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Plants under Climatic Stress

III. LOW TEMPERATURE, HIGH LIGHT EFFECTS ON PHOTOSYNTHETIC PRODUCTS

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ABSTRACT

An investigation has been made of the combined effects of low temperature and high light on the level of several photosynthetic products in the leaves of a group of plants differing widely in their tolerance to this stress. Starch levels in these plants after chilling are dependent on the time of day that temperatures are lowered and seem related to rates of CO_2 assimilation under this stress. Prolonged low-temperature, high-light treatment (10 C at 160 wm^{-2}) of *Sorghum bicolor* induced a rapid starch hydrolysis after a lag of some 24 hours. Differing rates of starch loss at the cellular level and a rapid migration of chloroplasts toward the base of upper mesophyll cells were also seen in leaves of this stress-sensitive species.

Chilling increased the level of almost all free amino acids in tolerant and in semi-tolerant species, while amino acids related to intermediates of the C_4-pathway show a sharp or transitory decrease in *Sorghum*. These and other changes observed in *Sorghum* suggest that some time- and temperature-dependent blockages develop in the interconversion of C_4-pathway intermediates and possibly in the flow of other intermediates to and from the sites of C_4-photosynthesis.

Levels of ATP in the leaves of *Sorghum*, *Paspalum*, and *Amaranthus* increased at night and following chilling and did not fall until pronounced necrosis of the leaves commenced.

Previous reports (21, 22) have shown that high light levels combined with low temperatures can cause necrotic lesions to develop on the leaves of several plants. Lower levels of light prevent or slow the onset of these lesions. Some species of both C_3- and C_4-photosynthetic pathway (7) plants damage rapidly, while others are damaged slowly or not at all. This type of lesion differs from the "Faris" bands noted in sugar cane (3) and other plants (17), which appear to be caused by chilling of the leaf meristems.

Chloroplast ultrastructural changes (22) and reductions in photosynthesis (21) which precede cellular necrosis under high light, low-temperature stress have been documented. In this paper, a group of plants differing in chilling sensitivity and in mechanisms of photosynthetic carbon assimilation have been used to investigate some further aspects of this environmental stress. *Lolium multiflorum* is a chilling-tolerant C_3-pathway (7) grass, *Glycine max* is a semi-tolerant C_3-pathway legume; *Paspalum dilatatum* is a semi-tolerant C_4-pathway tropical grass, *Amaranthus lividus* is a chilling-sensitive C_4-pathway dicotyledon, and *Sorghum bicolor* is a very sensitive C_4-pathway tropical grass (21). These plants have been exposed to chilling conditions and the levels of starch, soluble sugars, ATP, amino acids, and malate measured at what appeared to be significant times before and during stress.

MATERIALS AND METHODS

Plant Species and Stress Conditions. Plants used were *Sorghum bicolor* hybrid NK 145, *Paspalum dilatatum* Poir, *Amaranthus* ann. *lividus*, *Glycine max* L. Merr. cv. Merit, and *Lolium multiflorum* L. var Grasslands "Tama" Westerwolds.

Potting media, plant nutrients, and controlled environment cabinets were as described previously (21). Plants were given three phases of treatment. They were first preconditioned at a constant day-night temperature of 25 C for 11 to 14 days. Temperatures were generally lowered at the start of a photoperiod to a constant 10 C day and night for 3 days and then returned to 25 C. Photoperiods were 12 hr with light intensities maintained at 160 wm^{-2} (400–700 nm).

Soluble Sugar, Starch, and Malate Assays. Several leaves with most recently emerged ligules (youngest-mature) were harvested from different plants 3 hr before the completion of 12-hr photoperiods. Leaves were lyophilized and ground, and aliquots were used for subsequent analyses. Soluble sugars extracted in 80% (v/v) ethanol and starch extracted from the alcohol-insoluble residue according to Hassid and Neufeld (6) were assayed with phenol-sulphuric acid (1). Malate was extracted from aliquots (250 mg) of the same dry leaf powder in 5 ml of 6% $HClO_4$ at 0 C and determined enzymically (8).

Similar assays were also used to determine the levels of leaf starch at more frequent intervals before and during low-temperature treatment.

