Water stress and day-to-day variation in apparent photosynthetic acclimation of field-grown soybeans to elevated carbon dioxide concentration

J.A. BUNCE and R.C. SICHER

Climate Stress Laboratory, USDA-ARS, Beltsville Agricultural Research Center,
10300 Baltimore Ave., Beltsville, MD 20705-2350, U.S.A.

Abstract

Midday measurements of single leaf gas exchange rates of upper canopy leaves of soybeans grown in the field at 350 (AC) and 700 (EC) $\mu mol(CO_2)$ mol^{-1} in open topped chambers sometimes indicated up to 50 % higher net photosynthetic rates (P_N) measured at EC in plants grown at AC compared to EC. On other days mean P_N were nearly identical in the two growth [CO_2] treatments. There was no seasonal pattern to the variable photosynthetic responses of soybean to growth [CO_2]. Even on days with significantly lower P_N in the plants grown at EC, there was no reduction in ribulose-1,5-bisphosphate carboxylase/oxygenase, chlorophyll, or soluble protein contents per unit of leaf area. Over three years, gas exchange evidence of acclimation occurred on days when either soil was dry or the water vapor pressure deficit was high (n = 12 d) and did not occur on days after rain or on days with low water vapor pressure deficit (n = 9 d). On days when photosynthetic acclimation was evident, midday leaf water potentials were consistently 0.2 to 0.3 MPa lower for the plants grown at EC than at AC. This suggested that greater susceptibility to water stress in plants grown at EC caused the apparent photosynthetic acclimation. In other experiments, plants were grown in well-watered pots in field chambers and removed to the laboratory early in the morning for gas exchange measurements. In these experiments, the amount of photosynthetic acclimation evident in the gas exchange measurements increased with the maximum water vapor pressure deficit on the day prior to the measurements, indicating a lag in the recovery of photosynthesis from water stress. The apparent increase in susceptibility to water stress in soybean plants grown at EC is opposite to that observed in some other species, where photosynthetic acclimation was evident under wet but not dry conditions, and may be related to the observation that hydraulic conductance is reduced in soybeans when grown at EC. The day-to-day variation in photosynthetic acclimation observed here may account for some of the conflicting results in the literature concerning the existence of acclimation to EC in field-grown plants.

Additional key words: chlorophyll; *Glycine max*; leaf protein; ribulose-1,5-bisphosphate carboxylase/oxygenase; saccharides; starch.

Introduction

Acclimation of photosynthesis to EC, such that photosynthetic rates measured at AC are lower in plants grown at EC compared to AC has long been recognized (*e.g.*, Hofstra and Hesketh 1975). The decreased photosynthetic capacity of EC plants can limit the increase in plant growth caused by EC (*e.g.*, Bunce 1997). However, CO_2 enrichment studies commonly have been performed with plants grown in pots and photosynthetic acclimation was considered unlikely to occur in herbaceous crop plants grown in well fertilized soils in the field (Long and Drake 1992). The interpretation of growth [CO_2] effects on photosynthesis can be compli-

cated by the fact that plants grown in pots can have restricted root growth (Arp 1991, Thomas and Strain 1991). Restricted rooting volume can lead to sink-limited or feedback inhibited photosynthesis due to inadequate rates of utilization of photosynthate. Acclimation of photosynthesis to CO_2 enrichment has frequently been reported in soybeans grown in controlled environment chambers and glasshouses (*e.g.*, Clough *et al.* 1981, Bunce 1992, Xu *et al.* 1994, Sicher *et al.* 1995) and even in "large pot" experiments (Reid *et al.* 1998, Sims *et al.* 1998, Ziska *et al.* 1998). Although most field studies with soybean have not specifically examined photosynthetic

acclimation, evidence of photosynthetic acclimation to growth EC in field experiments with soybean is contradictory. Two field studies that specifically tested for photosynthetic acclimation in soybean found no evidence of it (Jones et al. 1985, Chen et al. 1995). On the other hand, Sionit et al. (1984) observed no increase in P_N when measured under the growth $[CO_2]$ conditions in soil-grown soybean over the range of 330 to over 600 μmol mol^{-1}. However, P_N did increase in plants grown outdoors in pots in the same study. This suggests that photosynthetic acclimation to EC occurred in the soil-grown plants. Havelka et al. (1984) observed increased P_N in field-grown soybean under EC growth conditions on many measurement dates, but on several other measurement dates no differences occurred. It was unclear whether differing photosynthetic responses to EC in soybean were related to cultivar variability or to growth environment, or both. Clearly, field-grown plants encounter a number of physical and biotic stresses not typically associated with plants raised in more closely regulated environments.

