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Daily irradiance and feedback inhibition of photosynthesis at elevated carbon dioxide concentration in *Brassica oleracea*

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Abstract

The fundamental cause of down-regulation of photosynthesis at elevated carbon dioxide concentration (EC) is thought to be a slower rate of utilization of saccharides than their stimulated rate of production, but there are few studies directly supporting this idea under field conditions. We hypothesized that within *Brassica oleracea*, down-regulation would not occur in kohlrabi because it has a large sink for saccharides in an enlarged stem, but would occur in collards, which lack this sink. Field tests were consistent with this hypothesis. In collards, the degree of down-regulation of photosynthesis in plants grown at EC varied depending on the daily integral of photosynthetically active radiation (PAR) of the day prior to the measurement of photosynthetic capacity, as did leaf saccharide content. However, EC did not result in lower leaf contents of chlorophyll, soluble protein, ribulose-1,5-bisphosphate carboxylase, or nitrate in collards, nor was there any evidence of a triose phosphate utilization rate limiting photosynthesis. Experiments in controlled environment chambers confirmed that there was a threshold response for the down-regulation of photosynthesis in collards at EC to the PAR of the previous day, with down-regulation only occurring above a minimum daily integral of PAR. Down-regulation of photosynthesis could be induced in plants grown at ambient carbon dioxide by a single night at low temperature or by a single day with high PAR and EC. In the controlled environment study, the degree of down-regulation of photosynthesis was highly correlated with leaf glucose, fructose, and sucrose contents, and less well correlated with starch content. Hence down-regulation of photosynthesis at EC in collards in the field represented feedback inhibition from the accumulation of soluble saccharides and day-to-day variation in its occurrence was predictable from the weather.

Additional key words: collard; irradiance; kohlrabi; saccharides; stomatal conductance; variety differences.

Introduction

Long-term exposure of plants to elevated concentrations of carbon dioxide (EC) in some cases results in a reduction in net photosynthetic rate (P_N), termed down-regulation of photosynthesis. Despite concerns that down-regulation was an artifact of small pot size and/or inadequate nutrient supply (*e.g.* Arp 1991), it occurs in the field in fertile soils (*e.g.* Sicher and Bunce 1997, 1999, Adam *et al.* 2000). There are at least three aspects of down-regulation of P_N at EC, which may occur independently in different species, a reduction in quantum efficiency measured at low photosynthetically active radiation (PAR), and reductions in CO_2-limited and CO_2-saturated P_N at high PAR (Bunce and Ziska 1999). Ability to predict P_N as atmospheric CO_2 concentration (AC) continues to rise is currently limited by our inability to predict the occurrence and magnitude of these various aspects of down-regulation of photosynthesis. The fundamental cause of down-regulation of photosynthesis at EC is thought to be inadequate sink capacity relative to source activity (Stitt 1991). There are at least four biochemical mechanisms for down-regulation of photosynthesis caused by source-sink imbalance at EC which have received experimental support under laboratory conditions: (*1*) sugar repression of gene expression (Sheen 1990, Van Oosten and Besford 1995), (*2*) insufficient N uptake (Stitt and Krapp 1999), (*3*) triose phosphate utilization rate limitation (Socias *et al.* 1993), (*4*) direct inhibition by saccharide content (Sasek *et al.* 1985, Sawada *et al.* 2001, Lewis *et al.* 2002). However, there are no studies directly supporting the idea that source-sink balance controls down-regulation of photosynthesis at EC under field conditions, although seasonal patterns of photosynthetic responses to EC in trees have been ascribed to changes in source-sink balance (*e.g.* Tissue *et al.* 1997, Lewis *et al.* 1999). In the field, one would expect that source-sink balance might change on the time scale of days, for example with periods of cloudiness, or warmer or cooler temperatures, as well as seasonally with changes in the

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relative amount or activity of source and sink tissues.

Reekie *et al.* (1998) found that the stimulation of growth by EC was larger in species and cultivars of *Brassica* with larger sinks, but did not test for down-regulation of photosynthesis. Collard and kohlrabi are members of the same species and are cultivated similarly. Collard is a leafy vegetable but the harvested portion of kohlrabi is a swollen stem. These plants offer a unique opportunity to study effects of source-sink balance on photosynthetic responses to EC in the field. We hypothesized that within the species *Brassica oleracea*, down-regulation of photosynthesis at EC would not occur in kohlrabi after it developed an enlarged stem, but would occur in collards, which lack this sink, and that it would occur in kohlrabi prior to the development of an enlarged stem. Furthermore, we hypothesized that the extent of down-regulation in collards would vary with the amount of storage saccharides in the leaf tissue as this varied with the irradiance and possibly temperature conditions of the preceding day. These hypotheses were examined under field conditions, and experiments in controlled environments were used to confirm conclusions drawn from the field data.

