

PROJECT TO USE COMMON BAKER'S YEAST, *SACCHAROMYCES CEREVISIAE*, TO MITIGATE COTTON STICKINESS**David T.W. Chun****USDA-ARS, Cotton Quality Research Station
Clemson, SC****Introduction**

Compared to 20 or even 10 years ago, the number of presentations about sticky cotton at the Beltwide Conferences is fewer, which suggest that the sticky cotton problem has lessened. But sticky cotton is a serious economic concern in the Cotton Industry when it occurs (Elliott, 2002). As the name implies, the cotton is sticky. The condition of sticky cotton arises from high levels of natural plant sugars or from insect honeydew (Perkins, 1971, 1993). Stickiness from naturally occurring plant sugars result in the stickiness being more uniformly distributed on the cotton and the stickiness problem is usually not considered too serious. But when insect honeydew falls directly on the cotton in the bolls or from coming in contact with contaminated leaves before or during harvest, the spotted and sticky residue are usually not uniformly distributed. This can then cause acute problems in the gin and mills; and in cases of heavy stickiness, production interruption can result which will 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. For this reason, field and laboratory investigation of sticky cotton problems have been ongoing.

Prevention has always been the most effective solution to the problem of sticky cotton. This usually involves adoption of an effective IPM program and the application of insecticides in preventing the build up of aphids and whiteflies. But control of insects causing contamination can be extremely difficult. Many variables including unpredicted weather factors, delays in farming practices, or even insect movement from surrounding neighbors can reduce effective prevention measures so that late-season whitefly or aphid infestation result in honeydew deposition on the lint. For this reason, post harvest measures have been proposed and studied (Perkins, 1993). Several strategies have been suggested to improve the processability of the contaminated cottons. Some strategies include lowering the relative 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 and can be time consuming to get just the right blend level. Another stratagem is to allow the cotton to fluff and dry out, often with the aid of some heat, by opening the cotton in advance to processing. Many mills will also use processing aid sprays (Perkins et al., 1992).

These attempts to permit processability of sticky cotton can be viewed as unnecessary steps by the mill. Worst yet, to engage any of these strategies require foreknowledge that the cotton is sticky which means that prior testing for stickiness must have been performed on the cotton. Since testing 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.

Field approaches to remedy cotton already contaminated with honeydew have been suggested. The simplest was to simply wait long enough for rain to wash the honeydew off the fiber or to use overhead irrigation to do the same thing. Related to this 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 observed as reduction in stickiness without having caused damage to the lint (Heur and Plaut, 1985). But this approach does not appear to have been adopted for field reduction of honeydew. A recent approach collects indigenous yeasts from aerial plant surfaces that degrade sugars from insect honeydew (Elliot, 2002). The hope is to eventually identify specific 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. In that paper, the author cautions 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., 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 a point not included in the abstract of that paper was that *Saccharomyces cerevisiae* would also reduce stickiness to the point where the treated cotton became spinnable. Because our location is involved with studies related to stickiness, we have often been queried about this approach. So in order to be knowledgeable of this approach we decided to study it. *Saccharomyces cerevisiae* is considered a relatively safe microorganism and is commonly sold as baker's yeast. Baker's yeast is used in the home and in industry so no serious regulatory problem to its use is expected. 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. Our findings will be presented as well as a description of a simple method, presented as a second paper elsewhere (Chun, 2008), that we developed to have cottons of increasing levels of stickiness available on demand for our studies.

Materials and Methods

Measurement of Stickiness

The minicard test was chosen for measuring stickiness 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 detailed description of the minicard test process and minicard stickiness rating has been described (Barton et al., 2005; Brushwood & Perkins, 1993). In the minicard test, four minicard ratings are used: 0, no stickiness; 1, light stickiness; 2, moderate stickiness; and 3, heavy stickiness. These four index ratings are limited and since a skilled operator can subjectively grade levels of stickiness between these four broad categories, an in-house rating system was adopted. For example, if a cotton was judged to be a light stickiness cotton a rating of '1' was used; but if the cotton was 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 sample, the average of 2 or 3 minicard readings from the sample was used as the MCI value of that sample.

