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Testing the “source–sink” hypothesis of down-regulation of photosynthesis in elevated [CO_2] in the field with single gene substitutions in *Glycine max*

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Abstract

Acclimation of photosynthesis to elevated atmospheric carbon dioxide concentration was tested in lines of soybean (*Glycine max*) that differed by single genes that altered either the capacity to nodulate or growth habit (determinate or indeterminate growth). Both genetic changes provided, within a uniform genetic background, a test of the “source–sink” hypothesis that down-regulation of photosynthesis in elevated carbon dioxide is a result of inability to form sufficient “sinks” for the additional photosynthate. Plants were grown under ambient and elevated [CO_2] (550 μmol mol^{-1}) in the field, using free air gas concentration enrichment (FACE). Mutation of the determinate cultivar, Elf, to an indeterminate form did not result in increased responsiveness to elevated [CO_2]. This may reflect a large sink capacity in the selection of determinate cultivars. In elevated [CO_2] only the determinate isoline of the indeterminate cultivar (Williams-*dt1*) and the non-nodulating genotype showed down-regulation of photosynthesis. This resulted from decreases in apparent *in vivo* Rubisco activity ($V_{c,max}$) and maximum rate of electron transport (J_{max}). Increase in total non-structural carbohydrate (TNC) content, which is often correlated with down-regulation of photosynthesis, in Williams-*dt1* was 80% greater in elevated [CO_2] than in ambient [CO_2] controls, compared to 40% in the indeterminate line. The results from mutations of the Williams line are consistent with the hypothesis that genetic capacity for the utilization of photosynthate is critical to the ability of plants to sustain increased photosynthesis when grown at elevated [CO_2].

Keywords: Soybean; *Glycine max*; Carbon dioxide; Climate change; Photosynthesis; FACE

1. Introduction

The CO_2 concentration of the atmosphere ([CO_2]) is predicted to rise from its present 370 to 550 μmol mol^{-1} by the middle of this century (IPCC, 2001). For C_3 plants this will substantially increase potential net leaf photosynthetic CO_2 uptake (A) because the oxygenase reaction of Rubisco will be inhibited and because carboxylation velocity will increase, since Rubisco is not CO_2 saturated in the present atmosphere (Drake et al., 1997). However, this increased potential is rarely realized fully in the long-term, due to down-regulation of photosynthetic capacity. Much circumstantial evidence, primarily from studies in enclosed environments, suggests that this

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down-regulation results from inadequate "sink" capacity. "Sink" is defined as the parts of the plant that at a given stage of development are utilizing photosynthate in storage, construction, or respiration. Under such conditions non-structural carbohydrates will accumulate and, possibly through increased hexose cycling within the leaf, expression of genes coding for the photosynthetic apparatus is suppressed resulting in decreased photosynthetic capacity; most notably a decrease in the amount of Rubisco (reviewed: Sheen, 1994; Drake et al., 1997; Moore et al., 1999). The issue has broader implications than atmospheric change, since it also concerns the question of whether increasing photosynthetic capacity through genetic manipulation may be translated into increased productivity. While many studies have inferred "sink" limitation as the cause of down-regulation of photosynthesis with growth at elevated [CO_2], single gene mutations providing defined alteration of sink capacity have not been used to test this hypothesis.

