THE USE OF GLYCOCONJUGATE ANALYSIS TO MONITOR GROWTH AND ENVIRONMENTAL STRESS IN DEVELOPING COTTON FIBERS

Allen K. Murray
Glycozyme, Inc.
Irvine, CA

Abstract

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. Aberrations in the normal sequence of appearance and disappearance of these compounds can be taken as indicators of abnormal cell wall synthesis and consequently abnormal growth and development. Such aberrations in the developmental sequence of glycoconjugates have been observed for both low temperature stress and drought stress. 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. 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.

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

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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

In the studies on DPL5415, PIMA S-6, and DES119, cotton was grown at Mississippi State in 1992 and 1993. First position bolls from tagged white flowers were removed from plants at the indicated number of days post anthesis (DPA) 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 the irrigation studies with MAXXA, cotton was grown near Fresno, CA. Developmental samples were from 1995 and mature samples were from a 1992 experiment as well. First position 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 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. Retention times are expressed in minutes and detector response is expressed in µCoulombs. Chromatographic analysis was performed using the Dionex AI-450 software. All chromatograms were 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.

Glycoconjugates were extracted from mature fibers obtained from an irrigation experiment using MAXXA. The low stress plants received their final irrigation one week earlier than the high stress plants. The week interval was characterized by unusually hot weather. Several glycoconjugates had different concentrations in the extracts from of fibers from high stress and low stress plants. A chromatogram of glycoconjugates from the low stress fibers was subtracted from a chromatogram of the glycoconjugates from high stress fiber using the Dionex Data Math program. The resultant chromatogram is characterized by peaks representing GC1-4 & 1-5, GC2-1, GC2-2, and GC2-3 indicating that these compounds are elevated in the fibers from the high stress plants as shown in Figure 4. Conversely, the resultant chromatogram had a negative peak for GC1-2 indicating that it is elevated in the fibers from the low stress plants. As a result of this relationship of the glycoconjugates differing between fibers from low stress and high stress plants, the content of these glycoconjugates was plotted for extracts from fibers obtained at various stages of development.

The glycoconjugate content of developing fibers of DPL5415 and PIMA S-6 is shown in Figures 5 and 6 respectively. The area under the peaks for GC1-2, GC1-3-5, GC1-6, GC2-2, GC2-3, and GC2-5 was plotted against the mg fiber dry weight. At 28 DPA, GC1-6 was not detected so the value for GC1-6 at 28 DPA was arbitrarily set at 100 to permit plotting on the log scale with the software used. These samples are from 1993, which was considered to have normal weather.

The glycoconjugate content of developing fibers of DES119 is shown in Figure 7. The DES119 samples are from 1992, which was not considered to be a normal year due to cool morning temperatures. The overall content of several glycoconjugates is different than for either DPL5415 or PIMA S-6 in 1993. Most striking was the content of GC1-6 which did not fall dramatically at 28 DPA and the content of GC2-3 is higher.

The glycoconjugate content of developing fibers of MAXXA with and without irrigation are shown in Figures 8 and 9 respectively. In Figure 10 the chromatograms from Figures 8 and 9 are superimposed to facilitate comparison. Extracts of fibers from plants with irrigation, 8-10 DPA taken 4 days after irrigation contain sucrose, GC2-1, GC2-2 and GC2-3. In contrast, extracts of fibers from plants without irrigation have a different composition. Sucrose is barely detectable and GC2-1, GC2-2 and GC2-3 are present in reduced concentrations while GC2-7.2 and GC2-7.3 are present in increased concentrations.

