SUGAR COMPOSITION OF COTTON APHID AND SILVERLEAF WHITEFLY HONEYDEWS

D. L. Hendrix
Western Cotton Research Lab, USDA, ARS
Phoenix, AZ

Abstract

Many of the sugars in the honeydew from the silverleaf whitefly (*Bemisia argentifolii*) and cotton aphid (*Aphis gossypii*) feeding upon upland cotton plants were identified. Both honeydews have been found to consist of several dozen oligomers of glucose and fructose. Both organisms produce these sugar mixtures from sucrose in their diet of cotton phloem. In both insects, most of the sugars in their honeydew are nonreducing and the vast majority of monosaccharides which make up these oligosaccharides are glucose. The sugar composition of these two honeydews are distinctly different. The most abundant sugar in silverleaf whitefly honeydew is the disaccharide trehalulose, an oligomer of sucrose. Cotton aphid honeydew contains only trace amounts of trehalulose but it contains large amounts of the trisaccharide melezitose. Neither of these sugars occurs in the cotton plant. Both insects create these sugar oligomers to counteract the osmotic stress of their diet and environment. One mechanism both insects utilize to overcome such stress is the conversion of some of the fructose from ingested sucrose into six carbon polyols. Whiteflies manufacture sorbitol from dietary fructose; aphids manufacture mannitol. Both honeydew formation and polyol formation are distinctly different in male and in female whiteflies.

Introduction

Silverleaf whiteflies and the cotton aphid are both homopteran insects which feed upon cotton phloem sap. They can be very destructive pests in cotton. They excrete honeydew which causes cotton fiber to become sticky and covered with sooty mold. Mold-contaminated cotton is frequently discolored and therefore lower in value. Honeydew-contaminated cotton also contains higher trash than clean cotton. Stickiness due to honeydew can make contaminated cotton fiber difficult to impossible to process in gins and textile mills. Both insects also transmit a large number of plant viruses.

Honeydew from both organisms contains several dozen sugars, each created by rearrangements of the monosaccharides in sucrose in their phloem sap diet called transglycosylation reactions (Edelman, 1956; Duspiva, 1955; Hendrix and Wei, 1991; Hendrix *et al*., 1993; Hendrix *et al*., 1996). In whiteflies, the enzymes necessary to carry out these sugar rearrangements are expressed in all life stages, even in freshly laid eggs (Hendrix *et al*., 1994). In aphids, transglycosylating enzymes appear to be localized in the midgut (Duspiva, 1955; Rhodes *et al.*, 1998). Some of these sugar rearrangements in whiteflies may be due to the insect's microbial flora (Davidson et al., 1994).

Polyols such as mannitol and sorbitol are known to protect organisms from a variety of stress. Sorbitol, for example, protects bacteria and mammalian renal cells from osmotic stress (Bagnasco *et al.*, 1987; Miller and Smith, 1975). Polyols also protect proteins against denaturation at high temperatures (Eraslan, 1995). Sorbitol, mannitol and glycerol, created by certain cold-hardy insects from stored glycogen, serve as protection against cold stress (Sømme, 1969; Storey and Storey, 1981).

Materials and Methods

Silverleaf whiteflies (*Bemisia argentifolii* Bellows and Perring) and the cotton aphid (*Aphis gossypii*, Glover) were reared in glasshouses on upland cotton plants (*Gossypium hirsutum* L., var. Coker 100A glandless) as described previously (Salvucci *et al.*, 1997). Cotton plants upon which insects were reared in the greenhouse were watered daily. Honeydew was collected by placing aluminum foil beneath insects feeding upon cotton leaves. Honeydew droplets were removed from the foil with hot deionized water and the sugars recovered from the water by lyophilization. Sugars in these honeydew samples were determined by anion HPLC as described previously (Hendrix and Wei, 1994).

