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Microbial Flora and Fauna of Respirable Grain Dust from Grain Elevators

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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ABSTRACT

Whidden, W. P., E. B. Smalley, R. W. Caldwell, J. K. Phillips, S. H. Mai, and W. E. Burkholder. 1980. Microbial flora and fauna of respirable grain dust from grain elevators. Final Report - NIOSH Contract 210-77-0150.

The importance o fungus spores, bacteria, mite and insect body parts and pesticide residues in grain elevator dust as causal agents in chronic, non-specific lung disease has not been well defined. Respirable dust samples (<10μm) obtained with personal air samplers taken during standard work shifts for more than 250 individual workers in eight different grain elevators in the Duluth-Superior area were subjected to detailed microbial and entomological analysis. The total number of fungal or bacterial propagules/m^3 of sampled air varied widely between samples. Viable individual sample counts as high as 3.4×10^5 propagules/m^3 were recorded. When bacteria and yeasts were excluded, the highest viable individual count recorded was 9.4×10^4 propagules/m^3. Viable counts for Duluth city workers were one-sixteenth the maximum encountered for grain workers. The predominant fungi present in respirable grain elevator dust as determined by viable counts were species of Penicillium and Aspergillus. Aspergillus glaucus was omni-present and outnumbered the other Aspergilli and Penicillia by a wide margin. Pencillium cyclopium was the most frequently encountered species of Penicillium. Direct (non-viable) fungal propagule counts for grain worker samples ranged up to 3.6×10^6 spores/m^3 while city worker samples ranged up to 1.4×10^5 spores/m^3 of air. Grain worker samples also had high concentrations of Ustilago (smut) spores ($2.5 \times 10^5/m^3$). while city worker samples contained none. The eight elevator environments could be ranked in terms of their relative contamination levels. Two were "cleaner" and had mean total viable spore concentrations of less than 1.0×10^4 propagules/^{3}m of sampled air. In the others, viable means exceeded 1.0×10^4 propagules/m^3 of sampled air with the "dirtiest" elevator exceeding 7.6×10^4 mean propagules/m^3. Elevators ranked in approximately the same order when compared by fungus species or by groups of species. In one of the more heavily contaminated elevators, viable means for bacteria and yeasts combined exceeded 6.4×10^4 propagules/m^3; none of the other elevators had means for yeasts and bacteria combined that exceeded 2.6×10^4, and four were less than 1.0×10^4. Major differences were less apparent when mean fungal and bacterial counts were contrasted with the various job categories from all elevators. "Annex workers," "equipment operators," and "laborers" as groups were exposed to the "highest" overall spore concentrations. Residues from pure cultures of Penicillium cyclopium, P. viridicatum, P. urticae, Aspergillus flavus, A. nidulans, and A. fumigatus, the most commonly encountered fungi in grain dust, were lethal to incubated weanling rats. The major fungi present in respirable grain dust were clearly mycotoxin producers, and no doubt contributed to the health problems of grain elevator workers. Very few whole insects or insect fragments were present in the bulk grain samples collected from the various elevators. No insect species predominated, and most of the insect fragments belonged to general in the orders Orthoptera and Coleoptera. The few whole insects detected were mostly common storage insects (Sitophilus granarius, Rhizopertha dominica) but no indication of heavy insect infestation was evident. Of the few settled

dust samples containing insects (7%), most contained larvae of the yellow mealworm (*Tenebrio monitor*).

KEYWORDS

Grain dust, microbial flora, fungi, bacteria, yeasts, *Penicillium*, *Aspergillus*, *Cladosporium*, *Ustilago*, grain insects, *Sitophilus*, *Rhizopertha*, *Tenebrio*.

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I. INTRODUCTION

Airborne and settled dust derived from grain elevators or storage/transfer installations is a heterogenous substance consisting of particles abraded from cereal grains, soybeans, sunflower seed. Large numbers of fungus and actinomycete spores are present as well as bacterial cells; pollen grains; various living mites; insects or their body parts; mammalian debris; and various chemical additives such as pesticides and herbicides (Kowlowski, 1972; Krantz, 1970; Farant, et al., 1973; Christensen, 1970; Cotton and Soman, 1978a & b; Chan-Yeung, et al., 1978). The concentration of suspended dust may be very high. In one study of grain elevators in Canada, Farrant and Moore (1978) reported levels ranging from 0.18 to 781 mg/m^3 of air. In spite of the fact that many existing grain elevators are old and lack adequate dust control equipment, the technology does exist to control dust to a short-term mean.average concentration for total dust of 10 mg/m^3 (Farant and Moore, 1978).

Prolonged exposure to grain dust has long been associated with respiratory disorders. Ramazzini (1713) described conjunctivitis, rhinitis, cough, shortness of breath, and cachexia as being common among grain sifters and measurers. Blackley (1880) incriminated the spores of Penicillium as sensitizing agents in severe bronchitis following inhalation of air from Penicillium-infested straw. Wittich (1940) suggested that smut allergy and grain dust allergy were closely related, but did not have the same allergens. Fawcett (1938), Harris (1939), and Ordman (1958) also implicated fungi present in cereal grain dust as the cause of respiratory and skin allergies.

Three major respiratory diseases have been attributed to grain dust inhalations: asthma, chronic bronchitis, and grain fever (Cotton and Dosman, 1978a). In the past silicosis and extrinsic allergic alverolitis (farmer's lung) have also been considered dust related health problems of grain elevator workers. However, in recent studies of grain industry workers, indications of alveolar damage and fibrosis characteristic of silicosis have been absent (Warren, et al., 1974; Davis, et al., 1976, do Pico, et al., 1977). In fact only a single confirmed case of silicosis has been reported which involved a grain elevator worker (Heatley, et al., 1944).

Farmer's lung, while usually associated with moldy hay, may also be associated with grain that has undergone heating (Dennis, 1971). However, the symptoms of farmer's lung, which include alveolar damage and diffuse nodules, have been absent in the most recent studies of grain workers (Warren, et al., 1974; Davies, et al., 1976; do Pico, et al., 1977). Therefore, silicosis and farmer's lung do not now appear to be major problems in grain workers.

The prevalence of grain dust asthma in grain workers has been difficult to estimate. Chan-Yeung, et al. (1978) and Warren, et al. (1974) have reported both immediate and delayed asthmatic reactions with bronchial challenges of grain dust extracts. However, only a relatively low percentage of workers examined had such asthmatic reactions. It has been suggested that those new workers experiencing asthmatic reactions left the job soon after the onset of symptoms to take other less hazardous employment. My personal delayed asthmatic reaction following a one-day tour of a Duluth-Superior grain

elevator would support this conclusion (Smalley, 1979). Therefore, it can probably be concluded that the number of long-time grain elevator workers with job related asthma is very low, since they really constitute a surviving population (Chan-Yeung, et al., 1978). While asthma may not be a major contributing factor to chronic lung disease in the surviving population, asthma attacks are occasionally observed in some workers and could be a potential hazard in others (Cotton and Dosman, 1978a).

Chronic bronchitis constitutes a major occupational health problem in grain industry workers. Surveys indicate that from 1/3 to 1/2 of those grain workers polled complained of a chronic, "productive" cough (Chan-Yeung, et al., 1978; do Pico, 1978; Cotton and Dosman, 1978a). Smoking proved to be a major contributing factor to this incidence of chronic bronchitis and bronchial obstruction (Sheridan, et al., 1978; do Pico, et al., 1978; Cotton and Dosman, 1978a). In one study, chronic bronchitis was 22% more prevalent in smokers than non-smokers who worked in grain elevators (Chan-Yeung, et al., 1978).

The occurrence of grain fever, the other major occupational health problem, is more variable. In one study, Williams, et al. (1964) reported an overall incidence of about 6% of grain elevator workers; later studies, however, indicated incidences varying from 19% to 40% (Muica and Teculescu, 1977; do Pico, et al., 1978). Grain fever is a febrile illness associated with dyspnea which occurs several hours after the grain workers leave the work environment (Chan-Yeung, et al., 1978). Additional symptoms such as an increased prevalence of grain fever after time away from work have also been attributed to grain fever. Such symptoms indicate a similarity to metal fume fever and byssinosis (Skoulas, et al., 1964). However, these latter symptoms have not been observed by other investigators (do Pico, et al., 1978). The exact cause of grain fever is unknown, but is currently the topic of intensive research (do Pico, et al., 1978).

Direct irritant effects, toxic reactions, and allergic responses have been suggested as the three causal mechanisms for occupational lung diseases observed in grain workers. Irritant receptors have been demonstrated in airway mucosa, which respond to stimulation by increasing mucous secretions (do Pico, 1979). Chronic non-specific stimulation of these receptors by grain dust may play a role in the pathogenesis of grain dust disease (Cotton and Dosman, 1978a). Alternatively, these changes may be due to a toxic reaction involving the complement system. Grain dust has been demonstrated to activate complement by the alternate pathway in vitro (Olenchock, et al., 1978b). Complement activation by grain dust was shown to be dose dependent and this suggested a capability to incite inflammatory sequelae in the lung, which could result in the respiratory problems in grain workers. Additionally, endotoxin has been detected in grain dust, which might also contribute to complement activation (Olenchock, et al., 1978a). Toxic compounds such as residual pesticides, grain fumigants and mycotoxins may also play a role in these problems, but their role has not been elucidated (Cotton and Dosman, 1978b).

Some evidence exists which indicates that allergic responses may produce grain fever and asthmatic-like reactions in grain workers. Grain mites,

Glycyphagus destructor and Glycyphagus domesticus, and the grain weevil, Sitophilus granarius, have caused positive skin reactions in a farmer who suffered with grain dust fever (Davies, et al., 1976). When challenged with S. granarius, millworkers showed reduction in FEV_1 (forced expiratory volume in one second) (Lum, 1966). Since the grain weevil and mites are antigenic, they could play a role in grain dust related diseases.

Bacteria have also been implicated in allergic responses in grain workers. Grain handlers with respiratory symptoms in Poland showed a higher incidence of positive precipitation and skin reactions to Erwinia herbicola than asymptomatic workers (Dutkiewicz, 1978b). Tests with Micropolyspora faeni and Aspergillus fumigatus gave much fewer positive reactions. Since a highly significant correlation existed between skin reactions to grain dust and E. herbicola, it should be considered a factor that increases the risk of respiratory problems in grain handlers.

Grain workers have been tested for allergic responses to numerous fungi. Workers harvesting grain may be exposed to as many as 20 million fungal spores/m^3 air (Darke, et al., 1976). Positive precipitin reactions were present in 64% of those workers tested, 43% of these also responded with positive skin reactions. Extracts from Paccilomyces fabrinosis, Aphanocladium album, and Verticillium lecanii caused reactions most frequently. When workers with skin reactions inhaled extracts of each individual fungus and a mixture of all three, all felt a tightness in their chests and their FEV_1 significantly decreased (Darke, et al., 1976). Commercial extracts of Alternaria, Aspergillus, Candida, Fusarium, Penicillium, yeast, rust, and smut, however, produced few if any positive skin reactions on grain workers (Warren, et al., 1974; do Pico, et al., 1977; Chan-Yeung, et al., 1979). However, Skoulas, et al. (1964) found 78% of grain handlers with respiratory symptoms had positive skin reactions to a mixed extract of Aspergillus, while 36% without symptoms also reacted; 43% with symptoms reacted to mixed Penicillium extracts but only 14% without symptoms reacted. With the exception of the study by Darke, et al., (1976), extracts have not apparently been made from species of fungi actually isolated from the work environment. A mixture of several fungus species in general common to cereals may not actually contain the antigens to which the workers were exposed. Further tests need to be conducted using fungi isolated from the grain workers' particular environment in order to determine the role of fungi in grain dust disease.

The microbial flora in grain elevators is diverse, and is in part a reflection of the type of grain being handled, its original growing conditions, and the prevailing storage practices. Some organisms contaminate the grain in the field while others are acquired during storage. Field fungi are those that invade or contaminate the developing or mature seed while it is still on the plant (Christensen, 1957). These include both parasitic and saprophytic species. Major saprophytic general of field fungi present in non-weathered grain include Alternaria, Fusarium, and Helminthosporium. Weathered seed may also be invaded by Cladosporium, Diplodia, Chaetomium, Rhizopus, and Absidia. Penicillium oxalicum, P. funiculosum, and in lesser numbers, P. cyclopium, are consistently isolated from freshly harvested corn (mislivec and Tuite, 1970). The more virulent parasitic fungi encountered are mainly species of rusts (Uredinales) and smuts (Ustilaginales). Bacteria

often form 90-99% of the microflora of freshly harvested grain (Wallace, 1973). Fifteen plant pathogenic species of bacteria have been associated with cereal kernels. Sixty-four other saprophytic bacterial species have also been detected on cereal grains or their commercial by-products. Some of the more common bacteria detected include Erwinia herbicola, various Pseudomonas spp., Bacillus spp., and Streptomyces (Wallace, 1973). However, not all field organisms actually reach the storage facilities. More than 90% of the epiphytic microflora can be expected to be removed during routine grain cleaning operations (Dutkiewicz, 1978a). Additionally, most field fungi do not survive very long after exposure to dry storage conditions. Fusarium and Helminthosporium may not be recoverable from only a small percentage of infected seeds after being stored for a year. Even in heavily invaded barley, most field fungi can be killed by storage at 14% moisture content and 20-25C for several months (Christensen and Kaufmann, 1965). Therefore, surviving field flora on grain in storage varies greatly in response to many factors.

Storage fungi are those organisms that generally develop on or in grain at relatively low moisture content after being stored for some time (Christensen, 1957). Under normal storage conditions, Aspergillus and Penicillium species then become the predominant organisms. Grain that is moist or has been heated often contains Aspergillus fumigatus, Micropolyspora sp., Thermoactinomyces vulgaris, Candida sp. and other yeasts, Mucor pusillus, and Absidia spp. The species of Aspergillus common to stored grains include: A. restrictus, A. glaucus (group), A. candidus, A. versicolor, A. ochraceus, A. flavus, and A. halophililcus. Penicillium species have often been lumped into one group rather than being counted individually because of the difficulty in their identification. However, Mislivec and Tuite (1970) identified the species of Penicillium from both freshly harvested and stored corn kernels. The most frequent, consistently occurring species on stored corn in their studies proved to be P. cyclopium, P. brevi-compactum, and P. viridicatum.

From the few studies available, the microflora of grain dust includes some of the wide variety of microflora of grain, as might be expected. Again, the type and condition of the grain determines in large part the diversity of species present. Williams, et al. (1964) isolated Penicillium oxalicum, Mucor sp. and Rhizopus sp. from settled grain dust in elevators handling mainly wheat, oats, or barley. However, Aspergillus flavus was isolated only from those elevators handling barley or oats; Penicillium chrysogenum, Aspergillus glaucus, and Mucor racemosus only from those dealing in wheat or barley, Rhizopus nigricans from elevators handling wheat or oats; and Aspergillus fumigatus and Aspergillus repens from those handling wheat. Thus, the species isolated can be expected to differ depending upon the grain being handled.

Similar findings were reported by Farant and Moore (1978) from grain elevators in Canada. The mean, range, and frequency of species encountered were higher if barley was being handled as compared to wheat. They also suggested that seasonal variations in weather affect the number and species of fungi present as well as increasing the possibility of heating during storage following very wet seasons. Thirty species of fungi in 15 genera were isolated from 113 samples. Penicillium expansum was found in 69% of the samples. Aspergillus repens and Hormodendrum cladosporiodes in nearly 45%, and Alternaria in 29%. Ustilago spores occurred in 88% of the samples when

barley was handled, but was only in 69% when wheat was handled (Farant and Moore, 1978). Additional differences existed in the numbers of fungus spores detected at various locations within the elevators. The dust control measures and level of activity at the individual sites seemed to affect the dust level and therefore the number of spores (Farant and Moore, 1978).

In the air of grain elevators in Poland, bacteria were the predominate micoorganisms present. Counts averaged 50 to 100 thousand Erwinia herbicola propagules/m^3 of sampled air and 10 to 50 thousand Staphpylococcus epidermidis and Streptomyces sp./m^3 pf sampled air in the elevators. Fungi were less numerous. Aspergillus glaucus, Aspergillus fumigatus and 30 other fungi species averaged less than 10,000 spores/m^3 of sampled air (Dutkiewicz, 1978a).

Our report contains the results of a detailed analysis of fungi, thermophilic actinomycetes, bacteria, insects, and mites in the dust from grain elevators or storage transport facilities of the Duluth-Superior area collected in October and November, 1977. The primary purpose of the study was to identify and quantify the organisms in grain dust. Additionally, the major organisms identified were examined for their ability to produce toxic substances when grown in pure culture in the laboratory. These studies were carried out to provide technical support, as a separate and autonomous study, to National Institute for Occupational Safety and Health (NIOSH) contract (No. 210-76-0175) on the prevalence of chronic, nonspecific lung diseases and related health problems in grain elevator or storage-transfer installations associated with maritime shipment in the Superior-Duluth area of the United States. This study was carried out by the staff of the Department of Preventative Medicine at the University of Wisconsin - Madison in cooperation with NIOSH personnel.

II. MATERIALS AND METHODS

A. Sources of Samples and Sampling Procedures

1. Shift Study Samples and Controls. - Eight-hour grain elevator worker shift dust collections were obtained during the related Phase II - Work Related Study of NIOSH Contract #210-76-0175. Grain worker-environment sampling, conducted by NIOSH personnel was begun on October 3, 1977 and concluded on November 29, 1977. Air sampling procedures utilized a worker-borne portable air sampling pump equipped with two samples/participant (one for total dust and one for respirable dust KL[10 µm]). Volunteer participants were primarily day-shift personnel (0800-1630) although approximately 20 night-shift (1600-2330) workers also cooperated. Participating workers were employed by various Duluth-Superior terminal grain elevator companies including: Archer - Daniel - Midland (ADM), Cargill, Continental Grain Elevator Co., Farmers Union (G.T.A.), General Mills, Globe (Peavy), International Multifoods (Capital), McMillan and Osborne (M & O), or by the States of Minnesota or Wisconsin. Their job descriptions ranged from Weighers, Annex Workers, Laborers, Transport within Elevators, Maintenance, Grain Inspectors, Equipment Operators or Supervisors.

Respirable, personal dust samples were collected utilizing an air sampling train consisting of a 10-mm nylon Bendix Cyclone Vortex respirable-dust lapel sampler assembly (BDX-99, Bendix Corporation, Baltimore,

MD) connected to a personal air pump by a 60.96 cm (2 ft.) length of 0.635 cm (1/4 in.) dia. tygon tubing. Each pump (BDX-44, Bendix Corporation) was calibrated to provide an air sampling flow rate of 1.7 $\pm$ 0.1 l/min. Personal total dust samples were collected in the same manner as the respirable dust samples except that the cyclones were not used. Pump flow rates for all total dust samples were approximately 2.0 $\pm$ 0.1 l/min.

The dust samples were collected on pre-weighed Gelman Metricel^{R} DM-800 (37mm dia., 0.8 µm pore size #64530) membrane filters supported on a Gelman absorbant pad (pure cellulose fiber #6474) in a two-piece polystyrene filter cassette holder (Gelman field monitor #4338) (Gelman Instrument Co., Ann Arbor, Mich.), sealed after weighing and assembly with cellulose "shrink" bands (#625415, Mine Safety Appliance Co., Pittsburgh, Pa.). Final results are expressed in terms of mg/m^3 of sampled air.

