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Effect of Growth Temperature and Temperature Shifts on Spinach Leaf Morphology and Photosynthesis[1]

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ABSTRACT

The growth kinetics of spinach plants (*Spinacia oleracea* L. cv Savoy) grown at 5°C or 16°C were determined to allow us to compare leaf tissues of the same developmental stage rather than chronological age. The second leaf pairs reached full expansion at a plant age of 32 and 92 days for the 16°C and 5°C plants, respectively. Growth at 5°C resulted in an increased leaf area, dry weight, dry weight per area, and leaf thickness. Despite these changes, pigment content and composition, room temperature *in vivo* fluorescence, and apparent quantum yield and light-saturated rates of CO_2 exchange or O_2 evolution were not affected by the growth temperature. Furthermore, 5°C expanded leaves were found to be more resistant to photoinhibition at 5°C than were 16°C expanded leaves. Thus, it is concluded that spinach grown at low temperature is not stressed. However, shifting spinach leaves from 5°C to 16°C or from 16°C to 5°C for 12 days after full leaf expansion had occurred resulted in a 20 to 25% reduction in apparent quantum yields and 50 to 60% reduction in light saturated rates of both CO_2 exchange and O_2 evolution. This was not accompanied by a change in the pigment content or composition or in the room temperature *in vivo* fluorescence. It appears that leaf aging during the temperature shift period can account for the reduction in photosynthesis. Comparison of cold-hardened and non-hardened winter rye (*Secale cereale* L. cv Muskateer) with spinach by *in vivo* fluorescence indicated that rye is more sensitive to both short term and longer duration temperature shifts than is spinach. Thus, susceptibility to an abrupt temperature shift appears to be species dependent.

The study of plant stress and the capacity to acclimate to a particular stress under controlled environment conditions generally involves a comparison of physiological and molecular changes that occur as a consequence of shifting plants from some optimal control growth regime to another suboptimal regime for a predetermined period of time. Interpretations of results from such studies are complicated by the fact that, inevitably, one is comparing plants that have grown and developed at different rates. This results in comparison of tissue of the same chronological age but different developmental state. Peet (16) and Krol *et al.* (11) have emphasized that tissue of similar developmental age must be employed in order to separate developmental effects from environmental effects.

Krol *et al.* (11, 12) showed that the prehistory of the leaf tissue of winter rye, in large part, determines the biochemical, physiological, and morphological response of rye leaves to a change in growth temperature. More recently, Öquist and Huner (15) showed that only rye leaves fully expanded at low temperature exhibit the capacity to develop a resistance to low temperature-induced photoinhibition. Leaves expanded at 20°C do not develop any resistance to low temperature photoinhibition even after exposure to low temperatures for 3 weeks (15).

Spinach is a cold-tolerant plant that has been used frequently in studies of cold acclimation and freezing tolerance (5, 6, 8, 9). Photosynthetic properties (10) as well as the susceptibility to low temperature-induced photoinhibition (17) have been compared in cold-hardened and nonhardened spinach. From much of the published data on cold acclimation, it is difficult to determine relative developmental ages of tissues being compared and to ascertain whether the leaf tissues being examined had actually developed at the low temperature or whether the leaf tissue merely had been subjected to a temperature shift. Thus, in this report, we describe, first, the effects of leaf development at low temperature as well as the effect of a temperature shift on leaf anatomy and cold tolerance in spinach. Second, we relate these effects to the *in vivo* photosynthetic and photoinhibitory response of spinach. Last, we compare the response of cold-tolerant spinach to growth and development at low temperature as well as to a temperature shift with that reported for cold-tolerant rye.

MATERIALS AND METHODS

Plant Growth

Spinacia oleracea L. cv. Savoy was germinated at 16°C for 4 d, at which time the cotyledons had just emerged from the potting mix. After 4 d, half of the plants were transferred to 5°C with all other conditions kept constant. Thus, all leaf expansion occurred at 5°C or 16°C. Winter rye (*Secale cereale* L. cv Muskateer) was grown at 5°C and 20°C as described elsewhere (11). Alternatively, spinach plants were subjected to a growth temperature shift after the second leaf pair was fully expanded. Thirty-two-day-old 16°C spinach was shifted to 5°C for 12 d (16°C → 5°C) and 92-d-old 5°C spinach was shifted to 16°C for 12 d (5°C → 16°C) with all other conditions held constant. Fluorescent and incandescent lights provided 250 μmol m^{-2} s^{-1} (PPFD) with a 16-h d. Only the second pair of leaves to emerge were utilized for experiments described here. Leaf area was determined using a Li-Cor portable area meter (LI-3000). Leaf dry weights were determined following

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48 h of drying at 80°C. Growth coefficients were calculated as described previously (11).

