BEMISIA WHITEFLIES, STICKY COTTON AND STICKY COTTON SAMPLING

T. J. Henneberry and S. E. Naranjo
USDA-ARS, Western Cotton Research Laboratory
Phoenix, AZ

Abstract

Significant progress has been made in understanding many of the components of cotton lint stickiness related to infestations of *Bemisia tabaci* (Gennadius) strain B (= *B. argentifolii*). Research suggests that *Bemisia* honeydew contains over 20 sugars, but over 58% of all the sugars are made up of trehalulose and melezitose. These two sugars are significantly correlated to cotton lint stickiness. Field studies have investigated the relationships between whitefly population densities and development of sticky cotton. Manual thermodetector counts of lint at periodic intervals during the open boll cycle increased with increasing adult and nymph whitefly densities. Thermodetector counts of 5 (the tentative upper limit of nonsticky cotton) did not occur until average pest densities during the open boll period exceeded [illegible] 9 adults per leaf or [illegible] 3 nymphs per cm^2 of leaf disk. These results suggest that established action thresholds to protect cotton yield will also effectively prevent sticky cotton development. Generally, > 95% of all bolls have opened by 15 September. Early harvest (20 September) prevented development of late-season sticky cotton, whereas extending the season, without insecticide protection, exposed open cotton to increasing whitefly populations and the associated development of lightly sticky lint by harvest (25 October).

Further research has begun to develop standardized sampling methods for the precise estimation of lint stickiness using the manual thermodetector (SCT) and an automated sticky cotton thermodetector (H2SD). Preliminary studies indicate that the within-field frequency distributions of thermodetector spots for the SCT and H2SD systems differed. SCT and H2SD thermodetector counts showed random and clumped distributions, respectively. Thus, more field samples may be needed when using the H2SD system. Examination of different sample units (1, 2 or 10 plants, 20 or 200 randomly picked bolls) suggest that thermodetector counts of subsamples of lint from these units do not vary significantly. Results to date suggest that a 1-plant sample unit is most cost efficient. H2SD assays consistently detected fewer thermodetector spots than the SCT. Regardless of the thermodetector system used, further analyses suggest that more emphasis should be placed on collection of additional field sample units rather than replicate assays on a single sample unit. Additional sticky cotton sampling studies are being conducted with lint samples having a wider range of lint stickiness.

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Introduction

Sticky cotton is a worldwide problem in the textile industry (Hector and Hodkinson 1989). The nature and causes of lint stickiness are complex. Plant-produced sugars that are not utilized fully in cellulose production as well as sugars produced by cotton plant nectaries, and other extraneous contaminants have been implicated in the sticky cotton problem. However, a high percentage of the documented lint stickiness problems have been associated with the occurrence of honeydew-producing insects such as aphids and whiteflies (Sisman and Schenek 1984, Watson *et al.* 1982, Rimon 1982). The potential for *Bemisia tabaci* (Gennadius) associated honeydew lint contamination was first suggested by Husain and Trehan (1942). *B. tabaci* has been present in the southwestern U.S. since the 1920's and population outbreaks have occurred periodically through the 1970's and 1980's. The consistent and severe outbreaks that have occurred since the early 1990's are associated with the introduction of a new biotype (Strain B) or a new species (*B. argentifolii* Bellows and Perring). Regardless, the magnitude of the sticky cotton problem in the U.S. has increased and considerable recent research has been initiated to begin to unravel this complex problem.

Here we briefly review some of the past and current research addressing cotton lint stickiness, including description and quantification of honeydew sugars, stickiness measurement systems, relationships between stickiness and pest populations, and progress in sampling methods for estimating lint stickiness.

Honeydew Analysis

Research has contributed much to our knowledge of *Bemisia* honeydew. Tarczynski *et al.* (1992) reported that sucrose (10.1%), glucose (5.3%), fructose (11.7%), trehalulose (43.1%), and oligosaccharides (primarily stachyose and raffinose [29.5%]) were found in honeydew produced by *Bemisia* feeding on cotton. Hendrix *et al.* (1992) identified honeydew sugars from *Bemisia* on the same host as 18.9% monosaccharides, 16% sucrose, 1% turanose, 43.8% trehalulose, 16.8% melezitose, approximately 3% of a novel trisaccharide later identified and named Bemisiose (Hendrix and Wei 1994), and several other oligosaccharides. The authors suggested that more than 20 sugars occur in *Bemisia* honeydew. Using an evaporative light-scattering detector and HPLC, Wei *et al.* (1996) reported that oligosaccharides larger than trisaccharides comprised greater than 13% of the total carbohydrates. Additional oligosaccharides were identified as bemisiotetrose, maltosucrose and diglucomelezitose as well as substantial amounts of the quaternary amine, glycine betaine (Wei *et al.* 1996, 1997). The percentages of known honeydew sugars (Wei *et al.* 1996) are shown in Table 1. There remain several additional unidentified sugars. However, trehalulose and melezitose make up over 58% of the total sugars. A number of field studies have shown cotton lint stickiness to be significantly correlated to numbers of *Bemisia* adults and nymphs and also to the *Bemisia*-produced trehalulose and melezitose (Henneberry *et al*. 1995, 1996, 1998a, 1998b).