Photosynthetic acclimation to EC has been associated with a number of alterations in physiological and biochemical plant growth factors (Stitt and Krapp 1999). In addition to reduced photosynthetic capacity mentioned above, CO_2 enrichment decreased foliar N content and this typically was observed in older rather than younger leaves (Conroy and Hocking 1993, Van Oosten and Besford 1995). Decreased concentrations of total soluble proteins, pigments, and associated pigment proteins, RuBPCO and RuBPCO activase and other chloroplast proteins per unit of leaf area have been frequently observed in EC studies (Stitt and Krapp 1999). Evidence that biochemical components of soybean leaflets are modified by growth EC is also controversial. Some authors (Campbell et al. 1988, Sicher et al. 1995) have reported that protein and RuBPCO were little affected by CO_2 enrichment, whereas other investigators (Xu et al. 1994, Sims et al. 1998) observed large responses of N-containing constituents during growth in EC. Moreover, accelerated growth rates in EC may induce nutrient imbalances, particularly with respect to N assimilation and utilization (Conroy and Hocking 1993, Stitt and Krapp 1999). Soybean is an N-fixing legume and photosynthate supply is a major factor limiting N-fixation rates (Hardy and Havelka 1975). Therefore, soybean may have different C/N interactions under CO_2 enrichment than other species.

The objective of the present study was to determine the effects of EC on photosynthesis and related gas-exchange properties of soybean grown in open top field chambers. We also correlated the effects of $[CO_2]$ enrichment on specific N-containing leaf constituents with the extent of photosynthetic acclimation. We present evidence that photosynthetic acclimation to twice AC in field-grown soybeans varied from day to day, and that this was related to specific environmental conditions rather than changes in biochemical leaf components.

Materials and methods

In one set of observations, soybean (Glycine max L. Merr. cv. Kent) was planted in the ground in six open topped chambers (2 m^2) in the field in Beltsville, Maryland, USA in early June of 1995, 1998, and 1999. The 1998 and 1999 summers were unusually dry for Beltsville. The soybean plots were grown in rotation with winter cereals that were fertilized with N, P, and K in early spring (Sicher and Bunce 1997). Soybeans were planted in rows 30 cm apart, and seedlings were thinned to a stand density of 25 plants m^{-2}. Outside air was circulated through all chambers at a constant flow rate, and mixing fans above the crop produced air speeds of about 1 m s^{-1}. The average ambient daytime $[CO_2]$ was 350 μmol mol^{-1}. Half of the chambers were supplemented with CO_2 to increase the $[CO_2]$ by 350±50 μmol mol^{-1} above the concentration of the outside air. The plants received only natural rainfall. Air temperatures inside the chambers averaged 1 °C higher than outside, and the chambers transmitted about 90 % of the incident photosynthetically active radiation. A weather station at the site recorded standard meteorological variables.

Leaf gas exchange rates of fully exposed, recently expanded upper canopy leaves of interior plants were measured near midday every few weeks during mid season. Steady state gas exchange rates of leaves from both $[CO_2]$ treatments were measured within 1 h of solar noon in full sunlight at a $[CO_2]$ of 700 μmol mol^{-1}, using a CIRAS-1 portable gas exchange system (PP Systems, Haverhill, MA, USA) with $[CO_2]$ control and a broadleaf chamber. On each measurement date, gas exchange rates were measured on leaves of two plants from each of the three chambers per $[CO_2]$ treatment. Differences in P_N between plants grown at AC and EC under the uniform EC condition were taken as evidence of photosynthetic acclimation to EC. Water potentials of leaf discs excised from three leaves in which gas exchange was measured per $[CO_2]$ treatment were determined each day. Excised discs were sealed and within 20 s of excision were placed into insulated C52 sample chambers (Wescor, Logan, Utah, USA) within an air conditioned shed at the field site. Water potentials were determined after equilibration periods of up to 2 h, using an HR-33 Dew Point

Hygrometer (*Wescor*, Logan, Utah, USA).