Leaf Morphology

The number of stomatal and epidermal cells per unit area and their ratios were determined from impressions of the leaf surface after cleaning with methanol. Peels were examined using ×400 total magnification. Leaf thickness was determined at ×100 magnification with the cell length measured at ×400 magnification from freehand and embedded sections cut perpendicular to the leaf surface.

Freezing Tolerance

Leaf discs, wrapped in damp cheesecloth and nucleated, were equilibrated at −2°C for 1 h before removal of samples at 2° increments. Samples were thawed overnight on ice. After 24 h, conductivity was measured as a percent of the total conductivity after 30 min of heating at 90°C. Osmotic content of the cell sap expressed following freezing was determined using a Wescor vapor pressure osmometer (model 5100C).

Pigment Analyses

Pigments were extracted by grinding in 90% (v/v) acetone. Chl was determined spectrophotometrically by the method of Arnon (2). Carotenoids were separated as described by Hager and Berthenrath (7) then measured spectrophotometrically according to the method of Davies (4).

Gas Exchange

Whole leaf CO_2 exchange was measured using a Li-Cor 6200 portable photosynthesis system. Light response curves were measured at ambient O_2 (21%) and CO_2 (350–450 ppm) at 5°C or 16°C using 10 light intensities starting at 0 and increasing to 900 μmol m^{-2} s^{-1} (PPFD). Q_{app}[2] were determined from six light intensities between 0 and 100 μmol m^{-2} s^{-1} (PPFD).

O_2 evolution from leaf discs was measured using a Hansatech (LD2) system. The computer-controlled light-emitting diode light source (Hansatech LH 36UB) was calibrated by integrating nine points in two perpendicular rows using Simpsons 3/8ths rule (3). A capillary matting was dampened with 180 mM bicarbonate solution and used as a spacer. Leaf discs were equilibrated for 15 min in the dark with the following gas mixture: 5% CO_2, 5% O_2, 90% N_2. Leaf discs were then pre-illuminated at 125 μmol m^{-2} s^{-1} (PPFD) for 6 min before the start of data collection to ensure attainment of maximum rates of O_2 evolution. The light response curves were determined at 5°C and 16°C using 20 light intensities starting at 0 and increasing to 600 μmol m^{-2} s^{-1} (PPFD). Ten points from 0 to 90 μmol m^{-2} s^{-1} (PPFD) were utilized for determination of Q_{app} of O_2 evolution.

[2] Abbreviations: Q_{app}, apparent quantum efficiency of CO_2 exchange or O_2 evolution; F_v/F_m, variable fluorescence/maximum fluorescence.

In Vivo Fluorescence

The F_v:F_m was determined at room temperature using a PAM Chlorophyll Fluorometer (Walz, FRG) following 1-h dark adaption at room temperature. Measuring beam intensity was 1.2 μmol m^{-2} s^{-1} (PPFD) with the saturated pulse intensity of 3200 μmol m^{-2} s^{-1} (PPFD) and 400 ms duration.

Photoinhibition Treatments

Two high pressure sodium vapor lamps were mounted on a frame in a cold-room. A 3-inch water bath filter was placed between the light source and the samples. This set-up provided 1200 μmol m^{-2} s^{-1} (PPFD) at the sample surface. Leaf discs from fully expanded leaves developed at 5°C or 16°C were placed on a thin layer of water in a tray over ice. The leaf temperature did not exceed 6°C. Photoinhibition was measured as a reduction in the F_v/F_m.

