GLYCOCONJUGATE ANALYSIS OF DEVELOPING COTTON FIBERS FROM SEVERAL VARIETIES GROWN ON THE SAME SITE

Allen K. Murray[1] and Judy Brown[2]
[1]Glycozyme, Inc.
Irvine, CA
[2]Finch Ranch
Orland, CA

Abstract

Glycoconjugate analysis is being used to monitor growth and development of cotton fibers. Environmental stress has been detected by glycoconjugate analysis. The present study was undertaken to compare several varieties under identical environmental conditions to assess varietal differences. Prior to this study developmental samples had only been obtained from two varieties grown on the same site. The differences in glycoconjugate analysis between several varieties grown on the same site indicate that significant varietal differences do not exist and the differences observed could have been due to differences in soil characteristics within the field. In this study glycoconjugate analysis documented the drought stress to which the plants were subjected and the delayed recovery following irrigation. Recovery did not produce a normal distribution of glycoconjugates indicative of a sequence of cell wall synthetic events which is not resumed upon recovery.

Introduction

Cell Wall

Plant cells are surrounded by a rigid cell wall consisting of polysaccharides and a small amount of protein. The primary cell wall is typically synthesized during cell elongation and consists primarily of hemicelluloses and pectic substances. The secondary cell wall is synthesized later, typically during wall thickening, and consists primarily of cellulose. Any growth of a plant cell requires simultaneous expansion and or thickening of the cell wall. Consequently, growth of a plant cell is directly linked to cell wall synthesis. This relationship is presented in a simplified diagram in Figure 1.

Carbohydrates and Boll Development

The plant is faced with the problem of getting the sugars produced by photosynthesis in the leaves to support all of the ongoing processes in all parts of the plant. Of particular interest to the study of fiber production is the problem of getting the carbohydrates, in the form of sucrose, the transport sugar, from the leaves to the developing bolls and ultimately into the developing seed and fiber cells. The term "boll loading" is a general term applied to boll development and the massive transport of carbohydrate to the bolls. This quantitative interconversion of carbohydrate is an example of bioglycology, a term used to describe the interconversion of carbohydrate by biological organisms. The carbohydrate translocated to the developing fibers, which is not used to fuel the cellular processes, ultimately resides in the cell wall of the fiber cell and quantitatively in the greatest concentration in the secondary cell wall of the fiber which consists primarily of cellulose. During the translocation of carbohydrate material to its ultimate destination of the fiber cell wall, the sugars must traverse several membrane barriers, the last being the cell membrane of the fiber cell in a chemical form that can be useful to the wall synthetic apparatus.

Cell Wall Synthesis

The cotton fiber is unique from the standpoint of its development since it is a plant cell that usually does not divide or store starch. During the period of time the fiber cell elongates it is generally synthesizing primary cell wall. Following the period of cell elongation, the fiber cell thickens as the fiber synthesizes secondary cell wall which consists of cellulose.

Cell wall synthesis is a very complex process that has been the subject of a great deal of study. However the various components in the process of cell wall synthesis have not been completely described. It has been known for over 20 years that the best precursor of cotton fiber cell wall is sucrose(Pillonel, *et. al.*, 1980). Recently, the enzyme sucrose synthase has been shown to be membrane bound and evidence suggests that it is a key enzyme in cell wall synthesis(Amor, *et. al.*, 1995, Delmer and Amor, 1995). However, the role of a lipid linked intermediate, analogous to that in bacterial cell wall synthesis, has not been demonstrated. The presence of such intermediates has been postulated for a number of years. Such an intermediate would facilitate the problem of transporting the carbohydrates across the cell membrane to a site of cell wall synthesis outside the membrane. The uniform flow of carbohydrate/biomass to cell wall results in a more uniform secondary cell wall and consequently, one would expect more uniform fiber.