Sticky Cotton Samples

The approach was simply to blend a non-sticky batch of cotton with a highly sticky batch of cotton, so that cotton samples of increasing levels of stickiness were created by this blending of different ratios of a non-sticky lot with a heavily sticky lot of cotton, details are presented elsewhere (Chun, 2008). A stepped series of 7 sticky cotton sample lots were used. The sticky cotton was a 50-gm sample, which was run 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 off-the-shelf 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 if available commercial yeast would reduce stickiness in cotton, a pilot test was done to determine if this yeast would utilize the sugars involved with stickiness. This was shown 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 diluent (Chun and Perkins, 1996) without gelatin or Tween-80 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 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 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 (without Tween-80 or gelatin) with baker's yeast or sprayed with diluent without baker's yeast. These cottons were incubated at room temperature (20°C; RT) 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 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 airbrush (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 airbrush 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. Each sample's stickiness was an average of two minicard determinations and reported as a minicard index.

Two tests of the incubation at RT were made. The first for 9 days and the second for 20 days of incubation. All 7 sticky lots of cotton were treated with yeast or without yeast, 4 50-g replicates were used for each yeast-treatment/lot treatment for a total of 56 50-g samples. The treatment assignment was completely random; however, all cotton lots sprayed with yeast were done 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 were 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 RT and ran parallel to the second incubation test. But these cotton lots were sampled periodically during incubation to determine the moisture content of the cotton during incubation. The oven-dried 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 yeast 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 was done 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 were 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 RT and ran 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

Early in this project, 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 is provided (Table 1). On the other hand, the increased yeast density after incubation is significantly greater than 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 whiteflies honeydew.

Table 1. The Initial Population density of *Saccharomyces cerevisiae* at the start of incubation and after 3 days incubation on trehalulose, melezitose, or the water controls at 37°C.

Treatment ^Z	Yeast density, cfu/ml ^Y
Trehalulose ^W	3.63 ^A
Melezitose ^W	2.89 ^B
Water ^X	3.05 ^B
Water ^W	2.74 ^B

^ZFor all sugar concentrations, 0.16%, 0.32% and 0.48%

^YMean separation within column by Duncan's multiple range test, 5% level.

Means with the same letter are not significantly different.

^XWater control, at start of incubation.

^WWater control, after 3 days incubation

The overall effect of using yeast to remediate stickiness was that no reduction in stickiness was observed (Table 2). The study began with RT (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 RT may not have been warm enough for the yeast to be effective as a bioremediation 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 2).

Table 2. 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

^ZThe 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.

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

In both cases, these were the overall results (Table 2) where lots of cottons with different levels of stickiness were analyzed together. Very possibly those cottons with high initial levels of stickiness would not be affected by the yeast application and may have overshadowed changes in the lesser sticky cottons. Very possibly the yeast may have been more successful in reducing the stickiness of lightly sticky cottons. To account for this, t-tests were done

for each level of sticky cotton (Tables 3 & 4). 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 and 7, all tended to be stickier than the non-treated lots. However, even though the differences are significant, the increased stickiness would probably not be practically discernible. Except for lot 6, the differences would be below a '+' in a minicard rating. The results from the 30°C incubation were more encouraging (Table 4). 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 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 then 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.

Table 3. 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	0.50	1.19
3	0.1550	2.56	2.13
4	0.0024	2.56	3.21
5	0.0412	3.12	3.27
6	0.0016	3.35	3.54
7	0.0492	3.87	3.97

^ZThe 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 4. 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

^ZThe 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.

In Table 5, the MCI values of the controls of the individual sticky lots and the MCI values of individual sticky lots, which had not been treated at all are shown. In general, the differences between the two are all very small changes in MCI. The controls (diluent spray without yeast) tend to have lower MCI than the corresponding sticky cotton lots that had been untreated. So it seems that just the effect of wetting the sticky cottons tends to reduce MCI; and the difference caused by 'wetting' the sticky cotton seem to obscure any effect produced by the addition of *S. cerevisiae* which made the value of a yeast treatment more in doubt.