Materials and methods

Field studies: Kohlrabi (*Brassica olearacea* var. *gongylodes* L. cv. Grand Duke) and collard (*Brassica olearacea* var. *acephala* DC. cv. Georgia Southern) were grown in open top chambers in field plots at the South Farm of the Beltsville Agricultural Research Center in the spring of 1999 and 2000 and in the fall of 2000 and 2001. In the spring plantings, the two varieties were grown simultaneously. In the fall, only collards were grown. Some chambers were ventilated with air drawn from outside the chambers, and other chambers were ventilated with outside air to which pure CO_2 was added at a flow rate sufficient to raise the $[CO_2]$ to 350±50 μmol mol^{-1} above that of the outside air. In all experiments there were three chambers per $[CO_2]$ treatment for each variety. The clear acrylic chambers transmitted about 90 % of the PAR and air in the chambers averaged 1 °C warmer than outside (Sicher and Bunce 1999). Plants were thinned to 10 m^{-2} for collards and 20 m^{-2} for kohlrabi, as recommended. Collard plants have about twice the leaf area of kohlrabi plants at maturity. Plots were fertilized with 25 g(N) m^{-2}, using an ammonia-based form of nitrogen. Periodic harvests were made to determine total leaf area and leaf and stem dry mass. The ratio of stem to leaf dry mass was used to determine the time during development that stem enlargement occurred in kohlrabi compared with collard. Air temperatures, PAR inside and outside the chambers, and $[CO_2]$ from ambient (AC) and EC chambers as measured by an absolute infrared analyzer were recorded every 15 min.

For the experiments in the spring, when both kohlrabi and collards were grown, leaf gas exchange characteristics were determined for both varieties and for both $[CO_2]$ treatments near midday on each of five clear days in both years. Gas exchange was measured on fully irradiated mature upper canopy leaves. P_N and stomatal conductance (g_s) were determined using a *CIRAS-1* portable photosynthesis system (*PP Systems*, Haverhill, MA, USA) with automatic $[CO_2]$ control and a broad leaf chamber. Steady-state rates of gas exchange were measured in full midday sunlight (PAR > 1 400 μmol m^{-2} s^{-1}), at the ambient air temperature and humidity, and at 700 μmol mol^{-1} external $[CO_2]$. A total of six leaves per variety and $[CO_2]$ treatment were measured each day, two from each open top chamber. These data were analyzed using a three-way analysis of variance to test for effects of date, variety, $[CO_2]$ treatment, and their interactions, with n = 3 replicate chambers. The date of variety by $[CO_2]$ treatment interaction term was significant, and the measurement dates were subsequently divided into three groups in which the three-way interaction term was not significant and the date term was also not significant. One division was the pre-planned division based on sink development of kohlrabi, to examine the hypothesis that kohlrabi would change from showing down-regulation of P_N at EC before it developed a swollen stem to lack of down-regulation later. The other division was a post-hoc division of the later sampling dates into days that followed sunny or cloudy days, which was based on contrasting responses to the $[CO_2]$ treatment in collards on such dates.

On dates when collards in the EC treatment had lower P_N than for the AC treatment, but no $[CO_2]$ treatment effect occurred in kohlrabi, additional gas exchange measurements were made on collard to further characterize those differences. P_N of plants grown at the higher $[CO_2]$ were also measured at an external $[CO_2]$ of 1 000 μmol mol^{-1} to test whether P_N increased from 700 to 1 000 μmol mol^{-1} in those plants, using a paired t-test. Lack of increase would indicate a limitation of P_N at 700 μmol mol^{-1} by the rate of utilization of triose phosphates (Sharkey 1985). P_N and g_s of plants from both $[CO_2]$ treatments were also measured at external $[CO_2]$ of 200 and 100 μmol mol^{-1}. These values were used to determine an initial slope of P_N to substomatal $[CO_2]$ for each leaf (termed carboxylation efficiency, CE). Mean values of CE for each treatment chamber were then used to test for $[CO_2]$ treatment effects on CE.