Glycoconjugates

Glycoconjugates are carbohydrates covalently linked to other carbohydrates, proteins or lipids. The glycoconjugates monitored in the present study appear to function as cell wall precursors or intermediates in the biosynthetic processes that produce the cell wall. Cotton fibers are unique as plant cells in that their primary function is the synthesis of cell wall material. A progression of appearance and disappearance of specific glycoconjugates has been observed in developing cotton fibers under "normal" conditions. Developing cotton fibers obtained from plants subjected to various forms of stress, which negatively impacted fiber development, demonstrate

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1205-1209 (1996)
National Cotton Council, Memphis TN

an abnormal or altered pattern of appearance and disappearance of the glycoconjugates monitored. Glycoconjugate analysis appears to be a sensitive method of monitoring cell wall synthesis which is directly coupled with cell growth. This analysis is applicable to roots, stems, leaves and fruits. In this case, the analysis has been applied to a fruit. The presence of these glycoconjugates has been demonstrated in a number of plants which leads to the conclusion that they will be found in virtually all plant cells. Structural studies on the glycoconjugates analyzed in this work is currently in progress. In addition to the monitoring of fiber growth and development, glycoconjugate analysis will demonstrate the presence of trehalulose or melizitose, oligosaccharides present in whitefly honeydew, if they are present.

Methods

Fifteen varieties were grown at Finch Ranch, Orland, CA. Planting was on 4/25 or 4/23 with irrigation on 6/30, 7/22 and 8/12/95. Spacing between rows was 30in with each variety in three row increments. First position white flowers were tagged on 7/25/95. Bolls were removed from plants at the indicated number of days post anthesis (DPA) and frozen as quickly as possible. Bolls were shipped to the laboratory on dry ice. Frozen bolls were kept in a freezer at -80° until lyophilized. Frozen bolls were cracked open in a vise with a section of angle-iron attached to one jaw by epoxy. Lyophilization was carried out at 200 millitorr at dryness and usually required up to 48 hours to achieve constant weight.

In the studies on DPL5415 cotton was grown at Mississippi State in 1993, first position bolls from tagged white flowers were removed from plants and frozen upon reaching the laboratory. Bolls were lyophilized, dissected and fibers were ground in a Wiley Mill and pressed into disks for calcium X-ray fluorescence studies at the Southern Regional Research Laboratory. The disks were then cut in half and one half sent to this laboratory. In a typical experiment 5mg of cotton fibers were extracted with a volume of 0.5 ml. Following extraction the contents of the vial were centrifuged in a Z-Spin cartridge, 0.2µm, prior to chromatography by HPAEC-PAD. HPAEC-PAD (High pH Anion Exchange Chromatography - Pulsed Amperometric Detection) was performed on a Dionex Bio-LC. Chromatographic analysis was performed using the Dionex AI-450 software. All chromatograms have been normalized for the amount of cotton fiber tissue extracted. It should be noted that all carbohydrates do not give the same detector response with pulsed amperometric detection. Quantitative differences between different carbohydrates can only be established after the detector response for each carbohydrate has been determined. However, the relative quantitation of the same glycoconjugate is possible between runs.

Results

A typical chromatogram of the glycoconjugates extracted from a sample of DPL5415 at 21DPA is shown in Figure 2. Peaks have been given arbitrary numerical assignments for the purpose of identification. A normal pattern of glycoconjugates and their changes through development is shown for DPL5415 in Figure 3. At 21 DPA a number of the glycoconjugates in both the GC-1 and GC-2 groups of peaks are present. At 28 DPA the GC-2 group is somewhat reduced as is the GC-1 group while at 42 DPA both groups are decreased. At maturity more peaks in the GC-1 group are present and the GC-2 group is almost undetectable. The relative amounts of the glycoconjugates as determined by the area under the peaks appears to be GC2-1< GC2-2 < GC2-3.