Grain dust samples taken from October 3, 1977 to October 27, 1977 were collected on randomly selected, weighed pre-numbered (#1 to 194), gas sterilized filters (within their plastic housings). Gas sterilization was carried out with ethylene oxide in a standard hospital sterilizer (Model 4040, Castle Corporation, Chicago, IL). Because this procedure was suspected to have introduced problems in dust weight determinations and possible latent toxicity to microorganisms being collected (Tessler, 1961), which were greater than the need for initial filter sterility, all cassettes used after October 27, 1977, were prepared with non-sterile filters (Numbers>194) used as they came from the sealed manufacturer's package. After sampling, filter housing orifices were sealed to exclude unrelated contaminants and packaged for shipment in such a manner as to minimize disturbance of filter surfaces prior to analysis.

Upon receipt of the "hand" carried dust samples in Madison, the filters were removed from their housings, weighed on an electronic analytical balance (Sartorius Model No. 2605 precision 0.1 mg) Brinkmann Instruments, Inc., Westbury, N.Y.), cut in half, reweighed, and placed in separate sealed sterile vials. Filters were stored at 4C until processed. Laboratory sampled air controls were handled in the same manner.

Filter cassettes for the non-grain industry worker controls were prepared in a similar manner by NIOSH personnel at Morgantown, West Virginia, with the exceptions that the unused filter was pre-dessicated prior to weighing and following sampling was again dessicated prior to re-weighing. Following receipt in Madison the control filters were again weighed, cut in half, reweighed and stored as previously indicated. Ten percent of the filters used on a sampling day were used as controls and treated identically to sampling filters except no air was drawn through the filter.

2. Causes of Sample Variability. - Several trials were carried out to determine the possible cause or causes of respirable grain dust weight variability (particularly the occurrence of occasional negative weights) and to develop usefuly correction factors. Potential causes to be explored included: (1) residual water or gas changes during weighing, gas sterilization and sampling, (2) balance malfunctions, (3) human errors, or (4) combinations thereof. The major error seemed to be associated with the process of ethylene oxide sterilization, since 9% negative values were

observed with filters handled in this manner, while only 2.3% were negative in the non-sterile filters. In the first trial, 40 filters taken directly from the manufacturer's sealed package, were weighed, using a different and newer electronic balance (Mettler Type H16 0.1 mg precision, Mettler Instrument Company, Hightstown, N.J.). They were then placed in a dessicator over anhydrous $CaCl_2$ and after 2, 8, 9, 10, 11, 15, 21, 22, 23, and 24 days individual filters were removed from the dessicator and assembled in cassettes. Laboratory air was then drawn through the cassette for eight hours, as in the procedure for grain dust, and then the filter was reweighed. The remaining 20 filters were reweighed again after two weeks, and 10 of these were again reweighed after four months.

In the second more comprehensive trial, 100 filters and their accompanying cellulose back-up pads were numbered and weighed twice, once on the older Sartorius balance and again on the newer Mettler balance. Ten aluminum foil squares of varying weights (0.5 to 327 mg) and ten additional filters were double weighed each day as non-absorbent and untreated filter controls. Temperature and relative humidity were continuously recorded during the weighings. Filters and pads were then assembled into new two-piece cassettes and treated as follows:

1. Blue and red caps were inserted tightly into the front and rear air flow orifices of 40 randomly selected loaded filter cassettes. Treatments A and E). These cassettes were then stored in closed plastic bags while the other treatments were completed.

2. The 60 remaining filter cassettes (Treatments B, C and D) were then packaged in a large paper bag, closed and gas sterilized in a Castle Model 4040 hospital sterilizer (Castle Co., Chicago, IL), with ethylene oxide (Linde-Freon 88% Ethylene oxide 12% for 2 hours) followed by a 4-hour exhaustion period. The treatment was identical to that carried out previously with the grain dust filters.

3. Thirty of the ethylene oxide sterilized cassettes were then sealed with the blue and red caps and stored as above (Treatments B and C).

4. The remaining 30 sterilized cassettes (Treatment D) were then disassembled and the filters and pads reweighed (once on each balance). The cassettes were then reassembled and the blue and red caps inserted.

5. Cellulose shrink bands were placed around each cassette, allowed to dry for several days and then all cassettes (with each treatment in separate labelled polystyrene cassette holders), were shipped via United Parcel Service to NIOSH at Morgantown, West Virginia.

6. Eight-hour air samples (Morgantown air) were then drawn through cassettes in treatments C, D, and E, and all cassettes were returned to Madison for completion of the gravimetric analysis.

Following completion of the gravimetric analysis, representative filters were bioassayed for the presence of possible toxic residues resulting from the ethylene oxide treatment. Selected filters and accompanying pads from each of the treatments were plated on Penicillium cyclopium seeded (10^3 or 10^5 spores/ml) potato dextrose agar or plated and sprayed with conidia of P. cyclopium. Other filters were extracted individually in 25 ml of a sterile 0.1% solution of tergitol NPX (Union Carbide Chemical Corporation, N.Y., N.Y.), the volume reduced to 1 ml and assayed to toxicity against P. cyclopium using the filter paper disc method (Vincent and Vincent, 1944).

3. Settled Dust and Bulk Grain Samples. - In addition to the worker - environment respirable and total dust samples, we received settled dust samples as collected by NIOSH personnel in one new one-pint (0.473 1) paper-plastic milk cartons. These samples were derived from various surfaces at random locations within the eight cooperating grain elevator or storage/transfer facilities in the Duluth-Superior area. Similarly derived bulk grain samples contained in new quart (.946 1) milk cartons consisted of samples of barley, spring wheat, durum wheat, winter wheat, oats, corn, flax and sunflower seeds. Settled dust and bulk grain samples were stored at 6 C prior to entomological and/or microbiological analysis.

B. Microbiological Analysis of Grain Dust

1. Respirable and Total Dust Samples. - A detailed microbiological analysis was performed on aq total of 231 respirable dust samples collected as described from grain elevator workers; 168 other analyses were performed on samples obtained in a similar manner from non-grain industry workers (City of Duluth, Minnesota employees) as well as 10 from our laboratory taken periodically during the time the other filters were being processed; and 44 other filters sent to Duluth-Superior, but which for one reason or another were returned unused. Additionally, 38 total dust samples (most of the total dust samples collected were sent elsewhere for silica determinations) from grain elevator workers and 15 from non-grain industry workers were processed in the same fashion as the respirable dust samples.

The microbiological analysis of the respirable and total dust samples was carried out in three phases. (1) Qualitative and quantitative viable propagule counts of the microflora present on half filter samples were done using pure culture methods similar to those described by Christensen (1946) and Marases, et al. (172). (2) The identity and total number of fungal spores (living or dead) present per half filter were calculated from direct microscopic counts made from each filter sample using methods similar to those described by Farant, et al. (1973). Direct sample counts were important in determining the presence of obligate fungal parasites such as rusts (Uredinales) or smuts (Ustilaginales) which will not readily develop on common culture media. (3) Selected filters from 12 different workers were examined by Scanning Electron Microscopy (SEM) to reveal in greater detail the nature of the material comprising respirable dust in grain elevator environments.

(a). Viable counts. - The dust from each filter half for the viable count determinations was suspended in 25 ml of a sterile 0.1% aqueous solution of tergitol NPX by agitation with a vortex mixer for 30 seconds. From this stock solution various dilutions were prepared to provide at least one set of plates in which the resulting colonies were not overcrowded and could be counted with one colony representing each spore or propagule. Usually the

suspension was diluted to yield final concentrations of 1/100, 1/200, 1/300, and 1/400 when 0.5 ml was spread over the agar plate surface. Further dilutions were utilized in the case of heavily contaminated filters. Five different agar media were used:

1. Czapek agar amended with corn steep liquor, tergitol, and aureomycin (CS).
2. Acidified potato dextrose agar (PDA).
3. Malt salt agar (MS).
4. Pridham's yeast malt agar (A).
5. Tryptic soy agar (TS).

Constituents and media preparation details are given in the Appendix (Table I). Czapek and potato-dextrose agar were chosen for counting and identifying most of the common fungi encountered in grain dust. The addition of tergitol NPX to CS agar limited the spread of fast growing organisms such as Mucor and Rhizopus while allowing rapid identification of the Penicillia and Aspergilli. Aureomycin and lactic acid served to limit excessive growth of bacteria. Malt salt agar was selected to count those organisms that grew slowly on regular media but tolerated high osmotic stress. Pridham's A agar media was utilized for the isolation and counting of thermophilic and thermotolerant bacteria and fungi following incubation at 50aC, while Tryptic soy agar, a rich organic culture medium was used to count the mesophilic bacteria. Four replicates for all dilutions were prepared on each medium for each dust sample. Plates utilizing CS, PDA, or MS were incubated at room temperature (20-24C) for 7 days prior to counting. The A medium plates, in plastic bags, were incubated in the inverted position at 50C for 7 days, while the TS plates (Also inverted) required two days incubation at room temperature. The major fungi developing from the isolation plates were isolated in pure culture and stored at 4C in vials on sterile silica gel. Where necessary (particularly in the case of the Penicillia), further transfers were made to the most suitable culture media for positive identification to species.

The number of viable propagules (to genus and species) per half filter was calculated by averaging the colony counts in the four plates multiplied by the inverse of the dilution factor. The total number of viable propagules was calculated by multiplying the half filter count by the ratio of the weight of the entire filter to the weight of the half filter (these calculations were accomplished as part of the computer program). The total number of viable propagules/m^3 of sampled air was then derived by dividing the number of propagules/whole filter by the volume of air sampled during the workers' approximate 8-hour shift.

(b). Direct (non-viable) counts. - Spores of certain fungi, such as the results or smuts failed to grow on conventional culture media even though they might have been present in high concentration in the grain elevator environment. Likewise, many other fungal spores present in these environments may have been no longer viable. In order to include these in the counts of the numbers of microorganisms present in the sampled air, they were counted by direct microscopic observation. For these counts, the remaining half of the filter was washed with approximately 50 ml of 0.1% tergitol solution. The suspension was decanted and filtered through a Millipore filter (Aa, 0.8 µm

pore size, 37mm in diameter) (Millipore Corp., Belford, MA). The filter was allowed to dry overnight before coating it with 1% Lucite (methyl methacrylate) in chloroform. Five discs, 8mm in diameter, were cut from each filter, placed on microscope slides, and cleared by adding a drop of triacetain (glyceryl triacetate) before covering it with a cover slip (Farant, et al., 1973).

Five fields per disc were examined under 400 X magnification. The spores were counted, identified to genus and the average number of spores/field calculated. Multiplying this average by a factor of 8.1×10^3* fields/filter yielded the total number of spores present on the original half of the filter. The number of spores on spores/half filter was then multiplied by the ratio of the weight of the entire filter to the weight of the half filters (=spores/filter) (included in the computer program). This value was then divided by the volume of sampled air to yield the number of spores/^{3} of sampled air.

(c). Grain dust challenge studies. - Twenty-seven similar additional total or respirable grain dust samples were also subjected to microbial analysis by the viable counting procedure. Because of the extremely heavy dust concentrations collected on the filters, no direct spore counting procedure was possible with these filters. These samples were derived from three different worker challenge studies carried out under controlled conditions inf the U.W. Department of Preventive Medicine (under the direction of Dr. G. do Pico and associates). Dust collections were made with coal mine filter plastic cassettes equipped with polyvinyl chloride (PVC) 5 µm pore size filters (Mine Safety Appliances Co., Pittsburgh, PA, Model No. 457193) in a similar fashion to those used in the grain elevator dust studies. The dust utilized in the first trial was derived from fresh, finely milled cereal grains, and the other trials utilized pooled settled grain dust collected from five different elevators in the Duluth-Superior area. (See report of NIOSH Control No. 210-76-0175 for details on worker reactions to these challenges.)

(d). Scanning electron microscopy. - Filter surfaces to be examined by Scanning Electron Microscopy (SEM) were prepared from small portions of the filter halves used for dust counts. Filter pieces were oven dried at 50C for 4-5 hours without fixatives. Small dried filter pieces were then fixed to standard SEM mounting pegs with silver adhesive, critically point dried with CO_2 and coated with gold-pallidium (60:40). Specimens were examined with a JEOL JSM-U3 scanning electron microscope and appropriate fields photographed.

2. Settled Dust Samples. - A detailed microbiological analysis was performed on two samples from each of six elevators cooperating in the study. The number of viable organisms present in the settled dust samples was determined generally as described previously for the respirable and total dust samples. Because the dust had been collected in bulk in milk cartons, the

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*This factor (8.1×10^3) was derived by determining the area of a field at 400 X magnification (1.257×10.3 cm^2) and the area of the filter upon which the spores had been redispersed (6.36×10^{-1} cm^2). The number of fields/filter was then obtained by dividing the area of the filter by the area of a field.

data was expressed as numbers of organism/g of dust; 0.5 g of dust was resuspended in 50 ml of 0.1% tergitol NPX solution and diluted to obtain culture plates with less than 300 colonies/plate. Direct counts of spores in the settled dust were not made because microscopic fields prepared from this material contained large quantities of large size particles.

C. Pure Culture Toxicity Determinations on Small Animals

1. Toxin Production on Sterilized Rice. - Two strains from each of the nine most commonly encountered fungus species present in grain dust from this study were grown in pure culture on autoclaved rice to determine their potential for roxin production. Rice cultures were prepared by autoclaving 100g of Uncle Ben's converted rice (Uncle Ben's Foods, Houston, TX), moistened with 50 ml distilled water in 1-1 Erlenmeyer (or 300g rice with 150 ml distilled water in 2.8-1 Fernbach flasks) for 15 min. (15 psi), followed by fast exhaust to minimize browning. The cooled rice then was either seeded with 2-3 ml of sterile Tween 80 solution of prepared from one of the selected fungi. In general cultures were incubated at room temperature for two weeks prior to extraction. Additional flasks of rice inoculated with either the Penicillium cyclopium or Aspergillus versicolor isolates were incubated for one week at room temperature followed by two weeks at 12C. This procedure was used for production of low temperature metabolites known to be produced by these species.

2. Toxin Extraction. - 500 ml Erlenmeyer flasks with a ground glass stopper containing 100 g of fermented rice culture in 150 ml of ethyl acetate was agitated vigorously for 30 min. on a G10 Gyrotory^{R} rotary shaker (New Brunswick Scientific, Edison, N.J.) at room temperature. The ethyl acetate was then poured through coarse filter paper and the remaining rice residue was re-extracted with an additional 100 ml of ethyl acetate by shaking for another 30 min. Following filtration the ethyl acetate extract was combined with the firsts extract and evaporated by Buchi Rotovapor^{R} (Brinkmann, Westbury, NJ). The residue was then redissolved in a small quantity ethyl acetate, transferred to a 50 ml flask and dried under a stream of air. Dried rice culture extracts were stored at 4C until utilized.

Settled dust samples were extracted with ethyl acetate in much the same manner as the rice cultures. One hundred g of dust were extracted in 200 ml of ethyl acetate in the first extraction and in 100 ml of ethyl acetate in the second time. The combined filtrates were then evaporated to dryness on the rotovapor, the residue transferred to a small flask with additional ethyl acetate and evaporated to dryness under an air stream. All extracts were stored at 4C.

3. Toxin Administration to Rats. - To determine the possible presence of toxic products each extracted culture or dust residue was dissolved in 2 ml of absolute alcohol then mixed with 6 ml of corn oil (Mazola CPC International, Englewood Cliffs, NJ). Following evaporation of the ethanol from the residue under an air stream, 1 ml of suspension was administered orally via stomach tube to individual weanling Sprague-Dawley rats. Extracts from each rice culture, dust sample or control (corn oil) were administered to four rats (two male and two female). Following sample administration rats were returned to individual cages and received no food until the morning after treatment. Each

rat was weighed before the extract was administered, two days later, and again after five days.

D. Entomological Analysis of Grain Dust and Bulk Grain

A detailed entomological analysis was performed on a total of 144 settled dust samples and 246 bulk grain samples. Two weeks after their receipt, bulk grain samples were allowed to warm at room temperature for 24 hours. The contents of each sample carton was shaken on a No. 18 mesh sieve and then sifted material examined under a stereo-binocular microscope. Insects (living and dead) and insect parts were removed, identified is possible, and stored in labeled vials. Grain was returned to the original cartons, sealed and stored again for ca. 4-6 weeks at 25C and 70% R.A. After this period, the grain was again examined as described above with particular attention given to newly maturated living insects. Specimens were again placed in labeled vials.

Eight weeks after receipt of settled dust, samples were removed from storage, allowed to warm to room temperature, emptied into a 18" x 24" white enameled pan and examined under high illumination. Insect specimens and parts were identified and stored in labeled vials.

E. Data Tabulation and Analysis

All data from the various pore or propagule counting procedures were supplied to a data base of the University of Wisconsin WISAR System*, written in the MIIS dialect of MUMPS, for the Data General Eclipse computer, as means of the half filter counts. Data was coded by filter or sample number, dust weight, worker number, job title, and job location for the various analysis procedures and correlations. Equations for each sample in the computer program, which upon tabulation and analysis yielded data expressed as total numbers/m^3 of sampled air are as follows:

$$WRTS = WT1 + WT2 - WTF$$
$$WTV = WT1 \div (WT1 + WT2) \times WTS$$
$$WTD = WT2 \div (WT1 + WT2) \times WTS$$
$$SVN = VN \times (WTS \div WTV) \div V \text{ (where N = 1 to 8)}$$
$$\underline{TV} = SV1 + SV2 \ldots + SV8$$
$$SDM = DM \times (WTS \div WTD) \Delta V \text{ (where M = 1 to 7)}$$
$$\underline{TD} = SD1 + SD2 + \ldots + SV7$$

Symbols used in the above equations are described as:

WTF = weight in grams of the whole filter before use in air samples
WT1 = weight in grams of half of the filter, spores and debris from which viable counts were made
WT2 = weight in grams of half of the filter, spores and debris from which direct counts were made
WTV = weight in grams of spores and debris on the half of the filter from which viable counts were made

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*WISAR is a modified version of MISAR, a general purpose interactive data base system, for small and medium size data bases developed at Beth Israel Hospital, Boston, MA, by Dr. Howard Bleich and Mr. Dan Gregg.

WTD = weight in grams of spores and debris on the half of the filter from which direct counts were made
WTS = weight in grams of spores and debris on the whole filter
V = volume of air samples expressed in m^3
SVN (where N = 1 to 8) = viable spores/m^3 of genus VN (where N = 1 to 8)
TV = total number of viable organisms/m^3 as determined by viable count
SDM (where M = 1 to 7) = number of spores/m^3 of genus DM (where M = 1 to 7) that were seen microscopically
TD = total number of spores/m^3 as determined by direct microscopic count

The following categories contain the average number of viable spores in each genus. Values are an average of three of four petri plates and represent the flora collected on a portion, approximately half, of the membrane filter. All counts were made from CS media, except for bacteria, which were counted on TS media.

