

ENGINEERING AND GINNING

Project to Use Common Baker's Yeast, *Saccharomyces cerevisiae*, to Mitigate Cotton Stickiness

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ABSTRACT

Honeydew contaminated cotton can interfere with ginning and cause interruption of production in the mill until the problem is corrected. Cleanup is expensive and time consuming. This study attempts to use a yeast spray application to remediate this sticky cotton condition. First pilot tests were done to determine if baker's yeast can utilize trehalulose and melezitose, the two major sugars causing cotton stickiness. In order to conduct this project, a method to create on demand levels of sticky cotton was developed. This method of blending sticky and non-sticky cottons to create a graded series of sticky cotton can be useful for other studies involving sticky cotton. For this test, *Saccharomyces cerevisiae* found in baker's yeast was used as a safe and readily available bioremediator. Sticky cottons were sprayed with the yeast and after incubation at room temperature (20°C), and at 30°C, the treated sticky cottons were compared with non-treated controls using the minicard test and rating index to measure stickiness. While a few treatments showed statistically significant reductions in stickiness, there were cases where the yeast treatment resulted in a statistically higher level of stickiness. Furthermore, when reductions were observed, they were not practically useful to improve processability in the gin or textile mill.

INTRODUCTION

Compared to the 1980's and 1990's, the problem of sticky cotton may have abated if the fewer number of presentations on the subject in the recent Beltwide Conferences is any indication. Possibly the rewards of judicious use of insecticides and aggressively followed integrated pest management (IPM) programs are being reflected in fewer incidences of sticky

cotton; and that the gins and textile mills have become more adept at coping with the problem. However, sticky cotton is still a serious economic concern in the cotton industry when it occurs (Elliott, 2002). The condition of sticky cotton arises from high levels of natural plant sugars or from insect honeydew (Perkins, 1971, 1993). When stickiness results from naturally occurring plant sugars, the sugars are usually more uniformly distributed on the cotton, and the problems are subtle. This kind of sticky cotton problem results in an accumulation of lint and residue buildup on textile machinery and roll laps. But such problems by naturally occurring plant sugars are easily remedied by an accelerated cleaning schedule of the rolls and machine parts. In contrast, insect honeydew falls on the leaves and bolls, and can further contaminate the fiber during harvest, leaving the cotton with spotted areas of sticky residue which can additionally become discolored due to sooty mold. Insect caused non-uniform distribution of stickiness can result in acute problems in the gin and mills; and in cases of heavy stickiness, can cause production interruptions that require immediate correction (Brushwood & Perkins, 1993; Perkins, 1993). As a consequence, sticky cotton is considered lower quality cotton and the grower is penalized when the cotton is discounted.

The most effective solution to the problem of sticky cotton is prevention. This is where the application of insecticides and adoption of an effective IPM program have been the most successful in preventing the build up of aphids and whiteflies, the insects most responsible for honeydew deposition on cotton. However control of insects causing contamination can be extremely difficult. Many variables including unpredicted weather factors, delays in farming practices, or even insect movement from neighboring fields can reduce effectiveness of prevention measures so that late-season whitefly or aphid infestation result in honeydew deposition on the lint. For this reason, many after the fact measures have been proposed and studied (Perkins, 1993). In the textile mills, several strategies have been suggested to improve the processability of the contaminated cottons. Some strategies include lowering the rela-

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tive humidity in yarn manufacturing areas. The most successful strategy is blending sticky cottons with non-sticky cottons to obtain a mix that will process satisfactorily; but this is largely a trial and error process which can be time consuming; and there are the problems of measuring the degree of stickiness, and the random highly variable isolated occurrences of stickiness. An even more time consuming stratagem is to allow the cotton to fluff and dry out, often with the aid of some heat, by opening the cotton in advance of processing. Many mills will also use processing aid sprays (Perkins et al., 1992).

These attempts to permit processability of sticky cotton require additional processing steps by the mill, and require foreknowledge that the cotton is sticky which means that prior testing for stickiness must have been performed on the cotton. Since testing is expensive, it is commonly not done unless a problem has already been experienced. The difficulties caused by sticky cotton may interrupt processing until the cause has been remedied. For this reason, other remedies have been suggested at the field, gin or bale storage stage, before reaching the mills.

