A RAPID TEST FOR HONEYDEW CONTAMINATION USING THE CLINITEST® REAGENT

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Abstract

The problems associated with honeydew contamination in cotton are well documented and understood within the cotton community. We reported last year, our attempt to develop a rapid test for the detection of honeydew contamination (Topping and Broughton, 1996). The work reported this year is a continuation, a new undergraduate student project by Wade Wallace, a senior in Textile Management and Technology at Auburn. The work documents our attempt to make sure that the procedure would detect the complex sugars which are most responsible for the stickiness associated with honeydew. It was found that hydrolysis of these sugars with dilute mineral acid, at elevated temperature, for a short period of time, would allow detection by the Clinitest® (registered trademark of Miles Inc.) reagent. The acid digestion selected did not produce a detectable quantity of reducing sugars from hydrolysis of cotton.

Introduction

Deposits of a solution of a sugary residue called honeydew, left by aphids and white flies can cause a major problem in cotton processing. Honeydew, or sticky cotton as it is often referred to, is different in its effect than other raw cotton contamination. Most trash can be removed and separated easily in the cleaning process. However, when honeydew contamination occurs, the removal which takes place is on to the machine parts. This sticky residue which collects on the machine parts then entangles other fibers as they pass and can result in poor quality, lost production due to cleanup, and can be sufficiently disruptive as to require changing to another source of cotton.

Obviously, detection of honeydew contamination prior to its dispersion throughout a mill is desirable. A number of tests have evolved, all of which suffer from inaccuracies and length of time to perform. Our purpose is not to review these previous procedures, but to improve the accuracy and simplify the Clinitest® techniques. We will focus on a modified Clinitest® procedure, originally reported last year, and try to determine and extend its effectiveness in estimating honeydew contamination (Topping and Broughton, 1996). The goal of our work is to develop a method is inexpensive, quick and simple and requires little technical training for an individual to conduct.

There are many sugars present in raw cotton. However, two of these sugars are unique to honeydew and are produced by the cotton aphid (*Aphis gossypii*) and the cotton white fly (*Bemisia tabaci*). Melezitose is principally produced naturally by the aphid and trehalulose, a sugar reported principally in white flies are found in large amounts in honeydew (Hendrix *et. al.*, 1991; Petelle, 1983; Davidson *et. al*, 1994 and Tarczynski *et. al.*. 1991). This research is done to determine if a modified Clinitest® procedure can detect these sugars in sticky cotton. The reagent actually works only on sugars which are easily oxidized (reducing sugars). Although trehalulose and melezitose are non-reducing sugars (under our previous test conditions), when hydrolyzed they form reducing sugars. The goal then is to devise a procedure to hydrolyze these two sugars without introducing artifacts or complications into the chemistry of detection. The effectiveness of the procedure in detecting honeydew sugars depends on the complications introduced by the hydrolysis step and the components (reducing or non-reducing sugars) which are formed when the compounds (along with cotton) are hydrolyzed during the test.

Literature Review

Although the problem of honeydew contamination has been around for some time, it has become increasingly serious in the past ten years. There are two reasons for the increase in the severity of this problem. The first is due to the aphids and white flies becoming more widespread and more difficult to control. The second reason is because of the advances made in the technology and sensitivity of processing machinery (Perkins., 1990). Processing of cottons severely contaminated with honeydew under normal conditions is virtually impossible (Perkins., 1983).

Not all stickiness in cotton is attributed to that of the sugars left by feeding insects. The insect stickiness is not always due to the usual aphid and white fly infestation. However, it has been estimated that 80-90% of the stickiness is attributable to insect honeydew. The remaining percent is often due to human factors such as grease and oil contamination during harvesting and processing (Miller *et. al.*, 1994).

Among the many components of honeydew, there are certain unique sugars produced by aphids and white flies feeding on cotton. High performance liquid chromatography shows high percentages of trehalulose in white fly honeydew and melezitose in aphid honeydew. The following table shows the percentages of sugars present in honeydew of aphids and white flies feeding on cotton (Hendrix et. al., 1991).

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Table 1: Honeydew Composition (Hendrix *et. al.*, 1991).

