PRELIMINARY EVALUATIONS OF AN ENZYME APPROACH TO REDUCE COTTON LINT STICKINESS

T. J. Henneberry, B. Blackledge, Terry Steele, D. L. Hendrix
USDA, ARS, Western Cotton Research Laboratory
Phoenix, AZ
H. H. Perkins
USDA, ARS, Cotton Quality Research
Clemson, SC
R. L. Nichols
Cotton, Incorporated
Raleigh, NC

Abstract

Laboratory and field studies were conducted to evaluate the potential of an experimental enzyme for degrading honeydew sugars on cotton lint and reducing cotton lint stickiness. Stickiness was measured with the sticky cotton thermodetector. Carbohydrate extractions from lint, in some experiments, were measured as percentages of total reducing sugars or individual sugars determined by high performance liquid chromatography. In the laboratory, (average temperature 85-100◦ F, 5 to 25% lint moisture on a wet weight basis) thermodetector counts decreased with increasing moisture percentages. Reduced counts occurred more rapidly when 1% of a proprietary enzyme was included in water solutions and seed cotton moisture percentages were 11% or higher. Significantly higher reductions in lint stickiness as measured by thermodetector counts occurred following incubation periods of 14 or more days as compared with 1 or 3 day incubation periods. In some instances, thermodetector counts increased following enzyme treatment, later decreasing to nonsignificant stickiness levels. This probably occurred because of the hydrolytic enzyme activity that resulted in degradation of the complex insect-produced sugars to one or more less complex sugars that were also sticky. Eventually these sugars were in turn reduced to nonsticky substances, probably by microbial activity. In the field, spray nozzles mounted in a spindle picker intake duct were more effective for application of water or water plus enzyme solutions than nozzles on a modified spray boom mounted in front of the picker.

Lower thermodetector counts for untreated cotton, water-treated, or water plus 1% enzyme B-treated cottons following commercial ginning suggested that gin processing reduced cotton stickiness. In a simulated spindle picker experiment, increased thermodetector counts occurred when spindle moisture pads were operated with moderately sticky but not lightly-sticky cotton. Ginning effects were minimal but reduced amounts of trehalulose and reduced thermodetector counts occurred following each lint process. Leaf trash from ginned seed cotton contained trehalulose and melezitose. Removal of leaf trash in ginning and lint cleaning probably accounts for some reduced lint stickiness

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:430-436 (1997)
National Cotton Council, Memphis TN

Introduction

Cotton stickiness has become a more pressing problem with increasing whitefly, *Bemisia* spp., populations (Hector and Hodkinson 1989). Aphids may also cause sticky cotton in some areas. Honeydew excreted by phloem feeding insects (adults and nymphs) when deposited on lint of open bolls remains localized. Discrete honeydew spots adhere to working surfaces of high speed lint processing machinery in the textile mill. Honeydew can also be a problem during mechanical harvest with spindle pickers (personal communications with growers) and during cotton ginning (Khalifa and Gameel 1982, Carlson and Mohamed 1986). Physiological plant sugars, primarily sucrose, that normally appear in mature cotton fiber are evenly distributed within the lint and generally do not cause stickiness (Elsner 1983). Bourley et al. (1984) suggested that healthy mature fiber containing 0.27 g of total physiological sugars per 100 g of fiber was not sticky but lint became sticky when total sugar exceeded 1 g per 100 g of fiber.

One approach to providing a solution to the lint stickiness problem is to reduce whitefly populations below threshold levels that result in sticky cotton. Considerable progress has been made in providing knowledge leading to our better understanding of whitefly population dynamics, fundamental physiological and biochemical processes, genetics, and potential for biological control approaches (USDA-ARS 1993, 1994, 1995, 1996). However, control at present is heavily oriented to the use of insecticides. Chemical control is expensive, difficult to achieve because of the underleaf whitefly habitat and resistance development and has the disadvantages of impact on nontarget organisms, induced secondary pests and cause for environmental concern (See Henneberry and Butler 1992, for review). Further, although effective chemical control has resulted in reduced cotton lint stickiness in some instances (Henneberry et al. 1995), it has failed in others (Chu et al. 1994). Explanations for the differences in results appear to be related to whitefly population densities.

A second approach being investigated to reduce the sticky cotton problem is the potential of enzymes for hydrolyzing honeydew sugars on cotton lint. Hendrix and Wei (1992) reported that Tempanil® (containing glucose oxidase, Gokak Patel Volkart Lmt., Bombay, India) significantly reduced sugars of sweetpotato whitefly, *B. tabaci* (Gennadius), honeydew contaminated lint. Also, an experimental proprietary product (Solvay Enzymes, Inc., Elkhart, Ind.) called enzyme A applied to sticky cotton lint significantly reduced minicard stickiness when applied to seed cotton during mechanical harvest in the field (Hendrix et al. 1993).

Based on the chemistry of the major sugars in whitefly honeydew, another proprietary enzyme product (called enzyme B) was suggested by Solvay Enzyme, Inc. to have greater potential for honeydew hydrolytic activity than enzyme A. Enzyme B applied to defoliated cotton in the field with conventional spray equipment and average seed cotton moisture of 3.5 to 5.1% did not reduce cotton lint stickiness (Chu et al. 1996). Because of the increasing importance of the sticky cotton problem we conducted a further series of laboratory and field tests to evaluate enzyme B for reducing cotton lint stickiness. We also investigated the effect of enzyme concentration, seed cotton moisture, incubation period following treatment, and effects of simulated spindle picker seed cotton processing, ginning and lint cleaning on cotton stickiness.

