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The Distribution of Gram Negative Bacteria and Endotoxin on Raw Cotton Components*

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The botanical composition of representative raw cottons from seven different growing regions was determined by manual removal and identification of all trash components greater than 50 μm in size. The number of gram negative bacteria (GNB) and the amount of endotoxin present in each of the separated raw cotton components were quantified. Low middling cotton contained significantly more bract-leaf trash than that found in higher quality cottons such as those in the middling grade division. Significantly more GNB and endotoxin were found in botanical trash components as well as lint of raw cotton derived from the southwest and southeast growing regions as compared to similar botanical components from far west cottons. For representative raw cottons from the 1980 USA crop we determined that 67% of the GNB and 89% of the endotoxin resided on white lint itself, from which all particulate larger than 50 μm in size had been removed manually.

Introduction

Gram-negative bacteria (GNB) and the endotoxin component of their cell walls are among the many biologically active agents in cotton dusts that may cause adverse health effects in man.[1] Endotoxin present in cotton dusts derived during cotton processing probably causes mill fever in both the textile and nontextile cotton industries. Studies from the model cardroom at Clemson, S.C., indicate that the decrease in FEV_1 over the work shift correlates best with the amount of endotoxin present both in raw cotton and its respirable dusts.[2] Our laboratory has been engaged in studying the microbial contaminants found on representative raw cottons from different USA growing areas. During successive crop years from 1976 to 1980, studies have been made on raw cottons, including a wide range of grades collected at United States Dept. of Agriculture-Agricultural Marketing Service (USDA-AMS) Classing Offices. A collection (N = 296) of classers' samples of all available grades of the 1980 American Upland cotton crop was studied for total content of GNB and endotoxin. We reported that raw cottons from the far west contained significantly less GNB and endotoxin than those from certain other geographical regions.[3] The current paper reports the results of analyses where botanical trash was removed by hand from 70 of the raw cottons studied in Reference 3. The separated cotton components then were analyzed for their GNB and endotoxin contents.

Previous studies from this laboratory have shown that amounts of GNB on cotton depend upon climatic conditions.[4] Raw cotton derived from cotton plants harvested late in the autumn contains significantly larger numbers of GNB and higher levels of endotoxin than cotton collected early in the harvest season. In one study the GNB content in raw cotton of one grade group increased from 0.7 million per g to 8.7 million per g when October and December harvested materials, respectively, were compared.[4] The endotoxin content of the October harvested cotton was 1.1 μg per g compared to 8 μg per g for December cotton. We also examined the GNB and endotoxin contents of the botanical trash components in a few raw cottons from the 1979 crop.[5] Larger amounts of GNB on a number per unit weight basis were found in the seed and in the bract-leaf trash than in the unsorted raw cotton. It is significant that the cleaned white lint (devoid of trash particles >50 μm size) from these cotton samples still contained large amounts of endotoxin. In the current study we have estimated the residual microbial contaminants present on the cleaned white lint from a representative number of raw cottons collected from all major USA growing regions.

Materials and Methods

Collection of Materials

From a larger group of 296 classers' raw cotton samples previously described,[3] 70 raw cottons from the 1980 crop were chosen. The 70 cottons were representative of the common USDA-AMS grades for 1980 from each of the five major growing regions in the United States, namely, the southwest, southeast, south central, south Texas and far west. Special collections of common grades were obtained also from the growing areas around Abilene and Lubbock, Texas. These latter two collections thus were subregional and both were located in the USDA-AMS southwest zone. The exact number of specimens analyzed from each region or subregion was as follows: southwest N = 9, Abilene N = 9, Lubbock N = 9, southeast N = 11, south central N = 9, south Texas N = 10, and far west N = 13. Grades 31, 41, 42 and 51 were represented in all regions. The other representative grades varied from region to region.