VN	Organism
V1 = VAS	(Aspergillus)
V2 = VPE	(Penicillium)
V3 = VCL	(Cladosporium)
V4 = VMU	(Mucor)
V5 = VAL	(Alternaria)
V6 = VYE	(yeast)
V7 = VBA	(bacteria)
V8 = VOT	(All others)

The following categories contain average numbers of spore types counted directly (non-viable) with aid of the microscope (400 X). A portion, approximately half, of the membrane filter was used and numbers refer to the quantity on that portion. Values are on average counts obtained from 25 microscope field counts.

DN	Organism
D1 = DAP	(Aspergillus)
D2 = DUS	(Ustilago)
D3 = DMU	(Mucor)
D4 = DFU	(Fusarium)
D5 = DCL	(Cladosporium)
D6 = DAL	(Alternaria)
D7 = DOT	(All others)

The following are the ranges utilized with data base for the various storage categories:

Category	Range
WTF	0 to 20,00000
WT1	0 to 0.00000
WT2	0 to 0.100000
V	0 to 1.10000
V1 (VAS)	0 to 200,000 (whole numbers only)
V2 (VPE)	0 to 200,000
V3 (VCL)	0 to 5,000
V4 (VMU)	0 to 500

V5 (VAL)	0 to 500
V6 (VYE)	0 to 1,000,000
V7 (VBA)	0 to 50,000,000
V8 (VOT)	0 to 2,000
D1 (DAP)	0 to 9,000,000
D2 (DVS)	0 to 900,000
D3 (DMU)	0 to 90,000
D4 (DFU)	0 to 50,000
D5 (DCL)	0 to 900,000
D6 (DAL)	0 to 70,000
D7 (DOT)	0 to 600,000

The following categories contain the average number of viable spores in each fungus species. Values are an average of three or four petri dishes and represent the major species of Penicillium and Aspergillus present on a portion, approximately half, of the membrane filter:

Category		Species
VAS	=	Aspergillus spp. (Total)
ASP VER	=	Aspergillus versicola
ASP NID	=	Aspergillus nidulans
ASP GLA	=	Aspergillus glaucus
ASP FUM	=	Aspergillus fumigatus
ASP FLA	=	Aspergillus flavus
VPE	=	Penicillium spp. (Total)
PEN CYC	=	Penicillium cyclopium
PEN VIV	=	Penicillium viridicatum
PEN URT	=	Penicillium urticae
PEN CHR	=	Penicillium chrysogenum
PEN BRC	=	Penicillium brevi-compactum

The data derived from the respirable and total dust samples were statistically analyzed using the previously described WISAR programs. The mean, range, and percent frequency for each category was determined for all grain workers and all municipal workers. Data from the individual samples were then analyzed in groups by elevator in which the worker worked on the day of sampling and also by job to which the worker was assigned on the day of sampling. Within these same groups, the samples were analyzed using the previously described WISAR programs. The mean, range, and percent frequency for each category was determined for all grain workers and all municipal workers. Data from the individual samples were then analyzed in groups by elevator in which the worker worked on the day of sampling and also by job to which the worker was assigned on the day of sampling. Within these same groups, the samples were analyzed by combing bacteria and yeasts (whose development is favored by similar environments) into one category and other fungi into another category. means, range, and percent frequency were similarly determined for all grain workers and all municipal workers as well as by elevators and by job.

Similar analysis was also carred out to contrast data derived from the viable counting procedures with that from the direct counting procedures. Grouped spore categories included Aspergillus types (Aspergillus and

Penicillium), Mucorales, Fusarium sp., Cladosporium sp., Alternaria sp., and "other" miscellaneous types. Ustilago sp. could not be contrased because it did not form identifiable colonies readily in the viable counting procedure.

Respirable and total dust weights from individual grain worker samples were also analyzed in groups by elevator in which the worker worked ont he day of sampling, and also by job to which the worker was assigned on the day of sampling. Results were also expressed as the frequency distribution (eg. % of samples) over the ranges: 0 to 0.100, 0.101 to 0.500, 0.501 to 1.000, 1.001 to 1.500, 1.501 to 2.000, 2.001 to 10.000, and 10.001 to 100.000 mg/m^3 as grouped by elevator and job.

Utilizing the Minitab Program (Statistics Department, Pennsylvania State University), regression equations and correlation coefficients were derived for total microflora of grain elevator airborne dust determined by viable (less yeasts and bacteria) as contrasted to non-viable (less Ustilago) counting modes. Sample regression equations and correlation coefficients were then calculated for respirable dust sample weights as compared to total dust sample weights, and for respirable dust sample weights as compared to total microflora derived by the viable counting modes.

III. RESULTS

A. Microbiological Analysis of Grain Dust

1. Respirable and Total Dust Samples.

a. Grain elevator worker and municipal source comparisons. - Microflora contained in airborne dust to which Duluth-Superior grain workers and Duluth municipal workers were exposed was drastically different. The mean concentration of viable fungi and bacteria in the municipal workers' samples was very much lower in every category than that present in the grain worker samples (Tables I & II). The Aspergillus and Penicillium species that were present in the municipal worker samples were not the same as the most prevalent species in the grain worker samples. Viable counts for grain worker samples ranged as high as 3.4×10^5 propagules/m^3 of sampled air. Excluding bacteria and yeasts, the maximum number of viable fungal propagules encountered in any grain worker sample was $9.4 \times 10^4/m^3$ of air. In contrast, viable bacteria and yeasts in municipal worker samples were not nearly so numerous nor so frequent. Viable fungi in municipal worker samples, however, ranged up to a maximum of 2.2×10^4 propagules/m^3 of air.

The predominant fungi (excluding the yeasts) present in respirable grain elevator dust as detected by the viable counting procedures were species of Penicillium and Aspergillus. Aspergillus glaucus was omnipresent and outnumbered the other Aspergilli and Penicillia by a wide margin. Penicillium cyclopium was the most numerous species of Penicillium encountered in the study, but its occurrence was highly variable. The most commonly encountered species of Penicillium and Aspergillus were isolated in pure culture and preserved on silica gel. The total number of species, isolates and their sources preserved in this way are indicated in Table 2. Cladosporium sp. were also frequently detected, but only occasional samples contained high concentrations (1.5×10^4).

Table 1. Concentration of microflora in respirable grain dust grouped by elevator - viable counting mode[a]

Rank	Elevator	Total[b,c]	Aspergillus	Penicillium	Cladosporium	Yeast	Bacteria
1	3(n=5)	76,098A	5,133A	2,406A	1,408AB	7,043AB	58,928A
2	8(n=4)	33,894AB	2,775A	4,382A	3,359A	6,756AB	14,401B
3	7(n=30)	33,734AB	2,785A	4,672A	555B	10,961A	14,175B
4	6(n=11)	27,712AB	2,184A	1,901A	1,705AB	2,235AB	19,053AB
5	1(n=35)	19,920AB	5,827A	2,170A	1,352AB	2,856AB	7,020B
6	4(n=2)	10,678B	575A	5,982A	782B	403B	2,621B
7	5(n=16)	8,848B	1,550A	1,286A	481B	2,146AB	2,454B
8	9(n=2)	5,594B	574A	1,169A	163B	1,097B	2,440B
9	2(n=4)	4,156B	442A	261A	80B	429B	2,794B
Mean	(n=109)[e]	24,962	3,460	2,775	1,037	5,091	11,866
10	10(n=65)[d]	766B	173A	482A	85B	4B	0B

a - Viable counts of aliquots from filters as mean propagules/m^3 of sampled air.

b - Includes other genera not illustrated.

c - Mean values in each line followed by the same letter do not differ significantly (P= 0.05) from each other by Duncan's Multiple Range Test.

d - 10 = municipal controls.

e - Mean = overall average of 109 samples.

Table 2. Summary of the numbers of *Penicillium* and *Aspergillus* isolates and their sources in the culture collection

Species source	Elevator number					Total
	1	5	6	7	8	
A. candidus	4	3		2	1	10
A. clavatus	2				2	4
A. fumigatus	5	2	2	4	2	15
A. flavus	4	2	2	3	4	15
A. glaucus	11	4	2	7	2	26
A. nidulans	5	3	2	3		13
A. niger	4	1	1			6
A. ochraceus	8	1	2	5	1	17
A. terreus	8		1	1		10
A. versicolor	10	4	4	5	1	24
P. brevi-compactum	2	8	2	2	1	15
P. capsulatum	1			1	1	3
P. chrysogenum	4		4	2	21	31
P. citrinum			2	1	6	9
P. claviforme	1		1	1		3
P. cyclopium	13	7	4	14	4	42
P. decumbens	1	1				2
P. frequentans	4	3	1	1	1	10
P. funiculosum			1			1
P. ochraceum	2	1				3
P. oxalicum	1	3	2			6
P. palitans	5	9		1		15
P. purpurogenum				2		2
P. rugulosum	4	2	5	2	4	17
P. urticae	2	1	2	3	2	10
P. variabile		1		1		2
P. viridicatum	12	13	8	4	4	41

Of the fungi grouped into the "other" category, Acremonium sp. were commonly encountered, while species of Fusarium, Papularia, Epicoccum, Neurospora, Trichoderma, and various members of the Sphaeropsidales such as Phyllosticta were occasionally detected. Dust samples rarely contained viable thermophilic Actinomycetes. One sample had a few colonies (≈100 colonies/m^3)) of Thermonospora viridis. Other thermophilic (or thermotolerant) organisms occasionally encountered included A. fumigatus, Humicola sp. and Absidia sp.

Respirable dust samples were collected periodically at various locations in our laboratory during the contract period. The mean propagule concentration was very low (Table 3). Penicillium sp. were most numerous while no Aspergillus sp. were present. In addition to the laboratory controls, other filters which had been numbered, weighed, and placed in the standard housing for dust sampling, but never used were also processed in the same way. Very few viable spores were present on any of the filters (Table 3).

Initial data processing of the viable counts of half filter samples numbered from 1 to 194 yielded extremely low counts; often the samples were sterile. This was unexpected since these numbers included most (31 or 38) of the total dust samples which should have had very high numbers of viable propagules. This was also in marked contrast with results obtained in sample numbers above 194, and also with the results obtained by direct counting procedures with the corresponding numbered filter-half. Since these filters had been sterilized with ethylene oxide prior to sue (the practice was discontinued for filters numbered above 194), it was assumed that the membrane filter, its support pad or plastic housing had retained lethal residues of the gas.

Comparisons of the presence or absence of fungal or bacterial propagules on filters as a function of the time of sampling regardless of the elevator, site or worker job, also indicated that the lowest number of viable propagules were always present on the samples collected prior to October 28 (Table 4). The percent of samples which were sterile or contained only small numbers of propagules (expressed as total counts for either Aspergillus (+) Penicillium) or bacteria were also highest in the earliest samples (October 4 to 6), fluctuated widely, but numbers of sterile samples gradually decreased with time up to October 28. Means of the total Aspergillus (+) Penicillium (the predominant fungi) and total bacterial counts were lower, correspondingly, for the early sampling times and gradually increased (while showing similar fluctuation) up to October 28. After October 28 all counts in these categories were very high (fluctuating with the site and worker job) but individual filters were rarely sterile. The filters used from October 4 to October 27 had all received the ethylene oxide treatment (#1 to 194) while none of those used from october 28through November 29 had been sterilized with ethylene oxide (#195 to 300). This gradual increase in viable counts over time suggested a loss of residual ethylene oxide or its reaction products was taking place over time from with the filter cassette, although even the last of the ethylene oxide treated filters used indicated considerable loss in viable counts (when contrasted with unsterilized filters).

Therefore, results of the viable counts for samples numbered 194 or below have not been included in any of the summaries or statistical analysis.

Table 3. Concentrations and frequencies of viable flora in laboratory control samples (spores/m^3 of air)

Organism[a]	Laboratory controls (n = 8) Mean±SEM	Range Min.	Max.	% Freq.	Unused controls (n = 25) Mean±SEM	Range Min.	Max.	% Freq.
Aspergillus (total)	0±0	0	0	0	2.52±2.05	0	50	8
versicolor	0±0	0	0	0	4.36±2.40	0	50	8
nidulans	0±0	0	0	0	0±0	0	0	0
glaucus	0±0	0	0	0	0±0	0	0	0
fumigatus	0±0	0	0	0	0±0	0	0	0
flavus	0±0	0	0	0	0±0	0	0	0
Penicillium (total)	108±44	0	393	90	4.36±2.40	0	50	4
cyclopium	0±0	0	0	0	0±0	0	0	0
viridicatum	0±0	0	0	0	0±0	0	0	0
urticae	0±0	0	0	0	0±0	0	0	0
chrysogenum	0±0	0	0	0	0±0	0	0	0
brevi-compactum	0±0	0	0	0	0±0	0	0	0
Cladosporium	14±9	0	60	20	0±0	0	0	0
Mucorales	0±0	0	0	0	0±0	0	0	0
Alternaria	4±4	0	36	1 10	0±0	0	0	0
Yeasts	4±4	0	36	10	0±0	0	0	0
Bacteria	0±0	0	0	0	1.36±9.4	0	17	8
Others	23±11	0	63	30	0±0	0	0	0
Total	153±72				12.6±7.79			

a - Total Aspergillus and Penicillium include other species in addition to the commonly occurring species listed in the table.

Direct count data from these filters were not affected and have been utilized in the analysis. The few total dust samples available were processed in the same manner as the respirable dust samples. However, because 31 of these were in the excluded numbers below 194, no grouping or analysis of the data derived from these filters was attempted.

Direct (non-viable) microscopic spore counting techniques also indicated major differences in the mean spore concentrations present in airborne dust between grain worker and municipal worker environments. Direct counts for grain worker samples range dup to 3.6×10^6 spores/m^3 of air while city worker samples only ranged up to 1.4×10^5 spores/m^3 of air (Tables 5 and III). Total mean spore concentrations were almost 10 times higher in grain worker environments than in corresponding city worker environments. Since identification of spores to species was not possible using the direct count method, comparison of the various microorganisms was limited to the generic level. The grain worker samples had high numbers of Ustilago (probably U. tritici) spores, while city worker samples had none in any of the samples.

The average concentration of fungal spores (excluding Ustilago) in grain worker environments as measured by the non-viable (direct) counting mode (excluding yeasts) (Table 6). Municipal worker non-viable total fungal counts were 8 times greater than the similar viable counts. The regression equation, however, derived form the relationship between viable counts and direct counts for fungi was $Y = -96989 - 1.45X$ with a non-significant ($P = 0.05$) correlation coefficient of -0.046.

Direct counts of spores were made of 30 total dust samples from grain workers and 15 from city workers. Mean concentrations and frequencies of spores in grain worker samples were also much higher than those in city worker samples (Table 7).

b. Elevator and job comparisons. - In addition to analyzing grain worker samples together, the data were grouped by elevator and by the job performed on the day the samples were collected. Dust samples from some of the elevators had relatively higher mean viable propagule concentrations than the rest. The highest mean counts, as well as the highest total counts were recorded in samples from workers form elevator #3 (Table 1 and IV).

Bacteria accounted for a large proportion of this high count, but even when bacteria and yeasts were excluded from the totals, workers from this high count, but even when bacteria and yeasts were excluded from the totals, workers form this elevator still were exposed to higher numbers of viable fungus propagules than workers from any of the other elevators (Table 8). These counts proved to be twice as great as the viable counts from the next highest elevators (#8, 7, 6) and almost seven times greater than the sixth ranked elevator (#4). Viable counts recorded for the seventh through ninth ranked elevators (e.g. #4, 5, 9 & 2) were distinctly lower than the others. In spite of these differences in means however the total number of propagules only differed significantly ($P = 0.05$) in degree of contamination between the first and second ranked elevators (Table 8). The Duncan's multiple range test indicated that the means between the second and ninth ranked elevators were not significantly different from each other or from the municipal worker controls (Table 1).

Table 4. Comparison of fungal or bacterial propagule means as a function of time of sampling regardless of elevator, site or worker job.

Sampling date	No. of samples	Penicillium (+) Aspergillus		Bacteria	
		Mean number viable propagules	% Sterile samples	Mean number viable propagules	% Sterile samples
		Filters ethylene oxide sterilized			
4 Oct 70	8	0	100	26	71
5 Oct 70	8	0	100	24	86
6 Oct 70	8	0	100	11	75
7 Oct 70	6	30	50	12	83
10 Oct 70	9	33	78	33	56
11 Oct 70	8	45	87	17	88
12 Oct 70	9	16	78	46	67
13 Oct 70	15	7	92	201	54
17 Oct 70	5	6	80	71	80
18 Oct 70	8	0	100	1,470	43
19 Oct 70	7	10	86	157	71
20 Oct 70	3	0	100	21	33
24 Oct 70	10	2,666	70	2,432	50
25 Oct 70	11	25	64	157	55
26 Oct 70	9	48	56	92	33
27 Oct 70	7	84	71	1,633	29
		Filters not sterilized			
28 Oct 70	6	23,196	0	43,184	17
1 Nov 70	6	3,811	0	30,471	0
2 Nov 70	7	3,810	0	15,559	0
3 Nov 70	5	17,611	0	19,410	0
4 Nov 70	8	11,297	0	4,912	17
7 Nov 70	7	1,742	0	3,390	0
8 Nov 70	6[a]	898	0	11,861	0

[a] - Sampling data through Nov. were similar and data beyond 8 Nov. 70 have been excluded.

Table 5. Concentration of microflora in respirable grain dust grouped by elevator - non-viable (direct) counting mode[a]

Rank	Elevator	Total[b,c]	Aspergillus[d]	Ustilago	Cladosporium	Mucorales[e]	Fusarium
1	7 (n=26)	149,222A	123,994A	18,340A	2,806A	656A	192A
2	1 (n=39)	115,514A	99,035A	9,284A	3,358A	1,107A	443A
3	3 (n=6)	39,056A	13,605A	22,029A	1,279A	1,210A	173A
4	6 (n=16)	39,012A	13,174A	19,411A	2,275A	865A	150A
5	4 (n=59)	36,800A	13,339A	19,890A	2,065A	519A	218A
6	2 (n=26)	18,210A	10,492A	4,497A	2,234A	37A	308A
7	8 (n=34)	15,962A	9,108A	4,403A	1,409A	299A	64A
8	5 (n=21)	10,987A	6,541A	1,672A	970A	232A	142A
9	9 (n=7)	8,265A	5,430A	1,090A	621A	369A	129A
Mean	(n=246)[f]	58,298	41,816	11,948	2,183	572	228
10	10 (n=65)	6,113A	5,346A	0A	400A	94A	70A

a - Counts made microscopically from millipore filter aliquots of original filters and expressed as spores/m^3 of sampled air.

b - Mean values in each line followed by the same letter do not differ significantly (P = 0.05) from each other by Duncan's Multiple Range Test.

c - Total includes other genera not illustrated.

d - Aspergillus type spores (Penicillium and Aspergillus species not distinguished in this procedure.

e - Genera of Mucorales not distinguished in this procedure.

f - Mean = overall average for 246 samples.

