normal half-strength Hoagland's nutrient solution before K-stress treatments were initiated. Removing or reducing K from the nutrient solution resulted in dilution of K in the plant tissues because of subsequent growth.

2.3. Measurements

Plant height, number of main-stem leaves and leaf lengths were recorded at 2-day intervals from 22 to 64 DAE on nine plants in each treatment. Main stem length was measured from cotyledonary node to the topmost, unfolded main-stem leaf. Leaf area was calculated based on measurements of leaf length using the following quadratic equation:

$$Y = -2.058 - 224.8X + 0.00805X^2$$

where Y is the leaf area in cm^2 and X is leaf length in mm. The equation was obtained by regressing the lengths and areas of 1010 leaves ($r^2 = 0.94^{***}$) measured from 90 plants in the 10 treatments at the second and third destructive harvests (30 and 85 DAE). Dates of first square and first flower stages were recorded in all treatments.

Plants were harvested on four sampling dates at 21, 30, 37, and 85 DAE. After each harvest, the pots were rearranged within the SPAR chambers to ensure all plants had the equal space in the growth chamber. Six (21 and 37 DAE) or nine (30 and 85 DAE) plants in each treatment were randomly selected for each harvest. Plant height and main-stem nodes of individual plants were recorded at all harvests. Leaf areas were determined using a LI-3100 area meter (LI-COR Inc., Lincoln, NE, USA). Then, plants were separated into leaves, stems, fruits (floral buds and bolls, if they were present), and roots. The plant parts were dried at 70 °C for 72 h and weighed. Specific leaf weight (SLW, mg cm^{-2} leaf) was also calculated as the ratio of dry weight to leaf area at the final harvest.

Canopy photosynthesis (Pn) was determined using a mass balance approach in each chamber throughout the experiment (Reddy et al., 1995b) and expressed as mg CO_2 m^{-2} ground area s^{-1} at 1500 μmol m^{-2} s^{-1} PAR. Each SPAR growth chamber and a fan-coil box formed a semi-closed system for the measurement of CO_2 fluxes. The Plexiglas chamber containing the plants, ducts, and cooling system was sealed. The chamber [CO_2] was measured with a dedicated infrared gas analyzer (LI-COR, model LI-6252, Lincoln, NE, USA) from the gas sample that was

drawn through the lines run underground from SPAR units to the field laboratory building at 10-s intervals and adjusted to predetermined levels. Moisture was removed from the gas sample by running the sample lines through refrigerated water tap (4 °C) that was automatically drained and through a column of magnesium per chlorate. Chamber [CO_2] was maintained by supplying pure CO_2 from a compressed gas cylinder through a system that included a pressure regulator, solenoid and needle valves, and a calibrated flow meter (Reddy et al., 2001). The time intervals during which the solenoid valves are open were monitored by the computer recording the amount of gas injected. Flow rates of CO_2 were recorded three times a day and converted into mass quantity using gas corrections for temperature and pressure. A leakage test was performed each night to derive a correction factor for losses of CO_2 from each SPAR chamber (Acock and Acock, 1989). All data of CO_2 exchange rates were obtained every 10 s and integrated over 900-s intervals throughout the day-lit period. The corresponding incident PAR was also measured by monitoring with a LI-190SA quantum Sensor (LI-COR Inc., Lincoln, NE, USA) and summarized with a data acquisition system at 900-s intervals. Canopy net CO_2 exchange rates were summarized over the same time intervals. The curve lines of canopy net CO_2 exchange rates versus PAR (i.e. photosynthetic light response curves) for each SPAR were fitted with a quadratic equation. Canopy Pn, expressed on a ground area basis at 1500 μmol m^{-2} s^{-1} PAR, was estimated from the photosynthetic light response curves during the experimental period. Canopy CO_2 exchange rates on a leaf area basis during the boll development (50–59 DAE) were estimated by dividing canopy Pn on a ground area basis by total leaf area at the same dates for each treatment.

Chlorophyll contents of the uppermost, fully expanded main-stem leaves were determined on nine plants in each treatment at 42, 51, and 74 DAE using a Minolta SPAD-502 chlorophyll meter (Minolta Corp., Osaka, Japan). Additionally, three uppermost fully expanded main-stem leaves were sampled from each treatment weekly starting from K treatment initiation (23 DAE) until the final harvest (85 DAE) between 10:00 and 11:00 h. The three leaves were combined and dried at 70 °C for 72 h, weighed, and ground to determine leaf K concentration. Leaf K was quantified in the Soil Testing Laboratory, Mississippi State University, according to methods of Donohue and Aho (1992) using an ICP spectrophotometer (Perkin-Elmer Instuments, Moonwalk, Connecticut, USA).