Three pairs of leaf discs (3.5 cm^2 each) from the same trifoliolates used for gas-exchange were snap frozen and immediately transferred to aluminum bags in liquid N_2 in the field. Samples were either analyzed immediately or were stored at –80 °C until use. One pair of leaf discs was extracted with solvent containing methanol : chloroform : water (5 : 3 : 1) and total chlorophyll (Chl) was quantified in 80 % acetone after partitioning the organic and aqueous-alcohol phases (Lichtenthaler 1987). Leaf starch was obtained from the pellet fraction, hydrolyzed with a crude α-amylase preparation (*Mylase 100, GB Fermentation Industries*, Charlotte, NC, USA), and quantified according to Hendrix (1993). Sucrose, glucose, and fructose were recovered in the aqueous fraction and were quantified in coupled enzyme assays according to Bergmeyer *et al.* (1974).

A separate pair of leaf discs was used to measure initial and total RuBPCO activity in crude extracts (Sicher and Bunce 1999). Assays were performed in stoppered vials at 25 °C before and after activating the enzyme with CO_2 and Mg^{2+} (Sicher and Bunce 1999). Soluble protein was determined on these enzyme extracts using a dye-binding procedure according to Bradford (1976). Additional protein extracts were separated by SDS-PAGE on 12 % acrylamide mini-gels and protein bands were stained with Coomassie brilliant blue R. The large sub-unit of RuBPCO was excised and RuBPCO protein was quantified spectrophotometrically after extracting the dye at 50 °C with formamide according to Makino *et al.* (1986)

In a second set of observations, plants were grown in the field, but with plants in 2 000 cm^3 pots filled with vermiculite and flushed once or twice a day with a complete nutrient solution. The enclosures were semi-open topped, with transparent acrylic lids held 1 cm above the side walls. Because of the lids, midday air temperatures in full sunlight averaged 5 °C above outside air in these chambers. Successive weekly plantings were made each of two years. There were two chambers with AC and two at 350±10 μmol mol^{-1} above ambient. Gas exchange measurements were made on third trifoliolate leaves a few days after full expansion. Early in the morning before the gas exchange measurements, plants were moved to a controlled environment chamber in the laboratory set at 23 °C and a dew point temperature of 18 °C, with 1 000 μmol m^{-2} s^{-1} photosynthetic photon flux density, and 700 μmol(CO_2) mol^{-1}. After about 2 h under these conditions, P_N was measured at 25 °C and 2 000 μmol m^{-2} s^{-1} photosynthetic photon flux density, at 700 μmol(CO_2) mol^{-1}. Samples for measurement of soluble protein content were taken from the same leaflets immediately after the gas exchange measurements as described above. Leaf gas exchange and protein content measurements were made on three plants from each chamber.

Results and discussion

Seasonal patterns of P_N measured at 700 μmol mol^{-1} for 1998 and 1999 (Fig. 1*A,B*) indicated that the effects of growth [CO_2] on P_N of soybean leaflets varied with measurement date. On some sampling dates there were no differences between AC and EC plants, while on other dates the plants grown at EC had significantly lower P_N than the AC controls. A significant treatment by measurement date interaction was detected for each year using analysis of variance (not shown). The dates when there were no treatment differences were mostly those that occurred shortly after rainfall events (see *arrows* in Fig. 1) and photosynthetic acclimation correlated with lack of rain rather than time of season. The day-to-day variation in photosynthetic acclimation observed here was in many ways similar to the results of Havelka *et al.* (1984). They noted larger variation among days in P_N of EC than AC soybeans, with a large net stimulation in rates measured at the growth [CO_2] on many days, but little or no stimulation on several other days scattered throughout the season. They did not report weather data nor explore correlation with meteorological variables.

