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INFLUENCE OF COTTON LEAF SUGARS ON COTTON APHID, *APHIS GOSSYPII* GLOVER, POPULATIONS

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Abstract

The study was conducted at the Texas Agricultural Experiment Station, Chillicothe, in the northern Texas Rolling Plains for four years from 1997 to 2000. The objective was to determine the relationship between nonstructural carbohydrates (glucose, fructose, sucrose) in cotton leaves and change in cotton aphid, *Aphis gossypii* Glover, numbers during late summer. The two whole plot treatments were cotton grown without irrigation (dryland) and irrigated cotton with last irrigation in late August. The two subplot treatments within each whole plot were an untreated check and a plot treated twice during the growing season with lambda-cyhalothrin to stimulate aphid population increase. The sugar ratio (glucose + fructose/sucrose concentrations) was higher in irrigated plots compared with levels in dryland plots. Regression analysis indicated that change in aphid numbers was influenced by numbers of aphids per leaf, temperature, leaf moisture, and sugar ratio. A negative linear relationship was observed between change in aphid numbers and sugar ratio; population growth was limited by high levels of glucose and fructose in cotton leaves, especially when temperatures were high and leaf moisture low.

Introduction

All aphid species that have been reared on artificial diets require sucrose (Srivastava 1987), but the optimum concentration varies with species. Auclair (1967a) reported that cotton aphids can tolerate large variations (10-40%) in sucrose concentrations in artificial diets, but that a diet with 30% sucrose was about optimum for survival and reproduction, while survival was lower on diets with a 10-15% sucrose concentration. While sucrose is generally acknowledged to be an important dietary component for aphids, the role of glucose, fructose, and other sugars is varied. Survival and reproduction of cotton aphids on diets containing 15% glucose or 15% glucose + 15% fructose was considerably lower than for aphids on diets containing 30% sucrose, and Auclair (1967b) concluded that glucose was detrimental to growth and survival.

The objective of this study was to establish the relationship between soluble sugars (glucose, fructose, sucrose) in cotton leaves and change in aphid numbers during late summer and early fall.

Materials and Methods

Experimental Design

This study was conducted for four years, 1997 - 2000, at the Texas Agricultural Experiment Station at Chillicothe, TX. The cultivar TAMCOT Sphinx was planted 2 May 1997, 30 April 1998, 28 April 1999 and 24 April 2000. Seeding rate varied between 17 - 19 seeds per m of row in 102 cm row spacings, and row direction was E-W. Fertilizer was applied immediately before planting at 33.6 kg N/ha in dryland plots and 67.2 kg N/ha in irrigated plots. Subplot size was 10 rows wide by 21.3 m long. Irrigated and dryland plots were maintained in the same location all four years, but chemical treatment subplots were randomly assigned each year.

A split-plot experimental design, arranged as randomized complete blocks, with three replications was used. The two whole plot treatments were dryland, with no supplemental irrigation during the growing season, and irrigated, with last application in late August. Irrigation dates were 16 July, 7 and 29 August 1997; 24 April, 25 June, 16 and 29 July, 13 and 27 August 1998; 15 and 29 July, 12 and 27 August 1999; and 19 July, 7 and 25 August 2000. The dryland treatment was irrigated on 24 April 1998 to obtain sufficient soil moisture for planting.

The two subplot treatments were an untreated check or an application of lambda-cyhalothrin (Karate® EC at 0.045 gm [AI] per ha, Zeneca; Wilmington, DE) during anticipated periods of increased bollworm, *Helicoverpa zea* (Boddie), activity. Lambda-cyhalothrin was applied on 29 July and 28 August 1997, 8 July and 12 August 1998, 2 and 25 August 1999, and 17 July and 24 August 2000. The water management and chemical treatments reported here represent a subset of the treatments reported previously by Slosser et al. (2001, 2002) who discussed the influence of these treatments on aphid populations and on sticky lint in cotton, respectively. Chemicals were applied with a John Deere Hi-Cycle® sprayer (Deere and Company, Moline, IL) with drops to provide three nozzles per row. Total solution applied was 101 - 109 l/ha.