Soybean (*Glycine max* (L.) Merr) provides a model C_3 species for such a study. Growth habit (determinate or indeterminate growth) and the ability to assimilate atmospheric N_2 through the symbiotic association with Bradyrhizobia affect "sink" capacity and are significantly associated with reported variation in the response of soybean to growth at elevated [CO_2] (Ainsworth et al., 2002a). Indeterminate soybean cultivars continue growth after flowering (Hoeft et al., 2000), while determinate cultivars have a shorter flowering period and mainstem growth is approximately 80% complete once flowering has begun (Lin and Nelson, 1988). By restricting vegetative growth after flowering, the determinate growth may potentially limit the size of the sink for carbon. Root nodules are strong sinks for carbohydrate (Walsh et al., 1987); the respiratory rate of a nodulated root system may be an order of magnitude greater than that of an equivalent non-nodulated root system (Vessey et al., 1988). Therefore, the absence of root nodules should accentuate the potential for carbohydrate feedback under elevated [CO_2] (Arnone and Gordon, 1990). A large "sink" capacity has been suggested as a critical factor for maximizing plant production in elevated [CO_2] (Drake et al., 1997). This hypothesis has been examined by comparing different species or cultivars within a species with obvious differences in storage organ sizes (Ziska and Bunce, 1995, 2000; Ziska et al., 2001). In these cases, however, there are multiple and undefined genetic differences between cultivars and thus differences in down-regulation are not necessarily the result of differences in sink-size. Further, most studies have used controlled environments or enclosures, which could also restrict the response to elevated [CO_2]. A survey of the 111 prior studies of the effects of elevated [CO_2] on soybean showed that the average increase in yield in large pots (>9 dm^3) was only one-third of that when grown in the field (Ainsworth et al., 2002a). The significance of genetic restrictions on sink capacity to acclimation is therefore most appropriately evaluated under field conditions, without restriction of rooting volume.

Isogenic soybean lines that vary by a single gene altering either growth habit or nodulating capacity provide an opportunity to test how acclimation of photosynthesis to elevated [CO_2] is affected by "sink-strength" (Nakamura et al., 1999). Indeterminate genotypes have the *Dt1* allele, determinate genotypes the *dt1* (Bernard, 1972). Effects of the *Dt1* versus *dt1* allele can be accurately assessed only in isolines or near isolines that are otherwise genetically identical (Desclaux and Roumet, 1996; Robinson and Wilcox, 1998; Kilgore-Norquest and Sneller, 2000). The effect of the allele at the *Dt1* locus on productivity is dependent on the genetic background of line. Very productive determinate cultivars adapted to the northern growing regions of the US have been developed with the *dt1* allele, but that was not possible without also introgressing other genes to complement the modified stem termination (Cooper, 1985). An unbiased test of the effect of determinate growth on photosynthetic acclimation to elevated [CO_2] would be conversion of an indeterminate genotype to determinate by a single gene substitution. Mutant soybean genotypes that lack capacity for nodulation provide another tool for testing the effect of decreased sink capactiy on the [CO_2] response within a uniform genetic background (Arnone and Gordon, 1990; Nakamura et al., 1999).

In this study, the response of soybean lines to elevated [CO_2] is examined in an open-air, field environment within the soybean free air gas concentration enrichment (SoyFACE) experiment. SoyFACE is located in central Illinois, in one of the world's most productive soybean growing areas (American Soybean Association, 2001), and allows the crop to be grown in the field under elevated [CO_2] without alteration of microclimate. Specifically, this study tests the following predictions in the field: (a) determinate forms of indeterminate cultivars and non-nodulating lines grown at elevated [CO_2] will show a decrease in *in vivo* Rubisco activity (Sage, 1994; Drake et al., 1997; Rogers and Humphries, 2000); (b) acclimation of determinate and indeterminate genotypes will correlate to increased total non-structural carbohydrate (TNC) (Drake et al., 1997; Rogers et al., 1998). Inability to nodulate is likely to have most effect at the beginning of flowering, before pod formation starts. At this time canopy development is complete, but the major sink, i.e. the pod, has not been initiated. Determinate growth habit is likely to have most effect during pod-filling. Effects on photosynthesis were therefore investigated at the start of flowering for non-nodulating mutants and during pod-filling for lines that differ in stem termination. In each case the mutant was compared with the germplasm from which it was developed.