RESULTS

Growth Kinetics and Pigment Composition

In this study, we selected the second leaf pairs for investigation because they were the youngest leaves to become fully expanded before initiation of stem elongation. At 16°C, these leaves became fully expanded 32 d after planting with no stem elongation or flowering occurring before 48 d. At 5°C, the second leaves became fully expanded 88 to 92 d after planting with stem elongation being initiated after 120 d. No flowering occurred even after 200 d of growth at 5°C. Spinach leaves that expanded at 16°C had growth coefficients for leaf expansion of 0.184 compared with 0.118 cm^2/d for 5°C leaves. However, 5°C leaves attained final areas that were threefold greater and dry weights that were fivefold greater than spinach leaves expanded at 16°C (Fig. 1). This resulted in a doubling in the dry weight per unit area for 5°C leaves (10 mg cm^{-2} compared with 16°C leaves (4.4 mg cm^{-2}). These findings are consistent with the work of Moustafa (14).

Measurements of osmotic potential of the cell sap revealed a twofold increase from −0.99 MPa in 16°C leaves to −1.9 MPa for 5°C leaves. This is consistent with decreased H_2O contents in 5°C leaves (5.3 g H_2O/g dry weight) compared with 16°C leaves (9.3 g H_2O/g dry weight). Furthermore, growth at 5°C increased the freezing tolerance from −3°C to −9°C as determined by electrolyte leakage of the second leaves of 16°C and 5°C grown plants.

Growth temperature had no significant effect on Chl or carotenoid content on a leaf area basis (Table I). Similarly, growth at either 5°C or 16°C did not influence the ratios of Chl *a*/*b* or the carotenoid:Chl ratio. However, Chl and carotenoid contents were twofold higher in 16°C than in 5°C leaves on a dry weight basis (Table I). Exposing 16°C leaves to 5°C or 5°C leaves to 16°C for 12 d did not affect these results (data not shown).

Leaf Anatomy and Morphology

Growth at low temperature resulted in a twofold increase in leaf thickness from about 290 μm for 16°C leaves to 567 μm for 5°C leaves (Fig. 2; Table II). This was due to a 1.3- to

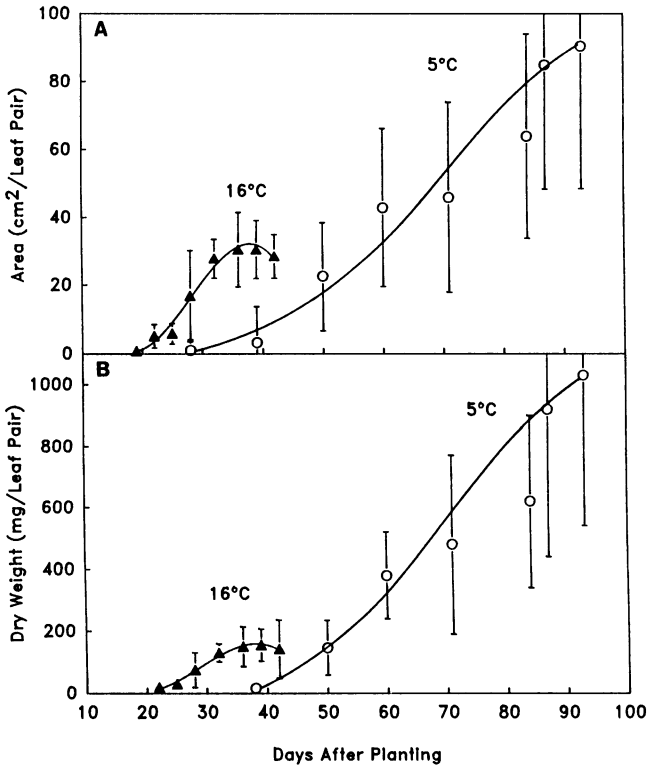

Figure 1. Growth kinetics of the second leaf pair in spinach expanded at 5°C or 16°C. Growth was based on leaf area (A) and dry weight (B). All data are the means of eight determinations ± SD from at least three different experiments.

1.4-fold increase in the mean lengths of both the palisade and the spongy mesophyll cells. Furthermore, leaves developed at 5°C exhibited two to three palisade layers, whereas leaves expanded at 16°C exhibited one to two palisade layers. It is interesting to note that leaves that fully expanded at 16°C and then shifted to 5°C for an additional 25 d resulted in an intermediate leaf thickness of 377 μm.

