A MODIFIED CLINITEST® PROCEDURE FOR ESTIMATION OF HONEYDEW CONTAMINATION IN COTTON

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Abstract

A review of the origins of “honeydew” on cotton is given, along with traditional chemical methods of testing for this contaminant. Difficulties with traditional testing methods are discussed. A new, more quantitative test for sugar is proposed.

Introduction

Near harvest time, a cotton field is white, speckled with the green of the plants, as the cotton bolls open in readiness for harvesting and ginning to be sent to mills and factories. Before the cotton is harvested, though, multiple minor and major disasters can occur. Among them is insect infestation. Flying insects (generally whiteflies) fly around the field and rest on the open bolls. Other varieties of insects, such as aphids, climb up the plant and rest on (or fall into) the cotton boll. Their secretions begin to collect in small droplets on the cotton. The insects and their secretions are small enough that the collections are not noticeable, yet they are very insidious. By the time the cotton is harvested, the entire crop can be contaminated to varying degrees with "honeydew". The problems don't really appear until the crop has reached the mill, where the sticky cotton causes dramatic effects which often end in shutting down production to clean machines that have stopped, and switching to another source of cotton. Identifying this "stickiness" problem is highly desirable, especially as insects become more and more resistant to pesticides and other chemicals, but deciding how much honeydew is on a particular bale is not easy. Highly contaminated cotton may have random fluorescent, sometimes colored spots (1), but cotton that is almost as sticky often as not has few or no colored spots. A number of test methods have been developed (2), but not "quick-and-easy" ones. Honeydew is a complex mixture of reducing and non-reducing sugars (3); determining exact concentrations therefore becomes more difficult. The tests that have been developed are time-consuming and impractical for large quantities of cotton waiting in a warehouse at a mill. Measuring the amount of sugar (or the amount of reducing sugar) present in such cotton provides some indication of cotton stickiness (4,5), but one which is subject to some false positives and false negatives. One should note that honeydew is not responsible for all sticky cottons that cause problems in mills. Some cottons simply have a naturally high sugar content. Whatever the source, a good rule of thumb is that a sugar content above 0.3 % means the cotton may cause problems; above 0.5 % means that the cotton will probably create problems (4). The uncertainties exist because of the random dispersion of honeydew and the possibility that the sugary syrup may have dried out, but the uncertainties are minimized if the sugar levels are very high or very low.

One of the older tests often used to measure reducing sugar is the ferricyanide reagent test (2,6). Sodium carbonate and potassium ferricyanide are diluted in distilled water. A 'A gram sample of cotton is taken in small tufts from over the larger cotton sample. The cotton is placed in a flask or beaker with a measured quantity of water and fericyanide reagent and boiled for 3 minutes with some agitation to wet out the sample. A color change from yellow to tan indicates the presence of honeydew (reducing sugars). This test is concentration specific but difficult to detect visually. If the solution remains its original yellow color, it indicates little honeydew, colorless to light brown means some honeydew, and a darker brown indicates a heavy concentration of honeydew on the sample. Determining the exact concentration of honeydew in a sample can be accomplished by a titration of the reacted fericyanide solution (2,6). While the qualitative test can be run quickly, not all the solutions begin boiling at the same time. Wetting the cotton is not easy in the absence of an effective wetting agent. The titration adds a significant amount of time and complication to the procedure.

Other sugar tests include spraying cotton with an acid/base indicator to detect the presence of acid metabolic residues from microorganisms growing in the honeydew (2,4), as well as chromatography of the sugars in water extracts of the cotton (2,3). Tests for stickiness include the minicard and thermally induced sticking of the sugar solutions and associated fibers to a clean foil (2,4).

In our experience, one of the most easily run chemical tests for semi-quantitative estimation of honeydew is the Clinitest® procedure which measures the amount of reducing sugars present on the cotton sample (2,5,7). Glucose is a common reducing sugar, and closely monitored in diabetics. Multiple tests have been developed to determine the amount of glucose present in a diabetic's blood. Some of these are enzyme based and therefore glucose specific. Others are, like the Clinitest®. procedure, general tests for reducing sugars. This procedure consists of a reagent tablet based on the classic Benedict's copper reduction reaction. The blue copper sulfate reacts with reducing substances to convert cupric sulfate to orange cuprous oxide. The resulting solution/suspension color varies from blue to green to brown to orange as the concentration of reducing sugar increases. The dramatic color change provides a good visual calorimetric indication of the concentration of reducing sugar present in the solution. A hot, basic medium is required for the reaction to work. The heat is provided by the sodium hydroxide reacting with water and citric acid.