Methods and Materials

All laboratory and field experiments were conducted with cotton harvested from fields or cotton treated in fields heavily infested during the season with silverleaf whiteflies, *B. argentifolii* Bellows and Perring. Cotton lint thermodetector counts were determined as described by Brushwood and Perkins (1993) at the USDA-ARS Cotton Quality Research Station, Clemson, SC. Thermodetector analysis is accomplished by spreading 2.5 g lint samples between aluminum foil sheets followed by heating under pressure. The aluminum foil sheets are subsequently separated and the numbers of sticky spots counted. Less than 5 spots indicates nonsticky cotton, 5 to 14 light stickiness, 15-24 moderate stickiness, and above 24, heavy stickiness (Perkins and Brushwood 1995). In all studies, seed cotton moisture content on a wet weight basis was estimated by repetitive weighing methodology (ASTM 1987). Seed cotton samples were weighed, oven dried at 212° F for 10-12 h, reweighed and loss in weight expressed as a percentage of the seed cotton weight before oven drying.

Laboratory experiments. Seed cotton moisture content (w/w) was adjusted by spraying an amount of water, with or without enzyme B, calculated to bring the determined moisture content after oven drying to the desired experimental level. Enzyme B in all laboratory studies was applied as a 1% solution to deliver approximately 1 ml of enzyme/g of seed cotton. Water or water plus enzyme B was applied on seed cotton with a compressed air sprayer. Triton X-100®, a wetting agent, was included at 0.5% (v/v) in all sprays.

Following treatment, seed cotton samples were placed in Ziplok plastic bags to prevent moisture loss and the bags were tightly rolled to compress the seed cotton within the bag. Seed cottons in the bags were held for varying lengths of time (incubation periods) in an outdoor laboratory at 115 to 125° F. Following the experimental incubation periods, seed cotton was removed from the plastic bags. A subsample was taken from each sample and seed cotton moisture content determined as described. After 24 h drying periods in the 110 to 130° F greenhouse, a second subsample was machine-ginned and lint analyzed for stickiness using the thermodetector.

Experiment 1 was conducted to determine the effect of seed cotton moisture alone on cotton stickiness. Seed cotton samples of 120 g each were sprayed with amounts of water estimated to result in moisture contents of 5, 10, 15, 20 and 25%. Untreated samples were controls. The treatments were replicated 5 times in a randomized complete block design. The incubation period in plastic bags was for 5 days.

Experiment 2 was a repeat of experiment 1 with the exception that seed cotton samples at all moisture levels were treated with 1% enzyme B solutions.

Experiment 3 was conducted to further define the role of seed cotton moisture and enzyme B on cotton stickiness. Seed cotton samples were treated with water alone or water plus 1% enzyme B to result in estimated moisture levels of 8, 10, and 12%. Untreated cotton samples were controls. The experiment was replicated 5 times in a randomized complete block design. Seed cotton samples were incubated for 1 day in the greenhouse as described before ginning and thermodetector analysis.

Experiment 4 was conducted to determine the effect of incubation period following enzyme treatment on cotton stickiness. Amounts of water were sprayed on seed cotton samples to result in estimated 10 and 12% moisture levels in each case with 0.25 or 0.50% enzyme solutions. Samples were incubated for 1, 2, 14 or 28 days. Air drying in the greenhouse and ginning following incubation periods were as described.

Field Experiments - General. Water or water plus enzyme treatments were made with spray equipment mounted on a mechanical cotton spindle picker. Triton X-100, as described, was included in all sprays. Two types of spray equipment were used. For the first (boom applications), 4; 40-inch long, vertically oriented booms were mounted on the front of a spindle picker. Two booms, in each case, about 18 inches apart, straddled a cotton row. There were 3 nozzles mounted on each boom. The top nozzle on each boom was about 6 inches down from the cotton plant terminal. The 2nd and 3rd nozzles on each boom were about 12 inches apart with the bottom nozzle about 10 inches from the ground. A 4th nozzle for each cotton row was directed downward to the plant tops and was mounted on a cross piece connecting the spray booms. For the second type of application (duct application), 4 nozzles were mounted on the sides of the seed cotton duct intake leading to the picker basket. Two nozzles about 2 feet apart were located on each side of the duct. All hoses were outside the ducts with nozzles on fittings mounted inside the duct. A 12 volt electric compressor was used to maintain

constant spray delivery pressure from a 14 gallon spray tank. T-Jet® nozzles were used in all cases, applications were made at 40 psi.

In all cases field plots were 2 cotton rows wide and ranged from 200 to 400 feet long. Seed cotton samples (≅ 2 lbs.) were hand picked from each plot prior to treatment. A subsample was placed in one-quart double lip seal paint cans, lids tightly sealed and held for laboratory seed cotton moisture determinations. Seed cotton picked with the mechanical picker during treatment was collected in 2 X 4 foot nylon-mesh bags attached to an extension of the picker seed cotton intake duct. Seed cotton subsamples were taken for moisture determination. Also, subsamples were placed in plastic bags and held for various incubation periods (1 d to 4 wks) prior to thermodetector analysis as previously described for the laboratory studies.

Field Experiments - Application Methods. Experiment 1 was conducted to determine seed cotton moisture percentages and thermodetector counts following 60, 40, or 20 gal/acre of water with the picker front-mounted spray boom or with nozzles mounted in the seed cotton intake duct. Seed cotton samples from the field plots were hand picked prior to treatment and, in each case, in the picker basket following treatment. Seed cotton moisture determinations were made and thermodetector counts, samples were incubated for 2 or 7 days before analysis. The study was conducted in a randomized complete block design with 4 replications.