Although growth temperature appeared to affect the shape of the epidermal cells (Fig. 2, C and D), growth temperature did not alter the number of epidermal cells per area, stomata per area, or epidermal cells per stomata on either the top or bottom of the leaves.

Effect of Growth Temperature on *In Vivo* Photosynthesis

Although leaves that expanded at 5°C or 16°C exhibited significant alterations in leaf morphology and anatomy, their photosynthetic properties were very similar when calculated on a leaf area basis (Tables III and IV). First, a comparison between 16°C and 5°C control plants indicated that the Q_{app} and light saturated rates of CO_2 exchange were similar regardless of the measuring temperature (Tables III and IV). Second, light saturated rates of CO_2 exchange in both 16°C and 5°C control plants were relatively insensitive to measuring temperature in the range of 5°C to 16°C. However, when CO_2 exchange was measured on a dry weight basis, 16°C control leaves exhibited a twofold greater light saturated rate of CO_2 exchange (0.58 μmol CO_2·g dry weight^{-1}·s^{-1}) than 5°C leaves (0.22 μmol CO_2·g dry weight^{-1}·s^{-1}).

Exposure to low temperature can predispose plants to photoinhibition even at moderate to low light levels (15, 17, 18). *In vivo* room temperature fluorescence is a rapid method for monitoring photosynthetic efficiency. F_v/F_m has been shown to correlate with quantum efficiency of PSII (1), which is reduced by a photoinhibitory treatment (15, 17). Measurements of F_v/F_m for spinach leaf discs subjected to photoinhibitory treatments for 0, 3, 6, or 9 h indicated that leaves fully expanded at either 5°C or 16°C are susceptible to photoinhibitory conditions (Fig. 3). However, leaves expanded at 5°C were less susceptible than those expanded at 16°C. Nine hours of photoinhibitory treatment resulted in a 40% reduction in F_v/F_m of 5°C expanded leaves compared with a 59% reduction for the 16°C expanded leaves (Fig. 3). Leaf discs kept in the dark for 9 h at 5°C resulted in no decline in the F_v/F_m for either 5°C or 16°C expanded leaves, indicating that the decline in F_v/F_m was light dependent. Thus, growth at 5°C appears to impart some resistance to low temperature photoinhibition.

Effect of Temperature Shift on *In Vivo* Photosynthesis

CO_2 exchange in fully expanded second leaf pairs of both 16°C and 5°C plants did appear to be inhibited after a 12-d exposure to an abrupt temperature shift (Tables III and IV). First, a 20% reduction in the Q_{app} for CO_2 exchange was observed when 16°C plants were shifted to 5°C (Table III, 16°C→5°C) or when 5°C plants were shifted to 16°C (Table IV, 5°C→16°C), regardless of the measuring temperature. Second, 5°C plants exhibited approximately a 50% reduction in light saturated rates of CO_2 exchange when shifted to the higher temperature. This was observed when CO_2 exchange was measured at either 5°C or 16°C (Table IV). However, light-saturated rates of CO_2 exchange in 16°C plants that were shifted to 5°C exhibited a 60% reduction only when measured at 5°C (Table III). Thus, both 16°C and 5°C spinach plants appeared to be stressed photosynthetically when exposed to a prolonged and abrupt temperature shift.

However, additional control measurements were required to separate stress effects from the possible effects of developmental age. First, 16°C plants that exhibited maximum leaf

Table I. *Total Chl and Carotenoid Contents of the Second Leaf Pair from Spinach*

All data are means of six determinations ± SD from two different experiments.

Pigment Content	Growth Temperatures	
	5°C	16°C
mg Chl/g dry wt	5.19 ± 1.2	14.4 ± 2.9
μg Chl/cm²	58 ± 12	64 ± 17
Chl *a*/*b*	2.88 ± 0.17	2.81 ± 0.15
mg Car[a]/g dry wt	0.91 ± 0.0	2.15 ± 0.32
μg Car/cm²	10.2 ± 1	9.43 ± 1.3
mg Car/mg Chl	0.19 ± 0.05	0.16 ± 0.04

[a] Car, carotenoid.