2. Methods

2.1. Site description and plant material

The study was conducted at the soybean free air gas concentration enrichment facility in Champaign, IL, USA (40°02′N, 88°14′W, 228 m above sea level). The SoyFACE facility, situated on 32 ha of Illinois farmland, consisted of four blocks, each containing two 20 m diameter octagonal plots. The soil is a Drummer (fine-silty, mixed, mesic Typic Endoaquoll), which is very deep and formed from loess and silt parent material deposited on the till and outwash plains. No fertilizer was added to the soybean crop, according to standard regional agronomic practice. Measurements for this study were made from 22 to 27 July 2001 and from 23 to 26 August 2002. The mean minimum and maximum temperature for the 2001 measuring period were 20.3 and 29.8 °C, respectively, and 19.2 and 28.4 °C for 2002. The average relative humidity was 85.0%, total precipitation was <0.001 mm for the 2001 measuring period, compared to 85.7% and 0.03 mm for the 2002 measuring period. Within each block, one plot was at current ambient [CO_2] of 370 μmol mol^{-1} and one plot was fumigated to an elevated [CO_2] of 550 μmol mol^{-1}, using the FACE design of Miglietta et al. (2001). The plots were separated by at least 100 m, which has been demonstrated sufficient to minimize cross-contamination of CO_2 (Lewin, 1992). In 2001, the actual [CO_2] averaged across the growing season and four soybean plots was 548.6 μmol mol^{-1}. One-minute averages of [CO_2] within the plots were within ±10% of the 550 μmol mol^{-1} target 86% of the time. In 2002, the mean elevated [CO_2] in the FACE plots was 551.8 μmol mol^{-1}, and 1 min averages of [CO_2] were within 10% of the target 85% of the time (Tim Mies, personal communication). Each plot was divided into 52 1.9 m × 1.9 m subplots. Soybeans were planted on 27 May 2001 and 1 June 2002. One side of each ring (26 subplots) was planted with a common soybean cultivar together with the rest of the field. The other side of each ring was planted with a range of genotypes including those described in Table 1 and included for this study. Each genotype occupied the same position in each ring. NN5 is a non-nodulating mutant of Williams that was found in a population mutagenized with NMU. This line carries two recessive genes (*rj*5 and *rj*6) that condition the non-nodulation response (Pracht et al., 1993). L85-2029 is a backcrossed-derived, determinate isoline of Williams, an indeterminate variety, and L81-4274 is a backcrossed-derived indeterminate isoline of the determinate Elf cultivar (Bernard et al., 1991). Seed was obtained from the USDA Soybean Germplasm Collection in Urbana, IL.

Table 1
List and description of soybean genotypes used in the experiments

Genotype	Denoted in text as	Nodulation	Growth habit
Williams		Nodulating	Indeterminate
NN5	Williams-NN	Non-nodulating	Indeterminate
L85-2029	Williams-*dt1*	Nodulating	Determinate
Elf		Nodulating	Determinate
L81-4274	Elf-*Dt1*	Nodulating	Indeterminate

2.2. Photosynthetic gas exchange

Measurements of leaf CO_2 and water vapor exchange were performed on Williams and Williams-NN (non-nodulating) during the flowering growth phase from 22 to 27 July 2001 as the first experiment, and on Williams, Williams-*dt1*, Elf, and Elf-*Dt1* during pod-filling, from 23 to 26 August 2002 as the second experiment. Typically three to four leaves per cultivar per ring were sampled for gas exchange from every plot for each experiment. The response of assimilation (A) to intercellular CO_2 concentration (c_i) provides an *in vivo* measurement of $V_{c,max}$ and J_{max}. The objective was to infer changes in the underlying photosynthetic capacity of leaves. Transient decrease in water potential, decrease in chloroplast inorganic phosphate concentration, and decrease in maximum photosystem II efficiency can all occur after a few hours of sunlight, and may all alter the A/c_i response. To avoid this complication, gas exchange analysis was conducted on leaves, sampled pre-dawn with petioles cut under water, and then kept immersed in water. Leaves were maintained in low light prior to measurement. Leaves were sampled from the upper canopy. Rates of photosynthesis measured on leaves, sampled in this way, equaled or exceeded those measured in situ, suggesting that this procedure did not lower photosynthetic capacity.