Table 6. Concentration of microflora in respirable dust (propagules or spores/m^3) in grain worker and municipal worker environments as determined by viable and non-viable (direct) counting modes

Organism	Grain workers (n=109) Mean±SEM	Range Min.	Max.	Municipal workers (n=65) Mean±SEM	Range Min.	Max.
Viable mode						
Bacteria & yeasts	21,473±4,857	0	302,750	4±2	0	95
All other fungi	8,008±1,376	0	94,021	764±360	0	22,126
Total	30,480±5,902			768±362		
Non-viable mode						
Ustilago	11,948±1,798	0	250,717	0±0	0	0
All other fungi	46,351±19,243	0	3,571,012	6,113±2,320	0	140,478
Total	58,299±19,355			6,113±2,320		

Table 7. Concentrations and frequencies of microflora in total dust samples (spores/m^3 of air) as determined by non-viable (direct) counting modes

	Grain workers (n=30)				Municipal workers (n=15)			
		Range		%		Range		%
Organism	Mean±SEM	Min.	Max.	Freq.	Mean±SEM	Min.	Max.	Freq.
Aspergillus-type	13,948±4,036	0	108,445	67	1,993±830	0	11,235	67
Ustilago	8,982±1,969	0	46,752	63	0±0	0	0	0
Mucorales	2,142±1,052	0	29,674	37	109±74	0	864	13
Fusarium	84±47	0	890	10	215±96	0	898	27
Cladosporium	2,624±660	0	13,636	50	1,092±506	0	7,876	60
Alternaria	187±84	0	1,701	17	109±74	0	827	13
Other	1,422±504	0	10,426	37	712±207	0	2,418	53

The mean Aspergillus and Penicillium counts while ranging from almost 6.0 x 10^3 propagules/m^3 to less than 5.0 x 10^2 among the various elevators were not significantly different (P = 0.05) (Table 1). Elevator 8 (ranked second) had significantly higher Cladosporium counts than the lower ranked elevators, and elevator #7 (ranked third) had significantly higher yeast counts than the lower ranked elevators - but in both cases some overlap in significance was present among the upper and middle rankings.

The ranking of elevators as determined by direct counts differed somewhat from that derived by viable counting. Measured in this fashion elevator #7 (ranked third by viable count) and elevator #1 (ranked fifth by viable count) had the highest and next highest direct counts (Table 5 & V). They were four and three times more contaminated than the third ranked elevator (#3) (highest by viable count). Direct count contamination levels did not differ significantly between the third (#3), fourth (#6) and fifth (#4). Elevator #2 (sixth) and #5 (eighth) were distinctly less contaminated than the others, as was also the case when using the viable counting method. The Aspergillus + Penicillium type spores (apparently mainly non-living) were major contributors to the high direct counts observed with samples from elevator #7 and #1. Interestingly, elevators #7, 3, 4 and 5 had very similar direct counts for Ustilago spores ($\simeq$1.8 x 10^4 to 2.2 10^4 spores/m^3 of air) (Table 9). Ustilago counts for the remaining elevators, however, were lower and ranged from 9.2 x 10^3 to 1.1 x 10^3 spores/m^3 fo air. The highest Ustilago count (2.5 x 10^5 spores/m^3 of air) was present on a sample from elevator #4. All of the elevators examined had similar Cladosporium direct counts ($\simeq$6.2 x 10^2 to 3.4 x 10^3 spores/m^3 of air).

The non-viable counting mode for determining the concentration of microflora in respirable grain dust when grouped by elevator, while indicating trends was considerably more variable than the viable counting mode and the Duncan's multiple range test failed to show significance between means. However when the elevator rankings made by two methods for total count were compared, they exhibited similarities (Table 1 & 5). Elevator #7 and #3 were in the lowest (1-3) rankings (most contaminated) in both methods. Elevators #8 and #9 were likewise in the highest (7-0) rankings (least contaminated) in both methods, while elevator #6 ranked fourth in both. Municipal controls were at the tenth rank (least contaminated) position in both.

When the data were regrouped and analyzed by job category the annex workers as a group appeared to be exposed to this highest mean concentration of viable spores or propagules (e.g. 1.2 x 10^5 propagules/m^3 of air) (Table 10 & VI) and were significantly higher than the other categories (P = 0.05). This exposure was seven times greater than the next highest category, the equipment operators. Individual worker samples had viable counts exceeding 3.4 x 10^5 spores/m^3. Bacteria and yeasts made up a large proportion of these counts, although the same job categories also had the highest mean viable fungal propagule counts (Table 11). Counts for Aspergillus and Penicillium accounted for a high proportion of the mean fungal propagule count for the annex workers (Table 10 & IV).

Mean viable counts for the other job categories ranged from 2.6 x 10^4 propagules/m^3 (inspectors) to 9.76 x 10^3 propagules/m^3 (supervisors). Individual workers sampled had occasional high total counts (e.g. maintenance =

Table 8. Concentrations and frequencies of microflora in respirable dust samples as determined by viable modes when grouped by elevators (spores/m^3).

Organism	Mean±SEM	Range Min.	Range Max.	% Freq.	Mean±SEM	Range Min.	Range Max.	% Freq.
	Elevator 1 (n=35)				Elevator 7 (n=32)			
Bacteria & Yeasts	9,877±3,954	0	100,413	100	40,297±12,473	240	302,750	100
All other fungi	10,046±3,175	68	94,021	100	8,593±2,813	31	62,560	100
Total	19,920±6,148				52,327±15,202			
	Elevator 2 (n=4)				Elevator 8 (n=4)			
Bacteria & Yeasts	3,223±1,251	36	5,454	100	21,157±12,456	1,784	57,334	100
All other fungi	935±562	0	2,415	75	12,739±5,047	3,198	26,698	100
Total	4,156±1,763				33,893±16,900			
	Elevator 3 (n=5)				Elevator 9 (n=2)			
Bacteria & Yeasts	65,971±58,608	518	300,038	100	3,536±1,499	2,037	5,036	100
All other fungi	10,131±8,132	396	42,456	100	2,060±655	1,405	2,716	100
Total	76,098±66,727				5,594±843			
	Elevator 4 (n=2)							
Bacteria & Yeasts	3,023±2,842	181	5,865	100				
All other fungi	7,657±7,294	362	14,951	100				
Total	10,678±10,136							
	Elevator 5 (n=17)							
Bacteria & Yeasts	4,600±3,634	0	58,714	69				
All other fungi	4,250±1,647	116	23,181	100				
Total	8,848±5,035							
	Elevator 6 (n=11)							
Bacteria & Yeasts	21,288±8,587	426	86,114	100				
All other fungi	6,427±1,437	609	17,630	100				
Total	27,712±9,632							

Table 9. Concentrations and frequencies of microflora in respirable dust as determined by non-variable modes with combined categories grouped by elevator (spores/m^3).

Organism	Mean±SEM	Range Min.	Range Max.	% Freq.	Mean±SEM	Range Min.	Range Max.	% Freq.
	Elevator 1 (n=44)				Elevator 7 (n=33)			
Ustilago	9,284±1,958	0	47,553	93	18,340±4,139	0	95,896	96
All other fungi	106,232±70,632	0	3,111,102	98	130,884±107,620	0	3,571,012	82
Total	115,514±70,678				149,222±107,159			
	Elevator 2 (n=26)				Elevator 8 (n=34)			
Ustilago	4,497±1,771	0	39,508	76	4,403±1,100	0	31,704	87
All other fungi	13,714±1,761	0	32,876	92	11,561±2,243	0	64,587	94
Total	18,210±3,034				15,962±2,782			
	Elevator 3 (n=6)				Elevator 9 (n=7)			
Ustilago	22,029±12,530	1,480	81,929	100	1,090±608	0	4,238	43
All other fungi	17,028±13,926	0	86,078	67	7,176±1,414	3,606	11,558	100
Total	39,056±26,042				5,265±1,610			
	Elevator 4 (n=59)							
Ustilago	19,890±6,083	0	250,717	93				
All other fungi	16,912±4,161	0	237,860	93				
Total	36,800±9,466							
	Elevator 5 (n=21)							
Ustilago	1,672±695	0	11,503	42				
All other fungi	9,317±2,331	0	31,820	81				
Total	10,987±2,670							
	Elevator 6 (n=16)							
Ustilago	19,411±9,424	1,674	155,994	100				
All other fungi	19,604±4,162	1,728	55,180	100				
Total	39,012±12,188							

Table 10. Concentration of microflora in respirable grain dust grouped by job category - viable counting mode[a]

Rank	Job[b]	Total[c,d]	Aspergillus	Penicillium	Cladosporium	Yeast	Bacteria
1	2(n=5)	120,786A	15,277A	9,869A	2,869A	8,574A	82,635A
2	7(n=13)	29,403B	5,455AB	8,343AB	2,300AB	1,295A	11,238B
3	6(n=23)	26,148B	2,710B	2,278AB	1,084AB	5,471A	13,741B
4	8(n=5)	24,636B	2,145B	3,673AB	767AB	3,993A	13,658B
5	3(n=18)	22,062B	2,265B	1,393AB	350B	10,781A	6,143B
6	5(n=24)	15,928B	2,474B	866B	738AB	4,635A	6,767B
7	4(n=6)	14,087B	3,658B	2,652AB	1,053AB	1,614A	4,660B
8	1(n=15)	9,747B	2,313B	808B	652AB	2,294A	3,255B
Mean	(n=109)[e]	24,962	3,460	2,755	1,037	4,091	11,866
9	9(n=65)	766B	173B	482B	85B	4A	0B

a - Viable counts of aliquots from filters as mean propagules/m^3 of sampled air.

b - Job categories: 1 - weighers, 2 - annex workers, 3 - laborers, 4 - transport within elevator, 5 - maintenance, 6 - inspectors, 7 - equipment operators, 8 - supervisors, 9 - municipal controls.

c - Mean values in each line followed by the same letter do not differ significantly (P = 0.05) from each other by Duncan's multiple range test.

d - Total includes other genera not illustrated.

e - Mean = overall average for 109 samples.

1.03×10^5 propagules/m^3) which were five times greater than the mean for their job category.

The mean spore concentrations by job category as determined by direct counts were all very similar with one exception (weighers) (Table 12 & VII). Individual weighers had maximum direct total counts as high as 3.57×10^6 spores/m^3 of air. Most of this total was made up of Aspergillus (+) Penicillium type spores (apparently non-living). Mean direct counts for the other job categories ranged from 1.6×10^4 spores/m^3 (supervisors) to 5.7×10^4 spores/m^3 (annex workers) (Table 13). Direct counting procedures indicated that transport workers, annex workers, and laborers were exposed to higher than average numbers of Ustilago spores (3.3×10^4, 2.3×10^4 and 2.1×10^4 spores/m^3, respectively. Sample means for all the other categories contained less than half this number of Ustilago spores. As was also the case with the elevator comparisons using the non-viable counting mode, although trends were indicated, the total spore counts from the various job categories did not differ significantly from one another. No correlation was present between job category ratings obtained by the non-viable mode and ratings obtained by the viable mode.

c. Dust weight comparisons

(1) Elevator and job comparisons. - Means of respirable dust concentrations derived from samples from the various grain elevator environments were ranked and compared with similar municipal worker controls.

Means ranged from 0.8984 mg/m^3 (elevator #1 - ranked #1) to 0.2060 mg/m^3 (elevator #9 - ranked ninth) and the municipal control (0.1786 mg/m^3 - ranked tenth) (Table 14). While the rankings indicated slight trends, mean values did not differ significantly among the elevators or municipal controls, although the mean for all elevators was four times greater than the control mean. Except for elevators #1 and 7 (ranked third) over half of the individual respirable dust samples were below 0.500 mg/m^3 (Table 15). Over half of the control samples were below 0.100 mg/m^3.

Ranking of individual elevator means for total dust samples indicated significant differences (P = 0.05) between the dustiest elevator (ranked #1) and the sixth through tenth ranks (Table 16). Elevator #3, the dustiest elevator (ranked 1), was 15 times dustier (9.475 mg/m^3) than the municipal control mean (0.598 mg/m^3). Over half of the individual samples int he dustiest elevators (ranked 1-6) were below 1.500 mg/m^3, while controls were below 0.500 mg/m^3 (Table 17).

Some correlation was evident between elevator rank for respirable dust concentration and total dust concentration (Table 14 & 16). Elevator #3 was among the two dustiest in both measurements while elevators #8 and 9 were least dusty by both measurements.

Ranking of respirable dust means grouped by job yielded significant differences (P = 0.05) between the dustiest (annex workers - 1.7156 mg/m^3) and the rest of the job categories (Table 18). Samples from municipal worker controls exposed to the least dust averaged 0.1786 mg/m^3. Over 50% of the

Table 11. Concentrations and frequencies for viable flora in respirable dust samples when grouped by jobs (spores/m^3)

Organism	Mean±SEM	Range Min.	Range Max.	% Freq.	Mean±SEM	Range Min.	Range Max.	% Freq.
	Weighers (n=15)				Maintenance (n=24)			
Bacteria & Yeasts	5,549±1,761	126	21,640	100	11,401±4,832	0	100,413	92
All other fungi	4,201±1,294	126	17,517	100	4,529±1,311	0	26,062	92
Total	9,747±2,414				15,927±5,237			
	Annex Workers (n=5)				Grain Inspectors (n=23)			
Bacteria & Yeasts	91,209±55,038	0	300,037	85	19,211±8,512	89	192,129	100
All other fungi	29,579±17,860	261	94,021	100	6,939±1,971	68	38,663	100
Total	120,786±63,332				26,148±10,315			
	Laborers (n=20)				Equipment Operators (n=13)			
Bacteria & Yeasts	41,993±18,296	181	302,750	100	12,533±6,139	0	76,346	85
All other fungi	5,140±2,104	31	32,668	100	16,873±6,154	116	62,560	100
Total	52,978±22,218				29,403±10,488			
	Transport within Elevators (n=6)				Supervisors (n=5)			
Bacteria & Yeasts	6,275±3,185	0	16,306	83	17,650±10,566	1,687	55,626	100
All other fungi	7,816±4,619	160	30,136	100	6,988±5,936	181	30,713	100
Total	14,087±7,127				24,636±16,045			

Table 12. Concentration of microflora in respirable grain dust grouped by job category - non-viable (direct) counting mode[a]

Rank	Job[b]	Total[c,f]	Aspergillus[d]	Ustilago	Cladosporium	Mucorales[e]	Fusarium
1	1(n=26)	280,276A	267,262A	6,162A	2,976A	1,112A	251A
2	4(n=15)	56,716A	13,010A	32,925A	3,122A	708A	205A
3	2(n=11)	52,702A	23,555A	23,181A	3,191A	1,302A	375A
4	3(n=40)	41,473A	16,086A	20,852A	2,123A	562A	273A
5	5(n=39)	34,797A	22,235A	8,312A	2,517A	406A	284A
6	7(n=25)	31,518A	17,069A	10,459A	2,349A	594A	184A
7	6(n=80)	20,668A	10,483A	7,130A	1,537A	368A	189A
8	8(n=10)	15,635A	10,761A	4,013A	1,073A	422A	70A
Mean	(n=246)[g]	58,299	41,816	11,748	2,183	572	228
9	9(n=65)	6,113A	5,346A	0A	400A	94A	70A

a - Counts made microscopically from millipore filter aliquots of original filters and expressed as spores/m^3 of sampled air.

b - Job categories: 1 - weighers, 2 - annex workers, 3 - laborers, 4 - transport within elevator, 5 - maintenance, 6 - inspectors, 7 - equipment operators, 8 - supervisors, 9 - municipal controls.

c - Mean values in each line followed by the same letter do not differ significantly (P = 0.05) from each other by Duncan's multiple range test.

d - Aspergillus type spores (Penicillium and Aspergillus spores not distinguished in this procedure).

e - Genera of Mucorales not distinguished in this procedure.

f - Total includes other genera not illustrated.

g - Mean = overall average of 246 samples.

Table 13. Concentrations and frequencies of direct counts with combined categories and grouped by job (spores/m^3)

Organism		Range Min.	Max.	% Freq.		Range Min.	Max.	% Freq.
	Weighers (n=26)				Maintenance (n=39)			
Ustilago	6,162±5,747	0	41,647	73	8,312±2,084	0	59,173	82
All other fungi	274,116±177,558	733	3,571,012	100	26,486±11,407	0	452,153	92
Total	280,276±177,301				34,797±12,616			
	Annex Workers (n=11)				Grain Inspectors (n=80)			
Ustilago	23,181±7,977	0	81,929	91	7,130±1,675	0	95,896	87
All other fungi	29,523±7,733	1,523	86,078	100	13,540±1,753	0	95,309	91
Total	52,902±14,531				20,668±2,825			
	Laborers (n=30)				Equipment Operators (n=25)			
Ustilago	20,852±7,362	0	250,718	88	10,459±3,460	0	78,104	92
All other fungi	20,623±6,427	0	237,860	88	21,061±4,831	0	117,050	92
Total	41,473±13,076				31,518±7,343			
	Transport within Elevators (n=15)				Supervisors (n=10)			
Ustilago	32,925±16,102	0	216,917	92	4,013±1,331	0	9,987	89
All other fungi	23,793±7,098	0	92,098	87	12,623±6,098	0	64,125	80
Total	56,716±18,184				16,635±6,031			

individual annex worker sample weights were 1.500 mg/m3 or lower while the other rankings were mostly below 0.500 mg/m^3 (Table 19).

Ranking of total dust sample means by job also yielded significant differences (P = 0.05) between the dustiest (annex workers - 10.733 mg/m^3) and the rest of the job categories (Table 20). Fifty percent of the sample weights for annex workers (ranked #1) were below 10.000 mg/m^3 while 50 percent of rank #2 to 6 samples were below 1.500 mg/m^3 (Table 21).

Good correlation between ranking of job category was apparent between the respirable and total dust measurements (Table 18 $ 20). Rank #1 to 3 (annex workers, equipment operators and laborers, respectively) (the dustiest), were the same in both measurements and the job categories weighers, inspectors and supervisors were ranked cleanest in both measurements.

Excellent correlation (P = 0.01) was apparent when individual respirable dust weights were compared with their corresponding total dust weight sample. The regression equation for the relationship was Y = .0951X + .470 with a correlation coefficient of 0.556 (df = 197). Excellent correlation (P = 0.01) was also apparent when individual respirable dust weights were compared with their corresponding total spore count derived by either viable or non-viable counting modes. The regression equation for the comparison between respirable dust weights and corresponding total viable propagule count was: Y = 6281 + 1954X with a correlation coefficient of 0.495 (df = 106). The regression equation for the relationship of respirable dust to the total direct (non-viable count was: Y = 21,366X + 16480 with a correlation coefficient of 0.363 (df = 234). In this latter calculation two sample spore counts which were 10 times greater than any of the other 237 values (indicating a major problem were deleted.

(2) Comparison of ranking techniques - Each of the methods (microorganism spore counts and dust concentration) ranked the level of elevator and job contamination in a slightly different order. The results of these various techniques were contrasted using a simple scoring technique and the "average" rank calculated for each elevator and job environment (Table 22). The method itself was also rated by assigning two points for each observed rank which coincided with the calculated position, and one point if the ranks occurred in the same division (upper or lower one-third). It was evident from this that elevators #3, 7 and 1 were relatively "dirtier" and elevators #2, 5 and 9 were cleaner. Among the various job categories annex workers, equipment operators, and laborers were exposed to the greatest concentrations of dust and spores, while the weighers, inspectors, and supervisors suffered the least exposure. The respirable dust measurements appeared to give the closest estimates of ranking to the calculated average score in both the elevator and job categories.

