Influence of Inverse Day/Night Temperature on Ozone Sensitivity and Selected Morphological and Physiological Responses of Cucumber

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Abstract. *Cucumis sativus* L. (cvs. Poinsett and Ashley) plants were grown from seed in a growth chamber at a +10C (28/18) or a -10C (18/28) difference (DIF) between day temperature (DT) and night temperature (NT) on a 12-hour photoperiod for 24 days prior to ozone (O₃) fumigation (3 hours at 0.5 $\mu\text{mol}\cdot\text{mol}^{-1}$). Negative DIF, compared to +DIF, reduced plant height, node count, fresh weight, dry weight, and leaf area in both cultivars. Photosynthetic rate (Pn), chlorophyll concentration, and variable chlorophyll fluorescence (F_v) were lower and O₃ injury and polyamine concentrations were higher at -DIF than at +DIF. Ozone fumigation generally increased leaf concentration of polyamines and reduced Pn, stomatal conductance, and chlorophyll fluorescence. 'Poinsett' generally had a higher specific leaf mass and higher concentrations of chlorophyll *a* and polyamines than did 'Ashley', but there was no cultivar difference in O₃ injury, growth response, Pn, or stomatal conductance.

Differences (DIF) between day temperature (DT) and night temperature (NT) have long been known to regulate stem elongation (Went, 1944). Plants grown under -DIF (NT > DT) are typically shorter than those grown under +DIF (DT > NT) or under a smaller -DIF (Berghage et al., 1991; Erwin et al., 1992; Pinthus and Meiri, 1979). Pinthus and Meiri (1979) reported that reversal of D/N temperature from 18/10C to 10/18C reduced leaf and stem elongation and promoted tillering in wheat (*Triticum aestivum* L.). Similar effects of -DIF have been observed in other species (Erwin et al. 1992).

In *Dendranthema grandiflora* Tzvelev 'Bright Golden Anne', *Fuchsia* $\times$ *hybrida* 'Dollar Princess', and *Pelargonium hortorum* L.H. Bailey 'Red Elite', Erwin et al. (1991) reported that both total chlorophyll concentration and the chlorophyll *a/b* ratio were affected by the difference between DT and NT and the average daily temperature. Total chlorophyll per unit of leaf area and per unit of leaf dry weight increased with increasing +DIF. The chlorophyll *a/b* ratio calculated on a per-unit area and per-unit dry-weight basis increased with decreasing DIF. Total chlorophyll and chlorophyll *a/b* ratio decreased as the average daily temperature under which the plants were grown increased.

Recent studies by Agrawal and co-workers (unpublished data) indicated that *Phaseolus vulgaris* L. ('Bush Blue Lake 290') plants seeded and grown for 1 week in the greenhouse and then grown in a growth chamber at -10C DIF (18/28C) for 2 weeks before O₃ fumigation were more tolerant of O₃ than those grown for 2 weeks at +10C DIF (28/18C). They found that reduction in O₃ sensitivity was accompanied by reductions in stem elongation and biomass

accumulation. The possibility that a-DIF regime might ameliorate air pollution injury in cucumber, a species known to be sensitive to O₃ and SO₂ (Beckerson and Hofstra, 1980; Hofstra et al., 1985), was examined in this study.

Plant response to O₃ and other air pollutants has been studied extensively (Heck et al., 1988; Krupa and Manning, 1988). Investigators have long recognized that environmental conditions before, during, and after fumigation are important in influencing plant sensitivity to air pollution injury (Heck et al., 1988; Kobriger and Tibbitts, 1985; Krupa and Manning, 1988). Relatively few studies, however, have examined the role of temperature pretreatment on O₃ sensitivity (Adedipe and Ormrod, 1974).

Krizek et al. (1986b) reported that coleus (*Coleus blumei* Benth.) plants grown at 13C for 5 days before SO₂ fumigation showed decreased stomatal conductance and SO₂ sensitivity in comparison to those grown at 20C. This was associated with increased endogenous abscisic acid (ABA) (Terry et al., 1986). Similarly, poinsettia [*Euphorbia pulcherrima* (Willd. ex Klotzsch)] plants subjected to water stress or given ABA showed reduced SO₂ damage (Krizek et al., 1986a). Application of inhibitors of gibberellin biosynthesis and other growth retardants were effective in reducing stem elongation and in ameliorating air pollution damage (Ormrod and Adedipe, 1974; Steffens et al., 1985).

