The *Journal of Cotton Science 15:52–60 (2011)*
http://journal.cotton.org, © The Cotton Foundation 2011

ENGINEERING AND GINNING

Effect of Fungal Spores on Cotton Color

David T.W. Chun and James E. Rodgers III*

ABSTRACT

We have observed that cotton can undergo color change when moisture conditions of the cotton sample would not be expected to support bacterial activity, which normally requires free water. Several fungi can grow under low moisture conditions. When fungal spores germinate, germ tubes are produced. These extensions from the spore might be large enough so that even if fungal growth stops, it might be possible for this larger fungal body to influence cotton color. The results from this study demonstrated that a spore presence alone can influence color change; for example, increasing application of spore density from 0.0X to 1.0X, of *Aspergillus niger* Tiegh. on cotton increased Rd from 0.6 to 11.2, but the impact on +b was small. The color changes were significantly greater for *A. niger* and *Penicillium* sp. spores when conditions permitted spores to germinate. Application of spores on cottons with different levels of stickiness usually resulted in small, but not significant, color changes associated with increased cotton stickiness.

During the 1980s and 1990s when lung disease, byssinosis, was a topic of great interest, much of the research was on bacteria and its effect on cotton (Chun and Perkins, 1996). Several reports associated color changes, especially graying and yellowing, with the presence of bacteria (Fischer et al., 1980; Morey et al., 1982). For this reason, bacteria are often considered when discussing color changes due to microbial activity, especially in regard to storing cottons of high moisture content (Chun and Anthony, 2004). However, it has been known that field exposure (weathering) can result in graying of the fiber and the presence of fungi can result in a lower grade classification (Simon and Harmon, 1954). Under certain conditions of cotton storage, especially storage under high humidities, color changes occur (Nickerson, 1964; Nickerson and Tomaszewski, 1958). Because bacteria usually require free moisture to grow, this suggested that under relatively high humidities fungi might be the leading agent of color change. It has been postulated that fungal spores under the right conditions would germinate and the increase of surface area covered would result in noticeable changes in cotton color.

Cotton color is classed by the AMS of the USDA with the Uster® High Volume Instrument (HVI). The HVI color of the cotton fiber is measured using two broad-band filters (wavelengths or wavelength regions), which yields two color parameters—Rd and +b. Rd is the reflectance of the sample, and +b is the sample's yellowness. Although the HVI performs well as a cotton colorimeter, Rd and +b are color parameters that are specific to cotton and are not normally used for other products and industries. The more common and globally recognized color systems are based on the use of three color parameters (three-coordinate system), whereas Rd and +b for cotton fiber represent only a 2-coordinate color system (Berns, 2000; Hunter, 1975; Judd and Wyszecki, 1975). When a human eye sees the color of a sample, one component that is observed is the reflectance of light from the sample's surface over the visible color spectral region of 400 nm (violet) to 700 nm (red). HVI measures the fiber's color at only two distinct regions of the color spectrum, whereas most modern spectrophotometers and colorimeters measure the fiber's color over the entire spectral region. Thus, a modern color spectrophotometer is capable of yielding additional fiber color information and results that might not be readily apparent or available with a HVI color measurement.

Several 3-coordinate color systems are based on tristimulus XYZ. Several color systems were developed based on modifications to tristimulus XYZ to improve the agreement between instrumental color measurement and visual color perception, to include the globally recognized International Commission on Illumination (or Commission Internationale d'Eclairage) L*a*b* (CIELAB) (Berns, 2000). L* relates to the total reflectance of the sample over the visual spectral range and a* (red-green) and b* (blue-yellow) are related to differences in the sample's reflectance between two

D.T.W. Chun, USDA-ARS Cotton Quality Research Station, P.O. Box 792, Clemson, SC 29633; and J.E. Rodgers III*, USDA-ARS, Southern Regional Research Center, 1100 Robert E. Lee Blvd., BLDG 001 SRRC, New Orleans, LA, 70124.
*Corresponding author: James.Rodgers@ars.usda.gov

color regions. The extent of color difference between L*a*b* color results is often expressed by the term dE*. dE* is given in Equation 1, where the Δ parameters indicate the differences between a reference unit or system and the unit or system being compared for the color parameters L*, a*, and b*.

$$dE^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

A color difference between samples is considered to be discernable by the eye and significant when dE* > 1.0. (Berger-Schunn, 1994)

A study was initiated where fungal spores were applied to the surface of cotton and permitted to germinate. Because it has been suggested that the higher sugar content associated with stickiness would favor microbial activity resulting in lower Rd and higher +b readings, cottons with different levels of stickiness were used. The color results of this study, by both HVI and spectrophotometer, are reported.

