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ESTIMATE OF BOTANICAL INGREDIENTS IN RESPIRABLE COTTON DUST. MOREY, P.R.,
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Research on the etiology of byssinosis has been confounded by the uncertain identity of botanical ingredients present in respirable ($<10 \mu\text{m}$) cotton dust. In this study estimates were made of botanical ingredients present in respirable dust associated with the following raw cotton grades: good middling (GM), strict middling, middling (M), strict low middling (SLM), low middling (LM), strict good ordinary, and good ordinary. All botanical trash materials $>50 \mu\text{m}$ were removed from replicate samples of raw cotton grades and identified as leaflike (bract, leaf, and weed leaf), seed (seedcoat and seedmeat), bark, exo-mesocarp, endocarp, or stem (petiole and wood) components. The average % weight content of each botanical material was determined on a per bale (217.7 kg) basis. For example, a SLM bale would contain 1.87 kg leaflike, 2.18 kg seed, 0.17 kg endocarp, 0.11 kg exo-mesocarp, 0.48 kg bark, and 0.39 kg stem trash materials. The amount of leaflike trash ranged from 87 g per bale for GM cotton to over 4 kg in the LM grade. All raw cottons irrespective of grade contained at least 1 kg of seed trash. The friability of each trash component was determined by (A) roller milling botanical material with an initial size $>500 \mu\text{m}$, (B) sifting the pulverized remains on a $10 \mu\text{m}$ mesh screen, and (C) recovering particulate that was $<10 \mu\text{m}$. The % weight of each milled plant material converted into respirable particulate was: wood=29%, bract=28%, exo-mesocarp=19%, petiole=16%, bark=12%, leaf blade=5.6%, nightshade weed=2.5%, endocarp=2.4%, and seedcoat=0.3%. Lint, linters, and seedmeat were not converted in measurable amounts to a size $<10 \mu\text{m}$. An estimate was made of the composition of respirable dust that would be generated if all botanical trash in a bale was milled. It was assumed that the proportion of bract:leaf:weeds in leaflike trash was 58:22:20, the ratio of seedcoat to seedmeat in seed trash was 99:1, and the ratio of wood to petiole in stem trash was 37:63 (AIHAJ 37, 409, 1976). For the M, SLM, and LM grades the following % composition of botanical ingredients ($<10 \mu\text{m}$) was predicted: leaflike=64-67, stem=12-18, bark=5-13, exo-mesocarp=4-9, seed=0.5-3.0, and endocarp=0.5-1.5. Thus, leaflike dust (largely bract) was most concentrated in respirable particulate. Stem, bark, and exo-mesocarp made smaller but significant contributions to $<10 \mu\text{m}$ dust, while the contribution of seed and endocarp was minimal. Although the relative % composition of each botanical ingredient in $<10 \mu\text{m}$ particulate did not vary greatly between the M, SLM, and LM grades, the total amount of botanical dust was predicted to vary with grade. For example, M raw cotton was estimated to generate only about 1/5 the botanical dust expected for the LM grade. Work supported by Cotton Incorporated and by NIOSH Grant No-5-R01-OH00667-02.

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INTRODUCTORY SUMMARY

The objective of this study was to determine the specific botanical ingredients present in respirable raw cotton dust.

Significant differences in content of gross leaflike (bract-containing) trash were found between several grade divisions of raw cottons. For example, higher grades of raw cotton (strict low middling = SLM) contained less leaflike trash than lower grade cottons (low middling = LM). The potential for production of fine particulate from botanical trash materials plus lint and linters was determined in the laboratory by an abrasive milling test. Bract and wood fragments were the most friable of all plant materials found in raw cottons whereas seed meat, lint, linters and seed coat were the least friable. Respirable ($<10 \mu\text{m}$) raw cotton dusts associated with the processing of middling, SLM and LM raw cottons were predicted to contain the following % weight composition of specific vegetable ingredients: leaflike = 70-72%, stem = 13-18%, bark = 3-8%, exocarp-mesocarp = 3-6%, endocarp = 1-2% and seed = 0.5-2%. Bract is the most abundant component in respirable raw cotton dust.

Botanically What is Raw Cotton Dust?