Polyamines accumulate under various stress conditions, including drought (Kandpal and Rao, 1985; Turner and Stewart, 1986), osmotic stress (Flores and Galston, 1982), UV-B radiation (Kramer et al., 1991b), chilling injury (Guye et al., 1986; Kramer and Wang, 1989), and O₃ pollution (Kramer et al., 1991a; Langebartels et al., 1991; Rowland-Bamford et al., 1988, 1989). Exogenous application of polyamines has provided protection against O₃ (Bors et al., 1989; Ormrod and Beckerson, 1986) and chilling injury (Kramer and Wang, 1989; Songstad et al., 1990). Amelioration of O₃ injury by polyamines has been linked to their ability to stabilize cell membranes (Altman et al., 1977), scavenge free radicals (Drolet et al., 1986), and reduce or prevent lipid peroxidation (Kramer and Wang, 1989, 1990; Tadolini, 1988).

The activity of arginine decarboxylase (ADC), a key rate-limiting enzyme involved in polyamine biosynthesis, increased in

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barley (*Hordeum vulgare* L.) (Roland-Bamford et al., 1989) and tobacco (*Nicotiana tabacum* L.) (Langebartels et al., 1991) plants following O₃ exposure. Application of DL- α -difluoromethylarginine (DFMA), a specific inhibitor of ADC, prevented the rise in ADC activity and was associated with increased visible O₃ injury (Rowland-Bamford et al., 1989). Based on these findings, polyamine induction in response to O₃ may be a defensive or adaptive response of plants to stress.

The objectives of this study were to: a) determine the influence of DIF on O₃ sensitivity in cucumber and to relate changes in polyamines and ozone sensitivity in DIF-treated plants, and b) quantify the effects of DIF on dry matter production and selected physiological and biochemical responses.

Materials and Methods

Plant material and cultural conditions. Two cultivars of cucumber found previously to differ in sensitivity to UV-B radiation were used—'Poinsett' (highly sensitive) and 'Ashley' (relatively insensitive) (Krizek, 1978). Seeds were planted in 15-cm green plastic pots containing 1500 cm³ of a peat-vermiculite mix (Jiffy Mix, Jiffy Products of America, West Chicago, Ill.). One week after germination, the seedlings were thinned to one per pot. Plants were watered one to two times daily and fertilized weekly with Peters (W.R. Grace & Co., Fogelsville, Pa.) (20N-8.7P-16.6K) nutrient solution at 386 mg N/liter.

Environmental conditions. Experiments were conducted from Aug. to Oct. 1990 in two adjacent Revco Model 511-38 (Revco Scientific, Asheville, N.C.) plant growth chambers at the USDA Climate Stress Laboratory, Beltsville, Md. Plants were seeded and grown on a 12-h (0830-2030 HR) photoperiod at 320 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of photosynthetic photon flux (PPF) provided by General Electric (GE) 1500-mÅ cool-white fluorescent and GE 52-W supplemental incandescent lamps (to provide $\approx 10\%$ of the total input wattage). Plants were grown at 70% $\pm$ 5% RH, ambient CO₂, and air temperatures specified below. Half of the plants were maintained in a growth chamber at +10C DIF (28/18C) and half was kept in a growth chamber at -10C DIF (18/28C). Differential temperature treatment was given for 24 days (from time of seeding) before O₃ fumigation.

Fumigation conditions. On day 25, the plants were fumigated in a Conviron Model PGW 36 (Conviron, Asheville, N.C.) plant growth chamber at 28 $\pm$ 1C, 70% $\pm$ 5% RH, and 320 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF provided by GE 1500-mÅ cool-white fluorescent lamps and GE 52-W incandescent lamps. Plants were equilibrated in the chamber for 1 to 2 h and then fumigated for 3 h at an O₃ concentration of 0.50 $\mu\text{mol}\cdot\text{mol}^{-1}$. This high O₃ concentration was used to induce visible injury symptoms based on our preliminary experiments. Non-fumigated plants were maintained in a separate Conviron growth chamber under similar environmental conditions, but without O₃. After fumigation, some plants were taken for physiological and biochemical measurements. The remaining plants were returned to their original growth chambers, where they were kept for 48 to 72 h, and then scored for O₃ injury.

