EFFECT OF NITROGEN FERTILITY ON COTTON-WHITEFLY INTERACTIONS

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Abstract

The impact of nitrogen fertility to cotton plant (*Gossypium hirsutum* L., c.v. Acala) on silverleaf whitefly (*Bemisia argentifolii* Bellows & Perring) population dynamics and the honeydew production and the related biochemical and physiological mechanisms were investigated in California. Five nitrogen levels were evaluated using urea in a randomized complete block design with five replicates. Treatments consisted of soil applications of 0, 100, 150 and 200 lbs nitrogen per acre, and a soil application of 100 lbs nitrogen together with a foliar application of 10 gal of low-biuret urea per acre. Applied nitrogen linearly increased densities of both adult and immature whiteflies during their peak population growth on cotton. Higher nitrogen treatments also resulted in higher densities of honeydew drops produced by the whiteflies. Also, the nitrogen treatments linearly enhanced cotton foliar photosynthetic rates and altered concentrations of soluble proteins, soluble amino acids and several soluble carbohydrates such as glucose, fructose and sucrose in cotton petiole. However, the applied nitrogen had no effect on seedcotton yield. Glucose levels were significantly correlated with densities of whitefly adults during the peak population size. Significant correlations between densities of adult or immature whiteflies and other cotton physiological parameters occurred on only a few sampling dates.

Introduction

The silverleaf whitefly (*Bermisia argentifolii* Bellows & Perring) is a major pest of cotton and other crops. The insect ingests plant phloem sap causing severe reduction in yield (Gerling et al., 1980; Bellows and Arakawa, 1998; Henneberry et al., 1995). In addition, this insect secretes honeydew that can fall onto lint to produce "sticky" cotton, resulting in problems during lint processing at textile mills (Perkins, 1986; Henneberry et al., 1996). The honeydew deposited on leaves provides suitable substrate for sooty mold development, which inhibits foliar photosynthesis (Yee et al., 1996).

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1135-1142 (2000)
National Cotton Council, Memphis TN

Dietary nitrogen is a limiting factor for growth and survival of phytophagous insects (White, 1984; Broadway and Duffey, 1986; Bi et al., 1994). The effect of cotton nitrogen status on silverleaf whitefly development and honeydew production was studied previously under greenhouse conditions (Blua and Toscano, 1994). The study indicated that subtle differences were found in whitefly development among the different levels of nitrogen fertilizer treatments. Early-instar whiteflies on higher nitrogen treated plants initiated production of honeydew earlier than those on plants treated with medium or low nitrogen but subsequently generated fewer droplets (Blua and Toscano, 1994). However, the effects of nitrogen fertility on cotton-whitefly interactions under field conditions are unknown. The present study was initiated to determine if different levels of nitrogen fertilizer treatment applied to cotton plants grown in the field increased whitefly densities and honeydew production, and determine the related biochemical and physiological mechanisms.

Materials and Methods

Experimental Plots

Cotton (*Gossypium hirsutum*, cv. Acala) was planted on 20 May at the Agricultural Experimental Station, University of California, Riverside. Five nitrogen levels were evaluated using urea in a randomized complete block design with five replicates. The plot size was 50 feet long and 25 feet wide with 10 feet of buffering area between neighboring plots in the same block. Each of the 5 blocks was separated by 4 rows of bare soil. Row spacing was 40 inches and there were 8 rows in each plot. Plants were thinned at the 4 node stage to a space of 4 inch intervals. Treatments consisted of soil applications of 0, 100, 150, and 200 lbs nitrogen per acre, and one treatment combining a soil application of 100 lbs nitrogen and a foliar application of 10 gal of low-biuret urea per acre. These treatments represented sub-optimal, optimal, and supra-optimal nitrogen fertility for cotton in California. Soil application of nitrogen was performed by side-dressing when the plants were at the 7 node stage (6 July). The foliar nitrogen was applied with a hand sprayer just prior to the flowering stage of the plants (27 August). Prior to planting, five soil samples (within 6 inches of top soil) across each experimental plot were analyzed for residual total nitrogen. Ten 3rd node cotton petioles in each plot were sampled twice during the cotton season to determine nitrate nitrogen levels. The sampling dates were 14 August and 11 September.

The field was furrow-irrigated. The frequency of irrigation was every two weeks prior to nitrogen fertilization and every week thereafter. The last irrigation date was 4 October.

Whitefly Densities

Densities of both adult and immature whiteflies were monitored throughout the cotton season. Sampling of adult whiteflies started in mid July and densities were determined

by counting numbers of whiteflies collected with an engine-powered vacuum over the whole 3rd or 4th row in each plot.

To sample for immature whiteflies, 20 5th main stem leaves were collected weekly from each of 20 randomly chosen plants across rows within each of the 25 plots. Sampling for immatures started in early September when population build-up started and densities on the underside of each leaf were determined using a microscope.