Amino Acid Assays. Amino acids were extracted from 1.5 g wet weight of youngest-mature leaf lamina in methanol-chloroform (2:1) followed by 0.1 N sodium citrate (pH 2.2). Extracts were combined, and the aqueous layer was concentrated and passed through 0.45-μ Millipore filters before being frozen. Aliquots were analyzed in a Beckman/Spinco Model 120 amino acid analyzer. Asparagine and glutamine were hydrolyzed with 1 N HCl (3 hr) and measured as increases in their corresponding dicarboxylic acids.

ATP Assay. Leaf discs (5 cm^3 total) were taken from the lamina of several youngest-mature leaves of each species at the times noted and immediately frozen in liquid N_2. Tissue was ground frozen, then resuspended in 3 ml of 0.3 M $HClO_4$ in methanol-water (2:1). Aliquots of 50 μl were assayed using liquid scintillation spectrometry with a standard bioluminescence system (18). Aliquots of extraction medium were added to ATP standards.

RESULTS

Starch and Soluble Sugars. Starch and soluble sugar levels in the leaves of several plants were changed substantially when

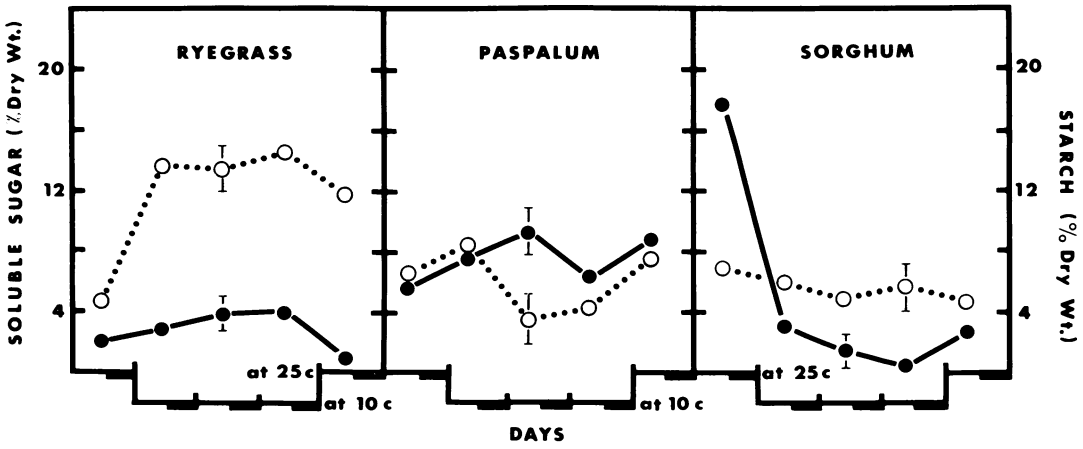

FIG. 1. Soluble sugar (○) and starch (●) levels in youngest-mature leaves of ryegrass, *Paspalum*, and *Sorghum*. Determinations were made 3 hr before the end of 12-hr light periods at 25 C, then during 3 days at 10 C, and again on returning plants to 25 C. Temperatures were changed at the end of 12-hr dark periods. Dark periods are shown as a thickening of the abscissa. One sample standard errors about the curves are shown.

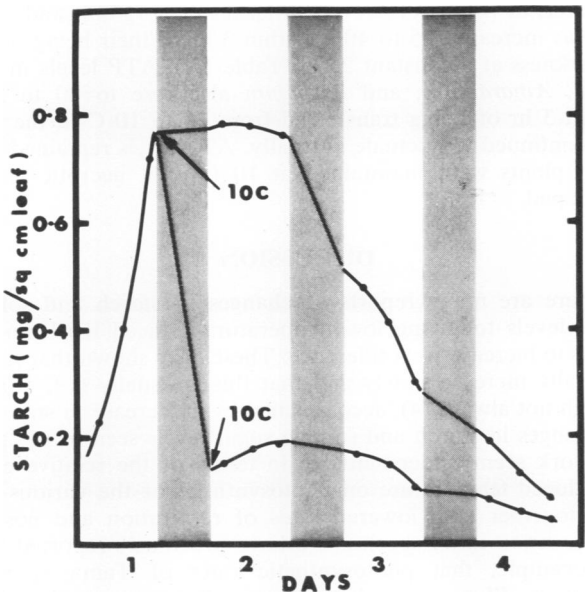

FIG. 2. Chilling effects on the diurnal variation of starch in youngest-mature *Sorghum* leaves. Plant temperatures were lowered from 25 to 10 C either at the start (upper curve) or end (lower curve) of the first dark period. Dark periods are shown as hatched areas. Light periods and dark periods were of 12-hr duration.

leaves were exposed to low temperature, high light conditions (Fig. 1). The type of change seemed to correlate with the degree of chilling tolerance of the various species. Levels rose in the stress-tolerant ryegrass, with soluble sugars showing the sharpest increase, while they fell in the stress-sensitive *Sorghum*, with starch being most affected. *Paspalum*, which is semi-tolerant, showed an intermediate response with levels rising initially, then falling.