2.4. Experimental design and data analysis

Frequent uniformity tests and various studies indicate that all SPAR chambers are finely controlled and identical (Acock and Acock, 1989; Reddy et al., 1995a,b, 2001; Zhao et al., 2003). Therefore, the experiment was a factorial treatment design (Kuehl, 1994) with six or nine replications. Ten treatments were randomly arranged in 10 SPAR units. Except for the two treatment factors of [CO_2] and K supply, the other growth conditions were same during the experiment for all treatments. Because each treatment had one SPAR unit due to limitation of SPAR chambers, the individual plants or pots within a treatment unit were used as replications. All growth and physiological measurements were made on six or nine replicate plants for each treatment, except for leaf K concentration that was determined from the three-leaf combinations without replication due to limited sample size.

The best-fit models of linear and non-linear regressions were used for plant growth parameters measured for each treatment against days after emergence. Specifically, additions of main-stem nodes followed a linear fashion and all other growth parameters (i.e. main-stem length, leaf area and total biomass) followed the patterns of sigmoid growth during plant growth in the experiment. The increment/expansion rates of these growth parameters were calculated via the first derivatives of the sigmoid equations. In order to determine responses of individual growth or physiological variables measured to K deficiency, the relative increment rates of the control plants of full K supply (the 100% K) at all nine sampling dates were set to 100%. The relative increment rates of each K-deficient treatment were further calculated by dividing their absolute increment rate by that of the control plants and multiplying with 100 at each sampling date.

Data were statistically analyzed by the ANOVA procedures in SAS (SAS Institute Inc., 1997) to determine the main and interactive effects of the two factors of [CO_2] and K nutrition on plant growth,

development, dry matter accumulation and partitioning, chlorophyll content, and canopy photosynthesis. If the hypothesis of equal means has been rejected by the ANOVA test, the Fisher LSD procedures at $P = 0.05$ probability level (SAS Institute Inc., 1997) were employed to distinguish among treatment means for the growth and physiological variables measured.

3. Results

3.1. Leaf K concentration

Overall, K concentrations of uppermost, fully expanded main-stem leaves did not differ between the two levels of [CO_2], but were considerably affected by K nutrient supply (Fig. 1). Leaf K increased slightly with days after emergence in the control (100% K), and almost kept the same level during growth in the 40% K treatment. In the first two weeks after K treatments were initiated, the leaf K concentrations of the 20, 5, and 0% K treatments declined rapidly. Between 44 and 83 DAE, leaf K concentrations in each of the three K-deficient treated plants did not change with the sampling time. When leaf K concentrations were averaged across the two CO_2 levels, the 40, 20, 5, and 0% K treatments had 21, 38, 69, and 80% lower leaf K, respectively, at 3 weeks (44 DAE) after initiation of K treatments. Leaf K concentrations of the control, 40, 20, 5, and 0% K treatments were 34.1, 26.3, 19.3, 11.2, and 9.0 g kg^{-1}, respectively, prior to the final harvest (83 DAE).

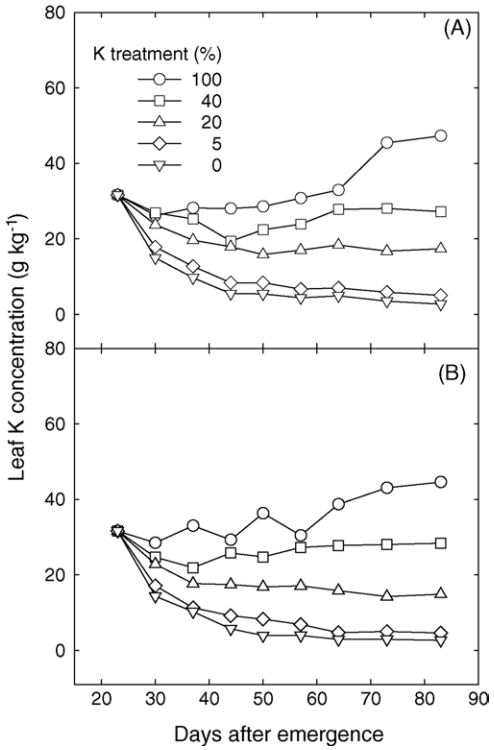

Fig. 1. Leaf potassium (K) concentrations for different K treated cotton plants grown under (A) 360 μL CO_2 L^{-1} and (B) 720 μL CO_2 L^{-1} during experimental period. The K treatments were initiated 23 days after emergence. Three replicated leaf samples were combined before determination of leaf K due to the limitation of sample size.

