Identification and Elimination of the Causative Agent of Byssinosis

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ABSTRACT

Separation and identification of possible causative agents of byssinosis have been high priority research activities at the Southern Regional Research Center since 1975. The Center serves as a focal point for several cooperating agencies also active in the search for this elusive agent. Several examples of recent research on proximate, elemental and microscopical analyses, as well as bioassays of cotton textile dust, washed cotton and dust obtained from selected cottonseed oil mills illustrate the multidisciplinary attack required by this area of research.

INTRODUCTION

Control and elimination of cotton dust continues to pose a serious problem to the cotton industry. In response, SEA-USDA has sponsored an extensive cotton dust research program since 1973 for which the budget has steadily increased to a current (1980) level of $4 million. The program at Southern Regional Research Center (SRRC) has been closely coordinated with other government- and industry-sponsored research programs to increase cooperation between the research groups under the direction of I.W. Kirk, Acting Center Director and the Southern Regional Coordinator for Byssinosis Research.

The various groups communicate with each other regarding receipt of dust samples for analysis, transmission of dusts and dust extracts for bioassays, and assimilation and interpretation of the analyses data (Fig. 1). Bioassays of dusts, and dust extracts, or body fluids from subjects exposed to cotton dust are performed by 3 universities under contract or cooperative agreement. Histamine content and release determinations using minced pig lung (1-3) are provided by M. Battigelli at the North Carolina School of Medicine. J. Fischer, also of North Carolina School of Medicine, furnishes gram-negative bacteria and endotoxin (Limulus crab assay) (4-6) level data and dusts and selected dust extracts. Recent studies at Tulane University Medical School under the guidance of B. Butcher are concerned with screening of cotton dusts and their extracts for biological activity (7-9) and the development of an improved animal model for cotton dust exposure (10). G. Greenblatt and associates at Texas A&M University have concentrated their efforts on alveolar macrophage studies (11,12) and improvement of existing histamine content/release bioassays (13-15) for cotton dusts and extracts. The SEA-USDA Human Panel Exposures at Clemson, SC, held in cooperation with K. Bragg, J. Cocke and the NIOSH group under J. Merchant, has been an extremely important common source of dust for all phases of the research. In a memorandum of understanding recently negotiated with Yale University and Cotton, Inc., SRRC will supply respirable ($\leq$ 20 μm) dust samples for human evaluation studies by M. Buck and high performance liquid chromatographic (HPLC) analyses of aqueous extracts of these dusts and bracts. G. Baker, Mississippi State, and S. Anthony, at the U.S. Cotton Ginning Laboratory, Stoneville, MS, have furnished innumerable dust and plant material samples for examination in our program.

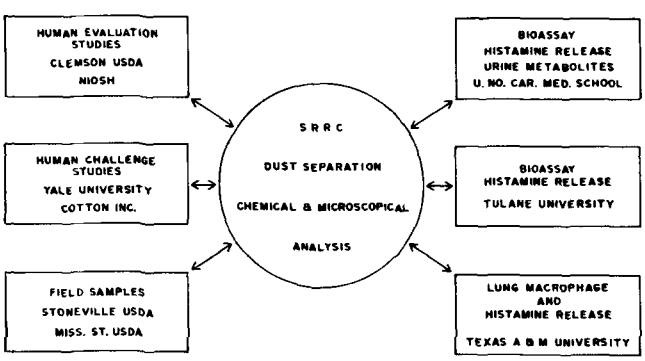

FIG. 1. Lines of communication and cooperation in cotton dust research.

This extensive cooperation in cotton dust research efforts has been concerned primarily with dust from the cotton textile industry. Information on the composition and physiological activity of cottonseed oil mill dusts is scant because the lung dysfunction associated with the dust in the cotton textile industry is not prevalent among workers in cottonseed oil mills. Among the publications that consider cottonseed oil mill dust, Noweir et al. discussed data on organic and ash content (16); Jones et al. detected a low prevalence ($\leq$ 6.5%) of chronic airways diseases among 172 employees of South American cottonseed oil mills (17); Matlock et al. reported total and respirable dust levels of southern Texas cottonseed oil mills (18); Brown et al. determined the proximate and inorganic compositions of Texas cottonseed oil mill dusts (19); and more recently, O'Neill et al. found 40 different antigens in the aqueous extracts of cotton textile mill dust and showed that cottonseed oil mill workers have specific IgG antibodies against at least 2 of these antigens (20). Further investigations are being conducted in an effort to correlate the antigen/antibody reaction of cotton textile mill and cottonseed oil mill workers.