Table 5. Average MCI of the lots of sticky cotton which had been prepared but untreated and the average MCI of the lots of sticky cotton used as controls to study the effect of spraying yeast to reduce stickiness, these controls only contained diluent with 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 ^{ZYX}	Average MCI ^{ZYW}
1	0.0 gm MCI 3 + 50.0 MCI 0	0.00 ^G	0.06 ^F
2	1.0 gm MCI 3 + 49.0 MCI 0	1.00 ^F	1.19 ^E
3	4.0 gm MCI 3 + 46.0 MCI 0	2.44 ^E	2.56 ^D
4	8.0 gm MCI 3 + 42.0 MCI 0	3.15 ^D	2.43 ^D
5	16.0 gm MCI 3 + 34.0 MCI 0	3.48 ^C	3.12 ^C
6	25.0 gm MCI 3 + 25.0 MCI 0	3.66 ^B	3.49 ^B
7	50.0 gm MCI 3 + 0.0 MCI 0	3.99 ^A	3.81 ^A

^ZThe 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.

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

^XUntreated sticky lots, the minicard index is averaged from 3 tests, where each sample lot in the test was assayed 3 times.

^WControl sticky lots used as the controls, these controls only contained diluent without yeast.

Cotton moisture was followed as 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 more 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. But when the moisture content was followed (Figure 2), moisture loss was much faster at 30°C incubation ($r^2 = 0.95$, $y = 11.51 - 0.47x$). 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.

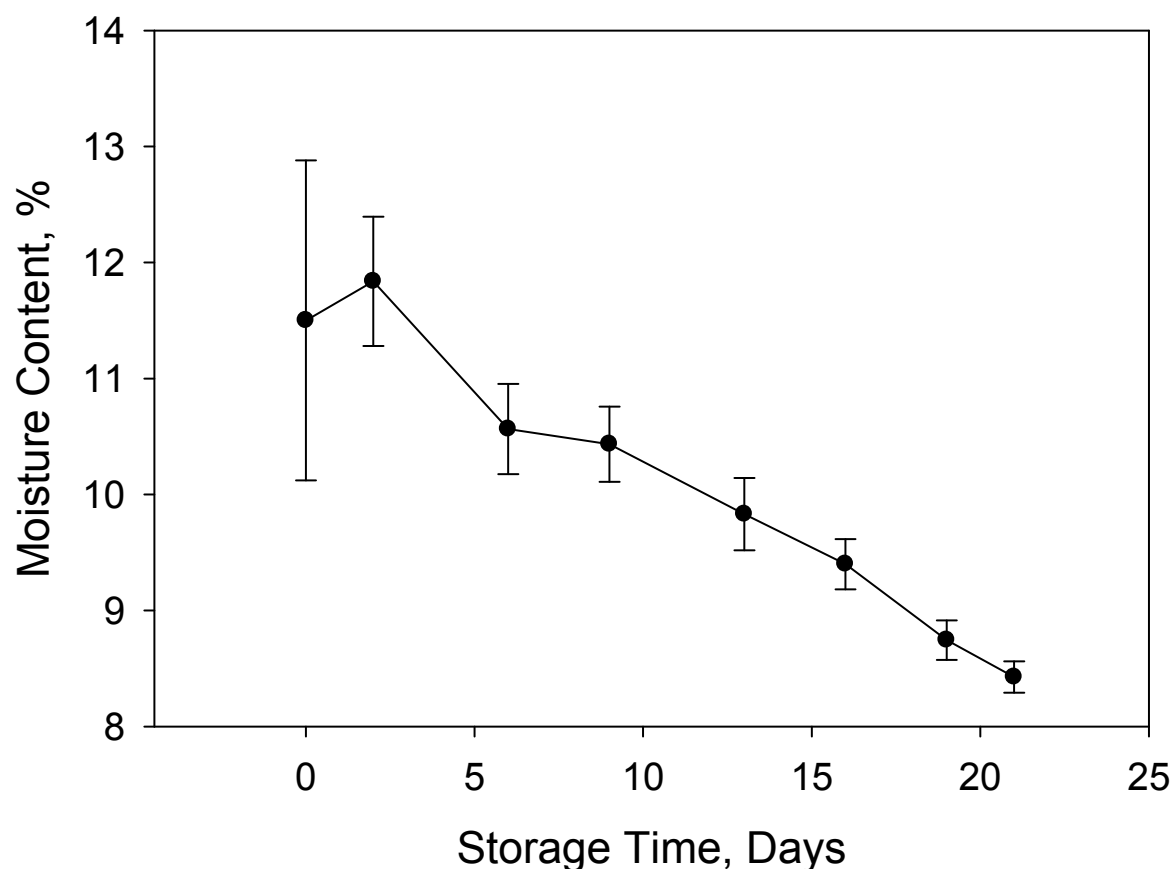


Figure 1. Average moisture content of 3 50-g cotton samples incubated at 20°C, each sample was initially sprayed with 5.0 ml of diluent without yeast.