Whitefly Honeydew Drops

Water-sensitive paper (Ciba-Geigy, Basel, Switzerland) was used to collect whitefly honeydew. Honeydew drops which fell onto the paper appeared as distinct blue spots that were easily seen. The paper (3.3 x 2.6 cm^2) was secured with a paper clip onto the 10th node petiole (counted from the terminal) of each of 5 plants chosen randomly in each plot. After being exposed for about 1 h in plant canopies, the papers were collected at 16.00 hours (Pacific Standard Time) and the honeydew droplets counted under a microscope. Because adult whiteflies were easily disturbed during attachment of the papers to plants, only honeydew drops produced by immature whiteflies were counted. The honeydew droplet counts were made weekly during the peak population growth of whiteflies.

Seed Cotton Yield and Plant Heights

Seedcotton was harvested twice - on 13 November and 10 December, respectively. Open bolls in a 30 row-feet of center row within each plot were hand-picked, dried and weighed. Heights of 30 randomly selected plants in the row chosen for harvesting from each plot were then measured.

Photosynthetic Rate and Stomatal Conductance

To search for the physiological and biochemical mechanisms of whitefly-cotton interactions affected by the nitrogen treatments, photosynthetic rate, stomatal conductance, soluble proteins, soluble amino acids and soluble carbohydrates were monitored in cotton throughout the season. Photosynthetic rates and stomatal conductance were measured every week after the plants were fertilized using a LI-6200 portable photosynthesis system (LI-COR Inc., Lincoln, NE) equipped with a 1-L stirred cuvette. Measurements were taken near the plant terminal between 11.00 and 13.00 hours when ambient photosynthetic active radiation (PAR) exceeded 1700 $\mu M\ m^{-2}\ s^{-2}$. One 3rd main stem fully expanded leaf randomly selected from each of the 25 experimental plots was used for the measurement.

Soluble Proteins, Soluble Amino Acids and Soluble Carbohydrates

Cotton petioles were sampled between the hours of 15.00-16.00 weekly. Ten cotton petioles, from 10 individual plants in each plot, were excised, wrapped in aluminum foil and immediately dropped into liquid nitrogen to transport to a -80 C freezer. The sample was freeze-dried and then ground to powder for assays of soluble proteins, soluble amino acids and soluble carbohydrates. The 5th main stem petioles were sampled because the 5th main stem leaves were used for whitefly density estimates (Naranjo, 1996).

Protein content was determined by the Bradford method (Bradford, 1976). Ten milligrams of the tissue powder was vigorously vortexed in 1 ml of 0.1 M ice-cold phosphate buffer, pH 7.0, containing 1% PVP. The resulting mixture was centrifuged at 10,000 g at -2 C for 10 min, and the supernatant was used immediately for soluble protein measurements. A 50 µl aliquot of the supernatant was mixed with 150 µl of Bio-Rad protein assay reagent (Bio-Rad, Richmond, CA). Absorbance of the reaction mixture was then read at 595 nm and protein content was determined from a standard curve established using bovine serum albumin (Sigma Chemical Co.).

Amino acids were extracted and quantified according to a method described by Mitchell et al. (1992). Two milliliters of ethanol extract (as described above for carbohydrate extraction) were dried under vacuum, resuspended in 100 µl of a drying reagent consisting of triethylamine:absolute ethanol:HPLC grade water (1:1:1, v/v), and dried again under vacuum. The amino acids were then converted to their PITC derivatives. Briefly, 200 µl of PITC reagent (phenylisothiocyanate : absolute ethanol:triethylamine:HPLC grade water 1:7:1:2, v/v) was added to each sample, and after 10 min the samples were dried to remove excess reagent. Samples were resuspended in 4.0 ml of resuspension buffer (15 mM sodium acetate, 3% [v/v] acetonitrile, and 0.025% [v/v] triethylamine, adjusted to pH 7.4 with phosphoric acid) and filtered through 0.2 µm syringe filters. A 20 µl aliquot of the filtrate from each sample was injected into a Rainin Dynamax ODS column (4.6 x 25 mm) held at 48 C. The column was connected to a Beckman binary gradient chromatography system and injections were performed by a Spectraphysics SP8780 autosampler. Amino acids from Sigma Chemical Co. were used as standards. Total level of soluble amino acids was the sum of all the detectable individual amino acid levels.