Experiments 2 to 5 were conducted to determine the feasibility of treating sticky cotton at the time of harvest with 1% enzyme in water to reduce thermodetector counts. Controls were treated with water alone. Seed cotton was treated in all experiments using the boom or duct method. All experiments were replicated 4 times in randomized complete block designs. Seed cotton moisture and thermodetector counts were determined following incubation periods ranging from 1 to 28 days.

Spindle Cotton Picker, Cotton Ginning, Lint Cleaning Experiment. Experiment 6 was conducted to investigate a possible effect of machine cotton harvesting on sticky cotton and also as a result of casual observations that enzyme B-treated and untreated cotton had lower thermodetector counts following seed cotton ginning and lint cleaning. Approximately 1 lb. samples of seed cotton were taken from 60 bags, in each case, from machine-harvested cotton in silverleaf whitefly infested fields at each of two locations. Samples were visibly different with respect to leaf trash content. Thermodetector counts for one location averaged 11 (n = 5) and 22 (n = 5) for the second location. Samples were thereafter designated as lightly sticky and moderately sticky, respectively. Samples were stored in 1 pint paint cans and seed cotton moisture determined. Ten subsamples of lightly-sticky and moderately-sticky seed cotton were weighed, ginned, and cotton seed and lint weighed and percentages of trash determined. Trash (parts of cotton leaves, stems, etc.) was collected from each seed cotton sample. Trash was soaked in water, charcoal filtered, freeze dried and analyzed for trehalulose and melezitose.

The effect of machine picking and spindle moisture pad operation on cotton stickiness and seed cotton moisture was determined by operating an International Harvester spindle picker in place. Twenty to 25 pound samples of the lightly-sticky or moderately-sticky seed cottons were manually fed individually into the operating spindle drum, in each case, with or without water flow to the spindle pads. Seed cotton samples were taken from each of the picker processed lots for moisture determinations. Control seed cotton was not processed in the spindle picker. Subsequently, control seed cotton and seed cotton processed through the picker with or without water flow, in all cases, were machine-ginned or hand delinted and, in each case, lint was not cleaned or processed through a lint cleaner. Thermodetector counts, percentages of total reducing sugars, trehalulose and melezitose determinations were made for lint samples from all treatments. The experiment was replicated 5 times.

Results

Laboratory - experiment 1. After 5 day incubation periods in plastic bags in the greenhouse, untreated seed cotton moisture content was 4.5% and the thermodetector count 26.3 (Table 1, experiment 1). Seed cotton moisture for samples treated with varying amounts of water ranged from 8.6 to 19.9%. The thermodetector count was 30.3 at 8.6% seed cotton moisture and decreased with each increasing level of seed cotton moisture. Thermodetector counts for all samples at 14.3% seed cotton moisture and above were significantly lower than the untreated seed cotton with an average moisture level of 4.5%. Thermodetector counts for 8.6 and 13.9% seed cotton moisture samples were not significantly different than for 4.5% moisture level seed cotton.

Experiment 2. Seed cotton sprayed with water-1% enzyme B solutions to an average moisture level of 8.4% had no effect on thermodetector counts following 5-day incubation periods (Table 1, Experiment 2). The same amount of enzyme with water resulting in seed cotton moisture levels from 11.3 to 19.7% dramatically reduced thermodetector counts during the 5 day incubation periods. The results suggest that the enzyme plus water treatment reduced cotton thermodetector counts more effectively at similar or lower seed cotton moisture percentages than occurred in experiment 1 with water alone.

Experiment 3. Untreated seed cotton thermodetector counts averaged 29.8 and seed cotton moisture 4.9% (Table 1, experiment 3). Water-treated seed cotton had thermodetector counts, after 1 day incubation periods, of 27.3, 23.3 and 15.0 at seed cotton moisture levels of 8.2, 9.3, and 12.1, respectively. Seed cotton treated with water

plus 1% enzyme B when moisture levels were 8.4, 8.5 and 9.1% had significantly lower thermodetector counts (19.5, 18.8 and 9.0, respectively) than untreated seed cotton, but not significantly different than seed cotton treated with water alone (15.0 to 27.3). The incubation period following all treatments was for 1 day only.

Experiment 4. The effects of incubation periods following water or water plus enzyme treatments on thermodetector counts are shown in Figure 1. Thermodetector counts averaged over all enzyme and seed cotton moistures decreased with increasing numbers of days of incubation (Figure 1A). Significantly greater reductions in thermodetector counts occurred following 14 and 28 day incubations than occurred following 1 and 3 day incubations. Thermodetector counts ranged from 0.7 to 14.7 for the 10% seed cotton moisture treatment (actual moisture content 11.5%) and decreased with increasing days of incubation (Figure 1B). Results were not significantly different for the 12% seed cotton moisture treatment (actual moisture averaged 13.0%) except for the 1 day incubation period when thermodetector counts for the 12% moisture treatment were significantly higher compared with the 10% moisture treatment. Seed cotton moisture percentages for 10 and 12% treatments were significantly different and averaged 11.5 and 13.0% over all enzyme concentrations and incubation periods, respectively. However, average thermodetector counts (7.8 and 7.4, respectively) were not significantly different at average moisture percentages of 11.5 and 13.0%. A significantly higher average (for all treatments) thermodetector count (8.7) occurred for 0.25% enzyme treated seed cotton than for 0.50% enzyme-treated seed cotton (6.4) (Figure 1C). However, the overall average seed cotton moisture content was higher (12.7%) for 0.50% enzyme-treated cotton than for the 0.25% treated cotton (11.9%) and may have influenced the results. Incubation periods had no effect on seed cotton moisture, 12.4, 12.3, 12.4 and 12.0%, respectively, for 1, 3, 14 and 28 days.