Many field approaches to remedy cotton already contaminated with honeydew have been suggested. Among the simplest was to merely wait long enough for rain or overhead irrigation to wash the honeydew off the fiber. Another field technique was the use of an experimental enzyme approach to degrade honeydew sugars on sticky cottons in the field and the laboratory (Henneberry, et al, 1997; Hendrix et al., 1993). Unfortunately, the ameliorating effect in these stratagems can be traced back to high moisture content (Henneberry, et al, 1997; Hendrix et al., 1993). When such high moisture contents are used, the potential of moisture damage to fiber quality can occur (Chun & Anthony, 2004; Chun & Brushwood., 1998; Chun et al., 1995). Another approach taken was to enrich the environment of the indigenous microbial organisms on cotton fiber by providing more optimal conditions by spraying ammonia compounds at different moisture contents. The results from laboratory and semi-industrial-sized experiments suggest that the microbial activity was increased and resulted in observable reductions in stickiness without causing damage to the lint (Heuer and Plaut, 1985). But this approach does not appear to have been adopted for field reduction of honeydew. A recent approach involves collection of indigenous yeasts from plant surfaces that degrade sugars from insect

honeydew (Elliot, 2002). The hope is to eventually identify specific yeasts, or groups of yeasts that can be used as powerful bioremediation agents to degrade insect honeydew.

An old report of a method for reducing sugar stickiness involved spraying *Beijerinckia mobilis*, a free living nitrogen-fixing bacterium, on sticky cotton (Balasubramanya et al., 1985). The results of that study showed significant reduction of stickiness, but the author cautioned against its use because *B. mobilis* is a gram-negative bacterium which may increase the endotoxin level of cotton. Since this paper was published, endotoxin has been recognized as the causative agent of byssinosis (Castellan et al., 1984; 1987). Since there is no correlation between cotton dust potential and stickiness (Chun, 2002), the addition of gram-negative bacteria to sticky cotton could increase the level of endotoxin and hence increase health risks. But not included in the abstract of that paper (Balasubramanya et al., 1985) was that the author found that *Saccharomyces cerevisiae* would also reduce stickiness to the point where the treated cotton became spinnable. *Saccharomyces cerevisiae* is considered a relatively safe microorganism and can be found as baker's yeast. Baker's yeast is used in the home and in industry so no serious regulatory problems for its use are expected. Furthermore, baker's yeast can be bought in bulk so it would be an easily acquired bioremedial agent that could be mixed and applied at the ginning stage.

MATERIALS AND METHODS

Measurement of Stickiness. There are many ways to measure stickiness: pH spray indicator test, Benedict test, USDA potassium ferricyanide test, thermodetector, rotor ring test, the minicard test and others (Barton et al., 2005; Brushwood and Perkins, 1993; Hector & Hodgkinson, 1989). Of these, the minicard test was chosen because of its widespread acceptance (Barton et al., 2005; Brushwood & Perkins, 1993; Chun, 2002; Frydrych et al., 1994; Hequet & Frydrych, 1992; Perkins & Brushwood, 1994; Watson, 1994). A recent detailed description of the minicard test process and minicard stickiness rating has been described (Barton et al., 2005; Brushwood & Perkins, 1993). Generally, four minicard ratings are used: 0, no stickiness; 1, light stickiness; 2, moderate stickiness; and 3, heavy stickiness. However, a skilled

operator can subjectively grade levels of stickiness between these four broad categories. For example, if a cotton was judged to be a light stickiness cotton, '1', but just not sticking or wrapping around the delivery rolls or leaving sufficient sticky residue specks on the delivery rolls enough to be rated as moderately sticky, '2', the operator may rate that cotton as a '1+', or a '1++', or even a '1+++'. For this study, a minicard index (MCI) was used which is based on the main rating number plus 0.33 for each subjectively assigned '+' given by the operator. For instance a '2++' or a '2+' rating would be given MCI values of 2.66 and 2.33, respectively. For each replicate sample, the average of 2 or 3 minicard readings from the sample replicate was used as the MCI value of that sample replicate.