Greater detail of these changes in starch level in *Sorghum* are shown in Figure 2 and Table I. Chilling temperatures almost stopped the normal diurnal increase and decrease in starch levels. If temperatures were lowered when starch levels were high at the end of a photoperiod, then they remained high for roughly 24 hr, after which they fell quite rapidly, showing a 90% reduction in 48 hr. If, however, temperatures were lowered when starch levels were low, then levels increased only marginally during the next photoperiod and subsequently

Table I. *Chilling and Leaf Orientation Effects on Starch Grains in Leaves of Sorghum*

Youngest-mature *Sorghum* leaves held horizontal during development at 25 C were continued in this orientation (normal) or were inverted under a chilling stress of 10 C at 160 wm^{-2}. Temperatures were lowered at the start of a dark period. The size and number of starch grains was estimated in 1.5-μ resin-embedded leaf sections viewed under phase contrast × 2000. Upper mesophyll and lower mesophyll refer to cell types present in these zones of the leaf when the leaf is in normal orientation.

Cell Type and Leaf Orientation	Size and Number of Starch Grains			
		Hours at 10 C		
	At 25 C midphotoperiod	18, midphotoperiod	23, end of photoperiod	39, midphotoperiod
Upper mesophyll				
Normal orientation	−[1]	−	−	−
Inverted		++	+	+
Middle mesophyll				
Normal orientation	+	++	++	+
Inverted		++	+	+
Lower mesophyll				
Normal orientation	+	++	++	+
Inverted		+	−	−
Bundle sheath				
Normal orientation	+++	++++	++++	+++
Inverted		++++	++++	+++

[1] ++++, large numbers (15 to 25 per chloroplast in sectional view) of large grains; +++, large numbers of medium-sized grains; ++, several (5 to 9 per chloroplast) large grains; +, several medium-sized grains; −, very small or nonexistent grains.

fell again. Microscopic observation of sections taken from *Sorghum* leaves exposed to this chilling stress in either inverted or normal orientation revealed that starch was lost most rapidly from chloroplasts in mesophyll cells more directly exposed to the light. Light also caused most chloroplasts in the uppermost mesophyll cells to migrate rapidly to the bottom of these cells. Chloroplasts in the uppermost cells did not take up this orientation at 25 C, nor did they in the lowermost mesophyll cells at 10 C.

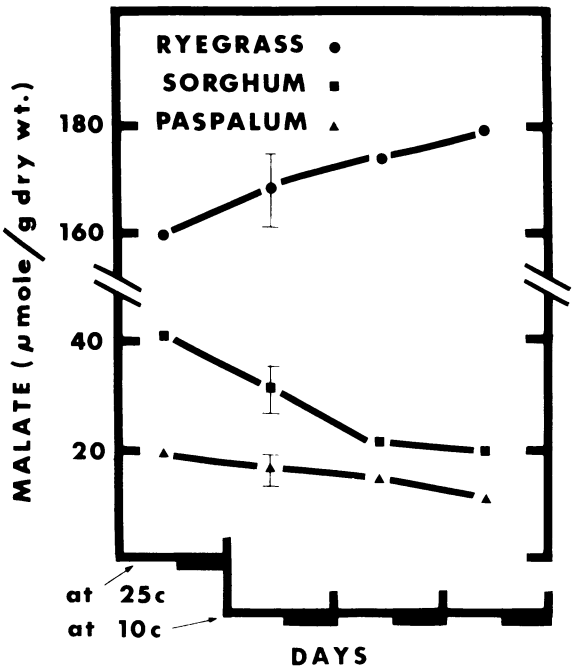

FIG. 3. Malate levels in youngest-mature leaves of ryegrass, *Paspalum,* and *Sorghum.* Determinations were made 3 hr before the end of 12-hr light periods at 25 C and then during 3 days at 10 C. Temperatures were lowered to 10 C at the end of a 12-hr dark period. Dark periods and standard errors are shown as in Figure 1.