In some experiments, adult whiteflies were allowed to lay eggs on clean cotton leaves for one day after which the adults were removed. The adults which emerged from these eggs were separated by sex and confined on the day of their emergence on clean cotton leaves using clip cages. The honeydew which was excreted by these males and females was collected daily and analyzed by HPLC.

In separate experiments, large amounts of whitefly and aphid honeydew were collected for detailed analysis of their sugar composition. Aphid honeydew was washed from contaminated cotton leaves collected in the San Joaquin valley. Whitefly honeydew was washed from a bale of honeydew-contaminated cotton grown in Arizona. For both of these preparations, sugars in the crude honeydews were purified by adsorption onto powdered charcoal suspended in deionized water, followed by elution of the sugars from the charcoal particles with 95% ethanol (Whistler and Durso, 1950; Wei *et al.*, 1997). Honeydew sugars isolated in this manner were fractionated by size by dissolving them in water and adsorbing them onto a charcoal-Celite column and then eluting them from this column with increasing concentrations of *n*-propanol in water (Whistler and Durso, 1950). Aliquots of the fractions containing the largest oligosaccharides (the 6% propanol fractions) were hydrolyzed to their component monosaccharides by heating to 100°C in 0.1 N HCl.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:47-51 (1999)
National Cotton Council, Memphis TN

Some of the honeydew sugar fractions isolated from the second charcoal columns were further separated by eluting them with deionized water through a large (4.8 X 120 cm) heated (Trenel *et al*., 1969) Bio-Gel P-2 column (Wei *et al.*, 1997). Fractions from the Bio-Gel column were finally fractionated into individual sugars using an amine HPLC column and a complex elution gradient consisting of a sequence of mixtures of methanol, acetonitrile and water (Wei *et al.*, 1997). The structure of sugars so isolated were determined by use of 2D-NMR, GC/MS, MALDI-TOF/MS and by analysis of their enzymatic, alkali and acid hydrolysis products (Hendrix and Wei, 1994; Wei *et al.*, 1996; Wei *et al.*, 1997).

Insects collected for body content polyol analysis were harvested by suction from cotton leaves and quickly transferred to either ice-cold 80% ethanol or liquid nitrogen for transportation to the laboratory. In the laboratory, these insects were extracted several times in hot (80°C) 80% (v/v) ethanol and aliquots of the pooled extracts were treated with activated charcoal to remove materials which interfered with subsequent chromatography (Hendrix and Peelen, 1987). After removal of the ethanol under N_2 the carbohydrates and polyols in these extracts were determined by the same HPLC procedures used for honeydew analysis (Hendrix and Wei, 1994).

Results and Discussion

Honeydew from the silverleaf whitefly and cotton aphid both consist of several dozen sugars (Fig. 1). The most abundant sugar in silverleaf whitefly honeydew is the disaccharide trehalulose [*O*-α-D-glucopyranosyl-(1→1)-D-fructofuranoside]; the most abundant sugar in cotton aphid honeydew is the trisaccharide melezitose [*O*-α-D-glucopyranosyl-(1→3)-*O*-β-D-fructofuranosyl-(2↔1)-*O*-α-D-glucopyrano-side]. The largest oligosaccharides in *B. argentifolii* honeydew are hexasaccharides, whereas the largest sugars in *A. gossypii* honeydew are decasaccharides. These sugars are created entirely from sucrose, the only sugar in the insect's diet of phloem sap (Tarczynski *et al.*, 1992). The extent of sucrose rearrangement is so great that sucrose is a minor component of both honeydews. The formation of oligomers larger than sucrose requires energy and this formation is apparently a function of the osmotic strength of the insect's diet, which changes during the day (Salvucci *et al.*, 1997; Fisher *et al.*, 1984; Rhodes *et al.*, 1998). Note also that the HPLC detector used in these experiments is more sensitive to monosaccharides than to larger sugars (Larew and Johnson, 1992) so that the larger sugars in these honeydews are more abundant than they appear in these chromatographs.