MATERIALS AND METHODS

Cotton Samples. The Pima cotton samples (from 2001 and 2005 harvest years from Bakersfield and San Joaquin Valley, CA, respectively) were prepared from a graded series of six sticky cotton lots that ranged from nonsticky to heavy stickiness (Chun, 2008a, 2008b). Stickiness increased from Lot 1, nonsticky cotton, to Lot 7, heavy sticky cotton; no Lot 5 was used. These sticky lots of cotton were prepared by blending different ratios of a nonsticky batch of cotton with a highly sticky batch of cotton. To prepare each sample, 10.0 g were taken from each lot of sticky cotton and spread as a thin, rectangular batting across carding cloth. The batting was removed and the two ends were folded one end over the other, to produce a thick, rectangular batting approximately 4 to 7 cm thick with an approximate area of 10 cm by 13 cm. These sample batts were stored in a conditioned room (20 ± 2°C; 55 ± 2% RH) on racks until used.

Fungal Spores. The fungal spores used in this study were the conidia from *Aspergillus niger* Tiegh., American Type Culture Collection No. 6275 (ATCC, Manassas, VA) and from a *Penicillium* sp. *A. niger* and *Penicillium* sp. were chosen because they were commonly found on cotton, the spores were visually different, and they could be used as a starting point for testing to see if spores would affect cotton color. The *Penicillium* sp. was isolated from spores of a penicillium colony growing on an old loaf of French bread. Both fungal cultures were maintained on plates or slants of potato dextrose agar (PDA) at room temperature (20 ± 2°C) (Chun et al., 2006). For spore collection, *A. niger* spores were spread over the surface of a PDA plate and incubated for 2 to 3 wk at 25°C in brown paper bags to reduce drying of the culture plates, instead of plastic bags, which encouraged spore production. The same spore collection procedure was use for *Penicillium* sp., except that the cultures were incubated for approximately a week.

For three of the four tests, the spores were collected by removing the top cover of the Petri dish, inverting the plate, and slamming it down on sterile weighing paper. After waiting for the spores to settle on the weighing paper, the culture plate was removed and the spores transferred into a bottle of diluent (Chun and Perkins, 1996). For this study, the normally present gelatin and Tween-80 components of the diluent were omitted. Normally, a single culture plate was sufficient to supply enough spores for each test. For the first test using *Penicillium* spores, the spores were collected using a sterile cotton swab that had been swept across the surface of the culture plate. The cotton portion of the swab was then broken off into a bottle of diluent and the spores suspended in the diluent by agitation. Swabs from two plates were needed when spores were collected this way. From this stock suspension of spores, a second suspension of spores was prepared as a 1:10 dilution, for the 1.0X and 0.1X spore density treatments, respectively. A magnetic stirring bar and laboratory stirrer were used to keep the spores suspended. An ice bath was used to keep the suspension chilled. Spore density was determined by using a phase counting chamber (hemacytometer) (Hausser number 3200; Hausser Scientific, Horsham, PA). The average of four sets of counts (top and bottom chambers) was used for determining spore density.

Experimental Design. Either 2.0 ml of a spore suspension or 2.0 ml of just the diluent was sprayed on one surface of each sample batt using an airbrush (276 kPa [40 psi] spray pressure). This lower pressure localized the sprayed material near the surface of the sample batt without deep penetration. The sprayed sample batt was either placed in a tray to air-dry overnight (approximately 24 h) or placed in a 22.9-cm x 30.5-cm ziplock 0.05-mm thick clear plastic bag (BCU Plastics & Packaging, San Marcos, CA) and incubated for 2 to 3 d at 25°C. At the end of 24 h, the air-dried sample batts were placed in plastic bags for storage. For the sample batts kept in plastic bags after incubation, these were removed to room temperature and held with the air-dried sample batts

until each was measured for color properties. In both cases, the sample batts were placed in the plastic bags so that the sprayed side could be distinguished from the unsprayed side of each sample batt. Previous trials indicated that the air-dried sample batts returned to original weight within 1 to 3 h and the sample batts in plastic bags returned to original weight within 1 to 2 d.

A total of four tests, two with each fungus were performed. In each test, sample batts were sprayed with either 2.0 ml of diluent or with 2.0 ml of the 0.1X or 1.0X spore suspension. The sprayed sample batts were either air-dried or incubated in plastic bags. For each of these treatments, three replicates were used from each of the six sticky lots of cotton for a total of 108 samples per test. The treatment identification numbers were randomly assigned to each sample; during treatment application, all the treatment samples sprayed with the diluent only were sprayed first, followed by the treatment samples sprayed with the 0.1X and then the 1.0X spore densities to reduce cross contamination. For analysis, the results from the two tests for the same spore type were combined.

Color Readings. HVI color readings from each sample batt consisted of reflectance (Rd) and yellowness (+b) measurements made by the HVI Lab in the Cotton Quality Research Station in Clemson, SC. An Uster HVI 900 A (Uster Technologies, Uster, Switzerland) was used for those readings. Spectrophotometer color readings were made with a portable HunterLab MiniScan XE Plus (Model No. D/8-L; Hunter Associates Laboratory, Inc., Reston, VA), using the Easy Match QC software from HunterLab. Spectrophotometer parameters were illuminant D65, 10 observer, large area of view (LAV; 25.4 mm), and specular component included (SCI). All color measurements were obtained "with glass," in which a 1-mm thick glass slide was placed on the cotton prior to measurement. The use of the glass yielded a smooth fiber surface for color measurement and prevented fiber contamination of the spectrophotometer. The primary spectrophotometer color parameters were L*a*b* and dE*. The HunterLab color results were transferred to an Excel spreadsheet for further data analysis.

