Interactions of CO₂ Enrichment and Temperature on Carbohydrate Production and Accumulation in Muskmelon Leaves

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Abstract. We examined how temperature and stage of vegetative growth affect carbohydrate production and accumulation in *Cucumis melo* L. 'Haogen' grown at various CO₂ concentrations ([CO₂]). Carbohydrate production was measured by net assimilation rate either on a leaf-area basis (NAR_a) or a leaf dry-weight basis (NAR_w); carbohydrate accumulation was measured by leaf starch plus sugar content. Twenty-four- and 35-day-old muskmelon plants were grown for 11 days in artificially lighted cabinets at day/night temperatures of 20/20 or 40/20C and at [CO₂] of 300 or 1500 µl-liter⁻¹. NAR_a and NAR_w both increased with increasing [CO₂], but the CO₂ effect was smaller at low temperature, especially for plants at the later stage of vegetative growth. NAR_w was a better indicator of total dry-weight gain than was NAR_a. Both suboptimal temperatures and CO₂ enrichment caused carbohydrates to accumulate in the leaves at both stages of vegetative growth. NAR_w was correlated negatively with leaf starch plus sugar content. The rate of decrease in NAR_w with increasing leaf starch plus sugar content was significantly greater for CO₂-enriched plants. Leaf starch plus sugar content >0.03 to 0.04 kg·kg⁻¹ of leaf residual dry weight at the end of a dark period may indicate that temperature is suboptimal for growth. Plants grown at the same temperature had higher leaf starch plus sugar content if they were CO₂-enriched than if grown in ambient [CO₂], suggesting that an optimal temperature for growth in ambient [CO₂] may be suboptimal in elevated [CO₂].

Carbon dioxide enrichment has been used by the greenhouse industry for many years to increase crop growth and yield (Wittwer, 1986). The decision to inject CO₂ into the greenhouse atmosphere will only make economic sense if atmospheric [CO₂] is the only important environmental variable that is limiting growth and if other important variables such as temperature are, if not optimal, at least nonlimiting. We can begin to quantify optimal combinations of temperature and [CO₂] for a particular crop by examining how these two factors affect the balance between carbohydrate production and use within the plant.

Carbon dioxide enrichment normally increases net photosynthesis of plants (Gaastra, 1959; Potvin and Strain, 1985), but the magnitude of this increase depends on temperature (Hopen and Ries, 1962; El Sharkawy et al., 1968; Hofstra and Hesketh, 1975) when other factors such as light do not limit photosyn-

thesis. NAR_a, which expresses dry-weight gain on a leaf-area basis, can be used to quantify photosynthetic efficiency of the plant. Its sensitivity to temperature has been reported for various crops (Warren Wilson, 1966b). The optimum temperature for MAR, is not the same for all species. Warren Wilson (1966a) found that the optimum temperature for rape, sunflower, and maize plants lay between 20 and 30C, with rape having the lowest optimum and maize the highest. The effect of a given temperature on NAR_a depends on its proximity to the optimum for that species. Optimum temperature may also change with CO₂ enrichment. Acock and Allen (1985), on the basis of numerous studies (Hofstra and Hesketh, 1969; Enoch and Hurd, 1977; Osmond et al., 1980; Jurik et al., 1984), suggested that the temperature optimum for photosynthesis increases with CO₂

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Abbreviations: CLW, weight of starch plus sugar; [CO₂], CO₂ concentration; LER, leaf area expansion rate; NAR_a, net assimilation rate, leaf-area basis; NAR_w, net assimilation rate, dry-weight basis; PAR, photosynthetically active radiation; PPFD, photosynthetic photon flux density; RGR, relative growth rate; RLW, residual leaf dry weight; SLA, leaf area/RLW ratio; TLW, total leaf dry weight.

enrichment up to a maximum of ≈ 35 to 40C. Temperatures above this maximum begin to damage enzymes, causing a rapid fall in photosynthetic rate.

