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Temperature Influence on the *in vivo* Hydrolysis of Cotton Seed Oil

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EXPOSURE of moist seed and seedlings during early stages of germination to a temperature of approximately 60° F. adversely influences germination behavior and seedling development of cotton (*Gossypium hirsutum* L.) (1, 2). The exact cause of the influence is improperly understood. The present authors postulated that enzymatic hydrolysis of seed oil might occur at temperature levels below the minimum required for free fatty acid utilization and germination, and that reactions resulting in increases in fatty acid adversely influence subsequent germination and seedling development.

The object of this study was to determine the influence of duration of suboptimum germination temperature, as applied to pregerminated and non-pregerminated seed, upon the catabolism of seed oil. Measurements of the accumulation of free fatty acids and decrease of oil were made.

REVIEW OF LITERATURE

Changes in the chemical composition of cotton seed lipids have been studied by Olcott and Fontaine (4) who reported a direct relationship between losses of both dry weight and lipid content and increasing levels of free fatty acid in germinating seed. In studying oil utilization in seed germinated under continuous light and continuous dark conditions, White (5) observed that the dry weight of seed germinated in darkness decreased only during the first 36 hours. Seedlings produced in light showed a more rapid rate of fat depletion than those grown in the dark. Free fatty acids remained at a low, but constant, level in the cotyledons of both types of seedlings. Linoleic acid and the saturated fatty acids were depleted more rapidly than oleic acid. No major temperature influence on oil catabolism was detected within a range of 21–39° C.

Considerable evidence is available which associates high free fatty acid levels in cotton seed with low seed viability. Hoffpauir et al. (3) cut excised cotton embryos perpendicular to the long axis, analyzed the nongerm end for free fatty acid, and germinated the portion containing the axial organs in sterile agar. The ratio of viable to nonviable embryos in seed with less than 1% free fatty acid in the oil was 21 to 1, while in seed containing greater amounts of acid the ratio was 1 to 14.

MATERIALS AND METHODS

The studies were conducted in temperature-control chambers which provided a ±0.5° F. control. The seed used was a selfed, doubled haploid strain of Delta Pine variety of *Gossypium hirsutum* L., M-8948. The seed was produced under irrigation at Iguala, Mexico, and was of extremely high quality, germinating 100% and having a free fatty acid content of less than 0.5%. All materials used in the studies were sterilized to reduce contamination of seed by organisms.

Three factors were investigated: level of temperature, duration of incubation, and pregermination. The levels of these factors were as follows:

A. Non-pregermination, and pregermination of hydrated seed for 24 hours at 75° prior to treatment.
B. Incubation temperatures of 50, 54, 58, 62, and 66° F.
C. Incubation for 0, 3, 6, and 9 days.

The experimental design was a randomized block factorial with four replications. Two tests were conducted: the first at temperature levels of 50, 54, 58, and 62° F., and the second at 58, 62, and 66° to supplement the results of the preceding experiment and to provide information on oil catabolism at 66°.

In the incubation treatments, seed were placed in germination paper rolls saturated with standard Knop's nutrient solution. The rolls were wrapped in aluminum foil and kept moist by additions of distilled water. Pregermination was effected by incubating seed in moist germination paper at 75° F. for 24 hours prior to placement at lower temperature. Tests were timed so that comparable pregerminated and non-pregerminated samples were simultaneously placed at assigned temperatures. Timing was accomplished by introducing the seed rolls into control chambers at 3-day intervals, commencing with the ninth day, followed by 6-day and 3-day entries.

At the completion of the treatments, the seed or resulting seedlings were killed by a 3-minute immersion in boiling water and dried at 120° F. for 48 hours. The entire seed or seedling material from each treatment was ground in a Wiley mill to pass a 40-mesh screen and the samples stored in sealed bottles.

Chemical Analysis Procedures

Prior to analysis, each ground sample was dried over calcium chloride in a desiccator for 24 hours. Total lipid content was determined by a 24-hour continuous extraction with petroleum ether (30–60° C. boiling point) in Soxhlet extractors. Sample weight loss was used as the basis for calculating lipid content.

Free fatty acid content was determined on lipid extractions obtained by soaking (with intermittent shaking) 25 g. of the ground seed material in 100 ml. of petroleum ether (30–60° boiling point) for 4 hours. The mixture was filtered and the residue washed several times with 15–25 ml. portions of ether. The filtrate was placed *in vacuo* at room temperature until the ether evaporated. Free fatty acid was determined by a modification of A.O.A.C. method 26.30 in the following manner: one gram of oil was mixed with 25 ml. of neutral 95% ethanol and the solution heated to boiling. The hot solution was titrated with a 0.02*N*

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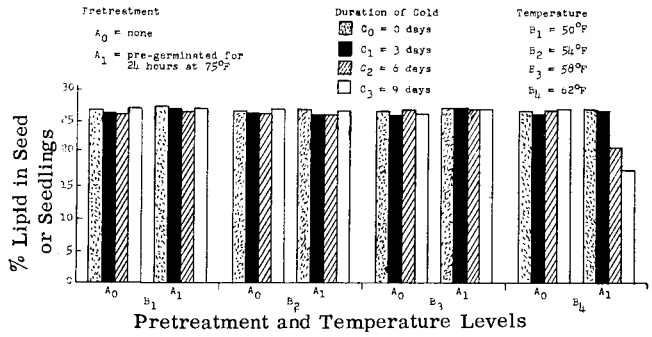

Figure 1—Influence of 3 durations of 4 temperatures applied to pregerminated and non-pregerminated seed upon the lipid content of seed and seedlings. Tukey's D at 0.05 = 0.492.