Extraction and quantification of carbohydrates were determined following a method described by Hendrix (1993) and Zhao and Oosterhuis (1998). Ten milligrams of the tissue powder were extracted three times, 8 min each time, in 1.2 ml of 80% ethanol in an 80 C water bath. Half a milliliter of the combined extract was pipetted into a centrifugal microfilter tube assembled with 20 mg of active charcoal to adsorb the colored pigments. The tube was covered and vortexed for 2 min and then centrifuged for 5 min to obtain a clear alcohol extract. Four 10 µl aliquots from each sample were pipetted into separate wells of a microplate and dried at 50 C for 15 min to remove alcohol. Thereafter, 20 µl of

deionized water, 100 µl of glucose-6-P dehydrogenase/iodonitrotetrazolium violet mixture (glucose kit 115A, Sigma Chemical Co.) and 10 µl of phosphoglucose isomerase (PGI enzyme, 0.25 units) were added into each well of the microplate under reduced room illumination. The sample plates were incubated at 37 C for 15 min, and then absorbance was read at 492 nm using D-glucose as a standard. Subsequently, 15 µl of invertase (83 units) was added to each well, the microplate was reincubated at 37 C for a further 15 min and absorbance was read again at 492 nm for sucrose concentration.

Statistics

The least significant difference (LSD) test in one-way randomized complete block general linear models procedure (GLM) in SAS was used to analyze the data. Densities of whitefly adults from vacuum samples were transformed using the formula $(y + 0.5)^{1/2}$ whereas densities of immature whiteflies and densities of honeydew droplets were transformed using the formula log (y + 1) before the analysis of variance and regression in order to normalize the data (Yee and Toscano, 1996). To determine the relationship between plant physiological factors such as photosynthetic rate, stomatal conductance, sugars or proteins and densities of adult or immature whiteflies, simple and multiple regression analyses were used.

Results

Residual Total Nitrogen in Soil and Nitrate Nitrogen in Cotton Petioles

Total soil nitrogen in all experimental plots prior to nitrogen treatments was consistent with a level around 0.04% ($P > 0.05$) (Table 1).

There was a positive linear response between the levels of nitrate nitrogen in petioles and nitrogen rate applied per acre on both sampling dates (Table 2). At 200 lbs N/acre, nitrate nitrogen levels were approximately 5-fold and 5.6-fold higher than in control (0 lbs N/acre) plots on 14 August and 11 September, respectively.

Whitefly Densities

There was a positive response between nitrogen treatments and densities of adult or immature whiteflies on most sampling dates during peak population growth (Figures 1 and 2, Tables 3 and 4). The population growth started in mid-September and declined at the end of October. The highest rate of nitrogen (200 lbs/acre) increased densities of whitefly adults by 50% on 23 October compared to the control (0 lbs/acre) (Figure 1). This treatment enhanced densities of immature whiteflies by as much as 170% at the end of October (Figure 2).

Whitefly Honeydew Drops

As a result of increased densities of immature whiteflies, there was also a significant increase in honeydew production associated with nitrogen treatments (Figure 3). Differences in drop densities among the different treatments varied by up to 140%.

Plant Heights and Seed Cotton Yield

Applied nitrogen linearly stimulated the vegetative growth of cotton (Figure 4). Plant heights ranged from 98 cm in the 0 N treatment to over 200 cm in the 200 lbs N/acre treatment. Higher rates of nitrogen (150 and 200 lbs/acre) slightly reduced seedcotton yield compared to the other treatments (Table 5), although the differences were not generally statistically significant ($P > 0.05$).

Photosynthetic Rate and Stomatal Conductance

The photosynthetic rates of cotton treated with different levels of nitrogen fertilizer are shown in Figure 5. Peak photosynthetic rates for all treatments were recorded from early August to early September. In general, the application of nitrogen significantly ($P < 0.05$) increased cotton foliar photosynthetic rates throughout the season. Increases in photosynthetic rates among the different levels of applied nitrogen (from 100-200 lbs/acre) were less striking. Results of regression analysis indicated that applied nitrogen linearly boosted cotton foliar photosynthetic rates on most of the measuring dates (Table 6). This trend was more apparent later in the season (Table 6).

The effects of nitrogen on stomatal conductance of leaves from different treatments followed a similar trend to those seen with photosynthetic rates (Figure 6). In general, applied nitrogen (from 100-200 lbs N /acre) significantly increased foliar stomatal conductance relative to the control (0 lbs N/acre) throughout the season. The nitrogen also linearly enhanced foliar stomatal conductance on most of the sampling dates (Table 6).

Soluble Proteins, Soluble Amino Acids, and Soluble Carbohydrates

Nitrogen fertilizer treatments affected levels of soluble proteins in cotton petioles (Figure 7). Early in the season, especially before peak flowering of the cotton plants (around the middle of August), the applied nitrogen linearly increased levels of soluble proteins. Later in the season, there was a linear decrease in proteins (Figure 7 and Table 6).

Levels of total amino acids were enhanced on most of the sampling dates with the application of nitrogen (Figure 8). Peak levels of amino acids in petioles occurred later in the season. This peak corresponds with the peak population growth of both adult and immature whiteflies (Figures 1, 2 and 8).