On six dates in the spring, during the period of each year when P_N differed between $[CO_2]$ treatments in collard but not in kohlrabi, leaf samples were taken for biochemical analysis. Samples were obtained from both varieties and both $[CO_2]$ treatments. Two of the dates when leaf samples were taken were also dates that gas exchange was measured. Leaf discs (1.8 cm^2) were quickly placed in liquid N_2 in the field to stop metabolism and

were then stored at –80 °C in aluminum foil bags until used. Chlorophyll (Chl), starch, glucose, fructose, sucrose, soluble protein, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO), and nitrate contents were determined, all expressed per unit of area. One to two frozen leaf discs were extracted at 0 °C in 2 cm^3 ice-cold 80 % aqueous ethanol and the homogenate was centrifuged at 800×*g* to remove particulate matter. The pellet fraction was washed with 1 cm^3 80 % ethanol and the supernatants were combined. The washed pellets were air-dried, suspended in 2 cm^3 of distilled water, and used for the determination of leaf starch according to Hendrix (1993). An aliquot of the ethanol extract was used to determine total Chl (*a*+*b*) in 80 % acetone according to Lichtenthaler (1987). A second aliquot of each extract was diluted 20- to 50-fold with distilled water, filtered with a 0.45 µm nylon syringe filter, and nitrate was measured by anion exchange high performance liquid chromatography (Thayer and Huffaker 1980). The remaining portion of the solvent extract was dried under a stream of N_2 gas at 37 °C, dissolved in 1 cm^3 of distilled H_2O, and glucose, fructose, and sucrose were determined spectrophotometrically in coupled enzyme assays (Sicher *et al*. 1995).

In the fall, when only collards were grown, midday measurements of P_N at 700 µmol mol^{-1} were made in full sunlight, at the ambient air temperature and humidity, as in the spring. When the plants grown at EC had lower P_N than those grown at AC, the plants grown at EC were also measured at 1 000 µmol mol^{-1} to determine whether P_N increased.

Controlled environment experiments: Collards were grown one plant per pot in 30 cm diameter plastic pots filled with vermiculite and flushed daily with a complete nutrient solution containing 14.5 mM nitrogen. Two chambers were used, with day/night air temperatures of 23/17 °C, 1 000 µmol m^{-2} s^{-1} PAR for 12 h per day from a mixture of metal halide and high pressure sodium lamps, with [CO_2] controlled at either 350±30 or 700±30 µmol mol^{-1} by injection of pure CO_2 or air scrubbed of CO_2. There were multiple successive plantings, with [CO_2] treatments and plants switched between chambers about every four weeks. Environmental changes and sampling of leaves for gas exchange characteristics and saccharide content were conducted when plants were 23 to 27 d old, with shoot dry masses of about 10 and 20 g per plant, for the 350 and 700 µmol(CO_2) mol^{-1} treatments, respectively.

Six plants from either the AC or EC treatments were moved just before the chamber lights came on to another controlled environment chamber programmed for various conditions, remained there for one 24 h period, and were then returned to the original chamber. Measurements of leaf P_N were made about 4 h after returning plants to the original chamber. P_N was measured on fully irradiated mature upper leaves. Comparisons were made with plants that had remained in the original chamber. In one set of experiments, EC plants were exposed for one day to PAR of 100, 200, 500, 700, 1 000, or 1 200 µmol m^{-2} s^{-1}, with same temperature, humidity, and [CO_2] conditions as in the original EC environment. In another experiment, EC plants were given one night at 23 °C rather than 17 °C. AC plants were exposed for one day to (*a*) a lower PAR of 200 µmol m^{-2} s^{-1}, (*b*) a lower night temperature of 11 °C, or (*c*) a higher [CO_2] of 700 µmol mol^{-1}. All other environmental conditions were the same as the original growth conditions.

For all of these 24 h treatments, and for control plants from both growth [CO_2] treatments, P_N of mature upper leaves was measured at a PAR of 2 000 µmol m^{-2} s^{-1}, a leaf temperature of 25 °C, 700 µmol(CO_2) mol^{-1}, and a water vapor pressure deficit of 1.5 kPa, using the *CIRAS-1* portable photosynthesis system. Photosynthetic properties of plants which had been grown continuously in the AC and EC treatment chambers were further characterized by measuring P_N over a wide range of substomatal [CO_2] at a PAR of 2 000 µmol m^{-2} s^{-1}, 25 °C, and vapor pressure deficit of 1.5 kPa, and by determining the quantum yield at 25 °C, 350 µmol(CO_2) mol^{-1} from measurements of P_N at several PAR between 50 and 150 µmol m^{-2} s^{-1}. For most of the 24-h treatment regimes, samples of the leaves in which gas exchange was measured were frozen in liquid nitrogen for determination of starch, glucose, fructose, and sucrose content per unit of area, using the methods described earlier.

For each environment to which plants were switched, *t*-tests were used to compare mean values of parameters for the six switched-plants with six plants from the same planting date that remained in the original chamber. For plants not switched between environments, comparisons of leaf gas exchange characteristics were made for single plants from each of six replicate experiments.