3.2. Plant height and main-stem nodes

Changes in plants height during crop growth and development followed a sigmoid growth pattern for all treatments (Fig. 2). Plants grown under 720 μL CO_2 L^{-1} were slightly taller than those grown under 360 μL CO_2 L^{-1}. The maximum main-stem elongation rates were 4.5–4.8 cm day^{-1} and the dates of achieving the maximum elongation rates were similar among the treatments between 36 and 41 DAE. From 40 through 80 DAE, the 0 and 5% K-treated plants had consistently lower stem elongation rates than the control.

In the first 60 DAE, main-stem nodes increased linearly ($r^2 = 0.99$) as plants aged in all treatments (data not shown). The node appearance rates (i.e. slopes of the regression lines) did not differ among treatments. It took about 3.0 days (slope = 0.33–0.34) to produce a main-stem node. Starting from 60 DAE, the rates of main-stem node addition were significantly reduced as plants aged. At the final harvest, the numbers of main-stem nodes did not differ between the two CO_2-treated plants, but the K-deficient plants had significantly fewer main-stem nodes (Table 1). Neither elevated [CO_2] nor K-deficient treatments affected plant major phenological events such as first square, 23 DAE, and first flower (47 DAE) dates in this study (data not shown).

3.3. Leaf area, total biomass and specific leaf weight (SLW)

Plants grown in elevated [CO_2] had significantly greater leaf area than plants in ambient [CO_2] at 30

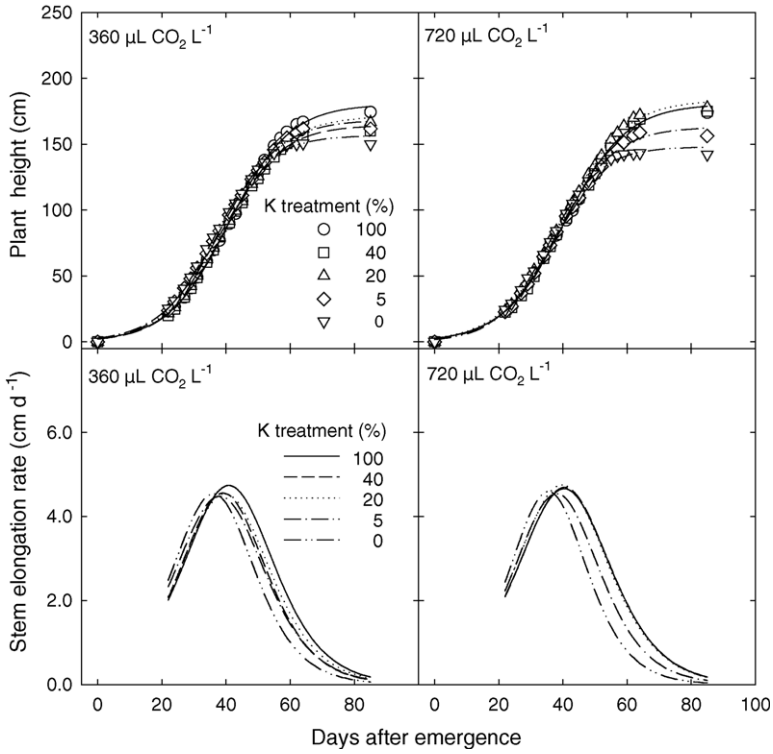

Fig. 2. Changes in plant height and main-stem elongation rates of cotton plants grown under 360 and 720 μL CO_2 L^{-1} as affected by K supply. Each data point represents the mean of nine replicate plants. The lines are regressions fitted to the data using a sigmoid equation for plant height and a first derivative of the sigmoid equation for main-stem elongation rate.

and 85 DAE (Table 1). Leaf area did not differ among K treatments at 30 DAE, but the differences in leaf area could be clearly observed with the development of K deficiencies. There was no $CO_2 \times K$ interactive effect on the leaf area development at 30 DAE ($P > 0.05$), but the interaction was significant ($P < 0.05$) at 85 DAE. At final harvest (85 DAE), plants grown under elevated [CO_2] had a 23% greater leaf area than plants grown under the ambient [CO_2], averaged across all K treatments. The plants grown in the 40, 20, 5, and 0% K treatments had 12, 16, 36, and 43% smaller leaf area, respectively, compared with the control plants of full K supply.