Measurement of Cotton Lint Stickiness

Hector and Hodkinson (1989) review many of the methods used to test for the presence of sugars and stickiness. Most direct measurements of cotton lint stickiness have been made with the minicard or manual sticky cotton thermodetector (Brushwood and Perkins 1993, Perkins and Brushwood 1995). The minicard method was adopted as the reference standard for measuring cotton stickiness by the International Committee on Cotton Methods of the International Textile Manufacturer's Federal (ITMF) in 1988 (Anonymous 1988). Because it is cumbersome to use, slow, expensive and because only a few minicards are in existence, ITMF recommended adoption of a method developed by the Institute for Research on Cotton and Exotic Textiles, Montpellier, France, the manual sticky cotton thermodetector (SCT) (Perkins and Brushwood 1995). The SCT involves spreading a thin web of lint between aluminum foil sheets, heating under pressure, separating the aluminum foil sheets, and counting the numbers of sticky spots (Brushwood and Perkins 1993, Perkins 1993). Thermodetector counts of individual sticky spots measure the overall contribution of all the sugar components in cotton lint. The manual thermodetector has been in use since the early 1990's and has been a useful tool for studying whitefly cotton lint stickiness relationships (see below). Results from SCT, minicard and processing quality tests were used to suggest a tentative scale for categorizing stickiness based on the number of thermodetector spots (Perkins and Brushwood 1995). Less than five spots indicate nonsticky lint; 5-14, 15-24 and > 24 spots indicate light, moderate and heavy stickiness, respectively. These ratings are frequently used to categorize stickiness for research purposes. However, it is unclear how these ratings relate to eventual ginning and/or milling problems and more research is urgently needed to clarify this issue.

Until recently thermodetector analysis has been impractical for large-scale industry implementation because of the time required per sample for the SCT (3-5 min. per sample) and the somewhat subjective process of counting sticky spots (e.g. Perkins and Brushwood 1995, Naranjo and Henneberry 1998). Automated high-speed systems for measuring sticky cotton have now been developed and provide the opportunity for more extensive sticky cotton testing in large cotton production areas. The High Speed Stickiness Detector (H2SD) (Hequet *et al*. 1997) is in use in the U.S. and France, and the Fiber Contamination Tester (Mor 1997) is currently in use in the U.S., Israel and Italy. For the most part these machines are in use at research institutions.

Cotton Lint Stickiness and *Bemisia* Populations

The seemingly simple relationship between *Bemisia* abundance and lint stickiness is complicated by many factors including the timing of pest presence with that of open bolls, the distribution of open bolls within the canopy, and rain and/or other degradative processes that can reduce or eliminate stickiness. In 1995 and 1996, we conducted field tests in replicated, randomized complete block designs to determine relationships between whitefly populations and cotton lint stickiness. Cotton plots were 0.1 acre or larger in size with standard 40 inch row spacing (Henneberry *et al*. 1998a, 1998b). Insecticides were applied, in accordance with university recommendations (Ellsworth *et al*. 1995) in some cases to create different levels of whitefly populations. Adult and nymph populations were sampled as described by Naranjo and Flint (1995) and Naranjo and Flint (1994), respectively.

In 1995 samples of 20 open bolls were taken weekly from all plots beginning on 27 August until harvest on 20 September. In 1996 weekly samples were taken from 15 August until harvest on 25 October. Lint analyses for all samples were done at the USDA-ARS Cotton Quality Research Station at Clemson, SC using a manual SCT system.