Results

Field studies: In the first measurements of each spring, when the kohlrabi plants were small, growth at EC reduced P_N measured at 700 µmol mol^{-1} both in kohlrabi and in collards (Table 1). Kohlrabi at this time had a total stem dry mass of 0.1 g or less, and a stem to leaf ratio no more than 1.2× that of collards. By the time kohlrabi had reached a stem dry mass of about 1 g and a stem to leaf ratio about 1.5× that of collards, no reduction in P_N of kohlrabi occurred in plants grown at EC on any of eight measurement days. On four of those days, significant reductions in P_N occurred in collard plants grown at EC (Table 1). These four days, when P_N was lower in collards but not in kohlrabi when grown at EC, were days after the mostly sunny days. The other four dates, when

Table 1. Net photosynthetic rate (P_N) and leaf constituents of collard and kohlrabi leaves grown in the spring at the current ambient (AC) or enhanced (EC; AC + 350 μmol mol^{-1}) [CO_2] in open top chambers in field plots. Mean values are presented for three conditions: (*1*) before stem swelling in kohlrabi (n = 2 d), (*2*) later in the season, after mostly sunny days (n = 4 d), and (*3*) later in the season, after mostly cloudy days (n = 4 d). P_N was measured in full midday sunlight (PAR > 1 400 μmol m^{-2} s^{-1}), at the ambient air temperature and humidity, and at 700 μmol mol^{-1} [CO_2] for two leaves in each of three chambers per [CO_2] treatment. Substomatal [CO_2] did not differ between varieties or growth [CO_2] treatments, and averaged 524 μmol mol^{-1}. "Sugars" is the sum of the glucose, fructose, and sucrose contents. Values are means for two sampling days when gas exchange and biochemical analyses were conducted on the same date. Values within rows followed by different letters were significantly different at p = 0.05, using analysis of variance. Values within parentheses are standard errors between measurement dates.

	Group	Collard		Kohlrabi	
		EC	AC	EC	AC
P_N [μmol m^{-2} s^{-1}]	Before stem swelling	44.2 b (2.2)	53.1 a (0.8)	43.8 b (1.0)	50.1 a (1.0)
	After sunny days	43.1 b (2.2)	50.3 a (2.7)	42.8 b (3.8)	42.8 b (3.3)
	After cloudy days	45.7 a (1.1)	47.0 a (0.3)	47.5 a (1.6)	47.2 a (1.0)
	Mean of 2 d	44 a (5)	51 b (6)	45 a (5)	45 a (5)
Soluble protein [g m^{-2}]		9.1 a (1.0)	9.3 a (0.9)	8.7 a (0.9)	8.7 a (1.1)
RuBPCO [g m^{-2}]		2.3 a (0.1)	2.5 a (0.2)	2.2 a (0.2)	2.4 a (0.2)
Chlorophyll [g m^{-2}]		0.38 a (0.04)	0.40 a (0.04)	0.35 a (0.05)	0.35 a (0.05)
Nitrate [g m^{-2}]		0.06 a (0.01)	0.04 a (0.02)	0.03 a (0.01)	0.08 a (0.06)
Starch [mmol(hexose) m^{-2}]		57 a (12)	23 b (8)	64 a (14)	27 b (10)
Sugars [mmol(hexose) m^{-2}]		35 a (6)	23 b (2)	35 a (13)	18 b (4)