Consistent with many earlier observations of increased saccharide content in plants grown at EC (*e.g.*, Sicher *et al.* 1995), mean leaflet starch and sucrose concentrations for samples collected in 1998 and 1999 were 86 and 67 % greater. respectively, in EC plots compared to AC ones when expressed per unit of leaf area (Table 1). There were no [CO_2] treatment effects on leaflet glucose and fructose per unit of leaf area in this study. The N-containing constituents, Chl, soluble protein, and RuBPCO protein, also did not differ between the AC and EC treatments. Initial and total RuBPCO activities displayed a significant [CO_2] treatment effect only on the final measurement date in 1999 (Fig. 2). RuBPCO activation state, expressed as the ratio of initial to total activity, averaged 81 and 79 %, in the AC and EC samples, respectively, and did not differ with [CO_2] treatment on any date. The above results, showing that leaflet constituents were little affected by CO_2 enrichment, were broadly in agreement with reports by Campbell *et al.* (1988) and Sicher *et al.* (1995). Reid *et al.* (1998) also reported that soybean leaflet RuBPCO and Chl contents were unaffected by EC. However, these authors did observe a decrease of initial RuBPCO activity and percent activation in response to CO_2 enrichment, most notably during seed maturation. Overall, the above evidence suggested that the day-to-day variation in apparent photosynthetic acclimation in soybean was not

the result of CO_2-dependent changes in leaflet N-constituents.

Further analysis was carried out separately for "wet" and "dry" sampling dates, defined as dates without and with significant differences between [CO_2] treatments in
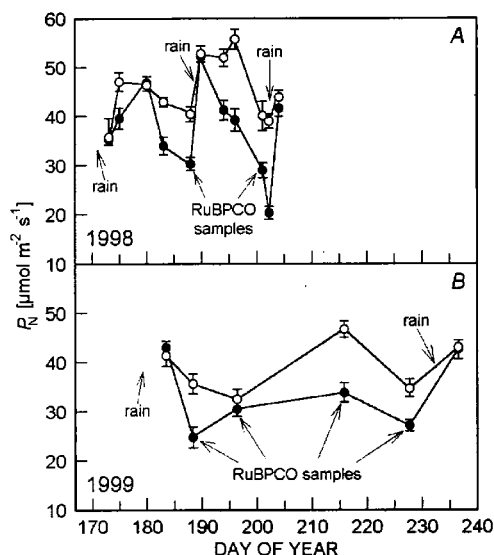

Fig. 1. Seasonal patterns of midday net photosynthetic rates measured at 700 μmol(CO_2) mol^{-1} for upper canopy leaves of soybean grown in field plots at ambient (o) and +350 μmol(CO_2) mol^{-1} (●) for 1998 (A) and 1999 (B). Each point represents a mean value for 6 leaves from 3 chambers per [CO_2] treatment, and bars represent SE for n =3. Precipitation events and dates when leaf samples for measurement of RuBPCO were taken are indicated.
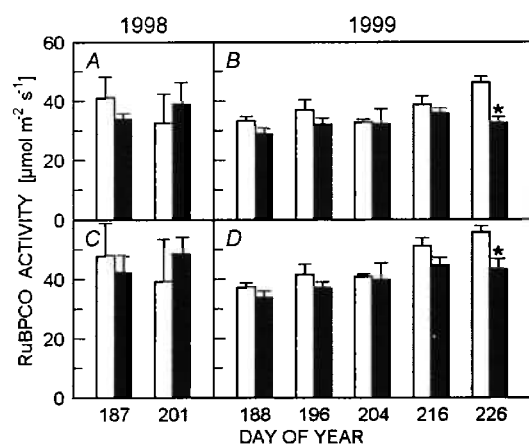

Fig. 2. Initial and total activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) from upper canopy leaves of soybean grown in field plots at ambient and + 350 μmol(CO_2) mol^{-1} for sampling dates in 1998 (A, C) and 1999 (B, D). Open bars represent plants grown at ambient [CO_2], and filled bars represent plants grown at elevated [CO_2]. Error bars represent SE, and * indicates a significant growth [CO_2] effect at p = 0.05.

