

# Cotton Aphid Biology and Honeydew Production

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## ABSTRACT

*Cotton aphid, Aphis gossypii Glover, fecundity, nymph development and honeydew production were studied in the laboratory. Apterous adult females produced an average of 1.7 nymphs per day and the nymphs (four instars) developed to adults in an average of 4.1 days at 26.7 °C in the laboratory. Average longevity of adults was 16.1 days. More honeydew drops were produced by one-day old nymphs than three- or four- day old nymphs. Numbers of honeydew drops produced on a day to day basis were highly variable and did not show a distinct pattern of production. More honeydew drops, sugars and progeny were produced by adults at 26.7 °C compared with 15.6 or 32.2 °C. Increasing times of exposure of clean cotton lint to aphids and the resulting increasing amounts of honeydew sugars under laboratory and field conditions were significantly related to increasing cotton lint stickiness as measured with a thermodetector.*

## Introduction

*Aphis gossypii* Glover, is the most common aphid species occurring on cotton, *Gossypium hirsutum* L., in the United States. It was first reported as an economic pest in South Carolina in 1854, followed by reports of economic pest status on cotton from much of the Southeast by 1880 (Slosser et al. 1989) and in Texas in 1916 (Paddock 1919). Cotton aphid outbreaks have been associated with reduced natural enemy populations as a result of insecticide use for boll weevil, *Anthonomus grandis* Boheman and bollworm, *Helicoverpa zea* (Boddie) control (Slosser et al. 1989). Additionally, cotton aphid resistance to several classes of insecticidal chemistry has been well documented and has been found to contribute to the difficulty of obtaining effective control (O'Brien et al. 1990, Grafton-Cardwell 1991, Kerns and Gaylor 1992). In 1986, cotton aphids were reported as a problem in Alabama, North Carolina, Louisiana, and California, presumably as a result of the use of pyrethroids for control of other insect pests (King et al. 1987). Slosser et al. (1989) suggested that additional factors such as bioclimate and cotton plant nutritional status interactions as well as other reasons may be responsible for initial cotton aphid population increases in the Rolling and High Plains of Texas. In 1991, cotton aphids were considered the number one cotton pest in the southwestern United States (Hardee and Herzog 1992).

The cotton aphid has a wide host range. Ebert and Cartwright (1997) reported over 90 plant families in which at least one species was listed as a host. Damage occurs as a result of direct feeding that reduces yield, contaminates plants and produce with honeydew, and results in associated fungal growth on honeydew and finally transmission of viruses causing more than 50 plant diseases. Cotton aphid honeydew contamination causes significant problems in lint processing at the gin and at the textile mill (Hector and Hodkinson 1989, Slosser et al. 1989, Ebert and Cartwright 1997). Cotton aphids were considered the main causal agents for the sticky cotton problems in Israel from 1983 to 1985 (Broza 1986), and in 1986 in California (Perkins and Basset 1988, Hector and Hodkinson 1989).

The pest status of the cotton aphid in some cotton growing areas of the United States (King et al. 1987) and the increasing concern for the sticky cotton problem prompted our studies on honeydew production by cotton aphids and investigations of the relationships between cotton aphid honeydew and cotton lint stickiness in the laboratory and in the field. We also determined the effect of temperature on aphid fecundity and rate of honeydew production.

## Methods and Materials

Cotton aphids used in the laboratory studies were originally collected on cotton plants in fields near Phoenix, AZ. Subsequent generations have been cultured in the laboratory on cotton plants for over five years. Studies were conducted, except for temperature effects tests, in constant temperature boxes maintained at  $26.7 \pm 0.5^\circ\text{C}$  and 14:10 h L:D conditions. Newly emerged aphid nymphs were obtained by transferring apterous adult females to leaves of uninfested cotton plants. Nymphs were collected after 12 h and individuals placed in 3.8-cm diameter x 0.85-cm high leaf clip cages on intact cotton leaves on plants in the two-to-four-leaf stage of development. For honeydew lint stickiness studies, all lint samples were analyzed at the USDA-ARS Cotton Quality Research Station, Clemson, SC, using the thermodetector method described by Perkins and Brushwood (1994).