Discussion

The glycoconjugate distribution in extracts of DPL5415 and PIMA S-6 indicate that there is a normal sequence of appearance and disappearance of a number of glycoconjugates during the development of the cotton fiber. Early on the GC1 group appears to be very prominent as well as the GC1 group of compounds. After 21DPA the number of peaks in the GC1 group is reduced until maturity is approached and the number of peaks in this group increases. The presence of the GC1 group of compounds steadily decreases as the fiber matures. The disappearance of GC1-6 at 28DPA and its reappearance in later samples was initially puzzling. However, in work not reported here, it has been determined that the compounds GC2-1, GC2-2, and GC2-3 can all be cleaved by mild acid hydrolysis to yield GC1-6 and monosaccharides. This hydrolysis data is indicative of a precursor/product relationship of the sequence GC1-6 GC2-1 GC2-2 GC2-3. If this is, in fact, the case, then the disappearance of GC1-6 at 28DPA would simply be indicative of a very small pool of this intermediate as cell wall synthesis proceeds. The DES 119 data, in which GC1-6 was not depleted at 28 DPA or any other time during development, would suggest that cell wall synthesis was inhibited at some step subsequent to the synthesis of these precursors so that they accumulated. This interpretation of the data would also explain the relatively higher concentration of GC2-3 in the extracts of DES119 fibers. Autolysis experiments, not reported here, have not demonstrated interconversion of the GC1 group of compounds. However, autolysis experiments do demonstrate the interconversion of the GC1 group of compounds, most notably the conversion of GC1-1 to GC1-2.

The analysis of extracts of fibers at maturity from plants subjected to low or high stress demonstrates the increased concentration of GC2-1, GC2-2, and GC2-3 which would be consistent with the inhibition of cell wall synthesis. The lower concentration of GC1-2 in the fibers from plants subjected to high stress is also consistent with this compound being associated with maturity or a normally completed cell wall. Yield from the plants subjected to high stress was considerably lower than yield from the low stress plants.

The extracts of fibers at 8 -10 DPA with irrigation demonstrate the presence of substantial amounts of sucrose GC2-1, GC2-2, and GC2-3. While extracts of fibers at 8 - 10 DPA without irrigation have a barely detectable amount of sucrose and reduced levels of GC2-1, GC2-2, and GC2-3. The most remarkable difference is the presence of relatively great amounts of GC2-7.2 and GC2-7.3. It is possible that the presence these two compounds may be an early indication of drought stress even though there may appear to be relatively normal amounts of other glycoconjugates.

These results demonstrate similarities which indicate that this type of analysis can be useful to monitor cotton fiber development. Structural studies currently in progress on the glycoconjugates will greatly facilitate our understanding of the role of these glycoconjugates in cell wall synthesis and their significance in normal and 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. Aberrations in the normal sequence of appearance and disappearance of these compounds can be taken as indicators of abnormal cell wall synthesis and consequently abnormal growth and development. 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. 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.

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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.

