DIFFERENCES IN POLYOL ACCUMULATION AND HONEYDEW EXCRETION IN SWEETPOTATO WHITEFLY AND COTTON APHID

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Abstract

Analysis of extracts of the sweetpotato whitefly *Bemisia tabaci* (Gennadius) (= *B. argentifolii* Bellows and Perring) and cotton aphid, *Aphis gossypii* Glover, showed that both accumulated very large amounts of polyhedric alcohols (polyols) in their bodies during the warmest part of the day. The sweetpotato whitefly accumulated sorbitol and the cotton aphid accumulated mannitol. Mannitol accumulation in the cotton aphid followed the temperature of its environmnent much closer than did sorbitol accumulation in the sweetpotato whitefly, which only appeared in large amounts in extracts of insects collected during the hottest part of the day. These polyols do not appear in the honeydew of either insect, suggesting that a significant portion of their metabolism is dedicated to both the creation and to the breakdown of these polyols during the course of a day. Results of experiments with the sweetpotato whitefly suggest that the reason for this major metabolic commitment is that this polyol accumulation protects these insects against desiccation and dietary osmotic stress. The production of honeydew from both insects was found to vary during the day and to be maximal in the early afternoon for insects feeding in fields of cotton. Both honeydews contain significant amounts of glucose, fructose, sucrose, trehalulose and melezitose, but the ratio of different honeydew sugars was substantially different for the two insect genera. Preliminary results from experiments involving spraying known sugars on clean cotton suggest that honeydew from the sweetpotato whitefly may be more sticky than that from the cotton aphid.

Introduction

Sweetpotato 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. Honeydew-contaminated cotton also contains a higher content of trash than clean cotton and spinning trash-contaminated cotton can lead to serious health problems among textile workers (Ayars *et al.*, 1986). Stickiness due to honeydew can make contaminated cotton fiber difficult to impossible to process in gins and textile mills and such fiber consequently receives a lower price.

The only sugar in cotton phloem sap is sucrose (Tarczynski *et al.*, 1992), which these insects convert to at least two dozen other sugars which they excrete as honeydew. The reason whiteflies and aphids convert sucrose into these sugars is not immediately obvious. The majority of the sugars in honeydew are larger than sucrose meaning that, on thermodynamic grounds, metabolic energy must be expended in their creation. Since each insect ingests more than their body dry weight in sucrose each day and most of this is converted to honeydew, a considerable proportion of the energy available to the insect from its food input is devoted to this process.

Why are Honeydew Sugars Formed From Sucrose?

An appealing hypothesis which explains why these aphids create this variety of honeydew sugars from sucrose has been put forward by Fisher *et al.* (1984). They found that the green peach aphid, *Myzus persicae*, altered the ratio of large to small oligosaccharides in its honeydew in response to changes in the osmotic strength (*i.e.*, sucrose content) of its diet. This hypothesis implies that the formation of honeydew sugars is necessary in these insects to achieve osmoregulation. This concept may also apply to honeydew formation in whiteflies (Salvucci *et al.* 1997).

Why do These Insects Create Polyols?

It has also been recently found that both the cotton aphid and sweetpotato whitefly manufacture polyols in their bodies during osmotically stressful periods. Hendrix and Salvucci (1998) showed that several species of whitefly, including *B. tabaci*, accumulated large amounts of sorbitol and several species of aphids, including *A. gossypii*, accumulated large amounts of mannitol in their bodies on a diurnal basis. Both whiteflies and aphids create these polyols from diet-derived fructose. As for honeydew formation, the large quantity of polyols formed by these insects suggests that considerable metabolic resources are devoted to their formation.

Polyols such as mannitol and sorbitol are known to protect organisms from a variety of stress factors. Sorbitol, for example, has been shown to protect bacteria and mammalian renal cells from osmotic stress (Bagnasco *et al.*, 1987; Miller and Smith, 1975). Polyols such as sorbitol, mannitol and glycerol, created by certain insect species from stored glycogen, protect them from freezing stress (Sømme, 1969; Storey and Storey, 1981). It has been shown that the polyol content of sweetpotato whiteflies increases with the increasing osmotic strength of their diet and also with increasing environmental temperature (Wolfe *et al.*, 1998; Salvucci *et al.*, 1999). These polyols may therefore serve to protect phloem-feeding cotton pests against temperature and water stess. Neither of these polyols occurs in the cotton plant and the metabolic paths for their formation are found

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only in these insects. Therefore, a method of inhibiting honeydew or polyol formation in whiteflies and aphids may provide a unique mode of action for their control.