It is possible to fractionate both of these honeydews into oligosaccharides of different size by eluting them from a charcoal column with a gradient of aqueous *n*-propanol. An HPLC chromatograph of a fraction of *B. argentifolii* honeydew which consisted primarily of trisaccharides through hexasaccharides is shown in figure 2. Analysis of acid digests of this fraction revealed that ninety percent of the sugars in this fraction were created from glucose, and ten percent from fructose (Fig. 3). An almost identical result was found when honeydew from *A. gossypii* was fractionated and hydrolyzed in the same manner. In those sugars from *B. argentifolii* honeydew which have been characterized thus far, the glucose and fructose moieties have been shown to be linked either by Glc(1,1)Fru, Glc(1,2)Fru or Glc(1,3)Fru bonds. The glucose to glucose linkages in these sugars are either Glc(1,1)Glc or Glc(1,4)Glc (Wei *et al.*, 1996, 1997). The anomeric carbons in these glucose to glucose bonds are always in the alpha configuration. Such data is important if enzymatic amelioration of honeydew stickiness is considered, since enzymes are quite specific about the bond positions and the anomeric carbon conformations they will attack (Hendrix *et al.*, 1993, 1996).

Honeydew secretion by whiteflies depends upon several factors, including the species of insect and plant, the water status of the host plant and the time of day (Hendrix, *et al.*, 1992, 1996). This secretion also varies with the sex and age of the insect (Fig. 4; Davidson *et al.*, 1994; Hendrix *et al.*, 1994). For example, nymphal *B. argentifolii* honeydew is characterized by a significant melezitose content; that from adults contains almost none of this trisaccharide. Unlike that excreted by adults, honeydew from first instar *B. argentifolii* contains a significant amount of sucrose, but very little trehalulose (Hendrix *et al.*, 1994). Newly emerged adult *B. argentifolii* females excrete honeydew which is very similar to that collected from cotton lint; that from males contains fewer sugars and little trehalulose (*cf.* Figs. 1 *vs*. 4).

Since these insects feed upon sucrose which they hydrolyze with sucrase into equal quantities of glucose and fructose (Duspiva, 1955), it is surprising that they excrete honeydew which is mostly glucose (Fig. 3). Higher animals readily metabolize glucose but the catabolic paths available for fructose in animals is quite limited. The enzymatic step which converts fructose to a component of the glucose metabolic path in animals begins with the phosphorylation of fructose:

$$\text{ATP} + \text{Fructose} \xrightarrow{\textit{hexokinase}} \text{Fructose-6-P} + \text{ADP}$$

The fructose-6-phosphate thus created readily can be isomerized to glucose-6-phosphate by *phosphoglucoisomerase* and then metabolized by glucose pathways. However, the proposed hexokinase step (above) would require the expenditure of enormous amounts of ATP because these insects ingest and metabolize sucrose equal to several times their fresh weight each day which would lead to very large amounts of fructose to be phosphorylated. One might expect, therefore, that large amounts of fructose would accumulate in the gut and would therefore predominate over glucose in the honeydews of these insects,

but it does not (Figs. 1,3). What happens to this excess fructose?

One reaction carried out by *B. argentifolii* involving fructose of dietary origin is the metabolism of fructose to sorbitol, which accumulates in the insect's hemolymph (Hendrix and Salvucci, 1998; Salvucci *et al.*, 1999; Wolfe *et al.*, 1998a,b). This conversion is strongly stimulated by increasing environmental temperature, dietary osmotic strength or changes in the water status of the host plant (Figs. 5,6; Wolfe *et al.*, 1998a; Hendrix *et al.*, 1998). If we make the assumptions that (1) these insects are 60% water (2) that 20% of their body water is in their hemolymph and (3) that this sorbitol is restricted to this compartment, we can calculate that sorbitol in the hemolymph of these insects varies from less than 50 mM at dawn to as much as 500 mM at noon (Wolfe *et al.*, 1998a). At noon, sorbitol would thus be the dominant osmotic component in these insects' hemolymph. Sorbitol in the insect's bodies could serve to prevent water loss to their hyperosmotic diet or to their very desiccating environment. Note that as with honeydew formation (Fig. 4) there is a significant sex component of sorbitol formation in *B. argentifolii*. Female silverleaf whiteflies manufacture considerably more sorbitol than males under the same degree of heat stress (Fig. 7).

