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CARBOHYDRATE COMPOSITION OF COTTON APHID HONEYDEW

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Abstract

Honeydew from cotton aphids (*Aphis gossypii)* has been collected from aphids feeding on Pima S-6 plants between the ages of 4 and 7 weeks. The sugars identified in the honeydew are the same as those identified from honeydew of aphids feeding on older plants of upland cottons with the exception that we did not find trehalulose and melizitose. The sucrosyl oligosaccharides and many larger oligosaccharides were also found. The quantity and and distribution of the larger oligosaccharides varies with the age of the plants with the greatest concentration being present at five and six weeks of age. The larger oligosaccharides are not reducing sugars consistent with their proposed biosynthesis from sucrose and sucrosyl oligosaccharides.

Introduction

The sugar composition of cotton aphid honeydew which feed on cotton phloem sap has been reported to contain a number of sugars and polyols (Hendrix, 1999, Henneberry, et. Al, 2000). Honeydew has been shown to contain several monosaccharides, disaccharides and a large number of glucose containing oligosaccharides which have not been identified. These studies have been done on the honeydew of aphids feeding on mature plants and the interest has been with respect to sticky cotton. The present work is part of a larger investigation however we report here on the sugar composition of cotton aphid honeydew and the differences in the sugar composition with young plants of different ages.

Materials and Methods

Cotton (cv. Pima S6) was grown from seed in 9-l tapered plastic pots (Treepot; Hummert International, Earth City, MO), filled with sintered clay (6-40 mesh; Quicksorb, A & M Products, Taft, CA). Pots were randomly assigned to outdoor chambers for exposure to one of three concentrations of ozone. Pots were automatically irrigated to run-through up to three times a day as required by the weather. A complete fertilizer (Miracle Gro; Scotts Miracle-Gro Products Inc., Port Washington, NY) was injected into the irrigation water at 1.3 g l^{-1} weekly.

A colony of cotton aphid (*Aphis gossypii*), was established from individuals obtained from the USDA/ARS aphid colony in Parlier, CA. The aphids were reared on young cotton plants in a greenhouse. Aphids were transferred to the field OTCs in small custom designed “clip cages”. These were 3 cm long made from 2.5 cm diameter plastic tubing. Air holes were cut in the sidewall and covered with fine mesh. One end of the tube was closed with a spring loaded clear plastic cover attached to the tube with a large hair clip. The other end of the container was the honeydew collection surface, a piece of aluminum foil formed tightly over the end of the tubing. This was easily removed and replaced during sample collection.

Approximately 10 aphids were transferred to each clip cage using a camel hair brush. Four clip cages were placed in each OTC, attached to the youngest fully expanded leaves of different plants. Containers were supported with a wire frame to maintain orientation with the aluminum foil at the bottom for efficient honeydew collection. Aphids were allowed to adapt to the experimental plants and to purge for 24 hours at which time the aluminum foil was discarded. A new piece of aluminum foil was attached and honeydew collected for 3 days. Containers were washed and dried prior to installation on a plant. The aluminum foil with deposited honeydew was immediately dried (40 C, 24 hours), then stored in individual Petri dishes sealed with Parafilm, until extraction.

Honeydew was eluted with water, taken to dryness in a SpeedVac and brought to known volume. Carbohydrate analysis was performed by HPAEC-PAD (High pH Anion Exchange Chromatography with Pulsed Amperometric Detection) on a Dionex Bio-LC, with a Dionex CarboPac PA 1 column. The eluent was 150mM NaOH, isocratic for 5 minutes, followed by a linear sodium acetate gradient from 0 to 500mM in 150mM NaOH, for 35 minutes . Retention times are expressed in minutes and detector response in µCoulombs (see Murray et al., 1997, 1999 in these Beltwide Proceedings, for further details). Chromatographic analysis of honeydew was performed with Dionex PeakNet software, transferred to an Excel spreadsheet. For known sugars, for which authentic standards were available, standard curves based on peak areas were used. peak areas for sugar amounts. Cellobiose was used as the internal standard.

Results

The honeydew samples were highly variable in their sugar content but there were consistent patterns of the distribution of sugars. The samples all contained fucose, arabinose, glucose, fructose and sucrose. The presence of larger oligosaccharides, including the sucrosyl oligosaccharides which consist of sucrose with increasing galactose residues on positon 6, raffinose, stachyose, verbascose, ajugose and the next two sugars in the series of sucrosyl oligosaccharides the heptasaccharide and octasaccharide was dependent on the age of the plants. A comparison of the chromatographic profiles of the sugars in honeydew from plants aged 4, 5, 6 and 7 weeks is shown in Figure 1. The presence of at least 30 larger oligosaccharides is clearly shown in the honeydew from plants aged five and six weeks. A lesser amount of these larger oligosaccharides is present in the honeydew from 4 week old plants but they were almost completely absent from honeydew from seven week old plants.

