2005 Beltwide Cotton Conferences, New Orleans, Louisiana - January 4 - 7, 2005

QUICK SUMMARY OF THE LATEST MOISTURE RESTORATION AT THE GIN STUDY AND OF A MICROBIAL CHECK STUDY ON THE POPULATION DENSITIES ON 'DISCOLORED' AND 'CLEAN' COTTON

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Abstract

As part of an ongoing investigation, a corroborative study was done to follow fiber quality, moisture content and microbial population of cotton bales that had been augmented with moderate amounts of moisture for target moisture contents of 5.1% (control untreated cotton), 6.5, 7.0, 7.5, 8.0, and 8.5% (0, 8, 10, 12 and 14 pounds of water added, respectively). The microbial population densities associated with the bales will be reported. In another study, cotton samples containing discoloration spots or smears were compared to areas free from discoloration. The microbial population densities associated with the discolored and the discoloration-free areas will be reported.

Introduction

The practice of adding water to ginned cotton to reduce bale packaging forces and increase the bale weight to make up for excessively dry cotton resulting from ginning has sometimes been extended to increase profitability. When excess moisture is added and the bales stored at higher than recommended moisture levels, microbial activity can be stimulated to such an extent that fiber quality decreases during storage and mold activity may increase to become an unnecessary health risk (Chun and Anthony, 2002; Lalor et al., 1994). However, if more moderate moisture levels where the microbial activity is not stimulated, perhaps the advantages of reducing excess bale packaging forces, of making up some of the moisture lost during ginning (Anthony and Griffin, Jr., 2001) and of providing the beneficial effects of moisture on fiber quality and processing as put forth by McAlister (1997), might be obtained at the same time so that the producer and end-user of the cotton would both gain advantages. This paper is part of an ongoing investigation that looked at the microbial activity of cotton bales that had additional moisture added (a control bale where no additional moisture was added, and bales with targeted moisture levels of 6.5%, 7.0%, 7.5, 8.0% and 8.5% moisture) and had been stored for approximately 6 and 7-8 months before opening. An interest of this laboratory is the general area of cavitoma (Perkins and Brushwood, 1997) so when cotton containing distinct gray to dark discoloration on the surface and interior of the samples were made available, we wanted to test if actual differences in microbial activities could be detected in these discolorations of suspected microbial origin. This paper also reports these observations.

Materials and Methods

Cotton Moisture Treatment Study

The methods and materials used in this study followed the methodology laid out in an earlier study (Chun et al., 2004). The Cotton was grown in New Mexico during the 2003 harvest year and sufficient cotton was ginned for 21 bales of cotton. The target moisture content were 5.1% (control or ambient moisture content bales), 6.5%, 7.0%, 7.5%, 8.0% and 8.5%, for which 0, 6, 8, 10, 12 and 14 pounds of water were sprayed on the cottons, respectively. Six bales had target moisture of 8.0%. The other target moisture treatments consisted of 3 bales each. The cotton was ginned and treated with moisture over a 4-day period from October 15 to October 18, 2003. The bales were transported to the Cotton Quality Research Station where they were stored at the USDA Warehouse in the Agricultural Servicenter on Old Cherry Road in Clemson, South Carolina. After a little more then 6 months of storage, 14 of the treated bales were sampled on April 22, 2004; and later after approximately another month of storage, the remaining 7 bales were sampled over a 2-week period from June 2 to about June 14, 2004. Samples were taken for microbial assays, and quality and spinning tests. When the last bale was sampled, all the samples from these remaining 7 bales were assayed for microbial populations. Only the microbial assays will be reported here.

Population Density on small dark smear shaped contaminated portions of lint vs. 'clean' areas of the same sample.
In May of 2004, 15 samples of an upland cotton was sent to the Cotton Quality Research Station for analysis to determine how well it could be spun and to determine the reason for small dark smears found throughout the cotton (Figure 1). A microbial analysis was performed which consisted of determining the microbial population density of the fiber discoloration; the spinning results will not be presented.

