EFFECT OF HIGH TEMPERATURE ON POLYOL METABOLISM IN THE SILVERLEAF WHITEFLY AND COTTON APHID

D. L. Hendrix, M. E. Salvucci and G. R. Wolfe
Western Cotton Research Lab, USDA, ARS
Phoenix, AZ

Abstract

The polyhydroxy alcohol, mannitol, was identified in the body of the cotton aphid, *Aphis gossypii* Glover. Mannitol accumulated in the bodies of aphids during the course of the day, analagous to the previously reported accumulation of sorbitol in the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring. In both organisms, polyol synthesis was stimulated by elevated temperatures. Hemolymph polyol during mid afternoon in these insects reached approximately 500 mM. Polyol concentrations during early morning hours was approximately 10-fold less than during mid afternoon. Enzyme assays of extracts prepared from adult insects showed that fructose was the substrate for mannitol synthesis in *A. gossypii*. This fructose originated in the insect's diet of sucrose. The enzyme catalyzing this reaction, an NADP(H)-dependent ketose reductase/mannitol dehydrogenase, is analagous to the NADP(H)-dependent ketose reductase previously shown to produce sorbitol from fructose in *B. argentifolii*. We did not find evidence of a glucose to sorbitol interconversion in whiteflies, and whitefly honeydew was found to contain only traces of this polyol. Likewise, cotton aphid honeydew did not contain mannitol. In addition, glucose was not converted to mannitol by aphid extracts in the presence of either NADH or NADPH. This suggests that in both insects polyols are converted back to fructose during periods when hemolymph concentrations decrease. Whiteflies living on water-stressed plants accumulated higher concentrations of sorbitol than those on well-watered plants. Whiteflies which were allowed to feed and thereby accumulate sorbitol were more resistant to elevated temperatures (35-50°C) than those prevented from feeding. These results suggest that polyol accumulation in these insects is a physiological adaptation for their survival in hot, dry environments.

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. Both insects also transmit a number of plant viruses which are very detrimental to crop plants.

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Polyols such as mannitol and sorbitol are compatable osmolytes which are known to protect proteins (Erarslan, 1995) and organisms (Chino, 1960; Miller and Smith, 1975; Storey *et al*, 1981; Storey and Storey, 1983) against thermal stress. Polyols are also known to protect mammalian renal cells (Bagnasco *et al.*, 1987), bacteria (Loos *et al*., 1994) and higher organisms (Garcia-Perez and Burg, 1991) against osmotic stress. Sorbitol is commonly found in animals (Chino, 1960; Storey *et al*., 1981). Mannitol is widespread in nature but in the animal kingdom it has only been reported in a few insects (Sømme, 1969; Srmme, 1982). The accumulation of sorbitol, mannitol or glycerol is known to protect insects from freezing stress (Sømme, 1969; Srmme, 1982; Storey and Storey, 1981).

We have recently found that silverleaf whiteflies (*Bemisia argentifolii* Bellows and Perring) accumulate sorbitol at when they feed at high temperatures (≥35°C). These insects were able to survive for hours at temperatures as high as 50°C, a temperature normally rapidly fatal to insects (Lighton and Wehner, 1993), if they were first allowed to accumulate sorbitol by feeding upon cotton leaves (Salvucci, *unpublished data*). Other insects which live in cotton fields, such as the pink bollworm (*Pectinophora gossypiella* Saunders), were found to not accumulate polyols at high temperature. The cotton aphid (*Aphis gossypii* Glover), also did not accumulate sorbitol under high temperatures. However, these aphids accumulated mannitol at high temperatures by a biochemical mechanism similar to that utilized by whiteflies to accumulate sorbitol.

Materials and Methods

Silverleaf whiteflies and the cotton aphid utilized in behavior studies 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. In some experiments, water was withheld from the plants for several days until their leaves were slightly wilted.

In feeding experiments where the dietary concentration of sucrose was varied, whiteflies were transferred to plastic tubes containing stretched Parafilm at their tops (Salvucci *et al.*, 1997). Liquid diet was placed on the upper surface of the Parafilm and the insects fed upon this diet by inserting their stylets through the membrane.

For biochemical experiments, silverleaf whiteflies were isolated from commercial cotton fields in Maricopa, AZ. using commercial vaccum harvesting equipment. After collection, insects were rapidly frozen and stored at 80°C until assay. For polyol and sugar analysis, 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).