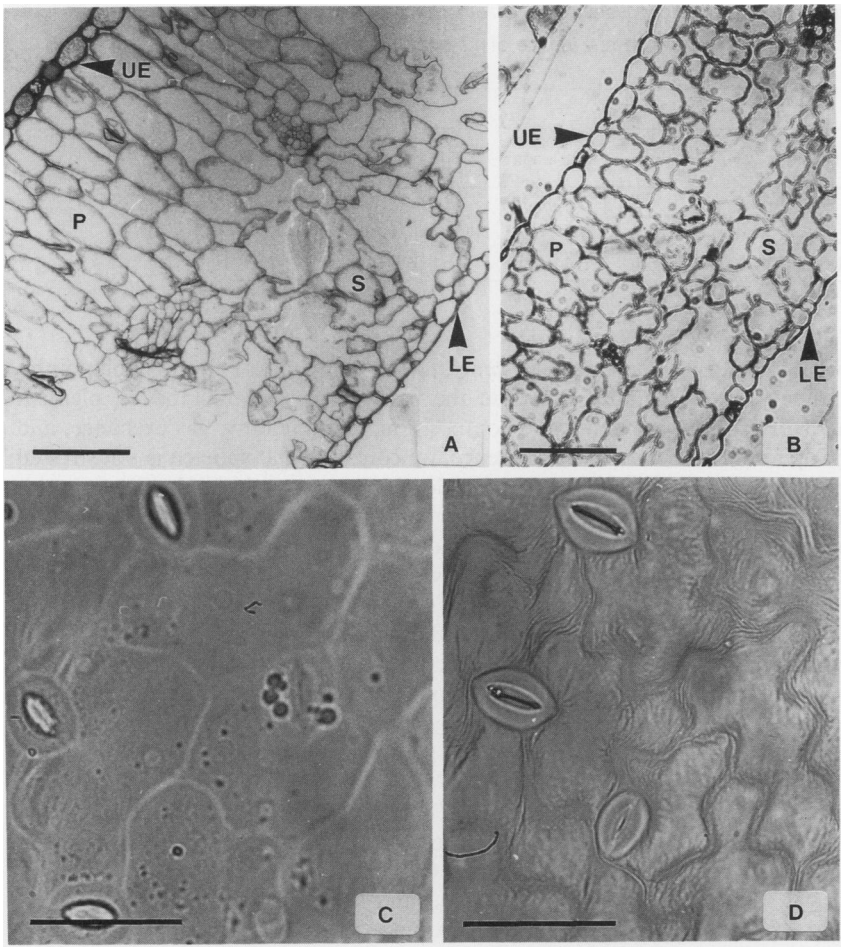

Figure 2. Cross-section (A, B) and impressions of the abaxial leaf surface (C, D) from fully expanded second leaf pairs of spinach. A, C: leaves expanded at 5°C; B, D: leaves expanded at 16°C. Scale bar indicates 100 μm for A and B and 50 μm for C and D. UE, upper epidermis; LE, lower epidermis; P, palisade mesophyll; S, spongy mesophyll.

expansion of the second pair after 32 d were exposed to an additional 12 d growth at 16°C. Since both 5°C and 16°C controls were at a similar developmental stage before the shift, 44-d-old 16°C plants would act as a control for 5°C plants, which were exposed to an additional 12 d at 16°C. Forty-four-day-old 16°C plants exhibited a 20% reduction in Q_{app} as well as a 50 to 60% reduction in light saturated rates of CO_2 exchange (Table III). These reductions were similar to those observed when fully expanded leaves of 5°C plants were shifted to 16°C for 12 d (Table IV, 5°C→16°C). Second, 5°C plants that exhibited maximum leaf expansion of the second pair after 92 d were exposed to an additional 12 d growth at 5°C. Thus, 104-d-old plants would act as an additional control for 16°C plants shifted to 5°C for 12 d. These plants exhibited similar Q_{app} and light saturated rates (Table IV, 5°C, 104 d) as fully expanded leaves of 16°C plants that had been exposed to an additional 12 d at 5°C (Table III, 16°C→5°C). Therefore, developmental aging during the 12-d shift period at either 16°C or 5°C can, for the most part, account for the reductions in Q_{app} and light saturated rates of CO_2 exchange. Similar trends in Q_{app} and light saturated rates of O_2 evolution were observed for leaf discs from 5°C or 16°C leaves or leaf discs from fully expanded leaves subjected to a temperature shift for a 12-d period. Q_{app} yields ranged from 0.08 to 0.10 μmol O_2/μmol quanta and light saturated rates ranged from 13 to 23 μmol $O_2 \cdot m^{-2} \cdot s^{-1}$ depending on the treatment (data not presented).

