2002 BELTWIDE COTTON CONFERENCES, ATLANTA, GA – JANUARY 8-12

RELATIONSHIP BETWEEN NUMBERS OF COTTON APHIDS PER LEAF AND STICKY LINT

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Abstract

The study was conducted in the northern Texas Rolling Plains in 1999 to define the relationship between number of cotton aphids, *Aphis gossypii* Glover, and resulting contamination of cotton lint by honeydew. Whole plot s were three furrow irrigation management treatments: cotton grown without supplemental irrigation (dryland), irrigated cotton with last irrigation in mid August, and irrigated cotton with last irrigation in late August. Subplots within each irrigation treatment included an untreated check, a plot treated with lambda-cyhalothrin to stimulate aphid population increase, a plot treated with lambda-cyhalothrin followed by pymetrozine after aphids began to increase, and a plot treated with lambda-cyhalothrin followed by thiamethoxam after aphids began to increase. Cotton aphids were counted on leaves picked from the top- and bottom-half of the plant. Cotton lint was analyzed for contamination by glucose, fructose, sucrose, and melezitose secreted by cotton aphids, and percentage leaf moisture and nitrogen and leaf sucrose concentrations were determined. The manual sticky cotton thermodetector was used to determine degree of lint stickiness. There was a significant relationship between thermodetector counts and melezitose contamination on lint, and a melezitose concentration of 90.9 μg/g of lint was associated with a thermodetector count of 10, the threshold for sticky lint problems in textile mills. An equation was developed to estimate melezitose concentration on lint as a function of average numbers of aphids per leaf and the interaction between percentage leaf moisture and nitrogen. The number of aphids per leaf associated with a melezitose concentration of 90.9 μg/g of lint ranged from 11.1 to 50.1, depending on percentage leaf moisture and nitrogen. The threshold for sticky lint problems occurred when aphid numbers ranged between 11.1 and 50.1 per leaf after bolls open.

Introduction

The cotton aphid, *Aphis gossypii* Glover, secretes honeydew that contaminates cotton lint after bolls open; however, there is little data on degree of sticky lint problems associated with cotton aphid populations. Problems associated with sticky lint include higher costs of insect control, increased trash in seed cotton, special handling requirements at cotton gins, reduced efficiency at textile mills, and reduced profits (Hector and Hodkinson 1989, Ellsworth et al. 1999). Cotton aphids were reported to be responsible for the sticky cotton problem in Israel from 1983 to 1985 (Broza 1986) and in California in 1986 (Perkins and Bassett 1988). The sticky cotton problem in the Texas High Plains in 1995 (Lloyd 1997) was the result of cotton aphid honeydew and plant-produced physiological sugars on lint resulting from an early frost.

After ingesting sucrose from phloem, the cotton aphid metabolizes sucrose into several dozen sugars that are excreted in the honeydew (Hendrix 1999), including glucose, fructose, sucrose, trehalulose, melezitose and other oligosaccharides, but melezitose constitutes 22 - 38% of the honeydew sugars (Hendrix et al. 1992, Hendrix and Henneberry 2000). Melezitose, a trisaccharide found primarily in cotton aphid honeydew, is relatively non-sticky but causes stickiness in water sprays at concentrations $\geq$0.60% (Miller et al. 1994).

The manual sticky cotton thermodetector, developed by the International Center for Agronomic Research and Development (CIRAD, Montpellier, France), is recommended by the International Textile Manufacturers Federation (ITMF) for measuring cotton lint stickiness (Perkins and Brushwood 1994). Results from the thermodetector and minicard (another ITMF recommended test for stickiness measurement) methods are highly correlated and both detect insect sugars on cotton lint effectively (Hector and Hodkinson 1989, Brushwood and Perkins 1993). Brushwood and Perkins (1993) described the procedure for measuring cotton lint stickiness potential using the thermodetector, and they reported that a reducing sugar concentration of 0.4% on lint produces a minicard rating of 1, which is near the threshold for causing sticky lint problems. A minicard rating of 1.5 was associated with a melezitose concentration of 0.6% on lint (Miller et al. 1994). A minicard rating of 1 is associated with a thermodetector count of 10 sticky spots on a 10 g sample of lint (Perkins and Brushwood 1994). Thus, a thermodetector count of 10 is near the threshold for sticky lint problems caused, in part, by melezitose in cotton aphid honeydew.