Monitoring of Leaf Carbohydrate Profile

Leaf discs were cut from cotton leaves for analysis of carbohydrates (glucose, fructose, sucrose) on the same dates that aphids were counted. Samples were taken once each week from untreated and lambda-cyhalothrin-treated plots in dryland and irrigated treatments beginning in late July. A leaf from the fifth main stem node below the terminal was selected, and six discs, each measuring 0.33 cm^2 in area, were cut with a cork borer. Discs were cut from only one leaf per plot in 1997-1998, but samples were taken from two leaves per plot in 1999-2000. If the leaf was contaminated with aphids and honeydew, it was thoroughly washed with distilled water and blotted dry with paper towels before cutting the leaf discs. The six discs from each leaf were placed into 2 ml of an 80% ethyl alcohol solution in a stoppered test tube (13 x 100 mm) and placed immediately into a cool chest containing ice. Sampling was conducted between 9:00 AM and noon. When sampling was completed, the test tubes with leaf disc samples were stored in a freezer (- 4°C) in the laboratory.

The leaf disc samples were sent to the USDA-ARS Western Cotton Research Laboratory, Phoenix, AZ, for carbohydrate analysis (Hendrix and Peelen 1987, Hendrix 1993). After removing the activated charcoal by centrifugation and filtration, a 200 µl aliquot of the supernatant was dried and analyzed by high performance liquid chromatography (Hendrix and Wei 1994).

Leaf Moisture

Ten leaves from the fifth main stem node below the terminal were collected to determine leaf moisture on the same day that plots were sampled for leaf carbohydrate content. Leaves were placed immediately into a plastic bag in an ice chest, and within an hour, leaf petioles were cut off with a sharp knife, and the leaves were weighed and then oven-dried at 50°C for 48 hours. Percentage leaf moisture was calculated using the gravimetric method. When leaves were infested with aphids, the leaves were washed and thoroughly blotted dry with towels before weighing.

Aphid Sampling

Aphids were sampled once per week beginning late July in all four years, and sampling continued until aphid populations peaked and then declined. Final samples were taken 22 September 1997, 8 September 1998, 13 October 1999, and 12 September 2000. Initially, aphids were counted on 10 leaves picked from the top and bottom half of the plant, for a total of 20 leaves sampled per plot, but sample size was reduced to 5 top-half and 5 bottom-half leaves when aphids exceeded about 100/leaf. Aphids were counted individually unless numbers exceeded about 100/leaf, when numbers were estimated by counting aphids in groups of five.

Climatic Data

Ambient air temperature data were obtained from the Texas Agricultural Experiment Station at Chillicothe.

Data Analyses

Data were analyzed using linear and stepwise regressions using Statistix 7 (Anonymous 2000). Average change in aphid numbers per week was calculated as the difference between aphid numbers per leaf on two consecutive weeks, and the difference was divided by the number of days between weekly samples. The change in aphid numbers calculation represents the change in average number of aphids per leaf (averaged over all three replications for each of the four treatments) per day per week. This value was transformed to Ln for linear and stepwise regression analyses (Statistix 7, Anonymous 2000); prior to transformation, 0.5 was added to rate of change to eliminate zero and negative values $\geq$ -0.4. The calculations were based on data collected beginning the last week in July and continuing until aphid populations reached peak numbers in September or October, and $n = 103$.

For linear and stepwise regression analyses, the relationships between average change in aphid numbers each week and the average weekly values for the following independent variables were investigated: number of aphids per leaf; concentration of leaf fructose, glucose, sucrose; sugar ratio (glucose + fructose/sucrose); leaf moisture (%); and daily high temperature (°C). The equations were developed by selecting from linear and quadratic functions for each independent variable. Following the recommendation of Gomez and Gomez (1984), we used treatment means averaged over replications.

Results and Discussion

Regression and correlation analyses indicated that change in aphid numbers per day was associated with numbers of aphids per leaf, sugar ratio, percentage leaf moisture and daily maximum temperatures (Table 1). These variables accounted for 87.2% of the variation in change in aphid numbers.