	Aphid	White fly
Monosaccharide	24.6	18.9
Sucrose	11.6	16.0
Turanose	0.0	1.0
Trehalulose	1.10	**43.8**
Melezitose	**38.3**	16.8
Other	2.9	2.2

As represented by the percentages above, trehalulose and melezitose made up of the highest percentages of the various sugars found in honeydew (Hendrix *et. al.*, 1991). Amounts of trehalulose and melezitose were significantly higher in the other research as well (Henneberry *et. al.*, 1996). It is also necessary to point out that these two sugars are present in both aphid and white fly honeydew. The combined percentage of these two sugars is 39.4 for aphid and 60.6 for white fly. Trehalulose alone may account for up to 45% of the total carbohydrate in the honeydew (Miller *et. al.*, 1994). Since trehalulose and melezitose alone or combined make up the highest percentage of sugar in honeydew of both aphids and white flies, the effectiveness of any test for honeydew must be measured by its ability to detect these sugars.

Research has also been conducted to determine if the specific sugars have different stickiness properties. The research has shown the trehalulose has a greater potential for causing stickiness than melezitose. This research was conducted on individual components as well as a complete artificial honeydew compound. The artificial honeydew compound consist of 45% trehalulose, 15% sucrose, 10% glucose, 10% fructose, 15% melezitose, and 5% turanose (Miller et. al., 1994). The compound is very close in percentages to the results of the high performance liquid chromatography (Hendrix *et. al.*, 1991).

The literature available indicates the degree of importance which trehalulose and melezitose have in the composition of honeydew contamination of cotton. The previous work to simplify and speed the Clinitest procedure (Broughton and Topping, 1996) still suffers from the fact that it does not detect (and give a semiquantitative estimate of) these important sugars in honeydew.

Experimental Procedure

Acid hydrolysis has been investigated as a way to convert the complex sugars in honeydew to a reducing form that will react with the Clinitest® reagent. The first step in the experimentation was to obtain quantities of these complex sugars in purified form. The melezitose was obtained through a Aldrich Chemical. Dr. William B. Miller, a professor at Clemson University, College of Agriculture (whose work has been cited in the literature review) kindly supplied trehalulose for ours experimentation.

After the compounds were obtained, standard solutions representing the concentrations expected from cotton extractions (1 g of cotton to 4 ml of extracting solution) were made. Selected concentrations (calculated as sugar on cotton) were 0.0%, 0.10%, 0.20%, 0.25%, 0.33%, 0.50%, and 0.66%. These concentrations were derived from a stock sugar solution composed of 0.10g sugar dissolved in 40 ml distilled water. A stock acid solution was prepared by mixing 1 g of concentrated sulfuric solution with 1 g of Griffwet® NP-9 (Grifftex Chemical, Opelika AL) in distilled water to make 100 ml of stock acid solution. Griffwet® NP-9 is an ethoxylated, non-ionic surfactant. The stock sugar solution was added to varying amounts of the stock acid, to make the desired concentrations. Table 2 shows the relationship of sugar and acid solutions to their corresponding concentrations.

Table 2 Standard Sugar Solutions.

		Sugar Concentration (%)	
Acid Solution	Sugar Solution	Actual	Equivalent on Cotton
9 ml	1 ml	.025	0.10
4 ml	1 ml	.050	0.20
3 ml	1 ml	.0625	0.25
2 ml	1 ml	.0833	0.33
1 ml	1 ml	.125	0.50
0.5 ml	1 ml	.166	0.666

In the original procedure(Topping and Broughton, 1996) acid/surfactant solution was heated to 80º C and then 4 ml was drawn into the 1 g cotton sample and allowed to stand for five minutes without additional heating (Table 3). These conditions were found to be inadequate for completely hydrolyzing the complex sugars. The sugar solutions were tested based on the original procedure but modified for use on the solutions rather than cotton. The experimental conditions for testing the trehalulose and melezitose sugar solutions were varied from the original procedure used for cotton samples. The procedure for testing the sugar concentrations is shown in Table 4.

Table 3: Honeydew Estimation Procedure from Previous Work.

A 1.0g sample of cotton using numerous pinches from over the sample
Put cotton into 10 cc syringe and compress the sample
Heat solution of 1% sulfuric acid and 1% NP-9 to 80º C
Draw 4 ml of the solution into the syringe covering cotton
Pump the solution in and out of the syringe several times to wet cotton
Allow to stand for 5 minutes - unheated
Squeeze out 1.0 ml of the extract into a test tube
Warm the solution in a beaker of hot water
Drop in the Clinitest® tablet
Compare the color developed

Table 4: Procedure for Hydrolysis and Concentration Estimation of Sugar Standards.