Carbon dioxide enrichment will not have its maximum effect on plant growth rate unless increases in carbohydrate production are accompanied by increases in carbohydrate use. Carbon dioxide enrichment and temperature both act on carbohydrate production, while temperature is the more influential of the two variables in increasing carbohydrate mobilization and/or use. Temperature has been shown to have an effect on mobilizing carbohydrate from leaves (Hartt, 1965; Potvin et al., 1984), on leaf expansion (Hesketh and Baker, 1969), on side shoot expansion (Chatterton et al., 1972), and on the development of other plant organs (Thorne and Koller, 1974; Hall and Brady, 1977). When CO_2 enrichment is combined with temperatures that are suboptimal for organ expansion, carbohydrate use may be reduced before carbohydrate production, causing the observed accumulation of starch and sugars in leaves at suboptimal temperatures (Warren Wilson, 1966a; Hatch and Glasziou, 1963; Chatterton et al., 1972). Also, the finding that CO_2 enrichment causes starch and sugars to accumulate in leaves (Hofstra and Hesketh, 1975; Nafziger and Koller, 1976) indicates that carbohydrate use can quickly fall behind carbohydrate production. Accumulation of starch and sugar is often associated with reduced photosynthesis (Warren Wilson, 1966b; Neales and Incoll, 1968; Warrington et al., 1977), a situation that helps restore a balance between carbohydrate production and use.

One way to determine how $[\text{CO}_2]$ and temperature affect carbohydrate production and use is to examine diurnal variations in leaf starch plus sugar content under various temperature and $[\text{CO}_2]$ regimes. Accumulations of starch and sugar still present in leaves at the end of 10 hr of darkness may imply that carbohydrate production is exceeding use. Low carbohydrate pools might indicate that production and use are well-balanced or that plant growth rate is being limited by insufficient carbohydrate production.

The experiment reported here examined how temperature and vegetative growth stage affected carbohydrate production and accumulation by 'Haogen' muskmelon plants grown at various $[\text{CO}_2]$. Carbohydrate production was measured as net assimilation rate and carbohydrate accumulation as starch plus sugar content of leaves.

Materials and Methods

Muskmelon seeds were germinated at 30C in coarse sand in a plant growth cabinet. After the appearance of the first true leaf, 50 seedlings of uniform size were transplanted to pots containing full-strength Hoagland no. 1 nutrient solution at a pH of 6.5 (Hewitt, 1952) and were grown in ambient $[\text{CO}_2]$, $30 \pm 1\text{C}$ and a PPFD of $208 \pm 12 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ (400 to 700 nm) during a 14-hr day until the start of treatment, which was either at 24 or 35 days after sowing.

At the start of each 11-day treatment, 25 plants were selected on the basis of plant height and allocated to one of four environments. One subset of these plants was destructively sampled to give initial values for NAR_a and NAR_w calculations. Mean total dry weight, leaf dry weight, and leaf area per plant were 0.64 g, 0.39 g, and 0.0164 m^2 , respectively, at 24 days, and 3.95 g, 2.58 g, and 0.1421 m^2 at 35 days. Environments were maintained in Saxcil (R.K. Saxton, Arc Works, Bredbury, Cheshire, United Kingdom) growth cabinets. Each cabinet had a plant floor area of $1.4 \times 1.4 \text{ m}$ and was illuminated by two banks of warm-white fluorescent lamps (Carpenter, 1966). Days

were at $20 \pm 0.5\text{C}$ or $40 \pm 0.5\text{C}$ with constant nights of $20 \pm 0.5\text{C}$ for all treatments. $[\text{CO}_2]$ within the cabinets was set at 300 ± 20 or $1500 \pm 60 \mu\text{l}\cdot\text{liter}^{-1}$. $[\text{CO}_2]$ was maintained during the light period by injection of CO_2 from a compressed gas cylinder through a solenoid valve. A conductimetric analyzer was used to measure and control CO_2 injection automatically (Acock et al., 1985). In all cabinets, the PPFD was $208 \pm 12 \mu\text{mol}(\text{photons})\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ (PAR) throughout a 14-hr day. Water vapor pressure deficit was $0.7 \pm 0.1 \text{ kPa}$ at 20C and $1.3 \pm 0.1 \text{ kPa}$ at 40C, representing 70% and 82% RH, respectively.

After 11 days of treatment, plants were sampled to determine the dry weight of their parts and specific leaf area. NAR_a was calculated from measurements at the beginning and end of each treatment period using the following equation (Evans, 1972): $\text{NAR}_a = (W_f - W_i)/(A_f - A_i) \times [\log_e (A_f/A_i)]/(t_f - t_i)$, where W_f is total plant dry weight at the end of the measurement period, W_i is total plant dry weight at the beginning of the measurement period, A_f is final leaf area, A_i is initial leaf area, and $t_f - t_i$ is the time period between measurements.

