COTTON PLANT SUGARS AND INSECT HONEYDEW CHARACTERIZED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Donald E. Brushwood, Chemical Engineerand
Henry H. Perkins, Jr., Research Chemist
USDA, ARS, Cotton Quality Research Station
Clemson, SC

Abstract

Identification of specific sugars and other components found on raw cottons related to stickiness has become increasingly important in recent years. This is mainly because of the spread of insect contamination into more cotton growing areas, including the Western United States. Cotton yields and quality are adversely affected and the sticky cottons cause problems in ginning and textile processing. Insect honeydew sugars are more complex than plant sugars and are generally randomly deposited on the lint in heavy specks. Insect honeydew is uniquely identified by the presence of the sucrose isomer trehalulose and the trisaccharide melezitose. Whitefly honeydew contains more trehalulose than melezitose; whereas, aphid honeydew also contains trehalulose and melezitose, but in different proportions. Recent work using anion high performance liquid chromatography has made it possible to separate, characterize, and quantify these sugars in cotton. Identifying specific insect contaminants is important to develop test methods and intervention treatments to alleviate cotton stickiness.

Introduction

Since the late 1980s, the problem of cotton stickiness has intensified on a worldwide basis. Prior to this time, particularly in the US, episodes of stickiness were sporadic and transient and, except for a few countries, were not a long term threat to cotton quality. This situation has changed mainly because of the spread of the whitefly into new areas and because of its increased vitality relative to tolerance to insecticides and adaptability to a wide variety of host plants. In 1992, whitefly infestations in certain areas of the western US cotton belt were so heavy that cotton yields were severely adversely affected and sticky cottons were produced that were difficult to gin and that caused serious textile processing problems. For the 1993 and 1994 US crops, the problems were minimized by controlling the insects by use of carefully conceived integrated pest management programs. Although this approach has been successful, it may not be the final solution, because of the adaptability of the insects. Therefore, treatments to eliminate or minimize cotton stickiness are needed. Identification of specific components of the sticky contaminants should lead to development of spot tests for detecting stickiness and will facilitate research to destroy or neutralize the components responsible for the stickiness.

Recent research using high performance liquid chromatography (HPLC) has shown that sugars and other carbohydrates can be identified and quantified effectively. Tarcynski, et al, Hendrix, et al, and Brushwood, et al have published reports showing how this technology can be utilized to analyze sugars and insect honeydews on several plants, including cotton [1,2,3,4,5,6,7,13]. We streamlined the HPLC technique to minimize testing time to enable us to look at large numbers of samples. The objective of the work reported here is to show how HPLC, sometimes supplemented by gas-liquid chromatography (GLC), can be used to characterize and differentiate natural plant sugars, insect honeydew, and other carbohydrates on cottons of diverse history including area of growth, variety, aphid contamination, and whitefly contamination.

Description of Test Methods

High performance anion exchange chromatography was performed using a Dionex Series DX-300 Chromatography System previously described [2,3] with slight modifications. Through supporting gas chromatography identification techniques [11,12] we found that when using the 200 mM sodium hydroxide and 0.5 M sodium acetate gradient, an unidentified component found in our cotton extracts peaked at the same time as the tri-saccharide melezitose. We modified the feed to exclusively use 200 mM NAOH as a single eluant. This change separated the unknown from melezitose, but with some reduced detector sensitivity to sugars of increasing molecular size such as trehalulose, sucrose, turanose, and melezitose. Earlier eluting monosaccharide peaks are larger than those of oligosaccharides of equal concentration. A typical chromatogram of twelve authentic carbohydrates and components found in cotton eluted at the following approximate times; a polyhydric alcohol probably inositol or glycerol peaks at 1.7 minutes, arabitol at 2.1 minutes, mannitol at 2.5 minutes, trehalose at 2.9 minutes, arabinose at 3.1 minutes, mannose at 3.3 minutes, glucose at 3.5 minutes, fructose at 3.9 minutes, trehalulose at 6.2 minutes, sucrose at 7.1 minutes, turanose at 8.0 minutes, and melezitose at 10.5 minutes. Carbohydrate standards were run daily to correct for possible day to day variations in column sensitivity. Peak height comparisons for the major sugar components of extracted cotton that elute after 3.5 minutes were also periodically run to insure detector repeatability. Figure 1 shows the average of 20 different such comparisons conducted over a 2-month period for glucose, fructose, trehalulose, sucrose, turanose, and melezitose using the 200 mM sodium hydroxide eluant. Correction factors for individual sugar peaks are based on an assigned a value of 1.0 for glucose. A peak height of glucose is 6.9 times the height of an equal concentration of melezitose. Estimates of unknown component