The objectives were to define (1) the relationship between melezitose concentration on cotton lint and thermodetector counts, and (2) the relationship between aphid numbers and melezitose concentration so that the relationships between the number of cotton aphids per leaf and the threshold for sticky lint problems could be defined.

Materials and Methods

TAMCOT Sphinx was planted 28 April 1999 at the Texas Agricultural Experiment at Chillicothe. Seeding rate was 5.7 seeds per foot of row in 40" row spacings. Fertilizer was applied immediately before planting at 30 lb N/acre in dryland plots and 60 lb N/acre in irrigated plots. Subplot size was 10 rows wide by 70' long.

A split-plot experimental design, arranged as a randomized complete block with three replications, was used. Whole plots were three irrigation treatments: (1) dryland - no supplemental irrigation during the growing season; (2) early termination of irrigation with last application in mid August; and (3) late termination of irrigation with last application in late August. The latter two treatments are referenced as irrigated-early termination and irrigated-late termination, respectively. Plots were furrow irrigated on 15 and 29 July and 12 August in both irrigated-early and irrigated-late termination treatments, with a final irrigation on 27 August in the irrigated-late termination treatment.

Subplots were four chemical treatments: (1) an untreated check; (2) an application of lambda-cyhalothrin (Karate® EC at 0.04 lb [AI] per acre, Zeneca; Wilmington, DE) during anticipated periods of increased bollworm, *Helicoverpa zea* (Boddie), activity; (3) an application of lambda-cyhalothrin followed by an application of pymetrozine (Fulfill™ 50 WG at 1.5 oz [AI] per acre, Syngenta, Greensboro, NC) when cotton aphid numbers began to rapidly increase; and (4) an application of lambda-cyhalothrin followed by an application of thiamethoxam (Actara™ 25 WG at 0.75 oz [AI] per acre, Syngenta, Greensboro, NC) when cotton aphids began to increase. Lambda-cyhalothrin was applied on 2 and 25 August, while pymetrozine and thiamethoxam were applied 8 and 17 September, respectively. Slosser et al. (2001) discussed the influence of these treatments on aphid populations. 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 10.8 gpa.

Although aphids were sampled weekly from mid July to late October, only the data taken on 22 and 28 September and 20 October were used in analyses. Aphids were counted on 10 leaves picked from the top-half and on 10 leaves from the bottom-half of the plant per plot on 22 September, but sample size was reduced to 5 top-half and 5 bottom-half leaves thereafter because of very high aphid numbers on 28 September.

Leaf discs were cut from cotton leaves for analysis of sugar content (glucose, fructose, sucrose, trehalulose, and melezitose) (Hendrix and Peelen 1987, Hendrix 1993) on the same dates that aphids were counted. A leaf from the fifth mainstem node below the terminal was selected, and six discs, each measuring 0.05 in^2 in area, were cut with a cork borer from each of two leaves per plot. The six discs from each leaf were placed into 0.07 oz of an 80% ethyl alcohol solution in a stoppered test tube (0.05 x 0.39 in) 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 (25°F) until analysis for sugar content.

Ten leaves from the fifth mainstem node below the terminal were collected to determine leaf moisture on the same day from the same plots that were sampled for leaf sugar content. Leaves were pulled from the stem of the plants and placed immediately into a plastic bag in a cool chest with ice. Within an hour of being picked, leaf petioles were cut off with a sharp knife, and the leaves were weighed and then oven-dried at 122°F for 48 hours. Percentage leaf moisture was calculated by subtracting dry weight from wet weight, dividing by wet weight, and multiplying by 100. The leaves that were sampled for leaf moisture content, after drying, were then used to determine percentage leaf nitrogen using the Kjeldahl procedure (AOAC 1980).