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INTRODUCTION

One of the most difficult problems associated with cotton dust research has been the absence of agreement on the nature of the etiological agent(s) in respirable particulate that causes acute symptoms of byssinosis in susceptible textile workers. The biologically active substance(s) appears to be localized in bract fragments which are a component of the gross trash in raw cottons processed by the cotton yarn manufacturer. (1,2) However the actual amount of bract fragments in respirable cotton dust in early steps of cotton yarn processing operations is unknown.

Pharmacological, chemotactic, antigenic and microbial agents present in or on raw cotton dust are all claimed to be byssinosis causatives. (3-6) Disagreement on etiology is not surprising in view of the nonanalytical descriptions that have been applied to cotton dust. This material has been defined by OSHA as particulate "... present in the air during the handling or processing of cotton, which may contain a mixture of many substances including ground up plant matter, fiber, bacteria, fungi, soil, pesticides, non-cotton plant matter and other contaminants which may have accumulated with the cotton during the growing, harvesting and subsequent processing or storage periods. Any dust present during the handling and processing of cotton through the weaving or knitting of fabrics, and dust present in other operations or manufacturing processes using new or waste cotton fibers or cotton fiber by-products from textile mills are considered cotton dust" (p. 27395 in reference 7). Because the toxic agent in this complex heterogeneous dust is unidentified and since there is no precise analytical method to measure risk of byssinosis (activity of the dust), OSHA has set permissible exposure limits for cotton dust exposure in terms of the mass of dust collected by the vertical elutriator cotton dust sampler or

equivalent sampling method.⁽⁷⁾

The objective of this study was to estimate the plant part composition (eg., % bract, % seed, etc.) of respirable raw cotton dust and thus to specify a standard reference material for continuing investigations on byssinosis etiology.

Raw cottons in the United States are classed for both staple and grade by the U. S. Department of Agriculture-Agriculture Marketing Service (USDA-AMS), Cotton Division under the authority of the Smith-Doxey Act.⁽⁸⁾ One of the major factors involved in determining grade is the amount of botanical trash seen by the classer in raw cotton samples. Raw cottons of the white color group are visually assigned by the classer to one of 7 grade divisions (eg., from the best to the poorest as follows: good middling = GM, strict middling = SM, middling = M, strict low middling = SLM, low middling = LM, strict good ordinary = SGO and good ordinary = GO) primarily on the amount of botanical trash entrained in the raw cotton lint. In order to estimate the botanical ingredients in raw cotton dust, it was necessary to determine both the % weight content of each gross botanical trash component (eg., bract, seed, etc.) in the 7 raw cotton grade divisions and the capacity of each type of plant trash material to produce respirable (<10 μ m) particulate during an abrasive milling process simulating early steps in raw cotton processing operations.

MATERIALS AND METHODS

Trash analysis of raw cottons. Botanical trash analyses were carried out on a total of 71 raw cottons including the following number of samples from each grade division: 3 GO (all from south west portion of cotton belt), 10 SM (7 from south west, 2 from far west and 1 from south central), 13 M (10 from south west, 3 from south central), 19 SLM (9 from south west, 9 from south central and 1 from south east), 17 LM (9 from south west, 5 from south central, 2 from south east and 1 from far west), 6 SGO (3 from south east, 2 from south west and 1 from south central) and 3 GO (2 from south west and 1 from south central). These raw cottons which had been classed by the USDA-AMS Cotton Division were from the 1974-1977 crop years.

A subsample of 0.9 to 3 g was made by removing 10 randomly selected plucks of lint plus entrained foreign matter from each 50-200 g raw cotton sample. All nonfibrous particles $>50 \mu\text{m}$ diameter were removed from each subsample manually with needles and forceps. Each nonfibrous fragment was then examined (stereomicroscope) and sorted into one of the following categories: (A) leaflike trash consisting of fragments of cotton leaf blades, cotton bract, vein material, weed leaves and grass strands. (B) seed trash including seed coat and seed meat (embryo) fragments. (C) endocarp, the innermost layer of the capsule (pericarp) wall. (D) exocarp-mesocarp, the outer 2 layers of the capsule wall. (E) bark consisting of sinuous, fibrous, longitudinally-oriented particles that are derived from the outer portion of cotton and weed stems. (F) stem trash consisting of wood (stem devoid of bark), petiole fragments and large veins devoid of lamina components. (G) miscellaneous trash including unidentified botanical trash $>50 \mu\text{m}$ diameter and all nonbotanical components such as metal, rock, paper and bale covering fragments. The

% trash component content in raw cotton samples was expressed as the weight of the trash component removed divided by the original weight of the sample (lint plus all trash materials).