In this report, we discuss the types of analyses used to characterize cotton textile dust, washed cotton and cottonseed oil mill dust, and attempt to discern the subtle differences in the nature of the dusts by using the energy-dispersive X-ray (EDX) techniques.

MATERIALS AND METHODS

Methods for collecting the high-volume air samples and recovering the dusts from the filters were described previously (18,19). Ash was determined gravimetrically after decomposition at 750 C in a muffle furnace. Elemental analyses were resolved by X-ray fluorescence (19). Other methods used in this study have been described previously: microelemental analyses by scanning electron microscopy (SEM) and EDX (21-23); endotoxin analyses by the Limulus crab assay (5); and dust extract analyses by HPLC methods (24-27).

RESULTS AND DISCUSSION

Table I shows typical proximate analyses data on dust collected during human evaluation studies of closed-boll cotton in the model card room at Clemson, SC, in 1978. Composition of Stoneville normal dust was typical, whereas dust from bract-removed, or bract-intact, closed-boll cotton shows decreases in ash content, water solubles and noncellulose organics, and a corresponding increase in cellulose content. However, values for the Lubbock cotton were atypical. Ash contents of Lubbock bract-removed, or bract-intact, closed-boll cotton were lower than those of the normally harvested cottons, but water solubles, noncellulose organics, and cellulose remained unchanged. Endotoxin levels showed little variation except for the bract-intact Lubbock sample which had a significantly higher level (35.7 ng/mg sample). Proximate analyses of cottonseed oil mill dusts obtained from linter and cleaning rooms of 2 Texas cottonseed oil mills are also shown in Table I. Although multiple-range tests show significant differences between individual cotton textile dusts and cottonseed oil mill dusts, variability in the textile dust and oil mill samples, respectively, preclude the use of proximate analysis data as a method of distinguishing between the two.

Table I also shows some elemental analyses of these same dusts. Of particular note are the values for silicon (Si). In the Stoneville cottons, the Si values are less for closed-boll cottons than for normal cotton. In contrast, the Lubbock closed-boll cottons showed no change from the normal Si values to the bract-removed samples, but bract-intact cotton showed an almost 2-fold increase over the normal. Lubbock closed-boll cottons gave quite different results in lung function tests than the Stoneville cotton. Lung dysfunction increased among patients exposed to Lubbock closed-boll cotton. Additional analyses of dusts from the more recent human evaluation studies will show whether these data are related. Elemental analyses obtained for the cottonseed oil mill dusts shown in Table II were of the same order of magnitude as the cotton textile dusts. Again, multiple-range tests show no significant differences between cotton textile and cottonseed oil mill dusts, except for the oil mill dust from cleaning room A which contained much larger quantities of Ca, Mg and Si (which are probably soil-related). Elemental analyses of cottonseed oil mill dust from Texas and Arkansas (unpublished data) show considerably more Al, Ca and Si in the Texas dust (also presumably soil-related). Growing location and variety reportedly have significant effects on dust characteristics (28,29).

Data on cotton and washed cotton obtained from Cotton, Inc., are shown in Table II. The high cellulose and low noncellulose organics contents suggest the absence of a great deal of vegetative matter even in the control cotton. Comparable data from 2 bract samples indicate the high amounts of ash, water solubles and noncellulose organics normally found in bract. Endotoxin analyses show that the unwashed cotton had a high gram-negative bacterial count with a small amount of endotoxin. However, even though the gram-negative bacterial count of the washed cotton had been greatly reduced, the endotoxin level remained unchanged.

Perhaps one of the most important conclusions from the washing studies is that dust concentrations emitted from cotton processed in the model card room have been significantly reduced (30).

Table III shows elemental composition obtained by X-ray fluorescence of parts of 15 cotton plants obtained from across the U.S. The raw data on these selected elements helped us discover the importance of the calcium/potassium (Ca/K) ratio, which is given in Table IV. Tables III and IV include seedcoat values not previously reported, and further supports the use of the Ca/K ratio for distinguishing between these important plant parts.

Figures 2 and 3 are micrographs of bract and pericarp with EDX maps of their K and Ca. The spectra in Figure 4 compare the relative intensities of elements in the bract and pericarp particles and indicate significant variations in Ca and K contents.

Dusts from a cottonseed oil mill (Sweetwater cleaning room high-volume sampler, and Sweetwater cottonseed dust from a tumbler box) were examined by SEM and EDX analyses in an effort to determine the source of individual dust particles. The tumbler box was built by C. Parrell, Jr., Texas A&M University, for removal and collection of dust particles from cottonseed samples. The SEM-EDX procedure previously developed for study of dusts collected in cotton textile mills was used (22,23).

