

## Effect of Pregermination Treatments on Germination and Growth of Cottonseed at Suboptimal Temperatures<sup>1</sup>

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### ABSTRACT

Various pregermination treatments were used to determine their effects on germination of cottonseed (*Gossypium barbadense* L.) at suboptimum temperatures and to determine whether chilling injury was reduced by these treatments.

'Pima S-4' cottonseed were preconditioned in water and gibberellic acid ( $10^{-3}M$ ) at 10 and 30 C or in adenosine-3':5'-cyclic monophosphate (cyclic AMP,  $10^{-4}M$ ) at 30 C, or were hot-water-treated at 70 C for 5 min. After being preconditioned, seed were chilled for 24 hours at 5 C in water. Seed were germinated over a temperature range of 15.5 to 30 C for 7 days on a thermogradient plate. Germination counts were made daily. The speed and percent of germination were higher for seed preconditioned at 30 C than at 10 C. Hot-water treatment reduced the sensitivity of seed to gibberellic acid and reduced germination at all temperatures. Chilling injury was reduced by preconditioning in either water, gibberellic acid, or cyclic AMP. Our data suggest that satisfactory cotton stands may be obtained at suboptimum temperatures (below 31 C) by elevating seed moisture and/or treating with a growth regulator prior to planting.

**Additional index words:** Seedling growth, Preconditioning, Chilling, Gibberellic acid, Cyclic AMP.

TEMPERATURES below 20 C reduce germination of cottonseed (*Gossypium hirsutum* L.) and emergence of seedlings. Temperatures of 5 to 10 C during imbibition kill the embryo or cause abnormal development of the seedling, such as sloughing of the root cortex or formation of nub-roots symptom, depending upon the time of chilling (2). Cottonseed are sensitive to chilling injury during imbibition especially at the time of initial hydration and 18 to 30 hours after imbibition begins (3). Christiansen (4) demonstrated that hydration chilling injury in cottonseed, genetic selection M-8, could be prevented by imbibing seed for 4 hours at 31 C. He also (5) indicated that the moisture content of the seed at the time of chilling determined the degree of injury to the seed. Seed with an initial moisture content above 13% were protected against chilling injury. Thomas and Christiansen (9) found that under field conditions preconditioned seed of low quality emerged at a faster rate than low quality seed that were not preconditioned, and that preconditioning enhanced overall crop performance throughout the growing season. Previous research (4,5) showed that chilling injury expressed at 31 C could be prevented by increasing the moisture content to 12%; however, no evidence is available that preconditioning protects seed from

injury when they are germinated in a range of temperatures below 31 C.

Our objectives were: 1) to determine the effect of preconditioning at different temperatures on rate and percent of germination of 'Pima S-4' (*G. barbadense* L.) seed over a range of suboptimal temperatures, 2) to determine the effect of imbibing seeds in water, gibberellic acid, or adenosine-3':5'-cyclic monophosphate (cyclic AMP) on rate and percent germination, seedling growth, and on reduction of chilling injury.

### MATERIALS AND METHODS

Thermogradient plates (8) were used to establish a range of 30 temperatures (15.5 to 30 C) in 0.5-C increments. Seed were planted on top of two layers of blue blotting paper. The blotting paper was placed on the plate and tap water was added as necessary. Seed were not exposed to light except during daily counting periods (4.6 lux).

**Experiment 1.** Two commercial lots of Pima S-4 (1971 seed, 7% moisture) were used in this experiment. Germination was 90 and 80% for lots 1 and 2, respectively (official germination methods). Seed had been acid-delinted, hot-water-treated, and fungicide-treated. The seed were separated into 10 sublots which were treated as indicated in Table 1. Immediately prior to each treatment, all seed which floated in water at 25 C and visibly damaged seed were removed. Treatments were timed so that all seed could be planted simultaneously on the thermogradient plates. Three thermogradient plates were planted with seed; each plate represented a replicate with all treatments being represented on each plate. Plates were divided into 10 areas ( $7.6 \times 76.2$  cm) and 10 seeds per treatment were planted at each of the 30 equally spaced temperatures. Each seed lot was tested separately.