(3) Causes of sample variation - Occasional filters from the grain work respirable dust collections when reweighed gave negative weights. These apparent negative values were most common (9% of the samples) on those filters sterilized with ethylene oxide prior to use, while fewer negative values (2.3) were obtained with the non-sterile filters. No negative values were obtained with dessicated, non-sterile control filters weighed at Morgantown, WV (.001 mg sensitivity). To examine the possibility that very

Table 14. Relative concentrations of respirable dust detected in Duluth-Superior grain elevator environments grouped by elevator[a]

Rank	Elevator	Dust weight (mg/m^3) Mean ± SEM[b]	Range Min.	Max.
1	1(n=42)	0.8984A ± 0.1270	0.0	8.2600
2	3(n=6)	0.8941A ± 0.8059	0.0	4.9090
3	7(n=32)	0.8224A ± 0.1275	0.0	2.4050
4	6(n=15)	0.6166A ± 0.1945	0.0	2.7640
5	4(n=58)	0.5606A ± 0.1112	0.0	5.3000
6	5(n=19)	0.5516A ± 0.1369	0.0	2.0360
7	8(n=34)	0.5127A ± 0.0839	0.0	1.6500
8	2(n=25)	0.3392A ± 0.0761	0.0	1.4590
9	9(n=7)	0.2060A ± 0.1085	0.0	0.8220
Mean	(n=238)[a]	0.6759 ± 0.0597		
10	10(n=65)[c]	0.1786A ± 0.0294	0.2	1.4800

a - Mean values in each line followed by the same letter do not differ significantly (P = 0.05) from each other by Duncan's multiple range test.

b - SEM = Standard error of mean.

c - Municipal controls.

d - Mean = overall average of 238 samples.

Table 15. Frequency distribution of respirable dust sample weights from Duluth-Superior grain elevator and Duluth municipal worker environments[a]

		Dust weight categories (mg/m^3)					
Rank	Elevator	0.000 to 0.100	0.101 to 0.500	0.501 to 1.000	1.001 to 1.500	1.501 to 2.000	2.001 to 10.000
1	1(n=42)	16.7	11.9	26.2	19.0	16.7	9.5
2	3(n=6)	66.7[b]	16.7	0.0	0.0	0.0	16.7
3	7(n=32)	18.8	25.0	18.5	15.6	18.5	3.1
4	6(n=15)	33.3	26.7	13.3	20.0	0.0	6.7
5	4(n=58)	41.4	18.0	22.4	10.3	1.7	5.2
6	5(n=19)	21.1	42.1	10.5	15.8	5.3	5.3
7	8(n=34)	32.4	17.6	32.4	11.8	5.9	0.0
8	2(n=25)	40.0	36.0	20.0	4.0	0.0	0.0
9	9(n=7)	42.9	42.9	14.3	0.0	0.0	0.0
Mean	(n=238)	39.5	23.1	21.4	12.6	8.0	4.6
10	10(n=65)	49.2	44.6	3.1	3.1	0.0	0.0

a - Distribution expressed as % of total samples for each elevation (n).

b - Figures underlined comprise the lower 50 percentile of samples.

Table 16. Relative concentration of total dust detected in Duluth-Superior grain elevator environments grouped by elevator[a]

Rank	Elevator	Dust weight (mg/m^3) Mean ± SEM[b]	Range Min.	Range Max.
1	3(n=5)	9.475A ± 7.420	0.727	38.947
2	4(n=43)	3.877AB ± 1.252	0.140	39.120
3	2(n=16)	3.369AB ± 1.340	0.250	20.160
4	7(n=30)	3.245AB ± 0.492	0.229	10.821
5	1(n=34)	2.857AB ± 0.952	0.196	30.184
6	6(n=16)	2.148B ± 0.669	0.220	10.048
7	5(n=16)	1.792B ± 0.557	0.268	8.285
8	8(n=33)	1.654B ± 0.604	0.170	20.192
9	9(n=8)	0.958B ± 0.405	0.200	3.610
Mean	(n=201)[d]	2.922 ± 0.403	0.140	39.120
10	10(n=64)[c]	0.598B ± 0.70	0.090	2.56

a - Mean values in each line followed by the same letter do not differ significantly (P = 0.05) from each other by Duncan's multiple range test.

b - SEM = Standard error of mean.

c - 10 = Municipal controls.

d - Mean = overall average of 201 samples.

Table 17. Frequency distribution of total dust detected in Duluth-Superior grain elevator environments[a]

		Dust weight categories (mg/m^3)						
Rank	Elevator	0.000 to 0.100	0.101 to 0.500	0.501 to 1.000	1.001 to 1.500	1.501 to 2.000	2.051 to 10.000	10.001 to 100.000
1	3(n=5)	0.0	0.0	40.0	20.0	0.0	20.0	20.0
2	4(n=43)	0.0	6.7	26.7	26.7	11.1	20.0	8.9
3	2(n=16)	0.0	25.0	12.5	18.8	6.3	25.0	12.5
4	7(n=30)	0.0	6.7	13.3	3.3	20.0	50.0	6.7
5	1(n=34)	0.0	14.7	32.4	23.5	5.9	17.6	5.9
6	6(n=16)	0.0	25.0	18.8	12.5	6.3	31.3	6.3
7	5(n=16)	0.0	18.8	31.3	25.0	0.0	25.0	0.0
8	8(n=33)	0.0	30.3	33.3	15.2	6.1	12.1	3.0
9	9(n=8)	0.0	83.3	0.0	16.7	0.0	0.0	0.0
Mean	(n=201)	0.0	17.9	24.9	18.4	8.5	23.9	6.5
Municipal controls	(n=64)	4.8	60.3	22.2	4.8	4.8	4.8	0.0

a - Distribution expressed as % of samples.

b - Figures underlined comprise the lower 50 percentile of samples.

Table 18. Relative concentration of respirable dust detected in Duluth-Superior grain elevator environments grouped by job[a]

Rank	Job[b]	Dust weight (mg/m^3) Mean ± SEM[c]	Range Min.	Range Max.
1	2(n=11)	1.7156A ± 0.5203	0.0	4.9090
2	7(n=25)	0.9030AB ± 0.3326	0.0	8.2600
3	3(n=38)	0.7691AB ± 0.1580	0.0	5.3000
4	4(n=15)	0.7525AB ± 0.1485	0.0	1.8400
5	5(n=39)	0.6309AB ± 0.0930	0.0	2.2160
6	1(n=24)	0.5885B ± 0.1246	0.0	2.1310
7	6(n=76)	0.4753B ± 0.0640	0.0	2.0210
8	8(n=9)	0.3756B ± 0.1956	0.0	1.5000
Mean	(n=237)[d]	0.6759 ± 0.0597		
9	9(n=65)	0.1786B ± 0.0294	0.2	1.4600

a - Mean values in each line followed by the same letter do not differ significantly (P = 0.05) from each other by Duncan's multiple range test.

b - Job categories: 1 - weighers, 2 - annex workers, 3 - laborers, 4 - transport within elevator, 5 - maintenance, 6 - inspectors, 7 - equipment operators, 8 - supervisors, 9 - municipal controls.

c - SEM = Standard error of mean.

d - Mean = overall average of 237 samples.

Table 19. Frequency distribution of respirable dust detected in Duluth-Superior grain elevator environments grouped by job[a]

		Dust weight categories (mg/m^3)						
Rank	Job[c]	0.000 to 0.100	0.101 to 0.500	0.501 to 1.000	1.001 to 1.500	1.501 to 2.000	2.001 to 10.000	10.001 to 100.000
1	2(n=11)	18.2	18.2	9.1	9.1[b]	9.1	36.4	0.0
2	7(n=25)	36.0	20.0	16.0	8.0	12.0	8.0	0.0
3	3(n=38)	21.1	34.2	15.8	15.8	7.9	5.3	0.0
4	4(n=15)	20.0	20.0	26.7	20.0	13.3	0.0	0.0
5	5(n=39)	23.1	25.6	30.8	12.8	5.1	2.6	0.0
6	1(n=24)	33.3	20.8	16.7	20.8	4.2	4.2	0.0
7	6(n=76)	38.2	22.4	23.7	7.9	6.6	1.3	0.0
8	8(n=9)	66.7	0.0	11.1	22.0	0.0	0.0	0.0
Mean	(n-237)	31.2	23.2	21.5	12.7	7.2	4.6	0.0
9	9(n=65)	49.2	44.6	3.1	3.1	0.0	0.0	0.0

a - Distribution expressed as % of samples.

b - Figures underlined comprise the lower 50 percentile of samples.

c - Job categories: 1 - weighers, 2 - annex workers, 3 - laborers, 4 - transport within elevator, 5 - maintenance, 6 - inspectors, 7 - equipment operators, 8 - supervisors, 9 - municipal controls.

Table 20. Relative concentration of total dust detected in Duluth-Superior grain elevator environments grouped by job[a]

Rank	Job[c]	Dust weight (mg/m^3) Mean ± SEM[b]	Range Min.	Range Max.
1	2(n=10)	10.733A ± 3.926	0.792	38.947
2	7(n=23)	3.847B ± 1.534	0.140	30.184
3	3(n=36)	3.503B ± 1.048	0.229	36.080
4	5(n=36)	3.305B ± 1.090	0.250	39.120
5	4(n=15)	2.579B ± 0.702	0.220	10.240
6	1(n=19)	1.534B ± 0.3188	0.196	5.470
7	8(n=6)	1.283B ± 0.485	0.428	3.466
8	6(n=56)	1.267B ± 0.168	0.170	6.717
Mean	(n=201)[d]	2.922 ± 0.403		
9	9(n=64)	0.598B ± 0.070	0.090	2.560

a - Mean values in each line followed by the same letter do not differ significantly (P = 0.05) from each other by Duncan's multiple range test.

b - SEM = Standard error of mean.

c - Job categories: 1 - weighers, 2 - annex workers, 3 - laborers, 4 - transport within elevator, 5 - maintenance, 6 - inspectors, 7 - equipment operators, 8 - supervisors, 9 - municipal controls.

d - Mean = overall average of 201 samples.

Table 21. Frequency distribution of total dust detected in Duluth-Superior grain elevator environments grouped by job[a]

Rank	Job[c]	Dust weight categories (mg/m^3)						
		0.000 to 0.100	0.101 to 0.500	0.501 to 1.000	1.001 to 1.500	1.501 to 2.000	2.001 to 10.000	10.001 to 100.000
1	2(n=10)	0.0	0.0	20.0	0.0	10.0	30.0[b]	40.0
2	7(n=23)	0.0	17.4	26.1	21.7	13.0	8.7	13.0
3	3(n=36	0.0	8.3	30.6	13.9	11.1	25.0	11.1
4	5(n=36)	0.0	11.8	20.6	17.6	8.8	38.2	2.9
5	4(n=15)	10.0	13.3	13.3	26.7	13.3	26.7	6.7
6	1(n=19)	0.0	22.2	22.2	27.8	5.6	22.2	0.0
7	8(n=6)	0.0	33.3	33.3	0.0	16.7	16.7	0.0
8	6(n=56)	0.0	30.4	28.6	17.9	3.6	19.6	0.0
Mean	(n=201)	0.0	18.2	25.3	17.7	8.6	23.7	6.6
9	9(n=64)	0.0	60.3	22.2	4.8	4.8	4.8	0.0

a - Distribution expressed as % of samples.

b - Figures underlined comprise the lower 50 percentile of samples.

c - Job categories: 1 - weighers, 2 - annex workers, 3 - laborers, 4 - transport within elevator, 5 - maintenance, 6 - inspectors, 7 - equipment operators, 8 - supervisors, 9 - municipal controls.

Table 22. Summary of the ranking of relative dust and microorganism contamination of grain elevator and job environments as determined by various techniques

Elevator rank	Point score	Elevator number: Total microorganisms Viable	Total microorganisms Non-viable	Dust concentration Respirable	Dust concentration Total	Final[a] position Score	Final[a] position Elevator
1	9	3	7	1	3	33	3
2	8	8	1	3	4	29	7
3	7	7	3	7	2	27	1
4	5	5	5	5	7	22	6
5	5	1	4	4	1	22	4
6	4	4	2	5	6	16	8
7	3	5	8	8	5	14	2
8	2	9	5	2	8	12	5
9	1	2	9	9	9	5	9
Technique[b] score		8	9	10	4		
Job[c] rank	Point score	Job category					
1	8	2	1	2	2	30	2
2	7	7	4	7	7	24	7
3	6	6	2	3	3	21	3
4	5	8	3	4	5	18	4
5	4	3	5	5	4	16	5
6	3	5	7	1	1	15	1
7	2	4	6	6	8	11	6
8	1	1	8	8	6	9	8
Technique score		5	7	14	12		

a - Rank score = sum of point scores for occurrences at each rank of the various techniques.

b - Technique score = 2 points if calculated rank = observed rank; 1 point if calculated rank occurs in the same division as (upper or lower 1/3) the observed rank.

c - Job categories: 1 - weighers, 2 - annex workers, 3 - laborers, 4 - transport within elevator, 5 - maintenance, 6 - inspectors, 7 - equipment operators, 8 - supervisors.

Table 23. Weight of dust on dessicated and non-dessicated filters following an eight-hour sampling of laboratory air[a]

Sampling location	Date	Initial weight	Final weight	Dust weight
		Non-dessicated Filters[f]		
1[b]	7/19/78	.03923	.03923	.00048(+)
2[c]	8/02/78	.04030	.04038	.00008(+)
2	8/18/78	.03598	.03612	.00014(+)
2	8/10/78	.03972	.03975	.00003(+)
2	8/16/78	.03505	.03505	0
3[d]	7/21/78	.03158	.03128	.00030(-)
3	7/31/78	.03288	.03108	.00180(-)
3	8/01/78	.03375	.03179	.00195(-)
$\bar{x}$				.00042(-)g
s = .00093g		$S_{\bar{x}}$ = .00033g		
		Dessicated Filters[e]		
1	10/18/78	.03291	.03255	.00036(-)
1	10/24/78	.03222	.03192	.00030(-)
1	10/25/78	.02860	.02892	.00032(+)
1	10/26/78	.02981	.02966	.00015(-)
1	10/27/78	.02986	.03000	.00014(+)
1	10/31/78	.02909	.02921	.00012(+)
1	11/16/78	.03098	.03098	0
1	11/07/78	.03063	.03065	.00002(+)
1	11/14/78	.03008	.03038	.00030(+)
1	11/15/78	.03045	.03000	.00045(-)
$\bar{x}$				.00004(-)
s = .0027g		$S_{\bar{x}}$ = .00009g		

a - Sample taken of air in Room 694 Russell Laboratory, UW-Madison.
b - Muriel Whidden's desk.
c - Rodney Caldwell's desk.
d - Transfer chamber.
e - Dessication began 10/6/78; weights determined by Sartorius balance.
f - Weights determined by Mettler balance.
g - Weights expressed grams.

light dust loads were below the sensitivity of our balance, we compared dust weights on the various non-sterilized, non-dessicated filters being used as our laboratory microflora-background controls. Three of the eight filters had negative dust weights, four were positive, and one did not change (Table 23). The mean dust weight was negative ((-) 0.00042 $\pm$ 0.00033g).

Since moisture changes may have played a role in sample variation, ten filters were weighed (using a newer Mettler analytical balance) and dessicated for various lengths of time before sampling laboratory air and reweighed (Table 23). Four filters lost weight, one did not change and five gained weight. The mean weight change was negative (0.00004 $\pm$ 0.00009g). Using the Mettler balance again, 210 filters were weighed, dessicated for two weeks and reweighed (without passing laboratory air through the filter). Ten of these were returned to the dessicator and reweighed after four months. After two weeks, 10 filters had lost weight, two remained the same and eight gained weight (Table 24). The mean weight change was again negative ((-) 0.00003 $\pm$ .00003g) after two weeks dessication. After four months dessication weight changes were still negative ((-) 0.00005 $\pm$ 0.00007g).

In a more comprehensive trial, this time using both balances, the variability of the respective balances was compared with the possible effects of ethylene oxide sterilization and eight-hour air flow (relatively dust free) upon subsequent filter weight changes. Aluminum foil standards were weighed six times on each balance over a nine-day period. Between weighings samples were held in clern sterile glass petri plates. Standard deviations, standard errors of the mean and coefficients of variation determinations were five times greater with the Sartorius balance than with the Mettler (Table 25). In addition the Sartorius balance, on the average, yielded 0.5 mg heavier values than did the Mettler. Non-dessicated filters, similarly handled, were weighed four times over a 13-day period. Standard deviations, standard errors of the mean and coefficients of variation were virtually identical to those obtained for aluminum foil (Table 26). The average difference between the two balances was again .05 mg.

Ethylene oxide treatment resulted in a significant loss in apparent filter weight as contrasted to the untreated controls (Treatments A and E) when weight changes were based on the initial determination made following ethylene oxide treatment and reweighed again after the eight-hour air flow (Treatment D_2).

This loss in weight was also apparent when comparisons were based on same filter weight determinations made after ethylene oxide treatment but before the eight-hour air flow (Treatment D_1) or between the initial weight (before sterilization and air flow) and the final weight (Treatment D_3). These conclusions were the same regardless of the balance utilized. However, when weight change observations were based on filter weighings, made before ethylene oxide sterilization, without aeration (Treatment D) prior to air flow, the weight changes as determined by the Sartorius balance indicated significant weight gains over the untreated controls (Treatment A.05 and Treatment E .01). However, no significant changes were observed in the results with the Mettler balance for this comparison (Table 27).

Table 24. Change in filter weight following dessication for two weeks and four months

Filter number	Initial weight	Final weight	Weight[a] change
		Two weeks	
1	.03096[b]	.03085	.00011(-)
2	.03159	.03142	.00017(-)
3	.03320	.03300	.00020(-)
4	.03297	.03310	.00013(+)
5	.03260	.03263	.00003(+)
6	.03075	.03077	.00002(+)
7	.03173	.03186	.00013(+)
8	.02961	.02968	.00007(+)
9	.03009	.03019	.00010(+)
10	.03192	.03187	.00005(-)
11	.02991	.02995	.00004(+)
12	.02889	.02873	.00016(-)
13	.02855	.02812	.00043(-)
14	.02894	.02903	.00009(+)
15	.03207	.03207	0
16	.03085	.03085	0
17	.03215	.03200	.00015(-)
18	.02921	.02908	.00013(-)
19	.02839	.02835	.00004(-)
20	.02921	.02891	.00030(-)
$\bar{x}$			.00003(-)
s = .00015g		$S_{\bar{x}}$ = .00003g	
		Four months	
6	.03075	.03070	.00005(-)
7	.03173	.03162	.00011(-)
8	.02961	.03000	.00039(+)
9	.03009	.03000	.00009(-)
10	.03192	.03170	.00022(-)
16	.03085	.03098	.00013(+)
17	.03215	.03180	.00035(-)
18	.02921	.02918	.00003(-)
19	.02839	.02852	.00013(+)
20	.02921	.02986	.00025(-)
$\bar{x}$			.00005(-)
s = .00022g		$S_{\bar{x}}$ = .00007g	

a - Weights determined by Mettler balance.

b - Weight expressed in grams.