On the day preceding final harvest, leaves of plants from all treatments were sampled for starch plus sugar content. Leaf disks were punched at the beginning of the light period (0 hr), then at 4, 9, 14, and 19 hr. Sugars were extracted from the oven-dried leaf disks with 80% (v/v) ethanol at 80C for 2 hr and then with water at 90C for 1 hr. The extracts were combined, hydrolyzed with 0.25 M HCl, and the resulting sugars were measured with an autoanalyzer system (Fuller, 1966). Starch in the leaf residue was hydrolyzed to reducing sugars with β -glucanase-free amyloglucosidase (EC 3.2.1.3) (MacRae, 1971) and measured in the same way.

Starch plus sugar contents were expressed on the basis of unit residue leaf dry matter to eliminate spurious effects caused by changes in nonstructural carbohydrates (Priestley, 1973). For this purpose, residual dry matter was taken as leaf dry matter minus starch and sugar, $\text{RLW} = \text{TLW} - \text{CLW}$, where RLW is the residual leaf dry weight, TLW is the total leaf dry weight, and CLW is the weight of starch plus sugar in the leaf sample. Starch plus sugar content is the ratio CLW/RLW .

RGR was calculated from the equation $\text{RGR} = \log_e(W_f/W_i)/(t_f - t_i)$; (Evans, 1972). Net assimilation rate was calculated on a RLW basis using $\text{NAR}_w = (W_f - W_i)/(\text{RLW}_f - \text{RLW}_i) \times [\log_e (\text{RLW}_f/\text{RLW}_i)]/(t_f - t_i)$. LER was calculated using $\text{LER} = \log_e (A_f/A_i)/(t_f - t_i)$. Leaf residue dry weight gain (LWG) was calculated using $\text{LWG} = \log_e(\text{RLW}_f/\text{RLW}_i)/(t_f - t_i)$.

Results and Discussion

Specific leaf area. All factors studied, including stage of vegetative growth, temperature, and CO_2 enrichment, influenced SLA, but there were no significant interactions among them. Young vegetative plants had a mean SLA value of $35.6 \text{ m}^2\cdot\text{kg}^{-1}$, whereas that for the older plants was $30.3 \text{ m}^2\cdot\text{kg}^{-1}$.

LER at 40/20C was at least 1.4 times that at 20/20C for both growth stages and for both $[\text{CO}_2]$ (Table 1). RLW did not keep up with LER at the higher temperature, so the high temperature produced less-dense leaves. SLA at 40/20C was $38.9 \text{ m}^2\cdot\text{kg}^{-1}$ and at 20/20C it was $27.0 \text{ m}^2\cdot\text{kg}^{-1}$.

Carbon dioxide enrichment produced a greater RLW per unit leaf area, giving SLA values 8% heavier for those in an enriched atmosphere than for those in an unenriched atmosphere. Anatomical studies have shown that more palisade cells are produced under CO_2 enrichment (Hofstra and Hesketh, 1975), increasing photosynthetic efficiency per unit leaf area but also increasing leaf dry weight per unit leaf area.

Table 1. Means $\pm$ SE for NAR_a , NAR_w , LER, leaf residual dry weight gain (LWG), and RGR of 'Haogen' muskmelon plants at two growth stages and held at various temperatures and CO_2 combinations.

Diurnal temperature (°C)	[CO ₂] (μl·liter ⁻¹)	Plant growth criteria				
		NAR _a (g·m ⁻² ·day ⁻¹)	NAR _w (g·kg ⁻¹ ·day ⁻¹)	LER (m ² ·m ⁻² ·day ⁻¹)	LWG (mg·g ⁻¹ ·day ⁻¹)	RGR (g·kg ⁻¹ ·day ⁻¹)
24 to 35 days after sowing						
20/20	300	8.28 ± 0.143	298 ± 15.2	0.143 ± 0.0066	169 ± 8.85	173 ± 8.57
40/20	300	9.17 ± 0.198	396 ± 22.2	0.207 ± 0.0048	211 ± 9.16	220 ± 10.1
20/20	1500	10.7 ± 0.550	383 ± 19.9	0.163 ± 0.0065	201 ± 10.2	206 ± 9.72
40/20	1500	13.5 ± 0.400	533 ± 10.9	0.247 ± 0.0070	250 ± 3.63	271 ± 2.91
35 to 46 days after sowing						
20/20	300	5.74 ± 0.373	237 ± 9.41	0.075 ± 0.0055	126 ± 2.95	137 ± 4.06
40/20	300	6.22 ± 0.529	295 ± 14.8	0.104 ± 0.0071	131 ± 9.45	156 ± 8.20
20/20	1500	6.50 ± 0.275	254 ± 3.67	0.077 ± 0.0070	146 ± 1.66	147 ± 1.11
40/20	1500	9.37 ± 0.405	418 ± 11.0	0.139 ± 0.0023	178 ± 3.09	207 ± 3.92