Effects of Temperature Shift on F_v/F_m for Spinach and Rye

Krol and Huner (12) have shown that fully expanded leaves of rye undergo progressive chlorosis and senescence when shifted from 20°C to 5°C for periods greater than 2 to 3 weeks. In contrast, pigment content and composition of spinach leaves were unaffected after exposure to a temperature shift for a comparable duration and were similar to those presented in Table I. This differential response of spinach and rye to a temperature shift was investigated further by room temperature fluorescence.

Neither the growth temperature nor a temperature shift had any significant effect on F_v/F_m for fully expanded second leaf pair of spinach (Table V). In contrast, both growth temperature and temperature shifts had a significant effect on F_v/F_m for fully expanded rye leaves. Growth of rye leaves at cold-hardening temperatures resulted in a 15% lower F_v/F_m compared with rye leaves developed at nonhardening temperatures (Table V). This was reversible since the F_v/F_m of cold-hardened rye recovered to normal levels after 90 h at 20°C. Furthermore, shifting nonhardened rye to 5°C for only 3.75 d caused a 32% reduction in F_v/F_m (Table V). After 18 d at

Table II. *Effects of Growth Temperatures on Leaf Thickness and Cell Size of Spinach*

Leaves were expanded at either 5°C or 16°C. All data are means of 17 determinations ± SD from three different experiments.

Growth Temperature	Leaf Thickness	Palisade Mesophyll			Spongy Mesophyll	
		Thickness	No. of layers	Cell length	Thickness	Cell length
	μm	μm		μm	μm	
5°C	567 ± 89	228 ± 92	3.1 ± 0.9	75 ± 35	271 ± 79	46 ± 18
16°C	289 ± 44	105 ± 90	1.8 ± 0.6	57 ± 19	151 ± 56	33 ± 15

5°C, the F_v/F_m was reduced to 0.44 (data not shown). It is at this time that visible signs of chlorosis and anthocyanin accumulation in the rye leaves became apparent. Nonhardened rye plants kept in continuous dark at 5°C for up to 6 d exhibited no change in F_v/F_m (0.68), indicating that the reduction in F_v/F_m at 5°C was light dependent. This indicates that nonhardened rye becomes photoinhibited when exposed to an abrupt low temperature shift. Thus, pigment analysis as well as the room temperature fluorescence are consistent and indicate that the response of spinach and rye leaves to different growth temperature regimes or to an abrupt temperature shift is quite different.

DISCUSSION

Since leaf growth of cold-tolerant spinach occurs at slower rates as the growth temperature is lowered, it follows that tissues grown at different temperatures would reach similar developmental stages at different chronological ages. The second leaf pairs of spinach compared in this study were at the same developmental stage, that is, full expansion, although they were of different chronological ages.

Despite changes in the leaf anatomy and morphology, growth temperature did not alter the photosynthetic characteristics of spinach leaves measured as CO_2 exchange or O_2 evolution. In addition, measurements of F_v/F_m also indicated that growth at low, cold-hardening temperatures did not affect spinach leaves adversely. In fact, 5°C spinach exhibited an increased resistance to low temperature induced photoinhibition. Thus, on the basis of the growth kinetics, pigment contents and composition, leaf anatomy, gas exchange, and *in vivo* fluorescence, we conclude that spinach is not stressed during growth and development at cold-hardening temperatures. However, the comparable photosynthetic capacity of spinach leaves expanded at 5°C and 16°C resulted in a twofold increase in the dry weight per area of the 5°C leaves. This may be because the sinks are more sensitive to low growth temperature than is the source.

