

# Physiological and biochemical responses of cotton following aphid feeding

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## ABSTRACT

The physiological and biochemical responses of cotton following aphid feeding were studied in a controlled environment. Nine days of aphid infestation significantly reduced stem, leaf and plant dry weights. An aphid infestation between 300 to 400 aphids per leaf did not significantly alter leaf photosynthetic and respiration rates, even though there appeared to be a trend for increased photosynthesis with aphids. The amount of individual carbohydrates found in the aphid honeydew varied significantly with time. In addition the total amount of sugars produced per aphid per 24 h period was 2.5  $\mu\text{g}$ . The number of honeydew droplets excreted per aphid varied significantly with time. Moreover, the pattern of honeydew excretion did not appear to be consistent. Aphids did not alter non-structural carbohydrates on a leaf area basis over the 24 h period. Aphid-infested leaves had significantly higher glutathione reductase activity after six days of exposure to aphids. In this study, cotton plants seemed to compensate for carbohydrates losses caused by aphids.

## Introduction

Insect pests are one of the major problems that a cotton producer confronts. In recent years, the cotton aphid (*Aphis gossypii* G.) has become a key pest across the U.S. Cotton Belt due to widespread resistance to different classes of insecticides (Mize *et al.*, 1993). Outbreaks on cotton (*Gossypium hirsutum* L.) have generally been associated with insecticide use for other pests (Slosser *et al.*, 1989). In 1991, the cotton aphid was the most serious pest of cotton in the U.S., reducing production by 360,209 bales (Head, 1992). Aphids may damage the crop by feeding on the phloem sap and indirectly through the production of honeydew. Sooty molds may develop on the honeydew and lead to reduced photosynthesis, reduced fiber quality of open bolls and hindered fabric production at the mill due to "stickiness" (Carter and Perkins, 1986). On tender terminals and young leaves, feeding may result in distorted growth, and excessive feeding may cause wilting, chlorotic leaves, and premature leaf loss (Sprenkel and Johnson, 1998). Large aphid populations may contribute to wilting of plants under water-deficit stress (Eastop, 1977). Large aphid populations have been shown to negatively affect cotton, however, scarce information exists relating aphid numbers and their physiological and biochemical impact on the cotton plant. The objectives of this research were: (1) to investigate the effect of cotton aphids on the physiology of single cotton leaves and on plant growth, (2) to investigate the diurnal changes in the physiology of single leaves and the patterns of aphid feeding, and

(3) to determine the activity of a foliar antioxidant enzyme.

## Experimental procedure

All the experiments were conducted in a growth chamber at the Altheimer Laboratory in Fayetteville, AR. The growth chamber was programmed for 14:10 hours (day/night), day/night temperatures of 28 °C/16 °C, and 75% relative humidity. Stoneville 474 seeds were sown in 2 l pots containing Sunshine mix (soil-less media). Pots were watered with half-strength Hoagland's solution to maintain a well-watered status. Cotton aphids were collected from aphid-infested fields at Lonoke, AR and reared in the laboratory. At 14 days after planting (DAP) the first unfurled leaf from the apex was tagged. Plants were divided in two groups, one group receiving aphids and the other one without aphids. One hundred aphids (wingless nymphs + adults) were individually transferred to the selected leaf with a moist paintbrush. In addition, the rest of the leaves (untagged) were infested with five aphids per leaf. Aphids were allowed to increase in numbers and 300 to 400 aphids per tagged leaf were recorded after nine days.

## Determination of dry matter

The experiment was conducted three times, each time with 24 plants. Plants were harvested before infesting the cotton plants (20 DAP) with aphids and after nine days of exposure to aphids. Dry weight of leaves, stems, and petioles were measured. The experiment was arranged in a split plot in time design with no treatment initially (day 0). Comparisons ( $\alpha=0.05$ ) between aphid-infested and non-infested leaves were made at day zero and at day nine.