Field Experiments - Application Methods. Hand-picked seed cotton (4 samples) averaged 5.3% (data not tabulated). On average the duct method of treating seed cotton increased seed cotton moisture significantly higher than the boom method (Table 2). Also, water applied at the rates of 60 and 40 gallons/acre increased, as expected, seed cotton moisture significantly greater compared with water applied at the rate of 20 gallons/acre. Seed cotton moisture (data not tabulated) were 10.7, 9.6 and 8.0%, respectively. Thermodetector counts were also significantly higher for boom-treated compared with duct-treated cotton. In each case, thermodetector counts were significantly higher after 7 days incubations compared with 2 day incubations. Rates of water applied did not significantly affect thermodetector counts (60 gal. = 22.8, 40 gal. = 23.3, 20 gal. = 29.7, ave. moisture 10.7, 9.6, and 8.0, respectively).

Experiments 2 to 5. For experiments 2, 3 and 4, gallons/acre were 30.8 and 22.0 for boom and duct application methods, respectively. The results of experiment 2 showed that seed cotton moisture percentages following treatment ranged from 8.0 to 10.5 and were not significantly different (Table 3, experiment 2). Thermodetector counts ranged from 12.5 to 29.8. The results were not significantly different except for the boom applied water-1% enzyme samples taken on the day of treatment which was significantly higher than duct applied water-1% enzyme on the day of treatment or boom or duct applied water plus enzyme on days 7 and 28 but not day 14 following treatment. For experiment 3, seed cotton moisture percentages ranged from 9.0 to 11.9% (Table 3, experiment 3). Thermodetector counts were significantly reduced for duct treated seed cotton compared with untreated seed cotton on the day of treatment and days 7 and 14 following treatment, but only on day 7 following treatment for the boom treated cotton. For experiment 4, seed cotton moisture percentages were not significantly different and ranged from 8.2 to 10.0. In general, no effects on thermodetector counts occurred (Table 3, experiment 4). However, increases in thermodetector counts occurred for boom-treated seed cotton for samples following 14 and 28 days incubation. For experiment 5, gal/acre for the boom application were increased from 30.8 to 50.4 and for duct application from 22.0 to 26.4 (Table 3, experiment 5). Seed cotton moisture percentages ranged from 9.3 to 10.9 for boom application and 14.8 to 15.7 for duct application. Thermodetector counts were significantly reduced compared with untreated cotton for duct applications after sample incubation for 1, 7 or 14 days and for boom application after 1 and 14 days sample incubation. Lint samples for boom application incubated 7 days had significantly higher thermodetector counts than the samples incubated for 14 days. Thermodetector counts for duct applications were significantly lower in all cases than for boom application.

Spindle Picker, Cotton Ginning Experiment. Percentages of leaf trash in lightly-sticky cotton and heavily-sticky cotton were 1.44 and 2.15, respectively (Table 4, Experiment 6). Amounts of trehalulose and melezitose in lint for the two samples were not significantly different. Also, lint thermodetector counts were significantly higher for cotton containing 2.15% leaf trash compared with cotton containing 1.44% leaf trash. Leaf trash contained significant amounts of trehalulose and melezitose with significantly higher amounts, in each case, per gram of leaf trash occurring in the highest leaf trash cotton compared with the lower leaf trash cotton. Seed cotton moisture before picker processing was higher for moderately-sticky cotton compared with lightly-sticky seed cotton (Table 5, Experiment 6). This probably occurred because of the higher percentage of trash in the moderately-sticky cotton. Spindle moisture pad operation during picker processing increased seed cotton moisture about 1%. Thermodetector counts for the moderately-sticky seed cotton was significantly higher than for lightly-sticky seed cotton. The overall mean thermodetector counts for seed

cotton processed through the picker with spindle moisture pads operating was significantly higher compared with seed cotton processed through the dry picker or control non picker processed cotton. There was a significant interaction for lightly-sticky or moderately-sticky seed cotton and picker processing. No significant differences occurred for picker processing of lightly-sticky seed cotton compared with significantly higher thermodetector counts for wet picker processed seed cotton compared with non picker processed or dry picker processed seed cotton. Percentages of total reducing sugars, and mg/g of lint of trehalulose and melezitose followed similar trends.

Effects of ginning were minimal although overall a reduced amount of trehalulose and a slightly lower thermodetector count occurred for machine-ginned vs. hand delinted cotton (Table 6). The overall mean thermodetector count for machined ginned seed cotton was 23.2 compared with 20.8 for hand delinted cotton (significantly different $P \leq 0.05$). Thermodetector counts for lint from moderately-sticky seed cotton and processed through the lint cleaner following ginning were significantly higher than non cleaned or cleaned lint from lightly-sticky seed cotton.

Discussion

The results of our studies demonstrate that artificially increasing moisture of honeydew contaminated seed cotton to 13.9 or higher percentages reduced thermodetector counts. Miller et al. (1994) found that trehalulose, a major component of *Bemisia* spp. honeydew, turanose, palatinose and sucrose solutions applied to cotton lint were very sticky. Melezitose, raffinose, glucose and fructose were relatively nonsticky. Hendrix et al. (1993) suggested that reduced lint stickiness at high seed cotton moisture levels may be a result of activation of microflora in seed cotton that affect sugars responsible for the stickiness. Klich (1986) isolated thirty-seven types of fungal flora from cotton seed from regional cotton variety trials in the southern United States. *Aspergillus niger* was commonly found, although low percentages of infected seed occurred. Solvay Enzymes, Inc. isolated the enzymes transglucosidase and pectinase from *Aspergillus* species (European Patent Application No. 94201168.5, 1994). The enzymes convert oligosaccharides to monosaccharides. Thus, the proposed microflora scenario is appealing and may explain reduced cotton stickiness under conditions of high seed cotton moisture.