The population density of the viable total bacteria, Gram-negative bacteria and fungal populations was assayed as usual (Chun et al., 2004) except instead of using 1-gm lint samples, 'pinches' of the lint containing the discolored areas were taken for assay. As a control, 'pinches' of 'clean' lint away from the discolored areas were taken. Each of the 15 samples was assay and considered as the 'discolored' treatment. For the control samples, 4 of the 15 original lint samples were randomly chosen and sampled. These four control samples consisted of 'pinches' of the lint from areas free of the gray-black smear contamination material. Since these 'pinches' of lint samples averaged 0.07 gm, each of the samples were individually corrected and the results reported on a gram dry weight basis.

Experimental Design and Statistical Analysis.
For the Cotton Moisture Treatment Study. Each bale was placed on its side and before the plastic straps were removed, divided into 10 layers or zones parallel to the compression layers. After the straps were cut, a rubber template was placed on the top surface of each layer. This template which had 14 15.24-cm diameter holes, each hole representing a fixed location and equidistant from one another, served to outline 14 fixed locations on the surface from which potential samples could be taken. One location was randomly chosen from each layer and enough lint was removed from that location to compactly fill a 0.95 L wide-mouth canning bottle. The sampled layer was then removed and the layer below it was exposed and sampled. This continued until each of the 10 layers were sampled. The samples were stored at room temperature in these tightly sealed canning bottles until the samples could be removed for fiber quality and microbial population measurements. The April 22 bales were sampled at the CQRS warehouse and yielded 140 samples for all of the moisture treatments. The remaining 7 Bales were used in a fiber property study and when that specific bale was to be processed it was brought to the CQRS pilot plant and sampled for microbial analysis. These remaining 7 Bales were sampled about a month later over an approximate 2-week period and provided 70 samples for all of the moisture treatments. A $\log_{10}$(cfu+1), where cfu = microbial population as colony forming units per gram lint (corrected for dry weight), transformation was used for the analysis dealing with the microbial data.

For the discolored cotton study, a t-test comparison was made to determine if the microbial population densities were different on the discolored compared to adjacent clean lint.

Data were analyzed using release 8.00 of SAS (SAS, Statistical Analysis System; SAS system for Windows NT, SAS Institute Inc., Cary, NC, USA) for Duncan mean comparisons. Microsoft EXCEL 2000 (Microsoft Corporation, USA) was used to randomize treatment assignments, to enter and store data, to sort data and prepare for SAS analysis, to correct data for dry weight, to transform data, to summarize and tabulate results, to obtain simple treatment statistics (means, t-tests, standard deviations, regressions, etc.), and to perform other spreadsheet functions. SigmaPlot 2002 Version 8.0 (SPSS, Inc., USA) was used for plotting the data.

Results and Discussion

After approximately 6 and 7-8 months of storage, the effect of moisture restoration appears to have been retained but at levels below the original target treatment (Table 1) which is in keeping with previous reports (Anthony, 2002; Chun and Anthony, 2004; Chun et al., 2003 & 2004). All of the moisture-augmented bales, even the bales with the target moisture contents of 8.0% and 8.5%, had moisture contents below the maximum recommended 7.5% level (Anonymous, 2003) by the end of 6 and 7-8 months of storage. The control and some of the treatment bales increased their moisture content after an additional 1-2 months of storage, but these increases were less than half a percent moisture and not unexpected considering the warmer and higher humidity during that time of year. Even though the treated bales were targeted as much as more than 3% moisture content higher than the control bales, at the end of 7-8 months the excess moisture over the control was at the most 1.3% higher.

The microbial observations indicate very little or no change in microbial population densities after 6 and 7-8 months of storage (Table 2 & 3). From the small difference between the control bales and treated bales, there was very little effect of

moisture on the microbial populations or there was very little moisture to influence microbial activity. From previous studies (Chun and Anthony, 2004; Chun et al., 2003 & 2004), moisture treated bales tend to decrease the bacterial population density significantly during storage so very possibly the moisture added to the bales was transitory and probably below the maximum recommended 7.5% early on during storage. Only the fungal populations, which tend to be more sensitive to low moisture content showed a significant drop in population density after 7-8 months of storage.