Open cotton bolls were pulled from 3 ft of row in a uniform stand of cotton in each plot on 23 and 30 September and 10 November 1999. Lint was picked from the burs, and a small laboratory bench-top gin was used to separate seed and lint. Lint from each plot was thoroughly mixed, and samples from each harvest date were sent to the USDA-ARS Cotton Quality Laboratory, Clemson, SC, for analysis using the manual sticky cotton thermodetector. Lint samples were also sent to the USDA-ARS Western Cotton Research Laboratory, Phoenix, AZ, and honeydew sugars were extracted from the lint for HPLC analysis following the procedures outlined by Henneberry et al. (2000).

Data were analyzed with a repeated measures analysis of variance and by linear and stepwise regression using Statistix 7 (Anonymous 2000). Main factors were irrigation treatment, chemical treatment, and sample date. Means were separated with protected least significant difference ($\propto = 0.05$).

Results and Discussion

The relationship between concentration of each sugar on lint and thermodetector counts was investigated (Table 1). While correlations between individual and total sugars and thermodetector counts were significant, the highest correlation was obtained with melezitose. A stepwise multiple regression selected only melezitose, indicating that melezitose was the primary sugar responsible for the thermodetector readings. Trehalulose was not detected in the HPLC analysis for sugars. The linear relationship (equation 1) between thermodetector counts (*TD*) and melezitose concentration on lint (*MC*) was $TD = -10.105 + 0.221(MC)$. Values used in this equation ranged from 55.1 to 277.7 µg/g of lint for melezitose and from 1.7 to 47.7 for thermodetector counts. By setting the thermodetector count to 10, which is associated with unacceptable levels of sticky lint (Perkins and Brushwood 1994), a melezitose concentration of 90.9 µg/g of lint was estimated to be the threshold for sticky lint caused by cotton aphid infestations.

Percentage leaf moisture, percentage leaf nitrogen, and sucrose concentration in leaves were not correlated with melezitose concentration on cotton lint, and the two-way interactions between these three variables were not correlated with melezitose on lint (Table 2). However, there was a significant correlation between aphid numbers on leaves and melezitose concentration on lint. A multiple stepwise regression indicated that aphid numbers (*AN*) and the percentage leaf moisture by leaf nitrogen interaction (*%M%N*) were significantly correlated with melezitose concentration (*MC*) on lint, and the equation (equation 2) for this relationship is MC = 176.660 - 0.589 (*%M%N*) + 0.642 (*AN*), where n = 18, Student's *t* and associated *P* values for %M%N interaction are -3.30 and 0.005, and for aphid numbers, 24.23 and <0.001. Percentage leaf nitrogen and percentage leaf moisture ranged from 2.4 to 3.0 and from 62.4 to 69.9, respectively, and aphid numbers ranged from 2.2 to 348.8 per leaf. The number of aphids per leaf estimated to cause an unacceptable level of melezitose contamination on lint could be determined (Equation 2) by setting melezitose concentration to 90.9 (from Equation 1). At 90.9 µg melezitose, aphid estimates ranged from 11.1 per leaf at 2.5% leaf nitrogen and 63% leaf moisture to 50.1 aphids per leaf at 2.9% leaf nitrogen and 69% leaf moisture (Table 3). By inference, a thermodetector count of 10 was attained when aphid numbers ranged from 11.1 to 50.1 per leaf. The lower range in our calculated values agrees with the threshold range of 10 to 15 aphids per leaf reported by Rosenheim et al. (1995) and Godfrey et al. (2000).

An unacceptable level of melezitose contamination was attained under conditions of plant stress, as reflected in lower aphid numbers at the lower levels of either leaf moisture or leaf nitrogen. Metabolism of melezitose may be a mechanism of osmoregulation enabling the cotton aphid to maintain internal water balance on stressed cotton plants. This could explain why the threshold for aphids was lower when percentage leaf moisture and leaf nitrogen were lower (Table 3).

The linear relationship (equation 3) between aphid numbers (*AN*) and thermodetector counts (*TD*) is $TD = 6.040 + 0.121\ (AN)$, where $r^2 = 0.783$, $F = 122.48$, $P < 0.001$, n = 36. When the thermodetector count was set to 10, the estimated aphid number was 32.7, which is intermediate between 11.1 and 50.1 illustrating the importance of host plant condition on the amount of melezitose secreted by cotton aphids onto cotton lint (Table 3). Equation 3 provides an estimate of thermodetector values when plant quality parameters (percentage leaf moisture and nitrogen) are not available.