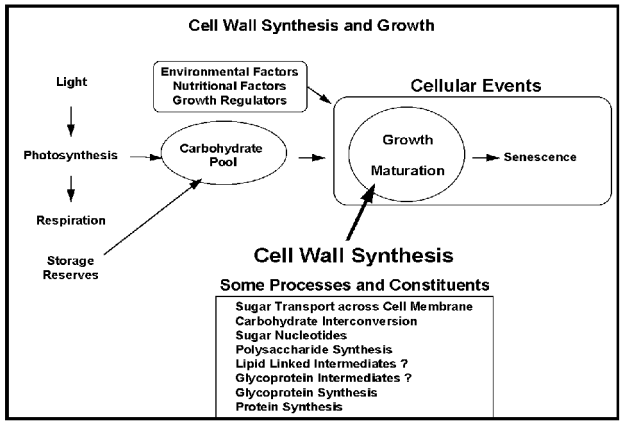

Figure 1. Cell Wall Synthesis and Growth

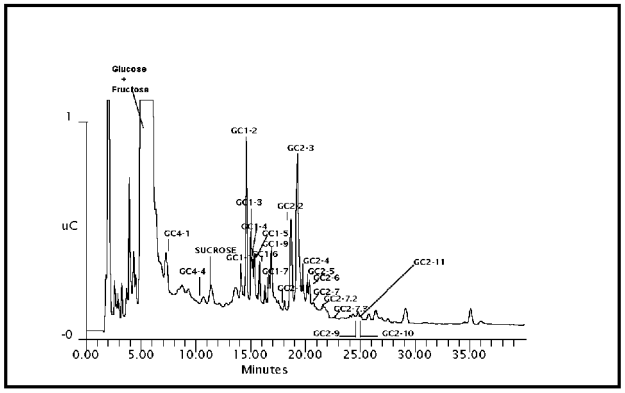

Figure 2. Peak Label Assignments
A typical chromatogram for DPL5415 at 21DPA. Peaks have been given arbitrary numerical assignments for identification.

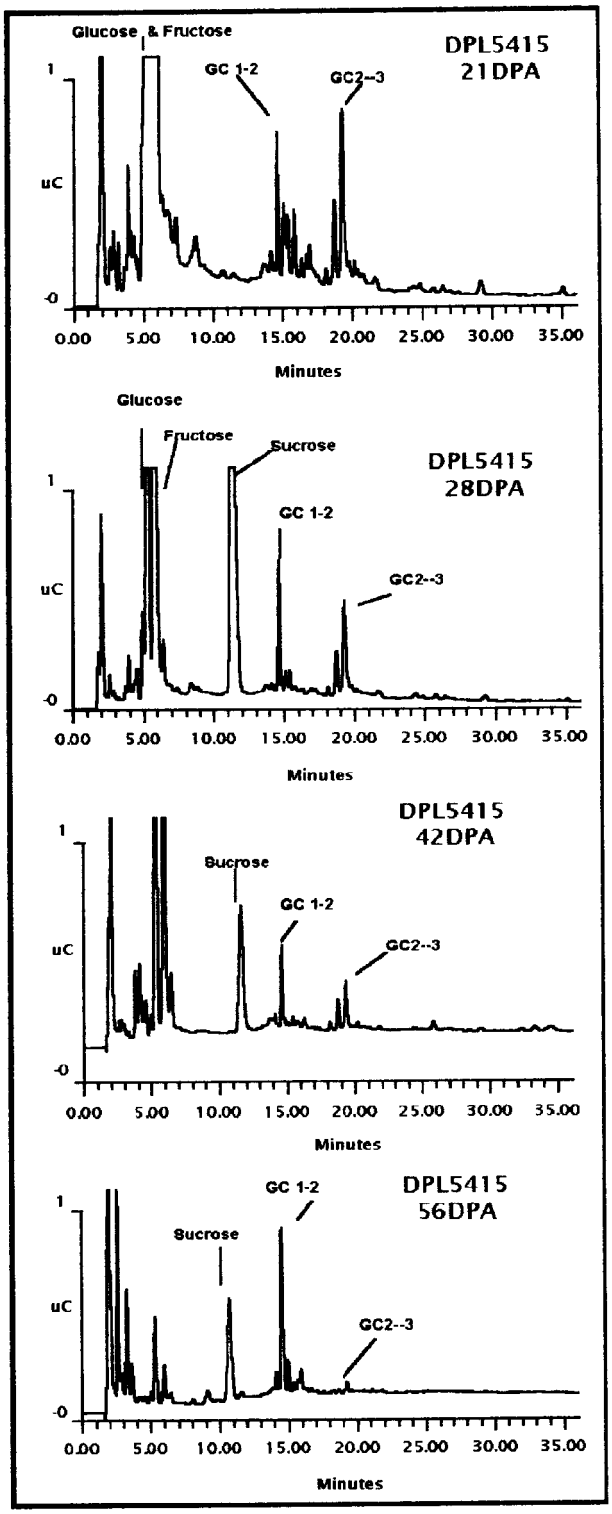

Figure 3. Normal Developmental Glycoconjugate Profiles
At 21 DPA a number of the GC 1 and GC 2 groups of peaks are present. At 28 DPA the GC 2 group is some what reduced as is the GC 1 group. At 42 DPA both groups are decreased. At maturity more peaks in the GC 1 group are present and the GC 2 group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