Similar to leaf area response to [CO_2] and K supply, elevated CO_2 significantly increased biomass production (Table 1). Total biomass did not differ among K treatments at 30 DAE, but was significantly affected by K treatments at 85 DAE and the interactive effect of $CO_2 \times K$ on total biomass accumulation was also observed ($P < 0.001$). The differences in total biomass at the final harvest (85 DAE) were not statistically significant among the control, the 40 and 20% K treatments within a CO_2 level. In contrast, biomass of the 5 and 0% K-treated plants, compared with the full K supplied control plants, was 17 ($P < 0.05$) and 41% ($P < 0.001$) lower, respectively for the plants grown under ambient [CO_2], and by 32 ($P < 0.01$) and 57% ($P < 0.001$), respectively for the plants grown under elevated [CO_2]. Under K sufficient (control) and slightly K-deficient (the 40 and 20% K treatments) conditions, plants grown in elevated [CO_2] had a 25–27% more biomass accumulation than plants grown in ambient [CO_2]. The evidence of elevated CO_2 stimulating biomass production did not exist when plants were subjected to severe K deficiency (the 5 and 0% K treatments).

Specific leaf weight (SLW) was sensitive to both [CO_2] and K treatments. Plants grown under elevated

Table 1
Effects of CO_2 levels and potassium (K) supply on the number of main-stem nodes (MSN), leaf area (LA), total biomass (TBM), and specific leaf weight (SLW) of cotton, 30 and 85 days after emergence (DAE)

Treatment[a]		30 DAE				85 DAE			
CO_2	K	MSN (no. plant^{-1})	LA (cm^2 plant^{-1})	TBM (g plant^{-1})	SLW (mg cm^{-2})	MSN (no. plant^{-1})	LA (cm^2 plant^{-1})	TBM (g plant^{-1})	SLW (mg cm^{-2})
360	100	9.7	1547	15.3	4.86	23.1	13798	244.2	4.07
	40	10.2	1571	15.8	5.00	22.1	11886	231.1	4.80
	20	10.2	1355	13.4	4.87	21.8	10885	212.5	4.67
	5	10.3	1456	16.1	5.20	22.2	11015	202.4	5.08
	0	10.3	1573	17.7	5.53	20.7	8475	144.7	5.85
720	100	10.5	1757	18.9	4.85	23.2	16316	332.7	4.44
	40	10.3	2122	21.3	4.84	22.7	14696	312.2	4.95
	20	10.7	1828	17.6	4.34	23.0	14451	281.5	4.79
	5	10.7	2040	21.0	4.66	21.0	11149	225.9	5.77
	0	11.0	1977	22.0	5.34	20.2	8789	144.3	6.29
CO_2		NS	***	***	***	NS	***	***	***
K		NS	NS	NS	***	***	***	***	***
$CO_2 \times K$		NS	NS	NS	NS	NS	*	***	NS

NS, not significant ($P > 0.05$).
[a] Ten treatments included two levels of CO_2 concentration (360 and 720 μL L^{-1}) and five levels of K supply (100 (control), 40, 20, 5 and 0% K). Details of the treatments are described in the text. The CO_2 and potassium treatments were initiated from 0 and 23 DAE, respectively through final harvest (85 DAE).
* Indicate significant at $P < 0.05$ proprtbility level.
*** Indicate significant at $P < 0.001$ proprtbility level.

CO_2 had lower SLW than the ambient CO_2-grown plants at 30 DAE, but results were reversed at 85 DAE (Table 1). The SLW significantly increased with increases in K deficiencies. No $CO_2 \times K$ interaction was detected for SLW.

3.4. Biomass partitioning

Elevated CO_2 and K deficiency affected not only plant biomass production, but also dry matter partitioning among plant components. The interactive effects of [CO_2] and K supply on plant tissue biomass production and partitioning were significant, except for leaf dry weight and root fraction (Table 2). When averaged across all K treatments, elevated [CO_2] significantly increased biomass accumulation in leaves, stems, roots and fruits determined at 85 DAE and the root dry weight had the greatest (40%) increase and stem dry weight had the smallest (17%) increase among the four plant components. The K deficiencies mainly decreased fruit biomass due to severe fruit abscission (data not shown), while leaf biomass had the least reduction. Averaged across the two CO_2 levels, severe K deficiency (the 0% K treatment at 85 DAE) had 19, 50, 43, and 66% less dry weight of leaves, stems, roots and fruit, respectively than the control of full K supply. As K deficiency became more severe, the fraction of leaf biomass to total biomass increased, the fruit biomass fraction decreased, and the fractions of stems and roots altered least (Table 2).