In the cotton textile mill study, cotton plant parts were

TABLE I

Proximate Analyses of Dust from Closed-Boll Cotton[a] and Cottonseed Oil Mills[b]

	Composition (%)								
Closed-boll cotton[c]	Ash	Water solubles	Noncellulose organic	Cellulose	Ca	Mg	Si	Al	Cl
Stoneville normal	14.5	13.1	30.9	29.9	2.0	0.4	2.3	0.9	0.1
Stoneville bract removed	3.0	7.2	13.3	68.0	0.6	0.2	0.2	0.1	0.2
Stoneville bract intact	4.2	10.0	18.1	61.2	1.5	0.5	0.5	1.8	0.5
Lubbock normal	16.0	6.3	19.2	55.3	1.2	0.4	5.9	1.2	0.1
Lubbock bract removed	3.8	9.4	21.4	55.7	1.1	0.4	5.8	2.2	0.2
Lubbock bract intact	6.6	9.2	17.7	57.4	1.1	0.5	9.1	3.0	0.2
Oil-mill dust									
Linter room A	10.3	9.8	29.6	44.6	2.0	0.6	2.4	ND[d]	0.04
Cleaning room A	20.6	6.6	16.2	48.3	3.4	1.2	7.1	0.3	0.03
Linter room B	10.3	12.0	33.5	35.0	1.8	0.6	3.2	ND	0.11
Cleaning room B	11.8	9.0	20.3	50.2	1.8	0.6	4.8	0.3	0.10

[a]Used in human evaluation experiments in the model card room at USDA-Clemson facilities 1978.

[b]Collected by C. Parnell, Jr., Texas A&M University, using a Hi-Vol sampler.

[c]The lipopolysaccharide (LPS) contents of these cottons ranged from 12-36 ng/mg dust.

[d]Not detectable.

TABLE II

Proximate Analyses of Cotton, Washed Cotton and Cotton Bract[a]

Sample	Composition (%)			
	Ash	Water solubles	Noncellulose organic	Cellulose
Unwashed cotton[b]	1.2	1.5	6.0	85.0
Washed cotton[c]	0.3	0.4	4.4	89.0
Bracts (Acala SJ-2)[d]	5.6	21.4	51.2	11.7
Bracts (Stoneville 256)[e]	12.3	30.0	38.3	13.9

[a]Samples supplied by P. Sasser (Cotton, Inc.).
[b]1,100 colony-forming units (cfu)/mg sample; 1.7 ng/mg LPS sample.
[c]33 cfu; 1.7 ng/mg LPS sample.
[d]Picked at Raleigh, NC.
[e]Picked at College Station, TX.

characterized by studying the relative content of elements present in individual particles of plant parts collected from a mature cotton plant. As shown in Table IV, leafy materials (leaf and bract) had a greater amount of Ca than K, whereas hard materials (pericarp, stem and seedcoat) contained more K than Ca. In addition, small amounts of Mg, Al, Si, S and Cl were detected by elemental mapping. Generally, the elemental content of leafy materials was greater than that of hard materials.

When dusts collected in cotton textile mills were studied, particles of both the leafy and hard materials were found, as well as smaller quantities of mineral salts such as silicates. Elemental signals from these mineral salts are much stronger than those from the biological material and are therefore easily distinguishable in the dust sample from the biological material.

TABLE III

Mean Chemical Composition of Cotton Plant Part Dusts from 15 U.S. Cotton Plants[a]

Element	Composition (%)				
	Leaf	Bract	Stem	Pericarp	Seedcoat
Magnesium	1.30	1.01	0.31	0.33	0.08
Aluminum	0.32	0.15	0.13	0.31	0.04
Silicon	0.75	1.19	0.10	0.40	0.08
Sulfur	1.25	1.12	0.24	0.34	0.26
Chlorine	1.13	1.09	0.64	0.65	0.11
Potassium	1.09	2.05	1.75	2.94	1.42
Calcium	4.11	3.37	0.95	0.37	0.22

[a]Values from X-ray fluorescence.

TABLE IV

Calcium and Potassium Contents of 15 U.S. Cotton Plants[a]

Plant part	Calcium (%)		Potassium (%)		Ca/K
	Mean	Standard deviation	Mean	Standard deviation	
Leaf	4.11	0.86	1.09	0.49	3.77
Bract	3.37	1.02	2.05	0.97	1.64
Stem	0.95	0.41	1.35	0.84	0.70
Pericarp	0.37	0.16	2.94	0.96	0.12
Seedcoat	0.22	0.09	1.42	0.37	0.15

[a]Values from X-ray fluorescence.