Aphids do not create sorbitol in their hemolymph from fructose but they do create mannitol, using a different but analagous metabolic path. As with the sorbitol formation in whiteflies, aphids create this mannitol from dietary fructose. In those aphids which create mannitol when they overwinter, this polyol is created from glycogen stores rather than from their diet (Sømme, 1969; Storey and Storey, 1981). The metabolic paths utilized by aphids and whiteflies to produce these hexitols in response to heat and osmotic stress are distinctly different (and they use completely different enzymes) from those utilized to produce these compounds during cold stress. The enzyme which converts dietary fructose to sorbitol in whiteflies (for a more complete discussion of these metabolic paths in *Bemisia* see Salvucci *et al*., 1998) has been found to be quite unique in nature and not antigenically similar to the analogous protein in aphids which creates mannitol during heat and osmotic stress (Hendrix and Salvucci, 1998; Wolfe *et al.*, 1998a,b; Salvucci *et al.*, 1997). However, this *Bemisia* protein is antigenically similar to the analagous protein in the greenhouse whitefly (*Trialeurodes vaporariorum*). Thus whiteflies seem to share the same mechanism but aphids use a separate metabolic path to create mannitol in response to heat and osmotic stress.

Acknowledgments

The author wishes to thank Cotton Incorporated for financial support and encouragement during this project.

References

Bagnasco, S.M., Uchida, S., Balaban, R.S., Kador, P.F. and M.B. Burg. 1987. Induction of aldose reductase and sorbitol in renal inner medulary cells by elevated extracellular NaCl. Proc. Natl. Acad. Sci. USA 84:1718-1720.

Davidson, E.W., Segura, B.J., Steele, T. and D.L. Hendrix. 1994. Microorganisms influence the composition of honeydew produced by the silverleaf whitefly, *Bemisia argentifolii*. J. Insect Physiol. 40:1069-1076.

Duspiva, F. 1955. Enzymatische prozesse bei der honigtaubildung der Aphiden. Deutschen Zool. Gesellshaft 18:440-447.

Edelman, J. 1956. The formation of oligosaccharides by transgly-cosylation. Adv. Enzymol. 17:189-232.

Eraslan, A. 1995. The effect of polyol compound on the thermostability of penicillin G acylase from a mutant of *Escherichia coli* ATCC 11105. Proc. Biochem. 30:133-139.

Fisher, D.B., Wright, J.P. and T.E. Mittler. 1984. Osmoregulation by the aphid *Myzus persicae*: a physiological role for honeydew oligosaccharides. J. Insect Physiol. 30:387-393.

Hendrix, D.L. and K.K. Peelen. 1987. Artifacts in the analysis of plant tissues for soluble carbohydrates. Crop Sci. 27:710-715.

Hendrix, D.L. and Y.-A. Wei. 1992. Detection and elimination of honeydew excreted by the sweetpotato whitefly feeding upon cotton. Proc. Beltwide Cotton Res. Conf. 671-673.

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

Hendrix, D.L., Blackledge, B. and H.H. Perkins, Jr. 1993. Development of methods for the detection and elimination of insect honeydews on cotton fiber. Proc. Beltwide Cotton Res. Conf. 1600-1603.

Hendrix, D.L. and Y.-A. Wei. 1994. Bemisiose: An unusual trisaccharide in *Bemisia* honeydew. Carbohydrate Res. 253:329-334.

Hendrix, D.L., Taysom, D., Steele, T., Davidson, E.W. and B. Segura. 1994. Enzymes forming honeydew sugars from cotton phloem sap are found in sweetpotato whitefly eggs. Proc. Beltwide Cotton Res. Conf. 1237-1238.