Our data suggest that treatment of seed cotton with water-enzyme B solution resulting in 9% or higher seed cotton moisture induced a more rapid reduction in cotton stickiness counts compared with water-alone treatments with similar seed cotton moisture percentages. This agrees with our results showing superior degradation of honeydew with enzyme B compared to enzyme A (Hendrix, unpublished data). The relationship between seed cotton moisture content and enzyme concentration is obviously a critical focal point for practical application in the treatment of sticky cotton. A vital concern in subsequent research should be the determination of optimum seed moisture percentage and concentration of enzyme required per unit of honeydew contaminated seed cotton. This issue cannot be resolved from the data obtained in the present study. Another issue of significant importance is the time between enzyme treatment and effects measurable using the thermodetector. Solvay Enzymes, Inc. (1994) suggested that enzyme hydrolytic activity on oligosaccharides occurs rapidly (minutes to hours) under laboratory conditions. In our studies, in some cases no significant reduction in thermodetector counts occurred during 1 to 14 day incubation periods following treatment. In fact, in some instances, within 14 days, thermodetector counts significantly increased. The precise chemistry involved in the enzyme degradation of sugars causing lint stickiness is not known. However, Hendrix et al. (1993), reported that Solvay Enzymes, Inc. proprietary product enzyme A dramatically reduced cotton stickiness without completely eliminating extractable sugars from the treated seed cotton. The enzyme A treatment, however, did reduce amounts of glucose, fructose, melezitose and a larger polymer carbohydrate fraction from the seed cotton extracts. The authors concluded that a large percentage of nonreducing sugar produced by whiteflies could be eliminated, but reducing sugars may increase because enzyme degradation converted nonreducing sugars to glucose, a reducing sugar. These conclusions were further substantiated by results presented in the Solvay Enzymes, Inc. (1994) patent application that demonstrated transglucosidase hydrolysis of sucrose, trehalulose and melezitose into glucose and fructose. Miller et al. (1994) found that glucose and fructose were sticky when artificially applied to cotton lint at high concentrations. The reactions described probably account for the increased thermodetector counts that sometimes occur within a few days to two weeks following enzyme treatment. The ultimate fate of these increased amounts of reducing sugars is unknown. Our results suggests that depending on seed cotton moisture, a significant decrease in thermodetector counts occur 2 to 4 weeks following the temporary increase as a result of the enzyme hydrolytic activity.

Temperatures following enzyme treatment of seed cotton is a critical factor which has not been well defined. However, tests to determine the honeydew hydrolyzing activity of transglucosidase were conducted under 122◦ F conditions for 18 hours (Solvay Enzymes, Inc. 1994). The results clearly showed an increase in glucose and fructose and a decrease in oligosaccharides. Our laboratory tests were conducted at temperatures ranging from 85 to 100◦ F with seed cotton incubation temperatures ranging from 110 to 130◦ F. Temperature was not controlled and night-day differences ranged from 20 to 30◦ F. Current cotton harvest technology involves storing picked seed cotton in cotton modules for unspecified periods of time. Storage time is largely a matter of scheduling for further processing at the gin. Seed cotton moisture and temperature, focal issues in

enzyme-honeydew hydrolytic activity, are also important issues in cotton moduling. Excess moisture and high temperatures causing biological activity in the modules result in deterioration of cotton lint and seed quality (Curley et al. 1988). The authors found that initial module temperature, generally about ambient air temperature, and seed cotton moisture are the critical factors influencing temperature fluctuations during module storage. For example, seed cotton with 9% moisture and harvested at 86◦ F was estimated to reach a maximum temperature of 95◦ F when the module was stored at ambient temperature of 55◦ F. Whereas, seed cotton with 16% moisture, harvested at 86◦ F and stored at 55◦ F could reach maximum temperatures of 149◦ F. Lint quality is adversely affected at moisture above 13 to 14% and high temperature increases cause significant increase in lint yellowness. Seed germination may also be affected. Generally, lower harvest and module building temperatures followed by low ambient temperatures during storage reduce the probability of adverse high module temperature development. These parameters will have to be addressed if acceptable enzyme treatments are to be developed for module-stored-enzyme-treated cotton.

Our data is limited but did show significantly lower thermodetector counts following commercial ginning and lint cleaning as compared with counts before ginning and cleaning. Our simulated studies with picked seed cotton rerun through a machine spindle picker, followed by ginning and lint cleaning suggest that these activities may indeed influence cotton stickiness. The effects occurred only with moderately-sticky cotton that also had a high trash percentage (2.15 %). No differences occurred with lightly-sticky cotton with low trash percentages. The results need to be verified in the field under actual harvest and seed cotton processing conditions. Leaf trash from both lightly and moderately-sticky cottons contained trehalulose and melezitose but higher amounts were found in the cotton with the highest trash content. The cottons were from different locations and results probably occurred because of heavier honeydew deposits on foliage and other plant parts at the location with the highest leaf trash in the seed cotton. Lint cleaning that physically removes leaf trash containing trehalulose and melezitose (also other sugars found in honeydew as well as the plant parts) probably accounts for reduced thermodetector counts in lint cleaned cottons in our studies. Further, emphasis should be given to determining the relationships between sticky cottons in the field and sticky cottons following harvest, module storage, ginning, lint cleaning, and at the textile mill.