Growth. Measurements of stem height, node count, fresh and dry weight of tops, and leaf area were determined after 24 days of temperature treatment. Plant height was measured from the cotyledonary node to the uppermost node. Leaf areas of individual leaves were determined with a LI-COR LI-3000 Leaf Area Meter (LI-COR, Lincoln, Neb.). After determination of top fresh weights, the samples were dried in a forced-draft oven at 70C for 48 h and then weighed. Percent dry weight and specific leaf mass (SLM, ratio of leaf mass to leaf area on a dry-weight basis) were obtained by calculation.

Ozone injury. Ozone injury of leaves was scored 48 to 72 h after O₃ fumigation from 0 (no injury) to 5 (100% injury) based on the leaf percentage showing necrosis. Only fumigated plants were included in the statistical analysis.

Gas exchange. Measurements of photosynthetic rate (Pn) and stomatal conductance were obtained on the second leaf from the base of the plant 21 to 24 h after fumigation with a LI-COR Model LI-6200 Portable Photosynthesis System under conditions described above.

Chlorophyll concentration. Chlorophyll extractions were made in 4 cm³ of 80% (v/v) acetone using six leaf discs (5 mm in diameter) per sample. Leaf discs were taken from the central portion of the leaf, avoiding the midrib. After 48 h in darkness at 4C, the leaf discs were washed with 4 cm³ of 80% acetone, and the volume was brought up to 10 cm³. Concentration of chlorophyll a, chlorophyll b, total chlorophyll, and the chlorophyll *a/b* ratio were calculated using equations provided by Arnon (1949), after measurement of the absorbance at 645 and 663 nm on a Shimadzu Model UV 160-A Recording Spectrophotometer (Columbia, Md.).

Chlorophyll fluorescence induction. Measurements of chlorophyll fluorescence induction kinetics were made at room temperature (23C) with a Model MF-1 portable chlorophyll fluorometer (Univ. of Missouri, Columbia) immediately following O₃ fumigation, as described by Miles (1990). This instrument was connected to a personal computer equipped with hardware and software to emulate a digital oscilloscope (Rapid Systems Inc., Seattle). The adaxial leaf surface was irradiated with a red light-emitting diode and the fluorescence signal was collected from the same surface. Leaves of all plants were dark-adapted for 6 min before measurements were carried out. Chlorophyll fluorescence characteristics such as initial fluorescence (F_o), maximum fluorescence (F_{max}), variable fluorescence (F_v = F_{max} - F_o), and the ratio F_v/F_o, were compared in the two cultivars.

Polyamine concentration. Leaf polyamine concentration was determined 24 h after O₃ fumigation. Tissue samples (≈ 0.6 g fresh weight) were taken from the second leaf from the base of the plant (after excising the midrib) and homogenized in 10 cm³ 5% (v/v) perchloric acid using a Polytron (Brinkmann, Westbury, N.Y.). Concentrations of free soluble polyamines were determined as dansylated derivatives by HPLC as described by Kramer and Wang (1989) and Kramer et al. (1991b).

Statistical analysis. Duplicate experiments were conducted during Aug. to Oct. 1990. A randomized complete block design was used. Data were collected and analyzed for three plants of each cultivar, temperature, and O₃ combination.

Results

Growth. Plants grown at -DIF produced one-half to one-third less growth than those grown at +DIF, based on top fresh weight (data not shown), stem height, leaf area, and top dry weight (Table 1). Plants grown at -DIF also produced one to two fewer nodes than those grown at +DIF (Table 1). Cultivar did not influence stem height, node count, leaf area, or top dry weight (Table 1), or fresh weight and percent dry weight of tops (data not shown). UV-sensitive 'Poinsett' had higher SLM than UV-tolerant 'Ashley' regardless of temperature (Table 1). There was no interaction between temperature and cultivar for any variable.

