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Periods of Chilling Sensitivity in Germinating Pima Cottonseed[1]

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ABSTRACT

Seed germination and seedling development are adversely affected in many crops by less than lethal chilling temperatures. Periods of chilling sensitivity have been identified in Upland cotton (*Gossypium hirsutum* L.) but little work has been reported in Pima cotton (*G. barbadense* L.). This study was conducted to evaluate the influence of temperature on the degree and timing of chilling susceptibility in germinating Pima cotton and to determine if genetic variability exists. Seed were germinated at 25 or 35 C for 72 hours with one additional 24-hour chilling exposure of 5, 7, or 10 C at various times during germination. Two periods of chilling sensitivity were noted. The first occurred at the beginning of germination. The second period showed maximum sensitivity between 28 and 32 hours when seed were germinated at 35 C and between 40 and 56 hours when seed were germinated at 25 C. Chilling at 7 C during both sensitive periods delayed emergence from a soil mix. In addition, final emergence was reduced by 26% when seed were chilled at the beginning of germination. Chilling at 5 C caused much more severe damage than chilling at 7 or 10 C. Some experimental lines showed greater resistance to chilling than the commercial cultivar, 'Pima S-4.' Two genetic lines that responded similarly when chilled at 5 C differed significantly when chilled at 7 and 10 C. This suggests that these higher temperatures may be best for evaluating genetic variation and seed treatments to impact chilling resistance.

Additional index words: **Chilling injury, Seed vigor, Rate of germination, *Gossypium barbadense*, *Gossypium hirsutum*.**

SEED germination and seedling development are adversely affected in many species, including cotton (*Gossypium hirsutum* L. and *G. barbadense* L.), by chilling temperatures which induce injury in the absence of freezing. This has been extensively investigated in Upland cotton (*G. hirsutum*), but little is known about temperature effects in Pima cotton (*G. barbadense*). Temperatures below 12 C inhibit cottonseed germination and seedling development (16, 21, 23, 24). Abortion of radicle meristems and a lag in growth occur in *G. hirsutum* if seed are given a short chilling exposure (5 to 10 C) at the beginning of germination. Cold exposure after radicles emerge from seedcoats causes a delay of growth and damage to radicle cortex tissue (4). The degree of damage is related to length of chilling exposure (5, 7).

Although chill-damaged seedlings may survive, subsequent metabolic activity and morphological development can be altered. Shallow secondary root systems commonly develop. Christiansen and Thomas (9) reported detrimental effects of chilling on plant height, fruit set, and quality of fiber.

Two periods of sensitivity to chilling have been characterized for the genetic line M-8 of *G. hirsutum* by Christiansen (6). The most susceptible period occurs during initial seed hydration. When germinated at 31 C, seedlings were susceptible again between 18 and 30 hours. Chilling at 5 C during initial water uptake caused root-tip abortion of all seedlings. The number of seedlings damaged at other chilling times was not reported. Visually undamaged seedlings were grown for 15 days and dry weights were used to assess the magnitude of chilling damage. Dry weight of normal-appearing seedlings exposed to 5 or 10 C after 24 hours of germination was reduced 66 and 29%, respectively.

Periods of sensitivity to chilling have not been previously reported for *G. barbadense*. Seed of some selections of this species imbibe water faster than seed of *G. hirsutum* (13) and physiological development of the germination process has been related to seed moisture level (23, 25). Thus periods of susceptibility to chilling most likely differ between the two species. This paper reports periods of chilling sensitivity of *G. barbadense* as an extension of the previous work with *G. hirsutum*. The influence of germination temperature on timing and duration of chilling sensitivity has not been previously reported for either species. More precise evaluation of this relationship should aid in making crop management decisions about replanting after chilling exposure and help in developing cotton lines more resistant to chilling injury.

The objectives of this study were to evaluate the influence of germination temperature on sensitivity to chilling in *G. barbadense* and to determine if genetic variation for resistance to chilling exists within lines of this species. Genotypic differences in germination at low temperatures have been observed by Ludwig (16).