The applied nitrogen altered sucrose levels in cotton petioles (Figure 9 and Table 6). The nitrogen generally increased sucrose levels before September, with levels decreasing thereafter.

Glucose levels also changed with the application of nitrogen (Figure 10 and Table 6). In all plots treated with nitrogen, there was a striking increase in glucose levels during periods of 24 July to 14 August and 25 September to 9 October.

The nitrogen generally enhanced fructose levels in cotton petioles in both early and late parts of the season (Figure 11). The increase was linear in about half of the sampling dates (Table 6).

Relationship Between Whitefly Densities and Plant Physiological Parameters

Tables 3 and 4 show the relationships between densities of adult or immature whiteflies and levels of glucose, fructose, sucrose, proteins, total amino acids, photosynthetic rate, or stomatal conductance. Glucose levels were significantly correlated with densities of whitefly adults during the peak population size. There was a significant correlation between densities of adult or immature whiteflies and other physiological parameters of cotton on only a few sampling dates.

Discussion

Nitrogen fertilizer treatments linearly increased densities of both adult and immature whiteflies on cotton at Riverside in California (Figures 1, 2, Tables 3 and 4). This result is consistent with earlier findings which showed that densities of cotton aphids were affected by nitrogen on cotton in California.

Glucose may be an important component determining whitefly population dynamics on cotton. It may function as a nutrient for whitefly development. The peak total amino acid production corresponds with the peak population growth of both adult and immature whiteflies. Amino acids may also function as nutrients for whitefly growth.

It is surprising that the applied nitrogen had no effect on seedcotton yield, although the vegetative growth was linearly increased. In 1998, the cotton season was delayed (planting occurred on 20 May, whereas normal planting is on 20 March) due to low spring temperatures. In addition to higher densities of whiteflies, it is likely that the applied nitrogen extended the growing season because there were still many immature bolls on plants treated with nitrogen (data not shown) when the plants were killed by frost. It was reported that cotton growers in California usually applied 200 lbs N/acre to their cotton field. Apparently, growers in 1998 had no benefit from nitrogen fertilizer applications; instead they suffered economic losses from nitrogen fertilizer costs, application costs, and whitefly outbreaks.

In summary, applied nitrogen had no effect on seedcotton yield but resulted in increased densities of whiteflies and the honeydew drops.

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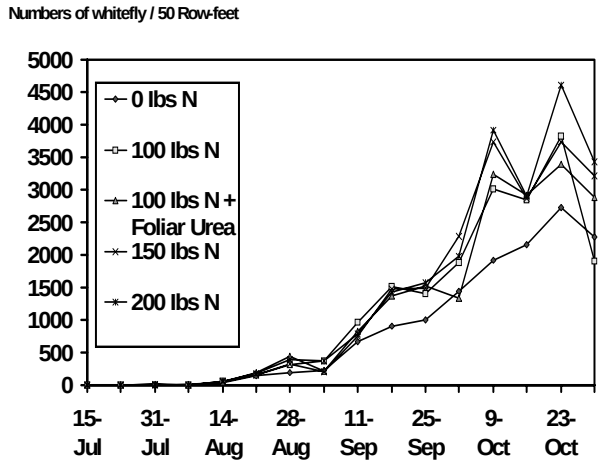

Figure 1. Effect of nitrogen fertilizer treatments on densities of adult whiteflies on cotton.

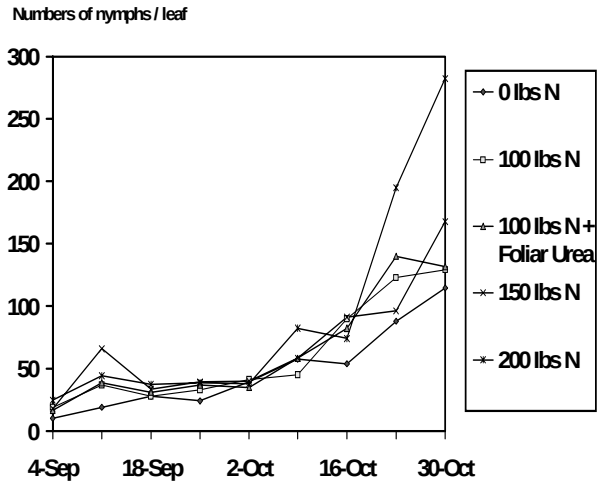

Figure 2. Effect of nitrogen fertilizer treatments on densities of immature whiteflies on cotton.