Hendrix, D.L., Steele, T.L. and H.H. Perkins, Jr. 1996. *Bemisia* honeydew and sticky cotton. *In*: D. Gerling and R. T. Mayer (eds.) Bemisia 1995: Taxonomy, Biology, Damage and Management. Intercept, Andover, UK pp 189-199.

Hendrix, D.L., Salvucci, M.E. and G.R. Wolfe. 1998. Effect of high temperature on polyol metabolism in the silverleaf whitefly and cotton aphid. Proc. Beltwide Cotton Res. Conf. 1077-1080.

Hendrix, D.L. and M.E. Salvucci. 1998. Polyol metabolism in homopterans at high temperatures: accumulation of mannitol in aphids (Aphididae: Homoptera) and sorbitol in whiteflies (Aleyrodidae: Homoptera). Comp. Biochem. Physiol. 120A:487-494.

Larew, L.A. and D.C. Johnson. 1988. Quantitation of chromatographically separated maltooligosaccharides with a single calibration curve using a postcolumn enzyme reactor and pulsed amperometric detection. Analyt. Chem. 60:1867-1872.

Miller, L.K. and J.S. Smith. 1975. Production of threitol and sorbitol by an adult insect: association with freezing tolerance. Nature 258:519-520.

Rhodes, J.D., Croghan, P.C. and A.F.G. Dixon. 1998. Dietary sucrose and oligosaccharide synthesis in relation to osmoregulation in the pea aphid, *Acrythosiphon pisum*. Physiol. Entomol. 22:373-379.

Salvucci, M.E., Wolfe, G.R. and D.L. Hendrix. 1997. Effect of sucrose concentration on carbohydrate metabolism in *Bemisia argentifolii*: Biochemical mechanism and physiological role for trehalulose synthesis in the silverleaf whitefly. J. Insect Physiol. 43:457-464.

Salvucci, M.E., Hendrix, D.L. and G.R. Wolfe. 1999. Effect of high temperature on the metabolic processes affecting sorbitol synthesis in the silverleaf whitefly, *Bemisia argentifolii*. J. Insect Physiol. (*in press*).

Sømme, L. 1969. Mannitol and glycerol in overwintering aphid eggs. Norw. J. Ent. 16:107-111.

Storey, K. B. and J. M. Storey. 1981. Biochemical strategies in overwintering in the gall fly larva, *Eurosta solidaginis*: Effect of low temperature acclimation on the activities of enzymes of intermediary metabolism. J. Comp. Physiol. 144:191-199.

Tarczynski, M.C., D.N. Byrne and W.B. Miller. 1992. High performance liquid chromatography analysis of carbohydrates of cotton-phloem sap and of honeydew produced by *Bemisia tabaci* feeding upon cotton. Plant Physiol. 98:753-756.

Trenel, G., John, M. and H. Dellweg. 1968. Gel chromatographic separation of oligosaccharides at elevated temperatures. FEBS Lett. 2:74-76.

Wei, Y.-A., D.L. Hendrix and R. Nieman. 1996. Isolation of a novel tetrasaccharide, bemisiotetrose, and glycine betaine from silverleaf whitefly honeydew. J. Agric. Food Chem. 44:3214-3218.

Wei, Y.-A., D.L. Hendrix and R. Nieman. 1997. Diglucomelezitose, a novel pentasaccharide in silverleaf whitefly honeydew. J. Agric. Food Chem. 45:3481-3486.

Whistler, R.L. and D.G. Durso. 1950. Chromatographic separation of sugars on charcoal. J. Am. Chem. Soc. 72:677-679.

Wolfe, G.R., Hendrix, D.L. and M.E. Salvucci. 1998a. A thermoprotective role for sorbitol in the silverleaf whitefly. J. Insect Physiol. 44:597-603.