## Physiological measurements

This experiment was conducted two times, each time with 42 plants. After nine days of exposure to aphids (31 DAP), net photosynthetic rate and dark respiration rates were recorded with a LI-6200 portable photosystem system at 4 h intervals for a 24 h period. Non-structural carbohydrate concentrations of the leaves were determined (Hendrix, 1993).

## Quantification of sugars in aphid-honeydew and droplets number

Honeydew sugar quantification was measured on only one of the two experiments. The aphid honeydew was collected on plastic sheets for four hours over a 28 h period. The honeydew collections were sent to the USDA Laboratory in Phoenix, AZ, and the amounts of carbohydrates were determined using the HPLC method of Hendrix and Wei (1994). Water-sensitive paper was utilized to count the number of honeydew droplets excreted by the aphids (Yee *et al.*, 1996; Costa *et al.*, 1999). The digitized image of the paper with droplets was processed using Kodak 1D image analysis software.

## Analysis of glutathione reductase (GR)

This experiment was conducted three times, and each time 12 plants were used. After the plants had been exposed to six days (16 DAP) and nine days (29 DAP) of aphid feeding, three leaves per treatment were sprayed with 1% (v/v) SDS, and then rinsed with deionised water. One hour later, the leaves were cut off, dipped in liquid nitrogen, and kept at -70 °C. The protein was extracted as described by Anderson *et al.* (1992). The glutathione reductase assay used by Schaedle and Abassham (1977) was followed.

## Results

### Dry matter

Before applying the treatments, there were no significant differences between both groups of plants (Table 1). However, after nine days of exposure to aphids, the dry weight of stems, leaves, and whole plants were significantly reduced (Table 1). This probably accounts for the stunted growth of aphid-infested plants. Petiole dry weights were not affected by aphids.

### Leaf photosynthesis and dark respiration

Figure 1 shows the diurnal variations of photosynthetic and dark respiration rates in aphid-infested cotton leaves. Photosynthetic rates during the dark cycle were assumed to be zero and were not included in the data analyses. The maximum photosynthetic rates in aphid-infested and non-infested leaves in experiment 1 were 24.7 and 22.0 mg CO<sub>2</sub> dm<sup>-2</sup>.h<sup>-1</sup>, respectively (Figure 1A) and in experiment 2 were 24.5 and 23.4 mg CO<sub>2</sub> dm<sup>-2</sup>.h<sup>-1</sup>. Overall, aphids did not significantly (P=0.05) alter photosynthesis. Dark respiration rates were significantly higher in experiment 1 in aphid-infested leaves than in non-infested leaves at 1400, 1800 and 0600 h (Figure 1B), probably due to the aphids' respiration, because aphids were not removed during the measurements. When aphids were removed in experiment 2, the differences between aphid-infested and non-infested leaves were not significant.

### Non-structural carbohydrates in leaves

The amounts of glucose and fructose were added together and expressed as hexose (Figure 2). The diurnal changes in hexose followed a similar pattern in aphid-infested and non-infested leaves. The peak for hexose occurred at noon and decreased with time probably due to variations in dark respiration rates (Figure 1B). Sucrose levels increased during the illuminated period and rapidly decreased during the dark period. Starch levels in aphid-infested and non-infested leaves did not vary much over time, except at 1600 and 0400 h there were two small peaks in non-infested leaves, but these were not significant. Starch in our experi-

ments constituted above 80% of the total non-structural carbohydrates in the leaves.

### Total carbohydrates in aphid-honeydew

The total carbohydrates produced by aphids over time were significantly different (Figure 3). The total quantity of sugars found in this study exhibited a bell-shaped pattern when plotted against time (Figure 3). The total amount of sugars produced per aphid per 24 h was 2.5 µg.