Botanical Analyses

All particles of trash greater than 50 μm in size were removed manually from cottons and were identified by microscopic

*Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

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TABLE I
Botanical Composition (% Wt.) of 70 Upland Raw Cottons From the 1980 Crop by Grade Division

USDA-AMS Grade Division and Number of Samples	Raw Cotton Components[A] Bract-Leaf	Stem	Seed	Bark	Total Trash[B]	Stained Lint
Strict Middling N = 5	0.24c	0	1.18	0.01	1.47b	0.11b
Middling N = 22	0.57bc	0.23	0.80	0.04	1.69b	0.24ab
Strict Low Middling N = 21	0.95ab	0.08	1.17	0.11	2.46b	0.28ab
Low Middling, Strict Good Ordinary N = 22	1.48a	0.20	1.44	0.09	3.44a	0.41a
Average of 70 Cottons[C]	0.93	0.16	1.14	0.07	2.45	0.30

[A]Means in vertical columns not followed by the same letter are different according to the Duncan's mean separation test at the 5% level of significance. Means in vertical columns without letters are not significantly different at the 5% level of significance.
[B]Total trash includes pericarp and weed materials, in addition to leaf, bract, stem, seed, and bark components.
[C]Clean lint represents 97.25% of average raw cotton sample weight.

examination as to botanical origin (see reference 6 for techniques). They were classified as bract-leaf, stem, seed, bark, pericarp, grass, weed, and other trash components. Lint devoid of gross trash particulate was separated into stained (brown, tan, yellow or green) and white or cream (herein referred to as cleaned lint) fiber. Definitions of cotton plant components have been given previously.[4,6] For sake of brevity, data on stem, bark, pericarp, grass, and weed trash have been excluded from most tables since these materials comprise only a small amount of the foreign matter in the 70 raw cottons examined.

Microbiology

Plant trash components and lints were weighed, suspended in sterile water, sonicated and vortexed. Suitable serial dilutions of extracts of lints and plant parts were made in water, and an aliquot of a tenth of a mL was spread on appropriate media. Cultures were made on trypticase soy agar with vancomycin and cycloheximide added and incubated at 25° C for GNB.

Endotoxin was estimated by a modification of the microtiter technique for the *Limulus polyphemus* amoebocyte lysate test.[7] Samples were extracted using endotoxin-free water. Serial tenfold dilutions were used to estimate the titration end point. This endotoxin-like activity was measured against an *E. coli* endotoxin standard obtained from Associates of Cape Cod (Woods Hole, MA). The source of the endotoxin material in the cotton is from many different GNB, and the use of an *E. coli* standard gives only a rough quantitation of the amount present. The microtiter method is a modification of the original *Limulus* test (see reference 7), and the method permits the detection of endotoxin in concentrations as little as 0.0125 ng per mL. Twenty μL of sample extract (or dilution) was added to 20 μL of Limulus amoebocyte lysate in a microtiter plate and incubated at 37° C for 60 min. Ten μL of 0.2% toluidine blue was added, and gelation was read by tipping the plates after 5 min. Positive and negative controls were included.

Results

Table I shows the botanical composition on a percentage weight basis of the 70 Upland raw cottons in which trash particles manually were separated from lint. Raw cottons are listed according to assigned USDA-AMS grade divisions. In general, cottons from the poorer grade divisions (low middling, strict good ordinary) contain greater amounts of bract-leaf, total botanical trash, and stained lint than cottons from better grades. It is interesting to note that while the amount of leaf and bract trash rises from about 0.25% in the best grade division studied to 1.5% in the poorest grades, the amount of the other major trash components with the exception of stained lint in these raw cottons does not vary significantly between grades. The average botanical composition of the 70 analyzed raw cottons is listed at the bottom of Table I. Thus, on average, bract-leaf and seed trash represent about 0.9 and 1.1%, respectively, of the weight of the 70 raw cottons examined. Stained lint accounts for only 0.3% of sample weight, whereas cleaned white or cream colored lint represents the bulk (97.25%) of the average raw cotton samples mass.

Table II shows the average number of GNB (log GNB per g) present in each of the sorted botanical components of the 70 raw cottons. Whereas cleaned lint contains only log 4.92 GNB per g, stained lint, bark and bract-leaf are contaminated by levels in excess of log 6 GNB per g. Other botanical trash materials are somewhat less contaminated with GNB. The 70 unsorted raw cotton samples contained an average GNB content of log 5.87 per g. This was almost one log higher than the level of GNB found on cleaned lint. The second line in Table II gives the correlation coefficient between the GNB content of each cotton component and that of the corresponding unsorted raw cotton sample. The bacterial content of both cleaned and stained lints and that of most trash components was positively and significantly correlated with levels of GNB measured in unsorted raw cottons. Note that about 70% of the variation in GNB content among unsorted raw cottons can be explained by variation residing in the cleaned lint fraction ($r^2 = 0.72$). Based on the average percentage weight content of each cotton trash component (Table I) and its average degree of contamination by GNB (Table II, first line), the final line of Table II gives the amount of GNB (in percentage of the total) estimated to be contained or carried on each trash or lint fraction. Thus, cleaned lint can be seen to account for most of the GNB in the examined samples. In this case, the great sample weight of clean lint (97.25% of raw cotton mass) far outweighs the relatively low degree (log 4.92 per g) of GNB contamination. By contrast, the more highly contaminated bract-leaf fraction comprises 0.93% of average raw cotton sample weight, and thus accounts for only 18.5% of the bacteria present in raw cottons.