In 1995 densities of adults and nymphs in untreated cotton increased in late July and August (Fig. 1A, B). A slight decrease in the density of adults occurred in mid-August, following rainfalls, and increases in adults and nymphs occurred again in early to mid-September. Open cotton bolls first occurred between 18 and 22 August (Fig. 1C). Numbers of open bolls accumulated thereafter and 98% of the total number produced for the season occurred by 15 September. Adults and nymphs were reduced in insecticide-treated compared with untreated plots for the entire season following the first application on 25 July.

Thermodetector counts for lint samples in untreated plots were in the slightly sticky range (5-14 spots) within 10 days after the first open bolls were observed (Fig. 1C) and numbers of adults and nymphs averaged 17.0/leaf and 9/cm^2 of leaf disk, respectively. In insecticide-treated plots thermodetector counts averaged less than 5 throughout the boll opening period.

In 1996 adult and nymph population densities in untreated cotton increased in late July (Fig. 2A, 2B). Densities decreased dramatically following a 2.21 cm rainfall on 25 July. First open bolls occurred about 7 August (Fig. 2C) and densities of adults/leaf and nymphs/cm^2 of leaf disk were < 1 during the entire first fruiting cycle. Ninety-five percent of the open bolls for the first fruiting cycle occurred by 10 September. Open bolls for the second fruiting cycle began about 25 September. Densities of whitefly adults and nymphs began to increase on 10 September and thereafter through the last sampling dates of 7 October for adults and 9 October for nymphs.

Thermodetector counts averaged < 3 through 6 October (Fig. 2C). A thermodetector count of 7 (lightly sticky cotton) occurred when cotton was harvested on 25 October.

In 1995, twenty boll samples were taken for thermodetector analyses when accumulated percentages of open bolls were 20, 75, 90 and 95%, respectively (Fig. 1). Thermodetector counts on the 1st, 2nd and 3rd dates were significantly correlated ($P \leq 0.05$) ($r = 0.68, 0.72$ and 0.75, respectively) to thermodetector samples for lint samples at harvest on 20 September. In 1996, open bolls began to occur about 11 days earlier than in 1995, but accumulated percentages of open bolls of 95% also occurred about 15 September. Thermodetector counts were less than 5 through 15 October. After 6 October, thermodetector counts on 9, 12 and 15 October were significantly correlated ($P \leq 0.05$) ($r = 0.36, 0.30$ and 0.37, respectively) to thermodetector counts for lint on the 25 October harvest.
A clear definition of the relationship between pest density and stickiness is problematic because, honeydew deposition by *Bemisia*, boll opening, and various degradative processes are cumulative. For both years data (Fig. 3), thermodetector counts were significantly correlated to adult leaf-turn counts ($r = 0.73$, $n = 27$, $P = 0.01$) and nymphs per square centimeter of leaf disk ($r = 0.80$, $n = 27$, $P = 0.01$). Thermodetector counts of 5 (tentative lower bound of lightly sticky range) were associated with mean densities of 8.9 adults per leaf and 3.2 nymphs per square centimeter of leaf disk over the boll-opening period. The results of several recent studies aimed at the development of action thresholds for *Bemisia* (Ellsworth and Meade 1994, Naranjo *et al*. 1998) suggest that thresholds that prevent economic yield loss (5-10 adults per leaf) also appear to generally prevent lint stickiness (< 5 spots).

Late season crop management can be a vital decision-making issue for avoiding development of sticky cotton. For the 1995 and 1996 studies reported here, 95% of the open bolls occurred by 15 September. In 1995, insecticide applications reduced whitefly populations during the season and associated sticky cotton thermodetector counts. Early harvest (20 September) avoided increasing whitefly populations and development of sticky cotton in late season. In 1996, weather (heavy rainfalls) reduced number of whiteflies during late July and continuing through mid-September. Extending the season, after 95% of the bolls were open, resulted in increasing cotton stickiness levels as late-season whitefly populations developed.

Sampling Methods for Estimating Lint Stickiness

As discussed above, considerable progress has been made in the development and testing of devices for the measurement of lint stickiness. However, there has been relatively little effort devoted to the development of standard methodologies for collecting lint samples from fields, bales, or modules to ensure that statistically precise estimates of stickiness will be obtained. With the support of Cotton Incorporated we have initiated studies to develop standardized, statistically valid sampling methods for the field (Naranjo and Henneberry 1998) as well as for other areas in the lint processing cycle. We briefly summarize some of our findings with the SCT and H2SD systems based on lint samples collected from a total of 38 field sites between 1995 and 1997 in Maricopa, AZ and Brawley, CA.