P_N, respectively. On "wet" sampling dates combined over all years, there were no differences between [CO_2] treatments in leaf water potentials or in P_N (Fig. 3), whereas on "dry" sampling dates, when P_N was lower in the plants grown at EC, leaf water potentials were lower than on "wet" dates and also were lower in EC than AC plants. In this soybean cultivar, EC increased leaf area relatively much less than it reduced stomata conductance (Wilson et al. 1999), so it is unlikely that soils dried more rapidly at EC in these well-ventilated chambers. Similar differences in stomata conductance occurred between [CO_2] treatments on both "wet" and "dry" sampling dates (Fig. 3), with overall lower conductances on the "dry" dates com-
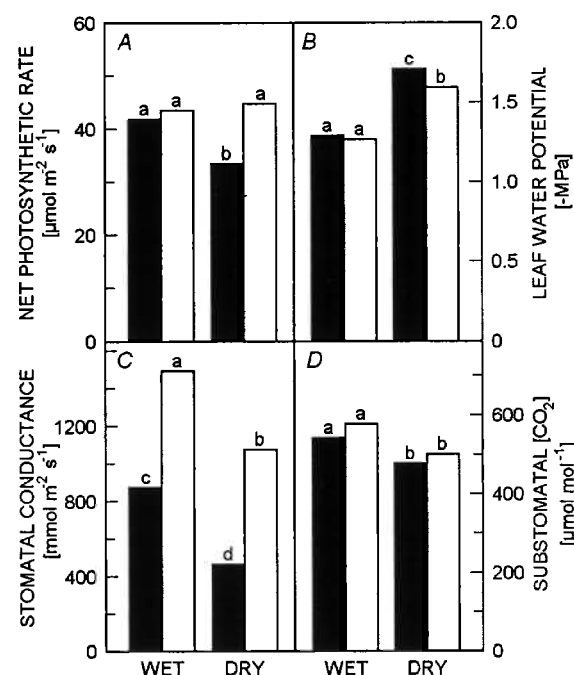

Fig. 3. Midday net photosynthetic rate (P_N) measured at 700 μmol(CO_2) mol^{-1}, leaf water potential, midday stomata conductances, and substomatal carbon dioxide concentrations for upper canopy leaves of soybean grown in field plots at ambient and + 350 μmol(CO_2) mol^{-1}on 9 "wet" and 12 "dry" sampling dates over three years. Values with different letters were significantly different at p = 0.05. "Wet" and "dry" days are defined in the text. The mean measurement temperature for the "wet" dates was 29 and 33 °C for the "dry" dates.

pared to "wet" dates. Substomatal [CO_2] was only slightly less for the EC treatment (Fig. 3), and these values also were lower on "dry" dates compared to "wet" dates. Total RuBPCO activities averaged over all years were 45.7 and 44.0 μmol m^{-2} s^{-1} over the five "wet" dates and 44.3 and 43.7 μmol m^{-2} s^{-1} over the five "dry" dates for the AC and EC grown plants, respectively. None of these means differed at p = 0.05. Therefore, our results are similar to those of Havelka et al. (1984) in indicating that gas exchange evidence of photosynthetic acclimation was

variable and may occur in field-grown soybean without any reduction in soluble protein or Chl per unit of leaf area.

Table 1. Comparison of response of soybean leaflets to ambient (350 $\mu mol \ mol^{-1}$) and elevated (ambient+350 $\mu mol \ mol^{-1}$) [CO_2]. Samples were collected from recently expanded upper canopy leaflets of plants grown in open top field chambers on two dates in 1998 and on five dates in 1999. The [CO_2] by date interaction for these leaf components was non significant at $p = 0.05$.

Leaf component	Harvests	CO_2 [$\mu mol \ mol^{-1}$]	
		350	700
Chlorophyll [g m^{-2}]	7	0.40±0.01	0.40±0.01
Starch [mmol m^{-2}]	7	29.0±2.3	53.9±3.5
Sucrose [mmol m^{-2}]	7	2.1±0.2	3.5±0.4
Glucose [mmol m^{-2}]	7	1.5±0.2	2.0±0.3
Fructose [mmol m^{-2}]	5	1.1±0.2	1.2±0.2
Soluble protein [g m^{-2}]	5	6.8±0.2	6.9±0.2
RuBPCO protein [g m^{-2}]	7	1.9±0.1	1.9±0.1

For plants grown in pots, where the rooting medium was never dry, evidence of photosynthetic acclimation to EC also varied from day to day. This effect was correlated with the water vapor pressure deficit to which the plants were exposed on the day prior to the photosynthetic measurements (Fig. 4). Day-to-day variation in water vapor pressure deficit resulted from differences in air temperature and air water content. These results suggest that high water vapor pressure deficits on the previous day had a carry-over effect on photosynthesis. Presumably, high water vapor pressure deficits resulted in low leaf water potentials, but leaf water potentials on the day prior to the P_N measurements were not determined in this experiment. In soybean, leaf gas exchange can be affected by low leaf water potentials without soil drying (Bunce 1999). Slow recovery of photosynthesis from water stress is common (e.g., Boyer 1971, Bierolai and Hopmans 1975). In our experiment, as in the soil-grown plants, leaves developed at EC had total soluble protein contents which were not significantly lower than those of AC plants on any measurement date (values not shown).