The average endotoxin content of botanical components of raw cottons is given in Table III. Highest levels of endotoxin occurred on stained lint and on bark trash. Cleaned lint was contaminated least with endotoxin. With the exception of bark, endotoxin contents of all botanical components were positively and significantly correlated with that of unsorted raw cotton samples. Over 50% of the variation in endotoxin content among unsorted raw cottons can be explained by variation residing in the clean lint fraction ($r^2 = 0.52$). Based on the average percentage weight content of each botanical component (Table I) and the average extent of its contamination with endotoxin (Table III, first line), calculations show that cleaned lint contains 89.1% of the endotoxin present in these raw cottons. The bract-leaf fraction contributes only 4.3% to the total endotoxin, while other botanical components are responsible for even smaller amounts of raw cotton endotoxin.

Table IV shows the content of endotoxin in major botanical components of the 70 raw cottons examined. In general, there is little or no difference in endotoxin content on botanical components of raw cottons when the middling through strict good ordinary grade divisions are compared. On the other hand, bract-leaf, seed, and cleaned lint from strict middling cotton contains significantly less endotoxin than poorer grade divisions. Significant differences in microbial contamination of other botanical trash materials such as bark, stem, and pericarp components were not observed when strict middling through strict good ordinary grade divisions were compared (data not shown). The number of GNB on cotton components by grade divisions follows the

TABLE II
Gram Negative Bacteria Present in Sorted Botanical Components of 70 Raw Cottons

Microbial Parameter	Cotton Component: Bract-Leaf	Stem	Seed	Bark	Stained Lint	Cleaned Lint
Average number of GMB (Log N per g) on cotton component from 70 samples	6.38	5.65	5.83	6.14	6.30	4.92[A]
Corr. Coef. between GNB content of cotton component and GNB content of unsorted raw cotton[B]	0.76***	0.35**	0.77**	0.27	0.54***	0.85***
% of total GNB derived from each cotton component[C]	18.5	0.6	6.4	0.8	6.0	66.9

[A]The average number of GNB in the 70 unsorted raw cottons was log 5.87 per g.
[B]*, **, *** = Correlation coefficients significant at 5%, 1%, and 0.1% levels, respectively.
[C]Total GNB in cotton as determined by multiplying the average % wt. content of each botanical trash or lint component (Table I) and average number of GNB present on each of these components.

TABLE III
Endotoxin Present in Sorted Botanical Components of 70 Raw Cottons

Microbial Parameter	Cotton Component: Bract-Leaf	Stem	Seed	Bark	Stained Lint	Cleaned Lint
Average endotoxin content (log ng per g) on cotton component from 70 samples	3.67	3.52	3.29	3.97	4.01	2.97[A]
Corr. coef. between endotoxin content of cotton component and endotoxin content of unsorted raw cotton[B]	0.62**	0.33*	0.57***	0.26	0.38**	0.72***
% of total endotoxin derived from each cotton component[C]	4.3	0.5	2.2	0.6	3.0	89.1

[A]The average content of endotoxin in the 70 unsorted raw cottons was log 3.09 ng per g.
[B]*, **, *** = Correlation coefficients significant at 5%, 1%, 0.1% levels, respectively.
[C]Total endotoxin in cotton was determined by multiplying the average % wt. content of each botanical trash or lint component (Table I) and the average content of endotoxin present on each of these components.

same pattern as illustrated for endotoxin in Table IV (data not shown).