Carbohydrates and polyols in these extracts were determined by gradient anion HPLC. Sugars and polyols which eluted from the HPLC column were detected by pulsed amperometry (Hendrix and Wei, 1994). The identity of sugars and polyols in these samples was confirmed by 1) their retention on HPLC columns with that of known sugar and polyol standards, 2) treatment of the extracts prior to HPLC with enzymes specific for these sugars and 3) NMR and mass spectrographic analyses (both MALDI-TOF and GC/MS) of compounds isolated by chromatography (Hendrix and Wei, 1994; Wei *et al.*, 1996; Wei *et al.*, 1997).

Honeydew excreted by these insects was collected by placing aluminum foil under insects feeding upon cotton leaves. After honeydew deposits accmulated on the foil for 24 h, they were dissolved in hot deionized water from which they were recovered by lyophilization. Polyols and sugars in these honeydew samples were determined by the same HPLC procedures used to analyze insect body extracts.

For enzyme analysis, extracts of adult whiteflies and aphids were prepared by homogenizing the insects in HEPES-KOH buffer, pH 7.9. Extracts were then centrifuged for 10 min at 10,000g and the supernatant was used for enzyme assays. The ketose reducase activity in these extracts was measured at 30EC as described previously (Wolfe *et al.*, 1998).

Results and Discussion

Sorbitol accumulated to relatively high levels in the hemolymph of the silverleaf whitefly. The amount of sorbitol in the insect's hemolymph was proportional to the environmental temperature in which the insects were feeding when collected (Fig. 1). Whiteflies feeding at 42 °C accumulated approximately 6 times as much sorbitol as whiteflies feeding at 25 °C (Table 1). Sorbitol accumulation was also proportional to the concentration of sucrose in the insect's diet. Insects feeding on 580 mM sucrose at 42 °C accumulated approximately 15 times as much sorbitol as those feeding upon 88 mM sucrose at the same temperature (Table 1).

Mannitol accumulation by the cotton aphid was also stimulated by temperature. Aphid mannitol content was at least 5-fold higher at 38 °C than at 23 °C (Fig. 1). Calculations based upon the assumptions that 20% of the insect body water was hemolymph and that mannitol was restricted to this compartment, gave a maximal daily hemolymph concentration of mannitol in these insects of 550 mM.

The body polyol content of both insects was greater at noon than in the early morning (Fig. 2). In both the cotton aphid and the silverleaf whitefly, polyols are created from dietary fructose by a ketose reductase (Table 2; Salvucci *et al.*, 1998). In both animals, this reaction is catalyzed by a protein using NADP(H) as a cofactor. The NADP(H)-requiring enzyme which creates sorbitol from fructose in *Bemisia* is unique. In nearly all other organisms the enzyme which carries out this reaction has been found to have an absolute requirement for NAD(H) (Barman, 1969; Salvucci *et al.*, 1998). A NADP(H)-requiring enzyme which converts fructose to sorbitol has been reported by Bischoff (1976) in *Drosophila melanogaster* L. but he failed to characterize the reaction products, so it is not clear whether or not this protein is really analagous to the enzyme in *Bemisia*. From the amino acid sequence of the protein in *Bemisia* we concluded that it is quite closely homologous to the NAD^+-requiring enzyme in mammals which converts fructose to sorbitol. It is far less homologous to the equivalent enzyme in the silkworm, *Bombyx mori* L. (Salvucci *et al.*, 1998; Wolfe *et al.*, *in preparation*).

Sorbitol and mannitol is created from glycogen reserves in those insects which survive freezing temperatures by manufacturing polyols in their hemolymph (Chino, 1960; Storey and Storey, 1983). At the beginning of diapause in these insects glycogen is converted to glucose, which is then converted to polyols. At the end of diapause, these polyols are converted to fructose which is, in turn, reconverted to glycogen. We find no evidence for a glycogen to polyol conversion in either the silverleaf whitefly or the cotton aphid. Further, extracts of both insects are unable to convert glucose to either sorbitol or mannitol. Labeling experiments using radioactive tracers confirmed that silverleaf whiteflies do not interconvert sorbitol and glucose (Wolfe *et al.*, 1998).