Seed were considered germinated when the radicle was at least 1 cm in length. Normal seedlings were counted and removed at 24-hour intervals for 7 days. Data from both lots were combined, and moving averages were calculated for each temperature with its adjacent two temperatures. From calculating a moving average, percent germination expressed at each temperature was the average for 180 seeds.

**Experiment 2.** Seed of Pima S-4 produced in 1972 in Arizona were used in this experiment. Seed were delinted for 3 min in concentrated sulfuric acid and washed for 5 min in tap water at 25 C. Seed which floated in water at 25 C and damaged seed were removed. Seed were divided into 10 sublots (7% moisture), and the sublots were treated as indicated in Table 1. Four thermogradient plates were planted as described for experiment 1. Moving averages were obtained as described for experiment 1 except that each moving average in experiment 2 represented the average for 120 seeds. Germination criteria and counting periods were the same as described for experiment 1. Data shown by moving averages were not statistically analyzed.

Twelve replicates of 25 seeds of each treatment were planted in rolled paper towels at 30 C to measure germination and seedling growth 4 days after planting. Means were separated by using Duncan's Multiple Range Test.

### RESULTS

**Experiment 1.** No differences between seed lots in response to treatments were detected. Earliest radicle emergence occurred over a wider range of temperatures from seed preconditioned at 30 C (Treatments 3, 4, 7, and 8) than from seed preconditioned at 10 C (Treatments 5, 6, 9, and 10) (Fig. 1A). Seed not chilled or

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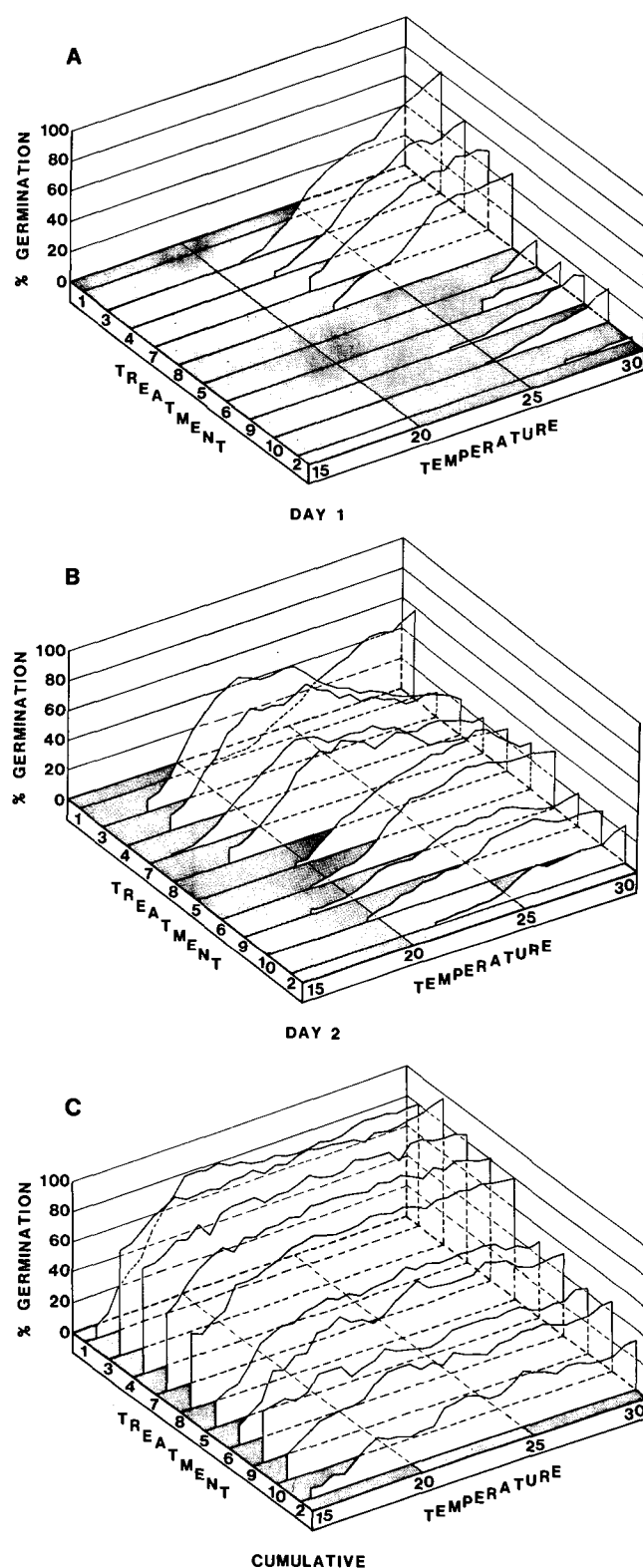