Sugar/acid solutions drawn into syringe
Heat syringe to 80ºC in a water bath for 5-10 minutes
Squeeze out 1 ml of solution into a test tube
Drop in the Clinitest® tablet
Wait 15 minutes for results

The solutions in Table 2 were held at 80º C for five, ten, and fifteen minutes in a water bath instead of being allowed to stand at room temperature for five minutes. This was done in order to select sufficient conditions for hydrolysis

of the complex sugars. Detection of sugars present and determining the color change is the final step in the procedure. After the sugar solution is added to the acid solution and the components are held at the appropriate temperature for the determined length of time one milliliter of the solution is transferred from the syringe to a test tube. Next the Clinitest® tablet is added to the test tube and allowed to stand for fifteen minutes. After the allotted time has elapsed the color change is observed and recorded. The results of the color changes in the varying concentrations can be used to determine the corresponding percent sugar on cotton tested under the same procedure.

After the melezitose and trehalulose sugar solutions were tested and appropriate conditions were chosen, samples of raw cotton were tested under the same conditions. Bleached and scoured cotton (no sugar) was also subjected to these same conditions and extract was tested for color change with the ® reagent. This was done to insure the extended exposure to heat did not degrade the cotton into reducing sugars that might be detected in the procedure. The experimental procedure finally selected for testing the cotton samples are given in the following table.

Table 5: New Procedure for Honeydew Estimation on Cotton.

A 1.0 g sample of cotton using numerous pinches from over the sample
Put cotton into 10 cc syringe and compress to remove the air
Draw 4 ml of 1% sulfuric acid and 1% NP-9 solution into syringe
Heat syringe to 80ºC for 5-10 minutes then remove
Draw solution in and out of syringe several times to insure wet-out
Squeeze out 1 ml of solution into a test tube
Drop in the Clinitest® tablet and observe results

The results of the color change for varying concentrations is the determining factor for the evaluation of the effectiveness of the Clinitest® procedure. The varying degree of color change corresponds to the varying concentrations of melezitose and trehalulose. This color change reflects the effectiveness of the procedure in detecting these sugars.

Results and Discussion

Hydrolysis for at least 5 minutes and 80 C were required to consistently hydrolyze Melezitose and Trehalulose to sugars which can be reduced by the Clinitest® reagent. Extending the time to 10 minutes made a slight difference in color developed but no change was noted after 10 minutes hydrolysis. Reducing the time and temperature showed restricted color development. It was decided to limit the time and temperature to the lowest possible values in order to minimize the degradation of cellulose during the reaction. The results (Table 6) show that the proposed procedure is effective in hydrolyzing and detecting the honeydew sugars melezitose and trehalulose.

Table 6: Clinitest® Results on Hydrolyzed Honeydew Sugars*.

% Sugar**	Melezitose	Trehalulose
0	Blue	Blue
0.1	Blue/Green	Blue/Green
0.2	Green	Green
0.25	Yellow/Green	Yellow/Green
0.33	Yellow	Yellow
0.5	Orange/Brown	Orange/Brown
0.666	Orange	Orange

*Solutions held at 80º C for 5 minutes.
**Equivalent concentration on cotton

As a result of these tests, the test procedures for cotton were modified as shown in Table 7. When bleached and scoured cotton was treated according to the procedure, no sugar was indicated, showing that the hydrolysis conditions were insufficient to liberate reducing sugars from the cellulose itself.

The test was then used on samples previously evaluated by both the Clinitest® and fericyanide procedure (Topping and Broughton, 1996). These results appear in Table 7. We chose to maintain our four level descriptors of no sugar (blue), light sugar (green), moderate sugar (yellow), and heavy sugar (orange)

Table 7: Comparison of Colorimetric Methods for Honeydew Detection.

Sample Bale #	Modified Clinitest® Procedure	Original Clinitest® Procedure	Fericyanide Procedure
669063	Light	Light	Light
669075	Light	Moderate	Moderate
657674	Moderate	Moderate	Light
666142	Moderate	Moderate	Moderate
669852	Light	Moderate	Negative
666185	Light	Moderate	Moderate
666978	Moderate	Moderate	Moderate
668906	Moderate	Moderate	Light
668905	Moderate	Moderate	Light
654945	Moderate	Moderate	Moderate
646368	Light	Negative	Negative
649867	Light	Light	Negative

Conclusions and Recommendations

The results demonstrate that complex sugars commonly found in honeydew can be detected, if not distinguished from simple reducing sugars which may be present in uncontaminated cotton. Design of a systematic layout to allow multiple tests to be performed simultaneously, and a substantial comparative trial with other test procedures are needed to determine the value of the proposed procedure.

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