The analyses of the different varieties indicated that the differences between varieties were minimal and could represent variability of soil conditions as well as slight differences in developmental time frames. Differences from the previously determined normal developmental pattern were demonstrated in all varieties. This abnormal developmental pattern correlates with reduced yield and fiber quality. Although unintentional, it is clear that all varieties were subjected to drought stress. The glycoconjugate analysis of Maxxa is shown in Figure 4. The most significant indicator of drought stress on 8/08/95 is the abnormally high concentration of GC2-7.2. Drought stress was not apparent from visual examination of the plants. However, significant drought stress was present in all varieties sampled on 8/11/95 as indicated by the near absence of GC2-1, 2-2, and 2-3 before irrigation on 8/12/95. Monosaccharides, sucrose and GC1-2 were present after irrigation on 8/22/95. The plants had recovered somewhat by 8/29/95 as indicated by increased levels of GC2-1 and GC2-3 but GC2-2 was not detected. In addition, sucrose levels were very low. However, the 35DPA glycoconjugate profile is not normal as shown by the relative concentrations of the peaks GC2-9, GC2-10, and GC2-11.

The glycoconjugate analysis for Royale is shown in Figure 5 and it demonstrates the same pattern as for Maxxa. The 14 DPA profile appears to have a normal amount of GC2-1, GC2-2 and GC2-3. The 17 DPA profile is deficient in the GC2-1, GC2-2 and GC2-3 cluster of peaks and it is also lacking in sucrose. The profiles at 21 and 28 DPA are also deficient. The 35 DPA profile demonstrates the presence of a number of the peaks as well as sucrose, indicative of recovery.

However, the 35DPA glycoconjugate profile is not normal as shown by the relative concentrations of the peaks GC2-9, GC2-10, and GC2-11.

The glycoconjugate analysis of DP51 before, after and without irrigation is shown in Figure 6. Significant

drought stress was present on 8/10/95 as indicated by the near absence of GC2-1, 2-2, and 2-3. before irrigation. Monosaccharides, sucrose and GC1-2 were still present after irrigation on 8/14/95. Fibers from plants without irrigation had much lower levels of monosaccharides and GC1-2 and sucrose was absent. These bolls were not from tagged flowers but were from first position bolls on equivalent fruiting branches.

Discussion

The purpose of this study was to determine if there are varietal differences in the glycoconjugates extracted from developing cotton fibers that would necessitate establishing a baseline for different varieties. In data not presented in this paper, it appears that the differences in glycoconjugate analyses between several varieties grown on the same site indicate that significant varietal differences are not significant and the differences observed could have been due to differences in soil characteristics within the field. The results demonstrate similarities which indicate that this type of analysis can be useful to monitor cotton fiber development. Although not intentional, it is apparent that the plants were subjected to drought stress. Following irrigation the plants did not recover for 12 to 19 days. The recovery period can not be more closely defined due to the fact that samples were routinely taken every 7 days. This period of drought stress on the plants coincided with the shift from synthesis of primary cell wall to that of secondary cell wall. Clearly this period is crucial to the development of cotton fibers. It is also apparent that when the plants did recover, the recovery did not resume the uninterrupted normal developmental sequence. This abnormal recovery could be explained by a sequence of reactions involved in the various phases of cell wall synthesis. If the sequence is interrupted by any form of environmental stress on the plant and the plant is subsequently allowed to recover, then the wall synthetic apparatus may not resume cell wall synthesis at the point at which it was interrupted but rather it may return to an earlier point in the sequence of cell wall synthetic reactions. This would account for the abnormal pattern of glycoconjugates present on recovery and it may explain poor fiber quality if the fiber synthesizes an inferior cell wall. Analysis of the fibers utilized in this study at the Southern Regional Research Center will follow to further document the fiber quality. Structural studies currently in progress on the glycoconjugates will greatly facilitate our understanding of the role of these glycoconjugates in cell wall synthesis and significance of abnormal developmental patterns.