MATERIALS AND METHODS

Six experiments were conducted using the G. *barbadense* cultivar, 'Pima S-4,' and other experimental lines of *G. barbadense*. Seed for the first five experiments were furnished through the courtesy of C. V. Feaster, ARS-USDA research agronomist, Phoenix, Ariz. These seed were harvested from a replicated yield trial at Phoenix where all genetic lines had a similar environmental background. For the sixth experiment, commercial seed of Pima S-4, treated with PCNB (pentachloronitrobenzene), captan [N-(trichloromethylthio) - 4 - cyclohexene-1, 2-dicarboximide], and methoxychlor [1,1,1-trichloro-2, 2-bis (p-methoxyphenyl) ethane] were used. The experiments were conducted during the spring and summer following harvest.

Seed for the first five experiments were acid-delinted in concentrated H_2SO_4 for 4 min. After rinsing in tap water for 6 min, floating seed, seed with cracked hulls, seed with light-colored seedcoats, and abnormally small seed were discarded. These seed normally show poor germination properties (1, 15, 20). Temperature-controlled water baths, maintained within 0.5 C of the desired temperature, were used during germination.

The first five experiments were conducted by placing 25 seed in rolled germination paper towels with the micropyle down.

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Water was added and the towels were placed in plastic bags attached to a rack designed to hold the open end up during germination. Chilled water was added to the germination towels of the treatments chilled at the start of germination. These experiments contained six replications and were conducted in the dark. Seed were germinated at either 25 or 35 C for 72 hours plus one additional 24-hour chilling exposure of 5, 7, or 10 C at various times during germination. The experimental design was completely randomized in a split-plot arrangement. The time of chilling was the whole plot and genotype the subplot. At the conclusion of the experiments, germination counts were made of seedlings without visual symptoms of chilling injury (damage to radicle tip or cortex disintegration) and with extending radicles longer than 1 cm. The plant axes of these seedlings were removed at the junction with the seed micropyle. The dry weight of the plant axis was expressed as a percentage of the total dry weight and is referred to as percent transfer which is similar to a term described by Christiansen (3). This value gives an indication of the rate at which visually undamaged seedlings developed. To characterize seed germination into a single term, germination index (GI) was determined as follows:

$$GI = (\%\ \text{germination})(\%\ \text{transfer})/(\text{days of germination})\,(100)$$

In the first experiment, three lines, including Pima S-4, were germinated at 25 C and chilled at 5 C after 24, 30, or 36 hours. The total germination time was 96 hours. The experiment also included a nonchilled control that was germinated for 72 hours.

The next two experiments were conducted to ascertain susceptible periods to chilling in Pima S-4 and an experimental line, P-23. In the second experiment, seed were germinated at 25 C and chilled at 5 C. The chilling temperature was the same in the third experiment, but seed were germinated at 35 C. In these two experiments chilling exposure was evaluated 18 times during the germination period. In the next two experiments, seed were germinated at 25 C and chilled at 7 or 10 C for 24 hours at seven different times during germination.

For the sixth experiment, with 12 replications in a completely randomized design, seed were placed in a clay loam topsoil, peat moss, and vermiculite (1:1:1) mix. Plastic cups with a diameter of 11 cm and a depth of 8 cm were filled with the soil mix to a depth of 3.5 cm. Five seed were laid on the soil, an additional 2 cm of soil mix added, and the soil mix brought to field capacity. When seedlings were chilled at the start of germination, chilled water was added to prechilled soil and seed. The cups were placed in the temperature-controlled water baths at 25 C for 72 hours with one additional 24-hour chilling exposure of 7 C at various times during germination. Seedlings were transferred to a growth chamber (programmed for 12 hour day-night cycles) for 14 days. The day temperature was 25 C with an irradiance of 110 W/m² (400 to 700 nm wavelength) and the night temperature was 15 C. Several emergence counts were taken and plants were harvested at the end of the period, dried, and weighed.

RESULTS AND DISCUSSION

In the first experiment, the genotype × chilling time interaction was significant for percent germination and GI, but not for percent transfer. The three genotypes generally did not differ dramatically in germination properties although some significant differences were noted (Fig. 1). When not chilled, GI of E-2 was significantly lower than for the other two lines. However. when chilled at 30 hours, GI of E-2 was significantly higher than P-25 with Pima S-4 being intermediate. The relative improvement in GI of E-2, compared to the other lines when chilled at 30 hours, occurred mostly because of chances in percent germination. In a field comparison with low soil temperature, E-2 had greater seedling emergence than Pima S-4 and other experimental *G. barbadense* lines tested (2).