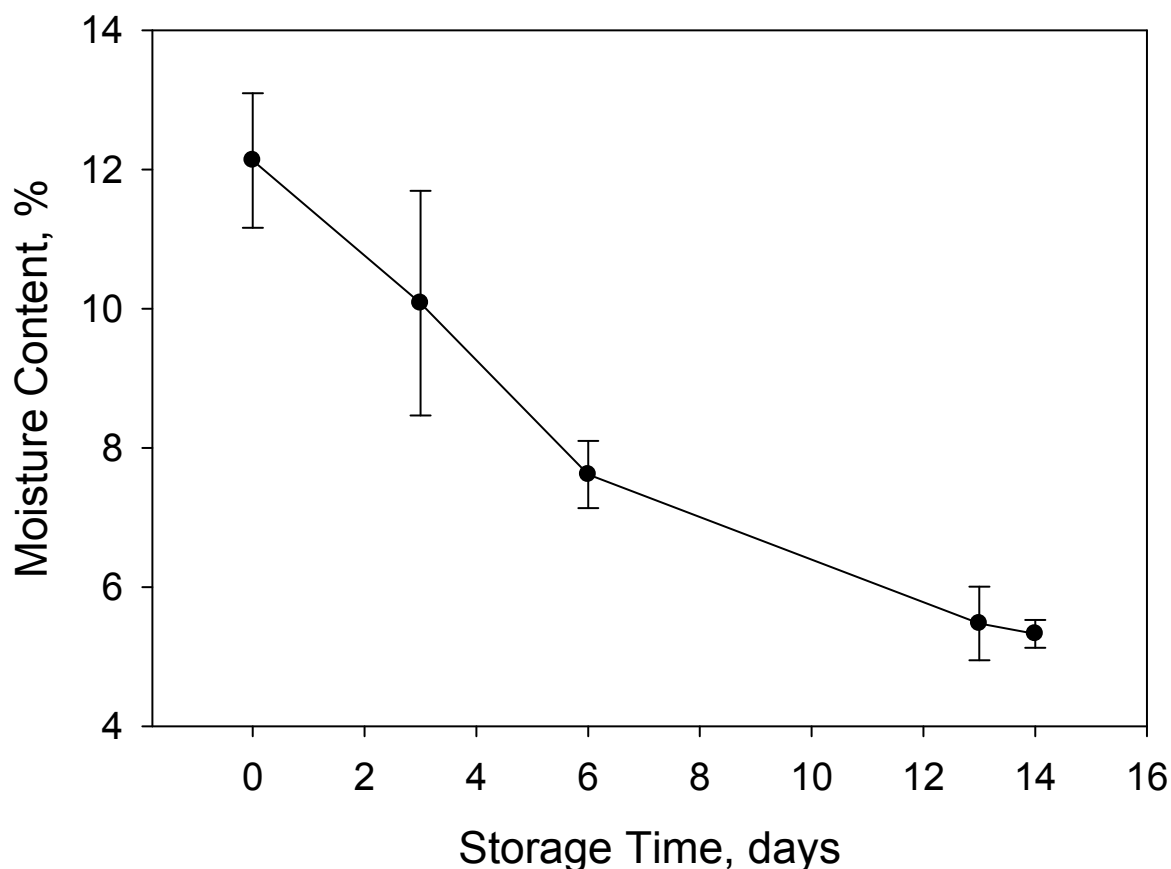


Figure 2. Average moisture content of 6 50-g cotton samples incubated at 30°C, each sample was initially sprayed with 5.0 ml of diluent without yeast.

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 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 won't be changed significantly.

In summary, laboratory trials using the yeast spray treatment to reduce cotton stickiness have 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.

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