*Aphid development and reproduction.* Cages were checked daily following infestation with less than one-day-old nymphs, and the days when the first new progeny occurred was recorded. These were designated as the days of development from nymphal births to adults. Following the occurrence of adults, numbers of new nymphs found each day were recorded until the adults died. New nymphs were removed from the cages each day.

*Honeydew drops: numbers, collection, and sugar identification.* Leaf clip cages had snap-on removable polyethylene bottoms. Cage bottoms were removed each day and numbers of honeydew drops were counted. Honeydew was washed from the leaf-cage bottoms with 3 ml of warm deionized water and frozen. Frozen honeydew samples were lyophilized and reconstituted in 125  $\mu\text{l}$  of deionized water. Sugars were determined using the high performance liquid chromatography (HPLC) methods of Hendrix and Wei (1994) and quantified by comparison with peaks of known sugar standards.

*Effects of temperature and host plant on aphid reproduction and honeydew excretion.* Individual adults were confined for ten days in leaf cages on plants and held in control temperature boxes at 15.6, 26.7 and  $32.2^\circ\text{C}$ . Progeny and honeydew drops were counted and honeydew collected daily and treated as precisely described for sugar analysis.

*Honeydew excreted by cotton aphids on lint in the laboratory.* Clear plastic boxes (93.5-cm long x 12.7-cm wide x 12.7-cm deep) were modified to enclose four- to six- leaf stage cotton seedlings growing in soil filled pots. Two, 5-cm diameter, muslin covered holes in the sides of each box provided ventilation. Openings of 0.63-cm wide x 2.54-cm long were cut in the middle and opposite each other in each of the bottom hinged halves of the boxes. The openings accommodated cotton-wrapped seedling stems when the box halves were closed. Each leaf had 50 to 100 aphids of mixed age nymphs and adults. Cotton lint samples (2.5 grams) were spread into a layer and equally distributed over the bottoms of the boxes. The cotton lint obtained from the Cotton Quality Research Station was selected based on consistent thermodetector counts of three or less (D. Brushwood, USDA-ARS, Cotton Quality Research Station, Clemson, SC; Personal Communication). Boxes with aphids were placed in randomized complete block designs in a  $26.7^\circ\text{C}$  constant temperature cabinet under 14:10 L:D conditions. Lint was removed from each of 10 boxes after 1, 3, 5, 7, and 9 d exposure periods. The lint from five of the boxes was analyzed using the thermodetector on each sampling date to determine the number of sticky spots.

*Statistical Analysis.* All data were analyzed using analysis of variance (ANOVA) (MSTAT-C 1988) for factorial treatments in randomized complete block designs. Means were separated, contingent on significant F tests, using the method of least significant differences at  $P \leq 0.05$ . Regression analyses were conducted to determine relationships of times of lint exposure to aphid infestations and relationships of honeydew sugar to thermodetector counts (lint stickiness).

## Results

*Aphid development and reproduction.* The average number of days from birth of nymphs to viviparous female adults as measured by the occurrence of newly born nymphs was  $4.1 \pm 0.1$  days ( $n = 51$ ) at  $26.7^\circ\text{C}$ . Adults, on average, produced  $1.7 \pm 0.1$  nymphs per day (Figure 1). On average,  $1.5 \pm 0.1$  nymphs per day were produced on

the first day of adult life. Fecundity increased thereafter. Production peaks of  $2.9 \pm 0.3$  nymphs per day occurred on days three and five. Fecundity decreased on day 11 to  $< 1$  nymphs per day, increased to  $> 1$  nymph per day on days 12 to 14 and decreased thereafter to  $< 1$  nymph per day for the remainder of the reproduction periods. Over 90% of the nymphs were produced from day one to day 18 of adult life. Nymph reproduction was sporadic after day 20 and numbers of nymphs produced ranged from 0 to 0.8 per day. The average longevity of adult females was  $16.1 \pm 1.6$  days (range 7 to 28 days).