Fig. 1. Effect of various pregermination treatments and a range of temperatures (experiment 1) on rate of germination of cottonseed after: (A) Day 1, (B) Day 2, and (C) total cumulative germination after 7 days. Treatment: 1, control; 2, 24 hours at 5 C in GA; 3, GA at 30 C; 4, water at 30 C; 5, GA at 10 C; 6, water at 10 C; 7, GA at 30 C plus chilled; 8, water at 30 C plus chilled; 9, GA at 10 C plus chilled; and 10, water at 10 C plus chilled.

Table 1. Treatments used in preconditioning cottonseed in experiments 1 and 2.

Preconditioning treatment	Temperature, C	Post-preconditioning treatment		Treatment number	
		Chilled†	None	Exp. 1	Exp. 2
None (control)	-			1	1
None	5	†		-	-
None	5	‡		-	10
Water	30	X		8	5
Water	30		X	4	4
Water	10	X		10	-
Water	10		X	6	-
GA ( $10^{-3}M$ )	30	X		7	7
GA ( $10^{-3}M$ )	30		X	3	6
GA ( $10^{-3}M$ )	10	X		9	-
GA ( $10^{-3}M$ )	10		X	5	-
Cyclic AMP ( $10^{-4}M$ )	30	X		-	3
Cyclic AMP ( $10^{-4}M$ )	30		X	-	2
Hot-water	70	X		-	9
Hot-water	70		X	-	8

\* Seed were preconditioned in the indicated solutions for 6 hours except for the hot-water treatments which were for 5 min; following preconditioning the seed were dried overnight (18 hours) at 25 C. † Following overnight drying, seed were chilled in water for 24 hours at 5 C and again were dried overnight (18 hours) at 25 C. ‡ Seed were chilled for 24 hours in gibberellic acid ( $10^{-3}M$ ) at 5 C and dried overnight (18 hours) at 25 C. § Seed were chilled for 24 hours in water at 5 C and dried overnight (18 hours) at 25 C.

Table 2. Effect of preconditioning on germination and seedling growth of Pima S-4 cottonseed (experiment 2) at 30 C 4 days after planting.

Treatment number	Preconditioning treatment	Germination	Seedling growth
		%	mm
3	Cyclic AMP + chilled	73.5 a*	257 ± 12†
2	Cyclic AMP	72.5 a	274 ± 12
4	Water at 30 C	71.5 ab	206 ± 14
6	GA	69.0 bc	266 ± 10
1	Control, no preconditioning	67.5 cd	233 ± 14
5	Water at 30 C + chilled	67.0 cd	187 ± 7
7	GA + chilled	65.5 d	268 ± 14
8	Hot water	43.0 e	235 ± 14
9	Hot water + chilled	3.0 f	133 ± 21
10	Chilled 24 hours	3.0 f	76 ± 11

\* Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's Multiple Range Test. † Means and standard error of mean of 10 seedlings selected at random from the germinated seed in rolled towels except for the last 2 treatments which are means of 6 seedlings.

preconditioned (Treatment 1) did not germinate until 2 days after planting (Fig 1B). Seed preconditioned at 30 C germinated faster over the entire range of temperatures than did the control seed and the seed preconditioned at 10 C. At either temperature no differences were detected between seed that imbibed gibberellic acid (Treatments 3, 5, 7, and 9) versus those that imbibed water (Treatments 4, 6, 8, and 10, Fig 1B,C). Preconditioning seed at 30 C (Treatments 3, 4, 7, and 8) increased the percent of germination over that of the control seed (Treatment 1) at temperatures below 20 C. The seed imbibed at 30 C (Treatment 3, 4, 7, and 8) were less subject to chilling injury (either abnormal radicle or dead) than those imbibed at 10 C (Treatments 5, 6, 9, and 10).