3.5. Chlorophyll and canopy photosynthesis

Leaf chlorophyll content (expressed as the SPAD readings) did not differ ($P = 0.4$) between plants grown under elevated and ambient [CO_2] (Fig. 3A). A significant difference ($P < 0.001$) was detected in chlorophyll content among K treatments. No interaction effect of $CO_2 \times K$ on chlorophyll was found. Averaged across CO_2 treatments, the 40 and 20% K treated plants had comparable chlorophyll with those of the control. However, plants receiving 5 and 0% K had 12 and 38% lower chlorophyll

Table 2
Effects of CO_2 levels and potassium (K) supply on biomass partitioning of cotton plants at final harvest, 85 days after emergence

Treatment[a]		Tissue biomass (g plant^{-1})				Fraction of total biomass (%)			
CO_2	K	Leaves	Stems	Roots	Fruits	Leaves	Stems	Roots	Fruits
360	100	56.2	61.4	15.4	111.3	23.2	25.4	6.3	45.1
	40	57.2	54.7	16.5	102.7	24.8	23.8	7.1	44.3
	20	51.0	49.1	13.5	98.9	24.0	23.5	6.3	46.2
	5	55.9	45.1	14.8	86.6	27.6	22.3	7.4	42.7
	0	49.5	35.2	11.7	48.3	34.2	24.3	8.1	33.4
720	100	72.4	73.7	24.7	161.9	21.9	22.3	7.4	48.8
	40	72.9	65.5	25.0	148.7	23.3	21.1	8.1	47.4
	20	69.3	66.4	21.5	124.4	24.7	23.7	7.6	43.9
	5	64.2	49.4	18.3	94.0	28.4	21.9	8.1	41.5
	0	54.8	32.9	11.2	45.5	37.9	22.9	7.7	31.5
CO_2		***	***	***	***	NS	***	**	NS
K		***	***	***	***	***	*	*	***
$CO_2 \times K$		NS	***	***	***	***	*	NS	**

NS, not significant ($P > 0.05$).
[a] Ten treatments included two levels of CO_2 concentration (360 and 720 μL L^{-1}) and five levels of K supply (100 (control), 40, 20, 5, and 0% K). Details of the treatments are described in the text. The CO_2 and potassium treatments were initiated from 0 and 23 DAE, respectively through final harvest (85 DAE).
* Indicate significant at $P < 0.05$ proprtbility level.
** Indicate significant at $P < 0.01$ proprtbility level.
*** Indicate significant at $P < 0.001$proprtbility level.

content than the control, respectively. Leaf chlorophyll data were pooled over all the treatments and dates of measurement and plotted against leaf K concentrations to determine the relationships between leaf K and chlorophyll (Fig. 3B). We found that when leaf K was higher than 15 g kg^{-1} dry weight (DW), there was no relationship between the two variables. When leaf K was lower than this level, however, leaf chlorophyll content sharply declined with the lower leaf K concentrations (Fig. 3B).

Both [CO_2] and K treatments significantly impacted the photosynthetic capacity of cotton plants during fruit development (63–72 DAE) ($P < 0.001$) and their interactive effects ($P < 0.001$) on canopy Pn were also detected (Fig. 4). In general, canopy Pn was increased by elevated [CO_2], while K deficiency decreased canopy Pn. The stimulation of canopy Pn due to elevated [CO_2] decreased as K deficiency became more severe. When canopy Pn was expressed on a ground area basis (Fig. 4A), plants grown under elevated [CO_2] with the full K (control), 40 and 20% K supply had a 65, 62, and 50% higher canopy Pn, respectively, compared to plants grown under ambient [CO_2]. Canopy Pn did not differ between the two CO_2 treatments under the two severe K deficiency (i.e. the 5 and 0% K) treatments. Under ambient [CO_2] conditions, canopy Pn of plants treated with the 5 and 0% K were 47 and 65%, respectively, lower than the control plants of full K supply; while under elevated [CO_2], the Pn of plants in these two K-deficient treatments were 67 and 77%, respectively, lower than those of the control plants (Fig. 4A). Therefore, elevated atmospheric [CO_2] increased the sensitivity of cotton plants to K deficiency.

The increase in ground-area based canopy Pn due to elevated [CO_2] was associated with both a larger leaf area (Fig. 4B) and a higher mean leaf Pn (Fig. 4C). The two variables almost had equivalent contributions to canopy Pn. Decreased canopy Pn (a ground area basis) due to K deficiency was mainly associated with a lower mean leaf Pn. The effect of the K deficiency on leaf area was less than the effects on mean leaf area-based Pn (Fig. 4B and C).