Wolfe, G.R., Smith, C.A., Hendrix, D.L. and M.E. Salvucci. 1998b. Molecular basis for thermoprotection in *Bemisia*: Structural difference between whitefly ketose reductase and other medium-chain dehydrogenases/reductases. Insect Biochem. Mol. Biol. (*in press*).

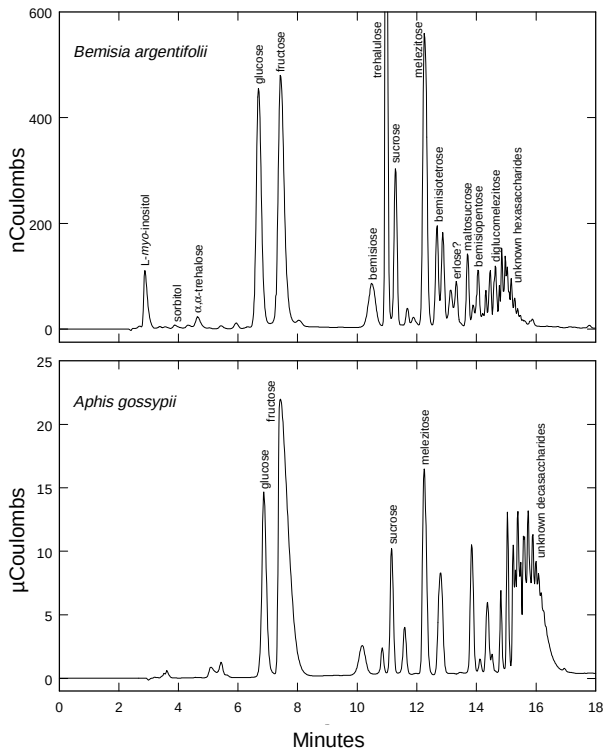

Figure 1. HPLC analysis of honeydew from *Bemisia argentifolii* (top panel) and *Aphis gossypii* (bottom panel), feeding upon upland cotton.

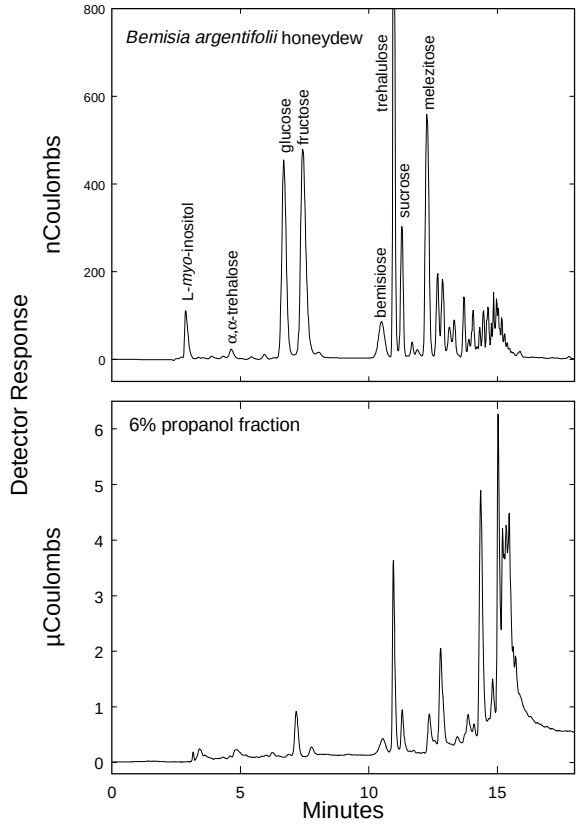

Figure 2. Fractionation of *Bemisia argentifolii* honeydew by elution from charcoal by *n*-propanol. The fraction in the bottom panel represents the largest oligosaccharides in this honeydew (primarily trisaccharides through hexasaccharides).