Table 25. Average weights of aluminum-foil test samples as measured with the Sartorius or Mettler analytical balances[a]

Sample number	Sartorius[b] (S)				Mettler[b] (M)				S-M	
	Average weight	s	$S_{\bar{x}}$	C.V.	Average weight	s	$S_{\bar{x}}$	C.V.	s	$S_{\bar{x}}$
1	60	8.8	3.6	14.7	49	0.5	0.2	0.9		
2	132	6.3	2.6	4.7	129	2.4	1.0	1.9		
3	274	16.3	6.7	6.0	259	1.5	0.6	0.6		
4	536	15.1	6.2	2.8	515	1.2	0.5	0.2		
5	1042	13.5	5.5	1.3	1024	1.8	0.8	0.2		
6	2137	12.7	5.2	0.6	2129	1.7	0.7	0.1		
7	4141	16.5	6.7	0.4	4125	2.9	1.2	0.1		
8	8159	14.1	5.8	0.2	8159	6.6	2.7	0.1		
9	16397	13.6	5.6	0.8	16405	5.1	2.1	0.0		
10	32711	20.6	8.4	0.6	32746	4.0	1.4	0.0		
$\bar{x}$		13.8	5.6	3.2		2.8	1.0	0.4	11.0	4.6

a - Foil was weighed on 12/19/79, 12/20/79, 12/21/79, 12/26/79, 12/27/79, and 12/28/79. Means are in average of the 6 weighings.

b - Filter weights, standard deviations (s, $S_{\bar{x}}$) expressed as 1/100 mg; coefficient of variability (C.V.) expressed as %.

Table 26. Average weight of non-dessicated filters as measured with the Sartorius or Mettler analytical balances[a]

Filter number	Sartorius[b] (S)				Mettler[b] (M)				S-M	
	Average weight	s	$S_{\bar{x}}$	C.V.	Average weight	s	$S_{\bar{x}}$	C.V.	s	$S_{\bar{x}}$
1	3322	17.5	8.8	5.5	3308	4.4	2.2	1.4		
2	3410	9.9	5.0	2.4	3394	5.5	2.8	1.4		
3	3344	5.6	2.8	1.6	3337	2.7	1.4	0.8		
4	3350	8.5	4.2	2.4	3343	2.5	1.3	0.7		
5	3365	6.9	3.4	1.9	3356	3.5	1.8	1.0		
6	3408	11.1	5.6	2.7	3414	1.4	0.7	0.3		
7	3390	25.1	12.5	6.4	3397	3.1	1.6	0.8		
8	3857	22.0	11.0	2.6	3853	4.4	2.2	0.5		
9	3451	24.3	12.2	5.4	3452	1.7	0.9	0.4		
10	3244	10.4	10.4	4.3	3237	6.2	3.1	2.6		
$\bar{x}$		14.1	4.1	3.2		3.5	1.8	1.0	10.6	5.3

a - Filters weighed on 12/20/79, 12/21/79, 12/26/79, and 2/2/80. Were stored in sterile glass petri dishes at room temperature. Means are an average of the four weighings.

b - Filter weights, standard deviations (s, $S_{\bar{x}}$) expressed as 1/100 mg; coefficient of variability (C.V.) expressed as %.

No differences were observed between the means for treatments B, D, and E when contrasted to the untreated control (A) with either balance. Treatment C (ethylene oxide + air flow) values are significantly greater than those for treatments A, D, and E (no ethylene oxide + air flow) with the Sartorius balance and suggested that the combined ethylene oxide sterilization plus eight-hour air flow resulted in an increase in weight. Without air flow (Treatment B) this effect was much less. These effects were only slightly significant with values obtained with the Mettler. Standard deviations, standard errors of the means and coefficients of variability for each of the balances in this trial were almost identical to those obtained with the aluminum foil and unused filter controls (Table 25 & 26).

Bioassays for possible residual toxicity of filters and filter pads sterilized with ethylene oxide against Penicillium cyclopium were entirely negative. This test was probably not conclusive, however, since it seems probable that low levels of ethylene oxide or its reaction products may have been present in or absorbed to the cassette, the filter or the filter pad, which, over the long period between sampling and processing, were sufficiently concentrated to cause the observed death of fungus spore and bacterial cells.

d. Grain worker challenge study

Dust weights in the challenge studies were very high. Respirable dust averaged 68 mg/m^3 of sampled air and total dust averaged 443 mg/m^3 of sampled air (Table 28). Dust weights in the second series were similar. The challenge dust levels exceeded by many fold the higher dust concentrations recorded in the elevator studies (e.g. 5 mg/m^3 for respirable dust or 10 mg/m^3 for total dust). Viable propagule counts were correspondingly high and total Aspergillus counts in one case exceeded 2.0 x 10^7 propagules/m^3 of sampled air (Table 28). Penicillium concentrations were generally lower than Aspergillus levels, and bacterial counts generally exceeded the fungus counts. Bacterial counts as high as 9.3 x 10^7 were recorded for one total dust sample. Of the Aspergillus species detected, A. glaucus generally exceeded the others by a wide margin (Table 29). Total dust counts as high as 4.2 x 10^7 A. glaucus spores/m^3 were recorded. In all samples P. cyclopium was the most commonly encountered Penicillium and total dust counts were recorded up to 9.9 x 10^6 spores/m^3. Counts of A. versicolor were more variable but occasionally were as high as 1.7 x $10^6/m^3$. In one instance, P. viridicatum spore counts of 2.7 x $10^6/M^3$ were found in a total dust sample.

e. Scanning electron microscopy

Scanning electron micrographs were prepared from a representative number of respirable dust, total dust or pure culture control samples (Table 30). A list of the total inventory of micrographs taken is given in the Appendix. Direct examination of grain dust by scanning electron microscopy, in some respects, proved to be the most revealing of all the studies. Problems of interpretation were large and many of the objects, particularly items of debris, were not readily identifiable. The irregular matrix of the Gelman Metricel filter (0.8mm pore size) is shown unobscured at high magnification (1 x 10^4) in Fig. 1. This view was taken of a clean portion of the relatively dust-free filter obtained during the shift of maintenance worker No. 138 and

Table 27. The effect of balance variability, ethylene oxide sterilization and eight-hour air flow upon changes in filter weights

Treatment[a]	Number filter	Sartorius[b] Weight change	s	$S_{\bar{x}}$	C.V.	Mettler[b] Weight change	s	$S_{\bar{x}}$	C.V.	S-M s	S-M $S_{\bar{x}}$
A	10	11.0(+)	16.9	5.4	15.3	1.0(+)	2.7	0.9	2.7		
B	10	16.4(+)	13.6	4.3	11.7	1.7(-)	3.0	0.9	2.9		
C	20	24.9(+)	14.7	3.3	11.7	1.5(-)	4.0	0.9	4.1		
D_1	30	5.9(+)	17.4	3.2	16.4	1.1(+)	6.0	1.1	5.7		
D_2	30	3.3(-)	16.8	3.1	17.3	3.4(-)	6.3	1.1	6.5		
D_3	30	2.6(+)	15.4	2.8	15.0	2.3(-)	6.6	1.2	6.7		
E	30	12.4(+)	15.8	2.8	14.0	0.3(-)	4.4	1.0	4.4		
$\bar{x}$			15.8	3.6	14.5		4.7	1.0	4.7	11.1	2.5

Analysis of variance -- LSD calculations of significant differences between Sartorius derived means[c]

Range	Range	Range	Range
A-B + 5.4ns			
A-C + 13.9**	B-C + 8.5ns		
A-D_1 - 5.1ns	B-D_1 - 10.5ns	C-D_1 - 19.0***	
A-D_2 - 14.3***	B-D_2 - 19.7***	C-D_2 - 28.2***	D_1-D_2 - 9.2**
A-D_3 - 8.4ns	B-D_3 - 13.8**	C-D_3 - 22.3***	D_1-D_3 - 3.3ns
A-E + 1.4ns	B-E - 4.0ns	C-E - 12.5***	D_1-E + 5.9ns
D_2-D_3+ 5.9ns			
D_2-E + 15.7***	D_3-E + 9.8**		

Table 27. (Continued)

Range	Range	Range	Range
A-B - 2.7ns			
A-C - 2.5ns	B-C + 0.2ns		
A-D_1 + 0.1ns	B-D_1 + 2.7ns	C-D_1 + 2.6**	
A-D_2 - 4.4***	B-D_2 - 1.7ns	C-D_2 - 1.9ns	D_1-D_2 - 4.4***
A-D_3 - 3.3*	B-D_3 - 0.6ns	C-D_3 - 0.8ns	D_1-D_3 - 3.4**
A-E - 1.3ns	B-E + 1.4ns	C-E + 1.2ns	D_1-E - 1.4ns
D_2-D_3+ 1.1ns			
D_2-E + 3.1***	D_3-E + 2.0ns		

a - Treatments were as follows:

A. Filter weighed, loaded in cassette, shipped to Morgantown, W.Va., returned to Madison.

B. Filter weighed, loaded in cassette, ethylene oxide sterilized, shipped to Morgantown, W.Va., returned to Madison, reweighed.

C. Filter weighed, loaded in cassette, ethylene oxide sterilized, shipped to Morgantown, W.Va., eight-hour air flow, returned to Madison, reweighed.

D. Filter weighed (1) loaded in cassette, ethylene oxide sterilized, reweighed (2), loaded in cassette, shipped to Morgantown, W.Va., eight-hour air flow, returned to Madison, reweighed (3).

D_1 = (1-2), D_2 = (2-3), D_3 = (1-3)

E. Filter weighed, loaded in cassette, shipped to Morgantown, W.Va., eight-hour air flow, returned to Madison, reweighed.

b - Filter weights, standard deviations (s, $S_{\bar{x}}$) expressed as 1/100 mg; coefficient of variability (C.V.) expressed as %.

c - ns = not significant
* = significant at .05
** = significant at .025
*** = significant at .01.

Table 28. Viable counts from respirable and total grain dust samples collected during 1979 grain worker challenge studies[a,b]

Patient	Date	Dust load (g/m^3)	Aspergillus	Penicillium	Cladosporium	Mucorales	Yeasts	Bacteria
				Respirable dust				
1	3/7	0.0871	378,118	49,706	0	7,101	335,513	252,073
2	3/13	0.0448	151,677	20,794	0	0	165,132	146,794
3	3/15	0.0532	120,748	42,461	0	10,615	152,328	533,430
4	3/20	0.0356	42,441	4,446	0	0	36,782	203,373
5	3/27	0.2048	206,446	67,051	0	0	268,204	1,207,557
6	3/27	0.0193	120,275	12,196	0	0	170,386	143,120
7	3/29	0.0193	114,171	34,051	0	8,012	180,270	70,504
8	5/22	0.0451	12,695,427	7,783,569	0	0	0	45,062,769
9	5/30	0.1422	20,903,872	5,143,016	165,903	0	0	20,737,968
10	6/5	0.0480	9,280,000	2,456,471	0	0	0	11,600,000
11	6/14	0.0247	4,820,530	3,104,748	0	0	0	10,621,506
	Mean	0.0678						
				Total dust				
1	3/7	0.5756	61,095	16,120	0	0	14,830	457,555
2	3/13	0.1634	1,246,304	82,729	0	4,298	224,550	3,076,924
3	3/15	0.3926	647,246	145,738	0	0	702,969	3,429,052
4	3/20	0.3177	146,280	41,400	0	0	263,111	144,596
5	3/27	0.7283	434,278	46,220	0	0	766,483	332,607
6	3/27	0.1503	132,875	48,014	0	0	14,830	457,555
7	5/22	0.2784	27,212,108	5,619,892	0	0	0	72,467,027
8	5/30	0.8391	43,533,382	13,326,545	0	148,072	0	51,085,091
9	6/5	0.4231	25,945,231	7,783,569	0	0	0	45,062,769
10	6/14	0.5600	31,208,129	13,269,600	163,882	0	0	93,378,667
	Mean	0.4429						

a - Patients received pooled airborne grain dust from Duluth-Superior grain elevators.

b - Challenge studies carried out with volunteer grain workers dosed under controlled laboratory conditions by do Pico, et al. in the Dept. of Preventive Medicine, University of Wisconsin, Madison.

Table 29. Viable Penicillium and Aspergillus species from respirable and total dust samples collected during 1979 grain worker challenge studies[a,b]

Patient	Date	Dust load (mg/m^3)	Penicillium[c]				Aspergillus[d]				
			bre	cyc	urt	vir	fla	fum	gla	nid	vir
						Respirable dust					
1	3/7	0.0871	0	31,954	1,775	10,651	31,954	21,302	277,381	15,977	1,775
2	3/13	0.0448	0	18,348	0	2,446	23,241	6,116	88,070	6,116	2,446
3	3/15	0.0532	0	35,826	0	1,327	14,596	9,288	72,980	13,269	1,327
4	3/20	0.0356	0	3,638	0	808	2,910	2,829	16,796	2,425	0
5	3/27	0.2048	0	52,935	1,764	10,587	19,762	8,822	144,692	22,938	7,058
6	3/27	0.0410	0	10,643	1,370	0	18,272	1,370	65,322	0	0
7	3/29	0.0193	0	22,434	0	2,003	22,033	8,012	44,066	10,015	4,807
8	5/22	0.0451	0	835,225	0	0	417,613	0	9,354,525	0	1,670,451
9	5/30	0.1422	0	3,318,075	165,904	1,493,134	331,807	0	18,581,219	0	0
10	6/5	0.0480	136,471	2,183,529	0	0	0	0	9,007,058	0	222,941
11	6/14	0.0247	0	2,451,116	0	490,223	0	0	4,657,122	653,631	1,307,262
Mean		0.4429									
						Total dust					
1	3/7	0.5756	0	10,962	3,224	0	12,251	0	38,527	8,382	0
2	3/13	0.1634	0	71,985	1,074	6,446	257,856	38,678	713,401	137,523	4,298
3	3/15	0.3926	8,573	94,301	17,146	25,718	120,019	0	390,062	34,291	25,718
4	3/20	0.3177	1,932	35,052	0	3,588	13,800	828	89,231	17,421	5,520
5	3/27	0.7283	0	37,935	0	2,287	80,868	13,859	240,249	30,005	2,287
6	3/27	0.1503	0	37,964	0	8,933	14,516	5,583	100,494	6,700	5,538
7	5/22	0.2784	0	5,176,216	295,784	0	0	0	26,768,432	0	295,784
8	5/30	0.8391	0	9,920,872	592,290	2,665,309	592,291	0	42,496,873	0	148,027
9	6/5	0.4231	0	6,008,369	273,108	1,228,984	409,662	0	25,399,015	0	0
10	6/14	0.5600	0	9,829,333	327,644	2,457,333	327,644	0	29,651,822	0	819,111
Mean		0.4429									

a - Patients received pooled airborne dust from Duluth-Superior grain elevators.

b - Challenge studies carried out with volunteer grain workers dosed under controlled laboratory conditions by do Pico, et al. in the Dept. of Preventive Medicine, University of Wisconsin, Madison.

c - bre = P. brevicompactum, cyc = P. cyclopium, urt = P. urticae, vir = P. viridicatum.

d - fla = A. flavus, fum = A. fumigatus, gla = A. glacus, nid = A. nidulans, ver = A. versicolor.

Table 30. Scanning electron micrographs of grain dust

Worker number	Elevator	Job[a]	Grain dust exposure	Weight of dust on filter (mg/m^3)
138[c]	1	5	General dust & sunflower	-0.78
38[c]	4	3	Durum & spring wheat	0.26
135[c]	4	3	Variety of grains	4.92
300[c]	4	3	Barley	0.80
252[c]	4	4	Vareity of grains	0.55
230[c]	4	4	General dust	0.63
168[b]	4	6	Sunflower & spring wheat	0.70
165[c]	5	6	Sunflower	0.00
94[c]	8	5	Durum & Spring wheat	0.05
264[b]	4	6	Variety of grains	0.97

a - Job categories: 3 - laborer, 4 - transport within elevator, 5 - maintenance, 6 - grain inspector.

b - Total dust sample.

c - Respirable dust.

serves as a control. Fig. 2 shows debris and spores (1×10^3) scattered over the moderately contaminated filter of worker No. 300 (laborer). Higher magnification of this same filter (3×10^3) shows a "T" shaped hyphae fragment (lower right) below conidia of Pestalotia (note the fine conidial appendages) (Fig. 3). Figures 4-6 show at increasing magnification (1×10^3, 3×10^3 and 1×10^4), the very heavily contaminated filter of worker No. 135 (laborer). The filter pores have been obscured by the collection of hyphal fragments, spores and miscellaneous debris.

The diverse nature of material collected on the filters is revealed in the series of micrographs taken of worker No. 252 (transport within elevator) filter with a moderate dust load (Fig. 7-12). Spiny Aspergillus spores are visible in Figures 7, 8 and 11 (see arrows). Cladosporium and Ustilago spores are present in Figures 9 and 12. The sharply angular "projectile" seen in Fig. 10 is unidentified, but could be an insect fragment (setae) or possibly a small higher plant trichome. Uneven distribution of spores and debris is apparent in Figures 13, 14 and 15 taken from the moderately contaminated filter of worker No. 230 (transport within elevator). A Ustilago spore (see arrow) is present in the center of Fig. 14. Also present are large particles of debris exceeding 15 µm in length (Fig. 14 and 15). Large particles are also present on the filters from the total dust samples of worker No. 168 (grain inspector) and worker No. 264 (grain inspector) (Fig. 16 and 17). A chain of Penicillium (or Aspergillus) spores appear disturbed, probably due to dessication (Fig. 18). Very fine particles of unknown origin are also present on this filter (Fig. 18) and can be seen at higher magnification in Fig. 19. Fig. 20 shows an unusual spiny particle found on the filter of worker No. 16 (laborer) at 3×10^3. A very fine particulate was also present on the filter of worker No. 38 (laborer) and is shown in Fig. 21 at 3×10^3. At higher magnification (1×10^4) these particles can be seen with as a spiny Aspergillus spore (left) and a collapse Ustilago spore (right) (Fig. 22).

For purposes of identification control filters were artificially seeded with conidia from pure cultures of the prinicipal fungi encountered in the viable counting procedure. These were processed for SEM in the same fashion as the grain dust filters. Spores of P. cyclopium (Fig. 23) and P. viridicatum (Fig. 24) are shown at 1×10^4 magnification. The similarity indicates the problems in separation of Penicillium species by the direct counting procedure.

2. Settled Dust Samples.

The mean concentrations and frequencies of spores and propagules from settled dust samples were extremely high for all categories (Table 31). Mean counts for bacteria, yeasts, Cladosporium and "others" were well over two million spores/g of settled dust. Individual samples contained as many as 2.4×10^8 bacterial cells/g while other samples contained as many as 4.0×10^7 viable Cladosporium spores/g of dust. The total propagule counts for the six elevators sampled, while variable, were not remarkably different from each other and differences between sampling sites within elevators were as large as between elevators.