### Number of aphid-honeydew droplets

Aphid honeydew excretion could be used to determine aphid-feeding activity. In our study, we used a mixed age aphid infestation, therefore the average number of drops excreted per aphid represents adults and/or nymphs. In experiment 1, the honeydew excretions appeared to follow a bell-shaped pattern (Figure 4). However, there was no clear pattern in experiment 2 (data not shown). At noon, there was significantly greater number of droplets excreted per aphid compared to early morning and after midnight periods.

### Antioxidant enzyme

The GR activity was significantly higher in aphid-infested leaves than in non-infested leaves on day six (Figure 5). Probably, the highest activities of this enzyme occurred at the beginning of the damage and then declined, with the activation of other antioxidant enzymes.

## Discussion and Conclusion

Aphid-infested plants experienced lower dry weight of leaves, stems and whole plant. This probably accounts for the stunted growth of aphid-infested plants. Fuchs and Minzenmayer (1995) reported that high aphid populations significantly reduced cotton plant height. Aphids did not alter photosynthesis and dark respiration. Different plant responses have been observed depending upon the aphid-plant combination. Overall, negative effects of aphids on plant photosynthesis have been reported in greenhouse-grown cotton (Shannag *et al.*, 1998), and field-grown cotton (Godfrey *et al.*, 1997; Godfrey and Wood, 1998). The non-structural carbohydrates in aphid-infested and non-infested leaves exhibited a similar pattern. Moreover, the amount of total carbohydrates found in aphid honeydew varied with time as well as the number of droplets excreted per aphid. Aphid-infested leaves had higher GR activity after six days of exposure to aphids. The main change of GR could be associated with a defensive mechanism of the plant against the physiological damage produced by the aphids as suggested by Argandoña (1994). This research demonstrated that cotton plants experienced some alterations due to aphid feeding, but they were able to compensate for carbo-

hydrate losses and tolerate the aphid infestations and feeding duration used in this study.