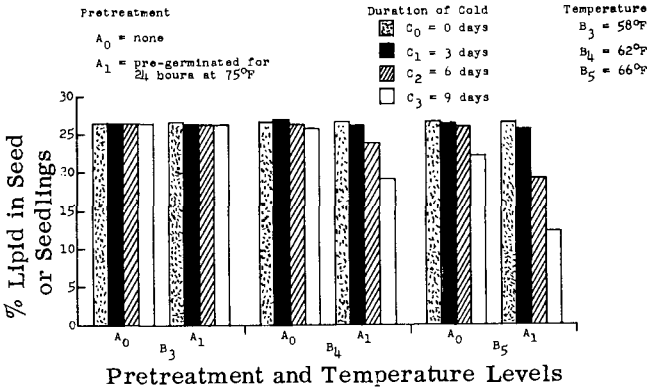

Figure 2—Influence of 3 durations of 3 temperatures applied to pregerminated and non-pregerminated seed upon the lipid content of seed and seedlings. Tukey's D at 0.05 = 2.088.

NaOH solution to a faint pink end-point, using phenolphthalein as an indicator. Free fatty acid was calculated as percentage of oleic acid.

RESULTS

A summary of lipid determinations is presented in Figures 1 and 2. Major reductions in lipid content occurred only at temperature levels of 62 and 66° F. At 62° lipid loss occurred only in the pregerminated seed. The loss was linear with time. At 66° lipid reduction occurred in both pregerminated and non-pregerminated seed. A significant loss occurred in 9 days in non-pregerminated seed and in 6 and 9 days in the pregerminated seed.

Summaries of free fatty acid data are presented graphically in Figures 3 and 4. No significant changes in free fatty acid occurred at temperatures of 50, 54, or 58° F. At 62°, significant linear increases occurred by the sixth and ninth days of incubation in pregerminated seed but not in the non-pregerminated seed.

At 66° significant increases in free fatty acid occurred in both pregerminated and non-pregerminated seed. Nine days of incubation were required to produce a significant change in the non-pregerminated seed while 6 days were sufficient for a significant change in free fatty acid level of pregerminated seed.

CONCLUSIONS AND SUMMARY

Lipid studies on high quality seed revealed that the minimum temperature for the *in vivo* hydrolysis of cotton

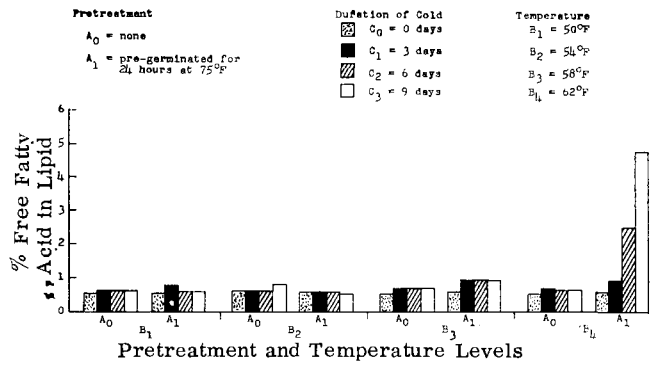

Figure 3—Influence of 3 durations of 4 temperatures applied to pregerminated and non-pregerminated seed upon the free fatty acid content in the oil of seed and seedlings. Tukey's D at 0.05 = 0.677.

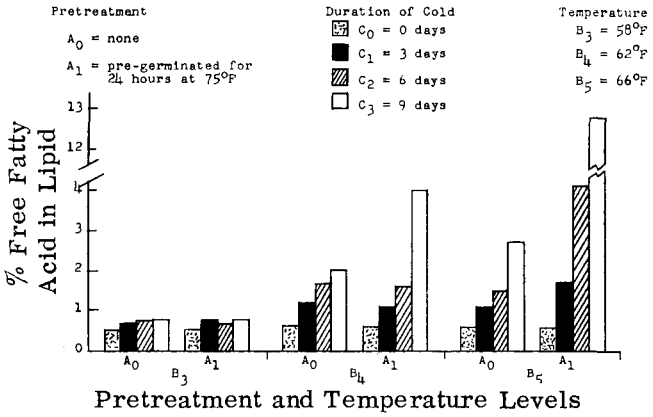

Figure 4—Influence of 3 durations of 3 temperatures applied to pregerminated and non-pregerminated seed upon the free fatty acid content in the oil of seed and seedlings. Tukey's D at 0.05 = 2.380.

seed oil occurs within the range of 58–62° F. This range is essentially the minimum level for germination, and it is also near the upper temperature limit at which chilling injury in germinating cotton seed becomes serious. Thus, the hypothesis that hydrolysis of lipid at suboptimum germination temperatures results in a toxic accumulation of free fatty acids appears to be invalid, at least at temperatures below 58–62° F. Blockage of germination by low temperature could be a direct result of temperature inhibition of oil hydrolysis; however, no direct seedling injury would result.

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