Sticky Cotton Samples. Cotton samples of increasing levels of stickiness were created by blending a non-sticky lot of cotton with a highly sticky lot of cotton. The highly sticky bale measured beyond a 3-minicard rating and was purchased in May of 2003 and was harvested during the 2001 harvest year. For the non-sticky cotton, initially non-sticky cotton from a previous study was used, which has been fully described (Chun & Brushwood, 1998). However as the study progressed, this cotton source became depleted and a new source of non-sticky cotton was found that was purchased in May 2005 and believed to be from the 2004 harvest year. When blended with the sticky cotton, the results appeared to be the same as the non-sticky cotton previously used. Both the non-sticky and sticky cottons were pima cotton.

Through trial and error, mixtures of sticky and non-sticky cotton were blended to create a stepped series of 7 sticky cotton sample lots (Table 1). The sticky and non-sticky cottons were blended by running each 50-gm sample twice through a Shirley Analyzer (Shirley Institute, Manchester, England). Each 50-gm sample was then kept in a 22.9 cm x 30.5 cm ziplock 0.05-mm thick clear plastic bag (BCU Plastics & Packaging, San Marcos, CA) until used.

Yeast Inoculum. The yeast used in the study was *Saccharomyces cerevisiae* from a commercial 455 g package of baking yeast (Instant Baker's Yeast, Fleischmann's Yeast, Fenton, MO). The baking yeast was transferred to a glass jar and stored in a refrigerator (4°C) until used. The viable population density of the baking yeast was determined using general microbial assay methods (Chun et al., 2006). The population density of the baker's yeast averaged $\sim 7.6 \times 10^9$ cells/gm.

Test of Utilization of Stickiness Sugars. Before investing resources into testing, a pilot test was done to determine if this yeast would utilize the sugars involved with stickiness. This was tested by growing baker's yeast on trehalulose and melezitose as the sole carbon source. The rationale was that both trehalulose and melezitose are the main sugars believed to be involved with cotton stickiness (Brushwood and Perkins, 1994 & 1995; Hendrix et al., 1993). A sugar solution was added to flasks containing 99-ml of an in-house diluent (Chun and Perkins, 1996), a weakly buffered salt solution made with deionized water usually containing gelatin and Tween-80 but made without either the gelatin or Tween-80 for this study, to make a series of solutions containing 0.16%, 0.32% and 0.48% (w/v) sugar concentrations. These flasks were then inoculated with a suspension of baker's yeast, *Saccharomyces cerevisiae*, to bring the solution density to approximately 100 cfu/ml. The amount of yeast to add was based on an earlier determination of the population density of the baker's yeast. The sugars used were trehalulose (from a 90% trehalulose syrup, Südzucker, Mannheim/Ochsenfurt, Germany) and melezitose (Sigma Chemical Co., St. Louis, MO). Water was used as the sugar controls. The flasks were stationary incubated at 37°C for 3 days before being assayed for yeast population density (Chun et al., 2006). Four replicates were used for each sugar concentration for a total of 40 samples, which included a time zero yeast density count. The test was repeated and the results combined before analysis.

Treating Sticky Cotton with Yeast. Sticky cottons were sprayed with diluent and baker's yeast or sprayed with diluent without baker's yeast. These cottons were incubated at room temperature (20°C) or 30°C. After incubation, the cottons were assayed for stickiness using the minicard. The sticky cottons were the 50-g lots of sticky cotton blended from non-sticky and sticky cotton stored in plastic ziplock bags. The yeast spray was made by suspending 1.0 gm of baker's yeast ($\sim 7.6 \times 10^9$ cells/gm) in 99 ml diluent; and then 1.0 ml was taken from this suspension and suspended in a second 99 ml diluent. From this second suspension of yeast, 5.0 ml was removed and sprayed on each cotton sample using an air brush (621 kPa [90 psi] spray pressure). The spray was applied until exhaustion. Two operators worked during spraying to maximize application of the spray to the cotton: one to move the air brush

and apply the spray; and the second to 'expose' the cotton surface to the spray. The sprayed cotton was immediately returned to the plastic bag and the bags were then sealed and all the samples incubated. At the end of the incubation period, the plastic bags were removed from incubation, the bags opened, and the cottons conditioned before assaying for stickiness with the minicard.