The discolored lint samples had much higher microbial densities than the lint samples taken away from the discolored regions of the lint (Figure 2). Even so, the bacterial counts found on the 'contaminated' regions appear to be lower than would be expected by almost one to two log values and so the bacterial densities found on the 'non-contaminated' regions are much lower than expected (Chun and Anthony, 2004; Chun et al., 2003, 2004; Chun and Perkins, 1996). However, this may be an anomaly of using such unusually small lint sample sizes and the generally high variability inherent in cotton. While the discolored area microbial densities appear to be much higher than the regions away from the discolored areas, the high variability, especially found in the non-discolored lint microbial densities, made it difficult to say that the differences were significant (Figure 2); so t-tests were run to determine if the differences in the microbial populations found on the 'contaminated' lint and the 'clean' lint were significant. The results indicated that for total and Gram-negative bacterial populations, the differences were highly significant (Total bacteria -- 4.3028 vs. 3.0503, $P = 0.002$; Gram-negative bacteria -- 2.9903 vs. 0.0000, $P < 0.001$, where the population density is reported as $\log_{10}(\text{cfu}+1)$, where cfu = microbial population as colony forming units per gram lint corrected for dry weight and P is the t-test probability). The fungal counts on the discolored regions, on the other hand, were unusually high (Figure 2). More often than not, viable bacterial populations exceed the fungal populations by one or more orders of magnitude; but here the fungal densities either were equal to or greater than the corresponding viable total bacterial populations. The differences between the fungal population found on the discolored and non-discolored lint had a t-test probability of 0.18 (5.1311 vs. 3.2266, where the population density is reported as $\log_{10}(\text{cfu}+1)$, where cfu = microbial population as colony forming units per gram lint corrected for dry weight). While this doesn't meet the $P = 0.05$ for significance, the highly variable nature of the fungal assay leaves one to believe that $P = 0.18$ may really indicate significant differences between the populations found on the discolored vs. non-discolored lint samples. During incubation, some of the colonies that appeared were obviously dark colored fungi such as *Alternaria* spp., *Rhizopus* spp., etc. Visually and numerically, the mold species may be the prominent microbial factor involved with the discoloration on the lint. However, molds will not grow without a food source and perhaps in regard to the problem of lint discoloration, the more pertinent question would be to determine what contaminant and how was this contaminant placed in this lot of cotton.

In conclusion, this moisture restoration study, which used moderate amounts of moisture for restoration, indicated that while the treated bales retained some of the moisture weight through storage, the amount retained was small and were well within the safe maximum recommendation of 7.5% moisture content. This probably accounts for the lack of microbial activity. In the lint discoloration study, which was done largely as a check for observing microbial activity, the discolored regions exhibited significantly higher microbial activity. But what stood out and is consistent considering the spotty nature of the discoloration, is that fungal species may play the pivotal role in such damage to cotton. The high fungal presence should really be considered as an indicator of some other contaminate, which nourished the furtherance of the fungal presence, that was dispersed in a spotty manner during some process of harvesting and processing the lint for storage.

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Table 1. Target moisture treatment and average moisture content after storage in the first sample collection and the second sample collection.

Treatment/Target Moisture, %	Actual Average Treatment Moisture at First Collection[1,2], %	Actual Average Treatment Moisture at Second Collection[1,2], %
5.1, control	5.19^{E}	5.38^{C}
6.5	5.77^{D}	5.41^{C}
7.0	5.92^{CD}	6.10^{B}
7.5	6.06^{C}	6.17^{B}
8.0	6.66^{A}	6.27^{B}
8.5	6.38^{B}	6.68^{A}

[1]First sample collection was made approximately 6.2 months after ginning and the second sample collection was made approximately 7.5 to 8 months after ginning.
[2]Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

Table 2. First sample collection[1]: microbial population density at the target moisture treatments.

Treatment/Target Moisture, %	Total Bacteria[1], Log_{10}(cfu+1)	G(-) Bacteria[1], Log_{10}(cfu+1)	Fungi[1], Log_{10}(cfu+1)
5.1, control	6.099^{A}	5.937^{A}	5.034^{A}

6.5	6.201[A]	5.942[A]	4.852[A]
7.0	6.113[A]	5.885[A]	4.962[A]
7.5	6.166[A]	5.983[A]	4.906[A]
8.0	6.103[A]	5.896[A]	4.883[A]
8.5	6.051[A]	5.914[A]	4.831[A]

[1]First sample collection was made approximately 6.2 months after ginning.
[2]Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

Table 3. Second sample collection[1]: microbial population density at the target moisture treatments.