Sampling Distribution

Within-field distributions of thermodetector spots for the 38 fields for the SCT and H2SD were different (Fig. 4). Regardless of sample unit size, SCT samples typically had coefficients of variation (CV = SD/mean) < 1 indicating a random (Poisson) distribution. In contrast, a large fraction of the samples assayed by the H2SD had CV's > 1 indicating a more clumped (aggregated) distribution. The reasons for the difference are unknown and further detailed investigations are underway. Accurate description of the sampling distribution is essential to defining sample sizes necessary to achieve precise estimates of stickiness. In general, samples sizes will increase with increasing degrees of aggregation.

Sample Unit Size

We investigated a number of different sample units (Naranjo and Henneberry 1998) including all lint from variable numbers of whole plants to random collections of variable numbers of bolls from all vertical strata in the canopy. In general, we found no statistical differences in estimates of stickiness among the various sample units examined nor any clear pattern in levels of variability in relation to size of the sample unit. Thus, it took much longer to collect larger sample units in comparison with smaller sample units, but this extra effort was not offset by less variable estimates (e.g. Table 2). Based on results to date the 1-plant sample unit appears most cost efficient. Additional observations are currently being collected for several boll-based sample units.

SCT/H2SD Comparisons

Overall our samples did not provide a good range of stickiness levels. Nonetheless, there were clear differences in the total number of thermodetector spots between SCT and H2SD assays of the same sample, with the latter being consistently lower. The relationship between the SCT and H2SD for our samples differed from those previously reported by Hequet *et al*. (1997). Other sampling considerations differed as well. Because thermodetector assays are conducted on subsamples from the field sample unit, the process of estimating stickiness is inherently a two-stage sampling problem with two sources of variation. We found that approximately one-third of the variation was attributable to differences among field samples while the remaining variation could be attributed to variability among SCT subsamples (replicate assays). This latter source of variation for the SCT includes variability due to subsampling and the SCT operator. Because the H2SD largely eliminates operator error we found that approximately 41% of the variability was attributed to subsampling with the H2SD system with the remaining 59% attributable to field-level variability. Taking field and assay costs into consideration our preliminary findings suggest that only one subsample should be assayed per sample unit on either machine and that more time should be devoted to collecting additional sample units from the field. Further research and analysis is needed to more accurately define the sampling distribution (particularly using the H2SD) before sample sizes can be clearly defined.

Discussion

Sticky cotton remains an important issue in the cotton industry. At the grower level, sticky cotton can result in significant lint price discounts, reduced mechanical picker efficiency and increased production costs that erode net profits. In lint processing, sticky cotton results in reduced cotton ginning efficiency and at the textile mill sticky cotton results in unacceptable machinery stoppages and increased downtime for maintenance. The cost accounting for these occurrences have not been well documented but they are obviously extremely expensive in terms of both time and manpower.

Although various methods of reducing cotton lint stickiness such as blending, lint additives, storage, microbial degradation, and enzyme treatment have been researched and shown to have potential, none are completely acceptable at all levels in the industry (Hector and Hodkinson 1989). The most obvious solution to the problem is to reduce pest (whitefly and/or aphid) populations to levels that mitigate yield losses and do not result in excessive levels of honeydew associated lint contamination. New and conventional insecticide chemistries, and application technology have been developed that help accomplish this objective (Ellsworth *et al.* 1996, 1997). Further, whitefly sampling (Naranjo and Flint 1994, 1995, Naranjo *et al.* 1996) and action thresholds have been developed and implemented (Ellsworth and Meade 1994, Ellsworth *et al.* 1995, Naranjo *et al*. 1998). These methods optimize insecticide use, help conserve natural enemies, and reduce or delay insecticide resistance. The results of the research presented herein suggest that chemical control within the framework of the established thresholds to protect cotton yield will also prevent the development of sticky cotton.

The accurate determination of cotton lint stickiness in the field could greatly aid management and research efforts, but additional work is needed to define crucial times for monitoring. For grower use, field sampling of stickiness could augment pest monitoring techniques and improve overall decision-making for pest suppression. This process may require the collection of samples at critical points in the open boll cycle that accurately reflect the level of lint stickiness at harvest. The utility of this information assumes that any pest control action used will prevent further honeydew lint contamination so that the overall accumulative effect at harvest will not result in sticky cotton. Further, field sampling that would identify unacceptable levels of stickiness at harvest informs the grower that he had a problem during the growing season and that additional remedial action may be needed. Some recent research suggests the possibility of developing an enzyme treatment to reduce honeydew caused lint stickiness in harvested cotton (Hendrix *et al.* 1993, Henneberry *et al*. 1997). Finally, standard methods for the estimation of lint stickiness in the field would also aid researchers developing mitigation and remediation methods to prevent or ameliorate lint stickiness. We are currently developing sampling plans which will define the number of field sample units and the number of subsamples to assay from these units. Our 1996 data exhibited a poor range of stickiness and additional data are needed to better characterize and compare the H2SD system with the manual thermodetector on which much of our database has been developed.