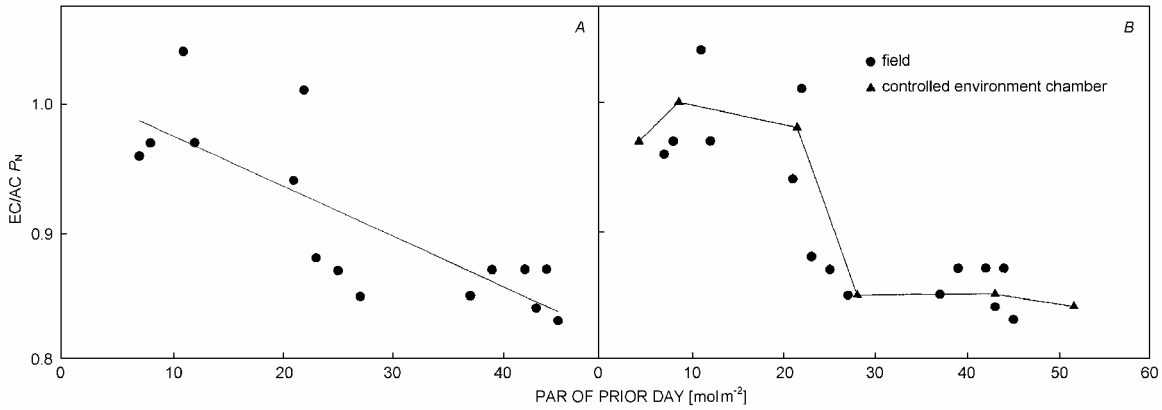

Fig. 1. Relationship between down regulation of net photosynthetic rate (P_N) and the photosynthetically active radiation (PAR) of the day prior to the P_N measurement in collards grown (*A*) in the field or (*B*) also at 700 μmol(CO_2) mol^{-1} in controlled environment chambers. In the controlled environment chamber, plants were switched to various PAR for one day, and P_N was measured on the following day. Plants were grown at the current ambient [CO_2] (AC) or at elevated (AC + 350 μmol mol^{-1}) [CO_2] (EC). P_N was measured at 700 μmol(CO_2) mol^{-1}. Each point represents the mean ratio of P_N on a single measurement date. Values below 1 indicate down regulation of photosynthesis in plants grown at EC. The r^2 value of the linear regression was 0.657.

no reduction in P_N occurred in collards at EC, were days that followed mostly cloudy days (Fig. 1*A*).

On the four days in which P_N was lower in collards but not in kohlrabi when grown at EC, P_N of collard leaves was higher when measured at 1 000 than at 700 μmol(CO_2) mol^{-1}, with mean values of 52.7 and 44.2 μmol m^{-2} s^{-1}, respectively. The increase in P_N was significant at p = 0.05, using a paired t-test. Responses of P_N to a wide tange of substomatal [CO_2] on one of these days (Fig. 2) indicated that leaves of EC grown collards had a CE 0.81× those of plants grown at AC, while P_N measured at 350 μmol(CO_2) mol^{-1} was 0.80× that of AC plants, and P_N measured at 700 μmol(CO_2) mol^{-1} was 0.86× that of AC plants.

Leaf starch and soluble sugar contents of leaves of both collard and kohlrabi were higher in plants grown at EC than at AC on the two dates when leaf gas exchange and constituents were sampled on the same day (Table 1). The EC treatment did not result in lower leaf contents of nitrate, Chl, soluble protein, or RuBPCO in either collards or kohlrabi (Table 1). The same lack of [CO_2] treatment effects on these constituents also occurred on the other four sampling dates (not shown). The [CO_2] treatment effects on saccharide content were consistent among sampling days, although the starch content increased significantly with the integral of PAR of the prior day (Fig. 3).

In collards grown in the fall, P_N was significantly

lower in plants grown at EC than at AC on two of the five measuring days each year. Those 2 d followed days with higher PAR, similar to the pattern found in the spring (Fig. 1*A*). No relationship between the degree of down-regulation of P_N at EC in collards and temperature could be established in either spring or fall.

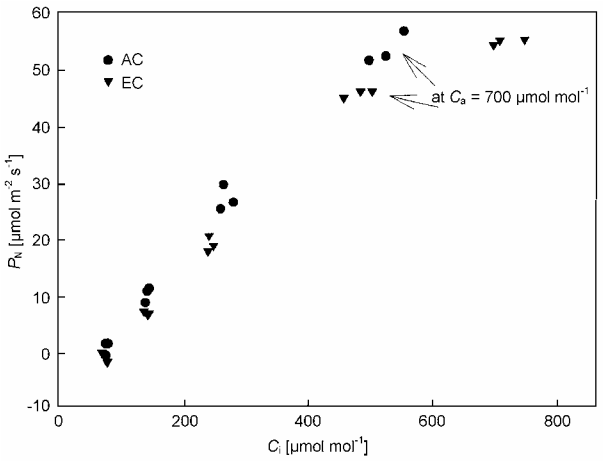

Fig. 2. Relationships between net photosynthetic rate (P_N) and internal [CO_2] (C_i) for collards grown at the current ambient [CO_2] (AC) or at elevated (ambient + 350 µmol mol^{-1}) [CO_2] (EC) in open top chambers. Measurements were made on June 2, 2000 on three leaves per [CO_2] treatment. Leaf temperature averaged 32 °C, PAR was > 1 400 µmol m^{-2} s^{-1}, and the leaf to air water vapor pressure difference averaged 2.5 kPa.

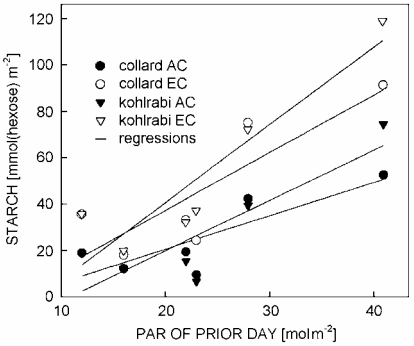

Fig. 3. Relationships between starch content and the photosynthetically active radiation (PAR) of the day prior to sampling for collard and kohlrabi plants grown at at the current ambient [CO_2] (AC) or at elevated (ambient + 350 µmol mol^{-1}) [CO_2] (EC) in open top chambers. Each point represents the mean value of six leaves. r^2 values of linear regressions were 0.684, 0.712, 0.737, and 0.845 for AC collard, EC collard, AC kohlrabi, and EC kohlrabi, respectively.