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## References

- Anderson, J.V., Chevone, B.I., and Hess, J.L. (1992). Seasonal variation in the antioxidant system of Eastern white pine needles. *Plant Physiology*, **98**: 501-508.
- Argandoña, V.H. (1994). Effect of aphid infestation on enzyme activities in barley and wheat. *Phytochemistry*, **35**: 313-315.
- Carter, F.L. and Perkins, H.H., Jr. (1986). Significance of insect honeydew contamination in textile processing. In Proceedings Beltwide Cotton Conferences, J.M. Brown and T.C. Nelson, eds., Vol. 1, pp. 214-215. National Cotton Council, Memphis, TN, 4-9 January 1986, Las Vegas, NV.
- Costa, H.S., Toscano, N.C., Hendrix, D.L. and Henneberry, T.J. (1999). Patterns of honeydew droplet production by nymphal stages of *Bemisia argentifolii* (Homoptera: Aleyrodidae) and relative composition of honeydew sugars. *Journal of Entomological Science*, **34**: 305-313.
- Eastop, V.F. (1977). Worldwide importance of aphids as virus vectors. In Aphids as virus vectors K.F. Harris and K. Maramorosch, eds., pp. 3-62. Academic Press, Inc, New York.
- Fuchs, T.W. and Minzenmayer, R. (1995). Effect of aphids on cotton development and yield in West Texas. In Proceedings Beltwide Cotton Conferences, Cotton Insect Research and Control Conference, D.A. Richter and J. Armour, eds., Vol. 2, pp. 890-892. National Cotton Council, Memphis, TN, 4-7 January 1995, San Antonio, TX.
- Godfrey, L.D., Fuson, K.J. and Wood, J.P. (1997). Physiological and yield responses of cotton to mid-season cotton aphid infestations in California. In Proceedings Beltwide Cotton Conferences, Cotton Insect Research and Control Conference, P. Dugger and D.A. Richter, eds., Vol. 2, pp. 1048-1051. National Cotton Council, Memphis, TN, 6-10 January 1997, New Orleans, LA.
- Godfrey, L.D. and Wood, J.P. (1998). Mid-season cotton aphid infestation in California: Effects on cotton yield. In Proceedings Beltwide Cotton Conferences, Cotton Insect Research and Control Conference, P. Dugger and D. Richter, eds., Vol. 2, pp. 1056-1058. National Cotton Council, Memphis, TN, 5-9 January 1998, San Diego, CA.
- Head, R.B. (1992). Cotton insect losses. In Proceedings Beltwide Cotton Conferences, D.J. Herber and D.A. Richter, eds., Vol. 2, pp. 621-625. National Cotton Council, Memphis, TN, 6-10 January 1992, Nashville, TN.
- Hendrix, D.L. (1993). Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. *Crop Science*, **33**: 1306-1311.
- Hendrix, D.L. and Wei, Y. (1994). Bemisiose: An unusual trisaccharide in Bemisia honeydew. *Carbohydrate Research*, **253**: 329-334.
- Mize, T.W., Mitchell, H.R. and Gage, E.V. (1993). Control of cotton aphid with Furadan 4F. In Proceedings Beltwide Cotton Conferences, D.J. Herber and D.A. Richter, eds., Vol. 2, pp. 728-729. National Cotton Council, Memphis, TN, 10-14 January 1993, New Orleans, LA.
- Schaedle, M. and Abassham, J. (1977). Chloroplast glutathione reductase. *Plant Physiology*, **59**: 1011-1012.
- Shannag, H.K., Thorvilson, H. and El-Shatnawi, M.D.K. (1998). Changes in photosynthetic and transpiration rates of cotton leaves infested with the cotton aphid, *Aphis gossypii*: Unrestricted infestation. *Annals of Applied Biology*, **132**: 13-18.
- Slosser, J.E., Pinchak, W.E. and Rammel, D.R. (1989). A review of known and potential factors affecting the population dynamics of the cotton aphid. *Southwest Entomologist*, **14**: 302-313.
- Sprengel, D. and Johnson, F. (1998). Insect monitoring and control in cotton. University of Florida, Cooperative Extension Service, Institute of Food and Agricultural Science, pp. 10-11.
- Yee, W.L., Hendrix, D.L., Toscano, N.C., Chu, C.C. and Henneberry, T.J. (1996). Diurnal field pattern of honeydew sugar secretion by *Bemisia argentifolii* (Homoptera: Aleyrodidae) nymphs on cotton. *Environmental Entomology*, **25**: 776-782.

**Table 1.** Dry weight of leaf, total leaves, stems, petioles and whole plant of aphid-infested (A) and non-infested (N) plants at two sampling times.