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The tablet is dissolved by sodium carbonate and citric acid. Any reducing sugar present will give a positive result.

Experimental

A test procedure for cotton, received from a local mill, involved weighing a 1 g sample, soaking it in 4 ml of water, squeezing out a 1 ml extract, dropping in a Clinitest® tablet, and noting the color development (7). The wetting out of the sample as well as squeezing of the extract are problems in this procedure that might have a simple solution. The use of a disposable syringe offered some promise for speeding the procedure and making it less tedious. In our procedure we have used a 10 ml disposable syringe into which is packed a 1 g cotton sample. The sample is then compressed and water is drawn up into the syringe. The syringe is convenient to squeeze out the extract for testing with the Clinitest® tablet. Of course, we are still left with the difficulty of wetting out the cotton for a good extraction as well as the problem that the test only indicates reducing sugars. In addition, extraction of cotton will generate a different dilution factor than that anticipated by the manufacturer of the tablet, so new color standards must be produced.

A minimum liquor ratio of 3/1 was anticipated for extraction of the cotton, so initial standards were made at the equivalent of 0 - 2 % sugar on 1 g of cotton diluted to 3 ml. In a glucose/water solution, with varying concentrations of glucose, the test lasts roughly twenty seconds. A single tablet is dropped in the test tube containing 1 mL of a glucose/water solution. As per the instructions on the Clinitest® package insert, the test tube sat still for 15 seconds, then was shaken, then let sit one minute, when any and all color change was complete. Color was recorded after the 15 see waiting period and after the one minute period. Some variance in color appeared in the two different times, though not entirely unexpectedly. The most rapid color change occurred after boiling finished to the 15 sec mark. After 15 see, color changed very little. The first experiments were run using the drop procedures described for urine but subsequently more accurate volume measurements were used.

A 2% stock solution was made by dissolving 5.0003 g of glucose in 250 ml of distilled water. From that solution, 1.5 %, 1%, .75 %, .5 %, and .25 % glucose stock solutions were made. A further dilution 5 ml of each stock solution was diluted with 10 ml of water, and 1 ml of that final solution was tested. After dropping in the tablet, the solution immediately began to boil for about 10 sec. Exact time varied with the concentration of glucose. 15 sec after boiling was complete, the test tube was shaken. After one minute, the color change was complete and the test tube was shaken again. Color was observed after 15 see and one minute. Only a few inconsistencies from expected color were observed. The final test procedure selected used 4 ml of water to extract I g of cotton so the concentrations are recalculated on that basis in Table 1.

Table 1: Color Standards With Glucose/Water

Glucose concentration (% by weight in water)		Color
Actual % tested	Equivalent % on cotton *	
0.66	0.5	Bright orange
0.50	0.375	A darker orange
0.33	0.25	Brownish /orange
0.25	0.188	Brown
0.167	0.125	Greenish brown
0.083	0.063	Greyish green
0.00	0.00	Blue

* Tested as if 1 g of cotton extracted with 4 ml of water

To test the effect volume on the reaction, 1.5 ml of the final test solution was tested for each concentration of glucose. The boiling for this reaction lasted 15 see, and the reaction continued to change color for about 15 min. Color was observed at these intervals. With a greater volume, the color differences are less apparent after the boiling is finished. After the reaction is complete, the color differences are more noticeable than with the 1 ml standards but colors continue to change after boiling is complete. One can speculate that because of the greater volume, the tablet cannot generate enough heat to make the reaction quick, and the colors are more erratic. Selection of suitable weight of cotton and volume of extraction liquid were followed by generation of a set of standards for percent reducing sugar on cotton.

