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Effects of Chilling on Protein Synthesis and CO_2 Fixation in Cotton Leaves[1]

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ABSTRACT

Biochemical changes resulting from chilling of cotton seedlings (*Gossypium hirsutum* L. cultivar Paymaster) that had been subjected to 5, 10, and 25 C for 36 hours were studied. Incorporation of ^{14}C amino acids into protein and fixation of $^{14}CO_2$ immediately after chilling showed a great increase in protein synthesis and CO_2 fixation. When protein synthesis was determined as a function of chilling duration at either 5 or 10 C, there was an increase in the amino acid incorporation following chilling up to 48 hours, but at 72 hours there was a sharp decrease. This result indicates that severe chilling injury caused sufficient damage to the metabolism of the plant to prevent the recovery processes from occurring. The observed stimulation in protein synthesis and CO_2 fixation after chilling may be related to processes associated with recovery of tissue from the chilling damage.

MANY plants indigenous to subtropical regions suffer physiological injury when subjected to low (0 to 10 C), but nonfreezing temperatures (3, 9). While the symptoms of this chilling injury have been adequately described in many sensitive plants (2, 6, 8), it seemed desirable to investigate biochemical aspects connected with chilling injury and recovery. Guinn (2) reported an increase in soluble nitrogen in the roots of chilled cotton seedlings. Razmaev (6) found that protein decreased while the soluble nitrogen increased in chilling sensitive plants, but not in nonsensitive plants. He assumed that the determining factor in the resistance of vegetative organisms to cold is the capacity to reverse catabolic processes. In previous work (4) we have reported that during a chilling period an increase in some of the free amino acids was found in okra leaves. All the above-mentioned studies were related to changes that occurred during the chilling period. Stewart and Guinn (8) reported that cotton plants returned to optimum conditions after a chilling period were able to restore the initial ATP concentration when chilled only 1 day, but not when chilled 2 days. Amin (1) showed a stimulation of respiration following chilling of cotton at 2.8 C. Preliminary work (4) with okra indicated that protein synthesis increased following chilling. The work described in this paper was undertaken to study protein synthesis and CO_2 fixation in the period immediately after chilling cotton seedlings.

MATERIALS AND METHODS

Seeds of cotton (*Gossypium hirsutum* L. cultivar Paymaster) were planted in washed sand in flats in a greenhouse and watered twice daily with Hoagland's nutrient solution. After 1 month the flats were placed in incubators at 5, 10, and 25 C (control) with continuous fluorescent light. The chilling period used is given in the data tables and figures. After the chilling treatment four leaf blades with 2.5 cm of petiole were excised from plants under each treatment. The petioles of two leaves were placed into 1 × 2.5-cm plastic vials containing 0.5 ml mixture of uniformly labelled ^{14}C amino acids[3]. This mixture contained L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-phenyl alanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine. The vials from each treatment were placed at 30 C under a 2,500 ft-c light for 1 hour, after which petioles were removed and discarded and the fresh weight of the blades was determined. Using a cold mortar and pestle, the leaf blades were homogenized by grinding with 0.1*M* Tris buffer pH 7.5 (1 w/2v). The homogenate was then transferred to 40-ml polyethylene tubes and centrifuged at 20,000 x *g* for one hour. The protein was precipitated from the supernatant by adding ammonium sulfate to give 90% saturation and centrifuged at 48,000 x *g* for 1 hour. The pellet was washed by centrifugation three times with saturated ammonium sulfate. The precipitate was dissolved in 2 ml 0.1*M* Tris buffer (pH 7.5) and transferred to vials, and the radioactivity was determined by liquid scintillation counting.

In the $^{14}CO_2$ fixation experiment seedlings were chilled as previously described. Following the treatment, leaves were excised and the petioles placed in vials in 0.25 ml $NaH^{14}CO_3$ containing 50 μc ^{14}C. After incorporation for 1 hour the leaf blades were removed, extracted four times with boiling 80% ethanol, and fractionated according to the scheme given in Fig. 1. The combined extracts were evaporated to dryness with formic acid in a flash evaporator. The residue was dissolved in water and ether, and the water-soluble and ether-soluble phases were separated and counted for radioactivity. The water-soluble extracts were then passed through Dowex-50 (H^+) resin columns, 1.0 x 15.0 cm, and the effluent from these columns were evaporated to dryness. The amino acids were eluted from the Dowex-50 columns with 50.0 ml of 1.0N NH_4OH, and this fraction was evaporated to dryness, dissolved in water and assayed for radioactivity. The dried effluent from this column was dissolved in water and passed through a Dowex-1 (formate) resin column, 1.0 x 15.0 cm. The effluent, which contained the ^{14}C neutral fraction, was evaporated to dryness, dissolved in water, and assayed for radioactivity. The acidic fraction was eluted from the Dowex-1 columns with 50.0 ml of 8*N* formic acid, evaporated to dryness, dissolved in water, and assayed for radioactivity.