Summary

Cell wall synthesis is an integral component of the events occurring during plant cell growth and development. Cell wall synthesis is sensitive to drought stress and other forms of environmental stress which affect cell growth and development. Several glycoconjugates have been identified which appear to function as cell wall precursors and their concentrations can be monitored by glycoconjugate analysis. Glycoconjugate Analysis (GCA) appears to be a sensitive method by which to monitor cotton fiber development and has the potential to identify abnormal development in cotton fibers.

Acknowledgment

We would like to thank Judith Bradow, USDA, Southern Regional Research Center and Gretchen F. Sassenrath-Cole, USDA, Mississippi State for samples of cotton, fiber data and stimulating discussions.

References

1. Amor, Y., Haigler, C.H., Johnson, S., Wainscott, M., and Delmer, D.P.,1995. A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants, Proc. Nat. Acad. Sci. USA 92:9353-9357.

2. Delmer DP. and Amor Y., 1995. CELLULOSE BIOSYNTHESIS, Plant Cell. 7(7):987-1000.

3. Pillonel, C., Buchala, A.J., and Meier, H.,1980. Glucan synthesis by intact cotton fibres fed with different precursors at the staages of primary and secondary wall formation Planta 149:306-312.

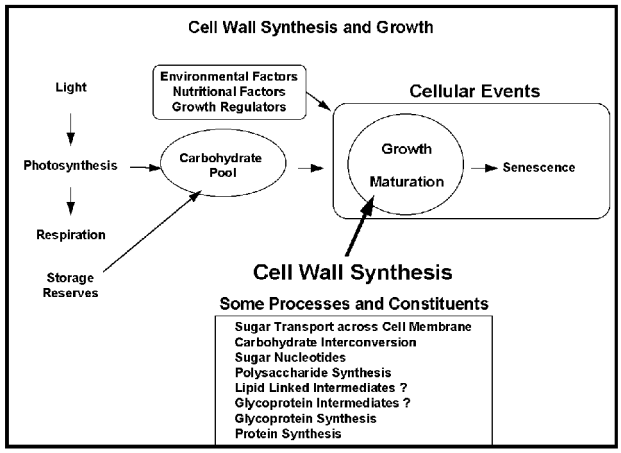


Figure 1. Cell Wall Synthesis and Growth

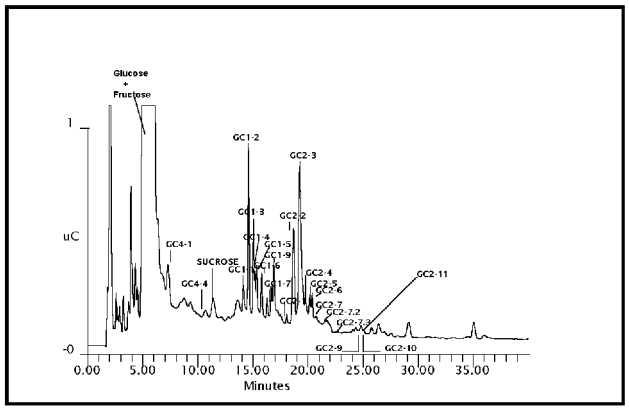


Figure 2. Peak Label Assignments
A typical chromatogram for DPL5415 at 21DPA. Peaks have been given arbitrary numerical assignments for identification.