Fig. 1. Daily net assimilation rate of muskmelon, expressed as NAR_a or NAR_w , calculated from measurements taken before and after 11 days of treatment at 20/20 or 40/20°C with [CO₂] at 300 or 1500 μ l·liter⁻¹ and plotted against total dry weight gain for the same period. (A) NAR_a , plants 24 to 35 days after sowing; (B) NAR_w , plants 24 to 35 days after sowing; (C) NAR_a , plants 35 to 46 days after sowing; (D) NAR_w , plants 35 to 46 days after sowing.

Net assimilation rate. Plant photosynthetic efficiency is sometimes measured as the rate of increase in total dry matter per unit photosynthetic leaf surface area. Under environmental conditions such as CO₂ enrichment, where the density of chloroplasts has been observed to increase within the leaf, it may be more appropriate to examine photosynthetic efficiency per unit leaf dry weight rather than per unit leaf area. Therefore, in this study, net assimilation rate was calculated both as NAR_w

and the more usual NAR_a . Both measurements were related to plant dry-weight gain.

NAR_a and NAR_w for young vegetative plants were higher than for older ones (Table 1). As plants grow, the leaves begin to shade each other from the light source, lowering the average intensity to which leaves are exposed. This change not only has the effect of lowering the value of NAR_a and NAR_w for older plants, but it may explain why the younger plants were more

Fig. 2. Leaf starch plus sugar content per unit leaf residue dry weight measured on leaf disks punched from attached muskmelon leaves. Leaf disks were taken at five intervals during the 24-hr period just preceding leaf harvest. (A) 34 days, (B) 45 days after sowing. (□) 20/20C, 300 $\mu\text{l-liter}^{-1}$ $[\text{CO}_2]$; (◆) 20/20C, 1500 $\mu\text{l-liter}^{-1}$ $[\text{CO}_2]$; (■) 40/20C, 300 $\mu\text{l-liter}^{-1}$ $[\text{CO}_2]$; (◇) 40/20C, 1500 $\mu\text{l-liter}^{-1}$ $[\text{CO}_2]$.

responsive to CO_2 enrichment (Table 1). If the photosynthetic efficiency of leaf area becomes limited by light rather than $[\text{CO}_2]$ in older plants, then CO_2 enrichment is not likely to have much effect. Young plants, having more light intercepted per unit leaf area, are therefore less likely to be limited by light and may be limited by ambient $[\text{CO}_2]$. In this case, CO_2 enrichment should have the larger positive effect that was measured.

Certain combinations of temperature and $[\text{CO}_2]$ can have a dramatic promoting effect on NAR_a and NAR_w (Table 1). Temperature alone made little difference to NAR_a for either the younger or older vegetative plants, but when the higher temperature was coupled with CO_2 enrichment, NAR_a increased synergistically for plants at both vegetative growth stages tested. The same general pattern was observed if net assimilation rate was measured on a leaf dry-weight basis, except that NAR_w was significantly higher at the higher temperature.

To determine which method of measuring net assimilation rate was the better indicator of biological yield, total plant dry-weight gain was regressed on NAR_a and NAR_w for each growth

Fig. 3. NAR_w plotted against leaf starch plus sugar content for muskmelon plants grown with $[\text{CO}_2]$ at (□) 300 $\mu\text{l-liter}^{-1}$ ($y = 0.0606 - 0.0000761x$; $r^2 = 0.69$) and (◆) 1500 $\mu\text{l-liter}^{-1}$ ($y = 0.174 - 0.000256x$; $r^2 = 0.90$) for all combinations of day/night temperature and stage of vegetative growth used in the experiment.

