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Periods of Sensitivity to Chilling in Germinating Cotton

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Summary. Cotton seedlings were subjected to a 96 hour chilling treatment (5° or 10°) after periods of germination at 31° ranging from 0 to 48 hours. Inhibition of subsequent growth at a favorable temperature by chilling was dependent on level of low temperature and stage of seedling development when chilled. Two periods of chilling hypersensitivity were observed during germination: 1) coincident with subjection of seed to a germination environment; and 2) after 18 to 30 hours of germination at 31°. Subsequent growth of seedlings chilled after 12 to 18 hours or 48 hours of germination at 31° was relatively unaffected. It is suggested that chilling alters specifically timed events that occur at the initiation of germination and after 18 to 30 hours of germination, and that alteration of these germination processes is visited on long term subsequent growth of the plant.

Immediate effects of low temperature are generally well recognized in current reviews of temperature influence on biological systems; however, references to delayed or sustained expression of low temperature influences are limited (9). Among the few reports concerning prolonged expression of cold lesion is that of Pollock and Toole (9) who showed chilling injury is induced in imbibing lima bean seed. Knapp (7) found inhibition of subsequent growth of *Senecio vulgaris* by chilling during germination. Highkin (5) reported that a low temperature-induced dwarfing in peas persisted for several generations after treatment. The long-term influence of chilling of germinating seed upon subsequent development of upland cotton (*Gossypium hirsutum* L.) has been studied by the author (2, 3). Chilling of cotton plants caused reduced growth at later favorable temperatures directly proportional to the duration of chilling at 10°. Two types of chilling injury symptoms in seedlings were described: A) a radicle tip abortion induced by chilling germinating seed from initiation of germination; and B) a root cortex disintegration induced by chilling the seedling after elongation of the embryonic axis had commenced (2).

The present paper provides evidence for the existence of discrete sensitive periods during germination when chilling adversely affects subsequent growth of seedlings and relates this to events that are known to occur during early germination.

Materials and Methods

The general experimental procedures involved application of low temperature treatments to cotton seedlings at various times after germination was initiated, and then transference of the seedlings to liquid nutrient culture in the greenhouse for evaluation of possible effects on subsequent growth.

The seed of *Gossypium hirsutum* L. used in all the investigations were from a single lot of self-pollinated M-8 genetic strain, which is a colchicine doubled-haploid derived from Delta Pine 14 cultivar. The seed coats were removed to eliminate variation in germination due to seed-coat-induced differential water uptake. The seed was germinated between 2 rolled 31 × 46 cm germination papers wet with 60 ml of a 0.5 strength modified Hoagland's (1) nutrient solution and covered with a waxed paper covering. Each seed roll contained 25 seeds.

Chilling consisted of temperature treatments at either 5° or 10° for a period of 96 hours. Treatments were applied to seeds in rolls after each of the following periods of germination at 31°: 0, 12, 18, 24, 30, 36, 42, and 48 hours. Each test included treatments at the 8 stages of germination and a control consisting of seedlings germinated 48 hours at 31°. Cold treated seedlings likewise received a total of 48 hours at 31° in addition to the 96 hours of cold. Treatments were scheduled so that all were completed simultaneously. Excess seedlings were included in each treatment so that only visually normal seedlings were transferred to the greenhouse and cultured in an aerated liquid nutrient solution. Seedlings with aborted root tips, cortical collapse, or other abnormalities were discarded.

The tests were arranged in a randomized block design with 5 replications. The plants were harvested after 15 days, divided into tops and root

system, dried at 60° and weighed. Treatment effects were computed on the basis of percentage of controls. The data reported are from 4 experiments.