To determine the role of sugar ratio, the six independent variables shown in Table 1 were examined using the individual main effects for year. Sugar ratio was not significant in 1997 or 2000, but ratio showed a significant negative influence on change in aphid numbers in 1998 and 1999 (Table 2). The year 1997 was mild climatically; percentage leaf moisture levels were highest and ambient temperatures were lowest of the four years. As a result, the weekly change in aphid numbers was significantly affected only by aphid numbers per leaf. The year 2000 was the most severe climatically with lowest leaf moisture levels and highest ambient temperatures, and aphid populations were severely suppressed, especially in dryland treatments. As in 1997, the only independent variable that influenced weekly change in 2000 was number of aphids per leaf. Leaf moisture levels in August-September 2000 averaged 60.0%, while leaf moisture levels averaged 71.9% in August-September 1997. In 1997, average maximum temperatures during August - September were 93°F, while in 2000, average maximum temperatures were 102°F.

The influence of sugar ratio was examined in the four individual treatments (Table 3). Sugar ratio did not influence change in aphid numbers in dryland treatments, but ratio did have a significant negative influence on change in aphid numbers in irrigated treatments. The fact that sugar ratio was not a significant variable in treated or untreated dryland plots, but had a significant negative influence in both treated and untreated irrigated treatments, indicates that lambda-cyhalothrin did not affect sugar concentrations in the cotton leaves.

When leaf concentration of glucose + fructose was greater than the concentration of sucrose, sugar ratio was greater than 1, but when concentration of glucose + fructose was less than the concentration of sucrose, sugar ratio was less than 1. Sugar ratios greater than 1 were observed in irrigated treatments in three (1998, 1999, 2000) of the four years, and sugar ratio was greater than 1 in dryland treatments in 1999 only (Table 4). These data indicate that population growth was limited by high levels of glucose and fructose (sugar ratio > 1.5) in cotton leaves. This interpretation regarding the negative influence of glucose is supported by the earlier work of Auclair (1967b) who concluded that high concentrations of glucose were detrimental to growth and survival of cotton aphids.

The following equation was used to develop the linear relationship between weekly change in aphid numbers per leaf and sugar ratio (Figure 1):

$$\text{Ln(Change)} = -20.379 + 0.029(\text{no. aphids/leaf}) - 5.426\text{E-05}(\text{no. aphids/leaf})^2 - 0.094(\text{sugar ratio}) + 0.025(\%\ \text{leaf moisture}) + 1.135(\text{max.ambient temperature °C}) - 0.017(\text{max. ambient temperature °C})^2.$$

Sugar ratio was varied between 0.2 - 11.2 (data range was 0.2 - 14.8), using 50 aphids per leaf which is the treatment threshold during the summer months,. When sugar ratio = 1.70, weekly change in aphid numbers = 1 under conditions of average percent leaf moisture (68.8%) and average temperature (37.4°C). When temperatures were high (68.8% leaf moisture, 40.6°C), maximum change in numbers = 0.51 when sugar ratio = 0.20. Weekly change in numbers became negative when sugar ratio was greater than 5.20. Under the most optimum conditions (78.2% leaf moisture, 31.1°C), weekly change in aphid numbers = 1.05 when sugar ratio = 6.20. When aphid populations are near the treatment threshold of 50/leaf, these relationships indicate that high levels of sucrose (sugar ratio < 1) are necessary to maintain a weekly change greater than 0 when environmental conditions are unfavorable (high temperatures and low leaf moisture), but when the environment is moderate to very favorable, population change remains positive and sugar ratio is not as critical.

Aphids feed within phloem sieve tubes (Auclair 1963), and sucrose constitutes over 90% of the carbohydrates in cotton phloem sap (Tarczynski et al. 1992). Our samples represented total nonstructural carbohydrates (glucose, fructose, sucrose) from whole-leaf extracts and not just from phloem. Therefore, our data do not accurately reflect the sugar composition where the aphids actually feed. However, aphid stylet penetration through the leaf may be inter- or intracellular, in which case the aphid would be exposed to higher concentrations of glucose and fructose than occurs in just the phloem. For example, Butler et al. (1972) reported that the percentage of glucose exceeded that of either fructose or sucrose in cotton leaf nectary secretions, which indicates that cotton aphids could encounter high levels of glucose during probing. In their discussion on silverleaf whitefly, *Bemisia argentifolii* (Bellows and Perring), feeding, Freeman et al. (2001) reported that host acceptance is made before the stylets reach the phloem. If the ratio of glucose : fructose : sucrose in leaf tissue sap is not acceptable, the aphid may perceive that the leaf is nutritionally unacceptable and cease to probe and feed, which in turn would influence change in aphid numbers.