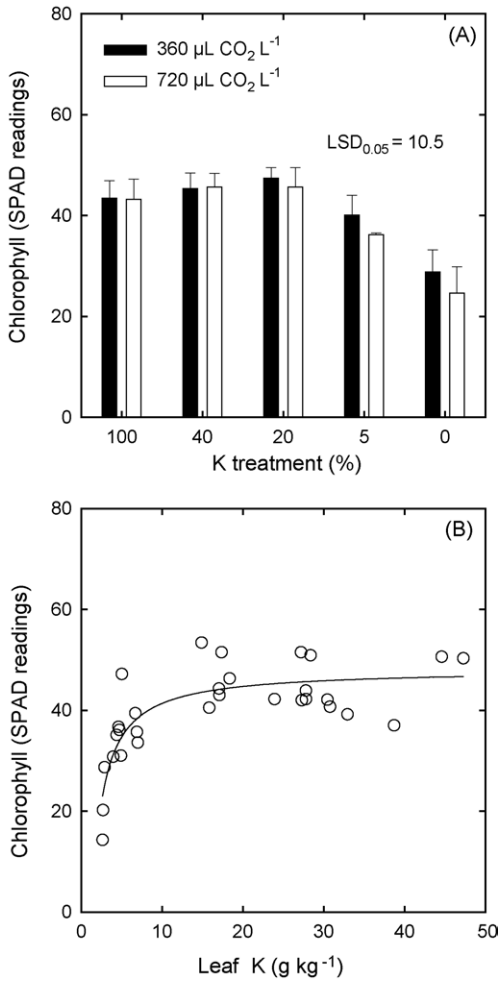

Fig. 3. (A) Chlorophyll content of uppermost, fully expanded main-stem cotton leaves as affected by elevated CO_2 and K deficiency and (B) relationships between cotton leaf chlorophyll and leaf K concentration. Data are mean ± S.E. of three measuring dates (42, 51, 74 DAE) with nine leaves each date.

3.6. Relationships between leaf K and plant growth and photosynthesis

The relative increment rates of plant main-stem elongation, leaf area expansion, biomass accumulation, and canopy photosynthesis could be best fit with leaf K concentrations ($r^2 = 0.71$–0.92^{***}, Fig. 5) using an exponential equation of $Y = a(1 - e^{-bX})$, where Y is relative increment rate of these growth or physiological variables, X is leaf K contents (g kg^{-1} DW), and a and b are constants. When leaf K was higher than

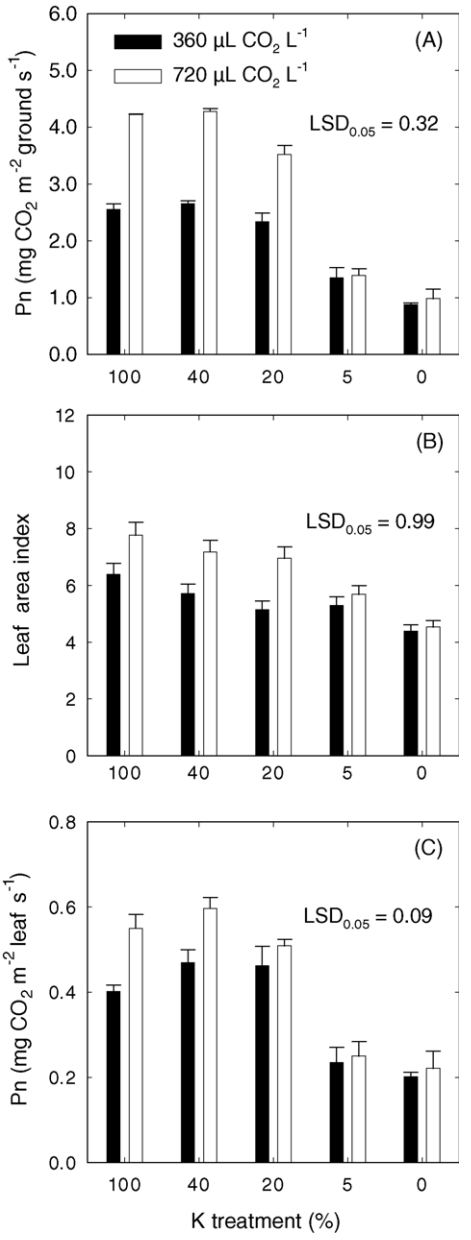

Fig. 4. Effects of elevated CO_2 and K supply on cotton (A) ground-based canopy photosynthesis (Pn) (B) leaf area index and (C) leaf area-based canopy photosynthesis during fruit development (50–59 DAE). Data are mean ± S.E. of 10-day measurements.