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1310-1313 (1996)
National Cotton Council, Memphis TN

concentrations eluting after glucose are possible using the linear relationship established in Figure 1. The previously mentioned compounds that elute before 3.5 minutes have the following height correction factors based on glucose as follows; 0.5 for the inositol/glycerol peak, 0.6 for arabitol, 0.8 for mannitol, 1.9 for trehalose, 0.9 for arabinose, and 1.1 for mannose.

Survey of Sugars Found in Cotton from 1994 and 1995 Crops

Twenty-six leading variety cottons (1994 crop) received at our station in late 1994 and early 1995 were analyzed for reducing sugars (five replicates) and specific sugars via HPLC (in duplicate). A summary is shown in Table 1. Averages from six major growing areas including, Southeastern, Mississippi Delta, Texas, Texas High Plains, Arizona, and California along with two California pima cottons are listed. Three of the original Southeastern cottons (one each from Alabama, Georgia, and Tennessee) contained high levels of arabitol and mannitol, sugar alcohols, that are diagnostic of microbial damage [3,12]. These three were not included in Table 1. The table lists the 5 highest percentage carbohydrates in these cottons plus any traces of trehalulose and melezitose. With the exception of pima cotton, these 7 compounds accounted for over 80 percent of all carbohydrate components. Whitefly honeydew influences were often seen in the cottons from Western growth areas. Trehalulose and melezitose, sugars previously identified as exclusively due to insect contamination, [6,9,10,13] were found in three of the 6 California, the single Arizona, and both California pima cottons. The highest trehalulose level in the California cottons was 9.4 percent with an overall average of 2.8 percent. The two Pima cottons averaged 3.7 percent, and the one Arizona cotton had 15.3 percent trehalulose. These were not what would be called "heavily contaminated" and may not cause ginning and processing problems at these concentrations. However, there should be heightened concern for the overall effects on future crop quality if contamination increases. Differences in reducing sugar content in uncontaminated cottons ranged from a high of 0.44 percent in the High Plains Texas area to a low of 0.22 percent in the Southeastern area. With the exception of the honeydew contaminated pima and Arizona cottons, glucose generally ranged from 32 to 40 percent of total sugars, fructose from 16 to 25 percent, and sucrose from 3 to 12 percent.

Table 2 is a summary of results from classing office cottons (13 locations) for the 1995 season. Five randomly selected cottons from each office were tested by HPLC in duplicate and the results averaged for the above six growing areas. Nine of the original 65 cottons had characteristics of microbial contamination and were not included in this data. Evidence of some degree of insect honeydew contamination was found in 38 percent of the surveyed cottons (25 total). Trehalulose and melezitose were found in 7% of the Mississippi Delta, 67% of the High Plains Texas, 80% of the Corpus Christi, and 100% of the Arizona and California cottons. The cottons from the Western growing areas (Arizona and California) had an overall average of about 2.5 percent trehalulose and about 1 percent melezitose. Further east, in Texas and the Central United States, contamination was spotty, but definitely present in some bales. Of the 6 growing areas, only the Southeastern classing office cottons were totally free of honeydew contamination. There is some indication that cotton honeydew contamination is spreading to new areas.

Reducing sugars contents of uncontaminated cottons followed the same general pattern as the 1994 crop survey with the High Plains Texas cottons averaging the highest and Southeastern the lowest. Sucrose levels averaged considerably higher than normal in the cottons from the Florence, SC (37 percent of total sugars), Macon, GA (18 percent), and the Greenwood, MS and Altus, OK (16 percent) classing offices. Across these growing areas glucose concentrations averaged slightly lower than in the 1994 crop (25 to 35 percent), fructose concentrations were about the same, and sucrose concentration were higher (4 to 18 percent). The low molecular weight glycerol/inositol concentrations averaged 2 to 3 percent higher in the 1994 cottons.