For the plants grown in soil, high water vapor pressure deficits also reduced P_N in the EC treatment more than the AC treatment, even when soil was wet (Fig. 4). This difference between [CO_2] treatments in the response of photosynthesis to water vapor pressure deficit for the plants grown in soil explains why, early in each season, differences between [CO_2] treatments occurred, then disappeared without rainfall (Fig. 1). The data points producing this pattern in Fig. 1 have been plotted in Fig. 4, and are consistent with the values for the plants grown in pots. For both sets of plants, the water vapor pressure

deficit shown in Fig. 4 is the maximum of the day prior to the gas exchange measurements. For plants grown in pots, the vapor pressure deficit during the gas exchange measurements was kept low, since the plants in pots were moved to the laboratory for measurement, whereas the plants in the soil were measured *in situ*. However, the field measurements were made near midday and peak water vapor deficits occurred late in the afternoon, so that the water vapor pressure deficits occurring during the measurements of plants in the field were considerably lower than the peak values of the prior day.

The pattern observed here for soybean, of apparent photosynthetic acclimation under dry conditions but not under wet conditions, is opposite from that observed in some other species. For example, Huxman *et al.* (1998) found evidence of photosynthetic acclimation in *Larrea divaricata* under wet conditions, but not under dry conditions. This was attributed to EC reducing transpiration rate by lowering stomata conductance, and reducing plant water stress under the dry conditions. We have observed a similar pattern in field-grown potato (Sicher
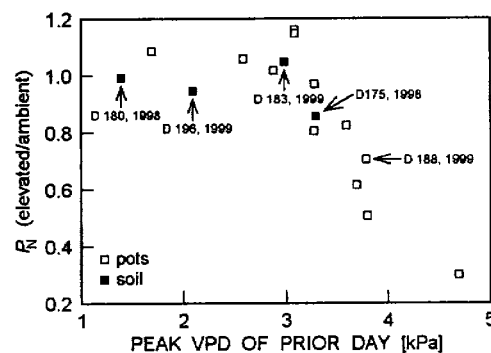

Fig. 4. The ratio of net photosynthetic rate (P_N) measured at 700 $\mu mol(CO_2) \ mol^{-1}$ for leaves of soybean plants grown at elevated [CO_2] relative to rates of plants grown at ambient [CO_2] as a function of the peak air water vapor pressure deficit on the day prior to measurement of P_N. Values are for plants grown in pots in semi-open chambers in the field and watered daily with nutrient solution, or for plants rooted in soil in open top chambers. The day of year and the year are indicated for plants rooted in soil, for reference to Fig. 1. Each point represents the ratio of means of 6 leaves per [CO_2] treatment.

and Bunce 1999) and in strawberry (Bunce, unpublished). This is the pattern expected when the EC treatment improves plant water status. However, in soybean the EC treatment did not improve plant water status, despite lower stomata conductance. Midday leaf water potentials were not higher for plants grown at EC even in wet conditions, and were consistently lower under dry conditions. Other studies with soybean have indicated that growth at EC results in lower hydraulic conductance expressed per unit of leaf area (Bunce 1996, Bunce and Ziska 1998), which is consistent with the values presented here. Estimating hydraulic conductance from water poten-

tial gradients and transpiration rates in the field is difficult because of fluctuating environmental conditions, and was not attempted here. In a survey of the literature, Bunce and Ziska (1998) found that it was common for EC to reduce transpiration rates without an increase in leaf water potential. The cause of lower hydraulic conductance in EC plants is unknown, and it is also unclear if lower hydraulic conductance is the cause of the apparent greater susceptibility to water stress in soybeans grown at

EC in this study.