Ozone injury. Because control plants were free of O₃ injury, no injury scores are shown for these plants (Table 2). Ozone injury in fumigated plants appeared as necrotic flecking within 24 h and was confined largely to the bottom three expanded leaves. Counting acropetally, leaf 2 of cucumber plants grown at -DIF before O₃ fumiga-

Table 1. Growth responses of 'Ashley' and 'Poinsett' cucumber plants grown for 24 days in a growth chamber at -10C (18/28; -DIF) or +10-C (28/18; +DIF) day/night difference.

Day/night temp (°C)	Cultivar	Stem ht (cm)	Node count	Leaf area (cm ²)	Dry wt of tops (mg)	Specific leaf mass (mg·cm ⁻²)
18/28	Ashley	20.4	4.6	195.7	739	28.9
	Poinsett	20.8	4.5	181.4	724	31.2
28/18	Ashley	58.5	6.2	540.4	1775	27.2
	Poinsett	50.5	6.7	522.9	1895	30.2
Source of variation		ANOVA summary (F values)				
Temperature		168.2***	34.8***	36.9***	55.8***	1.7 ^{NS}
Cultivar		2.1 ^{NS}	0.4 ^{NS}	0.1 ^{NS}	0.1 ^{NS}	6.5*
Temperature × cultivar		2.6 ^{NS}	0.9 ^{NS}	0.0 ^{NS}	0.2 ^{NS}	0.1 ^{NS}

^{NS},*,***Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

Table 2. Leaf injury of 'Ashley' and 'Poinsett' cucumber plants 48 h after fumigation with O₃ (0.5 μmol·mol⁻¹) for 3 h following 24 days of growth at -10C (18/28; -DIF) or +10C (28/18; +DIF) day/night difference. Leaves numbered acropetally.

Day/night temp (°C)	Cultivar	O ₃ injury ^z		
		Leaf 1	Leaf 2	Leaf 3
18/28	Ashley	0.6	2.7	0.2
	Poinsett	2.2	4.1	0.7
28/18	Ashley	0.5	0.3	0.1
	Poinsett	0.0	0.0	0.0
Source of variation ^y		ANOVA summary (F values)		
Temperature		4.0 ^{NS}	22.8***	3.2 ^{NS}
Cultivar		1.0 ^{NS}	0.8 ^{NS}	1.0 ^{NS}
Temperature × cultivar		3.8 ^{NS}	1.6 ^{NS}	2.2 ^{NS}

^z0 = no injury to 5 = 100% of leaf injury.

^yTwo-way and three-way interactions not shown were nonsignificant.

^{NS},***Nonsignificant or significant at $P \leq 0.001$, respectively.

tion showed greater injury than at +DIF (Table 2). Temperature had no effect on O₃ injury of leaves 1 or 3. Cultivar did not influence O₃ injury and there was no interaction between cultivar and temperature.

Gas exchange. Plants of both cultivars grown at +DIF had higher Pn than those grown at -DIF, but showed no difference in stomatal conductance (Table 3). Ozone fumigation reduced Pn of 'Ashley' cucumber following +DIF and -DIF by 44% and 65%,

respectively; but had little or no effect on 'Poinsett' (Table 3). Only O₃ fumigation affected stomatal conductance, with reduced values tending to be more pronounced following +DIF (Table 3).

Chlorophyll concentration. Plants grown at -10C DIF (18/28C) had 40% less chlorophyll a and 31% less total chlorophyll on a dry-weight basis than those grown at a +10C DIF (28/18C) (Table 3). Ozone fumigation had no effect on the concentration of chlorophyll a or total chlorophyll (Table 3). There was no cultivar difference in chlorophyll a and total chlorophyll concentration (Table 3). There was no effect of temperature pretreatment, ozone fumigation, or cultivar on chlorophyll *u/b* ratio (data not shown).

Chlorophyll fluorescence induction. Temperature pretreatment and O₃ fumigation affected chlorophyll fluorescence (Table 3). Variable fluorescence (F_v) was lower in O₃-fumigated than in nonfumigated plants, but was unaffected by DIF treatment. The ratio F_v/F₀ was sensitive to both temperature and O₃ stress. There were no cultivar differences in chlorophyll fluorescence (Table 3) and no interactions.