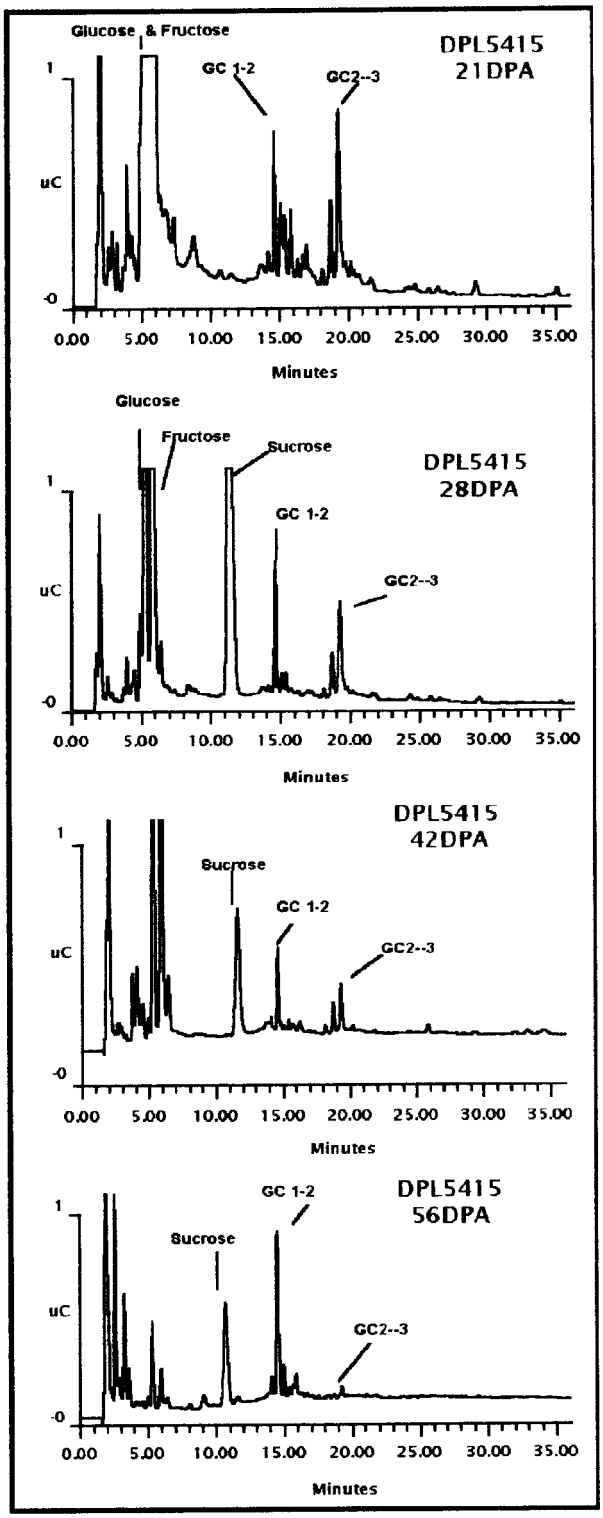


Figure 3. Normal Developmental Glycoconjugate Profiles
At 21 DPA a number of the GC 1 and GC 2 groups of peaks are present. At 28 DPA the GC 2 group is some what reduced as is the GC 1 group. At 42 DPA both groups are decreased. At maturity more peaks in the GC 1 group are present and the GC 2 group is almost undetectable.