The day-to-day variation in evidence of acclimation observed here may explain some of the conflicting reports concerning the existence of photosynthetic acclimation to EC in field-grown soybean, as the same leaf material may or may not show evidence of acclimation depending on the weather on the day prior to the measurement of gas exchange.

References

Arp, W.J.: Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. - Plant Cell Environ. **14**: 869-875, 1991.

Bergmeyer, H.U., Brent, E., Schmidt, F., Stock, H.: D-Glucose. Determination with hexokinase and glucose 6-phosphate dehydrogenase. - In: Bergmeyer, H.U. (ed.): Methods of Enzymatic Analysis. Vol. 3. 2nd Ed. Pp. 1196-1198. Academic Press, New York 1974.

Bielorai, H., Hopmans, P.A.M.: Recovery of leaf water potential, transpiration, and photosynthesis of cotton during irrigation cycles. - Agron. J. **67**: 629-632, 1975.

Boyer, J.S.: Recovery of photosynthesis in sunflower after a period of low water potential. - Plant Physiol. **47**: 816-820, 1971.

Bradford, M.M.: A rapid sensitive method for the quantitation of microgram quantities of protein using the principle of dye-binding. - Anal. Biochem. **72**: 248-254, 1976.

Bunce, J.A.: Light, temperature and nutrients as factors in photosynthetic adjustment to an elevated concentration of carbon dioxide. - Physiol. Plant. **86**: 173-179, 1992.

Bunce, J.A.: Growth at elevated carbon dioxide concentration reduces hydraulic conductance in alfalfa and soybean. - Global Change Biol. **2**: 155-158, 1996.

Bunce, J.A.: Variation in growth stimulation by elevated carbon dioxide in seedlings of some C_3 crop and weed species. - Global Change Biol. **3**: 61-66, 1997.

Bunce, J.A.: Leaf and root control of stomatal closure during drying in soybean. - Physiol. Plant. **106**: 190-195, 1999.

Bunce, J.A., Ziska, L.H.: Decreased hydraulic conductance in plants at elevated carbon dioxide. - Plant Cell Environ. **21**: 121-126, 1998.

Campbell, W.J., Allen, L.H., Jr., Bowes, G.: Effects of CO_2 concentration on Rubisco activity, amount and photosynthesis in soybean leaves. - Plant Physiol. **88**: 1310-1316, 1988.

Chen, X.-M., Alm, D.M., Hesketh, J.D.: Effects of atmospheric CO_2 concentration on photosynthetic performance of C_3 and C_4 plants. - Biotronics **24**: 65-72, 1995.

Clough, J.M., Peet, M.M., Kramer, P.J.: Effects of high atmospheric CO_2 and sink size on rates of photosynthesis of a soybean cultivar. - Plant Physiol. **67**: 1007-1010, 1981.

Conroy, J., Hocking, P.: Nitrogen nutrition of C_3 plants at elevated atmospheric CO_2 concentrations. - Physiol. Plant. **89**: 570-576, 1993.

Hardy, R.W.F., Havelka, U.D.: Photosynthate as a major factor limiting nitrogen fixation by field-grown legumes with emphasis on soybeans. - In: Nutman, P.S. (ed.): Symbiotic

Nitrogen Fixation in Plants. Pp. 421-439. Cambridge Univ. Press, Cambridge – London – New York – Melbourne 1975.

Havelka, U.D., Ackerson, R.C., Boyle, M.G., Wittenbach, V.A.: CO_2-enrichment effects on soybean physiology. I. Effects of long-term CO_2 exposure. - Crop Sci. **24**: 1146-1150, 1984.

Hendrix, D.L.: Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. - Crop Sci. **33**: 1306-1311, 1993.

Hofstra, G., Hesketh, J.D.: The effects of temperature and CO_2 enrichment on photosynthesis in soybean. - In: Marcelle, R. (ed.): Environmental and Biological Control of Photosynthesis. Pp. 71-80. Dr W. Junk Publ., The Hague 1975.

Huxman, T.E., Hamerlynck, E.P., Moore, B.D., Smith, S.D., Jordan, D.N., Zitzer, S.F., Nowak, R.S., Coleman, J.S., Seemann, J.R.: Photosynthetic down-regulation of *Larrea tridentata* exposed to elevated atmosopheric CO_2: interaction with drought under glasshouse and field (FACE) exposure. - Plant Cell Environ. **21**: 1153-1161, 1998.