In this project we determined the accumulation of polyols in sweetpotato whiteflies and cotton aphids and compared the carbohydrate composition of honeydews produced by these two species feeding on cotton in the field. We also determined the effect of cotton aphid's honeydew lint contamination on stickiness as measured with the thermodetector (Perkins and Brushwood, 1994) and compared these data to equivalent data collected earlier from sweetpotato whitefly honeydew.

Materials and Methods

Sweetpotato whiteflies (*Bemisia tabaci* Gennadius) and cotton aphids (*Aphis gossypii*, Glover) were reared in glasshouses on upland cotton plants (*Gossypium hirsutum* L., var. Coker 100A glandless) in screened enclosures as described previously (Salvucci *et al.*, 1997). Photosynthetically-active light intensity (PAR) at the leaf on which insects were feeding was measured with a photometer. The temperature of the air a few cm above the leaf upon which the insects were feeding was measured with a miniature K-type thermocouple; for aphid experiments, the temperature of the leaf was also determined by pressing the thermocouple to the bottom of the same leaf. Insects collected for body content polyol analysis were harvested by suction from cotton leaves and quickly transferred to ice-cold 80% ethanol for transport 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 anion HPLC (Hendrix and Wei, 1994).

Sweetpotato whiteflies were collected from cotton plants in the glasshouse and placed in small feeders where they fed through an artificial membrane on sucrose solutions buffered with 100 mM potassium phosphate containing 0.5% yeast extract (Salvucci *et al.*, 1997; Jancovich *et al.*, 1997). Each feeder contained about 200 adult insects. After feeding overnight in an illuminated room at 26.5°C, nonfeeding insects were removed and the remaining insects transferred to a thermostated glass chamber (Wolfe *et al.*, 1998). The temperature of the feeders was then raised slowly (#10°C/h) to 40°C and maintained at this temperature for 5 h. At the end of this period, the surviving insects were collected, counted and analyzed for sorbitol by anion HPLC.

In the field, cotton aphid or silverleaf whitefly honeydews were collected by exposing nonhoneydew-contaminated lint for different periods (0, 3 and 6 days) under heavily-infested cotton leaves (150 to 400 aphids/leaf). Plastic petri dishes (7.62 cm diameter) were mounted on a stiff (1.6 mm diameter) metal wire. Clean cotton lint (2.5 g) was attached to the top of the dish with Velcro strips and rubber bands. Lint on the dishes was placed under infested leaves and the dish height regulated to between 6.4 and 7.6 cm from the aphids on the bottom of cotton leaf surfaces by adjusting the penetration of the support wire into the soil. Ten lint samples were collected for each exposure period. Five samples on each sample day were analyzed by a thermodetector for sticky spots. In addition, honeydew sugars were extracted and identified from each of the five remaining samples as previously described (Hendrix and Peelen, 1987; Hendrix *et al.*, 1993; Hendrix and Wei, 1994).

Honeydew rain during the day for aphids and whiteflies feeding upon cotton in the field during a single day was determined by analyzing honeydew which collected on 5 cm diameter aluminum weighing pans placed on a metal platform located ca. 7 cm below feeding insects. These pans were replaced at 20 min intervals for *B. tabaci* collections and replaced every 15 min when collecting honeydew from *A. gossypii.* Honeydew on the pans was suspended in 0.2 ml deionized H_2O and this solution was analyzed for sugars by anion HPLC after filtering through a 0.2 µm filter.

Results and Discussion

Sweetpotato whiteflies feeding upon cotton in a glasshouse accumulated large amounts of sorbitol in their bodies but only during the warmest parts of the day (Fig. 1). Sorbitol existed in these insects only at very low levels before the air temperature in the glasshouse rose above 35°C. Whitefly sorbitol content fell sharply in the early afternoon even though the air temperature in the greenhouse was relatively constant during this period. Whitefly sorbitol content did not correlate well with solar radiation (PAR). The sorbitol shown in this figure is restricted to the bodies of these insects; it does not occur in their honeydew (Hendrix and Salvucci, 1998).

Unlike whiteflies, cotton aphids feeding upon cotton in a glasshouse accumulated significant amounts of mannitol from the earliest part of the morning, even when both the air and leaf temperature were well below 35°C (Fig. 2). The mannitol content of these insects followed the air temperature reasonably well. Aphids were also found to accumulate much greater amounts of mannitol per unit of body water than sorbitol accumulation in whiteflies (data not shown). This proportionally higher commitment of their metabolism to polyol production implies that aphids are more dependent upon this mechanism of stress amelioration than are whiteflies.