Because honeydew is not entirely reducing sugars, but contains cyclic and polymeric sugars some of which may be hydrolyzed to reducing sugars, a hydrolysis of these materials would add to the accuracy of the test. A test of solutions of 1% sucrose and 1% starch (both non-reducing "sugar') solutions tested negative, as expected. The 1% solutions were diluted (2/1) with 196 sulfuric acid and allowed to stand for five minutes. The results were a positive result for sucrose but still a negative result for starch. Sucrose is hydrolyzed into glucose and fructose, both reducing sugars. A hydrolyzed 0.66% sucrose solution gives the same calorimetric result as a 0.66% glucose solution. The sulfuric acid solution hydrolyzes at least some non-reducing sugars, and offers potential as a possible way of increasing the accuracy of the test.

Finding a wetting agent and conditions that thoroughly wetted the cotton in a reasonable amount of time without inhibiting the reaction of sulfuric acid or the Clinitest® reagent proved difficult. Several commercial surfactants were tested for their wetting ability and to see how much they interfered with the hydrolysis of, and color development in sucrose solutions. Simple kinetics of sucrose solution hydrolysis were determined. The final experimental conditions selected are:

A 1.0 g sample of cotton using numerous small 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 80° C.
Draw 4 ml of solution into syringe covering cotton.
Pump the solution in and out of the syringe several times to wet the cotton.
Allow to stand for 5 minutes 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.

A test of bleached and scoured cotton using this procedure was negative, but gave a slightly positive test at higher temperatures and times.

Local mills submitted bale samples of cotton for analysis of honeydew. Both the fericyanide and the modified Clinitest® procedures were used on the cotton samples, and representative results are compared in Table 2. Two 1.00 g samples of cotton were weighed out from each bale of cotton and tested. A clean plastic test tube received the final solution from the syringe, and one ml of extract was put in each test tube. The test tubes were placed into a hot water bath, and the Clinitest® tablet was dropped in and the color generated was recorded and compared against the color standards. Most of the bales provided matching results between the Clinitest® and the ferricyanide. There was some variation, as expected, since honeydew is a random contamination. The colors fell mostly in the green range. No bale generated an orange, and few generated brown. The gradations of the colors matched the qualitative amount of honeydew estimated from the fericyanide test. The Clinitest® results were recorded as follows: a blue color is negative; green to blue was recorded as light contamination; green to brown was recorded as a moderate amount; brown to orange was recorded as heavy contamination.

Table 2: Test of Honeydew on Cotton

Bale number	Fericyanide result	Clinitest® result
648316	moderate	moderate
657674	light	moderate
666142	moderate	moderate
666978	moderate	moderate
669063	light	light
669075	moderate	moderate
666152	light	light
669852	negative	moderate
668906	light	moderate
666964	light	heavy
648625	negative	negative
649867	negative	light
646368	negative	negative
654945	moderate	moderate
669869	moderate	moderate
668905	light	moderate
649927	light	moderate
666185	moderate	moderate

Conclusions

A procedure is demonstrated for the estimation of honeydew contamination on cotton which is reasonably rapid, offers reasonable accuracy, and has the potential for detecting some of the hydrolyzable, non-reducing sugars. Additional testing is needed to demonstrate hydrolysis of several known components in honeydew and to improve the speed of the procedure.

References

Jennings, Edward, "Rapid Test for Honeydew", *Textile Industries*, December 1953 pp 120-123.

Perkins, H. "A Study of Sugar and Sticky Cotton Test Methods" Proceedings of the 1993 Beltwide Cotton Conferences, National Cotton Council, Memphis, TN, pp 1136-1141.

Hendrix, D., and Wei, Y., "Detection and Elimination of Honeydew Excreted by the Sweet Potato White Fly Feeding Upon Cotton" Proceedings of the 1992 Beltwide Cotton Conferences, National Cotton Council, Memphis, TN pp 671 -673.

Brushwood, D., and Perkins, H., Cotton Stickiness Potential as Determined by the Minicard, Thermodetector and Chemical Methods, Proceedings of the 1993 Beltwide Cotton Conferences, National Cotton Council, Memphis, TN, pp 1132-1135.

Cook J. M., "Honeydew", *Textile Industries*, February 1953 pp 148- 151.

Perkins, Henry H., Jr., "Some Observations on Sticky Cottons", Textile Industries. 135 (3): 49-64, 1971.

Jason White, Personal Communication.