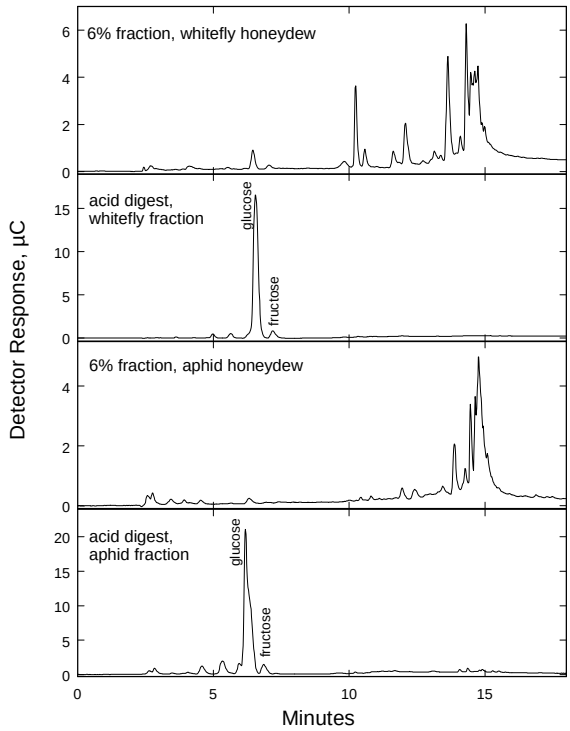

Figure 3. HPLC analysis of the 6% propanol honeydew fraction and its HCl digestion products from *Bemisia argentifolii* (top two panels) and *Aphis gossypii* (bottom two panels).

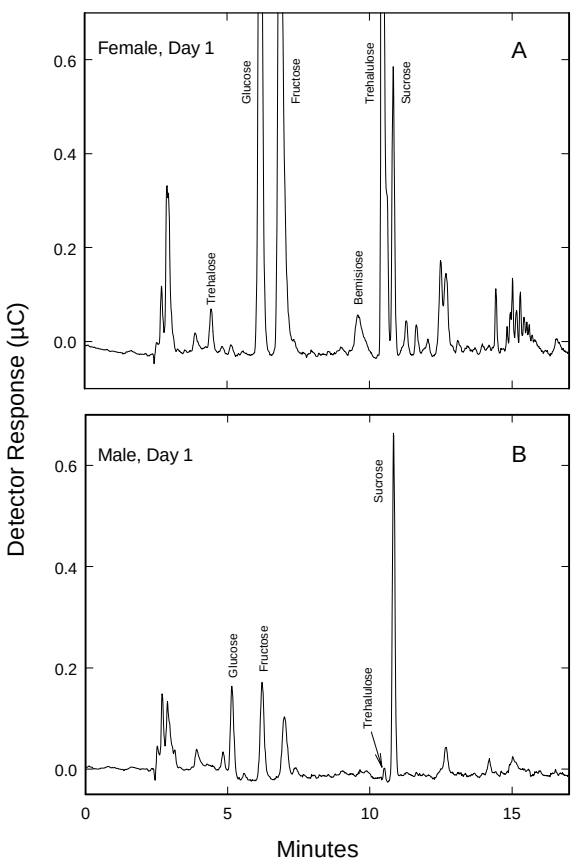

Figure 4. Honeydew from *Bemisia argentifolii* adult females (panel A) and males (panel B) during their first day of emergence on cotton leaves.