In both the cotton aphid and silverleaf whitefly fructose of dietary origin is converted to polyols. The decrease in hemolymph polyol content during the evening seems to be due to the reconversion of polyols to fructose during the night rather than polyol excretion, since the honeydew from both insects was never found to contain more than trace amounts of sorbitol or mannitol (Fig. 3). This was true even for insects feeding at high environmental temperatures.

Besides environmental temperature, the concentration of sucrose in the diet injested by the silverleaf whitefly is also capable of triggering sorbitol formation in its hemolymph (Table 1). The greater the sucrose in the diet, the greater the sorbitol content of the insect's hemolymph. The sugar content of plant phloem sap varies considerably during the course of a day (Mitchell *et al.*, 1992). The sucrose content of phloem sap could therefore be one of the triggers which stimulates polyol accumulation in these insects. However, laboratory experiments show that these insects can be made to accumulate polyols while feeding upon diets of constant and relatively low sucrose concentration if they are exposed to elevated ($\geq$35 °C) temperatures (Salvucci, *unpublished data*).

In previous work, we established that sorbitol accumulation in the silverleaf whitefly provides a mechanism for thermo- and osmoprotection (Wolf *et al.*, 1998). Evidence presented here suggests that mannitol functions in a similar capacity

in the cotton aphid. The creation of polyols in the hemolymph of these two pests helps them to survive in the hot, arid environment of cotton fields.

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Table 1. Effect of dietary sucrose content and temperature upon sorbitol accumulation by the silverleaf whitefly.

	Sorbitol content (nmol whitefly^{-1})[a]	
	25 C	42 C
88	0.03±0.00	0.09±0.014
220	0.04±0.002	0.47±0.002
580	0.23±0.17	1.37±0.20

[a]nanomoles sorbitol per whitefly±S.E.M.; results of two separate experiments each using 3 feeders with 25 whiteflies per feeder.

Table 2. Hexose reductase in *Aphis gossypii* and *Bemisia argentifolii*

Substrate	Coenzyme	Activity (IU[a] mg protein^{-1})	
		Aphis gossypii	*Bemisia argentifolii*
Glucose	NADPH	0[b]	0
Fructose	NADPH	0.61±0.04	1.06±0.07
Glucose	NADH	0	0
Fructose	NADH	0.19±0.01	0.12±0.02

[a]IU = mol min^{-1}
[b]Activity not detected

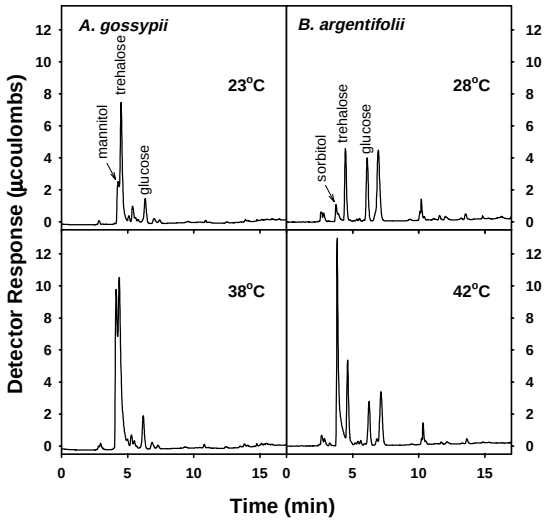

Figure 1. HPLC chromatograms of extracts from cotton aphids and silverleaf whiteflies feeding upon cotton plants at the temperatures indicated on the panels.

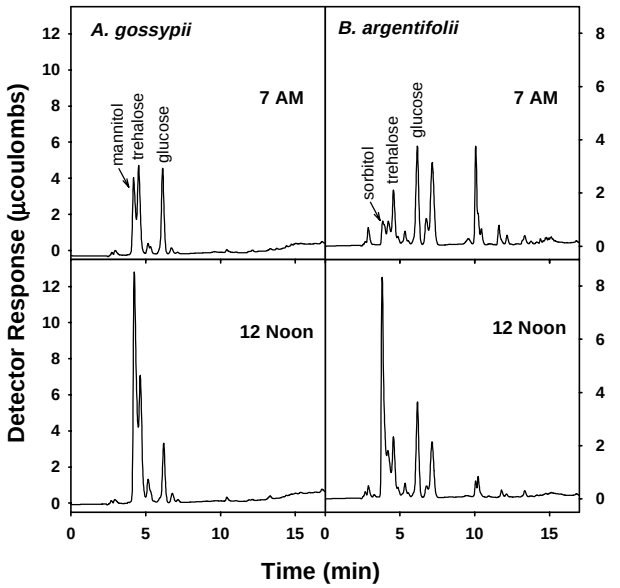

Figure 2. HPLC chromatograms of extracts from cotton aphids and silverleaf whiteflies feeding upon cotton plants at 7 AM and at noon..