Overall, the results of our studies indicate that artificially induced high seed cotton moisture alone can reduce cotton lint stickiness. Our data suggest that the level of seed cotton moisture required may be excessive in relation to development of adverse high temperatures in module storage that would decrease cotton lint and seed quality (Curley et al. 1988). Promising results for reducing cotton lint stickiness were obtained with water plus 1% enzyme-treatment of seed cotton during harvest. It appears that this may be accomplished within acceptable levels of added seed cotton moisture. Future research should define the optimum enzyme, seed cotton moisture, and temperature relationships for amelioration of cotton lint stickiness within acceptable levels of developing module temperatures.

Acknowledgments

The authors are particularly grateful to Cotton Incorporated for financial assistance to partially defray costs of this research. CI-ARS 5344-21220-009-02R, Sticky Cotton: Identification and Chemical Characterization of Insect Honeydew.

References

ASTM. 1987. Standard test method for moisture in cotton by oven-drying. pp. 394-399, *In* Testing and Materials, Annual Book ASTM Standard D. 2495-87. Amer. Soc. Testing and Materials.

Bourley, J., J, Gutknech, and J. Fournier. 1984. Etude chemique du collage des fibres de coton. 1re, partie: Role des sucres et des miellats dans le processus du collage. Coton et fibres tropicales 39: 47-53.

Brushwood, D. E., and H. H. Perkins. 1993. Cotton stickiness potential as determined by minicard, thermodetector, and chemical methods, pp. 1132-1135. *In* D. J. Herber and D. Richter [eds.], Proceedings of the 1983 Beltwide Cotton Prod. Res. Conf., National Cotton Council Am., Memphis, TN.

Carlson, G. A and A. Mohamed. 1986. Economic analyses of cotton insect control in the Sudan Gezira. Crop Protection 5: 348-354.

Chu, C. C., T. J. Henneberry, D. H. Akey, and N. Prabhaker. 1994. A study on control action thresholds for *Bemisia tabaci* (Gennadius) on cotton, pp. 1239-1241. *In* D. J. Herber, and D. A. Richter [eds.], Proceedings of the 1994 Beltwide Cotton Prod. Res. Conf., National Cotton Council Am., Memphis, TN.

Chu, C. C., T. J. Henneberry, H. H. Perkins, Jr., D. L. Hendrix, and T. Steele. 1996. Sticky cotton and reduction of lint stickiness, pp. 1020-1022. *In* P. Dugger and D. Richter [eds.], Proceedings of the 1996 Beltwide Cotton Prod. Res. Conf., National Cotton Council Am., Memphis, TN.

Curley, R., B. Roberts, T. Kerby, C. Brooks, and J. Knutson. 1988. Effect of moisture on moduled seed cotton. Paper #88-1049. Amer. Soc. Agric. Engineers.

Elsner, O., J. Hani, and E. Lubenevskaya. 1983. The sugar content in lint of growing bolls. Coton et Fibres Tropicales 38: 223-227.

Hector, D. J., and I. D. Hodkinson. 1989. Stickiness in cotton. C.A.B. International, Oxon, UK, 43 pp.

Hendrix, D. L., and Y. Wei. 1992. Detection and elimination of honeydew excreted by the sweetpotato whitefly feeding upon cotton, pp 671-673. *In* D. J. Herber, and D. A. Richter [eds.], Proc. Beltwide Cotton Res. Conf., National Cotton Council Am, Memphis, TN.

Hendrix, D. L., B. Blackledge, and H. H. Perkins, Jr. 1993. Development of methods for the detection and elimination of insect honeydews on cotton fiber, pp 1600-1602. *In* D. J. Herber, and D. A. Richter [eds.], Proc. Beltwide Cotton Res. Conf., National Cotton Council Am, Memphis, TN.

Henneberry, T. J., and G. D. Butler, Jr. 1992. Whiteflies as a factor in cotton production with specific reference to *Bemisia tabaci* (Gennadius), pp. 674-683. *In* D. J. Herber, and D. A. Richter [eds.], Proc. Beltwide Cotton Prod. Conf., National Cotton Council Am, Memphis, TN.

Henneberry, T. J., D. L. Hendrix, H. H. Perkins, S. E. Naranjo, H. M. Flint, D. H. Akey, L. Forlow Jech, and R. A. Burke. 1995. *Bemisia argentifolii* (Homoptera: Aleyrodidae) populations and relationships to sticky cotton and cotton yields. Southwestern Entomol. 20: 255-271.

Khalifa, H. and O. I. Gameel. 1982. Control of cotton stickiness through breeding cultivars resistant to whitefly, *Bemisia tabaci* (Genn.) infestation. Improvement of oil seed industrial crops by induced mutations, IAEA, Vienna, pp. 181-186.

Klich, M. A. 1986. Mycoflora of cotton seed from the southern United States: A three year study of distribution and frequency. Mycologia 78: 706-712.

Miller, W. B., M. E. Peralta, D. R. Ellis, and H. H. Perkins, Jr. 1994. Stickiness potential of individual insect honeydew carbohydrates on cotton lint. Test. Res. J. 64: 344-350.

Perkins, H. H., Jr., and D. E. Brushwood. 1995. Interlaboratory evaluation of the thermodetector cotton stickiness test method pp. 1189-1191, *In* D. A. Richter and J. Armoor [eds.] Proc. Beltwide Cotton Prod. Conf., National Cotton Council, Memphis, TN.

Solvay Enzyme, Inc. 1994. European Patent Application 94201168.5, 18 pp. European Patent Office.

USDA. 1993. Sweetpotato whitefly: 1993 Supplement to the 5-Year National Research and Action Plan. T. J. Henneberry, N. C. Toscano, R. M. Faust, and J. R. Coppedge, [Eds.], USDA-ARS- 1 12, U.S. Govt- Printing Office, Washington, D. C., 178 pp.

