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Effects of Acid Residue on Germination and Vigor of Acid Delinted Cottonseeds

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EFFECT OF ACID RESIDUE ON GERMINATION AND VIGOR OF ACID DELINTED COTTONSEEDS

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Delinting cottonseeds with dilute sulfuric acid is one of the primary methods used in the U.S. This process leaves an acid residue on the seeds. Methods of determining acid residue are not uniform. The effect of different levels of sulfuric acid residue on standard germination and other quality tests has been established. During investigations of the effect of neutralization vs. non-neutralization of cottonseeds delinted by the dilute sulfuric acid process (1, 6, 7), it was obvious that there were different amounts or levels of acid on the seeds and that seeds with high acid residues had lower standard germination percentages.

Since there was no information available as to an acceptable level of acid residue, and communications with cottonseed delinters indicated no uniform procedure among them for determining acid residue levels, this study was initiated to:

1. Develop a procedure for determining sulfuric acid residue on delinted cottonseeds; and

2. Study the effect of different levels of sulfuric acid on seed germination and vigor.

Seed delinting equipment was a modified clothes washer and dryer as developed by Cotton Incorporated and modified as described by Cabrera in the previous paper and McCarty et al. (8).

A McGill rice huller was used to remove seedcoats from the delinted cottonseeds when necessary.

A Beckman Model 76004 pH meter was used for all pH measurements. Standard 0.25N sodium hydroxide solution was used for titrations. A Blue M^{R} humidity cabinet set at 42^{o}C and 100% RH was used as an accelerated aging chamber.

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Three lots of cottonseeds were used; one lot each of 'DES 422', 'DPL 61', and 'HAS 44.'

Acid residue determination was conducted on 50 whole seeds, 10 seedcoats only, and 25 embryos of seeds without seedcoats. Fifty grams of whole seeds, 10g of seedcoats and 25g of embryos were soaked for 5, 15, 30, 60, 120 and 180 minutes in distilled water, the water was filtered off, checked for pH, then titrated to pH 7.

Effect of acid residue levels was investigated on both high vigor and low vigor seeds. Low vigor seeds were obtained by taking one half of each high vigor sample and placing it in the accelerated aging chamber at 42°C and 100% RH for 72 hours. After aging, the seeds were dried with forced air for 24 hours. Different acid residue levels were obtained by varying times for seeds soaked in the acid, stirring, centrifuging or drying. Apparent residue levels were as follows:

Level of acid residue	Range of acid residue (%)
I	0.00 - 0.05[2]
II	0.20 - 0.29
III	0.30 - 0.39
IV	0.40 - 0.49

The following laboratory seed quality tests were performed: (1) standard germination test, (2) cool germination test, (3) accelerated aging test, and (4) tetrazolium test. All tests were performed as described by appropriate authorities (2, 3, 4, 5,).

Determination of Acid Residue

Regardless of level of acid, titration indicated different levels of acid in the water for different soaking times (Figure 1). This should be expected since the seeds or seed parts were still in contact with the acid and could still react with it. For this reason, we do not know the exact amount of acid and will refer to results as "apparent acid residue."

On whole seeds, the apparent acid residue increased rapidly for the first 15 minutes, reached a maximum at 30 minutes, and

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[2]Neutralized with sodium bicarbonate.