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), using Proc GLM 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. SigmaPlot for Windows Version 10.0 (Systat Software, Inc., Richmond, CA) was used for graphical creations and regression statistics generation used to interpret data. Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA) was used to randomize treatment assignments, to sort data and prepare for SAS analysis, to transform data, to summarize and tabulate results, and to obtain simple treatment statistics (e.g., means, standard deviations, regressions, t-test comparison). These tests were applied to the results observed to determine if changes in color were significant.

RESULTS AND DISCUSSION

The *A. niger* spore suspensions used in the study were dark in appearance with the 1.0X spore suspension was darker than the 0.1X suspension. When sprayed on the surface of the sample batts, a noticeable darkening of the surface was observed, which was most noticeable with the 1.0X spore treatment. The sample batts sprayed with the 0.1X and 0.0X spore suspension had some graying to no visible change in appearance, respectively. Similar surface darkening was observed on cotton samples sent to CQRS and in moisture effect on cotton bale studies (Chun & Anthony, 2004). These visual observations were supported by the color changes measured by Rd, L*, +b, and b* (Tables 1a and 1b). As expected from prior observations (Chun & Anthony, 2004), Rd and L* for the sprayed samples decreased, and +b and b* increased significantly as the applied spore density was increased. The largest color changes were observed for Rd and L*. Because inherent differences in color of the individual sample batts were expected, color readings were made of the unsprayed side of each batt. Large color changes were not expected on the unsprayed side, however small color changes were observed for Rd, L*, +b, and b*. The color readings of the unsprayed surface tended to follow the same trend as the spore density application, but to a much lower extent. Even though some of average unsprayed surface color parameters were statistically significant, the averages did not vary as much as the averages on the sprayed surfaces. For example, in Table 1 the average L* for the unsprayed surface of the mattes related to the 0.0X spore application was 84.7, which was significantly different from the unsprayed surface of the mattes sprayed with the 1.0X spore application, 84.4. However, the 0.3 L* color change difference for the unsprayed surfaces was not of practical difference compared to the 5.4 L* color change difference of

the 0.0X and 1.0X sprayed surfaces (84.3 and 78.9, respectively). To fully appreciate the color change by the application of different spore densities to the cotton surface, Rd, L*, +b, and b* of the sprayed side for each sample were subtracted from the Rd, L*, +b, and b* from the unsprayed side to get an average Rd, L*, +b and b* difference. When the average differences were examined for Rd, L* +b, and b*, the color changes were significantly affected by the addition of spores to the surface. Thus, the observed trend in decreased reflectance/lightness and increased yellowness was related to higher spore density.

Table 1a. Overall effect of spore density on Rd and L* by applying *Aspergillus niger* spores on the cotton samples.

Spore Density[z]	Average Rd, Sprayed Surface[y]	Average Rd, Unsprayed Surface[y]	Average Rd Difference[y]
0.0X	73.4[a]	73.9[a]	0.6[c]
0.1X	71.0[b]	73.3[ab]	2.3[b]
1.0X	61.9[c]	73.1[b]	11.2[a]
Spore Density[z]	**Average L*, Sprayed Surface[y]**	**Average L*, Unsprayed Surface[y]**	**Average L* Difference[y]**
0.0X	84.3[a]	84.7[a]	0.4[c]
0.1X	83.3[b]	84.5[ab]	1.2[b]
1.0X	78.9[c]	84.4[b]	5.5[a]

[z] Spore density of 1.0X, 1-1.3 x 10^7 spores/ml; approximately 2-2.6 x 10^7 spores sprayed over the sample batt surface.

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

Table 1b. Overall effect of spore density on +b, and b* by applying *Aspergillus niger* spores on the cotton samples.

Spore Density[z]	Average +b, Sprayed Surface[y]	Average +b, Unsprayed Surface[y]	Average +b Difference[y]
0.0X	15.5[c]	15.7[a]	0.2[a]
0.1X	15.7[b]	15.6[a]	-0.1[b]
1.0X	16.3[a]	15.4[b]	-0.9[c]
Spore Density[z]	**Average b*, Sprayed Surface[y]**	**Average b*, Unsprayed Surface[y]**	**Average b* Difference[y]**
0.0X	12.6 [b]	12.6 [a]	-0.0 [a]
0.1X	12.8 [b]	12.6 [a]	-0.2 [a]
1.0X	13.06 [a]	12.4 [a]	-0.6 [b]

[z] Spore density of 1.0X, 1-1.3 x 10^7 spores/ml; approximately 2-2.6 x 10^7 spores sprayed over the sample batt surface.