**Experiment 2.** Seed preconditioned and chilled (Treatments 3, 5, and 7) germinated faster than seed which were only preconditioned (Fig 2A,B; Treatments 2, 4, and 6). Seed which were not preconditioned or chilled (Treatment 1) germinated on the second day as in experiment 1. Hot-water-treated seed (Treatments 8 and 9) and seed chilled for 24 hours (Treatment 10) had the slowest rate of germination. Preconditioning in cyclic AMP, gibberellic acid, and water increased the rate of germination at the lower temperatures (Fig 2C; Treatments 2 to 8). Hot-water treatment reduced percent germination at all temperatures (Treatments 8 and 9) and was least effective of all pretreatments in reducing injury due to chilling (Fig 2C). Significant differences among treatments were detected in germination and seedling

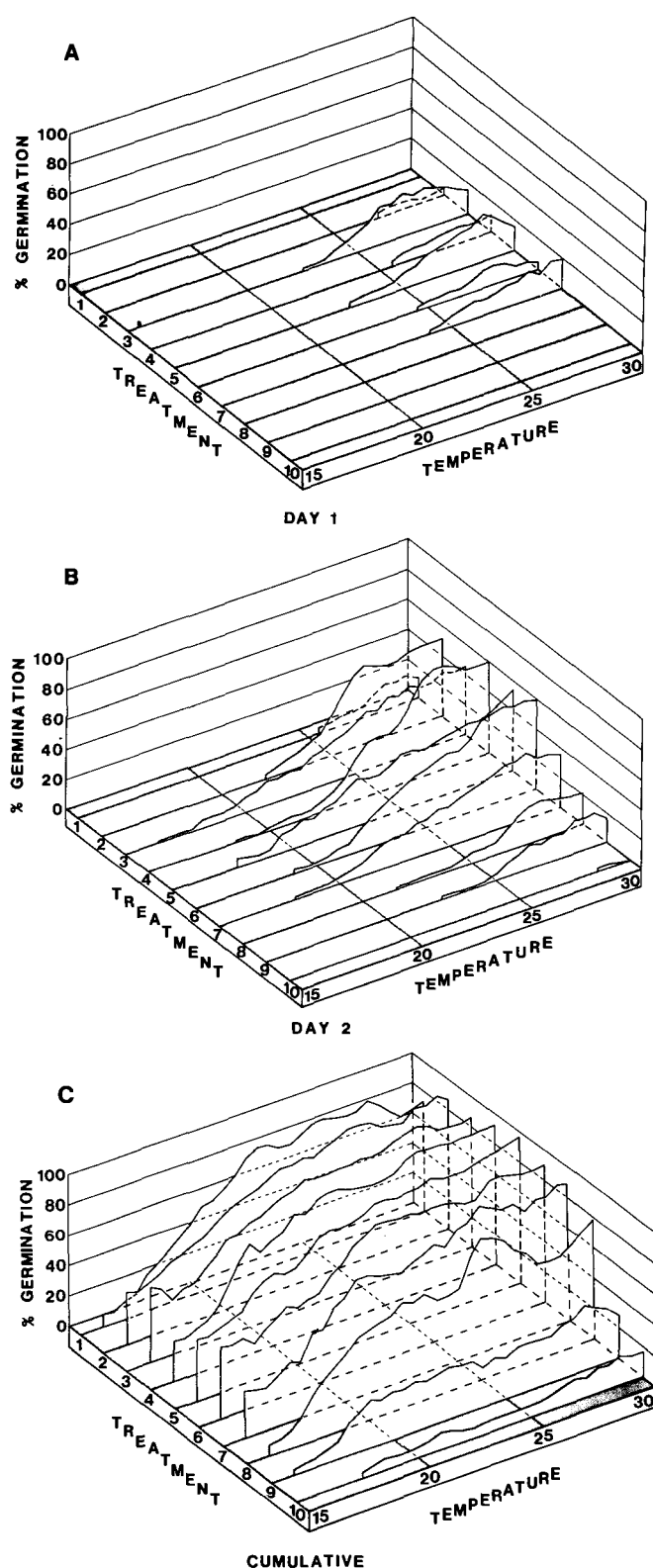


Fig. 2. Effect of various pregermination treatments and a range of temperatures (experiment 2) on rate of germination of cottonseed after: (A) Day 1, (B) Day 2, and (C) Cumulative germination after 7 days. Treatment: 1, Control; 2, cyclic AMP at 30 C; 3, cyclic AMP at 30 C plus chilled; 4, water at 30 C; 5, water at 30 C plus chilled; 6, GA at 30 C; 7, GA at 30 C plus chilled; 8, hot-water (70 C); 9, hot-water (70 C) plus chilled; and 10, water at 5 C for 24 hours.

growth (Table 2). Cyclic AMP and gibberellic acid increased seedling length (Treatments 2, 3, 6, and 7; Table 2). Preconditioning in water at 30 C followed by chilling (Treatment 5) and chilling the hot-water-treated seed (Treatment 9) reduced seedling growth (Table 2).

## DISCUSSION

Preliminary experiments using seed which had not been hot-water or fungicide treated indicated that gibberellic acid increased the rate and completeness of germination at all temperatures included in the study, especially at temperatures below 20 C. In experiment 1, with hot-water-treated seed, gibberellic acid did not increase the percent germination at either of the imbibing temperatures, whereas in experiment 2, by using seed that were not hot-water treated, an increase was noted. This suggests that hot-water treatment of cottonseed reduces their sensitivity to gibberellic acid.

Christiansen (4) showed that the seed of *G. hirsutum* L. genetic selection M-8, were either killed or performance of the seedlings was seriously decreased when seeds were imbibed at 5 C. Results from these experiments demonstrated that seed of *G. barbadense* were either killed or germination performance was reduced by hydration at 5 C. Seed representing the control were slower to germinate than seed which had been preconditioned.