The availability of automated sticky cotton detection systems makes feasible extensive sampling that may be useful during crop production and in harvested cotton. Automated, high-speed systems have the potential to provide consistent and accurate determination of lint stickiness. At what point or points in the crop production - lint processing cycle lint stickiness should be determined remains a critical issue. It appears that determinations during both phases would be the most logical approach. Monitoring during the season and use of decision-making tools to aid in determining the need for whitefly (aphid) control to prevent sticky cotton development would be useful and profitable for the grower to produce high quality lint and avoid price penalties. Sticky cotton determinations in harvested cotton would be an obvious consideration for the textile manufacturer to prevent costly machinery downtime and excessive machinery maintenance. Overall, the most critical issue, for the cotton producer and the textile industry is that wherever the sticky cotton determination is made, it accurately reflects the degree of the problem in processing the lint.

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Table 1. Elution of honeydew sugars from Amino HPLC Column (From Wei *et al.* 1996).

Peak	Retention Time (min)	% Total (peak area)
Fructose	16.22	10.48
Glucose	17.42	5.63
Glycine betaine	19.87	2.72
Sucrose	21.63	5.38
Turanose	23.13	1.29
Trehalulose	25.53	36.59
Melezitose	32.83	21.73
Bemisiose	39.07	2.83
Bemisiotetrose	48.33	4.67
All other peaks	--	8.68

Table 2. Summary of some sample units examined for estimation of lint stickiness.

Sample Unit	Per Unit Cost (min)	Relative Net Precision[a] SCT	Relative Net Precision[a] H2SD
All open bolls on indicated number of consecutive plants			
1-Plant	1.40	0.039	0.011
2-Plants	2.18	0.018	0.006
10-Plants	8.40	0.011	0.003
Open bolls (1 per plant) within a row			
20-Bolls	2.08	0.017	0.007
200-Bolls	15.20	0.008	0.0002

[a] Relative net precision measures the ratio between precision and cost. A higher value indicates a more efficient sample unit.

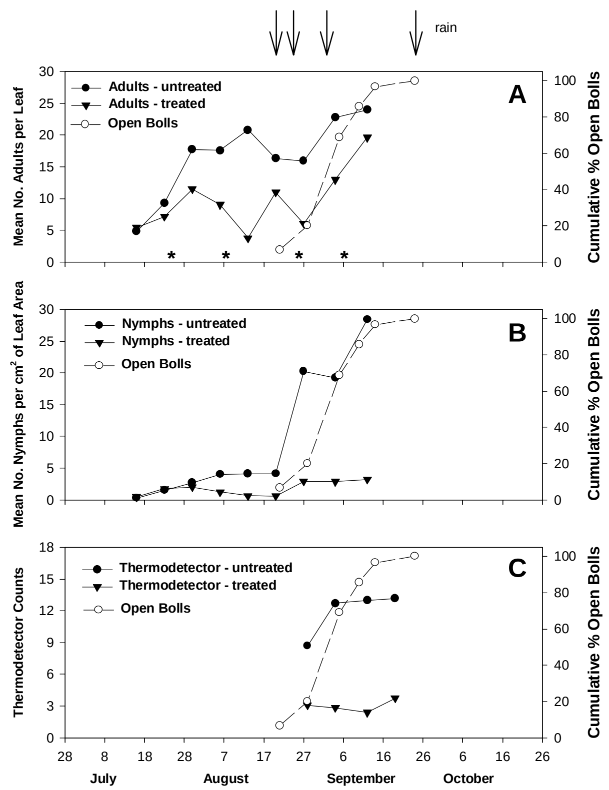

Figure 1. Mean numbers of *Bemisia* adults and nymphs and thermodetector counts in untreated and insecticide treated cotton plots in relation to accumulated percentages of open cotton bolls 1995. Arrows indicate rain. Asterisks indicate insecticide spray dates.