Much of the literature on cold-hardening involves short term acclimation protocols rather than leaf expansion at the low temperature as described above. For example, studies utilizing winter rape plants have reported a strong inhibition of CO_2 exchange in both the Q_{app} and light saturated rates following 2 or 4 d of cold pretreatment (13). Winter rape has a hardiness similar to that of spinach, and one might expect similar inhibition of CO_2 exchange. Indeed, we did observe a decrease in both Q_{app} and light saturated rates for CO_2 exchange when fully expanded spinach leaf tissues were subsequently subjected to an abrupt temperature shift. This occurred regardless of whether the temperature shift was to a warmer or colder temperature and despite the ability of the spinach leaf to change morphology when shifted to a lower temperature. Thus, fully expanded spinach leaves appear to be stressed only when exposed to a temperature shift. However, when fully expanded leaves were maintained at their respective growth temperature for an additional time period

Table III. *Apparent Quantum Efficiencies and Light Saturated Rates of CO_2 Exchange by Spinach Leaves Expanded at 16°C*

Plants were grown at 16°C for 32 d at which time the second leaf pair was fully expanded. Either the leaves were used at 32 d (control) or the plants were shifted to 5°C for an additional 12 d (16°C→5°C) before use. In addition, as another set of controls, plants were kept at the 16°C growth temperature for an additional 12-d period (16°C 44 d). All data are the means of four different experiments ± SD.

Treatment	Measuring Temperature	Q_{app}	Light Saturated Rate
	°C	$\mu mol\ CO_2/\mu E \cdot m^{-2} \cdot s^{-1}$	$\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$
16°C Control	16	0.047 ± 0.003	25.6 ± 5.0
	5	0.052 ± 0.003	22.2 ± 5.6
16°C→5°C	16	0.038 ± 0.003	21.6 ± 4.0
	5	0.041 ± 0.004	14.3 ± 0.9
16°C 44 d	16	0.040 ± 0.002	14.5 ± 1.1
	5	0.040 ± 0.002	15.3 ± 2.1

Table IV. *Apparent Quantum Efficiencies and Light Saturated Rates of CO_2 Exchange by Spinach Leaves Expanded at 5°C*

Plants were grown at 5°C for 92 d, at which time the second leaf pair was fully expanded. Either the leaves were used at 92 d (control), or the plants were shifted to 16°C for an additional 12 d (5°C→16°C) before use. In addition, as another set of controls, plants were kept at the 5°C growth temperature for an additional 12-d period (5°C 104 d). All data are the means of four different experiments ± SD.

Treatment	Measuring Temperature	Q_{app}	Light Saturated Rate
	°C	$\mu mol\ CO_2/\mu E \cdot m^{-2} \cdot s^{-1}$	$\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$
5°C control	16	0.043 ± 0.003	21.3 ± 4.0
	5	0.041 ± 0.003	18.2 ± 1.4
5°C→16°C	16	0.036 ± 0.001	12.7 ± 0.9
	5	0.033 ± 0.001	13.3 ± 1.9
5°C 104 d	16	0.043 ± 0.001	18.9 ± 3.8
	5	0.043 ± 0.001	12.8 ± 2.1

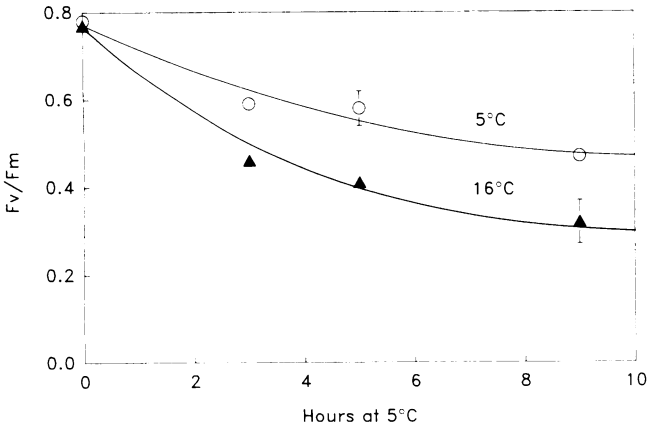

Figure 3. Effect of growth temperature on the susceptibility of spinach to photoinhibition. Photoinhibitory response was measured as a change in F_v/F_m. Measurements were made as a function of time at 5°C and 1200 μmol m^{-2} s^{-1} (PPFD). Leaf discs were from the second leaf pairs expanded at 5°C or 16°C as indicated. All data are the means of four determinations ± SD. Where not shown, SD are no larger than the symbols.

equivalent to the shift period, they exhibited photosynthetic rates reduced to the same extent as that of the plants shifted to that temperature. Thus, we conclude that aging during the shift period can account for most of the observed inhibition in photosynthesis.