Water vapor and carbon dioxide fluxes were measured with a portable open gas-exchange system, incorporating an infrared gas analyzer (LI-6400, Li-Cor, Lincoln, NE, USA). A and stomatal conductance to water vapor (g_s) were calculated in response to changes in c_i according to the method of von Caemmerer and Farquhar (1981).

Photosynthesis was initially induced, on transfer to the measurement photon flux density (1250 μmol $m^{-2}\,s^{-1}$) at the growth [CO_2]. The leaf cuvette [CO_2] was reduced stepwise to 50 μmol mol^{-1} and then increased stepwise to an upper concentration of 1200–1400 μmol mol^{-1}. Ten or 11 points were measured to construct each A/c_i curve. Leaf temperature was maintained at 25 °C with a vapor pressure deficit in the air of the cuvette of 1.25 ± 0.3 kPa.

The response of A to c_i was fit via the model of Farquhar et al. (1980) using maximum likelihood regression to predict $V_{c,max}$ and J_{max} (Sigmaplot, Jandel Scientific, Erkrath, Germany), as described in Ainsworth et al. (2002b). $V_{c,max}$ was determined from points below the inflexion of the A/c_i plot and J_{max} was determined from values above the inflexion.

2.3. Carbohydrate analysis

Leaf disks for carbohydrate analysis were collected on 30 August 2002 from 14:00 to 15:30 p.m. Leaf disks (3.14 cm^2) were sampled in the field, immediately frozen in liquid N, and stored at −80 °C. Ethanol–soluble carbohydrates were extracted from ground leaf tissue in six overnight incubations in 90% (v/v) ethanol at 60 °C. The six overnight incubations were necessary to recover >98% of the ethanol–soluble carbohydrate fraction. Bulk ethanol–soluble carbohydrate content was determined using the phenol–sulfuric acid assay described by Dubois et al. (1956). Following ethanol incubations, starch was extracted from ground leaves using 32% (v/v) perchloric acid as described by Farrar (1993) and assayed as described above.

2.4. Statistical analysis

The experimental design was a split-plot with [CO_2] as the main plot factor and cultivar as the split-plot factor ($n = 4$ blocks). A mixed model ANOVA (PROC MIXED; SAS Institute, 1996) was used for statistical analysis of gas exchange variables and leaf carbohydrate data. Each pairing of cultivar and its mutant was analyzed independently. Pre-planned comparisons of means were analyzed with linear contrasts.

3. Results

3.1. Nodulation

The light-saturated rate of photosynthesis (A_{sat}) was increased by 29% in elevated [CO_2] in Williams ($P < 0.05$). In sharp contrast, it was not affected by [CO_2] in Williams-NN when measured during flowering in 2001 (Table 2). The photosynthetic capacity of Williams-NN was significantly reduced under elevated [CO_2]. $V_{c,max}$ was reduced by 29% ($P < 0.05$) and J_{max} was reduced by 18% ($P < 0.05$). Again by contrast, the photosynthetic potential of Williams was unaffected (Table 2). The ratio of $V_{c,max}$:J_{max} was not significantly altered by growth at elevated [CO_2] in either Williams or Williams-*dt1* (Table 2).

3.2. Stem termination

There was no difference in photosynthetic capacity between Williams-*dt1* (the determinate Williams isoline), and Williams, or between Elf-*Dt1* (the

Table 2
The effect of [CO_2] on photosynthetic capacity of a nodulating and non-nodulating genotype

Genotype and growth [CO_2]	A_{sat} (μmol m^{-2} s^{-1})	$V_{c,max}$ (μmol m^{-2} s^{-1})	J_{max} (μmol m^{-2} s^{-1})	$V_{c,max}/J_{max}$
Williams				
370	17.9 a	84.6 a	137.9 a	0.62 a
550	23.1 b	85.2 a	150.5 a	0.57 a
Williams-NN				
370	20.6 a	101.2 a	160.4 a	0.64 a
550	19.6 a	78.6 b	131.1 b	0.60 a