Elemental analysis by X-ray flourescence spectroscopy of dusts collected in a cottonseed oil mill showed Ca contents to be higher than those expected in dusts that should have a greater content of seedcoat materials than leafy materials. Therefore, dusts from cottonseed oil mills were examined by SEM-EDX analyses to determine whether dust particles present were similar to those found in the study of individual cotton plant parts, and whether a reason for the high Ca content of the samples could be found.

The cottonseed oil mill dust samples that were examined had a high linter content. As much of this fibrous material as possible was removed from the samples to prevent complication in mounting and microscopic examination; the remaining dust was mounted on carbon planchets for SEM-EDX study. The samples were scanned and 2 types of obviously crystalline materials were found. One type was a flat platelet that exhibited only an Si peak on the EDX spectrum and for which only Si could be mapped (Fig. 5). The other crystalline particle was hexagonal, and produced K, Al and Si maps (Fig. 6). A third material that did not have any regular crystalline shape exhibited only a Ca peak (Fig. 7). As the EDX process used does not identify carbon, nitrogen, or oxygen, further study will be necessary for exact crystalline identification of these materials. However, they are present in very high quantities in relation to the amount of organic plant material present, and thus explain the higher-than-expected Ca quantities present in the cottonseed oil mill dust samples, as well as the very high Si contents.

Observation of the biological materials present indicated that they were generally more similar to the hard plant tissues than the leafy materials. Very fine particles of the

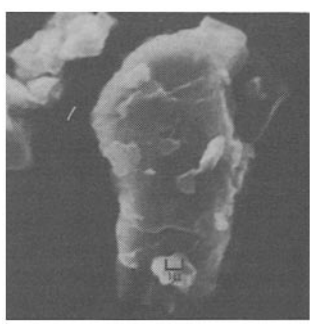

FIG. 2. (A) SEM of bract dust, (B) EDX potassium map, and (C) EDX calcium map.

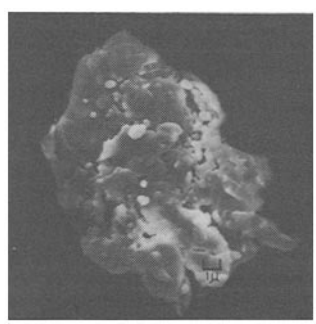

FIG. 3. (A) SEM of pericarp dust, (B) EDX potassium map, and (C) EDX calcium map.

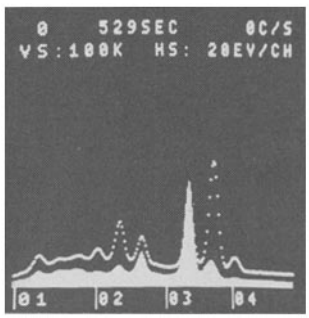

FIG. 4. Comparison of elemental intensities in pericarp (solid) and bract (broken line).

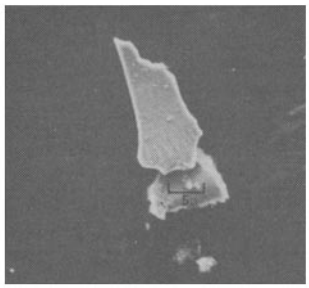

FIG. 5. (A) SEM of flat platelet in cottonseed oil mill dust and (B) EDX silicon map.

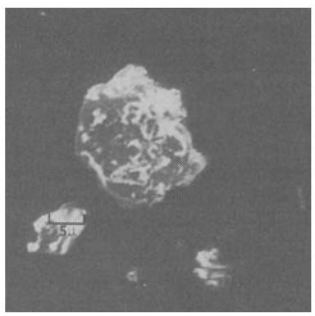

FIG. 6. (A) Hexagonal particle from cottonseed oil mill dust, (B) EDX potassium map, (C) EDX aluminum map, and (D) EDX silicon map.

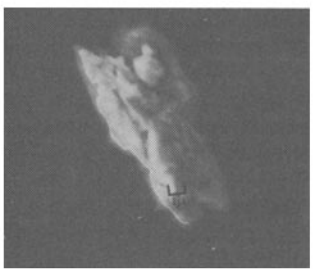

FIG. 7. (A) Particle from cottonseed oil mill dust and (B) EDX calcium map.

crystalline inorganics were often deposited on the surface of the organic particles, making a sharp elemental profile of the particle itself hard to obtain. Examination of a larger number of samples is necessary to determine the amount of leafy materials present, but from these results it appears to be low.