Controlled environment experiments: Collards grown continuously at 700 µmol(CO_2) mol^{-1} had P_N at 700 µmol mol^{-1} which averaged 0.85× that of plants grown at 350 µmol mol^{-1} (Table 2), which was similar to the growth [CO_2] treatment effect in field-grown plants measured after mostly clear days. Carboxylation efficiency and quantum yield were also lower in these leaves (Table 2), and P_N of EC plants was not saturated for [CO_2] at 700 µmol(CO_2) mol^{-1} (not shown). The responses of P_N to substomatal [CO_2] were very similar to those of plants grown in the field shown in Fig. 2, except that all rates were about 25 % higher, possibly because the measurement temperature was closer to optimal.

Table 2. Photosynthetic characteristics of leaves of collard plants grown at 350 and at 700 µmol(CO_2) mol^{-1} in controlled environment chambers. Net photosynthetic rate (P_N) [µmol m^{-2} s^{-1}] and carboxylation efficiency (CE) [mol m^{-2} s^{-1}] were measured at a PAR of 2 000 µmol m^{-2} s^{-1}. All measurements were made at temperature of 25 °C, and a leaf to air vapor pressure difference of about 1.5 kPa. Quantum yield [mol mol^{-1}] on an absorbed photon basis was measured at 350 µmol(CO_2) mol^{-1}, at PAR between 50 and 150 µmol m^{-2} s^{-1}. Substomatal [CO_2] did not differ between growth [CO_2] treatments, and averaged 226 and 455 µmol mol^{-1} for the measurements at 350 and 700 µmol(CO_2) mol^{-1}, respectively. Values within rows followed by different letters were significantly different at p = 0.05, using analysis of variance. Values within parentheses are standard errors for six replicate experiments.

	Growth CO_2 [µmol mol^{-1}]	
	350	700
P_N at 700 µmol(CO_2) mol^{-1}	65.1 a (1.3)	55.0 b (1.1)
P_N at 350 µmol(CO_2) mol^{-1}	25.5 a (0.5)	19.8 b (0.4)
CE	0.107 a (0.01)	0.082 b (0.01)
Quantum yield	0.070 a (0.002)	0.050 b (0.002)

Table 3. Net photosynthetic rate (P_N) measured at 700 µmol(CO_2) mol^{-1} for leaves of collard plants grown at 350 and at 700 µmol(CO_2) mol^{-1} in controlled environment chambers, with day/night temperatures of 23/17 °C, and a PAR of 1 000 µmol m^{-2} s^{-1} for 12 h per day. P_N was measured at a PAR of 2 000 µmol m^{-2} s^{-1}, a leaf temperature of 23 °C, and a leaf to air vapor pressure difference of about 1.5 kPa. Plants were either grown continuously at the two growth [CO_2] conditions (controls), or switched for one day to the specified altered condition. *indicates a value significantly different from the respective control [CO_2] value, using a *t*-test. Values in parentheses are standard errors for n = 6 plants for the altered condition treatments, and n = 18 and n = 6 plants for the unaltered condition treatments with the growth [CO_2] of 350 and 700 µmol mol^{-1}, respectively.

Growth [CO_2]	Altered condition	P_N [µmol m^{-2} s^{-1}]
350	none	65.3 (1.3)
700	none	54.8 (2.1)
700	night temperature = 23 °C	56.2 (2.2)
350	night temperature = 11 °C	59.3* (1.7)
350	PAR low	65.2 (2.5)
350	[CO_2] = 700 µmol mol^{-1}	57.5* (1.6)

When collards grown at EC were shifted for one day to a range of PAR, there was a threshold PAR below which their P_N the next day was not different from plants grown at AC, which was consistent with the response observed in the field-grown plants (Fig. 1*B*). Giving

plants grown at EC a single warmer night did not affect their P_N (Table 3). AC plants did not have increased P_N when given one day at lower PAR, but had lower P_N when given a single cooler night or when given a day at EC at high PAR (Table 3). Among these treatments, P_N varied with leaf glucose and fructose contents in a threshold pattern and approximately linearly with sucrose content, but was less clearly associated with starch content (Fig. 4).