The botanical composition of raw cottons separated according to geographical origin and the degree to which botanical components are contaminated by endotoxin are shown in Tables V and VI. A minimum of 9 and a maximum of 13 samples were examined for each geographical region. The variability in bract-leaf, seed, total trash, and stained lint contents within each region was so great that significant differences in the content of botanical trash components by region were not found (Table V). It is evident that cotton components from the far west contained lesser amounts of endotoxin than that found on similar components from other geographical regions (Table VI). This is especially evident for the cleaned lint fraction where endotoxin levels are at least one half of a log order less than those present on lint from other regions. It is interesting to note that cleaned lint from the southwest and southeast regions contained the same or greater amounts of endotoxin as found on the bract-leaf trash from far west samples. The number of GNB on cotton components separated according to geographical origin follows the same pattern as illustrated in Table VI for endotoxin (data not shown).

Discussion

In this paper we have described botanical and microbial analyses carried out on 70 raw cottons collected from seven USA cotton growing regions or subregions in 1980. Cotton samples chosen for analysis from each region were from representative grades only, and as such, extremely high quality and poor grades such as good middling, below grade, and yellow-stained samples were not examined. Our analyses then should be representative, at least for the 1980 crop year, of the types of botanical and microbial variables that can be expected among the typical raw cottons available for use by the cotton textile industry.

Bract is a major botanical component of trash found in raw cotton and is thought to be the major vegetable component of raw cotton dust.[6] As a result of analyses carried out on a group of 71 raw cottons from the 1977 crop, it was found that poorer grade divisions such as low middling contained significantly more bract-leaf trash (*e.g.*, 2.02% by wt.) than higher quality grades such as middling (bract-leaf = 0.40% by wt.).[6] Similar results also were obtained in the current study with 1980 crop raw cottons. Significantly more bract-leaf trash was found in the low middling (1.48%) versus the middling (0.57%) grade division. The USDA-AMS grade division classification system for raw cottons continues to provide an accurate and simple guide for determining the bract-leaf content of raw cotton samples. Progressively greater amounts of this botanical trash component occur as one numerically ascends the grade division quality ladder from strict middling to low middling (Table I). While the USDA-AMS system of classifying cottons by grade division also can be used to predict total botanical trash levels and amounts of stained lint, it is an unsatisfactory indicator of other botanical variables in raw cottons, such as amounts of seed and bark trash (Table I).

The geographical region from which raw cottons were obtained, at least for common grades as analyzed in this study, did not appear to influence the bract-leaf content of the sample (Table V). Variability within each of the seven growing regions was sufficiently great that significant differences in bract-leaf content, or the content of any other type of botanical trash material, were not observed. Thus, raw cottons from the Abilene and Lubbock areas, both within the overall southwest USDA-AMS region, contained widely divergent amounts of bract-leaf trash (an average of 0.69 *vs.* 1.32%, respectively, Table V).

Bract-leaf, stem, stained lint, and other cotton botanical components, on average, contain somewhat more GNB and endotoxin than that found on cleaned lint from which all particulate greater than 50 μm in size was removed (Tables II and III). However, because botanical trash and stained lint fractions account, on average, for just under 3% of the total cotton mass, the contribution of cleaned lint to the overall GNB and endotoxin contamination of raw cotton is overwhelming. Based on the data in Tables II and III it may be concluded that about 67% of the GNB and 89% of the endotoxin in samples of raw cotton studied are present in or on the lint itself or on the fine trash particles that we could not remove from fiber surfaces. Results of correlation analysis between GNB or endotoxin contents on cotton components and unsorted raw cottons also lead to the same conclusion. Highest coefficients of determination (r^2) were found between the cleaned lint and the unsorted raw cottons (Tables II & III). Damage to maturing lint by premature freezing or insect attack are among some of the environmental factors that can lead to the contamination of fiber by GNB and their endotoxins.[4,8]