USDA. 1994. Silverleaf Whitefly (Formerly Sweetpotato Whitefly, Strain B) 1994 Supplement to the 5-Year National Research and Action Plan. T. J. Henneberry, N. C. Toscano, R. M. Faust, and J. R. Coppedge, [Eds.], USDA-ARS, U.S. Govt. Printing Office, Washington, D.C., 224 pp.

USDA. 1995. Silverleaf Whitefly 1995 Supplement to the 5-Year National Research and Action Plan. T. J. Henneberry, N. C. Toscano, R. M. Faust, and J. R. Coppedge, [Eds.], USDA-ARS, U.S. Govt. Printing Office, Washington, D.C., 299 pp.

USDA. 1996. Fourth annual progress review of the 5-Year National Research and Action Plan for Development of Management and Control Methodology for Silverleaf Whitefly. T. J. Henneberry, N. C. Toscano, R. M. Faust, and J. R. Coppedge, [Eds.], USDA-ARS, U.S. Govt. Printing Office, Washington, D.C. 1996-01, 232 pp.

Table 1. Effects of seed cotton moisture and enzyme treatment on cotton lint stickiness. Laboratory experiments 1, 2, 3.

Treatment and estimated % seed cotton moisture	Actual % seed cotton moisture[a]	Thermodetector[a]
	Experiment 1[b]	
Untreated (no water)	4.5 d	26.3 ab
Water alone (%)		
5	8.6 c	30.3 a
10	13.9 b	16.5 bc
15	14.3 b	6.0 cd
20	17.2 a	3.5 d
25	19.9 a	3.3 d
	Experiment 2[b]	
Untreated (no water)	5.2 f	26.5 a
Water plus enzyme[c] 1%		
5	8.4 c	29.5 a
10	11.3 d	7.5 b
15	14.7 c	3.8 b
20	17.8 b	4.3 b
25	19.7 a	5.0 b
	Experiment 3[d]	
Untreated (no water)	4.9 c	29.8 a
Water alone (%)		
8	8.2 b	27.3 ab
10	9.3 b	23.3 a-c
12	12.1 a	15.0 cd
Water plus enzyme(1%)		
8	8.4 b	19.5 bc
10	8.5 b	18.8 bc
12	9.1 b	9.0 d

[a] Means of 5 replications in the same column not followed by the same letter are significantly different LSD.
[b] 5-day incubation periods.
[c] Solvay Enzymes Inc. proprietary product designated enzyme B
[d] 1-day incubation period.

Table 2. Effects of spray boom and cotton picker intake duct applied water and water plus 1% enzyme B sprays on seed cotton moisture and thermodetector counts. Field experiment 1.

Application Method/Gal./A.	Thermodetector Count		% seed cotton moisture[a]	
	Boom	Duct	Boom	Duct
2 days[b]				
60	14.5 a	16.8 a`	9.1 a	10.6 a
40	26.5 a	7.5 a	7.5 a	10.3 a
20	29.5 a	15.8 a	6.7 a	9.9 a
7 days[b]				
60	35.8 a	24.0 a	11.4 a	11.7 a
40	38.8 a	20.2 a	7.7 a	13.1 a
20	35.0 a	38.5 a	6.2 a	9.2 a
Means				
Boom vs duct	30.0 A	20.5 B	8.1 B	10.8 A
Means incubation (days)				
2	23.5 B	13.3 C	7.7 B	10.2 A
7	36.5 A	2 7 . 6 AB	8.4 B	11.4 A

[a] Means of 4 replications in a row or column not followed by the same letter are significantly different. Method of least significant difference $P \leq 0.05$.
[b] Incubation periods.

Table 3. Thermodetector (TD) counts and seed cotton moisture (SCM) percentages for machine-picked untreated or water plus 1% enzyme-treated cotton. Field experiments 2-5

	Experiment[a]							
	2		3		4		5	
Post Trmt Days[c]	TD	SCM (%)	TD	SCM (%)	TD	SCM (%)	TD	SCM (%)
1								
Untrd	21.0ab	8.2 a	13.3 a	10.5c	24.5bc	8.2a	21.5a	9.5b
Enzy.								
Boom	29.8a	8.0a	12.3ab	10.8a	--	--	15.0b	9.3 b
Duct	13.5b	10.1a	4.8c	11.6a	--	--	4.0cd	15. a
7								
Enzy.								
Boom	14.0b	9.5a	6.8bc	10.9a	18.5bc	8.4 a	26.0a	10.7b
Duct	16.5b	10.2a	6.3bc	11.0a	13.3c	9.4a	5.3cd	14.8a
14								
Enzy.								
Boom	23.0ab	8.1a	16.0a	9.0a	29.8b	9.2a	8.0c	10.9b
Duct	20.8ab	10.5a	5.3c	11.9a	15.3c	10.0a	1.3d	15.0a
28								
Enzy.								
Boom	18.0b	8.6a	--	--	48.5	--	--	--
Duct	12.5b	10.1a	--	--	17.0 c	--	--	--

[a] Means of 4 replications in a column not followed by the same letter are significantly different LSD $P \leq 0.05$.
[b] Solvay Enzymes Inc. proprietary product designated enzyme B
[c] Incubation periods

Table 4. Silverleaf whitefly honeydew sugars in cotton lint and leaf trash after ginning. Experiment 6