Holm and Miller (7) reported that cyclic AMP and gibberellic acid were effective in increasing speed and totality of germination in several plant species. Coats (6) reported that several growth regulators were not effective in increasing field stands of the cotton cultivar 'Stoneville 213.' Bird and Ergle (1) reported that cultivars differ in their response to GA and that the cultivars may vary in levels of natural gibberellin.

Our data indicate that seed of *G. barbadense* can be protected against chilling injury by preconditioning and that the seed are protected against chilling at temperatures far below the optimum germination temperature of 31 C. The data also show that treating the seed with a growth regulator will increase the speed and totality of germination at all temperatures used in these experiments. These results suggest that by elevating seed moisture in conjunction with growth regulator treatments, satisfactory cotton stands may be obtained at temperatures less than the optimum temperature for germination (31 C). Additional experiments are needed to test the effectiveness of these treatments in field conditions.

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## REFERENCES

1. Bird, L. S., and D. R. Ergle. 1961. Seedling growth differences of several cotton varieties and the influence of gibberellin. *Agron. J.* 53:171-172.
2. Christiansen, M. N. 1963. Influence of chilling upon seedling development of cotton. *Plant Physiol.* 38:520-522.

3. ———. 1967. Periods of sensitivity to chilling in germinating cotton. *Plant Physiol.* 42:431-433.
4. ———. 1968. Induction and prevention of chilling injury to radicle tips of imbibing cottonseed. *Plant Physiol.* 43:743-746.
5. ———. 1969. Seed moisture content and chilling injury to imbibing cottonseed. 1969 Beltwide Cotton Prod. Res. Conf., Proc., Publ. Nat. Cotton Counc., Memphis, Tenn. p. 50-51.
6. Coats, G. E. 1967. Effects of growth regulators on germinating cotton. *Miss. Agr. Exp. Sta. Bull.* 752. 8 p.
7. Holm, R. E., and M. R. Miller. 1972. Hormonal control of weed seed germination. *Weed Sci.* 20:209-212.
8. Larsen, A. L. 1971. Two-way thermogradient plate for seed germination research: Construction plans and procedures. USDA, ARS 51-41.
9. Thomas, R. O., and M. N. Christiansen. 1971. Seed hydration-chilling treatment effects on germination and subsequent growth of cotton. *Crop Sci.* 11:454-456.