At the heart of our analytical research on cotton dust, dust extracts and body fluids is the HPLC analysis. Previously, we concentrated on separation, identification and quantitation of lacinilene C 7-methyl ether (LCME) from extracts of cotton textile dust (Fig. 8) (24-26). Recently, the HPLC quantitation of lacinilene C (LC) and LCME present in ether-Soxhlet extracts of cotton plant parts was undertaken as a possible assay of the amounts of vegetative matter present in cotton dust (31). Using this method, analysis of an ether-Soxhlet extract of a Sweetwater high-volume cottonseed oil mill dust from cleaning room A showed the presence of 77 μg/g dust of LCME and no detectable LC. These results correlate closely with the findings of the HPLC analysis of the ether-Soxhlet extract of cottonseed (92 μg/g dust, LCME, no LC) and would suggest that the vegetative matter present in the cottonseed oil mill dust is predominantly from the seed.

We are, at present, concentrating on 2 areas of research. The first area involved an HPLC method to quantitate histamine present in aqueous extracts of cotton textile dusts and cotton plant parts for possible use as a bioassay (27). This study will be expanded to encompass quantitative HPLC determinations of histamine released in minced lung tissue upon exposure to physiologically active extracts and/or compounds for bioassay. The second is an attempt to simultaneously analyze histamine and one of its major metabolites, 1-methylimidazole 4-acetic acid, in urine

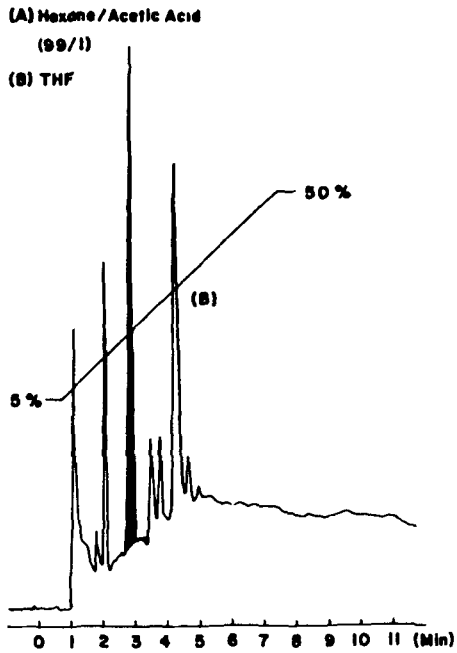

FIG. 8. HPLC profile of ether extract of cotton dust.

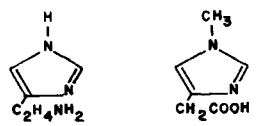

FIG. 9. Structures of histamine and 1-methylimidazole-4-acetic acid.

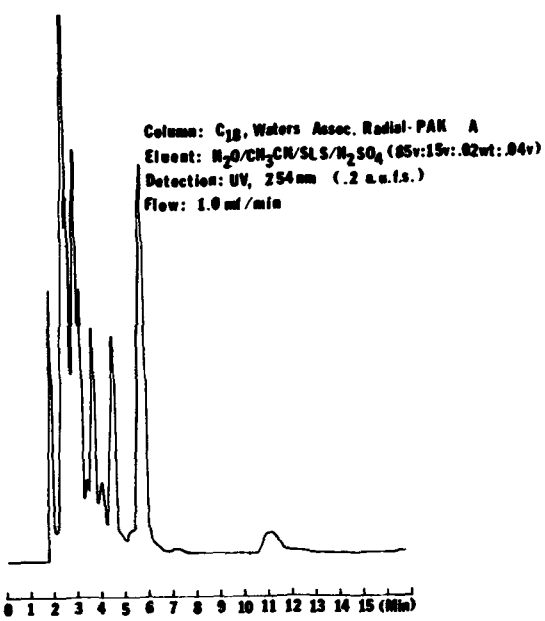

FIG. 10. HPLC profile of urine sample from a subject who reacts to cotton dust.

(shown in Fig. 9). The second area of research is respresented by Figure 10 which is an HPLC trace of a urine sample obtained from a subject who had experienced a sharp drop in lung function after exposure to cotton dust.

The profile indicates a large variation in catecholamines compared to urine obtained before exposure to cotton dust. However, many more samples must be analyzed to determine if these changes are related to byssinosis.

Pertinent information can be gained from cotton textile dust and cottonseed oil mill dusts, dust extracts and urine analyses. This information, especially data obtained from the human exposure, could help elucidate the causes of byssinosis.

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