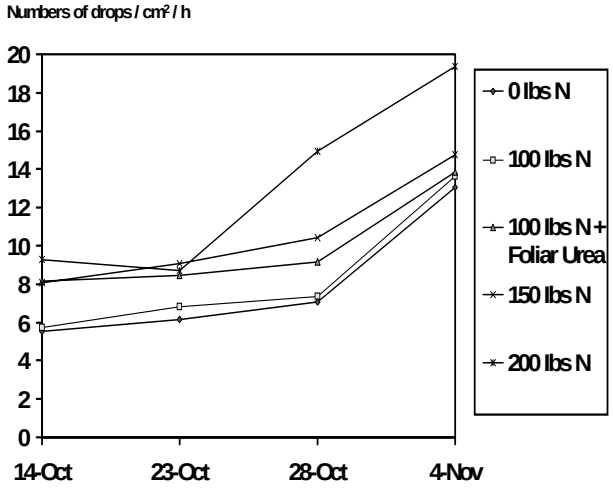

Figure 3. Effect of nitrogen fertilizer treatments on densities of honeydew drops produced by whiteflies.

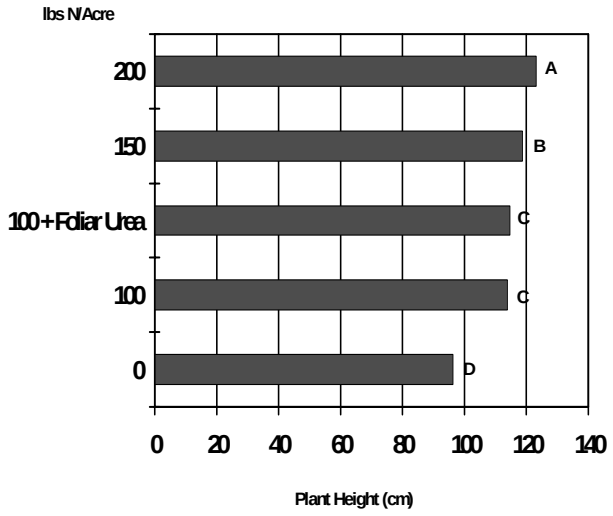

Figure 4. Effect of nitrogen fertilizer treatments on vegetative growth of cotton. Means followed by different letter are significantly different at $P < 0.05$.

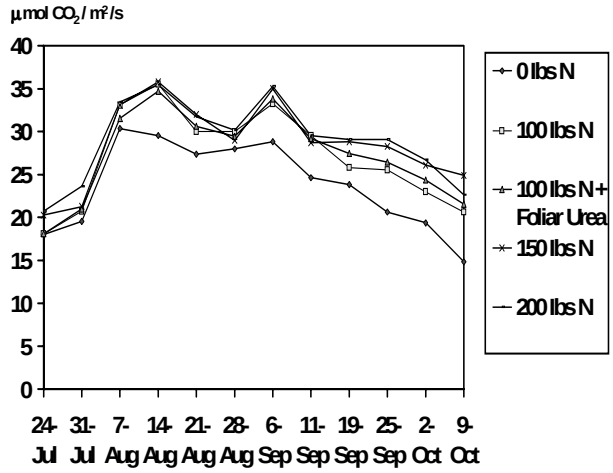

Figure 5. Effect of nitrogen fertilizer treatments on cotton foliar photosynthetic rate.

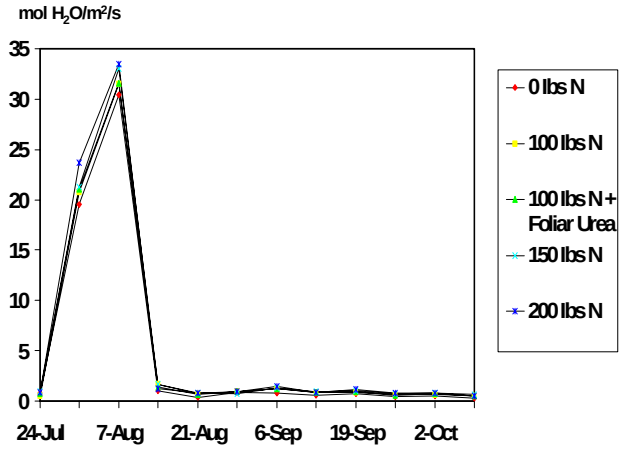

Figure 6. Effect of nitrogen fertilizer treatments on cotton foliar stomatal conductance.

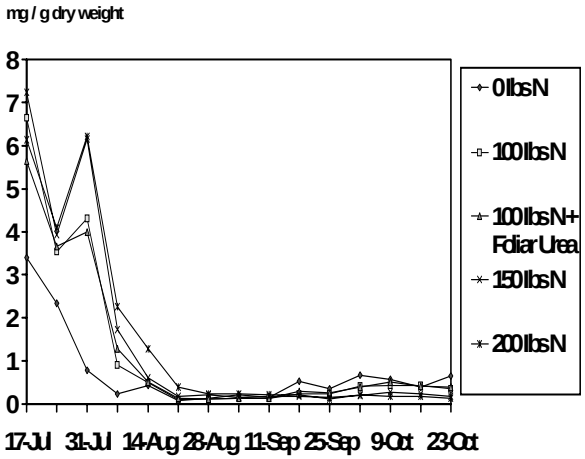

Figure 7. Effect of nitrogen fertilizer treatments on levels of soluble proteins in cotton petioles.

