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# Chilling Injury in Cotton Seedlings: Changes in Permeability of Cotyledons[1]

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## ABSTRACT

**Chilling at 5C caused cotton seedlings (*Gossypium hirsutum* L.) to wilt and apparently increased permeability of cotyledonary membranes. Chilling greatly increased the amounts of reducing sugars, ninhydrin-positive material, and ionic material which subsequently leached from cotyledons when they were floated in 0.2*M* mannitol after the chilling treatment. Permeability did not increase until seedlings were chilled for at least 3 hr. Moisture stress, caused by decreased water uptake at low root temperature, may have affected permeability of cotyledons. Cold-hardened seedlings, upon chilling, showed less visible injury and less leakage from cotyledons than control seedlings.**


***Additional index words:*** **Cold-hardening, Dehydration, Desiccation, Leakage, Membranes.**

FREEZING greatly increases membrane permeability in tissues which are injured by freezing (6, 11, 12), and membranes have been suggested to be the primary site of freezing injury in plants (11). Thermophilic plants can be damaged by nonfreezing temperatures, and limited evidence suggests that, as with freezing injury, membranes may be affected. For example, Lieberman, et al. (10) reported that slices of sweetpotato roots (*Ipomoea batatas*) showed approximately five times as much leakage during 10 weeks storage at 7.5C as they did at 15C. Christiansen found that cotton (*Gossypium hirsutum* L.) seedling radicals exuded sugars and amino acids when chilled or exposed to sodium azide (Christiansen, M. N. 1969. Exudation by cotton seedling radicles at low temperatures. Proc. 1969 Beltwide Cotton Production Research Conferences, pp. 127-130). In contrast to my results with cotyledons, however, exudation ceased when chilling was discontinued.

This paper reports the effects of chilling at 5C on permeability of cotton cotyledons as indicated by leaching of reducing sugars, ninhydrin-positive substances, and ionic material from cotyledons floated in a mannitol solution after the chilling treatment.

## MATERIALS AND METHODS

Three tests were conducted to determine the effects of chilling on permeability of cotton (*Gossypium hirsutum* L. 'Westburn') cotyledons. Cotton seeds were germinated and the seedlings cultured in a complete nutrient solution as reported earlier (4).

The first test was conducted to determine the effects of 24 hr at 5C on permeability of cotyledons. Seedlings were cultured during May in a fiberglass greenhouse at 25 to 40C and in a growth chamber maintained at 25C night and 30C day temperatures. Eleven days after planting, half of the seedlings were harvested and half were placed in a growth chamber at 5C, kept there for 24 hr, and then harvested. Light intensity was approximately 1,800 ft-c during 14 hr of illumination with a mixture of cool-white fluorescent and incandescent lamps. Immediately after harvest, cotyledons were removed and placed, one pair to a petri dish, in 10-ml portions of 0.2*M* mannitol and kept for 24 hr in the dark at approximately 25C. After 24 hr, the cotyledons were removed and the solutions were centrifuged to remove turbidity. Reducing sugars and ninhydrin-positive compounds which had leached from the cotyledons into the mannitol were measured by a ferricyanide test (3) and a ninhydrin test (2), respectively.

A second test was conducted to determine how soon chilling injury begins to develop. Seedlings were cultured in a greenhouse for 12 days from date of planting in late May. Greenhouse temperatures ranged from 25 to 40C during this period. They were then transferred to a growth chamber previously set at 5C. Plants were illuminated during the chilling period at a light intensity of approximately 1,800 ft-c. Cotyledons were harvested after 0, 1, 2, 3, 4, and 6 hr of chilling. (Unfortunately, the growth chamber was initially unable to maintain the temperature at 5C because of the warming effect of the nutrient solutions.) Cotyledons were floated in mannitol as reported above and the solutions were subsequently assayed for ninhydrin-positive material.

A third test was conducted to compare leaching from cotyledone of cold-hardened and nonhardened seedlings. (Previous unpublished tests had indicated that seedlings could be cold-hardened at 15 to 20C and that they were then much less sensitive to chilling at 5C.) Seedlings were cultured in a greenhouse in late January and early February and, because of shorter days and lower temperatures, developed more slowly than in the first two tests. Fifteen days after planting, half of the seedlings were transferred to a growth chamber to harden at 20C day and 15C night temperatures with 14 hr of illumination at 3,000 ft-c per day. After 3 days of hardening, half of the hardened and half of the control seedlings were harvested. The remaining seedlings were placed in a growth chamber and chilled at 5C for 24 hr. The seedlings were illuminated for 14 hr at 1,800 ft-c during the chilling treatment. Seedlings were harvested and cotyledons placed in mannitol as reported above. Amount of leaching was estimated by determining electrical resistance with a conductivity meter and content of ninhydrin-positive material in the mannitol solutions.

## RESULTS AND DISCUSSION

Chilling injury in cotton seedlings apparently involved increases in permeability of cotyledonary membranes because chilling increased the amount of material which subsequently leached from cotyledons floated in 0.2*M* mannitol.