Two tests of the incubation at room temperature were made, the first for 9 days and the second for 20 days of incubation. All 7 sticky lots of cotton were treated with or without yeast, 4 50-g replicates were used for each yeast-treatment/lot treatment for a total of 56 50-g samples and the minicard index for each sample replicate was an average of two minicard determinations. The treatment assignment was completely random; however, all cotton lots sprayed with yeast were treated at the same time and all lots sprayed without yeast were sprayed at the same time. In each case, the individual 50-g sample lot was sprayed sequentially based on its randomly assigned identification number. This was done to save time and reduce cross-contamination. The results of the two tests appeared to be unaffected by the different incubation times and the results were combined for analysis. Three 50-g lots of non-sticky cotton were sprayed with diluent and incubated at room temperature, and ran parallel to the second incubation test. These cotton lots were sampled periodically during incubation to determine the moisture content of the cotton during incubation. The oven drying method (ASTM, 1971) was used to determine moisture content.

Two tests at a 30°C incubation temperature were made. Both tests were incubated for 14 days. For the 30°C incubation tests, lot #5 of the sticky lots of cotton was not used in these tests. The sticky lots were treated with or without yeast, 4 50-g replicates were used for each yeast-treatment/lot treatment for a total of 48 50-g samples. The treatment assignment was completely random; however, all cotton lots sprayed with yeast were treated at the same time and all lots sprayed without yeast were sprayed at the same time, in each case the individual 50-g sample lots was sprayed sequentially based on its randomly assigned identification number. This was done to save time and reduce cross-contamination. The results of the two tests were later combined for analysis. Three 50-g lots of non-sticky cotton were sprayed with diluent and stored at room temperature and run parallel to

each of the main incubation tests. But these cotton lots were sampled periodically during incubation to determine the moisture content of the cotton during incubation.

Statistical Analysis. Data were analyzed with release 8.00 of SAS (SAS, Statistical Analysis System; SAS system for Windows NT, SAS Institute Inc., Cary, NC, USA) for Duncan mean comparisons when the analysis of variance analysis yielded significant 'F-values' to indicate a high degree of difference of the variable to the variation. Graphs and regression statistics generation were created using SigmaPlot for Windows Version 10.0 (Systat Software, Inc., Richmond, CA). Microsoft® Office Excel 2003 (Microsoft Corporation, USA) was used to randomize treatment assignments, to enter and store data, to sort data and prepare for SAS analysis, to transform data, to summarize and tabulate results, to obtain simple treatment statistics (means, standard deviations, regressions, t-test comparison, etc.), and to perform other spreadsheet functions.

RESULTS AND DISCUSSION

One of the difficulties of doing studies with sticky cotton is acquiring cottons with different levels of stickiness in sufficient quantities for research. The most common approach is to collect cottons from many sources over a long period of time (Barton et al., 2005; Chun, 2002). Then these samples must be tested and sorted to the criteria of the study. The approach taken here was to create sticky cottons of different levels of stickiness on demand. The approach was simply to blend a non-sticky batch of cotton with a highly sticky batch of cotton. As shown in Table 1, this approach and use of a minicard index successfully provided cottons of at least 7 levels of stickiness which are significantly different from one another. The specific mix of sticky to non-sticky cottons was by trial and error, but once a successful recipe for the particular cottons was made, approximate levels of the same stickiness could be made as needed. This approach to creating repeatable levels of sticky cottons can be adjusted for sources of sticky cotton less sticky than the one used here. This approach to creating different levels of sticky cotton can also be fine tuned and expanded to create extended levels between the minicard ratings of 0, 1, 2 and 3, for other types of studies.

Table 1. Composition of the lots of sticky cotton blended from non-sticky and sticky cotton.

Lot	Content	Average MCI ^{zyx}	MCI Range ^{yw}
1	0.0 gm MCI 3 + 50.0 MCI 0	0.00 ^G	0.00
2	1.0 gm MCI 3 + 49.0 MCI 0	1.00 ^f	1.00
3	4.0 gm MCI 3 + 46.0 MCI 0	2.44 ^e	2.00-3.00
4	8.0 gm MCI 3 + 42.0 MCI 0	3.15 ^d	3.00-3.33
5	16.0 gm MCI 3 + 34.0 MCI 0	3.48 ^c	3.33-3.66
6	25.0 gm MCI 3 + 25.0 MCI 0	3.66 ^b	3.66
7	50.0 gm MCI 3 + 0.0 MCI 0	3.99 ^a	3.99

^z The minicard index is averaged from 3 tests, where each sample lot in the test was replicated 3 times.