*Honeydew drops: numbers, collection, and sugar identification.* Nymphs on days one, two, three and four of their life (first to fourth instars) averaged  $14.3 \pm 2.8$ ,  $11.9 \pm 2.6$ ,  $7.7 \pm 2.5$  and  $7.6 \pm 1.6$  honeydew drops per day (Table 1). The numbers of drops produced by one-day-old nymphs was significantly greater compared with three- or four-day-old nymphs but not two-day-old nymphs. Differences in numbers of drops produced by two-, three-, or four-day-old nymphs were not significantly different. Total sugars produced by 1 and 2 day-old nymphs were higher than those produced by 4 day-old nymphs.

*Effects of temperature on adult aphid reproduction and honeydew excretion.* More nymph progeny ( $1.47 \pm 0.21$ ) per day were produced at  $26.7^\circ\text{C}$  compared with  $15.6^\circ\text{C}$  ( $0.27 \pm 0.06$ ) or  $32.2^\circ\text{C}$  ( $0.53 \pm 0.09$ ) (Table 2). The average numbers  $2.94 \pm 0.57$  and  $7.75 \pm 1.13$  honeydew drops produced per aphid per day at  $15.6$  and  $32.2^\circ\text{C}$ , respectively, were significantly lower compared with  $14.09 \pm 2.07$  drops produced at  $26.7^\circ\text{C}$ . These numbers include some unknown numbers of drops and sugars produced by nymphs during the 24-h periods between observations. Results for total sugars in honeydew were similar.

*Cotton aphid honeydew excretion and cotton lint stickiness in the laboratory.* The numbers of thermodetector spots increased with increasing numbers of days of lint exposure to cotton aphids in ventilated plastic corsage boxes (Table 3). Results were similar for sugars except for the 7 day exposure. Thermodetector counts ranged from  $2.00 \pm 0.54$  for unexposed lint to  $18.70 \pm 3.70$  for lint exposed to aphids for nine days. The regression for thermodetector spots and days of exposure was significant ( $r^2 = 0.93$ , Figure 2).

## Discussion

Developmental time from cotton aphid nymph birth to adult (4.1 days at  $26.7^\circ\text{C}$ ) in our studies was less than the five days at  $27.5^\circ\text{C}$  reported by Akey and Butler (1989). This is probably explained by the difference in temperature and because our nymphs could have varied from 0- to 12-h old when our tests were initiated. The first nymphs produced by adult females could have occurred any time during the 24-h intervals between observations. Our results for apterous cotton aphid fecundity agree with those reported by Akey and Butler (1989) at comparable temperatures. The fecundity results of O'Brien and Graves (1992) with alate cotton aphids also appear to be similar to our results. Additionally, as occurred in our studies, O'Brien and Graves (1992) noted high reproductive rate early compared to late in an aphid's life.

In the present studies, numbers of honeydew drops and associated honeydew sugars produced per individual aphid per day were highly variable. These results as well as those of the frequency of honeydew drop excretion per 24-h appear to agree with results of studies on many other aphid species characterizing aphid honeydew excretions as "continuous" and "discontinuous" with on and off excretion frequencies encountered (Auclair 1963). For eight aphid species, Büsgen (1891) reported 1.7 to 20 drops per aphid per 10-h and for four additional aphid species, Auclair (1963) found reports of 4.1 to 22.0 drops per aphid per 10-h. Our data for adult cotton aphids fall within these ranges.