Results and Discussion

The results presented in figure 1 show that sensitivity to chilling varies with stage of seedling development and level of temperature. The 5° treatment when applied at zero time killed all seeds. The same temperature caused only a moderate amount of subsequent growth inhibition when applied after 12 hours of germination at 31°. A second period of chilling hypersensitivity occurs after elapse of 18 to 30 hours of germination at 31°, which coincides with the period of rapid radicle elongation and ends with initiation of rapid hypocotyl elongation (table I). The response curve to 10° chilling is similar to the 5° curve, except that the amount of subsequent growth inhibition is not so great. Both periods of hypersensitivity (0 time and 18–30 hr) are exhibited by seedlings after 10° chilling; however, the seeds are not killed by the zero time 10° treatment as they are at 5°.

The second period of chilling hypersensitivity which occurs after 18 to 30 hours of germination at 31° is evident at either 5° or 10°. It presumably involves damage to the same system at either 5°

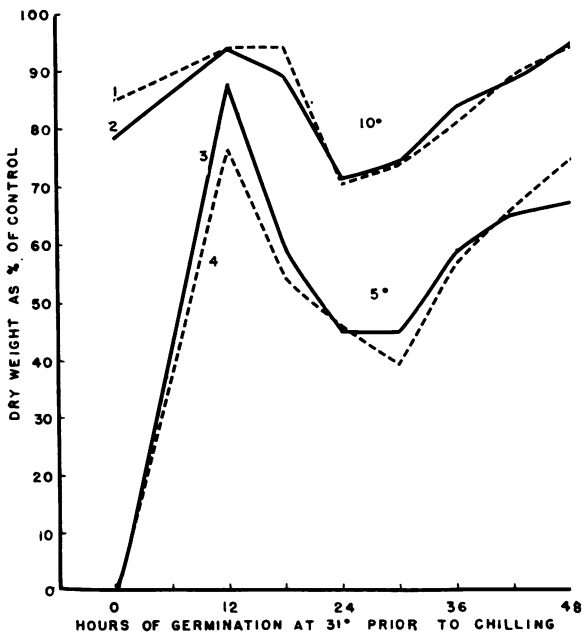

FIG. 1. Dry weight after 15 days greenhouse growth of roots (dotted line) and tops of seedlings chilled (solid line) at 5° or 10° after germination for the indicated increments of time at 31°. Difference required between points on curves for significance at 5 % level of probability; curve 1 = ± 4.1, 2 = ± 3.7, 3 = ± 6.3 and 4 = ± 5.8.

Table I. *Radicle and Hypocotyl Development after Germination for Several Periods of Time*

Germination at 31°	Length	
	Hypocotyl	Radicle
hrs	cm	cm
0	0.20	0.20
12	0.20	0.40
18	0.20	0.82
24	0.25	1.45
30	0.30	2.63
36	0.95	3.84
42	1.45	4.70
48	2.77	7.21

or 10°. The difference in the amount of inhibition induced by the lower temperature treatment is another example of the previously reported (2, 3) additive influence of either level or duration of low temperature. The fact that a 96-hour chilling at 5°, applied at the 24-hour stage, inhibited subsequent growth 66 %, as compared to half that amount (29 %) at 10°, supports the idea that chilling causes a quantitative disruption of a key germination process which is vital to the subsequent performance of the plant. Our earlier work (2) showed that chilling at the onset of germination induces a high incidence of root terminal abortion in cotton, which certainly would influence subsequent growth: however, atypical seedlings were discarded in the present study. The growth inhibition measured in seedlings from the zero time chilled at 10° treatment thus suggests a borderline injury to radicle tip meristem cells. As a result, subsequent root system development is altered or delayed.

Among the more significant events known to occur during seed hydration and early germination which might be influenced by chilling are the rapid activation of RNA synthesis, polysome formation, and an active protein synthesis system shown to occur by Marcus et al (8), Waters and Dure (10), and Fujisawa (4). Parallel evidence by Woodstock and Skoog (11) and more recently by Key (6) indicates that RNA and protein synthesis are essential for cell elongation in seedling tissue. The impact of chilling during early germination thus could be on RNA and protein synthesis, and cell elongation.

The present results suggest that: A) germination is a sequence of interrelated steps, some of which are more sensitive to low temperature than others; and B) interruption of the sequence of events with cold at rather precise stages may cause death, or long term growth inhibition.

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