Plant material used in friability tests. Lint samples (1977 crop) were obtained from SM and SLM raw cottons from the south central or south west portions of the cotton belt. First cut linters (1976 crop) were obtained from the far west, south west, south central and south east portions of the cotton belt. Bark, wood, endocarp and exocarp-mesocarp were removed from stem and capsule trash collected during seed cotton cleaning of Acala 1517 in NM in 1974. Leaf blade and petiole were collected in November 1976 from abscised Coker 4348 leaves which fell to the ground after the first frost in Lubbock, TX. Bract was hand-picked in November 1977 from a large number of cultivars one to 10 days before harvest in Lubbock, TX. Silverleaf nightshade (SLN) leaf was removed from cotton fields in Lubbock, TX in November 1976. Seed coat and seed meat from the 1977 crop were obtained from an oil mill processing TX High Plains cottonseed.

Abrasive roller milling. In each roller mill test approximately 1.0 g of plant material in the size range between 500 and 999 μm (no upper size limit however, for lint, linters and bark) was used. Prior to milling, all particles <500 μm diameter were removed from each plant material by extensive sifting on a 500 μm mesh screen in an ATM Sonic Sifter. The friability of different plant materials was determined by abrasive roller milling with 10 porcelain cylinders in a porcelain jar for an arbitrary 5 hr period at a speed of 70 RPM. Porcelain cylinders were 13 mm in diameter, 12 mm in length and weighed about 5 g. The jars, cylinders and gram lots of plant material were desiccated for at least 24 hours immediately prior to milling.

Fractionation of milled plant materials. Plant material was removed from jars and sifted in a Sonic Sifter (relative humidity 50-65%) through an arbitrarily selected set of screens with mesh openings of 250, 75 and 30 μm . Particle size classes $>250 \mu\text{m}$, 75-249 μm , 30-74 μm and $<30 \mu\text{m}$ were weighed and the % recovery was calculated. Material balances for lint and linters were nearly 100% or 100%. For other plant parts, material balances were from 70-95%. Notwithstanding extensive attempts at removal, plant material of all sizes was retained in the mesh of screens and also along the sides of porcelain jars. A 100% material balance was calculated for each plant material assuming that the % lost was distributed in each of 4 size fractions as a function of the weight recovered in each of the 4 fractions.

In order to determine the size of particles passing through the 30 μm mesh screen, a 10-50 mg subsample of the $<30 \mu\text{m}$ material from each roller mill test was processed on a 10 μm mesh screen in the Sonic Sifter. The % weight remaining on the 10 μm screen (10-29 μm size range) and the % passing through the screen ($<10 \mu\text{m}$) were recovered. Material balances ranged from 60-90%. A 100% material balance was calculated by distributing the % lost into the 10-29 and $<10 \mu\text{m}$ fractions as a function of the actual weight recovered in each fraction. Three or 4 roller mill tests were carried out for each type of plant material.

RESULTS AND DISCUSSION

The % weight content of leaflike, seed, endocarp, exocarp-mesocarp, bark and stem trash was determined for raw cotton samples from each of the 7 grade divisions (Table I). Significant differences in content of leaflike trash were found between several grade divisions. For example, SM raw cotton contained significantly less leaflike trash than SLM cotton. Raw cottons from the 3 poorest grade divisions (LM, SGO and GO) all contained significantly more leaflike trash than SLM cotton. Because of the highly variable amount of seed trash in separate cotton samples within each grade division, significant differences in the content of this botanical component between grade divisions were absent (Table I). Raw cottons from the LM, SGO and GO grade divisions tended to have greater amounts of stem and bark trash than cottons from the better grades (Table I).