Seedlings chilled at 24 hours often had higher percent transfer and GI than control seedlings. The total

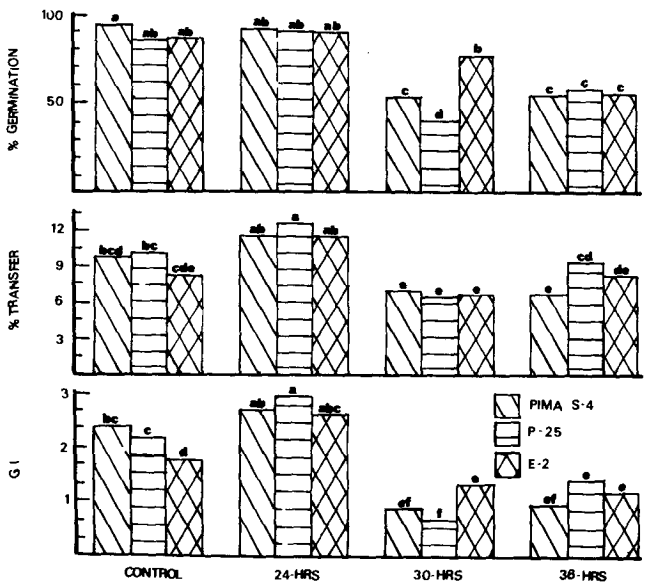

Fig. 1. Response of three genotypes to chilling at 5 C at various times after the start of seed germination. Letters show significant differences at 0.05 level.

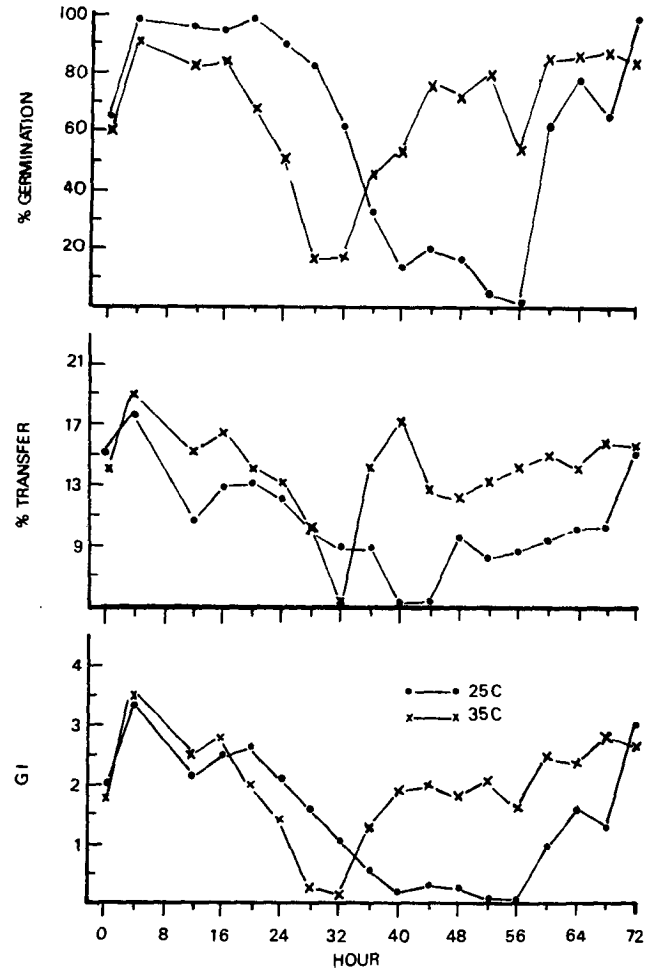

Fig. 2. Effect of chilling at 5 C at various times on cotton-seed germinated at 25 or 35 C.

Table 1. Effect of chilling at 7 or 10 C on cottonseed germinated at 25 C.