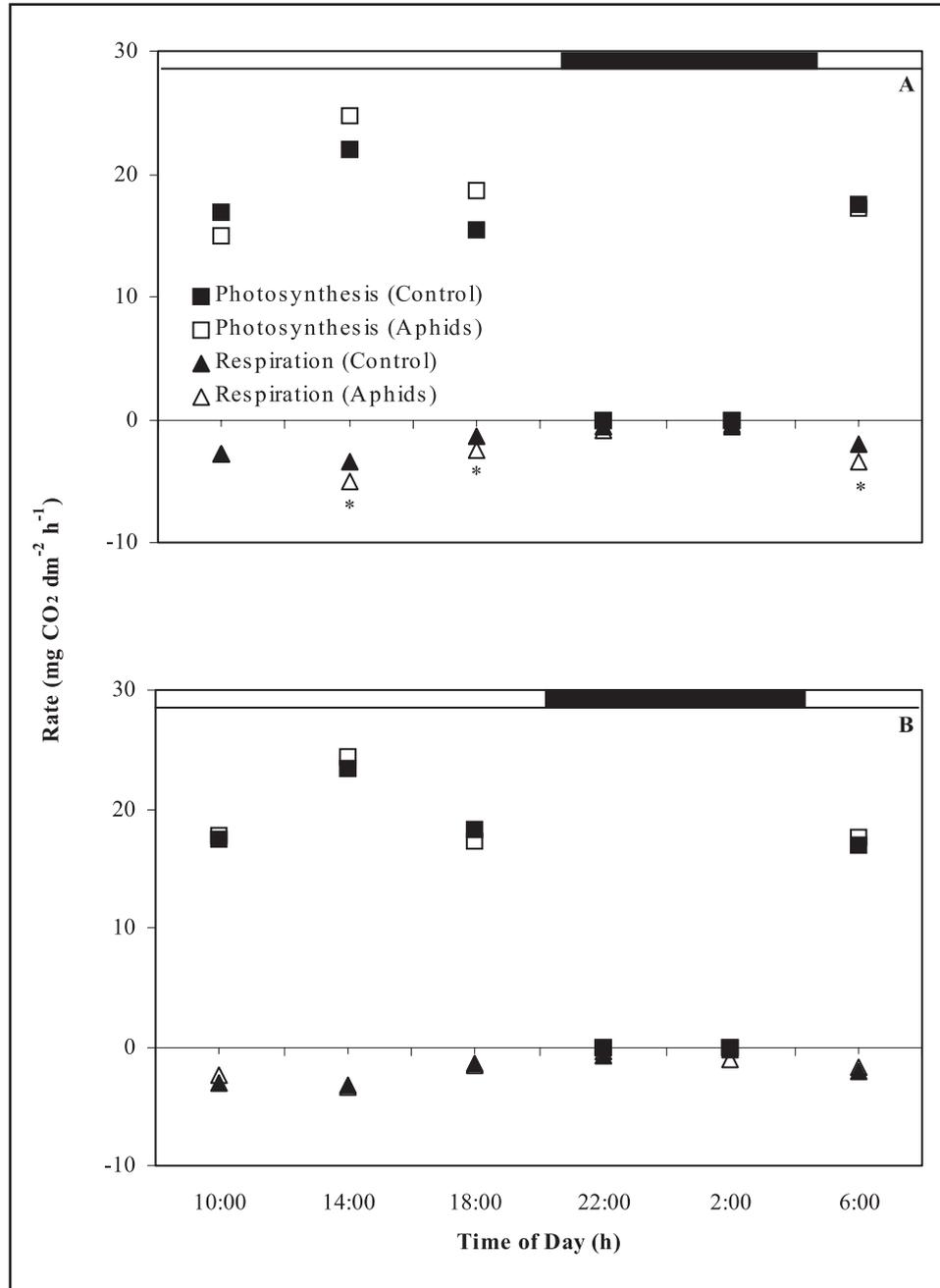
Variable	Sampling time			
	Initial		9 DAT <sup>z</sup>	
	A <sup>y</sup>	N	A	N
	Dry weight (g/plant)			
Leaf (mg)	245.18 <sup>z</sup>	232.45	512.95	507.82
	0.65 <sup>x</sup>		0.85	
Total leaves (mg)	835.57	853.05	3525.31	3774.05
	0.89		0.05	
Stems (mg)	172.31	181.21	822.15	909.87
	0.82		0.03	
Petioles (mg)	52.78	61.05	386.14	401.87
	0.54		0.25	
Whole plant (mg)	1060.66	1095.31	4733.60	5085.79
	0.84		0.05	

<sup>z</sup> = Days after treatment.