A standard method used by the yarn manufacturer to estimate the waste content of raw cotton is based on the use of the Shirley Analyzer Instrument.⁽⁹⁾ The Shirley nonlint content was determined for the raw cottons in Table I to see if this simple test was predictive of amount of botanical trash entrained in the lint. In general, cottons from poorer grade divisions contained significantly higher Shirley nonlint contents than better grade cottons (Table I). For all raw cottons examined in Table I, correlation coefficients between Shirley nonlint content and the content of the 6 botanical trash categories taken separately were highly significant ($P < 0.01$). Unfortunately, the coefficients of determination (r^2) between Shirley nonlint content and each of the 6 botanical trash categories was < 0.52 (eg., $r^2 = 0.40$ for

leaflike trash and Shirley nonlint contents). This indicated that variability in Shirley nonlint content was not highly predictive of variation in amount of specific botanical entities such as leaflike trash entrained in raw cottons. Manual separation of botanical trash materials from lint and identification of the former by stereomicroscopy remains as the only accurate quantitative procedure available for determining the content of specific kinds of vegetable trash (eg., leaflike trash) in raw cotton.

The potential for the production of fine particulate from botanical trash materials plus that of lint and linters was determined by abrasive roller milling. For this purpose it was assumed that (A) leaflike trash was composed of bract, leaf and weed leaves, (B) seed trash consisted of seed coat and seed meat components and (C) stem trash contained wood and petiole materials.

Lint, linters and seed meat were broken up during abrasive milling only in small amounts to a size $<250 \mu\text{m}$ and none of these plant materials were converted in measurable amounts into $<10 \mu\text{m}$ particulate (Table II). Seed coat material, while not readily broken apart, did produce a small amount ($<0.4\%$) of particulate $<10 \mu\text{m}$ diameter. SLN leaf (a representative weed), cotton leaf blade and endocarp were ground up into $<10 \mu\text{m}$ particulate in amounts ranging between 1 and 5% (Table II). Bark, exocarp-mesocarp, petiole and especially wood and bract fragments were the most friable of all botanical materials found in cotton (Table II). After abrasive roller milling $<10\%$ of each of these materials remained in the size class $>250 \mu\text{m}$. Approximately 30% of the bract and wood had been ground up into $<10 \mu\text{m}$ particulate. It was concluded that bark, exocarp-mesocarp,

petiole and especially bract and wood fragments in raw cottons likely make substantially greater contributions to dust generation than would otherwise be indicated by their relative weight among the gross botanical trash materials found in raw cottons.

Estimations (Table III) were made of the relative composition of botanical ingredients likely to be present in $<10 \mu\text{m}$ size cotton dust assuming that all botanical materials in raw cottons were subjected to abrasive roller milling as had been done for plant materials in Table II. In making these estimates it was further assumed that the proportion of bract to leaf to weed leaves in leaflike trash was 58:22:20, the ratio of seed coat to seed meat in seed trash was 99:1 and the ratio of wood to petiole in stem trash was 37:63 (Table II in reference 2). In estimating the botanical ingredients in $<10 \mu\text{m}$ particulate associated with for example, the processing of SLM raw cotton, the content of leaflike trash (0.85%, Table I) was partitioned in a 58:22:20 manner between bract, leaf and weed leaves, respectively. The absolute amount of respirable bract, leaf and weed dusts generated during raw cotton processing was estimated by multiplying the amounts of gross bract, leaf and weed leaf by the capacity of each of these plant parts to produce $<10 \mu\text{m}$ particulate in roller mill tests (Table II). In like manner the absolute contributions of seed coat, seed meat, petiole, wood, endocarp, exocarp-mesocarp and bark to cotton dust formation was calculated based on the amount of each material in raw cottons (Table I) and the capacity of each plant part to be ground up into $<10 \mu\text{m}$ particulate (Table II).

It was predicted (Table III) that 60-72% of the weight of raw cotton dusts was leaflike and the majority ($>90\%$ because bract comprises

58% of leaflike trash and is the most friable plant part) of this was bract particulate. Seed dust should account for only a very small fraction of raw cotton dust especially for the poorer grade divisions (Table III). For the M, SLM and LM raw cottons which are most commonly used in cotton yarn manufacturing, the following % composition of botanical ingredients is predicted: leaflike = 70-72%, stem = 13-18%, bark = 3-8%, exocarp-mesocarp = 3-6%, endocarp = 1-2% and seed = 0.5-2% (Table III). These estimations on relative % weight composition of botanical ingredients in <10 μ m size raw cotton dusts did not take into consideration contributions made by soil particles which may be considerable⁽¹⁰⁾ and possible contributions from the micronization of the small amounts of unidentified (miscellaneous components, Table I) foreign material also entrained in raw cottons.