Table II. *Chilling Effects on Amino Acids in Leaves of Ryegrass, Soybean, Paspalum, and Sorghum*

Samples were taken at midphotoperiod from youngest-mature leaves of a group of plants grown at 25 C. Temperatures were lowered to 10 C at the start of the following photoperiod, and further samples taken after 30 hr at 10 C.

	Amino Acid Concentration							
	Rye Grass		Soybean		*Paspalum*		*Sorghum*	
	25 C	10 C	25 C	10 C	25 C	10 C	25 C	10 C
	μmole/g fresh wt							
Alanine	0.55[1]	1.37	1.45	3.50	3.65	6.01	6.50	2.08
Serine	2.18	3.11	2.50	1.76	1.21	2.87	1.90	2.44
Glycine	0.21	0.45	0.33	0.44	0.35	1.45	1.72	1.10
Aspartate	1.05	2.77	3.05	5.71	0.60	0.61	2.06	2.76
Asparagine	0.15	0.25	1.90	5.53	0.04	0.20	0.71	1.14
Glutamate	4.32	5.89	4.00	11.09	2.42	3.74	2.26	2.51
Glutamine	1.25	4.20	0.42	7.72	2.36	3.25	2.43	0.82
Valine	0.11	0.22	0.17	0.33	0.08	0.28	0.19	0.37
Leucine	0.10	0.35	0.12	0.23	0.05	0.32	0.08	0.55
Tyrosine	0.01	0.17	0.13	0.15	0.07	0.28	0.09	0.22
Proline	0.07	1.14	0.30	1.20	0.05	1.10	0.24	0.38
Total	9.99	19.92	14.37	37.66	10.88	20.11	18.18	14.37

[1] Data are means of fully-duplicated samples. Mean CV ± 5.1% for all levels above 0.4 μmole/g fresh weight of amino acid.

Malate and Amino Acids. Levels of malate in leaves of *Sorghum* and *Paspalum* at 25 C were only 15 to 25% of those in ryegrass (Fig. 3). When these plants were exposed to low temperatures, malate levels fell marginally in *Paspalum,* quite significantly in *Sorghum,* and rose slightly in ryegrass.

The level of all major free amino acids and of several minor amino acids were changed significantly by chilling treatment (Table II). These together represent more than 90% of the free amino acids present in leaves of these species.

The total molar amount of free amino acids was approximately doubled by 1.5 days of low temperature treatment except in *Sorghum;* even in *Sorghum,* amino acids such as valine, leucine, and tyrosine, whose metabolism is somewhat removed from photosynthetic carbon assimilation and intermediary metabolism, increased as in other species. The most significant feature of the total reduction in amino acid levels in *Sorghum* was that it resulted from a sharp drop in the level of a few amino acids whose biosynthesis is closely related to intermediates of the C_4-photosynthetic pathway. Chilling effects on the level of some of these amino acids are shown in more detail in Figure 4. One striking feature was the rapid reduction in alanine levels in the stress sensitive species *Sorghum bicolor,* compared to its rapid increase in the semi-tolerant species *Paspalum dilatatum.* Another feature was the sharp drop in aspartate coincident with an equimolar rise in glutamate when *Sorghum* was returned to 25 C after 1.5 days at 10 C.

Levels of ATP. ATP levels in leaves of *Sorghum* and *Amaranthus* increased 25 to 40% within 3 hr of their being placed in darkness at a constant 25 C (Table III). ATP levels in *Sorghum, Amaranthus,* and *Paspalum* also rose to 20 to 30% within 3 hr of being transferred from 25 to 10 C in the light and continued to fluctuate diurnally. ATP levels remained high when plants were maintained at 10 C until necrotic lesions developed.

DISCUSSION

There are many reports of changes in starch and soluble sugar levels following low temperature-induced hardening of plants to increase frost tolerance. These have shown that sugars generally increase slowly and that this is usually (13, 16), although not always (4), accompanied by a decrease in starch.