The light-saturated rate of CO_2 uptake (A_{sat}) measured at growth [CO_2], maximum rate of RuBP-saturated carboxylation in vivo ($V_{c,max}$), maximum in vivo rate of electron transport contributing to RuBP regeneration (J_{max}), and the ratio of $V_{c,max}$:J_{max}. Williams and Williams-NN were measured during flowering, from 22 to 27 July 2001 ($n = 4$ blocks). Different letters indicate a significant difference within genotypes at the $P < 0.05$ level according to pre-planned linear contrasts.

indeterminate Elf isoline) and Elf under ambient [CO_2], suggesting that differences in sink capacity were insufficient to induce changes at the current [CO_2], but were at elevated [CO_2]. For three of the four genotypes (Elf, ELF-*Dt1*, and Williams), there was no difference in A_{sat} of plants grown in control and elevated [CO_2], when compared at a common measurement [CO_2] (Table 3). However, A_{sat} of Williams-*dt1* grown at elevated [CO_2] was significantly less than A_{sat} of control-grown plants, when both lines were measured at a common [CO_2] (550 μmol mol^{-1}; Table 3). This implies a loss of photosynthetic capacity with growth at elevated [CO_2]. Analysis of the A/c_i response of Williams-*dt1* revealed that this resulted from significantly lower $V_{c,max}$ with growth at elevated [CO_2] (Fig. 1). The decreased $V_{c,max}$ and J_{max} (data not shown) in the Williams-*dt1* eliminated any increase in photosynthesis by elevated [CO_2], such that photosynthesis of the plants grown and measured at elevated [CO_2] equaled that of those grown and measured at ambient [CO_2]. This contrasts sharply with Williams where plants grown and measured at elevated [CO_2] showed a significant ca. 30% increase (Table 3). There was a consistent reduction in the ratio of $V_{c,max}$:J_{max} in elevated [CO_2] for both Williams and Williams-*dt1*; however the reduction in the ratio was not significant for the Elf and Elf-*Dt1* genotypes (Fig. 2, Table 4).

Table 3
The effect of [CO_2] on the photosynthetic rate of determinate and indeterminate genotypes

Genotype	CO_2 concentration: grown, measured (μmol mol^{-1})		
	370, 370	370, 550	550, 550
Elf	17.0 a	24.0 b	24.1 b
Elf-*Dt1*	17.7 a	24.3 b	24.9 b
Williams	19.7 a	25.9 b	25.6 b
Williams-*dt1*	18.3 a	25.7 b	21.7 a

Light-saturated leaf CO_2 assimilation rate (μmol m^{-2} s^{-1}) of soybean genotypes grown under control (370 μmol mol^{-1}) or elevated [CO_2] (550 μmol mol^{-1}), measured during pod-fill (23–26 August 2002). Genotype abbreviations are listed in Table 1. Different letters for CO_2 treatments within a genotype indicate a significant difference at the $P < 0.05$ level according to pre-planned linear contrasts ($n = 4$ blocks).

Elevated [CO_2] significantly increased leaf ethanol–soluble carbohydrate content by 20%, starch content by 120%, and total non-structural carbohydrate content by 58% (Fig. 3, Table 4). Ethanol–soluble carbohydrates were increased by growth in elevated [CO_2] in the two determinate lines, Elf and Williams-*dt1* but not in the two indeterminate lines, Elf-*Dt1* and Williams. While all genotypes grown at elevated [CO_2] had significant increases in starch and TNC, Williams-*dt1* had the greatest absolute levels of TNC (Fig. 3). When grown at elevated [CO_2], TNC was significantly greater in determinate Williams-*dt1* than in indeterminate Williams ($P < 0.1$).