The presence of cotton aphids or sweetpotato whiteflies, *Bemisia tabaci* type B (Gennadius) (= *B. argentifolii*), has been associated with over 80% of the sticky cotton problems at textile mills (Hector and Hodkinson 1989). The major sugar components of the honeydew from these insects (fructose, glucose, sucrose, trehalulose, and melezitose) have been applied individually and in various combinations to cotton lint under laboratory conditions (Henneberry et al. 2000). Sucrose, trehalulose, melezitose, fructose and glucose produce varying levels of lint stickiness as measured with the thermodetector. The ratios of the insect-produced sugars in honeydew, trehalulose and melezitose differ between cotton aphids and sweetpotato whitefly. A higher percentage of the total sugars in cotton aphid honeydew occurs as melezitose compared with trehalulose whereas the opposite is true for sweetpotato whitefly (Hendrix et al. 1992). Also, the largest oligosaccharides of cotton aphid honeydew are twice the size of the largest oligosaccharides in *B. tabaci* honeydew. Difference in the amounts of other sugars also occur, but the

significance of these differences in relation to the biology of the insects remains unknown. However, excessive amounts of honeydew of either species when deposited on cotton lint in the laboratory and in the field produces sticky cotton.

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## Tables

Table 1. Mean ( $\pm$  S.E.) Numbers of Honeydew Drops and Micrograms of Honeydew Sugars Produced Per Cotton Aphid Nymph Per Day.

Nymph age (days)	Drops <sup>a</sup> (No.)	Total <sup>b</sup> Sugar
1	14.30 $\pm$ 2.80 a	4.33 $\pm$ 0.63 a
2	11.90 $\pm$ 2.60 ab	4.36 $\pm$ 0.59 a
3	7.70 $\pm$ 2.50 b	3.88 $\pm$ 0.67 ab
4	7.60 $\pm$ 1.60 b	2.99 $\pm$ 0.44 b
F <sup>c</sup>	2.96	2.85

<sup>a</sup> Means of 42 individual aphids. Means not followed by the same letter are significantly different. Method of least significant differences  $P \leq 0.05$ .

<sup>b</sup> Includes glucose, fructose, trehalulose, sucrose and melezitose.

<sup>c</sup> df = 3, 114;  $P \leq 0.05$  for all analyses.

Table 2. Mean ( $\pm$  S.E.) Numbers of Progeny and Honeydew Drops and Honeydew Sugars Produced Per Day by Cotton Aphids Feeding on Cotton at Different Temperatures.

Temp (°C)	Number per day <sup>a</sup>		Total Sugars ( $\mu\text{g}$ / day/aphid) <sup>a</sup>
	Progeny	Honeydew drops	
15.6	0.27 $\pm$ 0.06 b	2.94 $\pm$ 0.57 c	0.05 $\pm$ 0.01 b
26.7	1.47 $\pm$ 0.21 a	14.09 $\pm$ 2.07 a	3.39 $\pm$ 0.49 a
32.2	0.53 $\pm$ 0.09 b	7.75 $\pm$ 1.13 b	1.01 $\pm$ 0.40 b
F	28.0	18.4	18.1

<sup>a</sup> Means of 10 individuals for 7 days. Means not followed by the same letter are significantly different. Method of least significant differences  $P \leq 0.05$ .

<sup>b</sup> Includes glucose, fructose, trehalulose, sucrose and melezitose.

Table 3. Mean ( $\pm$  S.E.) Micrograms of Cotton Aphid Honeydew Sugars Per Gram of Cotton Lint Following Exposure to Infested Cotton Seedlings in Clear Plastic Boxes in the Laboratory.

Days Exposed	Total Sugars <sup>a</sup>	Thermodetector Counts
0	600.1 $\pm$ 24.4 c	2.00 $\pm$ 0.5 d
1	746.6 $\pm$ 16.4 c	7.50 $\pm$ 1.3 cd
3	719.4 $\pm$ 79.8 c	9.30 $\pm$ 1.5 bc
5	1003.9 $\pm$ 74.4 ab	15.00 $\pm$ 2.6 ab
7	779.2 $\pm$ 97.1 bc	16.30 $\pm$ 2.2 a
9	1135.4 $\pm$ 134.0 a	18.70 $\pm$ 3.7 a
F <sup>b</sup>	5.5	8.1

<sup>a</sup> Means of 10 replicates in the same column not followed by the same letter are significantly different. Method of least significant differences  $P \leq 0.05$ .