20 g kg^{-1} DW, changes in leaf K concentration did not affect any of these growth parameters. When leaf K was lower than 15 g kg^{-1} DW, however, plant growth and photosynthesis were impaired significantly

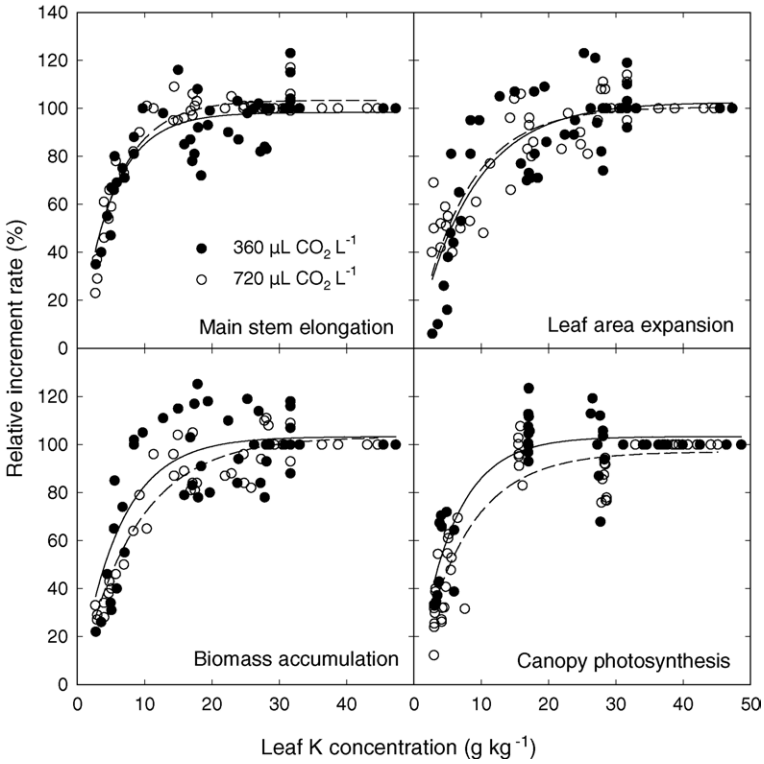

Fig. 5. Relationships between main-stem elongation, leaf area expansion, total biomass accumulation or canopy photosynthesis and leaf K concentration for cotton plants grown under 360 and 720 μL CO_2 L^{-1}. The solid and dash lines represent the exponential regression lines of the two CO_2 levels, respectively.

(Fig. 5). This estimation was based on the equations in Fig. 5 and varied with the growth variables measured and the CO_2 treatments. The leaf critical K content was 12 g kg^{-1} DW for stem elongation and 17 g kg^{-1} DW for leaf area expansion for both ambient and elevated CO_2 treatments. The leaf critical K levels for canopy photosynthesis and biomass production were 12–13 g kg^{-1} DW for plants grown under ambient [CO_2] and 18–19 g kg^{-1} DW for plants grown under the elevated [CO_2] (Fig. 5).

4. Discussion

The results of elevated [CO_2] stimulating growth and biomass accumulation of cotton plants grown under adequate K nutrition in the present study are in agreement with earlier reports (Reddy et al., 2000; Zhao et al., 2003). The enhancement of plant growth by elevated [CO_2] was closely related to both a larger leaf area and a higher leaf photosynthetic capacity (Fig. 4). Numerous studies have shown that elevated [CO_2] increases Pn in C_3 plants because higher [CO_2] can suppress RuBP oxygenase activity; decrease photorespiration; and increase carbon assimilates for plant growth and development (Lawlor and Mitchell, 2000). Our results clearly indicate that K deficiency depressed the stimulations of cotton plant growth and physiology due to elevated [CO_2]. Therefore, rapid growth and high productivity of field crops require more mineral nutrient supply, particularly K, in a future elevated CO_2 environment. Although strong evidence indicated that plants grown under elevated [CO_2] have significantly lower leaf N concentration in many crop species (Rogers et al., 1993; Sitt et al., 1999; Fangmeier et al., 2002; Reddy et al., 2004), reports of leaf K level responses to elevated [CO_2] are inconsistent. Fangmeier et al. (2002) documented that elevated [CO_2] significantly reduced K concentration in both aboveground biomass

and tubers of potato crops. In contrast, elevated [CO_2] did not affect leaf K in either sorghum or soybean plants (Reeves et al., 1994). The results of comparable leaf K concentration in the two CO_2 treated cotton plants in the present study are in agreement with Reeves et al. (1994). Therefore, faster growth for plants grown under elevated [CO_2] without the reduction in plant tissue K concentration may require additional K supply compared with cotton plants grown under current [CO_2].