Discussion

The hypothesis that down regulation of photosynthesis at EC would occur in collards but would not occur in kohlrabi after it developed a strong sink in its swollen stem was supported by the field data. A source-sink imbalance mechanism for the down regulation in collards at EC was further supported by the observation that the occurrence of down regulation was highly correlated with the amount of PAR experienced by the plants the day prior to the P_N measurement. Collards thus provide one of the first cases where the occurrence and the magnitude of the down-regulation of photosynthesis by EC under field conditions can be predicted using PAR data.

Prior to developing a strong sink, kohlrabi had the same degree of down-regulation of photosynthesis as collards. This indicates that even young developing plants may be sink limited. Similar to the results presented here for kohlrabi, Ziska *et al.* (1995) reported that the down regulation of photosynthesis at EC in sugar beet in controlled environment chambers decreased after the development of a large sink for saccharides. Lewis *et al.* (2002) reported experiments where photoperiod was used to separate age from developmental stage, and showed that source-sink imbalance was correlated with down regulation of photosynthesis at EC in cocklebur. With examples such as these, and the data presented here for collards, the idea that down-regulation of photosynthesis by EC can result from source capacity exceeding sink capacity is moving beyond a conceptual hypothesis and becoming a predictive model. All three aspects of down-regulation of photosynthesis observed at EC in other studies occurred in collards. These are, reduced quantum yield at limiting PAR, and reduced P_N at high PAR, measured both at limiting [CO_2] and at high [CO_2]. In collards, the relative reduction was largest for quantum yield, and least for P_N measured at high [CO_2].

It is unclear how common it is for the down-regulation of photosynthesis at EC to vary with changes in saccharide status induced by variations in weather. In contrast to collards, down regulation of photosynthesis at EC persisted after several cloudy days in strawberry (Bunce 2001) and wheat and barley (Bunce, unpublished data), and occurred in the dim light of forest understories in other species (Osborne *et al.* 1997, Takeuchi *et al.* 2001). In such cases it is difficult to envision source-sink imbalance as the cause of down-regulation of photosynthesis at EC. In soybean, day-to-day variation in the occurrence of down-regulation of photosynthesis at EC was related to water stress (Bunce and Sicher 2001), and was not correlated with leaf saccharide content. In strawberry, seasonal patterns of down-regulation of photosynthesis were not related to source-sink balance (Bunce 2001).

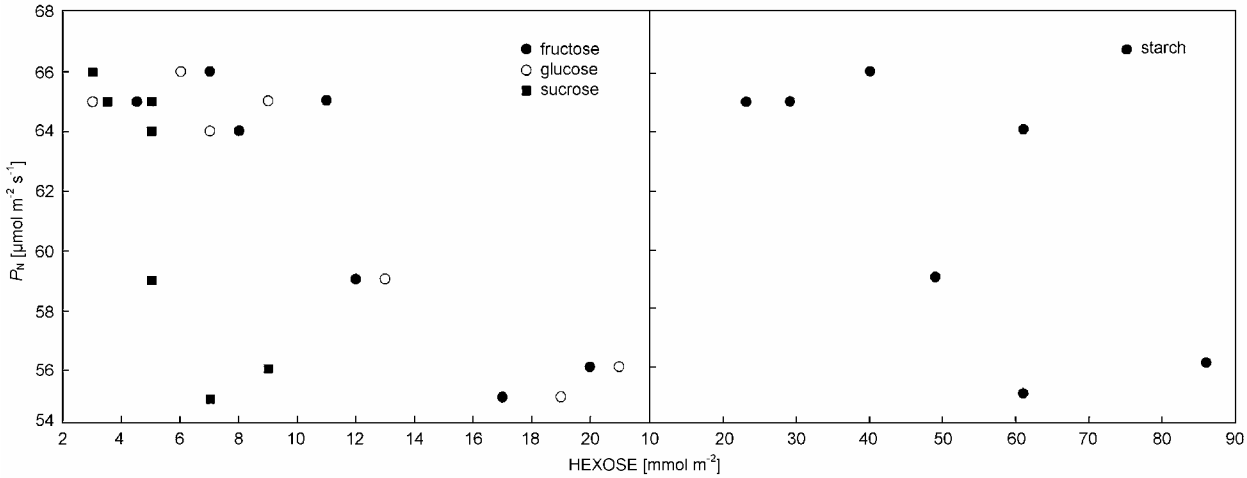

Fig. 4. Relationships between net photosynthetic rate (P_N) and the fructose, glucose, sucrose, and starch contents of collard leaves grown in controlled environment chambers at 350 and 700 µmol(CO_2) mol^{-1}, or grown under those conditions but switched to altered PAR, night temperature, or [CO_2] conditions for one day prior to measurement (see text for details). P_N was measured at 700 µmol(CO_2) mol^{-1}. Each point represents a mean of measurements on leaves of six plants.