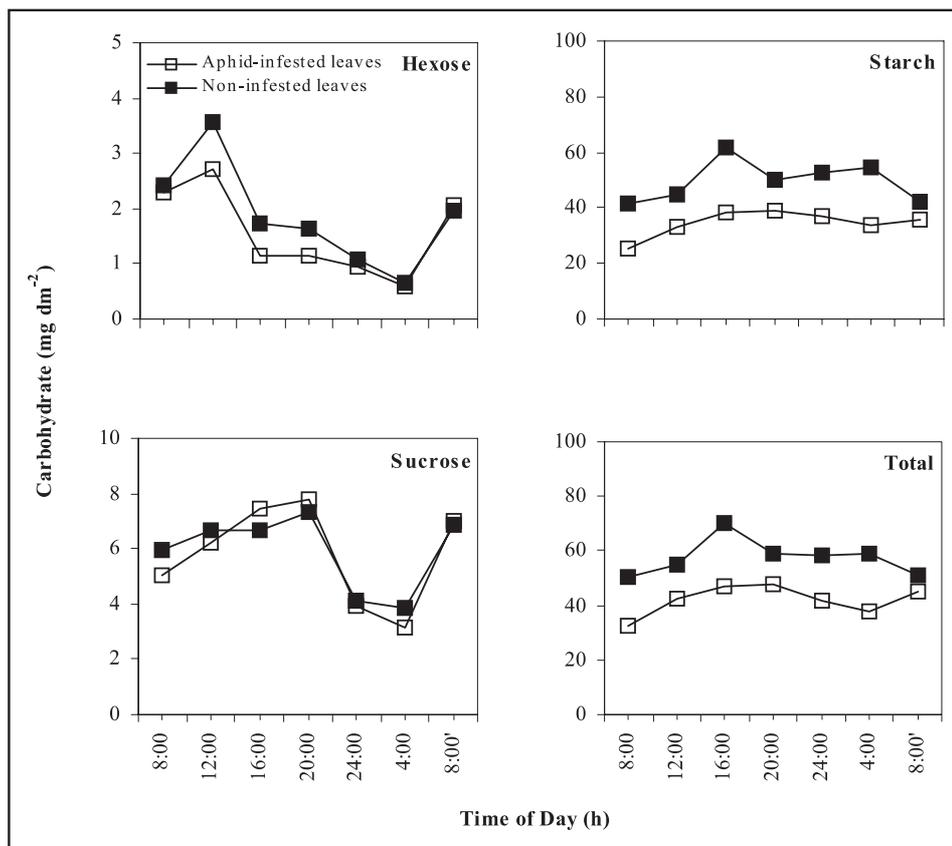
<sup>y</sup> Plants assigned to aphid treatment before applying the treatment.

<sup>x</sup> Probability (F-test, alpha=0.05).

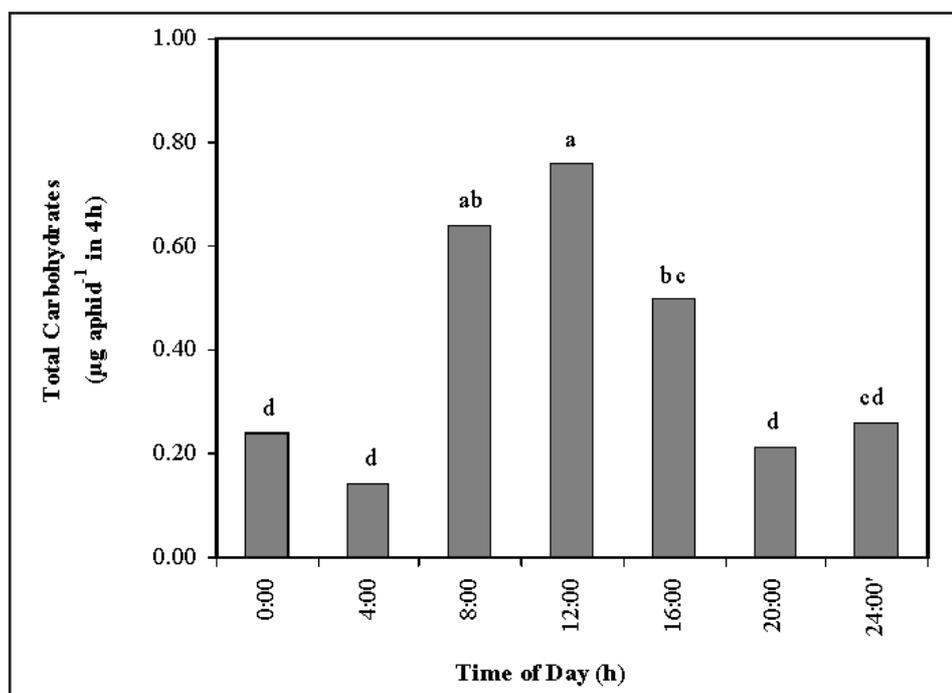
**Figure 1.** Diurnal variation of photosynthesis and dark respiration rates in non-infested and aphid-infested cotton leaves. A bar at the top of the figure indicates the light/dark cycle. Values are means of three replicates in experiment 1 (A), and four replicates in experiment 2 (B). Asterisk represents significant difference between treatment means at a time period ( $P=0.05$ ).