Treatment/Target Moisture, %	Total Bacteria[2], Log_{10}(cfu+1)	G(-) Bacteria[2], Log_{10}(cfu+1)	Fungi[2], Log_{10}(cfu+1)
5.1, control	5.966[A]	6.065[A]	5.016[A]
6.5	5.793[A]	5.953[A]	4.840[AB]
7.0	5.792[A]	5.906[A]	4.992[A]
7.5	5.879[A]	5.899[A]	4.594[BC]
8.0	5.791[A]	5.864[A]	4.816[AB]
8.5	5.764[A]	5.927[A]	4.477[C]

[1]Second sample collection was made approximately 7.5 to 8 months after ginning.
[2]Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

Figure 1. Examples of the small dark smeared areas picked for assay. These areas were on the surface and within the mass of cotton. The 'clean' areas for comparison were chosen from locations away from and without these visually darkened areas.

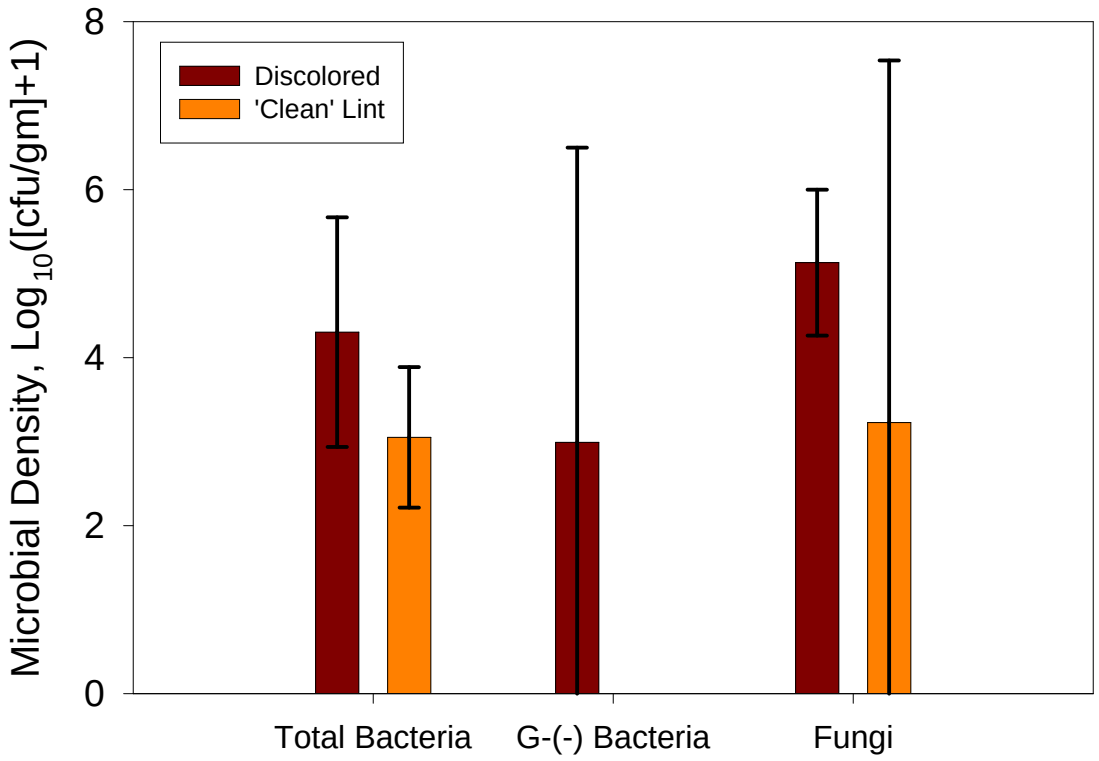

Figure 2. Average population density of the viable total bacterial, Gram-negative bacterial and fungal populations found on the discolored cotton samples and the 'clean' portions of the lint samples, each half bar represents 2 s.e.