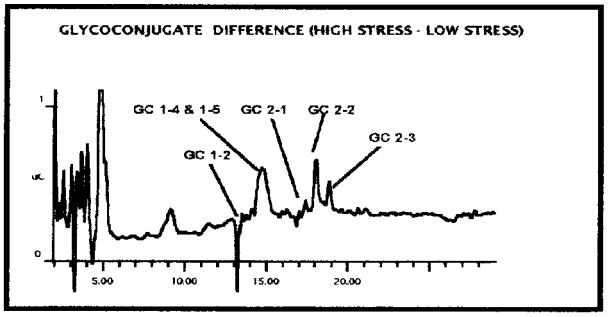

Figure 4. Glycoconjugate Content of Mature Fibers from Plants Subjected to High and Low Stress
Samples were taken at maturity from a 1992 irrigation experiment with Maxxa. The low stress field received its third irrigation one week before the high stress field. The chromatogram from the low stress extract was subtracted from the chromatogram of the high stress extract using the Dionex Data Math program. In this difference chromatogram, peaks (GC 1-4 & 1-5, GC 2-1, GC 2-2, and GC 2-3) were elevated in the high stress extract while the negative peak (GC 1-2) was elevated in the low stress extract.

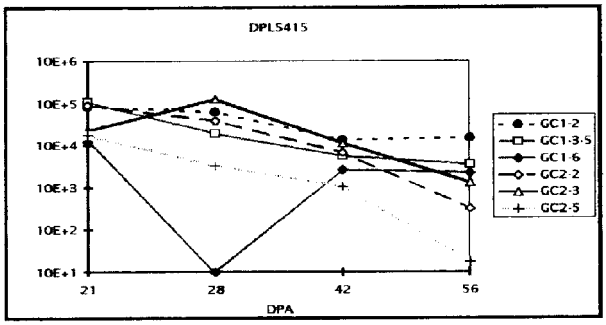

Figure 5. Glycoconjugate Content of Developing Fibers of DPL5415
The integrated area under the peaks indicated, GC 1-2, GC 1-3-5, GC 1-6, GC 2-2, GC 2-3, and GC 2-5 was plotted against the mg fiber dry weight. At 28 DPA, GC 1-6 was not detected so the value for GC 1-6 at 28 DPA was arbitrarily set at 100 to permit plotting on the log scale with the software used. These samples are from 1993, which was considered to have normal weather.

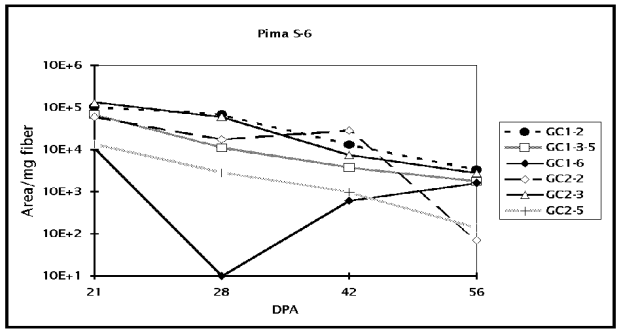

Figure 6. Glycoconjugate Content of Developing Fibers of Pima S-6
The integrated area under the peaks indicated, GC 1-2, GC 1-3-5, GC 1-6, GC 2-2, GC 2-3, and GC 2-5 was plotted against the mg fiber dry weight. At 28 DPA, GC 1-6 was not detected so the value for GC 1-6 at 28 DPA was arbitrarily set at 100 to permit plotting on the log scale with the software used. These samples are from 1993, which was considered to have normal weather.