Lint contamination by aphid honeydew is generally attributed to a late-maturing crop in the fall, accompanied by aphid infestations during boll opening and insufficient rainfall to cleanse the lint. However, rain typically cleanses the lint of honeydew prior to harvest in the Texas Rolling Plains. In California, Rosenheim et al. (1995) have shown that rain reduces lint stickiness. Aphid numbers in the range 11 - 50 represent a very low threshold for development of sticky lint problems. Avoidance of the problem may be a better alternative to using insecticides to control such low numbers, considering application expenses, a potential need for multiple applications, and extended re-entry intervals for further sampling and harvest. Slosser et al. (2001) reported that irrigations in late August, coupled with use of lambda-cyhalothrin for control of bollworms, consistently resulted in high aphid numbers during September when cotton bolls are opening. Irrigation management, insecticide selection, and timely harvest are key to reducing the threat of honeydew contaminated lint. If critical thermodetector values change, Equations 1 and 2 or 3 can be used to estimate aphid numbers (Table 3) associated with the threshold for unacceptable lint stickiness. Slosser et al. (2002) gave a detailed discussion of this study.

Acknowledgments

We thank Bobby Idol (Texas Agricultural Experiment Station, Vernon) for technical assistance, D. E. Brushwood (ARS, USDA, Cotton Quality Research Station, Clemson, SC), for sticky cotton analysis using the sticky cotton thermodetector, and W. E. Pinchak (Texas Agricultural Experiment Station, Vernon) for leaf nitrogen analysis. This research was supported by The Texas Agricultural Experiment Station (Project H-8136); ARS, USDA, Western Cotton Research Laboratory; Cotton Incorporated (Project 97-482), Texas State Support Committee (Project 98-553TX), and by Syngenta Crop Protection, Inc.

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Table 1. Correlation analyses of the relationship between thermodetector counts and sugar concentration on cotton lint.

Sugar	***F***	***P***	r^2
Glucose	69.56	<0.001	0.672
Fructose	81.98	<0.001	0.707
Sucrose	27.96	<0.001	0.451
Melezitose	390.77	<0.001	0.920
All Sugars	92.82	<0.001	0.732

Regression format: y = a + bx where y = thermodector count and x = sugar concentration (μg/gm of lint). For all regressions df = 1,35.

Table 2. Correlation analyses of the relationship between melezitose concentration on cotton lint and different cotton leaf parameters.

Equation No.	**Leaf Parameter**[a]	***F***	***P***	r^2
1	% leaf moisture (M)	0.79	0.389	0.047
2	% leaf nitrogen (N)	0.16	0.695	0.010
3	M x N interaction	0.36	0.557	0.022
4	sucrose concentration (SC)	1.77	0.202	0.010
5	SC x M interaction	1.82	0.196	0.102
6	SC x N interaction	1.52	0.235	0.087
7	aphid numbers (AN)	364.24	<0.001	0.958
8	M x N x AN interaction	300.22	<0.001	0.976

Format for equations 1-7 is y = a + bx, and for equation 8, $y = a + bx_3 + bx_7$; where y = melezitose concentration on lint (μg/gm) and x = leaf parameter. For equations 1-7, df = 1, 16, and for equation 8 df = 2, 15, *n* = 18.

[a] Sucrose concentration is $\mu g/cm^2$ and aphids are avg. numbers per leaf.

Table 3. Estimated number of aphids per leaf to cause a sticky lint problem (i.e., thermodetector count of 10) at different percentages of leaf moisture and nitrogen.

	% Leaf nitrogen (%N)		
% Leaf moisture (%M)	**2.5**	**2.7**	**2.9**
63	11.1	22.6	34.1
65	15.6	27.5	39.5
67	20.2	32.5	44.8
69	24.8	37.5	50.1

Melezitose (μg/gm) = 176.660 - 0.589 (%M%N) + 0.642 (Aphids); a melezitose concentration = 90.973 is associated with a thermodetector count = 10.