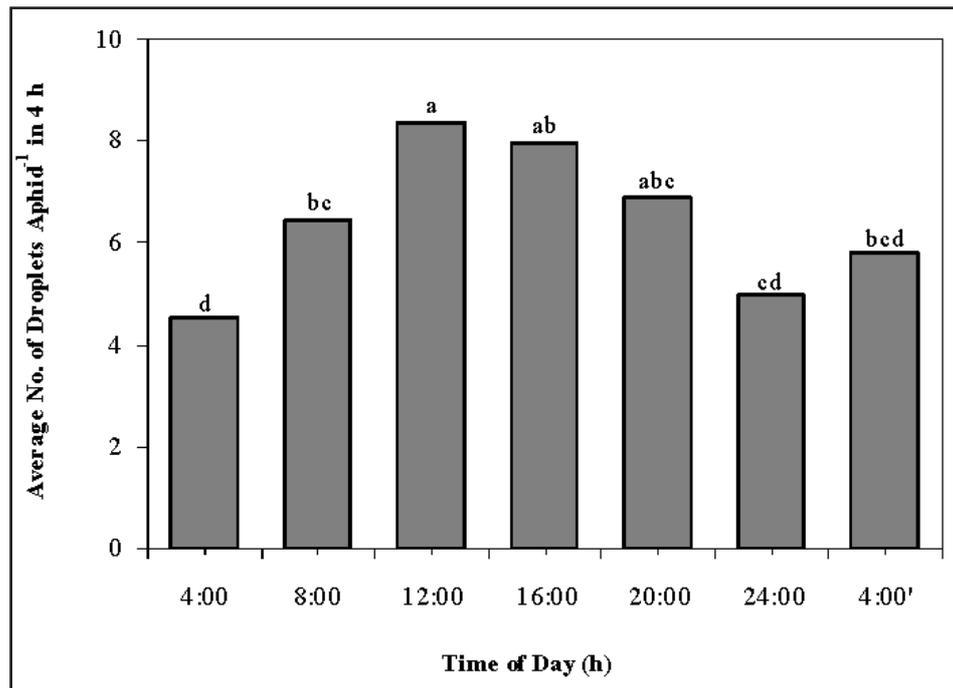
**Figure 2.** Diurnal changes in hexose, sucrose, starch and total non-structural carbohydrates in aphid-infested and non-infested cotton leaves. Values are means of four replicates in experiment 2. Treatment by time interaction was not significant ( $P=0.05$ ).



**Figure 3.** Total carbohydrates in honeydew produced per four hours per cotton aphid. Means of six replicates followed by the same letter are not significantly different ( $P=0.05$ ).



**Figure 4.** Diurnal honey-dew excretion pattern of *Aphis gossypii* feeding on cotton leaves. Values are means of five replicates in experiment 1. Means not followed by the same letter are significantly different ( $P=0.05$ ).



**Figure 5.** Glutathione reductase activity in non-infested and aphid-infested leaves, after six and nine days of exposure to *Aphis gossypii*. FW=fresh weight. Values are the means of twenty-seven observations.