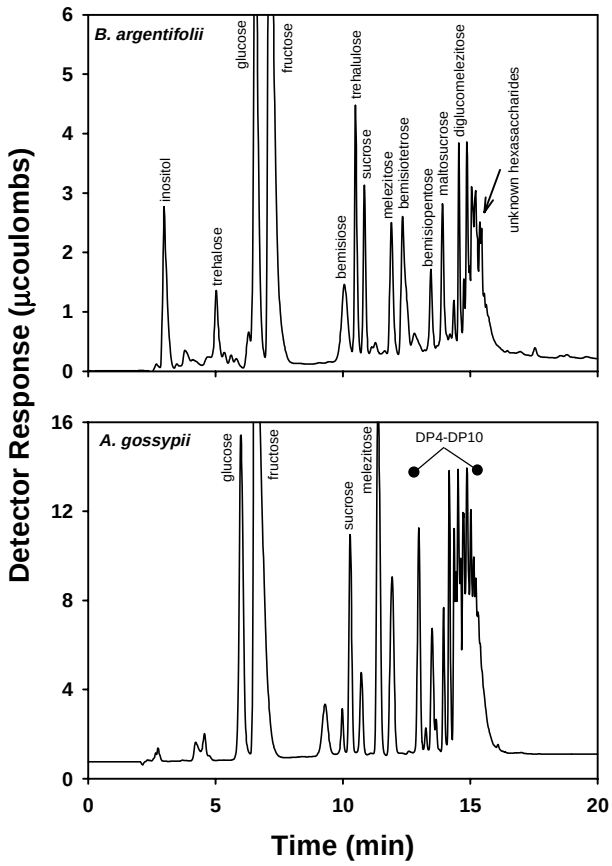

Figure 3. HPLC chromatographs of honeydew of the silverleaf whitefly (top panel) and cotton aphid (bottom panel) feeding upon upland cotton plants.

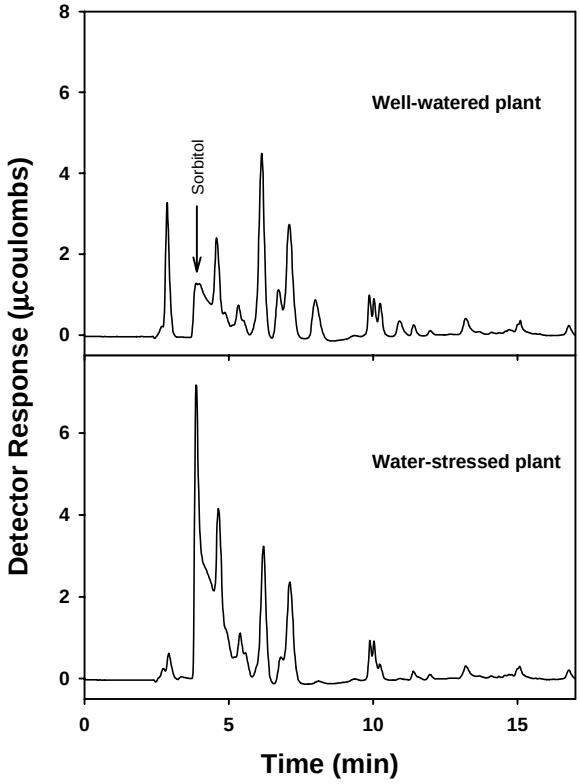

Figure 4. HPLC chromatograms of extracts of bodies of silverleaf whiteflies feeding upon well-watered upland cotton plants (top panel) and upon upland cotton plants from which water was withheld for several days (bottom panel). Both sets of plants were grown in the same greenhouse.