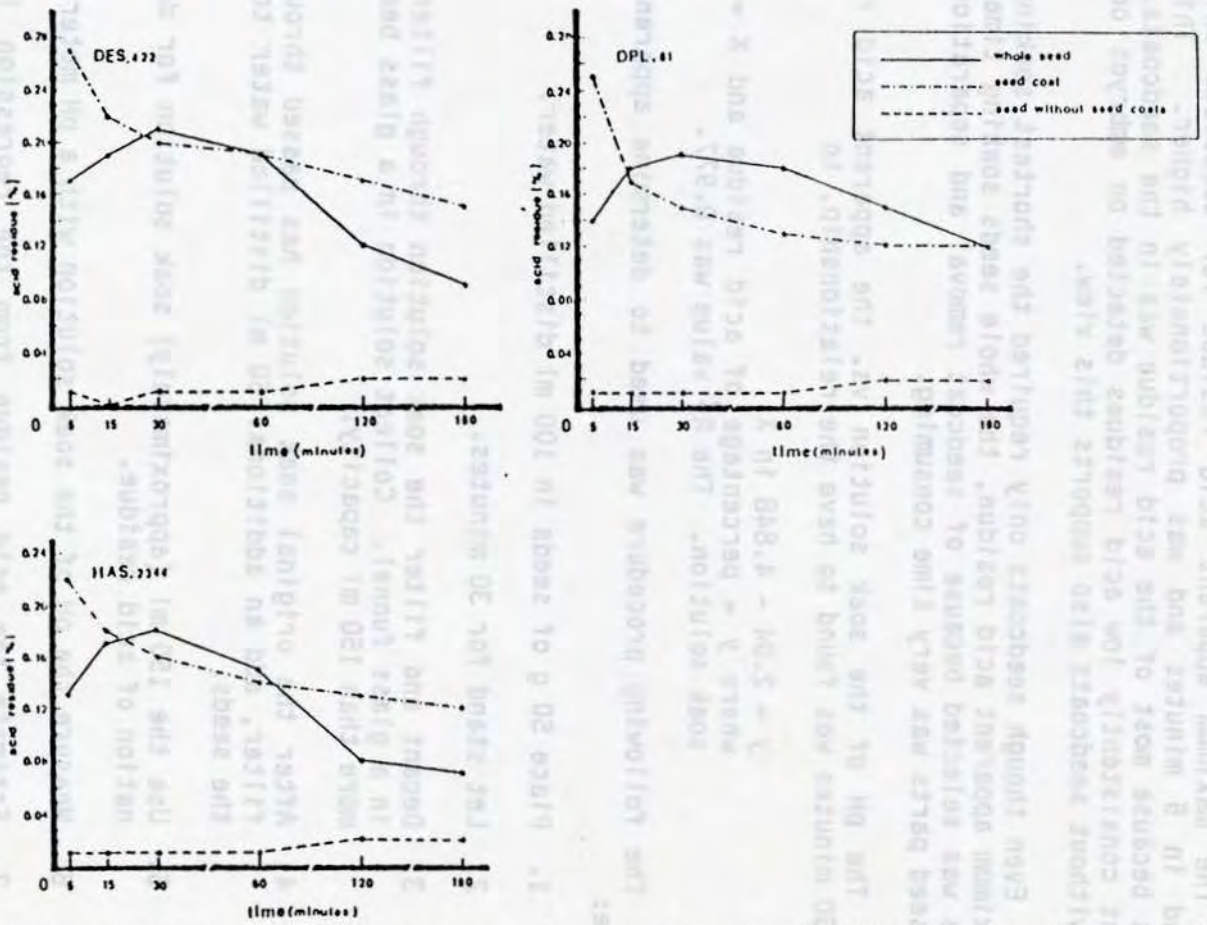

Figure 1. Percent sulfuric acid residue vs. soaking time in distilled water for acid detected on whole seed, seed coats, and seed without seed coats of three cultivars of acid delinted cottonseed.

declined slowly thereafter. However, there were no significant differences among 15 to 60 minutes.

The maximum apparent acid residue for seedcoats only was obtained in 5 minutes and was proportionately higher. This was obvious because most of the acid residue was in the seedcoats. The apparent consistently low acid residues detected on embryos only or seeds without seedcoats also supports this view.

Even though seedcoats only required the shortest soaking time for maximum apparent acid residue, the whole seeds soaking time of 30 minutes was selected because of seedcoat removal and separation from other seed parts was very time consuming.

The pH of the soak solution vs. the apparent acid residue after 30 minutes was found to have the relationship, in

$$y = 2.04 - 4.848 \text{ in } X$$

where y = percentage of acid residue and X = pH of soak solution. The R^2 value was 0.977.

The following procedure was used to determine apparent acid residue:

1. Place 50 g of seeds in 100 ml distilled water.
2. Let stand for 30 minutes.
3. Decant and filter the soak solution through filter paper in a glass funnel. Collect solution in a glass beaker of more than 150 ml capacity.
4. After the original soak solution has passed through the filter, add an additional 50 ml distilled water to rinse the seeds.
5. Use the 150 ml (approximately) soak solution for determination of acid residue.
6. Measure the pH of the soak solution with a pH meter.
7. Estimate the acid residue from the regression line in Figure 2.

Data on apparent acid residue were obtained by the above procedures. It is important to realize that apparent acid residue obtained by different soak times may be quite different.