Area of Growth Effects on Variety

Average carbohydrate concentrations for an uncontaminated Deltapine 50 variety grown in 4 areas (1994 crop) are shown in Table 3. Reducing sugar averages followed the same pattern, as seen in Tables 1 and 2, with the High Plains cotton being highest (0.39 percent) and the Southeastern cotton being the lowest (0.18 percent). In the 4 areas, the glucose concentrations averaged 33 to 40 percent, the fructose concentrations varied between 20 and 29 percent, and the sucrose concentrations were below 6 percent. The only exception was a high sucrose level of 20 percent in the High Plains Texas cottons.

Honeydew Contaminated Cottons

Whitefly Honeydew

Whitefly honeydew is specially characterized by the presence of trehalulose [2,3,5,6]. Reducing sugar levels are generally very high and the lint is very sticky for heavily contaminated cotton. Figure 2 is a chromatogram of a typical extract from a whitefly honeydew contaminated cotton. This particular sample is a moderately sticky cotton that contains equal amounts of glucose and trehalulose (16 percent) with a reducing sugar content of 0.50 percent . With the exception of cases where very low levels of trehalulose are detected (less than 0.5 percent), melezitose is also present. Both of these sugars have been associated with stickiness in ginning and textile processing of whitefly contaminated cotton. During the last two harvesting seasons we have collected a wide assortment of whitefly honeydew contaminated cottons from Texas, Arizona, and

California. Figure 3 is a comparison of the measured levels of the sugars trehalulose and melezitose in a total of 55 such cottons ranging from 1.0 to 36.2 percent trehalulose. On the average whitefly honeydew contaminated cottons contained 2.5 to 3.0 times more trehalulose than melezitose.

Residues of honeydew from a 1995 very sticky whitefly contaminated cotton (3+ on the minicard and 45+ on the thermodector) were collected from the minicard delivery rolls, extracted, analyzed for sugar composition, and compared to the composition of an equivalent fiber extract. Figure 4 shows overlaid chromatograms of the fiber and minicard residue extracts. Glucose, fructose, trehalulose, sucrose, and melezitose accounted for 85 percent of all known sugars in the fiber and 93 percent in the minicard deposit extracts. Fiber extract concentrations were 11, 20, 35, 7, and 12 percent for the above sugars, respectively. The minicard residue extract contained 4 times more sugar than the fiber extract. Glucose, fructose, and sucrose concentrations were approximately the same for both extracts. In the minicard deposit, trehalulose and melezitose levels increased from 36 to 46 and 10 to 13 percent, respectively. This is clear evidence that a majority of the stickiness found in whitefly honeydew is caused by trehalulose and melezitose. Another unique characteristic of whitefly honeydew is that the fructose concentration is generally about twice as high as that of the glucose; whereas, in uncontaminated cottons the glucose concentration is almost always greater than the fructose concentration.

Aphid Honeydew

The average very sticky aphid contaminated cotton is often not easily identified by a reducing sugar analysis (measuring less than 0.35 percent). HPLC sugar extracts indicate levels of trehalulose below 5 percent. Trehalulose/melezitose ratios for these extracts are lower than the whitefly honeydew ratios. A limited supply of aphid honeydew contaminated cottons has prevented us from establishing a good useable ratio. Current data, however, indicates a higher concentration of melezitose than trehalulose.