Polyamine concentration. Plants grown in-DIF for 24 days had higher concentrations of spermine (Spn) and spermidine (Spd) than those grown in +DIF when O₃ treatment and cultivar effects were pooled (Table 4). 'Poinsett' leaves had higher concentrations of Spn and Spd than those of 'Ashley' at both +DIF and -DIF, but putrescine (Put) concentrations were unaffected by DIF or cultivar (Table 4). Ozone fumigation increased the concentrations of Put and Spd, but not that of Spn (Table 4).

Table 3. Gas exchange, chlorophyll concentration, and chlorophyll fluorescence of 'Ashley' and 'Poinsett' cucumber plants 21 to 24 h after fumigation with O₃ following 24 days of growth at -10C (18/28; -DIF) or +10C (28/18; +DIF) day/night difference.

Day/night temp (°C)	Cultivar	O ₃ (μmol·mol ⁻¹)	Stomatal conductance (cm·s ⁻¹)	Photosynthetic	Chlorophyll <i>a</i> (μg·g ⁻¹)	Total chlorophyll (μg·g ⁻¹)	F _v	F _v /F ₀
				rate (Pn) (CO ₂ , μmol·m ⁻² ·s ⁻¹)				
18/28	Ashley	0	0.76	5.18	9.0	11.5	76.8	0.41
		0.5	0.59	1.79	9.8	12.8	69.3	0.26
	Poinsett	0	1.03	2.82	10.0	13.0	72.9	0.39
		0.5	0.95	2.15	10.0	13.4	67.2	0.36
28/18	Ashley	0	1.38	11.19	14.8	19.9	76.9	0.38
		0.5	0.73	5.94	12.3	16.8	56.4	0.27
	Poinsett	0	1.10	10.72	17.3	17.3	70.4	0.35
		0.5	0.58	11.03	19.8	19.8	54.0	0.26
Source of variation ^z				ANOVA summary (<i>F</i> values)				
Temperature			0.6 ^{NS}	52.4***	19.6***	22.3***	2.8 ^{NS}	8.6**
Cultivar			0.1 ^{NS}	0.5 ^{NS}	3.5 ^{NS}	3.8 ^{NS}	0.8 ^{NS}	0.5 ^{NS}
Ozone			6.3*	5.8*	0.6 ^{NS}	0.6 ^{NS}	8.5**	9.7**
Cultivar × ozone			0.1 ^{NS}	5.0*	0.2 ^{NS}	0.2 ^{NS}	0.1 ^{NS}	0.2 ^{NS}

^zTwo-way and three-way interactions not shown were nonsignificant.

^{NS},*,***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 4. Leaf concentration of soluble polyamines of 'Ashley' and 'Poinsett' cucumber plants 24 h after fumigation with O₃ following 24 days at -10C (18/28; -DIF) or +10C (28/18; +DIF) day/night difference.

Day/night		O ₃	Polyamine concn (nmol·g ⁻¹ fresh wt)			
temp. (°C)	Cultivar	(μmol·mol ⁻¹)	Spermine	Spermidine	Putrescine	Total
18/28	Ashley	0	46.8	334	55.5	436
		0.5	52.3	383	135.3	572
	Poinsett	0	58.0	381	42.0	481
		0.5	62.6	410	121.1	593
28/18	Ashley	0	37.5	183	54.2	274
		0.5	34.3	204	84.5	323
	Poinsett	0	45.0	193	44.5	283
		0.5	37.6	235	99.1	372
Source of variation ²			ANOVA summary (<i>F</i> values)			
Temperature			31.3***	173.9***	3.1 ^{NS}	99.0***
Cultivar			7.6**	4.8**	0.3 ^{NS}	2.2 ^{NS}
Ozone			0.0 ^{NS}	7.3**	36.0***	21.4***

²Two-way and three-way interactions were nonsignificant.

^{NS}, **, ***Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.

Discussion

Reduced stem elongation of cucumber plants grown at low-day and high-night temperature was accompanied by reduced leaf enlargement and dry matter accumulation (Table 1). This result contrasts with that for wheat grown in -DIF, in which internode length and leaf elongation were reduced, but biomass production was unaffected (Pinthus and Meiri, 1979).