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

The *Penicillium* sp. spore suspensions were much lighter in appearance than the *A. niger* suspension spore suspensions used in the study, even though the spore densities were about the same. The penicillium spore color was a bluish-green color, whereas the color of *A. niger* spore was black. In addition, the *A. niger* spore is larger than the *Penicillium* spore, 3.1 to 3.5 µm and 2.3 µm, respectively (Kanaani et al., 2007). Compared to the *A. niger* spores results, the color results for the cotton surfaces treated with spores of *Penicillium* sp. yielded smaller changes in reflectance (Rd and L*) and yellowness (+b and b*) (Table 2). The Rd and L* 0.0X treated batts were significantly different from the 0.1X and 1.0X treated batts for the sprayed surfaces. The average Rd and L* differences for the *Penicillium* sp. spores followed the same trend of decreasing Rd and L* with increased spore density, but these color differences were much smaller compared to color differences observed for the *A. niger* spores samples. Yellowness as average +b and b* exhibited only small color changes with increased *Penicillium* sp. spore density, and the differences between the unsprayed and sprayed surfaces were not large for the different spore densities. The smaller effect on color with the *Penicillium* sp. spores might be related to the inherent difference in color of the *A. niger* and the *Penicillium* sp. spores.

Table 2a. Overall effect of spore density on Rd and L* by *Penicillium* sp. spores on the cotton samples.

Spore Density[z]	Average Rd, Sprayed Surface[y]	Average Rd, Unsprayed Surface[y]	Average Rd Difference[y]
0.0X	70.4[a]	71.9[a]	1.6[b]
0.1X	69.7[b]	72.0[a]	2.3[a]
1.0X	69.4[b]	71.9[a]	2.5[a]
Spore Density[z]	**Average L*, Sprayed Surface[y]**	**Average L*, Unsprayed Surface[y]**	**Average L* Difference[y]**
0.0X	83.2[a]	84.0 [a]	0.8 [a]
0.1X	83.0[ab]	84.0 [a]	1.0 [a]
1.0X	82.8[b]	83.9[a]	1.1 [a]

[z] Spore density of 1.0X, 0.8-1.0 x 10^7 spores/ml; approximately 1.6-2.0 x 10^7 spores sprayed over the sample batt surface.

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

Table 2b. Overall effect of spore density on +b, and b* by *Penicillium* sp. spores on the cotton samples.

Spore Density[z]	Average +b, Sprayed Surface[y]	Average +b, Unsprayed Surface[y]	Average +b Difference[y]
0.0X	15.6[a]	15.6[a]	-0.1[a]
0.1X	15.5[a]	15.4[a]	-0.1[a]
1.0X	15.5[a]	15.6[a]	0.0[a]
Spore Density[z]	**Average b*, Sprayed Surface[y]**	**Average b*, Unsprayed Surface[y]**	**Average b* Difference[y]**
0.0X	13.1[a]	13.0[a]	-0.1[a]
0.1X	12.9[a]	12.7[a]	-0.2[a]
1.0X	13.0[a]	12.7[a]	0.2[a]

[z] Spore density of 1.0X, 0.8-1.0 x 10^7 spores/ml; approximately 1.6-2.0 x 10^7 spores sprayed over the sample batt surface.

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

Lower color grade followed increased stickiness (Tables 3 and 4), which supports the general observation made at CQRS that sticky cottons often have a lower color grade than non-sticky cottons. In this analysis, the average color of each lot was compared (0.0X, 0.1X, and 1.0X spore density). With the nonsprayed sides of the cotton batts from different sticky lots, Rd and L* decreased with stickiness and +b and b* increased with stickiness, and the differences between lots were statistically significant. The treated-sprayed side of the sample batts followed the same trend with Rd and L* as stickiness increased, and the decreases were statistically significantly different. In addition, +b and b* increased significantly as stickiness increased. Cottons with different levels of stickiness were included because the higher sugar content associated with stickiness and the moisture provided by the treatments could favor microbial activity, resulting in lower Rd and L* and higher +b and b* color results. The change in color readings between the unsprayed side (control) and spayed side of the same batt might relate more to the inherent color of the sticky cotton samples. Significant differences were not observed when the average Rd, L*, +b, and b* differences between the unsprayed and sprayed sides of the batts for the *A. niger* spore treatments were compared. The Rd and L* differences tended to increase with stickiness, but these differences were relatively small (Table 3a). With yellowing, the differences in +b did not follow a discernable trend, and significant differences were not observed. With b*, a weak trend and significant differences between the b* differences were observed, but the range of the difference only amounted to 0.4 of a b* unit. For the batts treated with *Penicillium* sp. spores, the same trends and statistically significant differences were observed for Rd, L*, +b, and b* on the sprayed and unsprayed surfaces, however the average Rd differences showed statistically significant differences (Table 4). With the Rd reflectance values, the Rd differences increased with stickiness, and the trend had significant differences between lot differences (Table 4a), but the range of Rd differences amounted to a 1.2 Rd unit. Significant differences were not observed for the L* differences, but the L* differences tended to increase with stickiness. Significant differences were observed when the average +b and b* differences were compared (Table 4b). As a whole, despite the trend with Rd and L* differences to increase with stickiness, as with the *A. niger* spores, the change in color readings might relate as much to the inherent color of the sticky cotton samples than to the associated sugar content to influence microbial activity to lower Rd and L* and increase yellowing.