stage (Fig. 1). Both measurements correlated well with total plant dry-weight gain. In the first growth stage, NAR_a could account for 77% of the variation in total dry-weight gain, while NAR_w could account for 88%. The relationship between dry-weight gain and NAR_w was further improved (92% of variation) by regressing $\log_e$ (dry-weight gain) on NAR_w . No improvement was achieved by regressing $\log_e$ (dry-weight gain) on NAR_a . In the older vegetative plants, NAR_a could account for 90% of the variation in total dry-weight gain, while NAR_w could account for 98%. Our results suggest that NAR_w may be a slightly better indicator than NAR_a for measuring carbohydrate production under the conditions of this experiment.

One possible explanation for higher NAR_w and NAR_a at 40/20C is that the optimal temperature for photosynthetic enzymes in muskmelon may be closer to 40/20C than to 20/20C. Another explanation is that leaf growth rate is strongly suppressed by the lower temperature, causing starch and sugars to build up in the leaves and that this suppresses photosynthetic activity. Certainly, the relative growth rate was much lower at 20/20C than at 40/20C (Table 1). If the lower NAR_w at 20/20C had been caused by limited growth, one would expect that CO_2 enrichment would have had no effect at 20/20C but, in fact, for younger plants it did increase NAR_w (Table 1). Photosynthesis and growth rate likely were reduced by the lower temperature.

To give some indication of how various treatments might affect the balance between photosynthesis and growth, we examined diurnal variation of leaf starch plus sugar content.

Leaf carbohydrate content. Starch plus sugar content of leaves over the course of a day was plotted for the two stages of vegetative growth (Fig. 2). The lowest levels of leaf starch plus sugar content were found near the end of the dark period. For treatments such as ambient $[\text{CO}_2]$ at 40/20C, where leaves had a carbohydrate content of 0.03 to 0.04 $\text{kg}\cdot\text{kg}^{-1}$ at the end of the dark period, the plants were using nearly all carbohydrate produced during the day and may have been source-limited. For treatments where carbohydrate content was >0.03 to 0.04 $\text{kg}\cdot\text{kg}^{-1}$ at the end of the dark period, the plants may have been sink-limited. Plants at 20/20C probably accumulated starch and sugar because temperature was limiting organ expansion.

Carbohydrate content tended to increase during the 14-hr light period for all treatments (Fig. 2). Accumulation was enhanced by low day temperature and by CO₂ enrichment and was greatest when CO₂ enrichment was combined with low temperature.

The tendency for plants grown at 40/20C to retain only small amounts of carbohydrate in their leaves, even when grown at high [CO₂] (Fig. 2), suggests that they either remobilized or used more carbohydrate than those grown at 20/20C.

To find out whether carbohydrate accumulation in leaves was associated with lower NAR_w values, we plotted NAR_w against leaf carbohydrate content for each [CO₂] treatment (Fig. 3), using the carbohydrate content at the end of the dark period when values were lowest. NAR_w was correlated negatively with leaf starch plus sugar content at the end of a dark period and the relationship was highly dependent on atmospheric [CO₂] (Fig. 3). The rate of decrease in NAR_w with increasing starch plus sugar content was significantly greater for elevated [CO₂] than for ambient [CO₂] (*t* test for homogeneity of regression coefficients = 4.92, df = 4). The points on the regression represent observations at 20/20C and 40/20C and at two stages of vegetative growth, suggesting that the relationship between NAR_w and leaf starch plus sugar content is robust for any given atmospheric [CO₂].

NAR_w was a slightly better indicator of total plant dry-weight gain than was NAR_a under the conditions of this experiment. Starch and sugar accumulations in leaves do not reduce NAR_w directly. High accumulations and high NAR_w were observed under CO₂ enrichment. The rapid decline in NAR_w with increased leaf starch plus sugar content suggests that accumulations of starch and sugar in leaves at dawn should be minimized to maintain highest NAR_w. To maintain high NAR_w under CO₂ enrichment, it may be necessary to adjust temperature to a level higher than is considered optimum in ambient [CO₂]. Using NAR_w values and carbohydrate accumulation in leaves as a guide, our results suggest that the best balance between carbohydrate production and use for 'Haogen' muskmelon plants during vegetative growth is achieved by not allowing leaf starch plus sugar content to exceed 0.04 kg·kg⁻¹ at dawn.

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