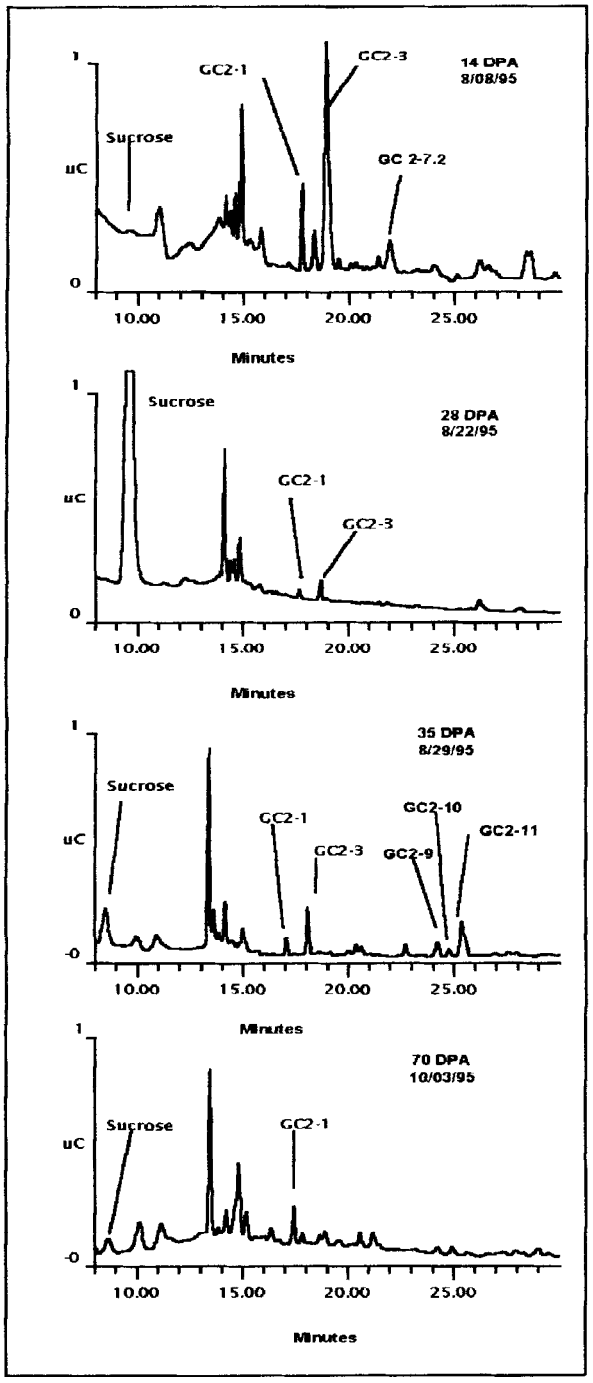
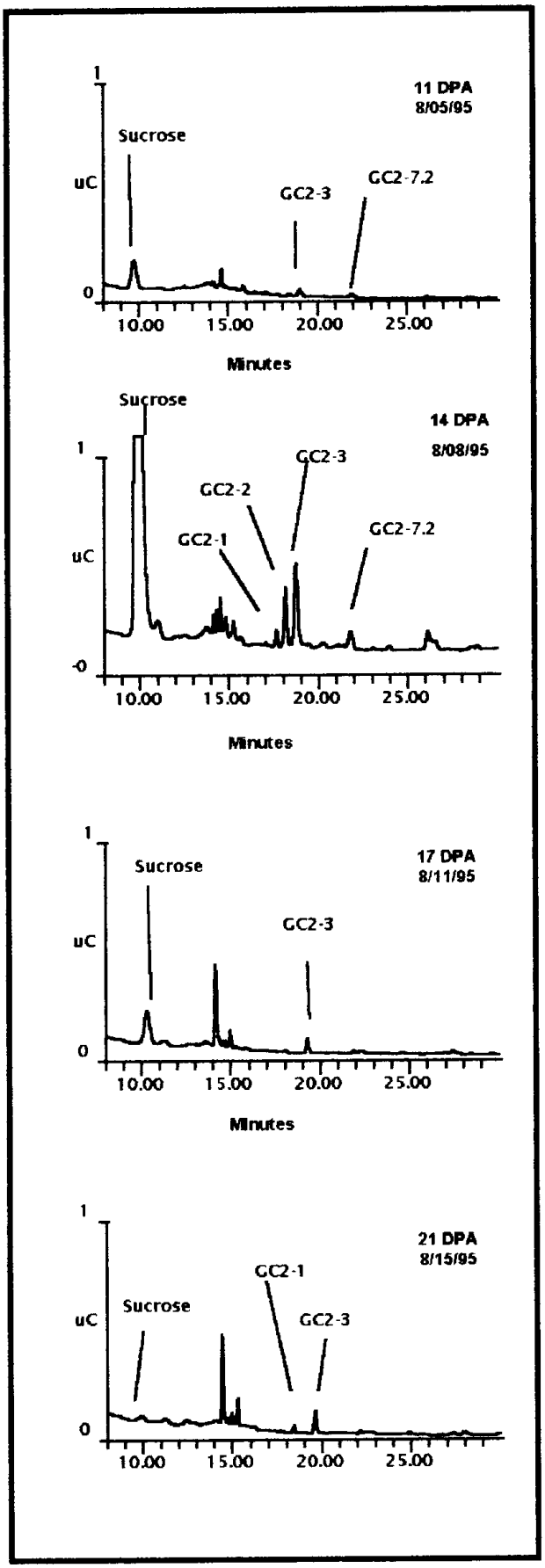