Hour of chilling	% Germination*		% Transfer		GI	
	Pima S-4	P-23	Pima S-4	P-23	Pima S-4	P-23
			7 C			
0	73.7 b	69.3 b	9.7 ab	10.0 a	1.82 cde	1.72 cde
4	98.0 a	97.3 a	10.1 a	10.4 a	2.47 ab	2.53 a
20	92.7 a	92.0 a	7.9 bcd	9.6 ab	1.85 cde	2.27 abc
28	93.7 a	95.3 a	6.7 def	8.1 bcd	1.58 de	1.92 be
40	73.0 b	77.3 b	5.3 f	7.8 bcd	0.97 f	1.48 e
64	91.8 a	96.7 a	5.9 ef	7.2 cde	1.35 ef	1.73 cde
72	96.7 a	96.0 a	8.1 bcd	9.0 abc	1.96 a-e	2.15 a-d
			10 C			
0	92.7 a	91.7 a	11.1 c	12.2 b	2.57 c	2.79 b
4	94.7 a	96.7 a	11.2 c	13.5 a	2.65 bc	3.27 a
20	98.7 a	98.7 a	5.5 def	6.0 de	1.35 d	1.48 d
28	95.3 a	98.0 a	6.2 d	5.4 def	1.47 d	1.34 d
40	95.3 a	94.0 a	4.2 g	4.8 f	0.99 f	1.11 ef
64	92.7 a	97.3 a	5.5 def	6.1 d	1.28 de	1.48 d
72	95.3 a	97.3 a	5.2 ef	5.8 de	1.24 de	1.41 d

* Values within a germination parameter for a chilling temperature followed by the same letter are not significantly different at the 0.05 level according to the Student-Newman-Keuls Test.

Table 2. Effect of chilling at 7 C on percent seedling emergence.

Hour of chilling	Days in growth chamber					
	0	1	2	3	4	7
Control	36 a*	74 a	82 a	82 a	82 a	84 a
0	0 b	26 b	36 b	42 b	44 b	54 b
4	24 a	72 a	84 a	86 a	88 a	94 a
40	0 b	56 a	84 a	88 a	90 a	92 a
72	0 b	56 a	82 a	88 a	88 a	90 a

* Values within a column followed by the same letter are not significantly different at the 0.05 level according to the Student-Newman-Keuls Test.

time of germination of chilled seedlings exceeded that of the control by the length of the chilling period. Early processes of germination are associated primarily with seed hydration which requires little metabolic energy. During early hydration at this low temperature, seedlings are probably sufficiently resistant to chilling injury that water uptake and germination advancement occurs. Later when imbibition is nearly complete, seedlings are more sensitive to chilling as reflected in a reduction in the germination parameters.

When Pima S-4 and P-23 were germinated at 25 or 35 C and chilled at 5 C, two major periods of sensitivity to chilling were found. The timing and duration of the periods were dependent upon germination temperature. The two genotypes responded similarly except for a significant genotype $\times$ chilling time interaction for percent transfer at 35 C. However, no consistent pattern was associated with the interaction, and data for the two genotypes are averaged (Fig. 2).

Seed were most resistant to chilling 4 hours after the beginning of germination. Percent germination was near 60% when chilled at the beginning of germination compared to over 90% when chilled at 4 hours. Percent transfer and GI showed similar relationships. Chilling at later times greatly reduced all germination parameters. This sensitive period began as radicles emerged from seedcoats and was maximal when they were 2 to 4 cm in length. Greatest susceptibility to chilling occurred between 28 and 32 hours at 35 C and between 40 and 56 hours at 25 C. All three germination parameters were similarly affected with GI values reduced to about 5% of the 4-hour values. In *G. hirsutum* maximum sensitivity during this susceptible period occurred 24 hours after initiation of germination at 31 C (6). Seedcoats were removed in that study which may have speeded the germination process and could account for maximum sensitivity occurring earlier than reported here.

Seedlings chilled at 72 hours had lower percent transfer and GI than seedlings chilled at 4 hours. Again, it is probable that the passive aspects of germination proceeded during chilling at the 4-hour resistant stage.