Whiteflies which fed upon concentrated (20%) sucrose solutions accumulated more sorbitol and survived a hot environment much better than those fed upon a dilute (2%) sucrose diet (Table 1). The sorbitol accumulation in their bodies during heat stress appears, therefore, to confer resistance to hot, dry environments. The same behavior was found in whitefly populations feeding upon cotton plants. Hendrix (1999) showed that whiteflies feeding upon water-stressed cotton plants accumulated much more sorbitol than those feeding upon well-watered cotton plants. This agrees with observations by Flint *et al.* (1995; 1996) who found that *B. tabaci* populations were significantly larger in water-stressed cotton fields than in adjacent well-watered cotton fields which were otherwise equivalent.

The honeydew rain due to both species living in fields of cotton was found to exhibit a similar pattern during the day (Figs. 3,4). Honeydew output from both insects was low at dawn and peaked in the afternoon. Note that a number of factors such as wind velocity, age of the insects (Costa *et al.*, 1999), and changes in population on the leaf with time can influence such data. In both of the experiments summarized here the insect populations were relatively constant but wind velocity increased substantially in the early afternoon. Even given the scatter in these data due to these factors, it seems clear that honeydew rain from either insect species was significantly more intense in the afternoon than during morning hours.

The major sugars in the honeydew of both the sweetpotato whitefly and cotton aphid, were found to be glucose, fructose, sucrose and melezitose (Table 2). Trehalulose was the most abundant sugar in sweetpotato whitefly honeydew and fructose and melezitose were the most abundant sugars in cotton aphid honeydew. Sugars larger than these five exist in both honeydews (Hendrix and Wei, 1994; Wei *et al.*, 1996). These larger sugars make up about 20% of the total sugars in these secretions. Honeydew also contains a number of nonsugar components, such as glycine betaine (Wei *et al.*, 1996), but only the sugar components are considered to exhibit stickiness.

The more time cotton lint is exposed to honeydew from either insect, the greater the lint stickiness as measured by the thermodetector (Table 3). It would appear from the data in Table 3 that sweetpotato whitefly honeydew is more sticky per unit of weight than cotton aphid honeydew. An explanation for this difference in stickiness per unit weight of honeydew might be due to the fact that thermodetector and minicard readings for honeydews form two different insects species are not comparable. The size of the sticky spots produced by these two insects are also different. Honeydew droplets from *A. gossypii* are slightly larger than droplets form *B. tabaci*. (Henneberry *et al.*, in preparation). In addition, the thermodetctor measures the number of spots but the minicard measures stickiness directly. Research is currently underway to examine this possible difference in stickiness in these two honeydews.

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Table 1. Survival of *Bemisia tabaci* for 5 h at 40°C on two sucrose diets.

Diet Composition	Sobitol, nMol/insect	% survival[1/]
2% sucrose	0.061	2.3±0.9%
20% sucrose	0.286	71.4±4.8

1/ Survival, in three feeding chambers per treatment each containing ca. 200 adult insects feeding on sucrose solutions containing 100 mM potassium phosphate (pH 6.5) and 0.5% yeast extract. All chambers were incubated overnight at 26.5°C; their temperature was then raised (#10°C/h) to 40°C.

Table 2. Micrograms of honeydew sugar per cotton leaf and individual sugars as percent total sugar produced by cotton aphids or sweetpotato whiteflies feeding upon DeltaPine cotton in the field.

	Cotton Aphid[1/]		Sweetpotato whitefly[1/]	
Sugar	µg/leaf	% of these sugars	µg/leaf	% of these sugars
Glucose	312.1±62.6	11.7±1.1	4.2±1.1	13.7±1.8
Fructose	100.7±193.8	37.1±2.1	5.6±1.5	18.3±2.8
Sucrose	726.3±132.1	28.2±2.2	5.2±0.7	18.9±1.8
Trehalulose	24.4±4.4	1.2±0.4	9.9±1.9	34.7±3.9
Melezitose	749.8±189.4	21.8±2.8	3.8±0.5	14.4±1.7
Total	2819.5±517.2	100%	28.6±4.6	100%

1/ Means±standard errors of 8 to 20 replications.

Table 3. Means (±SE) of cotton lint thermodetector counts and total honeydew sugars following exposure in the field to cotton aphids and sweetpotato whiteflies.