A number of assumptions are inherent in estimating the vegetable ingredients in respirable raw cotton dust. First, there is the assumption that the laboratory milling of plant trash materials in porcelain jars is realistic in terms of forces applied to the same trash components in cotton processing operations such as carding. However, the roller mill test is at present the only procedure that has been successfully used to study the way in which plant parts are ground up into fine particulate.⁽¹¹⁾ Secondly, with the exception of lint and linters, plant materials used in roller mill tests were collected from sources other than the cotton bale (eg., leaf was obtained from the ground after frost induced defoliation). Thus, with the exceptions noted, plant materials utilized in roller mill tests were similar but not totally identical to the botanical trash fragments found in raw cottons. Another assumption inherent in these estimates is that bract does comprise 58% of the weight of all the gross

leaflike trash manually removed from raw cotton samples. The 58% figure, while being the best available in the literature,⁽²⁾ was taken from work wherein histological techniques were used to identify somewhat larger (840-2000 μm size range) gross trash fragments.⁽²⁾ The actual content of bract is leaflike trash in the 50-840 μm size range may differ from the 58% found in larger leaflike trash fragments.

Cotton dust generated in the raw cotton processing industry is primarily a vegetable particulate of heterogeneous composition. Bract,^(2,3,12) leaf,⁽²⁾ capsule,^(2,12) stem,^(2,13) seed fragments^(2,14) and weeds^(2,15) have all been observed as gross contaminants of raw cotton, and can be assumed to make some contribution to the generation of respirable particulate when raw cotton is processed by the yarn manufacturer. This study estimates for the first time the relative amounts of botanical ingredients in respirable raw cotton dusts (Table III). Leaflike material (largely bract) is the most abundant botanical component in cotton dust (Table III) and aerosolized extracts of bract cause symptoms of byssinosis similar to those elicited by cotton mill dust.^(1,16) While evidence suggests that the byssinosis causative(s) does not reside in the capsule wall⁽¹⁾ and in seed^(1,17) trash, the bioactivity of stem and bark components is unknown. Since bark and stem dusts are estimated to account for up to 25% of the weight of raw cotton dusts (Table III) it is important for long term strategy on solutions of the cotton dust problem to determine if bract alone, or bract together with other plant part dusts carries or contains the agent(s) which gives rise to acute byssinotic responses.

The estimation of exact botanical ingredients in respirable raw cotton dust has significance in terms of future experimental studies on

physiological and patho-physiological mechanisms associated with the development of byssinosis. No two laboratories have up to the present time used identical vegetable material as the source of the toxic agent (byssinosis causative) in in vivo and in vitro bioassays on disease mechanisms. Mixtures of dry bract and leaf fragments,⁽¹⁸⁾ aerosolized extracts of bracts,^(1,3) and a wide and variable range of cotton mill dusts and trash materials^(3,18,19) have all been used in studies on disease mechanisms. It is no wonder that there is so much disagreement on etiology of this rather well studied respiratory disease. This study provides an estimate (Table III) for preparation of a standard reference material for further investigations concerned with the acute and chronic effects of raw cotton dust.

Given the assumption that leaflike trash is the major carrier of the agent which causes acute symptoms of byssinosis, Table I provides an estimate of potential toxicity of raw cottons as classified by the USDA-AMS. Thus SM raw cotton contains only about 24% as much leaflike trash as SLM cotton (Table I). There is evidence that the acute symptoms of byssinosis are much reduced when a better grade division of raw cotton is processed.⁽²⁰⁾ The change in $FEV_{1.0}$ for a carefully selected test panel was -2.9% and -8.5% when SM and SLM raw cottons, respectively were processed (Table II in reference 20). Subjective symptoms of byssinosis were also reduced when the SM cotton was utilized. The USDA-AMS classing system for raw cotton when used with reference to the amount of leaflike trash in various grade divisions (Table I) may provide a rough prediction of potential toxicity before raw cotton is processed by the yarn manufacturer.