The influence of germination temperature on time and magnitude of sensitivity suggests an association with physiological stage of development. At the low germination temperature, slowed metabolic rates necessitated more time to reach and pass through stages of susceptibility and resistance. The second period of chilling sensitivity lasted longer at 25 than 35 C. In fact the recovery is likely to be over-estimated at the low temperature. The adverse effects of chilling did not have time to be completely manifested after the last cold exposure because the seedlings were immediately harvested. This accounts for the sharp rise in the germination parameters between the last two time periods at 25 C. Under field conditions with lower germination temperatures, seedlings are probably sensitive for an even longer period of time.

Research on adverse effects of chilling has resulted in some understanding of critical processes susceptible to chilling damage. Injury results in exudation of solutes from seedling radicles which suggests membrane damage (8). Maximal DNA synthesis in germinating seedlings of *G. barbadense* coincides with the second sensitive period (12). The majority of nuclear DNA synthesized at this time is apparently associated with nuclear membranes while that synthesized during the earlier resistant stage is not associated, or only loosely associated, with nuclear membranes. Chilling during the second sensitive period results in a marked reduction in DNA metabolism and membrane-associated DNA polymerase activity. In contrast, chilling during the resistant stage at 4 hours has little effect on DNA synthesis (10, 11).

Differences in chilling susceptibility among species has been related to fatty acid composition of membranes. Species with greater sensitivity to chilling generally have membranes with higher amounts of saturated fatty acids than more tolerant species (14, 17, 18, 19, 22, 26). Within several *G. barbadense* lines, including Pima S-4, dry seed of E-2 had the highest ratio of unsaturated to saturated fatty acids (2). In addition, DNA activity in E-2 is less sensitive to chilling than in Pima S-4 (11).

The next two experiments evaluated the influence of less-severe chilling temperatures on Pima S-4 and P-23. In both experiments, significant genotypic variation occurred for percent transfer and GI with a significant genotype $\times$ chilling time interaction at 10 C for these parameters. From a consideration of percent transfer and GI, P-23 was generally more resistant to injury than Pima S-4 (Table 1). When evaluated un-

der field conditions with low soil temperature, more P-23 seedlings emerged than Pima S-4 seedlings. P-23 also had a significantly higher unsaturated/saturated fatty acid ratio than Pima S-4 (2).

The slight reduction in severity of chilling from the previous experiments resulted in much less seedling injury. Few seedlings showed visual injury when chilled at 10 C, although percent transfer was markedly reduced for some exposures. When chilled at 7 C, visual damage was noted for seedlings chilled during initial water uptake and at 40 hours. Percent transfer was reduced more when chilled at 40 hours at 10 C than at 7 C. This unexpected relationship cannot be explained.

The fact that greater genetic differences were found when Pima S-4 and P-23 were chilled at 7 and 10 C than at 5 C suggests that the higher temperatures are more desirable for evaluating seed treatments and screening genetic lines. A 5-C chilling temperature is very severe and, based on our experience, rarely (if ever) occurs at planting depth with near-normal planting dates.

The final experiment was conducted to evaluate the effect of chilling on subsequent growth and development of seedlings. The 7 C chilling exposure resulted in a significant delay in seedling emergence except when applied at 4 hours (Table 2). Seedlings subsequently recovered from all but the 0-hour chilling treatment as shown by the final emergence. Seedlings chilled initially had a final emergence that was only 64% of the control. When seedlings were chilled at 0 hours, the time required for 50% of total emergence was delayed about 1 day compared to the control. When chilled at 40 or 72 hours, the delay was about 0.5 day.

By the end of the experiment, height, dry weight, and appearance of true leaves of emerged seedlings were similar and not significantly different among treatments.

Although chilling soon after radicles emerge from seedcoats resulted in greater initial growth delay and visual damage to seedlings than chilling during initial germination, recovery is faster. However, injury during the second period may be of greater concern under field conditions. Damage associated with the first sensitive period can be avoided by planting when soil temperatures are favorable or by elevating seed moisture prior to planting (7). An unexpected cold front after planting, however, can lower the soil temperature and produce chilling damage during the later period of chilling sensitivity. Although the laboratory studies indicate that emergence is merely delayed following chilling after radicles emerge, this could have serious consequences in the field. With deeper planting depths and marginal soil temperature, chilling injured seedlings may show reduced emergence and be more subject to disease. Since genotype was shown to influence susceptibility to chilling injury, it would appear that emphasis should be placed on selection of lines with improved chilling resistance.

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