Table 3a. Overall effect on Rd and L* by using lots of different stickiness and *Aspergillus niger* spores.

Cotton Lots[z]	Average Rd, Sprayed Surface[y]	Average Rd, Unsprayed Surface[y]	Average Rd Difference[y]
Lot 1	70.7[a]	75.2[a]	4.5[a]
Lot 2	70.0[ab]	74.6[ab]	4.6[a]
Lot 3	70.0[ab]	74.2[b]	4.2[a]
Lot 4	69.5[b]	74.3[ab]	4.8[a]
Lot 6	67.7[c]	72.7[c]	5.0[a]
Lot 7	64.8[d]	69.8[c]	5.0[a]
Cotton Lots[z]	**Average L*, Sprayed Surface[y]**	**Average L*, Unsprayed Surface[y]**	**Average L* Difference[y]**
Lot 1	82.7[a]	85.0[a]	2.3[a]
Lot 2	82.7[a]	84.9[a]	2.3[a]
Lot 3	82.7[a]	84.9[a]	2.2[a]
Lot 4	82.3[a]	84.8[a]	2.4[a]
Lot 6	81.8[b]	84.2[b]	2.4[a]
Lot 7	80.8[c]	83.3[c]	2.5[a]

[z] Stickiness increases from nonsticky cotton, Lot 1, to heavily sticky cotton, Lot 7 (Chun, 2008a, 2008b).

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

Table 3b. Overall effect on +b and b* by using lots of different stickiness and *Aspergillus niger* spores.

Cotton Lots[z]	Average +b, Sprayed Surface[y]	Average +b, Unsprayed Surface[y]	Average +b Difference[y]
Lot 1	15.6[b]	15.3[b]	-0.3[a]
Lot 2	15.7[b]	15.3[b]	-0.3[a]
Lot 3	15.6[b]	15.5[b]	-0.1[a]
Lot 4	16.0[ab]	15.5[b]	-0.5[a]
Lot 6	16.0[ab]	15.8[a]	-0.1[a]
Lot 7	16.1[a]	15.8[a]	-0.3[a]
Cotton Lots[z]	**Average b*, Sprayed Surface[y]**	**Average b*, Unsprayed Surface[y]**	**Average b* Difference[y]**
Lot 1	12.3[d]	11.9[d]	-0.4[ab]
Lot 2	12.5[cd]	12.2[c]	-0.3[ab]
Lot 3	12.5[cd]	12.2[c]	-0.3[ab]
Lot 4	12.8[c]	12.3[c]	-0.5[ab]
Lot 6	13.2[b]	13.0[b]	-0.2[ab]
Lot 7	13.6[a]	13.5[a]	-0.1[a]

[z] Stickiness increases from nonsticky cotton, Lot 1, to heavily sticky cotton, Lot 7 (Chun, 2008a and 2008b).

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

Table 4a. Overall effect on Rd and L* by using lots of different stickiness and *Penicillium* sp. spores.

Cotton Lots[z]	Average Rd, Sprayed Surface[y]	Average Rd, Unsprayed Surface[y]	Average Rd Difference[y]
Lot 1	72.0[a]	73.8[a]	1.8[bc]
Lot 2	71.5[ab]	73.0[b]	1.6[c]
Lot 3	70.6[bc]	72.7[bc]	2.0[bc]
Lot 4	70.1[c]	72.0[c]	1.9[bc]
Lot 6	68.5[d]	70.8[d]	2.4[b]
Lot 7	66.3[e]	69.3[e]	3.0[a]
Cotton Lots[z]	**Average L*, Sprayed Surface[y]**	**Average L*, Unsprayed Surface[y]**	**Average L* Difference[y]**
Lot 1	83.8[a]	84.6[a]	0.8[a]
Lot 2	83.7[a]	84.6[a]	0.9[a]
Lot 3	83.4[ab]	84.3[a]	0.9[a]
Lot 4	83.1[b]	84.0[a]	1.0[a]
Lot 6	82.5[c]	83.4[a]	0.9[a]
Lot 7	81.6[d]	82.8[a]	1.2[a]

[z] Stickiness increases from nonsticky cotton, Lot 1, to heavily sticky cotton, Lot 7 (Chun, 2008a, 2008b).

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

Table 4b. Overall effect on +b and b* by using lots of different stickiness and *Penicillium* sp. spores.