4. Discussion

Acclimation of photosynthesis to elevated [CO_2] in soybean has previously been described as complex, inconsistent, and difficult to understand (Xu et al., 1994). Soybean has been described as a species that shows no photosynthetic acclimation (Ziska et al., 2001), moderate reduction in Rubisco activity (Vu et al., 1997), and substantial reduction in Rubisco (Moore et al.,

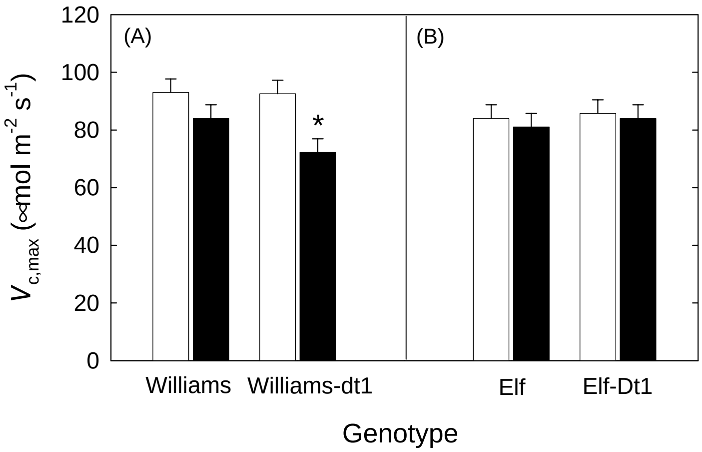

Fig. 1. The maximum rate of RuBP-saturated carboxylation *in vivo* ($V_{c,max}$) ±1 S.E. (A) Williams isogenic pair: Williams and Williams-*dt1*; (B) Elf isogenic pair: Elf and Elf-*Dt1*. Soybeans were grown at ambient [CO_2] (white bars) and elevated [CO_2] (black bars), and measured during pod-filling in August 2002 ($n = 4$ blocks). Abbreviations for cultivars are listed in Table 1. Pre-planned comparisons of [CO_2] treatments within genotypes were performed using contrast statements. $^{*}P < 0.05$.

1998). The range of reported responses likely stems from differences in CO_2 treatment levels, nutrient conditions, rooting volumes, and multi-allelic differences between varieties. In this study, acclimation of photosynthesis was studied for the first time under open-air elevation of [CO_2] without any restriction of rooting volume and, for the first time, using isolines with traits directly affecting "sink" capacity, i.e. nodulation and stem termination. Significant acclimation of photosynthesis was apparent only in the non-nodulating, Williams-NN, and the determinate Williams-*dt1*, both derived by single gene mutations of the nodulating indeterminate Williams (Table 2, Fig. 1). This is consistent with the expectation that acclimation only

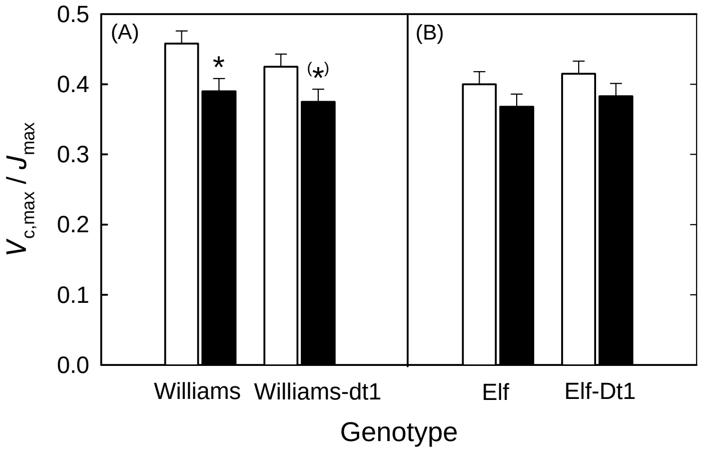

Fig. 2. The ratio $V_{c,max}/J_{max}$ ±1 S.E. (A) Williams isogenic pair: Williams and Williams-*dt1*; (B) Elf isogenic pair: Elf and Elf-*Dt1*, grown at ambient [CO_2] (white bars) and elevated [CO_2] (black bars), and measured during pod-filling in August 2002 ($n = 4$ blocks). Abbreviations for genotypes are listed in Table 1. Pre-planned comparisons of [CO_2] treatments within genotypes were performed using linear contrast statements. $^{*}P < 0.05$; $^{(*)}P < 0.10$.

occurs when sink capacity is lowered (Rogers et al., 1996, 1998; Drake et al., 1997; Morgan et al., 2001).