Chilling for 24 hr at 5C greatly increased the amount of reducing sugars and ninhydrin-positive material which leached from cotyledons (Table 1). Plants cultured in a greenhouse appeared to be more sensitive to chilling than those cultured in a growth chamber. Chilling caused 15-fold and 68-fold increases, respectively, in reducing sugars leached from cotyledons of seedlings cultured in a growth chamber and in a greenhouse. Similarly, chilling caused 14-fold and 48-fold

**Table 1. Reducing sugars and ninhydrin-positive materials leached from cotyledons before and after chilling for 24 hours at 5C.***

| Location of culture prior to treatment | Reducing sugars | | Ninhydrin-positive | |
|---|---|---|---|---|
| | Control | Chilled | Control | Chilled |
| | —— μg —— | | —— mμmoles —— | |
| Greenhouse | 14.5±1.1 | 983±85 | 29±4 | 1,385±104 |
| Growth chamber | 52±11 | 778±121 | 37±4 | 504±98 |

* Data are averages of eight samples and are expressed as micrograms of reducing sugars (equivalent to glucose) and millimicromoles of ninhydrin-positive material (equivalent to glycine) leached from one pair of cotyledons into 10 ml of 0.2 M mannitol in 24 hr at 25 C. Standard error of the means are shown.

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**Table 2. Ninhydrin-positive substances leached from cotyledons as influenced by duration of chilling treatment at 5C prior to leaching.***

| Duration of chilling treatment in hours | | | | | |
|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 | 4 | 6 |
| 135±58 | 348±129 | 92±21 | 306±83 | 819±271 | 1,329±264 |

* Data are averages of six samples and are expressed as millimicromoles of ninhydrin-positive material (equivalent to glycine) leached from one pair of cotyledons into 10 ml of 0.2 M mannitol in 24 hr at 25 C. Standard errors are shown.

increases in amount of ninhydrin-positive materials leached from cotyledons of seedlings cultured in a growth chamber or greenhouse, respectively.

Results of the second test indicate that more than 2 hr of chilling were required to cause changes in permeability of cotyledons (Table 2). Results were quite variable, probably because of excessive temperature variation with location within the growth chamber. However, the results do indicate that permeability started increasing after 3 hr of chilling.

Both low temperature and dehydration may be required to cause loss of differential membrane permeability in cotyledons and leaves. I found (unpublished) that chilling at 2C had no apparent detrimental effect when leaf discs were floated in 0.2*M* mannitol during the chilling treatment, but leaves were severely injured or killed when intact plants were chilled. Low root temperatures drastically decrease the rate at which cotton roots absorb water (1, 8, 13, and Guinn, unpublished) and wilting is an early symptom of chilling injury. Dehydration due to ice formation is thought to cause disruption of membrane structure and loss of differential permeability when cells freeze (6, 11, 12). It is also possible that dehydration caused by an excess of transpiration over water uptake (1, 8, 9, 13, 15) alters membrane structure in thermophilic plants at temperatures above freezing. The lag period prior to wilting and before cotyledons became leaky may have been due to the relatively slow cooling of roots in nutrient solutions. Once initiated, however, the dehydration process may accelerate because of progressive loss of differential permeability with consequent rapid loss of moisture. Chill-injured cotton leaves and cotyledons often become completely flaccid within 24 hr and subsequently become necrotic or develop necrotic areas, depending upon severity of the chilling injury (Guinn, unpublished).

Chilling injury and subsequent leaching were not as great in the third test as they were in the first, possibly because of lower temperatures and humidity in the greenhouse during the third test (January vs. May). Nevertheless, chilling at 5C caused a considerable amount of leaching in this test as indicated by increased electrical conductivity and increased content of ninhydrin-positive material in mannitol solutions in which cotyledons were floated (Table 3). Cotyledons of plants which were cold hardened prior to chilling showed less visible injury and also lost less material to the mannitol solutions than cotyledons of plants which were not hardened before chilling. Therefore, preconditioning treatment apparently affects sensitivity to chilling.

If chilling does disrupt membrane structure in thermophilic plants, serious consequences would likely result. The most obvious consequence is that affected tissue becomes desiccated when injury is extensive (13, 15). Although not investigated, it seems possible that

**Table 3. Leakiness of control and cold-hardened cotyledons before and after chilling for 24 hr at 5C as indicated by electrical resistance of and ninhydrin-positive material in the bathing medium.***

| Treatment | Electrical resistance K ohms | Ninhydrin-positive mμmoles |
|---|---|---|
| Control | 26.7±1.0 | 38±7.2 |
| Hardened | 26.9±1.2 | 45±7.4 |
| Chilled | 3.4±0.7 | 819±176 |
| Hardened and chilled | 13.8±1.9 | 420±152 |

* The bathing medium was 10 ml of 0.2 M mannitol per pair of cotyledons and leaching was continued for 24 hr at 25 C before measurements were made. Standard errors are shown. Data are averages of 24 samples.

leakage of metabolites might promote invasion by seedling disease organisms. It is also likely that membrane-dependent processes such as oxidative- and photo-phosphorylation would be impaired, as has been shown with freezing injury (5, 7). Chilling did cause a sharp decline in ATP content of unhardened cotton seedlings (14). Loss of differential membrane permeability would probably permit detrimental mixing of cell components. Phenolic materials could be released and inhibit or destroy enzyme activity or hydrolytic enzymes could be released from lysosomes and cause hydrolytic destruction of essential components such as RNA and protein. Preliminary results (unpublished) indicated a partial loss of high molecular weight RNA after chilling. Further work is needed to substantiate this apparent effect.

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