Figure 5 is an example of a 1995 aphid honeydew contaminated cotton. This particular cotton was extremely sticky on the minicard and thermodetector and had a 0.25 percent reducing sugar content. These overlaid chromatograms represent, as with the whitefly example (Figure 4), normal fiber and minicard residue deposit extracts analyzed by anion HPLC. An interesting difference in these chromatograms is that the 5 major sugars referred to above total only 49 percent for the fiber and 70 percent for the minicard deposit. This is considerably less than corresponding totals for whitefly honeydew extracts. Fiber extract concentrations were 15 percent for glucose, 13 percent for fructose, 1 percent for trehalulose, 19 percent for sucrose, and 1 percent for melezitose. The concentrated minicard residue extract contained twice the amount of total carbohydrates as the fiber extract. Proportionately, the glucose concentration was slightly lower (12 percent), the fructose higher (21 percent), and the sucrose lower (6 percent) in composition. Major differences were seen in the concentrations of trehalulose and melezitose in the fiber and minicard samples. In the minicard residue, melezitose concentration was more than twice that of trehalulose. Melezitose represented 22 percent and trehalulose 9 percent of the sugars. Compared to the fiber extract, the level of trehalulose increased 9 times and the level of melezitose 22 times in content in the minicard residue. In contrast to whitefly honeydew residues, where trehalulose appears to be the major component and contributor to cotton stickiness, melezitose is dominant in aphid honeydew. Melezitose is a non-reducing sugar and will not be detected by the universally used Perkins test for reducing sugars. Although melezitose has been reported to have a low stickiness potential (8), the combination of high melezitose and some moderate levels of trehalulose on cotton could cause heavy stickiness.

Summary

Research using high performance liquid anion chromatography has been refined to quickly analyze sugars and other carbohydrates from cottons. A complete analysis of these components can be accomplished in less than 30 minutes. This is particularly important in identifying whitefly and aphid contaminated cottons. Characterization of specific natural plant and honeydew sugars and the differentiation between types can lead to protocols to minimize the effect of the components responsible for stickiness. Surveys of recent beltwide crops show a possible increase in spread and influence of the whitefly in new areas.

Disclaimer

Trade names are used solely to provide specific information. Mention of a trade name does not constitute a warranty or an endorsement of the product by the US Department of Agriculture to the exclusion of other products not mentioned.

Reference

1. Bates, R.B., D.W. Byrne, W.K. Vinayck, W.B. Miller, and S.R. Taylor. 1990. N.M.R. characterization of trehalulose from the excrement of sweet potato whitefly, Bemisia tabaci. Carbohydrate Research. 201:342:342-345.

2. Brushwood, D.E. and H.H. Perkins, Jr. 1994. Characterization of sugar from honeydew contaminated and normal cottons. Proc. Beltwide Cotton

Conferences, National Cotton Council, Memphis, TN. pp 1408-1411.

3. Brushwood, D.E. and H.H. Perkins, Jr. 1995. Variations in insect honeydew composition and related effects on test methods and processing quality. Proc. Beltwide Cotton Conferences, National Cotton Council, Memphis, TN. pp 1178-1181.

4. Byrne, D.N. and W.B. Miller, 1990. Carbohydrates and amino acid composition of phloem sap and honeydew produced by Bemisa tabaci. Journal of Insect Physiology. 36:433-439.

5. Hendrix, D.L., Y.A. Wei, and L.E. Leggett. 1992. Homoteran honeydew sugar composition is determined by both insect and plant species. Comp. Biochem Physicol. 101B:23-27.

6. Hendrix, D.L., B. Blackledge, and H.H. Perkins, Jr. 1993. Development of methods for detection and elimination of insect honeydews on cotton fiber. Proc. Beltwide Cotton Conferences, National Cotton Council, Memphis, TN. Pp 1600-1602.

7. Hendrix, D.L. and Y.A. Wei. 1994. Bemisiose: an unusual trisaccharide in Bemisia honeydew. Carbohydrate Rec. 253:329-334.

8. Miller, W.B., E. Peralta, D.A. Ellis, and H.H. Perkins, Jr. 1994. Stickiness potential of individual insect honeydew carbohydrates on cotton lint. Textile Res. J. 64:344-350.

9. Perkins, Jr., H.H. 1991. Cotton stickiness - a major problem in modern textile processing. Proc. Beltwide Cotton Conferences, National Cotton Council, Memphis, TN. pp 523-524.

10. Perkins, Jr., H.H. 1993. Controlling cotton stickiness - summary of progress. Proc. 21st International Cotton Conf., Bremen, Germany. pp 219-224.

11. Roberts, C.W., H.S. Keoing, R.G. Merrill, P.S.R. Chueg, and H.H. Perkins, Jr. 1976. Implications of monosaccharides in sticky cotton processing. Textile Res. J. 46:374-380.