Cotton Lots[z]	Average +b, Sprayed Surface[y]	Average +b, Unprayed Surface[y]	Average +b Difference[y]
Lot 1	15.3[b]	15.2[c]	-0.1[a]
Lot 2	15.4[b]	15.3[c]	-0.1[a]
Lot 3	15.4[b]	15.4[bc]	0.0[a]
Lot 4	15.4[b]	15.3[c]	-0.1[a]
Lot 6	15.5[b]	15.6[b]	0.1[a]
Lot 7	16.3[a]	16.3[a]	-0.1[a]
Cotton Lots[z]	**Average b*, Sprayed Surface[y]**	**Average b*, Unprayed Surface[y]**	**Average b* Difference[y]**
Lot 1	12.4[d]	12.3[d]	-0.2[a]
Lot 2	12.6[cd]	12.5[cd]	-0.1[a]
Lot 3	12.7[cd]	12.4[cd]	-0.3[a]
Lot 4	12.8[c]	12.6[c]	-0.2[a]
Lot 6	13.2[b]	13.0[b]	-0.2[a]
Lot 7	14.3[a]	14.0[a]	-0.3[a]

[z] Stickiness increases from nonsticky cotton, Lot 1, to heavily sticky cotton, Lot 7 (Chun, 2008a, 2008b).

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

When the spores were sprayed on the surface and immediately air-dried, the moisture content dropped precipitously, so that conditions did not favor germination and growth of the fungus. The treated sample batts that were placed in plastic bags to retard moisture loss should have had sufficient time for the fungal spores to swell and to germinate, possibly to a germling state that would be many times larger than the original size of the spore before evaporation reduced the water content of the cotton and halted further growth. The color readings for Rd and L* suggested that this happened; and even when the color readings of the surfaces with just the presence of resting spores alone were compensated for, the differences indicated the reflectance was decreased significantly (Tables 5 and 6). For both *Penicillium* sp. and *A. niger*, Rd, L*, and +b were statistically different. The differences between the unsprayed and the sprayed surfaces for the air-dried and incubated samples were not significant for +b and b*, suggesting that yellowness changed only slightly (Tables 5 and 6). Overall, spore germination tended to reduce Rd and L* beyond just the presence of the spore. The +b and b* yellowness value differences were small. The results in Table 5 show the overall color averages

that included all the *A. niger* spore treatments in the averages, including the controls (0.0X spores). To examine the effect of just the germinating spores, dE* values were compared for the different spore density treatments of the air-dried and the incubated cotton batts (Fig. 1). The L*a*b* color results for the air-dried, 0.0X samples were used as the reference values for dE*. The dE* results indicated significant increases in the dE* for all spore densities of the incubated cotton batts when compared to the air-dried batts for both fungi, and those dE* results tended to increase with spore density. The largest influence on dE* were changes in L*. The average dE* for the incubated batts was higher than the air-dried batts.

Table 5. Overall effect on Rd, L*, +b, and b* by presence of *Aspergillus niger* spores alone on the surface vs. the presence of spores that have germinated.

Aspergillus niger spores, Air-Dried vs. Incubated[z]	Average Rd, Sprayed Surface[y]	Average Rd, Unsprayed Surface[y]	Average Rd Difference[y]
Air-Dried	70.5[a]	74.2[a]	3.7[b]
Incubated	67.1[b]	72.8[b]	5.7[a]
Aspergillus niger **spores, Air-Dried vs. Incubated[z]**	**Average L*, Sprayed Surface[y]**	**Average L*, Unsprayed Surface[y]**	**Average L* Difference[y]**
Air-Dried	82.9 [a]	84.7 [a]	1.8[b]
Incubated	81.5 [b]	84.4 [b]	2.9[a]
Aspergillus niger **spores Air-Dried vs. Incubated[z]**	**Average +b, Sprayed Surface[y]**	**Average +b, Unsprayed Surface[y]**	**Average +b Difference[y]**
Air-Dried	16.0[a]	15.7[a]	-0.2[a]
Incubated	15.7[b]	15.4[b]	-0.3[a]
Aspergillus niger **spores Air-Dried vs. Incubated[z]**	**Average b*, Sprayed Surface[y]**	**Average b*, Unsprayed Surface[y]**	**Average b* Difference[y]**
Air-Dried	12.7 [a]	12.4[b]	-0.3[a]
Incubated	12.9 [a]	12.6[a]	-0.3 [a]

[z] Air-dried samples refer to samples sprayed with fungal spores and the spores are in the resting spore stage; the incubated samples refer to samples sprayed with fungal spores but were given time and conditions for the spores to have germinated; and the spores are in different stages of germination.

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

Table 6. Overall effect on Rd, L*, +b and b* by presence of *Penicillium* sp. spores alone on the surface vs. the presence of spores that have germinated.