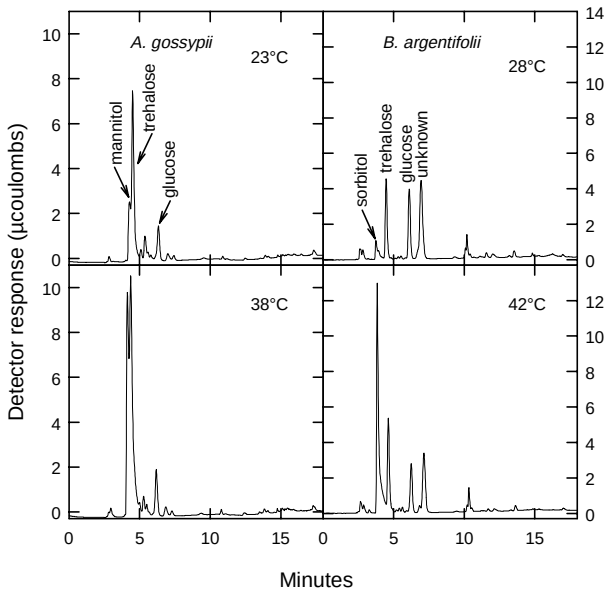

Figure 5. Body sugar and polyol content of cotton aphids and silverleaf whiteflies living on cotton plants at low and high temperatures.

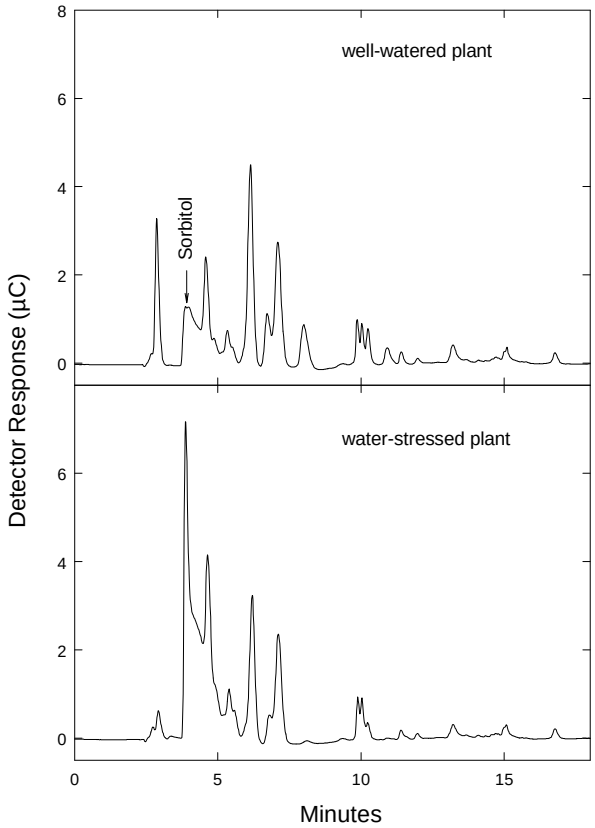

Figure 6. HPLC chromatograms of extracts of bodies of silverleaf whiteflies feeding upon well-watered cotton plants (top panel) and upon cotton plants in the same greenhouse from which water was withheld for two days (bottom panel).

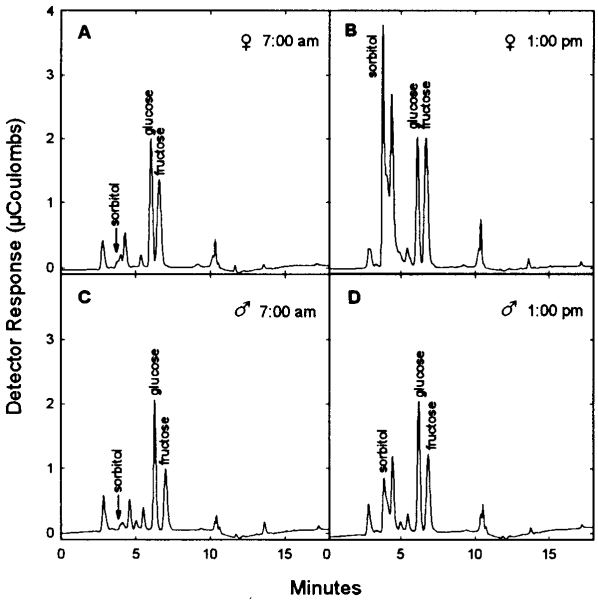

Figure 7. Body sugar and polyol content of silverleaf whitefly males and females reared on cotton in a glasshouse during the course of a day.