Discussion

The carbohydrate composition of cotton aphid honeydew reported here is very similar to that reported by Hendrix (Hendrix, 1999) with the major difference being the absence of trehalulose and melizitose in these samples. The presence of large oligosaccharides was also reported by Hendrix. In this report we employed a more shallow sloped salt gradient to resolve the larger oligosaccharides. The earlier work was focused on the role of honeydew from older plants and its role in sticky cotton. In this case, the honeydew was collected from much younger plants as part of a larger study. We did not anticipate the observed difference in honeydew composition with plants of different ages. These differences may reflect differences in phloem constituents with developmental age of the plants. At this time it is unclear which of the larger oligosaccharides may be the result of polymerization of sucrose by the aphids and which of the oligosaccharides may arise from the polymerization of oligosaccharides larger than sucrose in the phloem. Most of the larger oligosaccharides are not reducing sugars.

References

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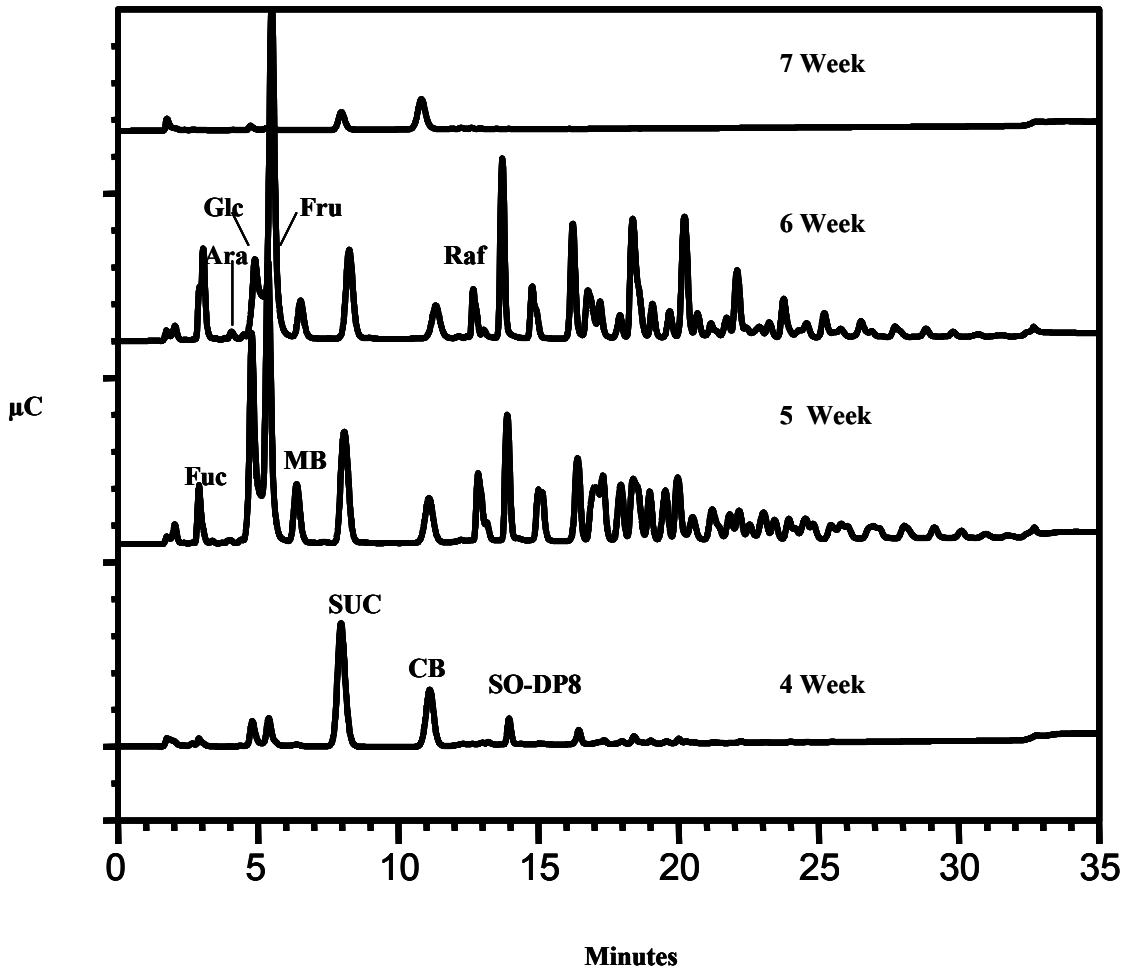

Figure 1. Representative chromatograms demonstrating the presence of known and unknown carbohydrates in honeydew from cotton aphids feeding on Pima S-6 plants at ages 4, 5, 6 and 7 weeks. Abbreviations: Ara:arabinose, Glc:glucose, Fru:fructose, MB:melibiose, Suc:sucrose, CB:cellobiose(internal standard), Raf:raffinose and SO-DP8:sucrosyl oligosaccharide degree of polymerization 8.