Plant growth is enhanced by EC and there is a corresponding increase in the demand for soil nutrients. Stitt and Krapp (1999) have argued that changes of photosynthetic capacity and of leaf constituents in response to CO_2 enrichment are primarily the result of an induced N insufficiency. Increased leaf starch and decreased leaf contents of soluble protein, RuBPCO, and Chl contents have all been associated with plant growth at suboptimal N. Also, photosynthetic acclimation to EC was greater in experiments that employed limiting compared to sufficient N (*e.g.* Geiger *et al.* 1999). One important and still unsettled question is whether or not the inhibition of photosynthesis by EC can occur when the N supply fully meets the demand. Farage *et al.* (1998) showed that acclimation to EC was not observed in young wheat plants grown hydroponically in controlled environments with a constant level of optimal N. These findings contrast with the present field study using collard and kohlrabi. Lack of a decrease in nitrogenous compounds, including RuBPCO protein, soluble protein, and Chl, during down-regulation of photosynthesis at EC in collards suggests that neither nitrogen deficiency nor saccharide-induced suppression of gene expression occurred in response to the source-sink imbalance in this case. Triose phosphate utilization rate also did not limit P_N in collards at EC under the conditions in which down-regulation of photosynthesis was observed. These three proposed mechanisms for the down regulation of photosynthesis by source-sink imbalance did not seem to be operating. The field data suggested a more direct role of saccharide accumulation in causing the down regulation of photosynthesis.

In the controlled environment studies with collards, saccharide sampling was more closely coordinated with the P_N measurements, and there were clear relationships between the inhibition of photosynthesis and glucose, fructose, and sucrose contents. Both the response of photosynthetic down regulation at EC and the relationships between P_N and glucose and fructose suggest threshold rather than gradual responses.

Saccharide accumulation in source leaves has been correlated with an inhibition of photosynthesis in a number of plant species. Azcón-Bieto (1983) observed reduced P_N in wheat leaves shortly after saccharide contents were increased either in response to CO_2 enrichment or when chilling the base of the leaf inhibited export. Blechschmidt-Schneider *et al.* (1989) also observed an inhibition of photosynthesis and a 5- to 6-fold increase of soluble sugars when petioles of the C_4 plant *Amaranthus edulis* were cold girdled. In the latter study the inhibition of photosynthesis disappeared when excess soluble saccharides were removed during a 14-h dark period. Although limitation of photosynthesis by triose phosphate utilization rate has been proposed as a mechanism for feedback inhibition (Sharkey 1985, Socias *et al.* 1993, Winder *et al.* 1998), Bagnall *et al.* (1988) demonstrated that feedback inhibition of photosynthesis may occur without the $[O_2]$ and $[CO_2]$ insensitivity symptomatic of triose phosphate utilization rate limitation. Several prior studies have suggested a direct correlation between the inhibition of photosynthesis at EC and increased leaf saccharide content, but have varied in the saccharide fraction implicated in the inhibition. For example, in rooted single leaves of soybean, Sawada *et al.* (2001) concluded that EC reduced photosynthesis measured at limiting $[CO_2]$ by the accumulation of starch granules which slowed the diffusion of CO_2 to the sites of fixation. Starch content was highly correlated with the inhibition of photosynthesis in cotton (Sasek *et al.* 1985), beech (Rey and Jarvis 1998), and potato (Sicher and Bunce 2001). On the other hand, Lewis *et al.* (2002) found in cocklebur that the inhibition of photosynthesis at EC caused by source-sink imbalance was highly correlated with soluble saccharides. A mechanism for the inhibition of photosynthesis by soluble saccharides has not been established, although a direct osmotic effect has not been ruled out. For example, the changes in sugar concentrations observed in collards between days with and without down-regulation of photosynthesis (Fig. 4) would result in changes in osmotic potential of approximately 0.2 to 0.3 MPa if the sugars were evenly distributed in tissue water. Equivalent changes in osmotic potential would result from roughly a reduction in of 20-30 % in relative water content, which could be sufficient to inhibit RuBPCO and photosynthesis (*e.g.* Bunce 1986, Parry *et al.* 2002). Inhibition of photosynthesis by starch and soluble saccharides are not mutually exclusive and their relative importance may vary with species.

Overall, the results suggest that down-regulation of photosynthesis at EC in collards in the field represented feedback inhibition from the accumulation of soluble saccharides which was attributable to an imbalance between sources and sinks for saccharides. Day-to-day variation in the occurrence of feedback inhibition of photosynthesis at EC was predictable from PAR.

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