Schöner and Krause (17) have reported a decrease in the total Chl content per fresh weight and an increase in the Chl *a/b* ratio in spinach as a function of a 10-d acclimation at cold-hardening temperatures. Similarly, the total carotenoid, lutein and zeaxanthin, and violaxanthin contents increased on a Chl basis with cold-acclimation. Chl content per area, however, was unaltered by acclimation (17). They suggest that the carotenoid pigments may help protect against photoinhibition. Somersalo and Krause (18) have shown that cold-hardening of spinach results in increased resistance to photoinhibition at low temperature. In contrast, our results on spinach leaves expanded at 5°C and 16°C or subjected to a temperature shift indicated no significant change in pigment composition or content on a Chl or leaf area basis, yet we still observed a reduced susceptibility to low temperature photoinhibition upon cold-hardening (Fig. 3). Thus, we conclude that changes in pigment content and composition are not associated with increased resistance to low temperature photoinhibition in spinach. However, low temperature growth does increase leaf thickness, which is due to an increase in the number of palisade cell layers. This may aid in resistance to photoinhibition because attenuation of absorbed light by the palisade cells may reduce the proportion of cells exposed to the high light intensity. The importance of developmental history and developmental stage of the tissues used for photoinhibitory studies will be the subject of a forthcoming paper.

Fully expanded second leaf pairs of spinach exhibited reduced rates of CO_2 exchange and O_2 evolution when they were subjected to an abrupt temperature shift. However, neither growth temperature nor a temperature shift appeared to affect *in vivo* room temperature fluorescence as measured by F_v/F_m. As F_v/F_m reflects photosynthetic efficiency of PSII, one would have expected a concomitant change in Q_{app} of O_2 evolution and F_v/F_m. We observed a 20% reduction in Q_{app} of O_2 evolution with no apparent reduction in F_v/F_m. This was probably due to the fact that the relationship between F_v/F_m and Q_{app} of O_2 evolution was not linear for these treatments. The Q_{app} of O_2 evolution was more sensitive than F_v/F_m (SR Boese, NPA Huner, unpublished results). A nonlinear response between Q_{app} of O_2 evolution and F_v/F_m has been reported for other species and treatments (1).

Based on our comparisons of temperature effects on photosynthesis between leaves developed at low temperature and those subjected to an abrupt temperature shift after full leaf expansion, we make the following conclusions. First, growth and development at low temperature is not strictly comparable with the effects of an abrupt temperature shift. Second, if a temperature shift treatment is to be tested, we suggest the need for appropriate developmental controls to separate stress effects from the effects of plant aging. Last, the reduction in photosynthetic efficiency, as measured by F_v/F_m, by an abrupt temperature shift is species dependent. Thus, our results illustrate the need for comparing tissues of the same developmental stage rather than chronological age if one is to distinguish between temperature effects and developmental effects.

Table V. *Effect of Growth Temperature and Temperature Shift on F_v/F_m of Spinach and Rye*

Nonhardened spinach was grown at 16°C for 32 d with cold-hardened spinach grown at 5°C for 92 d. Nonhardened rye was grown at 20°C for 25 d while cold-hardened rye was grown at 5°C for 56 d (11). Cold→warm temperature shift represents data of cold-hardened spinach and rye shifted to 16°C or 20°C, respectively. Warm→cold temperature shift represents data from nonhardened spinach and rye shifted to 5°C. Spinach plants were shifted for 12 d, while rye plants were shifted for 3.75 d. All data represent means ± SD. For spinach $n = 30$ and for rye $n = 15$.

Treatment	F_v/F_m	
	Spinach	Rye
Nonhardened	0.78 ± 0.04	0.79 ± 0.01
Cold-hardened	0.78 ± 0.02	0.69 ± 0.03
Cold→warm	0.77 ± 0.05	0.76 ± 0.02
Warm→cold	0.80 ± 0.02	0.60 ± 0.04

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