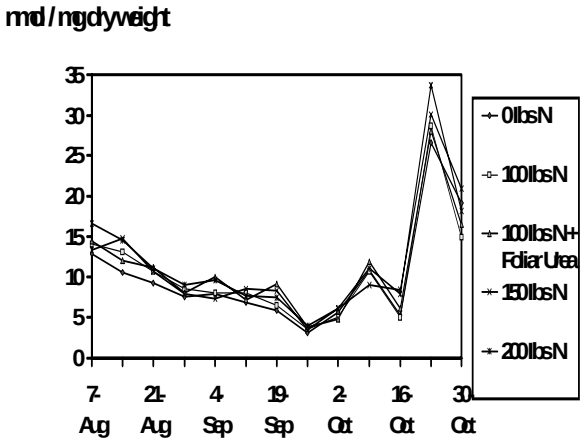

Figure 8. Effect of nitrogen fertilizer treatments on levels of total amino acids in cotton petioles.

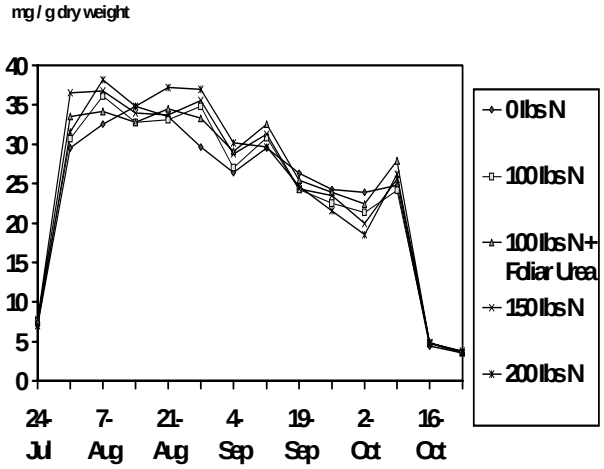

Figure 9. Effect of nitrogen fertilizer treatments on sucrose levels in cotton petioles.

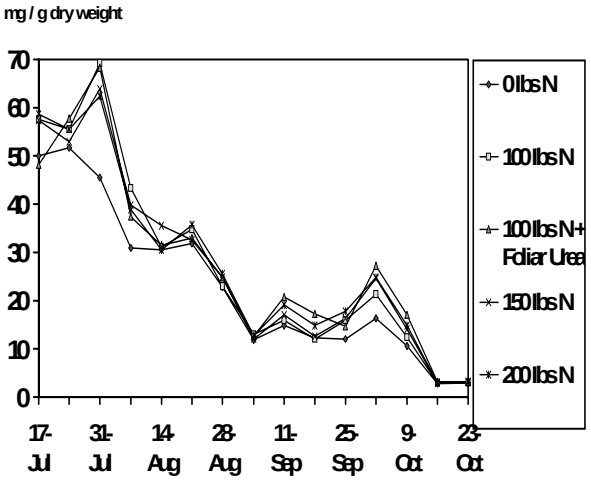

Figure 10. Effect of nitrogen fertilizer treatments on glucose levels in cotton petioles.

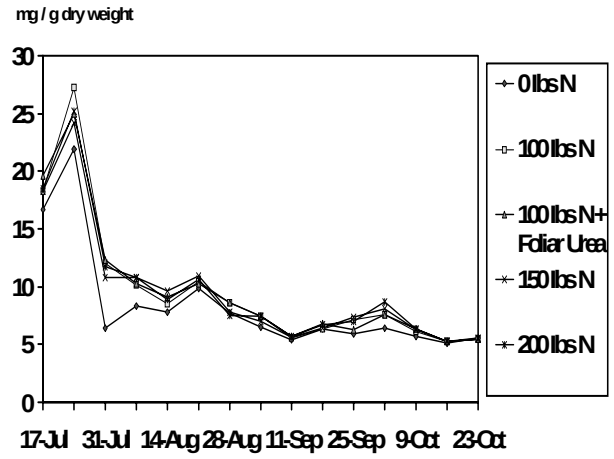

Figure 11. Effect of nitrogen fertilizer treatments on fructose levels in cotton petioles.

Table 1. Residual soil nitrogen levels in experimental plots prior to nitrogen treatments

Experimental Plots Prior to Nitrogen Treatments (lbs/acre)	Residual Soil Nitrogen[1] Levels (%)
0	0.040 (0.001) a[2]
100	0.040 (0.001) a
100 plus foliar urea	0.041 (0.001) a
150	0.042 (0.001) a
200	0.039 (0.001) a

[1]Soil nitrogen was analyzed for total Kjeldahl nitrogen.
[2]Means in columns followed by different letter are significantly different at $P < 0.05$. Numbers in parentheses are standard errors.