Days of Exposure	Thermodetector Count	Total Honeydew[1/] Sugars Measured (µg/g of lint)
	Aphis gossypii	
0 (control)	2.2±0.5	677±1.50
3	23.8±2.5	1457±253
6	54.2±7.9	6153±1573
	Bemisia tabaci[2/]	
0 (control)	3.5	20.9±1.9
3	7.3	32.9±3.0
6	16.1	39.1±4.6

1/ Total of glucose, fructose, sucrose, trehalulose and melezitose; means of 5 to 10 replications.
2/ From Henneberry *et al.*, 1996, using the relationship between thermodetector and minicard in Perkins and Brushwood (1994).

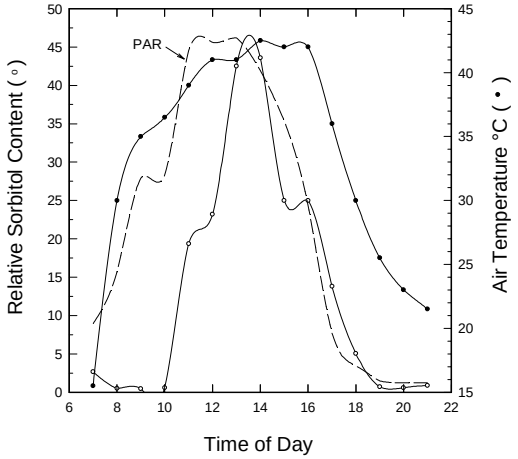

Figure 1. Sorbitol in the bodies of sweetpotato whiteflies feeding upon upland cotton in a glasshouse. Air temperature and photosynthetically-active radiation (PAR) are also shown.

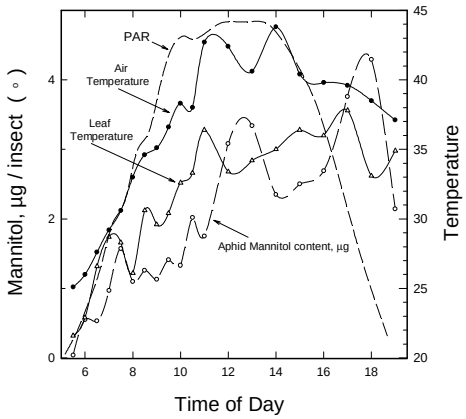

Figure 2. Mannitol in the bodies of cotton aphids feeding upon upland cotton in a glasshouse. Air temperature, leaf temperature and photosynthetically-active radiation are also shown.

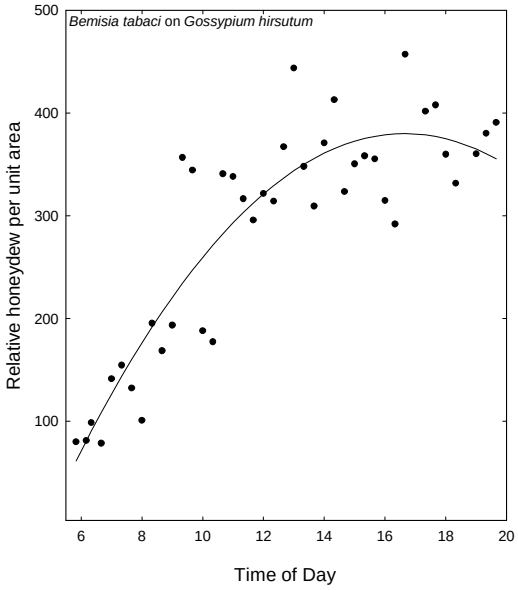

Figure 3. 'Rain' of honeydew from sweetpotato whiteflies feeding on leaves of upland cotton in a field. Samples collected on metal discs which were changed every 20 min.

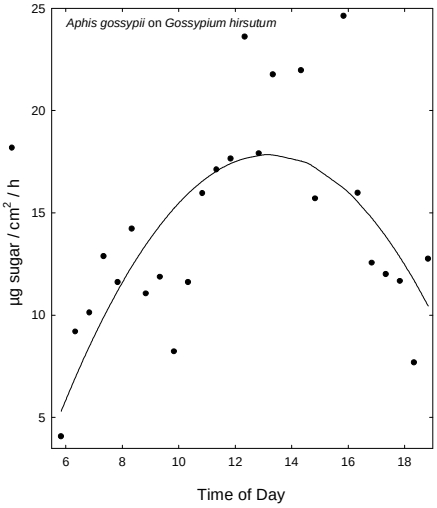

Figure 4. 'Rain' of honeydew from cotton aphids feeding upon leaves of upland cotton in a field. Samples collected on metal discs which were changed every 15 min.