The amount of radioactivity in the aqueous samples was assayed in a Beckman liquid Scintillation system (model 15-200B). Each 0.2 ml radioactive sample was added to 15.0 ml of scintillation fluid containing 5 g of DPO (diphenyl oxazole), 100 g of napthalene, 10 ml of water, and dioxane to 1 liter. The scintillation vials were dark-adapted for several hours, and the counts were assayed with ±2% error. Each experiment was repeated at least once with comparable results.

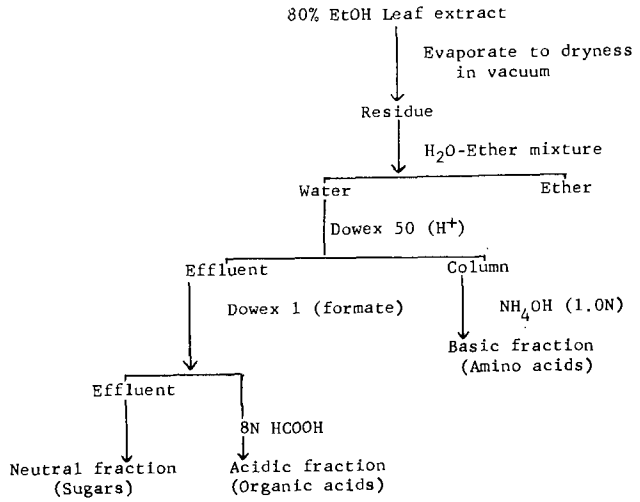

Fig. 1. A schematic diagram of the fractionation of leaf material exposed to $^{14}CO_2$ following chilling treatments.

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[3] All radioactive chemicals used in this work were purchased from New England Nuclear Co.

RESULTS

Under the conditions of these experiments the cotton seedlings exhibit the symptoms of injury illustrated in Fig. 2. The degree of injury depended upon the duration and severity of chilling.

Protein synthesis as evidenced by ^{14}C amino acid incorporation increased following a chilling period of 36 hours at 5 and 10 C when compared to the control kept at 25 C (Table 1). The 10 C chilled plants incorporated ^{14}C amino acid into protein to a larger extent than the plants treated at 5 C. The incorporation of $^{14}CO_2$ after the 36-hour chilling treatments is shown in Table 2. There was a considerable increase in all fractions when the chilled plants were compared to the control. The distribution between the amount of ^{14}C in the various fractions was the same in plants treated at all three temperatures.

When protein synthesis was determined as a function of chilling duration (Fig. 3), there was an increase in amino aicd incorporation into protein following chilling periods of 24 and 48 hours. Incorporation following the 48-hour chilling was much increased over that following a 24-hour exposure. After 72 hours of chilling amino acid incorporation was decreased sharply below that obtained after 48 hours. Greater amino acid incorporation was found in plants following chilling at 10 than at 5 C.

DISCUSSION

The cotton seedlings used in these experiments showed injury with decreased temperatures and increased time of exposure to either 5 or 10 C. Previously, Powell and Amin (5) showed that with less severe injury there was a greater recovery from chilling damage. It is possible that recovery involves metabolic changes. For example, Stewart and Guinn (8) showed that chilled cotton plants had a reduced ATP level that was restored following chilling provided the chilling period was limited to 1 day. We have found that the incorporation of amino acids into protein was greatly stimulated following chilling. The data from the $^{14}CO_2$ incorporation experiment indicated that amino acid synthesis was also stimulated, since there was a great increase in the ^{14}C level of the basic fraction, which contained mostly amino acids. There was also a great increase in other fractions, indicating a general increase in metabolism. Amin (1) has shown that respiration of cotton leaf disks as measured manometrically increased following chilling at 2.8 C for 12 hours, but that extended chilling at this temperature reduced respiration. As the exposure to chilling