Jones, P., Allen, L.H., Jr., Jones, J.W., Valle, R.: Photosynthesis and transpiration responses of soybean canopies to short- and long-term CO_2 treatments. - Agron. J. **77**: 119-126, 1985.

Lichtenthaler, H.K.: Chlorophylls and carotenoids - pigments of photosynthetic biomembranes. - In: Colowick, S.P., Kaplan, N.O.: Methods in Enzymology. Vol. **148**. Pp. 350-382. Academic Press, San Diego – New York – Berkeley – Boston – London – Sydney – Tokyo – Toronto 1987.

Long, S.P., Drake, B.G.: Photosynthetic CO_2 assimilation and rising atmospheric CO_2 concentrations. - In: Baker, N.R., Thomas, H. (ed.): Crop Photosynthesis: Spatial and Temporal Determinants. Pp. 69-103. Elsevier Science Publ., Amsterdam 1992.

Makino, A., Mae, T., Ohira, K.: Colorimetric measurement of proteins stained with Coomassie Brilliant Blue R on sodium dodecylsulfate polyacrylamide gel electrophoresis by eluting with formamide. - Agr. biol. Chem. (Tokyo) **50**: 1911-1912, 1986.

Reid, C.D., Fiscus, E.L., Burkey, K.O.: Combined effects of chronic ozone and elevated CO_2 on Rubisco activity and leaf components in soybean (*Glycine max*). - J. exp. Bot. **49**: 1999-2011, 1998.

Sicher, R.C., Bunce, J.A.: Relationship of photosynthetic acclimation to changes of Rubisco activity in field-grown winter wheat and barley during growth in elevated carbon dioxide. - Photosynth. Res. **52**: 27-38, 1997.

Sicher, R.C., Bunce, J.A.: Photosynthetic enhancement and conductance to water vapor of field-grown *Solanum*

tuberosum (L.) in response to CO_2 enrichment. – Photosynth. Res. **62**: 155-163, 1999.

Sicher, R.C., Kremer, D.F., Bunce, J.A.: Photosynthetic acclimation and photosynthate partitioning in soybean leaves in response to carbon dioxide enrichment. – Photosynth. Res. **46**: 409-417, 1995.

Sims, D.A., Luo, Y., Seemann, J.R.: Comparison of photosynthetic acclimation to elevated CO_2 and limited nitrogen supply in soybean. – Plant Cell Environ. **21**: 945-952, 1998.

Sionit, N., Rogers, H.H., Bingham, G.E., Strain, B.R.: Photosynthesis and stomatal conductance with CO_2-enrichment of container- and field-grown soybeans. – Agron. J. **76**: 447-451, 1984.

Stitt, M., Krapp, A.: The interaction between elevated carbon dioxide and nitrogen nutrition: the physiologiocal and molecular background. – Plant Cell Environ. **22**: 583-621, 1999.

Thomas, R.B., Strain, B.R.: Root restriction as a factor in photosynthetic acclimation of cotton seedlings grown in elevated carbon dioxide. – Plant Physiol. **96**: 627-634, 1991.

Van Oosten, J.J., Besford, R.T.: Some relationships between the gas exchange, biochemistry and molecular biology of photosynthesis during leaf development of tomato plants after transfer to different carbon dioxide concentrations. – Plant Cell Environ. **18**: 1253-1266, 1995.

Wilson, K.B., Carlson, T.N., Bunce, J.A.: Feedback significantly influences the simulated effect of CO_2 on seasonal evapotranspiration from two agricultural species. – Global Change Biol. **5**: 903-917, 1999.

Xu, D-Q., Gifford, R.M., Chow, W.S.: Photosynthesis acclimation in pea and soybean to high atmospheric CO_2 partial pressure. – Plant Physiol. **106**: 661-671, 1994.

Ziska, L.H., Bunce, J.A., Caulfield, F.: Intraspecific variation in seed yield of soybean (*Glycine max*) in response to increased atmospheric carbon dioxide. – Aust. J. Plant Physiol. **25**: 801-807, 1998.