^y The minicard index is based on the minicard rating plus .33 for each subjective '+' assigned; for example, 3+++ will be valued at 3.99 and 2+ will be valued at 2.33.

^x Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

^w Lowest and highest MCI values of the sample replicates from each lot – Three test, three sample replicates per test for each lot; and the minicard rating is the average of two minicard determinations for each sample replicate.

Early in this project to attempt reduction of stickiness by applying readily available yeast to sticky cotton, we needed to first determine if baker's yeast would degrade trehalulose and melezitose. This was a concern because trehalulose and melezitose were considered difficult sugars to be utilized by microorganisms (Thompson et al., 2001). In addition, where indigenous yeast's ability to reduce sugars from insect honeydew was studied (Elliot, 2002), it was not clear if baker's yeast would utilize either trehalulose or melezitose. When both sugars were provided as the sole carbon source, melezitose did not appear to be utilized at a rate to show noticeably significant growth compared to when no sugar was provided (Table 2). On the other hand, the increased yeast density after incubation is significantly greater with sugar than with the water controls at the beginning of incubation and at the end of incubation which suggests that trehalulose supports yeast growth and is presumed to be utilized by baker's yeast. When comparing the starting yeast populations in water alone and after 3 days of incubation, the population densities are not significantly different. However, the population after incubation was about half what it was at the start which suggests that without a suitable energy source some of the yeast were dying off during incubation. Regardless, this project was

continued even though only trehalulose appears to be degraded by the baker's yeast since most of the sticky cottons that have been sent to CQRS have been found to be from whitefly honeydew.

Table 2. The Initial Population density of *Saccharomyces cerevisiae* at the start of incubation and after 3 days incubation on trehalulose (sugar associated with whitefly sticky cotton), melezitose (sugar associated with aphid sticky cotton), or the water controls at 37°C.

Treatment ^z	Yeast density, cfu/ml ^y
Trehalulose	3.63 ^a
Melezitose	2.89 ^b
Water ^x	3.05 ^b
Water ^w	2.74 ^b

^z For all sugar concentrations, 0.16%, 0.32% and 0.48%

^y Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

^x Water control, at start of incubation.

^w Water control, after 3 days incubation

The overall effect of using yeast to remediate stickiness was that no reduction in stickiness was observed and treatment actually appeared to increase stickiness at 20°C from a statistical perspective (Table 3). The study began with room temperature (20°C) incubation as a reasonable first approach since baker's yeast could grow at that temperature and warehouses were kept at that temperature for parts of the year. However, when the yeast was sprayed on the cottons there were concerns that room temperature may not have been warm enough for the yeast to be effective as a bioremediator since Brushwood and Perkins (1994) observed slow microbial breakdown of honeydew sugars at room temperature. However, even at 30°C incubation for 14 days, no stickiness reduction was observed (Table 3).

Table 3. Overall effect on stickiness by spraying yeast on sticky cotton, after incubation at room temperature (20°C) for 9 and 20 days and after incubation at 30°C for 14 days.

Treatment	Average MCI ^{zy} , 20°C	Average MIC ^{zy} , 30°C
No Yeast	2.28 ^a	2.38 ^a
Yeast	2.47 ^b	2.34 ^a

^z The mini-card index is based on the mini-card rating plus .33 for each subjective '+' assigned; for example, 3+++ will be valued at 3.99 and 2+ will be valued at 2.33.

^y Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

One possible explanation for the results of table 3 is that those cottons with high initial levels of stickiness, any small reduction in stickiness would not be noticeable with the high levels of stickiness present, and may have overshadowed changes in the less sticky cottons. To determine if this was the case, t-tests were done for each level of sticky cotton (Tables 4 & 5). The t-test probabilities at RT incubation showed many sticky lots with significant differences between the lots treated with and without yeast. But where significant differences were shown, the sticky lots treated with yeast tended to have higher MCI than the non-treated lots (Table 3). Sticky Lots 2, 4, 5, 6,

Table 4. Overall effect on stickiness by spraying yeast on sticky cotton with different levels of stickiness, after incubation at room temperature (20°C) for 9 and 20 days.