B. Small Animal Toxicity Trials

Table 31. Concentrations and frequencies of viable flora from settle dust (spores/g of dust)

Organism	Settled dust (n = 12) Mean ± SEM	Range Min.	Max.	% Freq.
Aspergillus (total)	544,167 ± 159,324	10,000	1,600,000	100
versicolor	11,667 ± 8,242	0	100,000	33
nidulans	833 ± 833	0	10,000	8
glaucus	425,000 ± 131,111	60,000	1,400,000	100
fumigatus	10,000 ± 8,257	0	100,000	25
flavus	33,333 ± 12,268	0	100,000	67
Penicillium (total)	671,667 ± 235,902	50,000	2,700,000	100
cyclopium	496,667 ± 217,720	30,000	2,600,000	100
viridicatum	87,500 ± 32,570	0	400,000	83
urticae	10,833 ± 8,207	0	100,000	33
chrysogenum	0 ± 0	0	0	0
brevi-compactum	20,000 ± 10,372	0	100,000	33
Cladosporium	4,143,600 ± 3,336,535	10,000	40,600,000	100
Mucorales	23,033 ± 16,521	0	200,000	33
Alternaria	80,833 ± 25,627	0	300,000	83
Yeasts	2,355,000 ± 1,838,920	800,000	6,800,000	100
Bacteria	107,558,333 ± 18,581,648	31,700,000	238,000,000	100
Others	2,075,000 ± 1,327,807	0	16,300,000	92
Total	117,451,633 ± 25,522,284			

1. Settled Dust.

With its extremely high fungus and bacterial population, settled grain dust should have proved to be highly toxic when extracted and fed to experimental animals. However, this proved not to be the case. No deaths resulted when rats were administered extracts of three typical settled dust samples or extracts from combined settled dust samples (from all the elevators) (Table 32). These same combined settled dust samples had induced grain fever-like symptoms in exposed grain workers in the challenge studies carried out in the University of Wisconsin Medical School by do Pico, et al. (Table 28 & 29).

To determine the mutagenic potential of settled grain elevator dust, one sample was extracted with ethyl acetate and the extracted residue subjected to the Ames - Salmonella test (Ames, et al., 1975). Four tester strains (TA 98, TA 100, TA 1535, and TA 1538) of Salmonella typhimurium were utilized in combination with activation using rat lung microsomes. The solvent-free residue was dissolved in either ethanol or dimethyl sulfoxide (DMSO) at concentrations of 1:10, 1:100, and 1:10,000. Mutagenic activity above the background counts were only observed with strain TA 98. High order mutagenicity was therefore not indicated, at least for the dust sample selected for this trial.

2. Pure Cultures.

Pure cultures of the major fungi isolated from grain dust, however, proved to be somewhat more toxic than the grain dust. Extracts of both isolates of Penicillium viridicatum and P. utricae were lethal to all four incubated rats in the trial (Table 32). Extracts of one isolate of Aspergillus nidulans killed all four rats, while extracts from the other isolate killed three of four rats. All rats that received extracts of A. fumigatus began trembling within 15 minutes after administration. These tremors had subsided by the following morning, but began again if any movement was attempted. Two of three rats administered one isolate and two of four rats administered the other subsequently died. Extracts from A. glaucus and A. flavus were less toxic. Only one of four rats, respectively, died following administration of extracts from one isolate of each and the other isolates proved to be non-toxic. The extracts from the two isolates of P. cyclopium were not lethal to rats when the cultures were incubated at room temperatures. However, when incubated at 12C, the extract of one isolate killed three of four rats.

Although, not all rats were killed by the extract of A. glaucus 88, and none were killed by either isolate of P. brevi-compactum, the rats failed to gain weight during the test period.

In preliminary studies concerning the possible identity of mycotoxins present in extracts from the various cultures from grain dust, we were not able to confirm the presence of known mycotoxins by standard TLC chromatography. We have not continued this line of investigation because of the absence of toxicity in the settled grain dust.

Table 32. Effects of grain dust and fungal extracts on rats

Extract	Number Died/Total	Mean weight change	SD
Settled dust 15	0/4		
Settled dust 17	0/4		
Settled dust 20	0/4		
Control	0/4		
P. cyc 4	0/5	23.0 ±	40.5
P. cyc 194	0/4	29.1 ±	2.0
P. vir 63	4/4	- 3.9 ±	0.6
P. vir 99	4/4	- 3.0 ±	4.0
Asp nid 68	4/4	- 5.4 ±	4.6
Asp nid 420	3/4	- 0.4 ±	0.6[1]
Asp vers 66	0/4	26.7 ±	17.2
Asp vers 147	0/4	21.3 ±	8.0
Control	0/4	28.3 ±	1.2[2]
Asp fum 35	2/3	-10.9 ±	34.9[3]
Asp fum 153	2/4	- 7.5 ±	0.5[4]
Asp glaucus 88	1/4	6.9 ±	47.1[5]
Asp glaucus 366	0/4	29.8 ±	44.0
P. urt 25	4/4	-10.8 ±	30.5
P. urt 54	4/4	- 8.4 ±	7.2
P. brevi 69	0/4	4.5 ±	193.3
P. brevi 99	0/4	7.8 ±	1.6
Control	0/4	34.4 ±	10.6
Asp flav 23	1/4	22.2 ±	32.2[6]
Asp flav 164	0/4	31.6 ±	19.8
Reacting dust[8]	0/4	28.4 ±	23.8
Control	0/4	32.8 ±	6.1
P. cyc 4*	3/4	- 3.6 ±	1.4[7]
P. cyc 194*	0/4	33.6 ±	27.2
Asp vers 66*	0/4	29.7 ±	42.1
Asp vers 147*	0/4	34.3 ±	7.1
Control*	0/4	31.9 ±	17.6

1 - Average of the 3 rats that died; 4th rat gained 11g. Average of all 4 rats = 2.4±25.0.
2 - One rat was killed on day 1 for comparison to those dying in other groups. Average is of the 3 rats at the end of the experiment.
3 - Average of the 3 rats that died; 4th rat gained 27.7g. Average of all 4 rats = 4.2±184.5.
4 - Average of 2 rats that died; 2 rats survived and gained 9.9g. 0.01. Average of all rats = 4.3±62.0.
5 - Average of 3 rats that survived; 4th rat died and lost 14.2g. Average of all 4 rats = 1.6±119.1.
6 - Average of 3 rats that survived; 4th rat died and lost 8.8g. Average of all 4 rats = 14.4±203.9.
7 - Average of the 3 rats that died; 4th rat gained 27.7g. Average of all 4 rats = 4.2±184.5.
8 - Grain dust challenge study.
* - Incubation was at room temperature for 1 wk and 12C for 2 wks rather than 2 wks at room temperature.

C. Entomological Analysis of Grain and Grain Dust

Very few whole insects (either adults or larvae) or insect fragments were present in the 246 bulk grain samples collected from the various elevators. No insect species predominated in the 11 elevator grain samples which contained whole adult insects or larvae. Most were common storage insects belonging to the order Coleoptera and included the granery weevil (Sitophilus granarius), the rice weevil (Sitophilus oryzae), the lesser grain borer (Rhizopertha dominica), the thirteen-spotted lady beetle (Hippodamia tredecimpunctata). Clearly none of the bulk grain samples showed any indication of heavy insect infestation and no doubt reflected the heavy frequent use of grain fumigants (Table 33 * VIII). About one-fourth (66) of the bulk grain samples contained small numbers of whole insects, mites or insect fragments (varying from 1 to 5/sample). Of the 129 whole insect or insect fragments found in these samples 32% belong to species in the order Orthoptera, 24% belong to representatives of the Coleoptera, while 41% (mostly small fragments) could not be classified. Clearly most of the insect fragment contamination was of field origin and not associated with storage insect infestation.

Durum wheat, oats and sunflowers appeared to be somewhat more contaminated with insect fragments than the other grains, possibly reflecting the fact that these commodities had just/or were just entering the elevators from the field. Spring wheat while containing the most fragments also comprised the largest number of samples examined and individual sample contamination was relatively lower. Only 10 of the 144 settled dust samples contained live insects or their fragments and seven of the 10 contained larvae of the yellow meal worm (Tenebria monitor) (one the three larvae per 1/2 liter sample). Dust samples with live insect contamination were restricted to five of the eight elevators sampled (Table VIII).

The following insects and mites were furnished as pure cultures (contained fresh frozen in petri dishes) to the University of Wisconsin Department of Preventive Medicine for use in their immunological studies related to grain worker health:

1. Sitophilus granarius (granary weevil)
2. Tribolium confusum (confused flour beetle)
3. Trogoderma glabrum - adults
4. Trogoderma glabrum - larvae
5. Attagenus megatoma - adults (black carpet beetle)
6. Attagenus megatoma - larvae
7. Tyrophagus putrescentiae (mold mite)

These and some other stored product pest species are available in pure culture from the University of Wisconsin Department of Entomology.

IV. DISCUSSION

Bacteria and yeasts were frequently the most numerous organisms present in respirable grain dust in our studies as determined by the viable counting procedures. These results were consistent with those reported for grain

Table 33. Summary of whole insect or insect fragments detected from 1977 Duluth-Superior grain elevator bulk grain samples[a]

Grain	Total no. of samples	Insect order[b]						
		Orthoptera	Coleoptera	Hemiptera	Diptera	Lepidoptera	Not classified	Total
Spring wheat	104	20	11	1	0	1	19	52
Barley	48	3	7	0	0	0	8	18
Durum wheat	36	7	6	0	0	0	13	26
Oats	30	8	3	0	0	0	11	22
Sunflower	11	0	3	1	1	0	3	8
Flax	10	0	0	0	0	0	0	0
Rye	4	4	0	0	0	0	0	4
Corn	2	0	0	0	0	0	1	1
Mixed	1	0	0	0	0	0	0	0
Total	246	42	30	2	1	1	55	131
%	100	17.1	12.2	0.8	0.4	0.4	22.4	53.3

a - Bulk 0.946 ℓ samples were collected at various places within the elevators during the work day.

b - Individual samples often contained several fragments and frequently were from more than one order.

elevators in Poland (Dutkiewicz, 1978a & b). Their presence in such abundance is a reflection of the occurrence of moist grain or moist storage conditions at some point from harvest, transport, cleaning to storage. Since Dutkiewicz (1978a) reported that 90% of the epiphytic microflora on grain could be removed during routine cleaning, it is possible that these high counts reflect the intense grain cleaning operations in progress in October and November at the times our samples were collected. The role of bacteria and yeasts as contributing factors in grain elevator worker health problems clearly cannot be discounted and further studies on their role in the complex problem are indicated.

The most prevalent fungi in grain dust in our studies were species of Aspergillus, Penicillium and Cladosporium and they occurred in more or less that order of frequency. Most of the species were common storage fungi, such as A. glaucus, P. cyclopium whose occurrence might have been predicted on the basis of earlier studies on the mycoflora of stored grain (Christensen, 1957; Mislivec and Tuite, 1970). In their study of elevators in Canada, Farant and Moore (1973) reported that Ustilago type spores were the microorganisms most frequently found in grain elevator air samples, and accounted for 70% of the total spores sampled (2.8 to 56.0 x 10^6 spores/m^3). In another report they found that Alternaria was present in 29% of the 113 samples examined (Farant and Moore, 1977). In the Duluth-Superior elevators in our studies, 17% of 103 samples contained Alternaria. Farant and Moore (1973) also found that Penicillium expansum was the most frequently isolated species and was found in 69% of the samples. The next most frequently isolated fungi were A. repens and Cladosporium (hormodendron) cladosporiodes which occurred in nearly 45% of the samples. In our study, A. glaucus (group) occurred in 83% of the samples, Cladosporium sp. in 80% and P. cyclopium in 54%. In a recent taxonomic revision of the subsection Fasciculata of Penicillium, Samson, et al. (1976) noted that P. viridicatum, P. cyclopium, and P. expansum merge imperceptibly into one another morphologically. They observed that strains classified in these species cannot be distinguished on significant morphological characters and therefore should be considered as one variable species, for which the name P. verrucosum is re-introduced. The high frequency of P. expansum over P. cyclopium as reported by Farant and Moore (1973) may be a reflection of this confusion over nomenclature.

The increased frequency of A. glaucus in our samples was probably due to the use of malt salt agar, which selects for those types of osmophilic fungi. In the Canadian studies, neither Sabouraoud nor Czapek agar would have created the osmotic stress necessary to select for osmophilic organisms. Their values no doubt underestimate the frequency of occurrence of members of the A. glaucus group. in general, however, the mean concentrations of all types of spores were less in our studies than in the Canadian study. Our dust weights were also substantially lower than those reported in the Canadian studies (Farant and Moore, 1978). They reported airborne dust levels which varied from 0.18 to 781 mg/m^3. In our studies the maximum respirable dust level encountered was 8.3 mg/m^3 and even the heaviest total dust sample encountered was only 39.1 mg/m^3. Our averages were clearly much lower, as problems with low or negative values for respirable dust weights attest. The only dust levels in our study which were close to those reported in the Canadian studies were in the grain worker challenge studies where certain workers were experimentally exposed to respirable dust levels as high as 205

mg/m^3, and total dust levels as high as 839 mg/m^3 of sampled air (Table 28 & 29).

The large difference in frequencies of Cladosporium cannot be explained by the use of malt salt agar. However, in the Canadian study, the samples were collected during February through March, while our samples were collected in October and November. Since the variability of the field fungus is reduced during storage (Christensen and Kaufmann, 1965), many Cladosporium spores may not have survived the additional four months of storage that occurred in the Canadian study. There are differences between the findings of the two studies, but the differences in methodology may account for these and neither study contradicts the other.

Respirable dust samples collected in the laboratory gave indication of the presence of low level concentrations of airborne Penicillium spores (100spores/m^3. A few other fungi were also present at very low levels (e.g. 4, 15, 23 mg/m^3). The unused filters serving as a base-line control on the product were essentially negative. The level of contamination of the control samples was therefore not sufficiently great to interfere with the overall project results.

Mean concentrations of spores, as determined by direct microscopic counts, were higher for grain workers' samples than for city workers'. However, hiwle the concentrations differed, the number of samples having at least some spores was similar for both groups. While Ustilago spores were absent from city worker samples, the mean concentration of Ustilago spores in grain workers' samples varied from 1.3×10^3 to 2.7×10^4 spores/m^3. In the Canadian study, much higher concentrations (1.0×10^6 to 2.4×10^7 spores/m^3) were reported (Farant and Moore, 1977). These differences probably reflected differences in the relative incidences of cereal smut diseases at the respective farm locations during the crop years related to the year of dust sampling.

It seems likely that a major "clean-up" took place in the various Duluth-Superior grain elevator facilities prior to the initiation of the studies on October 3, 1977. Comments by NIOSH and the University of Wisconsin Preventive Medicine personnel who had inspected the grain elevator facilities before the time of the actual sampling also indicated that this might have been the case. Similar grain dust samples from various grain elevators which we received for exploratory studies in 1975 from NIOSH had dust levels more closely resembling those present in the challenge studies (Table 28 & 29). Some of the filters we examined microbiologically at that time possessed dust layers two or more millimeters in depth. We noted, at the time, our concern that for quantitative studies, a much shorter collection time would probably be required to obtain manageable quantities of dust for microbiological analysis.

We can therefore conclude that the results of this study may not be entirely typical of the every day airborne dust levels and microflora concentrations encountered by grain elevator workers. Our results seem to reflect the airborne dust levels of relatively clean grain elevator situations. Certainly year-round sampling studies would be required to more precisely define the average airborne dust conditions encountered by grain elevator workers.

In general, the microflora in air to which grain workers and municipal workers were exposed was different as determined by both the viable and non-viable counting procedures. In both municipal and grain worker samples, the mean concentration of fungal spores was lower in viable counts than in the direct (non-viable) counts. This seemed to indicate that there were many spores present which were either no longer living, or were no longer able to grow on artificial media, and, thus, remained undetected by viable counting methods.

In our studies, it was apparent that the direct counting method had additional drawbacks. Burge, et al. (1977) in contrasting the comparative recoveries of airborne fungus spores using viable and non-viable methods had similar results. They found that as air spore levels rose, culture plate data progressively underestimated the prevailing concentrations--viable recoveries fell below 5% at concentrations above 500 spores (Cladosporium)/m^3. Although they offered no explanation for these results they concluded that even when interest (in the survey) is confined to the most prevalent taxa, neither sampling technique is adequate. It was unable to detect the large numbers of bacteria and yeasts that were frequently in samples present and detected by viable counting methods. Additionally, in direct counts the fungi could not be assigned to species (or in some cases even to specific genera with assurance) in most cases. Combining the data gathered by both methods probably provided the more accurate picture of what organisms were present in grain dust.

Our third method for evaluating the airborne contamination of grain elevator environments further supplemented the information derived from the viable and non-viable counting procedures. Scanning electron micrographs revealed in great detail the complex nature of grain dust and served to illustrate the problem encountered in making light microscopic counts of spores in the presence of large quantities of grain debris. Many particles could be recognized as spores and could even be identified to genus by their distinctive shape. However, much of the dust and debris was not identifiable as being of fungus, plant, or insect origin. Larger particles of debris were observed in the total dust sample (Fig. 16, 17). However, there were also particles present in respirable dust samples that were obviously larger than 10 µm in diameter (Fig. 10, 14 & 15). Due to the angular nature of some particles (Fig. 10 and 20) and even the spiny walls of Aspergillus spores (Fig. 11), it is quite conceivable that a direct irritant effect, as suggested by do Pico (1979), may play a role in the pathogenesis of grain worker occupational disease.

In addition to analyzing grain workers' samples as a group, the samples were grouped by the elevator in which they worked and by the job they performed on the day the samples were collected. The data unfortunately represent only one sample that was collected on one day out of the year. The sample size was small in most cases and the variation between sample size was great. Therefore, the data indicate broad general tendencies but many other factors no doubt affected the data besides the elevator job in which the person worked. Such factors would include the volume and type of grain being handled, the dust control measures in practice, and how long the worker remained in particularly dusty areas. In any case, certain elevators appear in all the various measurements (viable and non-viable counts, respirable

dust, total dust) to be relatively dirtier or relatively cleaner than the rest (Table 22). Likewise, weighers, annex workers and laborers were clearly exposed to dustier environments than their supervisors. Correlations between these data and actual worker health as determined in the companion NIOSH contract will be of interest. The direct correlation between individual respirable dust weights and total spore concentrations (derived by either method) suggested that the simpliest general indicator of potential grain worker health hazard would probably be the measurement of exposure to respirable dust. It ranking as the technique which matched most closely the final "average" rank of elevator and job (Table 22).

In previous studies, grain workers have been tested for allergic responses to fungi, insects, and mites (Darke, et al., 1976; Warren, et al., 1974; Davies, et al., 1976; Lum, 1966). However, very few recognizable mites or insect parts were found in the dust from the Duluth-Superior elevators in this study. It thus appears unlikely that these mites and insects would play a major role in their problems.

The role of fungi in grain worker occupational disease is more likely. Some previous studies have found that Alternaria, Aspergillus, Candida, Fusarium, Penicillium, yeast, rust, and smut extracts produced few if any skin reactions in grain workers (Warren, et al., 1974; do Pico, et al., 1977; Chan-Yeung, et al., 1979). However, all of these studies used commercially prepared antigens which were probably not representative of the fungus species that predominate in the grain dust that a worker actually inhales. Use of antigens prepared from the actual fungus species present in grain dust might produce more positive results.