The suggestion that almost 90% of the endotoxin in raw cotton is found in or on the lint has some implications with regard to possible approaches for making available raw material with the lowest possible amount of bioactive, potentially byssinotic agents. Endotoxin is recognized as a potential bioactive agent in raw cotton.[9] If, after removal of all foreign matter greater than 50 μm in size, the bulk of endotoxin still remains associated with the fiber, then it is questionable if mechanical cleaning at the gin or in early stages of textile processing will significantly decrease amounts of this microbial material below a certain baseline level. Thus, in preliminary experiments it was found that the use of seven gin gleaners did not significantly lower the endotoxin content of cotton below that attained by two cleaners.[10] Removal of endotoxin by solubilization, *e.g.*, in water washing[11] would seem to be the best alternative to mechanical cleaning. Alternatively, it is possible that some form of the mechanical cleaning of fiber may be effective in removing microdust (<50 μm) attached to fiber surfaces, and microdust may contain significant amounts of endotoxin. It should be noted that in our studies we manually removed trash particles down to a size of only about 50 μm, and we did not attempt further lint cleaning. Further experiments are needed both to confirm that the bulk of endotoxin in raw cotton is located on the lint, and to determine if microdust present on fiber surfaces contains significant amounts of endotoxin.

TABLE IV
Endotoxin (Log ng per g) in Sorted Components Of Upland Cotton by Grade Division

USDA-AMS Grade Division	Cotton Component[A] Bract-Leaf	Seed	Cleaned Lint	Stained Lint
Strict Middling	2.69b	2.28b	1.88b	3.33a
Middling	3.47a	3.29a	2.92a	4.16a
Strict Low Middling	3.78a	3.20a	3.14a	4.08a
Low Middling, Strict Good Ordinary	4.00a	3.62a	3.11a	3.93a

[A]Means in vertical columns not followed by the same letter are different according to the Duncan's mean separation test at the 5% level of significance.

TABLE V
Botanical Components (% wt.) of 70 Raw Cottons According to Geographical Regions

Geographical region and number of samples	Cotton Component[A] Bract-Leaf	Seed	Total Trash	Stained Lint
Abilene, N = 9	0.69	1.23	2.08	0.50
Lubbock, N = 9	1.32	1.09	3.08	0.39
Southwest, N = 9	0.75	0.98	2.22	0.36
Southeast, N = 11	0.84	0.97	2.05	0.24
South central, N = 9	1.13	1.29	2.86	0.18
South Texas, N = 10	1.19	0.94	2.49	0.27
Far west, N = 13	0.64	1.42	2.47	0.19

[A]Means in vertical columns are not significantly different at the 5% level of significance according to the Duncan's mean separation test.

We could not demonstrate any significant differences in amounts of botanical trash components and stained lint between representative grades of raw cottons from each of the seven growing regions listed in Table V. Although this may have been due to the small number of samples (N = 9 to 13) examined from each region, it is interesting that regional differences in levels of microbial contamination among the cottons of the same grades were found (Table VI). Bract-leaf, seed trash and stained lint from the southwest (including Abilene and Lubbock) and the southeast, for the most part, contained significantly higher levels of microbial contaminants including endotoxin (Table VI) than far west samples. Cleaned lint from the far west also contained lower amounts of endotoxin than that found on cleaned fiber from other growing regions. Lower numbers of GNB, gram positive bacteria, and fungi, and a consistently reduced level of endotoxin are also characteristic of intact raw cottons from the far west versus those from other USA growing regions.[3] The underlying cause of regional variation in microbial contamination of cotton cannot be ascribed to botanical composition, since in this study regional differences in the content of plant trash materials were not observed (Table V). Differences in numbers of GNB and amounts of endotoxin found in raw cotton, however, previously have been reported to be caused by such climatic variables as the occurrence of a

TABLE VI
Endotoxin Content (log ng per g) of Sorted Cotton Components by Geographical Region

	Cotton Component[A]			
Region	Bract-Leaf	Seed	Stained Lint	Cleaned Lint
Abilene	4.11a	3.97ab	4.66a	3.76a
Lubbock	3.82ab	3.26bc	3.98ab	3.09ab
Southwest	3.80ab	3.26bc	4.19ab	2.89bc
Southeast	4.01ab	4.45a	4.44a	3.41ab
South central	3.35bc	2.80cd	3.65ab	2.74bc
South Texas	3.81ab	3.14c	3.94ab	2.97b
Far west	2.90c	2.24d	3.20b	2.18c

[A]Means in vertical columns not followed by the same letter are different according to the Duncan's mean separation test at the 5% level of significance.

freeze in which immature capsules are damaged, and extensive weathering of cotton plants in the field prior to harvest.[4] Since mitigation of a potential contaminant at its source generally is accepted to be the most effective means of contaminant control, it would appear that further study to define those environmental-climatic conditions associated with minimal contamination of cotton by GNB and their endotoxins is needed.

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