Table 2. Effect of nitrogen treatments on NO_3-N levels in cotton petiole

Nitrogen Treatments (lbs/acre)	NO_3-N Levels (ppm)	
	14 August	*11 September*
0	432 (58) c	26 (10) b
100	992 (115) b	58 (26) b
100 plus foliar urea	1072 (130) b	38 (8) b
150	1458 (187) b	70 (23) ab
200	2048 (247) a	146 (48) a

Means in columns followed by different letter are significantly different at $P < 0.05$. Numbers in parentheses are standard errors.

Table 3. Results of regression analyses between [1]densities of adult whiteflies and amounts of N applied to cotton, or cotton physiological status

	Adults &N		Adults & Glucose		Adults & Fructose		Adults & Sucrose		Adults & Protein		Adults & Total Amino Acids		Adults & Photosynthetic Rate		Adults & Stomatal Conductance	
Date	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**
7/24	0.5949	0.0130	0.6140	0.0112	0.9716	0.0001	0.1809	0.0765	0.1714	0.0797	---	---	0.6940	0.0069	0.3693	0.0352
7/31	0.2154	0.0650	0.1520	0.0871	0.0625	0.1428	0.1713	0.0798	0.3148	0.0439	---	---	0.8824	0.0010	0.6707	0.0080
8/7	0.0589	0.1453	0.8391	0.0018	0.8922	0.0008	0.8033	0.0028	0.2789	0.0508	---	---	0.0675	0.1380	0.9141	0.0005
8/14	0.1363	0.0978	0.0511	0.1555	0.2850	0.0495	0.0554	0.1504	0.3874	0.0326	0.1366	0.0780	0.9463	0.0002	0.1812	0.0764
8/21	0.3624	0.0378	0.0191	0.2163	0.8579	0.0014	0.0640	0.1413	0.4023	0.0307	0.4977	0.0211	0.2033	0.0694	0.9212	0.0004
8/28	0.0448	0.1671	0.1819	0.0761	0.9541	0.0002	0.0432	0.1660	0.7371	0.0050	0.9831	0.0000	0.0017	0.3548	0.6053	0.0118
9/4	0.3220	0.0437	0.5606	0.0149	0.3704	0.0350	0.7791	0.0035	0.5851	0.0132	0.4826	0.0216	0.0899	0.1200	0.0251	0.1998
9/11	0.5302	0.0166	0.5207	0.0182	0.2893	0.0487	0.8827	0.0010	0.6120	0.0114	0.5116	0.0198	0.2161	0.0657	0.4287	0.0274
9/19	0.0152	0.2240	0.6523	0.0090	0.7831	0.0034	0.9580	0.0001	0.1217	0.1009	0.0341	0.1877	0.4409	0.0260	0.4697	0.0230
9/25	0.0380	0.1786	0.0550	0.1509	0.6525	0.0090	0.0890	0.1236	0.7530	0.0044	0.8084	0.0027	0.0810	0.1265	0.1698	0.0803
10/2	0.0522	0.1605	0.0750	0.1313	0.0205	0.2123	0.2016	0.0699	0.4847	0.0215	0.0008	0.3777	0.2305	0.0619	0.5605	0.0149
10/9	0.0094	0.2677	0.0782	0.1288	0.1423	0.0912	0.0429	0.1664	0.1821	0.0761	0.2204	0.0659	0.1471	0.0892	0.3863	0.0328
10/16	0.1625	0.0854	0.0762	0.1303	0.1175	0.1031	0.0295	0.1898	0.0343	0.1805	0.6585	0.0090				
10/23	0.0576	0.1542	0.5998	0.0122	0.9049	0.0006	0.9420	0.0002	0.1039	0.1108	0.8077	0.0026				
10/30	0.0550	0.1529	0.4605	0.0239	0.8119	0.0023	0.3715	0.0349			0.9896	0.0000				

[1]Densities of whitefly adults were transformed using $(y + 0.5)^{1/2}$.