Down-regulation of photosynthesis in Williams-*dt1* corresponded to a significant increase in leaf TNC, as would be expected with decreased sink capacity. Such an increase in TNC has been phenomenologically linked to acclimation of photosynthesis and decreased $V_{c,max}$ (Xu et al., 1994; Rogers et al., 1998; Isopp et al., 2000). Williams-*dt1* was developed from the indeterminate variety Williams with a single gene substitution (*dt1*). Elf is also a determinate variety, yet by contrast to Williams-*dt1* did not show any loss of photosynthetic capacity with growth at elevated [CO_2]. The disparity between those two determinate

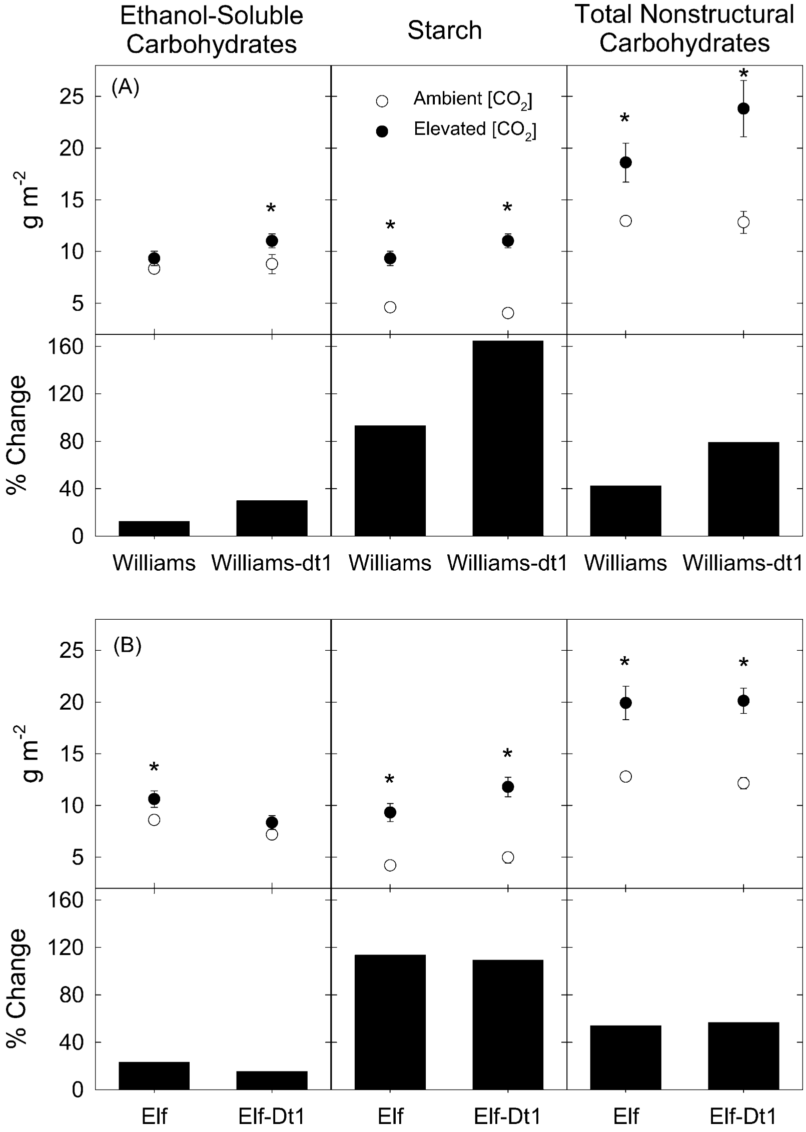

Fig. 3. Ethanol–soluble carbohydrate content, starch content, and total non-structural carbohydrate content of soybean genotypes ±1 S.E., sampled during pod-fill in August 2002. (A) Williams isogenic pair; (B) Elf isogenic pair. Abbreviations for genotypes are listed in Table 1. The bar charts show percent change in carbohydrate with growth at elevated [CO_2] (((FACE − control)/control) × 100). Pre-planned comparisons of [CO_2] treatments within genotypes were made using linear contrast statements. $^{*}P < 0.05$.