Lot	t-Test, P ^y	AVG MCI ^z	
		No Yeast	Yeast
1		0.00	0.00
2	0.0203	0.00	1.00
3	0.1550	2.50	2.00
4	0.0024	2.50	3.33
5	0.0412	3.17	3.33
6	0.0016	3.33	3.66
7	0.0492	3.83	3.99

^z The mini-card index is based on the mini-card rating plus .33 for each subjective '+' assigned; for example, 3+++ will be valued at 3.99 and 2+ will be valued at 2.33.

^y The probability associated with a Student's t-Test, 2-tailed distribution paired test.

Table 5. Overall effect on stickiness by spraying yeast on sticky cotton with different levels of stickiness, after incubation at 30°C for 14 days.

Lot	t-Test, P ^y	AVG MCI ^z	
		No Yeast	Yeast
1	0.1379	0.13	0.62
2	0.0016	1.88	1.31
3	0.0136	2.56	1.88
4	0.0162	2.31	2.96
6	0.0000	3.62	3.35
7	0.1114	3.76	3.89

^z The mini-card index is based on the mini-card rating plus .33 for each subjective '+' assigned; for example, 3+++ will be valued at 3.99 and 2+ will be valued at 2.33.

^y The probability associated with a Student's t-Test, 2-tailed distribution paired test.

and 7, all tended to be stickier than the non-treated lots. However, even though the differences are statistically significant, the increased stickiness would probably not be practically discernible at the textile mill. Except for lot 6, the differences would be below a '+' in a minicard rating. The results from the 30°C incubation were more encouraging, but still inconsistent (Table 5). Sticky lots 2, 3, 4, and 6 had significantly different MCI averages between the yeast and non-treated cotton lots. In this case, sticky cotton lots 2, 3 and 6 had average MCI lower in the yeast treated lots. However, the reductions in stickiness, while statistically significantly different, were small. What is puzzling is the higher MCI from both the RT and 30°C incubation. Possibly the presence of the yeast may have contributed to some of the stickiness: the approximate yeast density applied was 7.6×10^4 CFU/gm cotton and if unequal distribution occurred than localized areas with higher number of cells may occur. Another, though unlikely, source of added stickiness may have been carry over of the emulsifying agents used in baker's yeast. Listed as ingredients of baker's yeast are: yeast, sorbitan monostearate, and ascorbic acid. Even though the amount of carry over is expected to be very small, what may have been carried over and not utilized by the yeast may have interacted with the yeast and surface material of the cotton to make the surface stickier.

Cotton moisture was found to be an important influencing variable. During early preliminary testing, the moisture content immediately after spraying the 5-ml diluent alone or with yeast was found to be approximately 13.7%. This moisture content is considered high and was expected to be more than sufficient for microbial activity (Chun & Anthony, 2004; Chun et al., 2006). The concern was that this high moisture content would preclude this type of remediation method (Anonymous, 2003). The moisture content was followed at RT incubation for 21 days (Figure 1). Moisture loss was linear ($r^2 = 0.97$, $y = 11.76 - .16x$) and above 7.5% for the 21 days. From this, the moisture content was believed to be sufficient for yeast activity during the 30°C incubation. When the moisture content was tracked during the experiment, it was found to be more rapid at 30°C incubation ($r^2 = 0.95$, $y = 11.51 - 0.47x$; Figure 2). Moisture content was above 7.5% for less than a week which may have halted or slowed yeast activity early in the incubation process and prevented MCI reduction; so while the 0.05-mm thick clear plastic bags acted as a barrier, it was not impervious to moisture loss.