Penicillium sp. spores, Air-Dried vs. Incubated[z]	Average Rd, Sprayed Surface[y]	Average Rd, Unsprayed Surface[y]	Average Rd Difference[y]
Air-Dried	72.5[a]	72.5[a]	0.0[b]
Incubated	67.2[b]	71.4[b]	4.2[a]
Penicillium **sp. spores, Air-Dried vs. Incubated[z]**	**Average L*, Sprayed Surface[y]**	**Average L*, Unsprayed Surface[y]**	**Average L* Difference[y]**
Air-Dried	84.1 [a]	84.2 [a]	0.1[a]
Incubated	81.9 [b]	83.7 [b]	1.8[b]
Penicillium **sp. spores, Air-Dried vs. Incubated[z]**	**Average +b, Sprayed Surface[y]**	**Average +b, Unsprayed Surface[y]**	**Average +b Difference[y]**
Air-Dried	15.6[a]	15.8 [a]	0.1 [a]
Incubated	15.5[b]	15.3 [b]	-0.2 [b]
Penicillium **sp. spores, Air-Dried vs. Incubated[z]**	**Average b*, Sprayed Surface[y]**	**Average b*, Unsprayed Surface[y]**	**Average b* Difference[y]**
Air-Dried	12.7[b]	12.6 [b]	0.0 [a]
Incubated	13.3[a]	12.9 [a]	-0.4 [b]

[z] Air-dried samples refer to samples sprayed with fungal spores and the spores are in the resting spore stage; the incubated samples refer to samples sprayed with fungal spores but were given time and conditions for the spores to have germinated; and the spores are in different stages of germination.

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

In Tables 7 and 8, a* surface readings are summarized. The a* is the redness/greenness of the sample for which there is no HVI colorimeter equivalence. For both fungi, stickiness appeared to have less effect on spore presence to affect cotton color when the inherent color of the sticky cotton was accounted for. Spore density increased a* slightly with *A. niger* spores but not with *Penicillium* sp. spores. But for both fungi, a* was significantly greater when the spores were permitted to germinate and increase in surface area. The color results for a* were similar to those observed for b* and +b.

Table 7. Overall effect of spore density, stickiness, and the presence of spores vs. the presence of spores that have germinated on a* by applying *Aspergillus niger* spores on the cotton samples.

Aspergillus niger Spore Density[z]	Average a* Sprayed Surface[y]	Average a*, Unsprayed Surface[y]	Average a* Difference[y]
0.0X	1.8[c]	1.6 [a]	-0.1[a]
0.1X	2.0[b]	1.6 [a]	-0.3[b]
1.0X	2.5[a]	1.6 [a]	-0.9 [c]
Aspergillus niger **Cotton Lots[z]**	**Average a*, Sprayed Surface[y]**	**Average a*, Unsprayed Surface[y]**	**Average a* Difference[y]**
Lot 1	2.0[d]	1.5[d]	-0.5 [a]
Lot 2	2.0[cd]	1.6[cd]	-0.4 [a]
Lot 3	2.0[cd]	1.6[c]	-0.4 [a]
Lot 4	2.1[c]	1.6[c]	-0.5 [a]
Lot 6	2.2[b]	1.8[b]	-0.5 [a]
Lot 7	2.3[a]	2.0[a]	-0.4 [a]
Aspergillus niger **spores, Air-Dried vs. Incubated[z]**	**Average a*, Sprayed Surface[y]**	**Average a*, Unsprayed Surface[y]**	**Average a* Difference[y]**
Air-Dried	2.0[a]	1.6[a]	-0.4[a]
Incubated	2.2[b]	1.7[b]	-0.5[b]

[z] Spore density of 1.0X, 1-1.3 x 10^7 spores/ml; approximately 2-2.6 x 10^7 spores sprayed over the sample batt surface.

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

Table 8. Overall effect of spore density, stickiness, and the presence of spores vs. the presence of spores that have germinated on a* by applying *Penicillium* sp. spores on the cotton samples.

Penicillium sp. Spore Density[z]	Average a* Sprayed Surface[y]	Average a*, Unsprayed Surface[y]	Average a* Difference[y]
0.0X	2.0 [a]	1.8 [a]	-0.2[a]
0.1X	2.0 [a]	1.7 [a]	-0.2 [a]
1.0X	2.0[a]	1.7 [a]	-0.2 [a]
Penicillium sp. **Cotton Lots[z]**	**Average a*, Sprayed Surface[y]**	**Average a*, Unsprayed Surface[y]**	**Average a* Difference[y]**
Lot 1	1.8[c]	1.6[c]	-0.2 [a]
Lot 2	1.8[c]	1.6[c]	-0.2 [a]
Lot 3	1.9[c]	1.6[c]	-0.2 [a]
Lot 4	1.9[c]	1.7[cb]	-0.2 [a]
Lot 6	2.0[b]	1.8[b]	-0.2 [a]
Lot 7	2.3[a]	2.1[a]	-0.2 [a]
Penicillium sp. **spores, Air-Dried vs. Incubated[z]**	**Average a*, Sprayed Surface[y]**	**Average a*, Unsprayed Surface[y]**	**Average a* Difference[y]**
Air-Dried	1.7[a]	1.7[b]	-0.1[a]
Incubated	2.1[b]	1.8[a]	-0.3[b]

[z] Spore density of 1.0X, 1-1.3 x 10^7 spores/ml; approximately 2-2.6 x 10^7 spores sprayed over the sample batt surface.