Table 4
Statistical analysis of effects of [CO_2] on photosynthetic parameters of determinate and indeterminate genotypes

Source of variation (degrees of freedom)	Isogenic pair	[CO_2] (1); *F*, *P*	Genotype (3); *F*, *P*	[CO_2] × genotype (3); *F*, *P*
A_{sat} (growth [CO_2])	Williams/Williams-*dt1*	23.10, <0.01	3.31, 0.08	1.61, 0.25
	Elf/Elf-*Dt1*	22.30, <0.01	0.21, 0.65	<0.01, 0.99
$V_{c,max}$	Williams/Williams-*dt1*	13.68, 0.01	2.06, 0.20	2.08, 0.20
	Elf/Elf-*Dt1*	0.43, 0.54	0.39, 0.57	0.03, 0.87
J_{max}	Williams/Williams-*dt1*	0.77, 0.41	<0.01, 0.95	7.2, 0.04
	Elf/Elf-*Dt1*	1.57, 0.26	0.13, 0.75	0.48, 0.51
$V_{c,max}/J_{max}$	Williams/Williams-*dt1*	25.59, <0.01	1.11, 0.33	0.57, 0.48
	Elf/Elf-*Dt1*	2.44, 0.15	0.66, 0.44	<0.01, 0.95
EtOH–soluble CH_2Os	Williams/Williams-*dt1*	5.07, 0.04	2.26, 0.16	0.75, 0.40
	Elf/Elf-*Dt1*	10.03, 0.02	11.16, 0.02	0.72, 0.43
Starch	Williams/Williams-*dt1*	19.55, <0.01	0.76, 0.42	1.82, 0.23
	Elf/Elf-*Dt1*	113.09, <0.01	11.16, 0.02	0.72, 0.43
TNC	Williams/Williams-*dt1*	22.72, <0.01	2.13, 0.17	2.33, 0.15
	Elf/Elf-*Dt1*	67.71, <0.01	0.04, 0.86	0.22, 0.66

Analysis of variance of photosynthetic and leaf carbohydrate parameters measured in 2002, for plants grown at ambient [CO_2] (370 μmol mol^{-1}) and elevated [CO_2] (550 μmol mol^{-1}). A mixed model ANOVA with [CO_2] as the main plot factor and genotype as the split-plot factor was used for each isogenic pair.

lines may lie in the fact that Elf was developed as a determinate variety. Elf, although much shorter than Williams, was selected to be highly productive by producing nearly as many pods on branches as on the main stem (Beaver et al., 1985), thereby avoiding "sink" limitation (Kilgore-Norquest and Sneller, 2000). For determinate cultivars to be competitive it is likely that breeders will select lines with sufficient potential for pod formation to avoid "sink" limitation.

While intraspecific variation in the response of photosynthesis to elevated [CO_2] in soybeans has been reported previously (Ziska and Bunce, 1995; Ziska et al., 1998, 2001; Nakamura et al., 1999), these studies were not performed under open-air conditions nor did they compare isogenic lines. When determinate and indeterminate cultivars of various isolines are compared, there are multi-allelic differences. This prevents ascribing differences in [CO_2] response purely to growth habit (Robinson and Wilcox, 1998). By using single gene changes, this study has demonstrated that preventing nodulation and restricting main stem growth produce acclimation of photosynthesis to elevated [CO_2]. However, the results also show that the effect of changing stem termination type is dependent on the genetic background of the variety. Converting the indeterminate variety Williams to a determinate form resulted in down-regulation of photosynthesis in elevated [CO_2]. However, the reciprocal conversion of the determinate Elf variety to indeterminate did not result in an up-regulation of photosynthesis in elevated [CO_2].

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