Changes in starch and soluble sugar levels seen in the present work seem understandable in terms of the relative effect of reduced temperature on photosynthesis of the various species, together with lowered rates of respiration and possible translocation in all species. We have previously reported (21), for example, that photosynthetic rates of Tama ryegrass, *Paspalum dilatatum* and *Sorghum bicolor* are reduced by about 33%, 66%, and 90%, respectively, when temperatures are lowered from 25 to 10 C; levels of starch plus sugar are seen to increase in ryegrass, change little in *Paspalum,* and fall in *Sorghum* under this treatment. Why sugars should build up more rapidly than starch in ryegrass is an interesting problem, but it may be that starch synthesis is generally not favored at lowered temperatures.

There are several additional features of interest which were seen in the more detailed work on *Sorghum.* If starch levels are high when temperatures were lowered, then levels remained high during the succeeding dark period, suggesting that starch hydrolysis or starch utilization is much reduced. Starch levels also change little throughout the next photoperiod, which is consistent with the almost negligible increase seen if temperatures were lowered at the start of a photoperiod when starch levels were low. After this 24-hr period, starch levels begin to fall; light seemed to be involved, because starch was lost more rapidly from the most strongly illuminated chloroplasts. This is reminiscent of early reports on solarization (9), where very high light levels which initially promote starch synthesis ultimately cause its destruction. This preferential loss of starch from chloroplasts receiving most light may be caused by damage to their CO_2 assimilatory power (21) or membranes (22),