Growing cucumber plants at -DIF proved highly effective in regulating growth. The use of -DIF has been recommended in the commercial production of several important greenhouse crops, including poinsettia, chrysanthemum, and Easter lily (*Lilium longiflorum* Thunb.) (Berghage et al., 1991; Erwin et al., 1992; Heins and Erwin, 1990). Negative DIF also has been used in the culture of vegetable transplants (Erwin et al., 1992).

In potato (*Solanum tuberosum* L.), Bennett et al. (1991) found that an alternating D/N temperature of 22/14C increased the tuber weight of 'Denali' by 25% compared to those obtained at a constant 18C, but had no consistent effect on 'Norland'. In contrast, when plants were grown at a -8C DIF (14/22), the tuber dry weights of 'Denali' and 'Norland' were decreased 78% and 51%, respectively, compared with those from plants grown at a constant 18C (T. Tibbitts, personal communication). This was true even though the total degree hours were the same for each group of plants.

Our O₃ injury results differed from those obtained by Agrawal and co-workers (unpublished data) for 'Bush Blue Lake 290' snap bean. In their study, bean plants pretreated for 2 weeks under -10C DIF (18/28C) showed reduced O₃ damage, while, in our study, preconditioning at 18/28C failed to protect cucumber plants against O₃ injury and actually increased the amount of injury to leaf 2. Differences in cultural handling of the plants in the two studies may account for the differences in results obtained. In the experiments with snap bean, the plants were grown for 7 days in a charcoal-filtered greenhouse and then held in a growth chamber for 14 days of temperature treatment. In our study, plants were given temperature treatment in a growth chamber 24 days from the time of seeding. Also, the O₃ concentration used in the current study (0.5 $\mu\text{mol}\cdot\text{mol}^{-1}$) was higher than that used for beans (0.3 $\mu\text{mol}\cdot\text{mol}^{-1}$).

Temperature preconditioning in -DIF increased polyamine accumulation in both cultivars, but failed to provide protection against subsequent exposure to O₃ (Table 4). Although similar

increases in polyamines were observed in *Phaseolus vulgaris* L. with -DIF (unpublished data), this was associated with decreased O₃ sensitivity. In both studies, however, O₃ exposure caused increased concentration of polyamines in leaves, irrespective of DIF treatment.

Because plants grown under inverted temperature were already under stress (based on their low chlorophyll concentration and reduced Pn), it was not surprising that increased concentrations of polyamines failed to afford protection against O₃ stress, unlike as reported by Bors et al. (1989), Ormrod and Beckerson (1986), and Rowland-Bamford et al. (1988, 1989). Elevated concentrations of polyamines following O₃ fumigation were consistent with results of others (Kramer et al., 1991a; Rowland-Bamford et al., 1988). However, because the O₃ concentration used in this study (0.5 $\mu\text{mol}\cdot\text{mol}^{-1}$) was very high, the polyamine pool available for scavenging free radicals may have been inadequate.

The reduced chlorophyll concentration of cucumber plants grown at -DIF was consistent with findings reported for other crops when a large -DIF was used (Berghage et al., 1991; Erwin et al., 1991; Heins and Erwin, 1990). Heins and Erwin (1990) showed that chlorophyll concentration in many species was affected by DIF and that, as -DIF increased, plants became progressively more chlorotic. At -5 to -10C DIF, they observed that seedlings of salvia (*Salvia splendens* F. Sellow ex Roem & Schult.) and gerbera (*Gerbera jamesoni* var. hybrida) were especially sensitive, becoming severely chlorotic and developing very slowly.

Further studies are needed to elucidate the basis for chlorosis in cucumber plants grown at 18/28C D/N. Chlorosis was apparent at the time of seedling emergence and persisted throughout the experiment, although the cotyledons and older leaves tended to become greener with time. Chlorosis of cucumber leaves was reversed within 24 to 48 h after moving selected plants to +DIF. Heins and Erwin (1990) observed that most of the chlorosis caused by -DIF occurred in the youngest leaves and also could be reversed within a few days of plant exposure to +DIF.