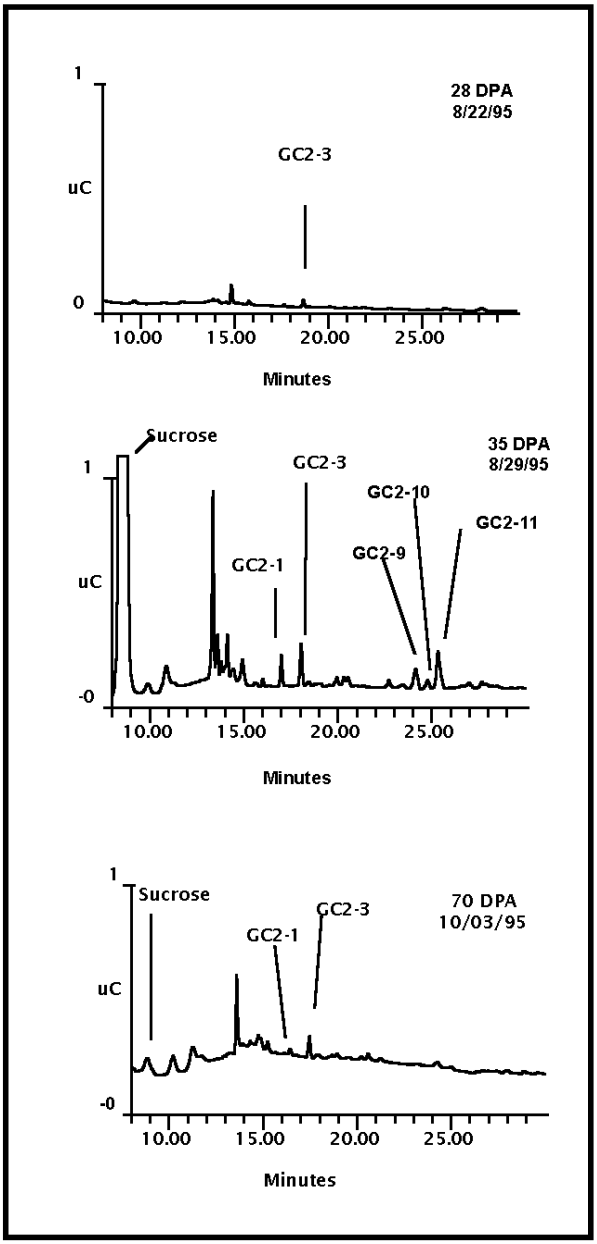