<sup>b</sup> df = 5,45;  $P \leq 0.05$  for all analyses. Includes glucose, fructose, trehalulose, sucrose and melezitose.

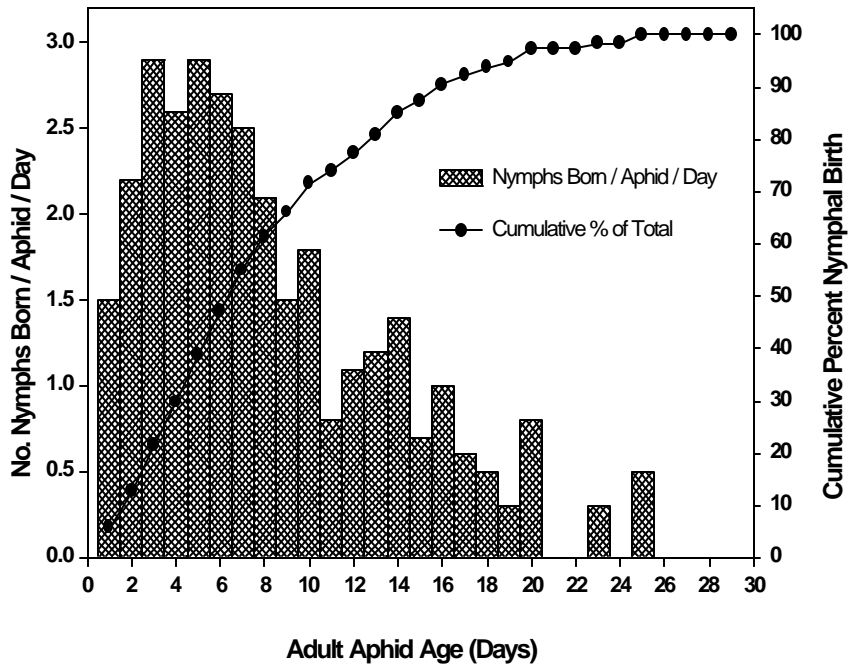


FIG 1. Mean numbers and accumulative percentages of cotton aphid nymphs born per adult aphid per day over their life spans.

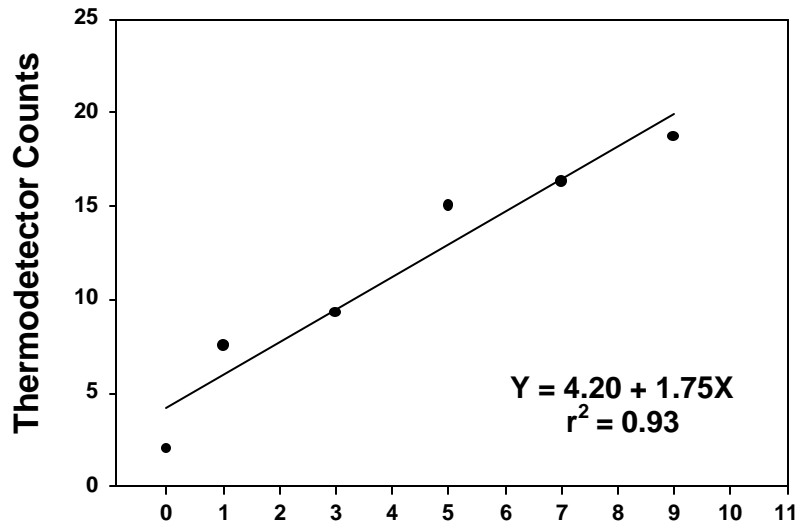


Fig. 2. Mean numbers of thermodetector spots on cotton lint following exposure for different numbers of days to cotton aphids feeding on cotton in the laboratory. Controls (0) were unexposed lint samples.  $F = 56.77$ ;  $df = 1,5$ ;  $P \leq 0.02$ .