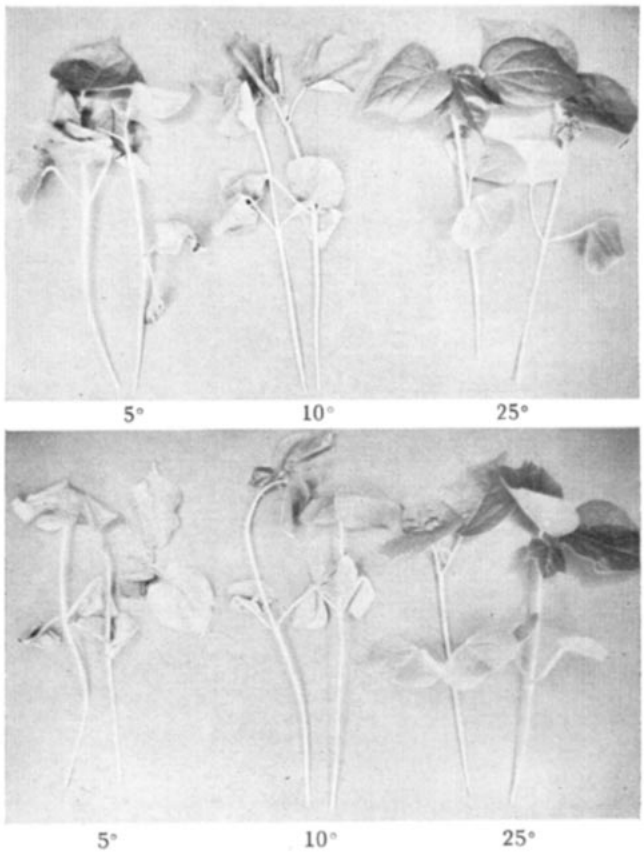

Fig. 2. Plants subjected to chilling for the temperatures indicated compared to similar plants kept at 25 C. Top exposed for 48 hours and bottom exposed for 72 hours.

Table 1. Effect of low temperature on the incorporation of ^{14}C amino acids into protein.

Treatments*	cpm/g fr wt	% stimulation
5C	10,971	140
10C	19,566	250
25C	7,824	---

* Chilling period of 36 hours followed by a one-hour incorporation time at 30C.

Table 2. Distribution of radioactivity from $^{14}CO_2$ fixation for 1 hour following 36-hour exposure to the specified temperatures.

	5C		10C		25C	
Fraction	cpm	%	cpm	%	cpm	%
Total (water extract)	357,210	100	354,794	100	214,390	100
Basic	1,119	0.31	2,166	0.61	719	0.33
Neutral	240,512	67.3	209,987	59.1	144,615	67.3
Acidic	49,075	13.7	52,471	14.7	32,962	15.3
% recovery		81.31		74.4		83.03

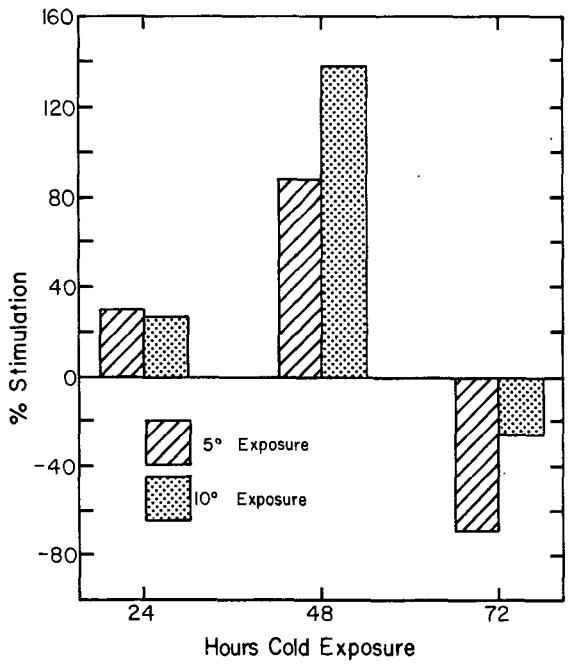

Fig. 3. Incorporation of amino acids into protein for a 1-hour period following cold treatment for the times and temperatures indicated. Results are given as the percentage of the control (250), which is the 0 line.

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increased beyond 48 hours at either 5 or 10 C, the amino acid incorporation decreased. Injury at this point was sufficiently severe to impair the recovery mechanism. With the 10 C treatment the incorporation of ^{14}C amino acids at both 48 and 72 hours chilling was greater than that at 5 C. It is likely that at 5 C greater injury occurred and the protein-synthesizing mechanism was affected.

It can be postulated that, following injury from chilling, recovery is initiated, and as a result, metabolic processes of the plant, including protein synthesis, are increased. If injury is sufficiently great, however, recovery does not occur. In this latter case the stimulation of metabolic processes, such as CO_2 fixation, and amino acid incorporation into protein do not occur.

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