12. Roberts, C.W., P.S.R. Cheung, and H.H. Perkins, Jr. 1978. Implications of monosaccharides in sticky cotton processing. Part II, Effects of growing conditions on fiber contaminants. Textile Res. J. 48:91-96.

13. Tarczynski, M.C., D.N. Byrnes, and W.B. Miller. 1992. High performance liquid chromotography lanalysis of carbohydrates of cotton phloem sap and honeydew produced by Bemisia tabacci feeding on cotton. Plant Physiol. 98:753-756.

Table 1. Summary of carbohydrate components in 1994 cottons

	Location						
	S.E.	Cent.	TX	TX-HP	AZ	CA	Pima
Reducing S (%)	0.22	0.30	0.31	0.44	0.47	0.33	0.28
Carbo. (%)							
Inositol	10.4	10.0	12.5	11.2	5.1	17.9	15.0
Trehalose	10.2	11.7	11.1	10.0	7.0	5.4	14.9
Glucose	38.5	40.2	36.5	35.7	16.1	31.5	25.9
Fructose	20.1	21.5	25.6	23.7	25.2	15.9	11.4
Trehalulose	0	0	0	0	15.3	2.8	3.7
Sucrose	5.7	6.4	3.4	12.1	6.9	8.5	4.4
Melezitose	0	0	0	0	10.8	1.2	2.0
Other	15.1	10.2	10.9	7.3	13.6	16.8	22.7

Table 2. Summary of carbohydrate components in 1995 classing office cottons

	Location					
	S.E.	Cent	TX	TX-HP	AZ	CA
Reducing S. (%)	0.19	0.32	0.33	.35	0.29	0.35
Carbo. (%)						
Inositol	9.7	7.5	8.9	7.8	8.1	12.1
Trehalose	11.4	10.4	5.5	10.7	12.3	12.5
Glucose	24.5	29.9	34.6	34.2	24.6	30.9
Fructose	14.9	23.3	24.9	23.8	21.6	15.2
Trehalulose	0	0.1	0.8	1.3	2.5	2.3
Sucrose	18.1	16.1	4.4	7.8	11.8	11.7
Melezitose	0	0	0.5	0.6	0.8	0.5
Other	21.4	12.7	20.4	13.8	18.3	14.8

Table 3. Area of growth effect on carbohydrate content of DPL50 variety - 1994 crop

	Location			
	S.E.	Cent.	TX	TX-HP
Reducing S. (%)	0.18	0.32	0.31	0.39
Carbo. (%)				
Inositol	10.4	10.4	12.7	10.5
Trehalose	10.2	12.3	9.3	10.9
Glucose	38.5	39.6	35.6	33.3
Fructose	20.1	20.9	28.8	19.5
Sucrose	5.7	5.7	3.0	19.9
Other	15.1	11.1	10.6	5.9