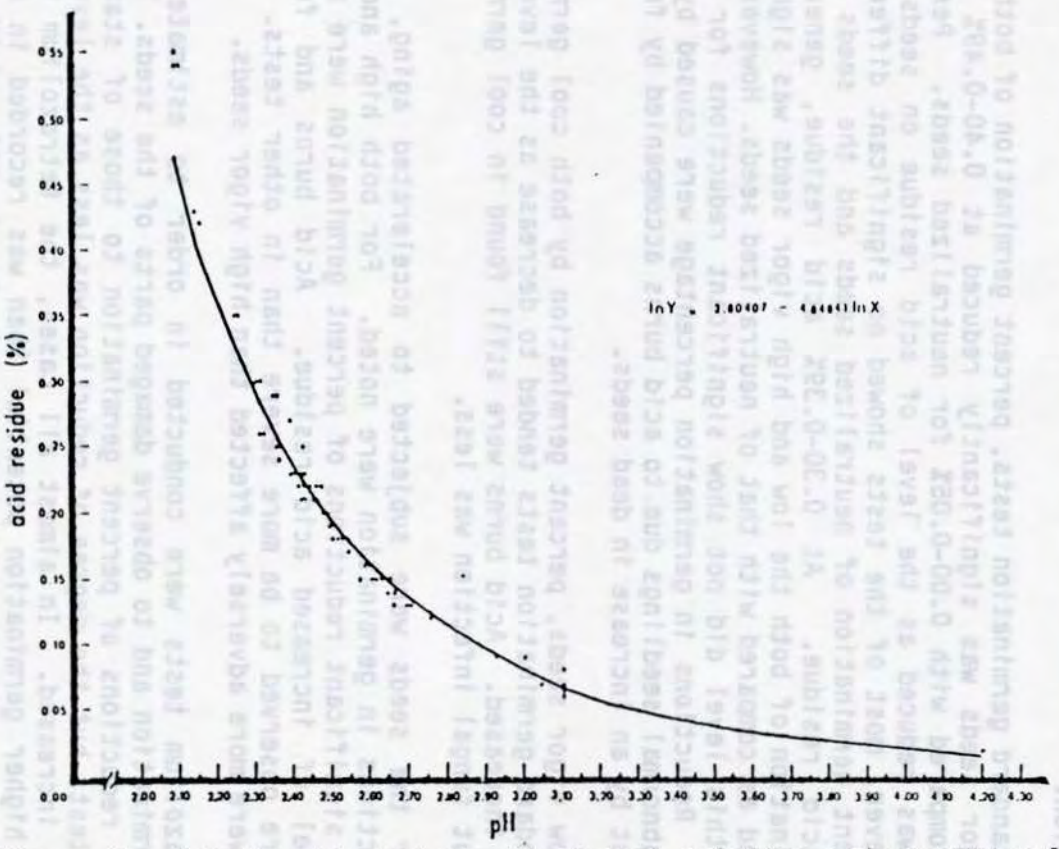

Figure 2. Relationship between pH of soak solution and percent sulfuric acid residue on acid delinted cottonseed after 30 minutes soak in distilled water.

Effect of Apparent Acid Residue on Laboratory Test Results

Levels of acid residue on the seeds affected germinability at all vigor levels and kinds of tests. Low vigor seeds were affected more by increasing levels of acid residue than were high vigor seeds. The pattern of effect of different acid residue levels varied with the severity of tests conducted on the seeds. The most severe test was accelerated aging followed by standard germination, tetrazolium, and cool germination.

In standard germination tests, percent germination of both low and high vigor seeds was significantly reduced at 0.40-0.49% acid residue as compared with 0.00-0.05% for neutralized seeds. Percent germination was reduced as the level of acid residue on seeds increased. However, most of the tests showed no significant difference between percent germination of neutralized seeds and the seeds with 0.20-0.29% acid residue. At 0.30-0.39% acid residue, generally percent germination of both the low and high vigor seeds was significantly reduced as compared with that of neutralized seeds. However, in some cases, this level did not show significant reductions for high vigor seeds. Reductions in germination percentage were caused by the increase in abnormal seedlings due to acid burns accompanied by fungal infection, not by an increase in dead seeds.

In low vigor seeds, percent germination by both cool germination and standard germination tests tended to decrease as the level of acid residue increased. Acid burns were still found in cool germination tests but fungal infection was less.

After the seeds were subjected to accelerated aging, more obvious reductions in germination were noted. For both high and low vigor seeds, significant reductions of percent germination were noted at each level of increased acid residue. Acid burns and fungal infection were observed to be more severe than in other tests. Low vigor seeds were more adversely affected than high vigor seeds.

Tetrazolium tests were conducted in order to estimate the potential germination and to observe damaged parts of the seeds. There were similar reductions of percent germination to those of standard germination tests but the degree of reduction was less as the level of acid residue increased. In almost all cases, the tetrazolium tests indicated a higher germination potential than was recorded in other tests. This was especially noticeable at acid levels III and IV. Most of the acid damage was observed on cotyledons and the tips of radicles.

In another study, the effects of acid concentration, neutralization vs. non-neutralization, and storability of neutralized vs. non-neutralized seeds were compounded. Monthly germination results of seeds delinted using 12% sulfuric acid and 15% sulfuric acid are

presented in Figure 3. There were no differences in germination due to acid concentration through 6 months in storage.

There was a difference in standard germination throughout the 6 months' storage period (Table 1). This difference was magnified as time in storage increased. This demonstrates the need to neutralize the acid residue on cottonseeds. Neutralization becomes more important when the logistics of delinting of planting are considered in the light of marginal seed quality. Often seeds from the mid-South, southeast high plains, eastern and southern Texas are marginal in germination to begin with, i.e., 80 to 85% standard germination is common. Seeds are delinted in January, February and March for the coming planting season. These seeds are in storage from 2 to 5 months. A decrease in standard germination of 5% or more during this time due to acid residue could cause germination to be below the 80% level which is the commonly accepted level of germination in the trade.