While average numbers of aphids per leaf were the most important variable affecting rate of change in aphid numbers, both plant nutrition (leaf moisture, sugar ratio) and temperature (physical environment) had a significant influence on change. Sugar ratio was the only independent variable in the above equation (also see Table 1) that has not been previously identified as influencing cotton aphid population dynamics.

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Table 1. Relationship between change in aphid numbers per day per week and biotic and abiotic variables. 1997-2000.

Independent Variable	t - value	P
x_1: aphids per leaf	12.51	< 0.001
x_2: (aphids per leaf)^2	- 7.88	< 0.001
x_3: sugar ratio (glu+fru/suc)	- 4.49	< 0.001
x_4: % leaf moisture	2.39	0.019
x_5: ambient temperature	4.12	< 0.001
x_6: (amb. temperature)^2	- 4.30	< 0.001

R^2 = 0.872, n = 103.

Table 2. Significant t-values for year main effects in multiple regression analysis for change in aphid numbers per day per week.

Independent variable	1997	1998	1999	2000
x_1: aphids per leaf	7.05	8.73	3.72	11.45
x_2: (aphids per leaf)^2	-4.02	-6.84	-2.49	-7.70
x_3: sugar ratio (glu+fru/suc)	--	-3.32	-3.65	--
x_4: % leaf moisture	--	2.75	2.52	--
x_5: ambient temperature	--	--	4.45	--
x_6: (amb. temperature)^2	--	--	-4.74	--
R^2	0.86	0.93	0.90	0.96
n	24	21	37	21

Table 3. Significant t-values for treatment effects in multiple regression analysis for change in aphid numbers per day per week.

Independent variable	Dryland Untreated	Dryland Karate	Irrigated Untreated	Irrigated Karate
x_1: aphids per leaf	10.13	8.09	7.97	8.58
x_2: (aphids per leaf)^2	-7.55	-6.16	-5.55	-5.36
x_3: sugar ratio (glu + fru / suc)	--	--	-2.42	-2.67
x_4: % leaf moisture	--	--	--	--
x_5: ambient temperature	--	5.33	--	--
x_6: (amb. temperature)^2	--	-5.29	--	--
R^2	0.88	0.94	0.85	0.91
n	27	27	26	23

Table 4. Yearly average sugar ratios in dryland and irrigated cotton.

Water Treatment	1997	1998	1999	2000
Dryland	0.62	0.92	2.93	0.68
Irrigated	0.73	1.65	5.14	1.18

Sugar ratio = concentration of (glucose + fructose / sucrose), µg/cm^2.

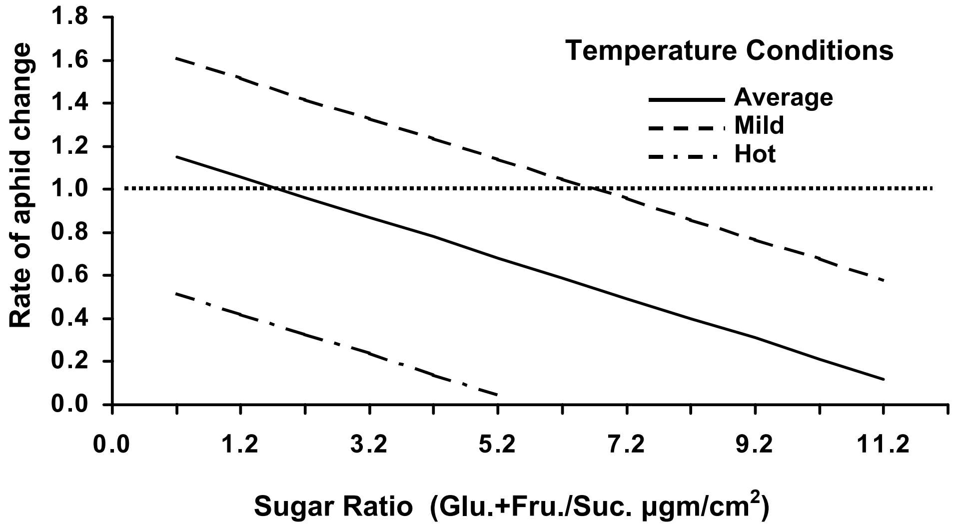

Figure 1. Change in aphid numbers per day per week versus sugar ratio, using 50 aphids per leaf and various temperature and leaf moisture conditions.