Figure 4. Glycoconjugate Analysis of MAXXA.
The most significant indicator of drought stress on 8/08/95 is the abnormally high concentration of GC 2-7.2. Significant drought stress was present in other varieties sampled on 8/11/95 as indicated by the near absence of GC2-1, 2-2, and 2-3. before irrigation. Irrigation began on 8/12/95. Monosaccharides, sucrose and GC1-2 were present after irrigation on 8/22/95. The plants had recovered somewhat by 8/29/95 as indicated by increased levels of GC 2-2 and GC 2-3. However, sucrose levels were very low and the 35DPA glycoconjugate profile is not normal as shown by the relative concentrations of the peaks GC2-9, GC2-10, and GC2-11.



Figures 5a. Glycoconjugate Analysis of Royale.



Figures 5a and 5b. Glycoconjugate Analysis of Royale.
Samples at 17 DPA were not from tagged flowers but they were from first position bolls on equivalent fruiting branches of tagged plants. The 14 DPA profile appears to have a normal concentration of GC 2-1, GC 2-2 and GC 2-3. The 17, 21, and 28 DPA profiles are deficient in the GC 2-1, GC 2-2 and GC 2-3 cluster of peaks and sucrose. The 35 DPA profile demonstrates the presence of a number of the peaks as well as sucrose, indicative of recovery. However, the 35DPA glycoconjugate profile is not normal due to the relative concentrations of the peaks GC2-9, GC2-10, and GC2-11

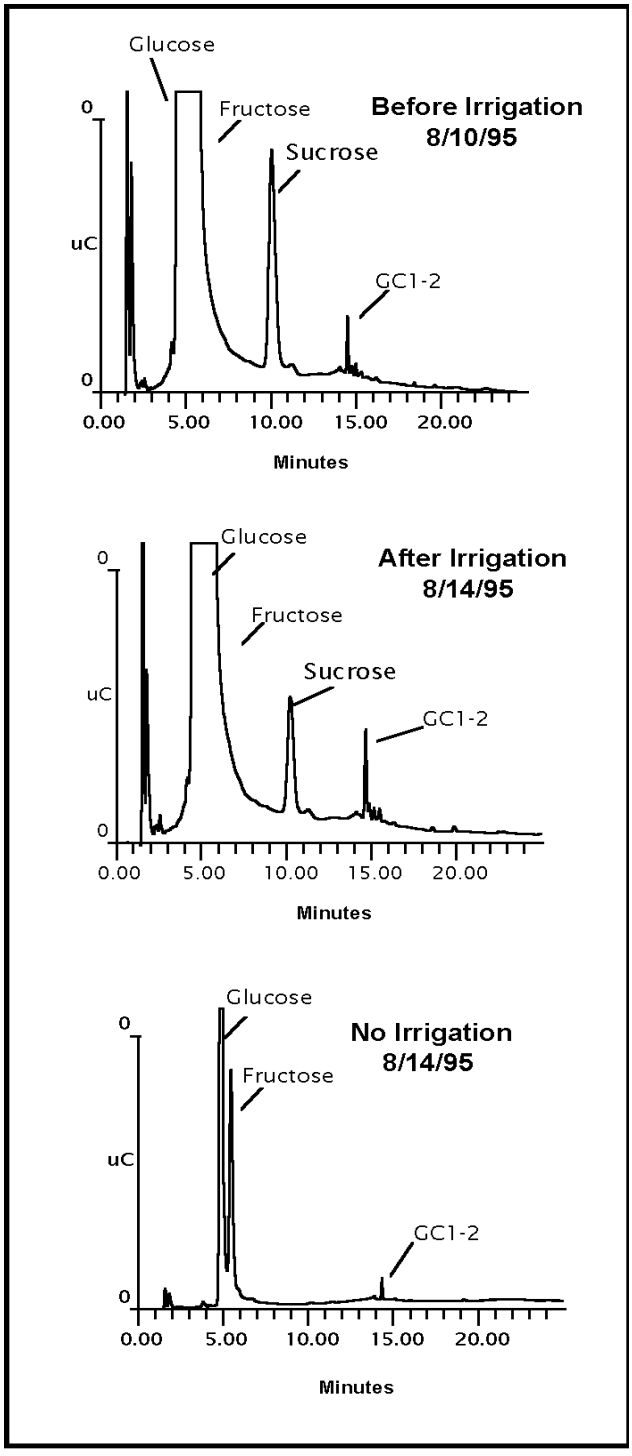


Figure 6. Glycoconjugate Analysis of DP51 Before, After and Without Irrigation.
Significant drought stress was present on 8/10/95 as indicated by the near absence of GC2-1, 2-2, and 2-3. before irrigation. Monosaccharides, sucrose and GC1-2 were still present after irrigation on 8/14/95. Fibers from plants without irrigation had much lower levels of monosaccharides and GC1-2 and sucrose was absent. These were not tagged flowers. Labels indicate sample dates. Irrigation began on 8/10/95. These samples were not from tagged flowers.