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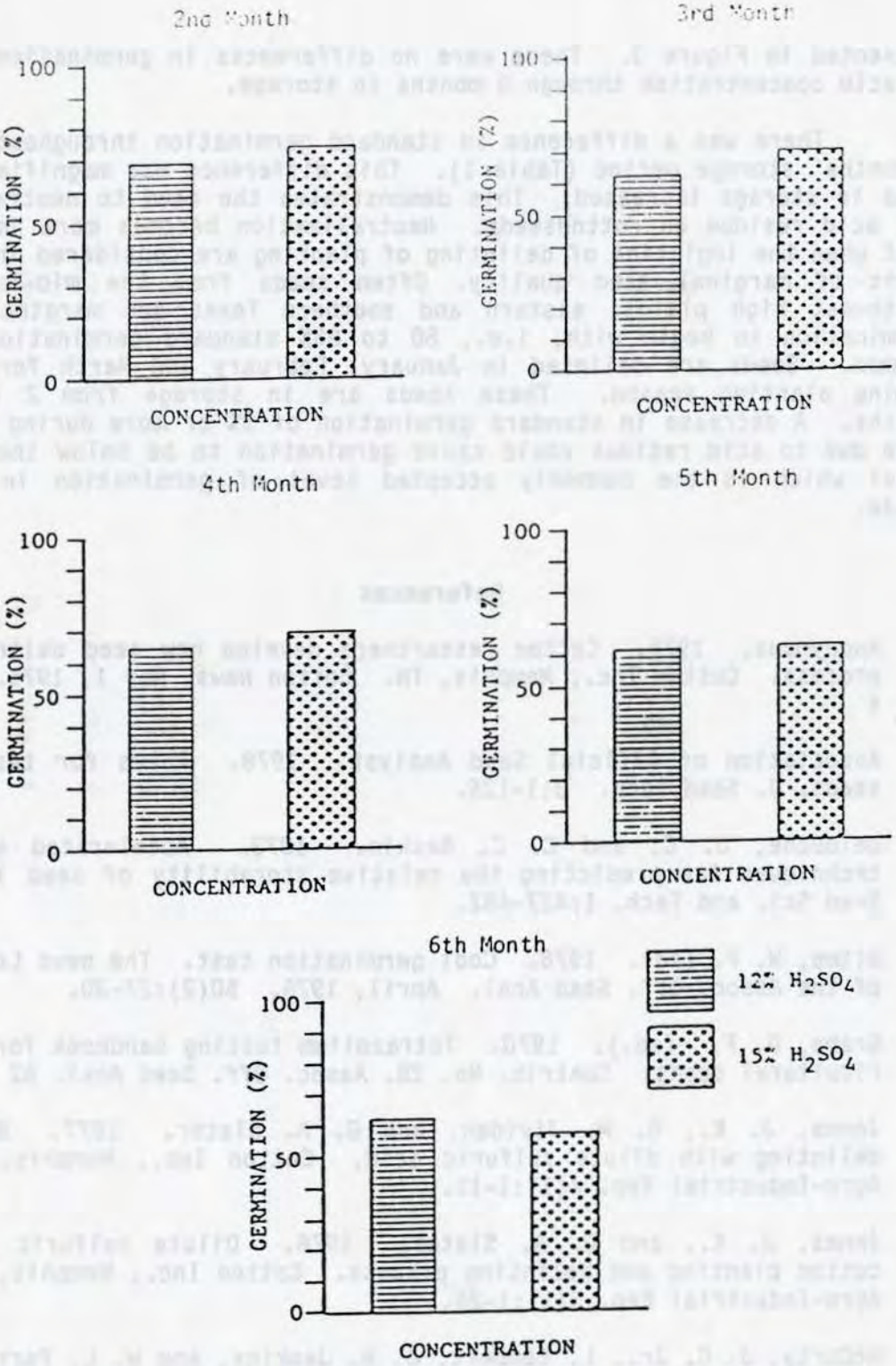

Figure 3. Monthly germination of cottonseed following delinting with 12% and 15% sulfuric acid solutions.

Table 1. Effect of neutralization on the germination of dilute acid delinted cottonseeds.

Month	Germination (%)	
	Neutralized	Not-neutralized
2	72.4 a	68.4 b
3	69.9 a	62.2 b
4	69.9 a	63.5 b
5	66.9 a	59.3 b
6	65.8 a	52.4 b

Means in the same row not sharing a common letter differ significantly at 0.05% level of probability as determined by DMRT.