Cotton leaf K concentration was closely associated with both K supply and plant growth stage (Fig. 1). Potassium deficiency significantly decreased leaf area expansion (Table 1) and canopy photosynthesis (Fig. 4), resulting in a lower biomass production (Table 1). These results are similar to reports by Bednarz et al. (1998) and Zhao et al. (2001). Additionally, K-deficient plants under both [CO_2] treatments had significantly greater SLW (Table 1). Increased SLW due to K deficiency was probably attributed to less intercellular space and higher leaf non-structural carbohydrate concentrations (Zhao et al., 2001) because photo-assimilate translocation in plant tissues was inhibited under K deficiency. Whether this was due to some aspects of the photo-assimilate translocation process or due to fewer fruit was not determined in the present study. In addition, our results indicated that K deficiency in cotton not only depressed total biomass accumulation, but also changed biomass partitioning among plant parts with the greatest reduction in fruit dry weight (Table 2) due to higher fruit abscission, compared with the K sufficient plants.

The interactive effects of elevated [CO_2] and K deficiency on growth and physiology of cotton plants depended on the specific variables measured. The interactions were not significant for leaf K concentration, main-stem elongation, and leaf chlorophyll content or leaf area development. However, significant interactive effects were detected on production and partitioning of biomass and canopy photosynthesis. Under severe K deficiency, the enhancements of these parameters by elevated [CO_2] were diminished (Tables 1 and 2; Fig. 4).

Cotton plants require large amounts of K for optimum growth and yield (Kerby and Adams, 1985; Oosterhuis, 1994). Better understanding of K requirement of cotton and monitoring of plant K status during crop growth are essential for K fertilizer recommendations and management. Potassium concentration in uppermost, fully expanded main-stem leaves is an important indicator of plant K status. Reuter (1986) suggested that adequate leaf K concentration for cotton growth and development was >32 g kg^{-1} DW during squaring. In order to improve cotton yield and to increase K fertilizer use efficiency, crop scientists have long been trying to find a realistic leaf critical K level in cotton for K diagnosis and for K fertilizer recommendation. Reports of the leaf critical K concentrations impacting plant growth, physiology and yield have been inconsistent. Singh et al. (1992) suggested that the critical K concentration in the topmost, fully expanded leaves affecting cotton yield was 8.5 g kg^{-1} DW at peak flowering. However, Pettiet (1994) found that the leaf critical K level at the same growth stage for cotton yield reduction was almost doubled (15 g kg^{-1} DW). Under an indoor growth chamber condition, Bednarz and Oosterhuis (1996) documented a leaf critical K level of 6.7–9.5 g kg^{-1} DW during squaring in influencing plant growth and physiology. More recently, Gopal et al. (2001) pointed out that the threshold values of leaf K deficiency and toxicity in cotton were 20 and 49 g kg^{-1} DW, respectively. Our results clearly indicted that the leaf critical K level depend upon the variables of plant growth and physiological parameters measured. Under ambient [CO_2], the leaf critical K levels during squaring and fruiting were 12 g kg^{-1} DW for canopy photosynthesis, main-stem elongation and total biomass accumulation (Fig. 5) and 17 g kg^{-1} DW for leaf area expansion (i.e. leaf area was more sensitive to K deficiency than the other parameters measured except for photosynthesis). The leaf critical K concentrations established in the present study are higher than that reported by Bednarz and Oosterhuis (1996), but lower than that of Gopal et al. (2001). When plants were grown under elevated CO_2 conditions, the leaf critical K levels increased to 18–19 g kg^{-1} from 12 g kg^{-1} DW compared to plants grown under ambient [CO_2] for both canopy Pn and biomass production. Therefore, elevated atmospheric [CO_2] increased not only the amount of K nutrient required but also the sensitivity of response to leaf K concentration.

In conclusion, elevated atmospheric [CO_2] stimulated cotton plant growth and biomass production

through increases in both leaf area and leaf net photosynthesis, but did not affect either leaf chlorophyll or K concentration. The primary effect of limited K on cotton plants was reduced canopy photosynthesis and leaf area development. As a consequence of lower Pn and reduced leaf growth, there were decreases in biomass production and shifts in biomass partitioning among plant organs. Plants grown under elevated CO_2 conditions required greater amounts of K and the plants were more sensitive to K deficiency compared to plants grown under ambient [CO_2]. Elevated CO_2 and K supply had significant interactive effects on canopy photosynthesis and biomass production and partitioning. The reduction in fruit biomass due to K deficiency was more than any other plant parts. Plants grown under elevated [CO_2] required a higher leaf critical K concentration to maintain optimum canopy photosynthesis and biomass production. Therefore, elevated [CO_2] did not mitigate the negative effects of K deficiency in cotton, but increased the sensitivity of plants to K deficiency.

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