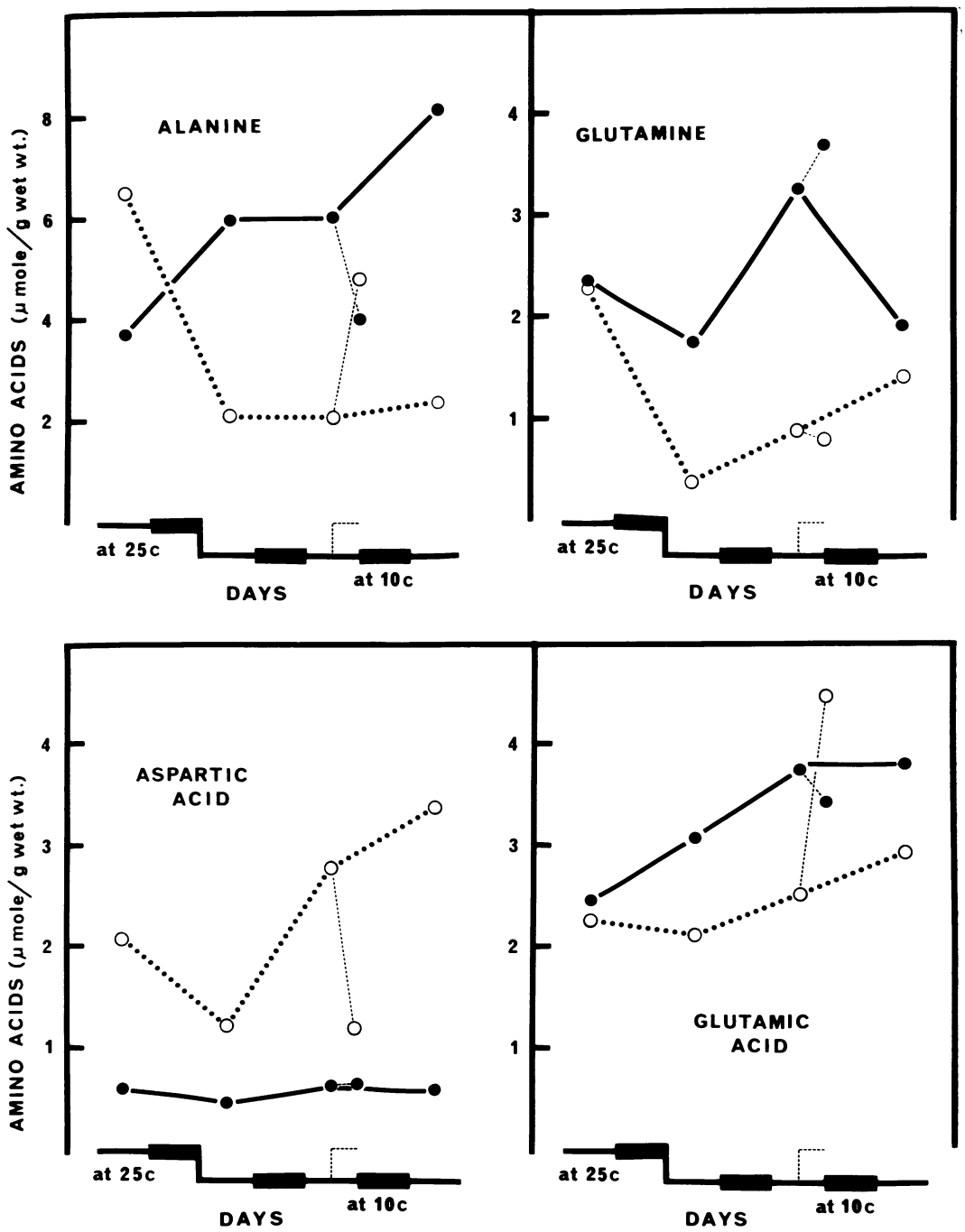

FIG. 4. Free amino acid levels in youngest-mature leaves of *Paspalum* (●) and *Sorghum* (○). Determinations were made at midphotoperiod before (at 25 C) and during 3 days of chilling treatment (at 10 C). Some plants (dashed lines) were returned to 25 C after 1.5 days of chilling, and amino acid levels were measured again 4 hr after their transfer.

since low temperatures are thought to induce starch hydrolysis in potatoes by damaging membranes surrounding the granules (15). Or, if a chilling-induced rise in pH of the stromal matrix (22) were to occur, then the equilibrium of the phosphorylase reaction (5) would be shifted towards starch hydrolysis.

Higher pool sizes of amino acid amides in mint which develop when these plants are exposed to low temperatures are considered to be general phenomena associated with stress conditions (19). Exposure of ryegrass, soybean, and *Paspalum* to low temperatures resulted in a general increase in the level of all the free amino acids, although species differences were seen, and the amides tended to accumulate in C_3- more than in C_4-pathway species. Even in *Sorghum*, many amino acids increased under low temperature treatment, but there was a sharp, though sometimes transitory, fall in the level of many amino acids associated with intermediates of the C_4-pathway. Exposure of *Sorghum* to this stress seems to cause a rapid drain of carbon from this photosynthetic pathway. Some stabilization then occurred, although aspartate levels soon began to rise. If temperatures were returned to 25 C after 1.5 days of chilling, alanine levels rapidly returned to normal (prestress), and an almost equimolar drop in aspartate and rise in glutamate levels was observed.

Interpretation of these chilling-induced changes is made

Table III. *Chilling Effects on ATP Levels in Leaves of Sorghum, Amaranthus, and Paspalum*

Leaf discs were from youngest-mature leaves of plants grown at 25 C. Prestress samples were taken 3 hr after commencement of a dark period and again before midphotoperiod the following day. Temperatures were then lowered to 10 C at midphotoperiod, and further samples were taken at the times noted. Figures in parentheses are dark levels.

	ATP Concentration							
	Prestress at 25 C	Time under chilling (10 C) in hours						
		3	9	24	33	48	57	72
	nmole/sq cm leaf							
Sorghum bicolor	5.8[1]	7.0		7.6		7.8		2.3
	(7.2)		(9.7)		(9.4)		(9.1)	
Amaranthus lividus	8.6	11.3		11.6		11.0		7.5
	(12.0)		(14.1)		(14.0)		(12.6)	
Paspalum dilatatum	4.5	5.9		6.1		6.8		5.7

[1] Values are means of triplicate determinations. CV was generally below ±4%, but rose as cell necrosis commenced.

difficult because these compounds may exist in several intracellular pools (11, 14) and vary diurnally (2). Nevertheless, some restrictions seem to develop in the interconversion of C_4-pathway intermediates and possibly in the flow of intermediates to and from the sites of C_4-photosynthesis. These chilling-induced problems do not develop in *Paspalum dilatatum*.

Reduced ATP supply under chilling has been considered a prime cause of chilling injury to plants (12), these concepts being based on a reported reduction in ATP levels following chilling of cotton seedlings at 5 C when plants were not hardened (20). Levels of ATP in the leaves of *Sorghum, Paspalum,* and *Amaranthus* rose quite rapidly during chilling treatment, and they remained high until pronounced cellular damage occurred (22). Elevated levels of ATP at lowered temperatures and at night have also been reported in leaves of *Chenopodium* and *Phaseolus* (10) and correlated with possible decreases in cytoplasmic viscosity. Chilling-induced increases in ATP seen in *Sorghum, Paspalum,* and *Amaranthus* could equally well develop because of much reduced anabolic metabolism, especially starch and protein synthesis.

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