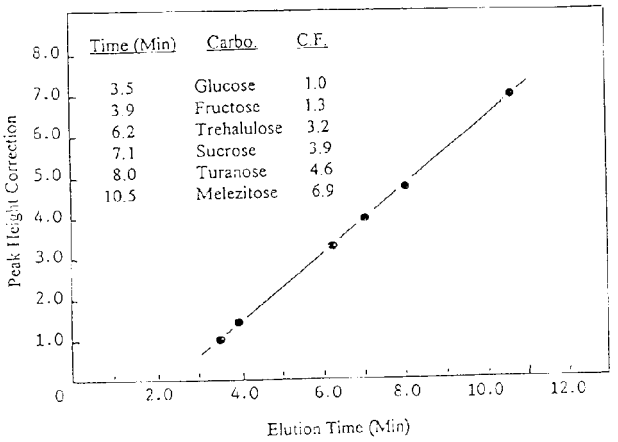

Figure 1. Calibration of Authentic Sugars by Anion HPLC Using 200 mM NAOH Eluant

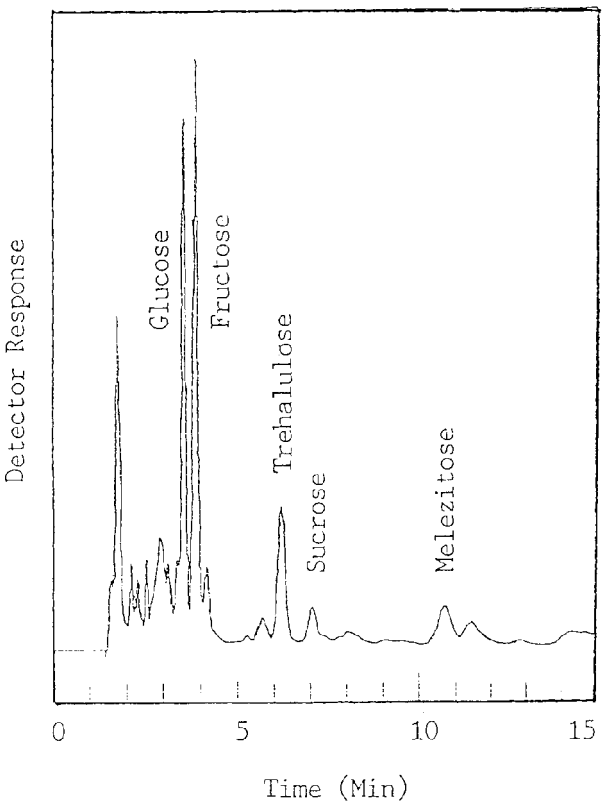

Figure 2. Typical Carbohydrate Extracts from a Whitefly Honeydew Contaminated Cotton

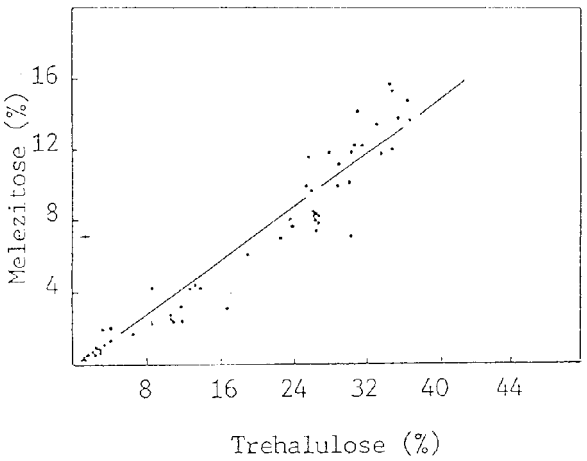

Figure 3. The Dependence of Melezitose Concentration on Trehalulose in Whitefly Contaminated Cotton

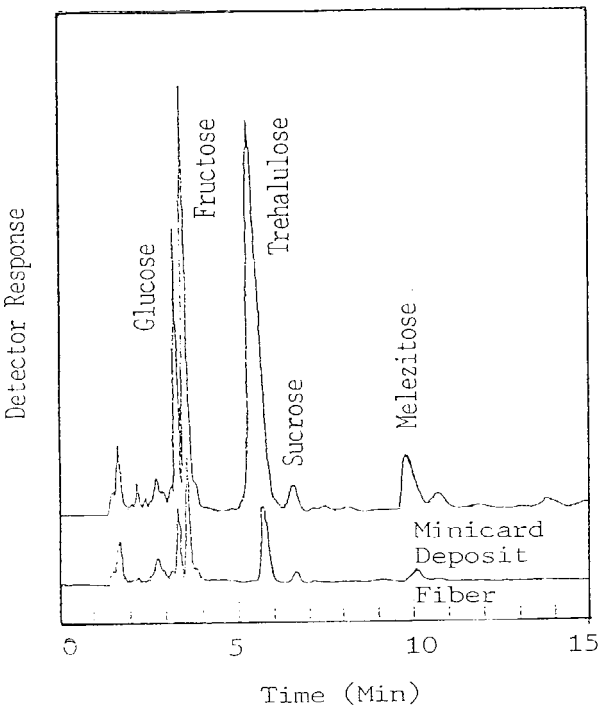

Figure 4. Sugar Extracts from Minicard Residue and Fiber of Whitefly Honeydew Contaminated Cotton