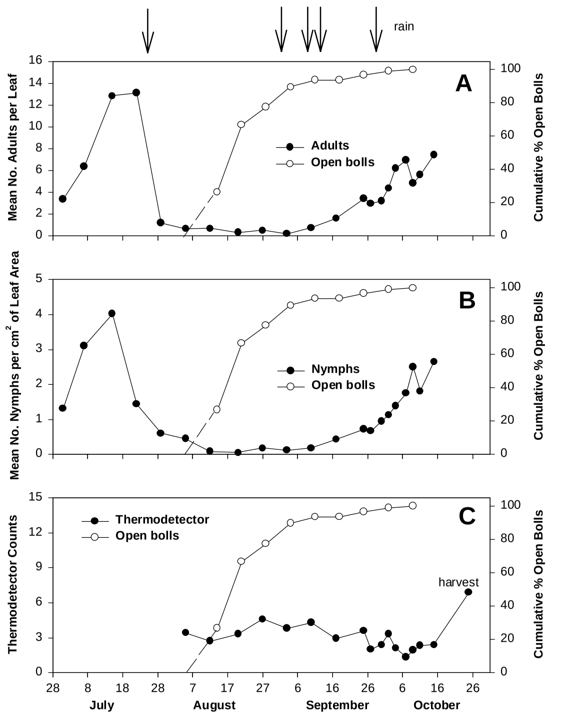

Figure 2. Mean numbers of *Bemisia* adults and nymphs and thermodetector counts in untreated cotton plots in relation to accumulated percentages of open cotton bolls 1996. Arrows indicate rain.

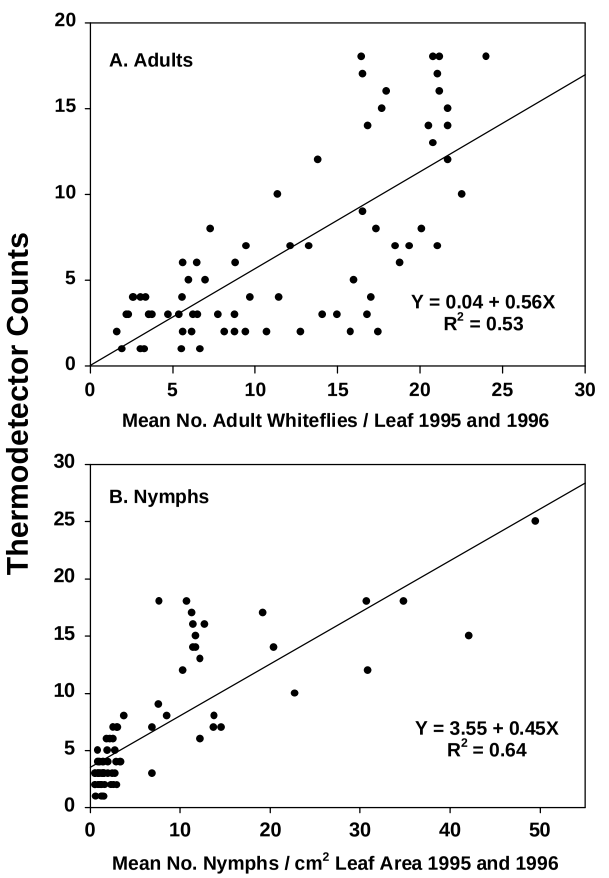

Figure 3. Mean numbers of *Bemisia* adults and nymphs in relation to thermodetector counts (1995 and 1996).

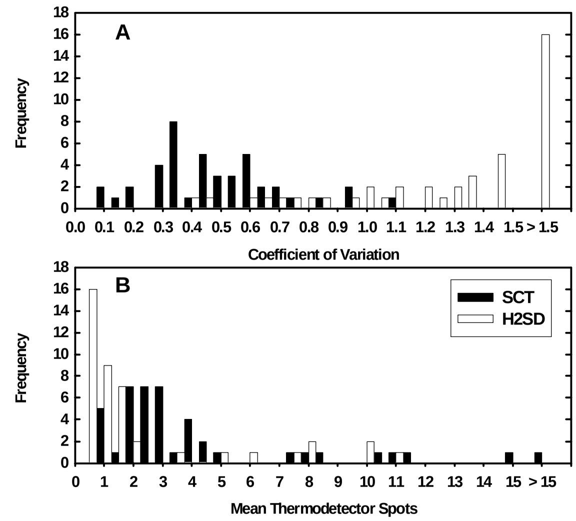

Figure 4. Distribution of (A) coefficients of variation (SD/mean) and (B) thermodetector spots from lint samples assayed using the SCT and the H2SD.