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TABLE I

Gross Botanical Trash in Raw Cotton Grade Divisions*

Grade Division	% Leaflike	% Seed	% Endocarp**	% Exocarp- Mesocarp**	% Bark	% Stem	% Misc.**	%Shirley Nonlint
GM	0.05a	0.46a	0	0	0.02a	0.01a	0.02	1.29a
SM	0.20a	0.63a	0.06	0.04	0.09a	0.05a	0.04	1.84a
M	0.40ab	0.92a	0.05	0.04	0.12a	0.07a	0.10	2.85ab
SLM	0.85b	1.01a	0.08	0.05	0.22a	0.18a	0.24	3.56bc
LM	2.02c	1.39a	0.24	0.25	0.23a	0.47b	0.23	5.64c
SGO	1.83c	1.62a	0.07	0.18	0.25a	0.58b	0.67	5.40c
GO	1.97c	1.71a	0.05	0.14	0.84b	0.47ab	0.22	5.74c

*Means in any vertical column followed by the same letter are not different at the 0.05 level of significance according to Duncan's Multiple Range Test.

**When analyzed separately, endocarp, exocarp-mesocarp and miscellaneous trash categories showed an absence of significant differences by grade divisions according to analysis of variance. Therefore the Duncan's means separation test could not appropriately be applied to these trash materials.

TABLE II
Friability of Trash Components and Fiber

Plant Part	% Weight by Size Class after Abrasive Roller Milling			
	250-500 μm	30-249 μm	10-29 μm	< 10 μm
Seed meat	99.45	0.55	0	0
Lint	98.39 ^a	1.24	0.32 ^b	0 ^b
Linter	97.53 ^a	2.13	- ^c	- ^c
Seed coat	88.40	9.48	1.73 ^d	0.22 ^d
Stem leaf	16.70	58.74	22.38	2.18
Leaf blade	14.78	52.43	29.08	3.71
Endocarp	20.47	53.70	21.50	4.33
Bark	4.79 ^a	49.81	38.32	7.08
Exocarp-mesocarp	4.94	30.05	50.47	14.54
Leaf petiole	6.72	41.95	32.99	18.34
Wood	0.53	25.61	44.47	29.39
Bract	1.73	31.06	34.36	32.85

^aNo upper limit on size.

^bTwelve mg of lint fragments passed through the 30 μm mesh screen during one of the 4 runs. None of these lint fragments passed through a 10 μm mesh screen. Lint fragments passed through the 30 μm mesh screen in such small amounts in 3 of the 4 runs that separation

into 10-29 μm and $<10 \mu\text{m}$ size classes could not be carried out. This accounted for the remaining 0.05% of the material balance.

^c Linter fragments passed through the 30 μm mesh screen in such small amounts during all 4 runs that separation into 10-29 μm and $<10 \mu\text{m}$ size classes could not be carried out. This accounted for the remaining 0.34% of the material balance.

^d During 2 of the 4 runs sufficient amounts of seed coat material passed through the 30 μm mesh screen so that segregation into the 10-29 μm and $<10 \mu\text{m}$ size classes was possible. However, during the other 2 runs only small amounts of seed coat material passed through the 30 μm mesh screen and further fractionation could not be carried out. This accounted for the remaining 0.17% of the material balance.

TABLE III
 Estimate of Botanical Ingredients Present
 in <10 μ m Raw Cotton Dusts

Raw Cotton Grade Division	Leaflike	Seed	Endocarp	Exocarp- Mesocarp	Bark	Stem
GM	68.43*	6.68	0	0	9.65	15.24
SM	59.72	2.00	3.83	8.55	9.38	16.52
M	70.39	1.76	1.88	5.04	7.36	13.57
SLM	71.47	0.92	1.43	3.01	6.45	16.72
LM	70.52	0.52	1.79	6.25	2.80	18.12
SGO	67.30	0.65	0.55	4.74	3.20	23.56
GO	67.66	0.64	0.37	3.44	10.06	17.83

*All numbers are % weight botanical ingredients estimated to be present in each raw cotton dust by grade division.