		Sugars in[a]				
		Lint (mg/g)		TD[b]	Trash (mg/g)[a]	
Seed Cotton Type	% Trash	Treh.	Mele.		Treh.	Mele.
Lightly Sticky Visible Trash[c]	1.44 b	0.31 a	0.16 a	11.0 b	1.41 b	0.33 b
Moderately Sticky Visible Trash[d]	2.15 a	0.36 a	0.17 a	22.0 a	2.37 a	0.57 a

[a] Means of 10 replications in a column not followed by the same letter are significantly different $P \leq 0.05$. Method of least significant differences.
[b] Thermodetector counts.
[c] Seed cotton samples of 97 to 207 grams containing lint of 40-98 and seed of 53-125 grams, respectively.
[d] Seed cotton samples of 70-117 grams containing lint of 25-43 and seed of 40-68 grams, respectively.
Treh. = Trehalulose; Mele. = Melezitose

Table 5. Effects of cotton spindle picker processing on seed cotton moisture (SCM), cotton stickiness and related sugars. Experiment 6.

	SCM			Mg/g of lint		
Treatment[a]	BP[b]	AP[c]	TDR[d] %	Treh.	Mele.	TD
Lightly-sticky cotton (1.44% leaf trash)						
No picker	5.7 b	--	0.33 c	0.29 d	0.37 c	16.2 d
Dry picker[e]	5.7 b	5.3 c	0.33 c	0.36 d	0.44 c	17.9 d
Wet picker[f]	5.8[b]	7.0 a	0.31 c	0.38 d	0.45 c	17.0 d
Moderately-sticky cotton (2.15% leaf trash)						
No picker	6.7 a	--	0.48 b	1.28 c	1.25 b	25.3 b
Dry picker[e]	6.6 a	6.0 b	0.50 b	1.48 b	1.26 b	22.1 c
Wet picker[f]	6.8 a	6.5 a	0.62 a	1.94 a	1.78 a	33.6 a
Mean cotton type						
Lightly sticky	5.8 b	6.2 a	0.33 b	0.34 b	0.42 b	17.0 b
Moderately sticky	6.7 a	6.3 a	.53 a	1.56 a	1.43 a	26.9 a
Mean picker treatment						
No picker	6.2 a`	--	40 b	0.79 b	0.81 b	20.7 b
Dry picker[e]	6.2 a	5.7 b	41 ab	0.92 b	0.84 b	19.9 b
Wet picker[f]	6.3 a	6.8 a	46 a	1.16 a	1.12 a	25.3 a

[a] Means of 5 replications, 4 observations per replication. Means in a row not followed by the same letter are significantly different $P \leq 0.05$.
[b] BP = before picker processing
[c] AP = after picker processing
[d] % of total reducing sugar.
[e] Spindle moisture pads not operating.
[f] Spindle moisture pads operating.
Treh. = Trehalulose; Mele. = Melezitose; TD = Thermodetector

Table 6. Effects of cotton ginning and lint cleaning on cotton stickiness and related sugars. Experiment 6.

Treatment[a]	% of total reducing sugars	Mg/g of cotton lint		Thermodetector counts
		Treh.	Mele.	
Lightly-sticky cotton (1.44% leaf trash)				
Lint cleaned	0.28 d	0.32 c	0.40 b	16.6 c
Lint not cleaned	0.36 c	0.36 c	0.43 b	17.4 c
Moderately sticky cotton (2.15% leaf trash)				
Lint cleaned	0.44 b	1.42 b	1.47 a	24.7 b
Lint not cleaned	0.62 a	1.71 a	1.40 a	29.2 a
Mean cleaning				
Cleaned	0.36 b	0.87 b	0.94 a	20.7 b
Not cleaned	0.49 a	1.04 a	0.91 a	23.3 a
Mean ginning				
Machine ginned	0.44 a	0.87 b	0.93 a	20.8 b
Hand delinted	0.42 a	1.03 a	0.92 a	23.2 a

[a] Means of 5 replications, 6 observations per replication. Means in a column not followed by the same letter are significantly different.Treh. = Trehalulose; Mele. = Melezitose

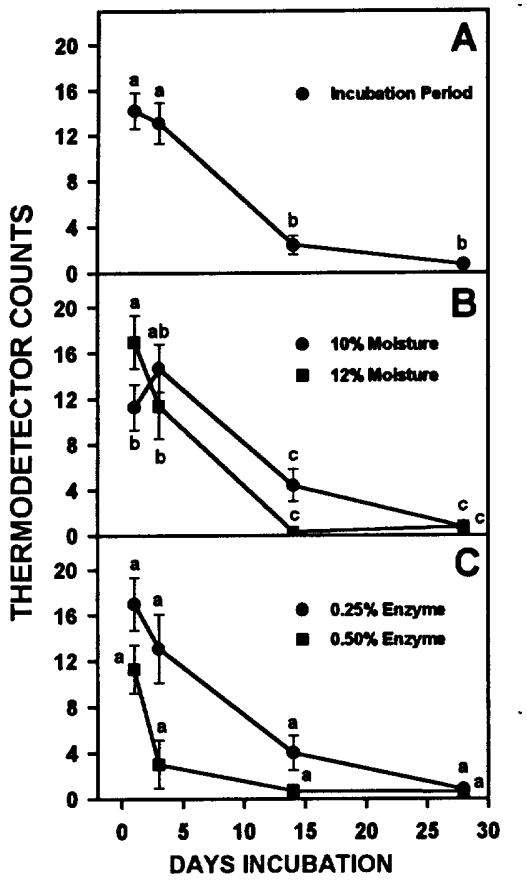

Figure 1. Overall mean thermodetector counts for incubation periods (A), seed cotton moisture (B), and enzyme treatments (C). Points within a graph not followed by the same letter are significantly different. Laboratory experiment 4.