Low-temperature induction of photoinhibition (characterized by inhibition of PS II activity and gradual loss of chlorophyll) is well-known (Powles, 1984). Hetherington et al. (1989) examined 15 tropical and temperate annual crop species with a wide range of chilling sensitivity and measured the ratio of variable fluorescence to maximum fluorescence (F_v/F_{max}) to determine the extent of photoinhibition. They found that leaves of chilling-resistant plants such as pea (*Pisum sativum* L.) and broad bean (*Vicia faba* L.)

began to develop photoinhibition at a moderate photosynthetic photon flux of $275 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, even near 18C. Other chilling-resistant crops, such as wheat and barley, also were susceptible to photoinhibition if the temperature was low enough. Although chilling-sensitive plants generally showed greater photoinhibition susceptibility, their chlorophyll fluorescence values showed that photoinhibition of cucumber and sesame (*Sesamum orientale* L.) was not confined to a chilling-inducing temperature of 12C, but was evident both above and below this point. Because low temperatures can induce photooxidation and bleaching of chlorophyll in the leaves of cucumber and other chilling-sensitive plants (Powles, 1984; Wang, 1990), it was quitelike that the 18C during the day we used had a photoinhibitory effect on cucumber.

Ozone fumigation caused a partial inactivation of PS II, as revealed by decreased variable fluorescence (F_v) and a decreased ratio of variable fluorescence to initial fluorescence (F_v/F_o) in 'Ashley' and 'Poinsett' cucumber (Table 3). Plants grown at +DIF generally showed a greater decrease in F_v and F_v/F_o ratio than those grown at -DIF, indicating differences in activity of the photosynthetic apparatus upon illumination.

The hypothesis that differences in UV-B sensitivity between 'Poinsett' and 'Ashley' cucumber (Krizek, 1978) might be associated with differences in O_3 sensitivity was not supported. Separate mechanisms of injury may be involved, even though both UV-B irradiation (Kramer et al., 1991 b) and O_3 fumigation induce oxidative stress and cause increased polyamine concentrations of leaves. The greater leaf area of cucumber plants in +DIF than in -DIF would be expected to increase sensitivity to O_3 fumigation. Because the opposite pattern was observed, plant size did not appear to have a confounding effect on O_3 sensitivity.

Pinthus and Meiri (1979) indicated that the inhibitory effects of low-day and high-night temperature on leaf blade and internode elongation in wheat were independent of those on dry-matter accumulation. Wheat plant dry weight at 18/24C exceeded that at 24/18C, but was less at 10/18C than at 18/10C. They attributed this response to temperature effects on the balance of endogenous phytohormones. In support of this hypothesis, they showed that application of gibberellic acid (GA_3) to plants grown at 10/18C restored their elongation, but did not affect their dry weight. Conversely, treating plants grown at 18/10C with the growth regulators chlormequat (2-chloroethyl trimethylammonium chloride, CCC) or indolebutyric acid (IBA) resulted in suppression of elongation similar to that at 10/18C.

Although we did not measure dark respiration, such rates would be expected to be higher in -DIF- than in +DIF-treated cucumber plants. This, plus the lower P_n in plants grown under -DIF compared to +DIF, would be expected to result in greater biomass differences than were obtained. Because -DIF plants were much more compact and had much thicker stems (visual observation) than +DIF plants, differences in biomass partitioning between the two treatments may have contributed to this disparity between growth and P_n measurements. There may have been greater photosynthetic allocation to the roots in -DIF plants than in +DIF plants. Another reason for this disparity may be a poor relationship between P_n measurements made on a single leaf and the P_n capacity of the plant owing to differences in physiological stage and PPF among leaves within a plant.

Failure to obtain phytoprotection against O_3 fumigation in cucumber may have resulted from an excessively large (-10C) -DIF that resulted in chlorosis and greatly reduced P_n . Reducing the -DIF to -2 to -5C may lessen damage to the photosynthetic apparatus, reduce sensitivity to O_3 treatment, and regulate stem length without reducing P_n and dry matter accumulation.

Further studies on -DIF effects should be of both academic and practical importance in view of growing interest in nonchemical approaches to regulating plant growth.

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