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

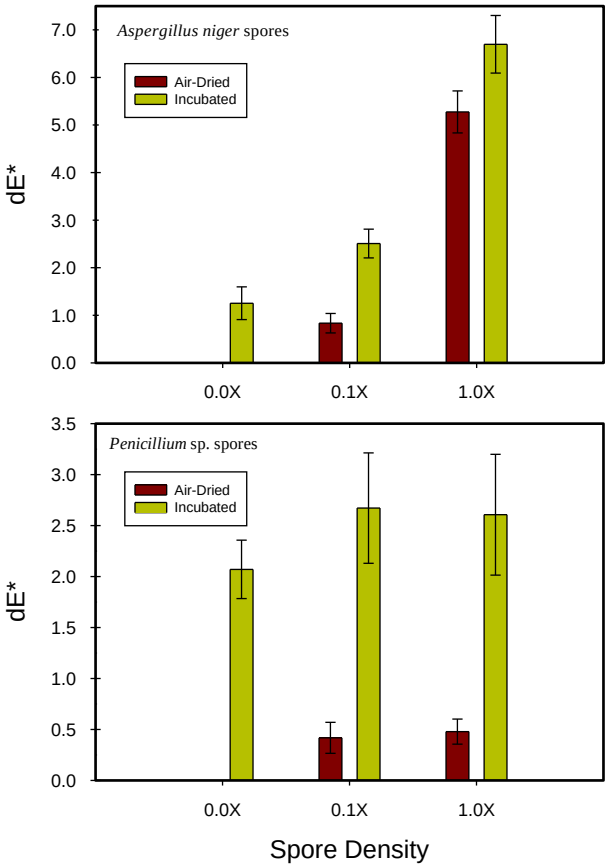

Figure 1. Average dE* color values for the cotton batts sprayed with spores or diluent alone. Each half-error bar represents 2 s.e., standard errors.

CONCLUSIONS

High spore deposition can result in significant fiber color changes (Rd, L*, +b, b*, and a*), and the magnitude of the color changes were most likely influenced by spore type (e.g., spore color) and size. Although the densities used in this study might appear high, in commerce these levels of contamination of cotton surfaces can occur. Cotton stickiness did not appear to affect spore presence on cotton color once the inherent color of sticky cotton was accounted for; as stickiness increases, Rd, and L* tended to decrease, and +b, b*, and a* tended to increase to a much lower extent. But beyond the simple presence of contaminating spores, color changes could also occur when conditions permit spores to germinate and grow. This work suggests that the germinated spores can significantly influence color changes for Rd, L*, +b, b* and a*.

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.

REFERENCES

Berger-Schunn, A. 1994. Practical Color Measurement. John Wiley & Sons, NY.

Berns, R.S. 2000. Billmeyer and Saltzman's Principles of Color Technology, 3rd ed. John Wiley & Sons, NY.

Chun, D.T.C. 2008a. Making sticky cotton for laboratory studies. p. 1551–1554. *In* Proc. Beltwide Cotton Conf., Nashville, TN. 8-11 Jan. 2008. Natl. Cotton Counc. Am., Memphis, TN.

Chun, D.T.C. 2008b. Project to use common baker's yeast, *Saccharomyces cerevisiae*, to mitigate cotton stickiness. J. Cotton Sci. 12:228–236.

Chun, D.T.W., and W.S. Anthony. 2004. Effects of adding moisture at the gin lint slide on cotton bale microbial activity and fiber quality. J. Cotton Sci. 8:83–90.

Chun, D.T.W., D.D. McAlister, III, S.E. Hughs, and D.R. Cobb. 2006. Microbial census and evidence for a direct temporal effect of bale moisture on color grade during six months of storage. J. Cotton Sci. 10:201–209.

Chun, D.T.W., and H.H. Perkins, Jr. 1996. Effects of conventional cotton storage on dust generation potential, bacterial survival and endotoxin content of lint and dust. Ann. Agric. Environ. Med. 3:19–25.

Fischer, J.J., P.R. Morey, and P.E. Sasser. 1980. Gram-negative bacterial content and fiber properties of raw cotton. Textile Res. J. 50:735–739

Hunter, R. 1975. The Measurement of Appearance. John Wiley & Sons, NY.

Judd, D., and G. Wyszecki, G. 1975. Color in Business, Science, and Industry, 3rd ed. John Wiley & Sons, NY.

Kanaani, H., M. Hargreaves, Z. Ristovski, and L. Morawska. 2007. Performance assessment of UVAPS: Influence of fungal spore age and air exposure. J. Aerosol Sci. 38: 83–96.

Morey, P.R., J.J. Fischer, and P.E Sasser. 1982. Endotoxin content and fiber properties of raw cotton. Textile Res. J. 52:524–528

Nickerson, D. 1964. Pattern of color change emerges from 3-year storage study of cotton grades. Textile Res. J. 34:645–647.

Nickerson, D., and J.J. Tomaszewski. 1958. Color change in raw cotton related to conditions of storage. Textile Res. J. 28:485–497 and 528–529.

Simon, S.A., and C. Harmon. 1954. Color in cotton. Textile Res. J. 2412–16