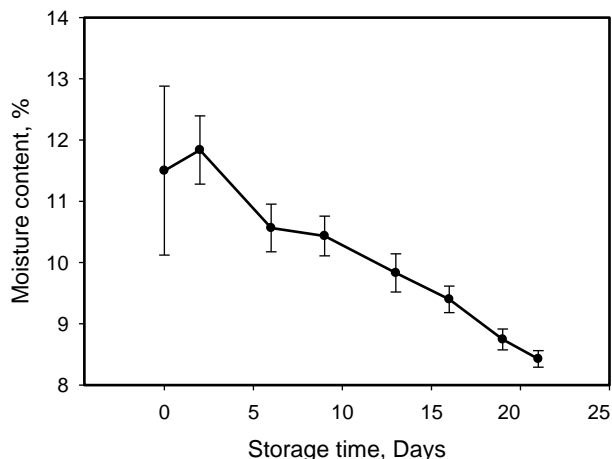


Figure 1. Average moisture content of 3 50-g cotton samples incubated at 20°C, each sample was initially sprayed with 5.0 ml of diluent without yeast.

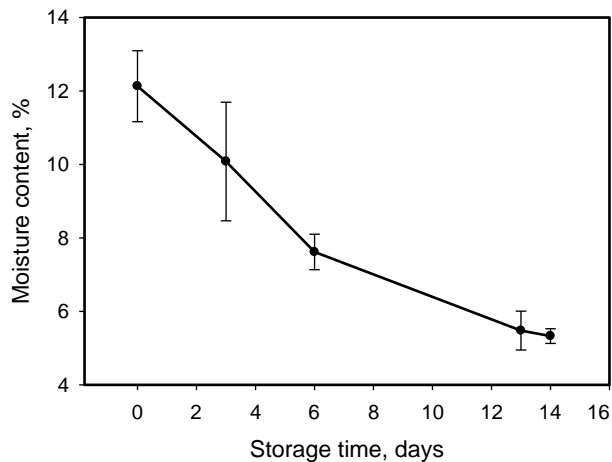


Figure 2. Average moisture content of 6 50-g cotton samples incubated at 30°C, each sample was initially sprayed with 5.0 ml of diluent without yeast.

In this study, yeast application to reduce stickiness does not appear to be a practical remediation practice. However, not all possible variables have been explored. While 7.6×10^4 CFU/gm yeast was used, this inoculum density could be increased without substantially increasing the cost of treatment since packaged baker's yeast is inexpensive. A higher density should improve coverage to improve chances of contacting sticky spots. A 5-ml carrier diluent was used to avoid excessive moisture. However, while moisture is retained for a long time at RT, a higher volume of carrier diluent may be a better choice for the higher incubation temperature where moisture is rapidly lost. In addition, moisture alone helps reduce stickiness (compare Tables 1 and 6) so perhaps more moisture would enhance yeast activity. The variables that could be changed to possibly improve the efficacy of the

yeast spray can be expanded, but the results obtained here probably will not be changed significantly.

In summary, laboratory trials using the yeast spray treatment to reduce cotton stickiness has shown that stickiness can be reduced, but where reductions occur, the improvement may not be of a practical nature to make the effort worthwhile. However, now that this approach has been tried, we are in a better position to discuss it along with other strategies of stickiness reduction. The benefit derived from undertaking this study, however, is that we have worked out a method of creating cotton of known levels of stickiness on demand which can be used in future studies.

Table 6. Average MCI of the lots of sticky cotton sprayed with diluent but no yeast and incubated at room temperature (20°C) for 9 and 20 days and at 30°C for 14 days.

Lot	Content	Average MCI ^{z,y}
1	0.0 gm MCI 3 + 50.0 MCI 0	0.06 ^f
2	1.0 gm MCI 3 + 49.0 MCI 0	1.19 ^e
3	4.0 gm MCI 3 + 46.0 MCI 0	2.56 ^d
4	8.0 gm MCI 3 + 42.0 MCI 0	2.43 ^d
5	16.0 gm MCI 3 + 34.0 MCI 0	3.12 ^c
6	25.0 gm MCI 3 + 25.0 MCI 0	3.49 ^b
7	50.0 gm MCI 3 + 0.0 MCI 0	3.81 ^a

^z The mini-card index is based on the mini-card rating plus .33 for each subjective '+' assigned; for example, 3+++ will be valued at 3.99 and 2+ will be valued at 2.33.

^y Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

DISCLAIMER

Mention of a trademark, warranty, proprietary product or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply approval or recommendations of the product to the exclusion of others that may also be suitable.

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