Table 4. Results of regression analyses between [1]densities of immature whiteflies and amounts of N applied to cotton, or cotton physiological status

	Nymphs & N		Nymphs & Glucose		Nymphs & Fructose		Nymphs & Sucrose		Nymphs & Protein		Nymphs & Total Amino Acids		Nymphs & Photosynthetic Rate		Nymphs & Stomatal Conductance	
Date	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**
9/4	0.0474	0.1652	0.2545	0.0561	0.1606	0.0837	0.0536	0.1525	0.8539	0.0015	0.0481	0.1611	0.4516	0.0249	0.6503	0.0091
9/11	0.0651	0.1390	0.0003	0.4437	0.0036	0.3135	0.0020	0.3445	0.1016	0.1123	0.0867	0.1201	0.0081	0.2627	0.1038	0.1109
9/19	0.2501	0.0593	0.0658	0.1396	0.0273	0.1946	0.0240	0.2024	0.0390	0.1724	0.2140	0.0687	0.2348	0.0608	0.4535	0.0247
9/25	0.0784	0.1274	0.2599	0.0548	0.5960	0.0124	0.5629	0.0148	0.8301	0.0020	0.6653	0.0085	0.0058	0.2867	0.0612	0.1442
10/2	0.4529	0.0257	0.6747	0.0078	0.2475	0.0577	0.1408	0.0919	0.0809	0.1266	0.0564	0.1548	0.0156	0.2288	0.0906	0.1195
10/9	0.3893	0.0338	0.1019	0.1121	0.5329	0.0171	0.3066	0.0454	0.8906	0.0008	0.4282	0.9531	0.1961	0.0716	0.4614	0.0238
10/16	0.1317	0.0967	0.2463	0.0580	0.9890	0.0000	0.3840	0.0331	0.7948	0.0030	0.5306	0.0181				
10/23	0.0422	0.1727	0.5454	0.0161	0.6173	0.0110	0.6824	0.0074	0.0725	0.1335	0.2519	0.0592				
10/30	0.0066	0.2810	0.5511	0.0157	0.0433	0.1659	0.1897	0.0736			0.1659	0.0854				

[1]Densities of immature whiteflies were transformed using log (y + 1).

Table 5. Effect of nitrogen treatments on seedcotton yield

Nitrogen Treatments (lbs/acre)	Seedcotton Yield (g/30 row-feet)
0	1657.6 (136.9) a
100	1612.4 (152.8) a
100 plus foliar urea	1646.0 (92.9) a
150	1573.2 (50.4) a
200	1532.0 (48.8) a

Means in columns followed by different letter are significantly different at $P < 0.05$. Numbers in parentheses are standard errors.

Table 6. Results of regression analyses between cotton physiological status and amount of nitrogen applied to the cotton

	Glucose & N		Fructose & N		Sucrose & N		Protein & N		Total Amino Acids & N		Photosynthetic Rate & N		Stomatal Conductance & N	
Date	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**	**R^2**
7/24	0.5113	0.0195	0.1966	0.0693	0.0010	0.8795	0.0001	0.4804			0.2787	0.0528	0.3696	0.0333
7/31	0.0094	0.2003	0.0074	0.2546	0.1167	0.1022	0.0000	0.7958			0.0476	0.1636	0.0947	0.1200
8/7	0.0213	0.1872	0.0074	0.0275	0.0901	0.1250	0.0000	0.8107			0.5907	0.0133	0.6050	0.0121
8/14	0.5898	0.0131	0.0315	0.1847	0.9474	0.0002	0.0000	0.4267	0.0022	0.3530	0.0081	0.2777	0.5255	0.0168
8/21	0.2758	0.0535	0.4196	0.0298	0.4207	0.0289	0.0000	0.4413	0.0689	0.1378	0.0061	0.2898	0.0039	0.3086
8/28	0.1187	0.1061	0.7434	0.0045	0.0015	0.3717	0.0097	0.2379	0.5743	0.0146	0.0936	0.1169	0.8751	0.0011
9/4	0.6342	0.0104	0.0338	0.1738	0.0029	0.3334	0.8217	0.0012	0.6993	0.0066	0.0016	0.3602	0.0022	0.3532
9/11	0.1620	0.0862	0.2646	0.0562	0.7332	0.0045	0.0256	0.1633	0.1848	0.0754	0.0081	0.2539	0.0074	0.2506
9/19	0.3145	0.0459	0.2987	0.0488	0.3016	0.0475	0.0002	0.4264	0.0788	0.1241	0.0005	0.4263	0.0028	0.3390
9/25	0.0441	0.1715	0.0152	0.2353	0.0383	0.1776	0.0074	0.2832	0.0737	0.1376	0.0000	0.7654	0.0000	0.5464
10/2	0.0097	0.2539	0.000	0.7221	0.0059	0.2958	0.0001	0.5184	0.3015	0.0406	0.0009	0.4007	0.0015	0.3703
10/9	0.0900	0.1215	0.0617	0.1478	0.3259	0.0438	0.0011	0.3758	0.0021	0.8326	0.0000	0.5063	0.0003	0.4272
10/16	0.0046	0.2817	0.0239	0.2035	0.1358	0.0920	0.0323	0.1714	0.0020	0.3455				
10/23	0.2293	0.0650	0.9126	0.005	0.7157	0.0060	0.0000	0.7442	0.2424	0.0609				
10/30	0.0971	0.1182	0.2696	0.0551	0.1107	0.1066			0.9487	0.0002				