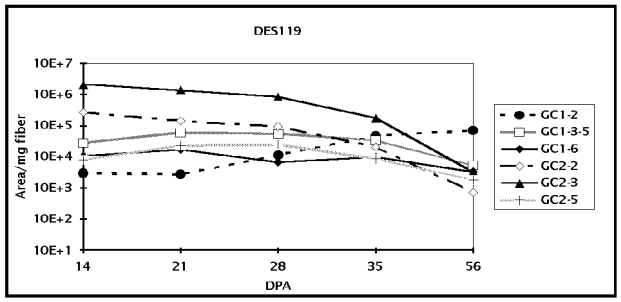

Figure 7. Glycoconjugate Content of Developing Fibers of DES119
The integrated area under the peaks indicated, GC 1-2, GC 1-3-5, GC 1-6, GC 2-2, GC 2-3, and GC 2-5 was plotted against the mg fiber dry weight. These samples were from 1992, which was not considered to be a normal year due to cool morning temperatures. In contrast to DPL5415 and Pima S-6 in 1993, the content of GC 1-6 did not fall dramatically at 28 DPA.

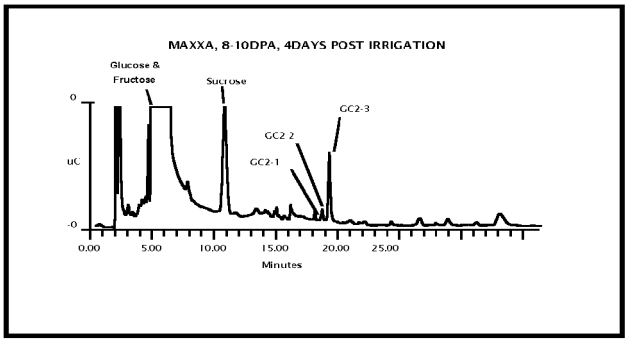

Figure 8. Glycoconjugate Content of Developing Fibers of Maxxa With Irrigation
Samples from a 1995 irrigation experiment, 8-10 DPA taken 4 days after irrigation. Sucrose, GC 2-1, GC 2-2 and GC 2-3 are present.

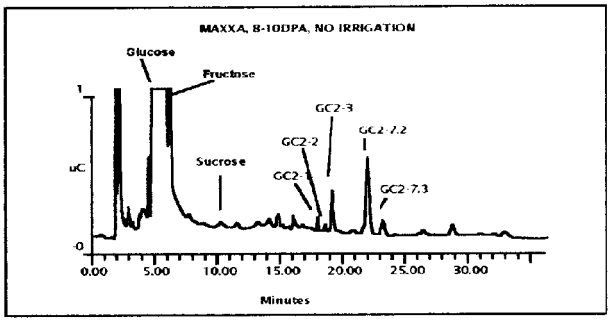

Figure 9. Glycoconjugate Content of Developing Fibers of Maxxa Without Irrigation
Samples from a 1995 irrigation experiment, 8-10 DPA taken 4 days after irrigation. Sucrose is almost not detectable and GC 2-1, GC 2-2 and GC 2-3 are present in reduced concentrations. GC 2-7.2 and GC 2-7.3 are present in increased concentrations.

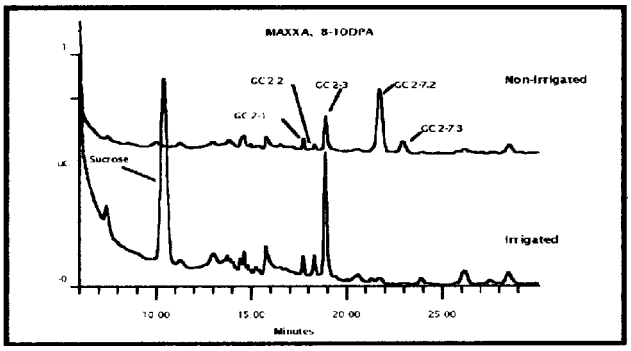

Figure 10. Glycoconjugate Content of Developing Fibers of Maxxa With and Without Irrigation

Overlay of chromatograms of Figures 8 and 9. Chromatograms of extracts from irrigated and non-irrigated Maxxa, 8-10DPA.