Residues from pure cultures of Penicillium clyclopium, P. viridicatum, P. urticae, Aspergillus flavus, A. nidulans, and A. fumigatus, the most commonly encountered fungi in grain dust were lethal to incubated weanling rats. The major fungi present in respirable grain dust were clearly mycotoxin producers and no doubt contributed to the health problems of grain elevator workers. However, experimental means to test these possibilities on human subjects were not available to this project. The grain dust challenge studies of do Pico, et al. attempted to resolve this problem, but really failed to narrow the causes of grain dust fever beyond identifying certain samples of mass-collected airborne grain dust. Inhalation studies with susceptible grain workers using controlled doses of conidia from pure cultures of grain dust fungi might have accomplished this. We also prepared protein fractions from each of the major grain dust species for use in immunological studies, but ultimately our cooperators in Preventive Medicine considered human testing with materials too risky. No doubt other immunological techniques, such as the skin reactions of susceptible workers, might be feasible in getting at this problem. Possibly passive immunological techniques might be utilized in which the skin reactions of nonhuman primates treated with protein fractions derived from susceptible grain workers would identify the causal agents. Interestingly, however, although causing symptoms in aritificially exposed grain workers, settled grain dust samples collected in large quantities from Duluth-Superior grain elevators and extracted in a similar fashion to the fungus cultures failed to induce observable signs when fed to weanling rats. Obviously rodents were not a particularly useful test animal for this purpose, and no doubt even rodent inhalation studies would not be relevant.

Several factors entered into the problems of dust weight variations and sample sterility. A major factor apparently resulted from the original protocol decision to sterilize filters, pads and cassettes with thylene oxide. The requirement for initial sterility proved unnecessary since controls run later indicated minimal background contamination in relation to the high concentration of microbial propagules present in grain dust. The ethylene oxide treatment resulted in an increase in filter weight which caused an excessively high initial weight determination. This added weight was then lost during the dust sampling process. In situations with very low dust levels, final weight determination resulted in occasional negative values (Table 27). Another causal factor, aside from occasional human error, was the variability factor inherent in the older Sartorius blance used in the weight determinations. Standard deviations, while consistent over many weight determinations, proved to be somewhat greater than the manufacturers original specifications (.1 mg). Control filter weight determinations made at Morgantown using a more sensitive blance (.001 mg) contained no negative dust weights.

Another consideration, however, may have played a greater role in the variation problems than either the ethylene oxide treatment or the over-age variation problems than either the ethylene oxide treatment or the over-age electronic balance. Because changes in humidity can lead to changes in observed sample weight, OSHA states in its Industrial Hygiene Field Operation Manual that "the standard procedure should be to desiccate all filters before pre-sample and post-sample weighing." This procedure was in fact carried out with the control filter samples in this study. This procedure is no doubt very reasonable with cotton or coal dust, but its effect on delicate living fungus and bacterial cells may be lethal, and may have accounted for a portion of the eight-fold difference observed between the low viable counts and higher direct counts recorded in the municipal control samples (Table 6).

Detailed studies by Hawley and Charell (1980), however, have shown that with dry (dessicated) filters being weighed at fairly high RH, a rapid weight gain occurs within 60 seconds and this gain depends on the percent concentration of moisture in the air rather than the relative humidity or temperature. The authors' conclusions are of great relevance to the current study. They state that, "the modern electrobalance will allow the technician to obtain sample weights within 15 sec. This is at the time when the dried filter is gaining weight (from air moisture) at its greatest rate." Their studies showed that the filter absorbs at least 80% of the total water in the first 30 sec after removal from the desiccator. They conclude that, "attempts to take a reading from a balance which is in the process of following a weight change is frustrating as well as experimentally worthless." Our experiences in trying to resolve the questions of apparent negative dust weights have been similar. Several of these authors' recommendations are noteworthy: (1) the filter should <u>not</u> be desiccated unless weighings will take place in a dry box; (2) the filter should be exposed to the same concentration of air moisture during pre- and post-sample weighing because of potential erros due to water absorption; (3) sampling times should be maximized to decrease the relative weighing error where water adsorption may be a problem.

It seems clear that if dust weights are being used as a critical measure of worker health hazard, then in situations of low level dust contamination,

as was the case with most of the respirable dust samples, much longer sampling times might be considered to bring sampled dust levels above the normal standard accuracy of commonly available analytical balances (e.g. 0.1 mg).

Although attempts to duplicate the apparent lethal effects of residual ethylene oxide on sterilized filters, filter pads, or cassettes were not successful, this treatment appeared to be the cause of the problem. Tessler (1961) in studies concerning the reaction of the sterilant ethylene oxide on plastics found that ethylene oxide in standard Freon formulations caused damage to both cellulose acetate butyrate and polystyrene resin plastics. Although he did not discuss the accumulation of potentially toxic residues in such damaged plastics, such accumulation appears probable. Major changes in viable spore counts after October 27, 1977 when the ethylene oxide treated filters were no longer used cannot be discounted (Table 26).

It seems likely, in retrospect, that a preliminary "run-through" of the proposed experimental procedures, prior to carrying out the major study would have been of advantage in eliminating problems in the protocol design, which proved quite detrimental to the outcome of the study and introduced, unnecessarily, considerable variation (and uncertainty) and considerable extra costs in producing the final research data.

V. CONCLUSIONS

1. Bacteria were the most prevalent microorganisms in airborne grain dust and accounted for half of the total mean viable propagules. Their role in grain worker health problems needs investigation.

2. The most prevalent fungi in airborne grain dust were species of yeasts (one-fifth of the viable total), Aspergillus (one-eighth), Penicillium (one-eighth) and Ustilago (one-fifth of the non-viable total). Aspergillus glaucus was the most commonly encountered fungus, while Penicillium cyclopium was the most common of the Penicillia.

3. Microflora in the airborne dust of grain elevator environments was different qualitatively and quantitatively from that of the municipal worker. Total mean viable propagule counts for grain worker samples were 33 times larger than municipal worker samples.

4. Grain elevators and grain worker jobs could be ranked in terms of exposure to airborne dust and microorganisms. Certain elevators were consistently dirtier or cleaner than the rest regardless of the measurement technique utilized. Among job categories, annext workers were exposed to the greatest contributions of dust and microorganisms, while supervisors suffered the least exposure.

5. Measurement of respirable dust concentrations as a single technique gave the closet ranking estimate to the average score of all methods in both the elevator and job categories.

6. Non-viable (direct) spore counts for grain workers averaged six times greater than similar viable spore counts. Similar differences were apparent in the data from unicipal worker environments.

7. Examination of grain dust by scanning electron microscopy (SEM) proved to be a most revealing technique for examination of airborne grain dust. This technique could prove to be an indispensible tool, along with the standard methods, for assessing the health hazards of grain dust.

8. Residues from pure cultures of the major fungi present in grain (Penicillium cyclopium, P viridicatum, P. urticae, Aspergillus flavus, A. nidulans, A. fumigatus [but not A. glaucus]) were lethal to incubated weanling rats. Experimental means to test the role of these mycotoxin producers on human subjects were not available to this project.

9. Very few whole insects, mites or insect fragments were present in bulk grain or settled grain dust samples. It seemed unlikely in this situation that mites or insects played a major role in grain worker health problems.

10. Results of this investigation, as contrasted to previous work, reflect the airborne dust levels of a relatively clean grain elevator situation. It can be expected that similar studies of grain elevators in other climatic locations will yield different results and in the case of souther U.S. elevators probably drastically different results. Year-round sampling would be required to more precisely define the average airborne dust conditions encountered by grain elevator workers.

11. Variations in the results of gravimetric determinations could be attributed in part to the ethylene oxide sterilization procedures (for those filter cassettes treated in this manner0, to very low dust weights which were often close to the standard deviation values of the available electronic balances, and to rapid moisture uptake during weighing. Attempts to correct the problem using standard OSHA procedures of pre- and post-sampling desiccation for filters used in microflora determinations are unacceptable micro-biologically and measures proposed by Hawley and Charell (1980) should be considered.

12. Where airborne respirable grain dust concentrations are being used as a critical measure of worker health hazard much longer samplings periods should be used.

VII. REFERENCES

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VII. APPENDIX

Table I. Culture media

1. Czapek Solution Agar Amended with Corn Steep Liquor, Tergitol and Aureomycin (CS)

$NaNO_3$	3.0 g	Stock Solutions:
K_2HPO_4	1.0 g	Aureomoycin - 750 mg/100 ml of sterile water
$MgSO_4 \cdot 7H_2O$	0.5 g	
KCl	0.5 g	
$FeSO_4 \cdot 7H_2O$	0.1 g	Tergitol NPX - 2.5 ml in 97.5 ml of water.
Sucrose	30.0 g	Autoclave.
Corn Steep Liquor	10.0 ml	
Agar	15.0 g	
Water (distilled)	1.0 l	

Dissolve the inorganic minerals in half the water and the sucrose and agar in the other half. Bring the gar to a boil to dissolve it. Combine the minerals and agar solutions. Add the corn steep liquor. Adjust the pH to 7.0 with 5% NaOH. Autoclave 15 minutes at 15 p.s.i. When the agar is cool enough to pour, add 4 ml of the stock solution of tergitol and 4 ml of the stock solution of aureomycin per 1 of medium. Mix well. The final concentration of tergitol and aureomycin are 100 and 30 ppm, respectively. Do not reheat the medium.

2. Acidified Potato Dextrose Agar (PDA)

Potatoes (white)	250 g
Dextrose	20 g
Agar	15 g
Water (distilled)	1 l

Slice the potatoes and steam then in half of the water for 1 hour. Drain the liquid from the potatoes and save the liquid. Boil the agar in the other half of the water. Add the dextrose and potato liquid to the molten agar. Autoclave 15 minutes at 15 p.s.i.

When the agar is cool enough to pour, add 2.5 ml of a half strength solution of 85% lactic acid to 500 ml of PDA. Do not reheat medium after adding acid.

3. Malt Salt Agar (MS)

NaCl	74 g
Malt extract	25 g
Agar	25 g
Water (distilled)	1 l

Dissolve NaCl in 300 ml of water. Autoclave it separately. Dissolve malt extract and agar in the remaining 700 ml of water. Autoclave 15 minutes at 15 p.s.i. When the agar is cool enough to pour, asceptically combine the salt solution and the malt-agar solution. Do not reheat the medium.

4. Pridham Yeast Malt Dextrose Medium (A)[1]

Yeast Extract	4.0 g
Malt Extract	10.0 g
Dextrose	4.0 g
Agar	15.0 g
Water	1.0 l

Adjust the pH with 5% NaOH to 7.3. Autoclave for 15 minutes at 15 p.s.i. The pH after sterilization drops to 6.8-7.0.

5. Tryptic Soy Agar (TS)

Difco Tryptic Soy Agar	40 g
(Tryptone	15 g
Soytone	5 g
NaCl	5 g
Agar	15 g)
Water, distilled	1 l

Heat to boiling. Autoclave for 15 minutes at 15 p.s.i.

[1]Pridham, T. G., P. Anderson, C. Foley, L. A. Lindenfelsen, C. W. Hesseltine, and R. G. Benedict. 1957. Antibiotics Ann. 1956-1957:947-953.

Table II. Concentrations and frequencies of viable flora in respirable dust samples (spores/m^3)

	Grain workers (n = 109)				Municipal workers (n = 65)			
		Range				Range		
Organism	Mean±SEM	Min.	Max.	Freq.	Mean±SEM	Min.	Max.	% Freq.
Aspergillus (total)[a]	3,460±685	0	54,204	94	173±91	0	4,511	43
versicolor	446±160	0	11,793	49	0±0	0	0	0
nidulans	338±150	0	14,035	34	0±0	0	0	0
glaucus	2,109±489	0	36,735	84	0±0	0	0	0
fumigatus	434±108	0	7,974	60	0±0	0	0	0
flavus	137±44	0	4,158	35	0±0	0	0	0
Penicillium (total)[a]	2,775±662	0	45,834	79	482±271	0	16,971	57
cyclopium	1,569±396	0	23,377	55	0±0	0	0	0
viridicatum	366±92	0	7,306	47	0±0	0	0	0
urticae	151±107	0	11,830	30	0±0	0	0	0
chrysogenum	53±24	0	2,215	15	0±0	0	0	0
brevi-compactum	24±7	0	560	15	0±0	0	0	0
Cladosporium	1,037±199	0	15,225	81	85±17	0	833	65
Mucorales	54±11	0	771	34	0±0	0	0	0
Alternaria	35±11	0	984	20	0±0	0	28	2
Yeasts	5,091±1,161	0	91,323	80	4±2	0	95	6
Bacteria	11,866±3,101	0	272,338	92	0±0	0	0	0
Others	646±160	0	13,475	73	0±0	0	0	0
Total	24,962±4,510				766±360			

a - Includes other species not listed in table.

Table III. Concentrations and frequencies of direct (non-viable) counts in respirable dust samples (spores/m^3)

	Grain workers (n = 246)				Municipal workers (n = 65)			
		Range				Range		
Organism	Mean±SEM	Min.	Max.	Freq.	Mean±SEM	Min.	Max.	Freq.
Aspergillus-type	41,816±19,078	0	3,565,959	96	5,346±2,326	0	140,479	86
Ustilago	11,948±1,798	0	250,717	86	0±0	0	0	0
Mucorales	572±80	0	7,260	30	94±34	0	1,093	11
Fusarium	228±34	0	3,037	20	70±43	0	2,148	5
Cladosporium	2,183±270	0	47,268	68	400±84	0	3,141	32
Alternaria	152±60	0	12,441	7	0±0	0	0	0
Other	1,400±374	0	77,000	33	204±58	0	2,356	20
Total	58,299±19,355				6,113±2,320			

Table IVa. Concentrations and frequencies of viable flora in respirable dust samples when grouped by elevators

Organism (spores/m^3)	Elevator 1 (n = 35) Mean ± SEM	Range Min.	Range Max.	% Freq.	Elevator 2 (n = 4) Mean ± SEM	Range Min.	Range Max.	% Freq.
Aspergillus (total)[2]	5,827 ± 1,806	0	54,203	97	442 ± 282	0	1,206	75
versicolor	917 ± 454	0	11,793	54	86 ± 86	0	344	25
nidulans	934 ± 450	0	14,035	63	0 ± 0	0	0	0
glaucus	3,495 ± 1,307	0	36,735	83	233 ± 137	0	586	75
fumigatus	631 ± 209	0	5,652	74	0 ± 0	0	0	0
flavus	348 ± 132	0	4,158	57	0 ± 0	0	0	0
Penicillium (total)[a]	2,170 ± 1,039	0	32,368	73	261 ± 156	0	624	50
cyclopium	1,625 ± 780	0	23,377	60	231 ± 144	0	597	50
viridicatum	231 ± 103	0	2,826	48	18 ± 18	0	73	25
urticae	59 ± 22	0	559	34	17 ± 10	0	35	50
chrysogenum	2 ± 2	0	73	3	0 ± 0	0	0	0
brevi-compactum	24 ± 10	0	242	20	0 ± 0	0	0	0
Cladosporium	1,352 ± 492	0	15,225	82	80 ± 48	0	220	75
Mucorales	80 ± 26	0	771	41	0 ± 0	0	0	0
Alternaria	32 ± 16	0	501	18	8 ± 8	0	33	25
Yeasts	2,856 ± 1,490	0	49,966	76	429 ± 248	0	1,139	75
Bacteria	7,020 ± 2,948	0	76,346	91	2,794 ± 1,257	36	5,088	100
Others	585 ± 217	0	5,867	73	144 ± 89	0	366	50
Total	19,920 ± 6,148				4,156 ± 1,763			

a - Includes other species not listed in table.

Table IVb. Concentrations and frequencies of viable flora in respirable dust samples when grouped by elevators

	Elevator 3 (n = 5)						Elevator 4 (n = 2)					
Organism (spores/m^3)	Mean	±	SEM	Range Min.	Range Max.	% Freq.	Mean	±	SEM	Range Min.	Range Max.	% Freq.
Aspergillus (total)[a]	5,133	±	3,957	60	20,710	100	575	±	575	0	1,150	50
versicolor	1,297	±	1,230	0	6,213	60	0	±	0	0	0	0
nidulans	0	±	0	0	0	0	0	±	0	0	0	0
glaucus	3,249	±	2,576	61	13,462	100	0	±	0	0	0	0
fumigatus	270	±	198	0	1,036	60	0	±	0	0	0	0
flavus	0	±	0	0	0	0	0	±	0	0	0	0
Penicillium (total)[a]	2,406	±	2,181	153	11,131	100	5,982	±	5,921	60	11,903	100
cyclopium	1,362	±	1,342	0	6,731	40	0	±	0	0	0	0
viridicatum	482	±	462	0	2,330	40	0	±	0	0	0	0
urticae	52	±	52	0	259	20	0	±	0	0	0	0
chrysogenum	0	±	0	0	0	0	0	±	0	0	0	0
brevi-compactum	8	±	39	0	39	20	0	±	0	0	0	0
Cladosporium	1,408	±	1,206	0	6,213	80	782	±	540	242	1,323	100
Mucorales	112	±	102	0	518	40	29	±	29	0	58	50
Alternaria	52	±	52	0	259	20	0	±	0	0	0	0
Yeasts	7,043	±	5,290	153	27,700	100	403	±	403	0	805	50
Bacteria	58,928	±	55,399	365	272,338	100	2,621	±	2,439	181	5,060	100
Others	1,018	±	662	125	3,624	100	289	±	229	60	518	100
Total	76,098	±	66,727				10,678	±	10,136			

a - Includes other species not listed in table.

Table IVc. Concentrations and frequencies of viable flora in respirable dust samples when grouped by elevators

Organism (spores/m^3)	Elevator 5 (n - 16) Mean	±	SEM	Range Min.	Range Max.	% Freq.	Elevator 6 (n = 11) Mean	±	SEM	Range Min.	Range Max.	% Freq.
Aspergillus (total)[a]	1,550	±	819	36	13,555	94	2,184	±	501	0	4,867	91
versicolor	49	±	23	0	266	31	365	±	155	0	1,582	72
nidulans	177	±	140	0	2,259	44	47	±	36	0	406	27
glaucus	561	±	172	0	2,259	94	1,060	±	226	0	2,147	91
fumigatus	604	±	492	0	7,974	69	339	±	265	0	2,974	45
flavus	48	±	32	0	532	31	65	±	37	0	351	36
Penicillium (total)[a]	1,286	±	488	0	7,700	70	1,901	±	463	402	5,850	100
cyclopium	598	±	295	0	4,379	50	990	±	322	0	3,276	73
viridicatum	395	±	164	0	1,939	44	205	±	105	0	1,170	64
urticae	22	±	18	0	285	12	54	±	32	0	351	36
chrysogenum	0	±	0	0	0	0	73	±	36	0	351	36
brevi-compactum	20	±	20	0	316	6	96	±	50	0	560	45
Cladosporium	481	±	170	0	2,406	70	1,705	±	675	0	7,798	91
Mucorales	6	±	6	0	95	12	76	±	27	0	560	54
Alternaria	4	±	4	0	61	6	21	±	12	0	113	27
Yeasts	2,146	±	2,071	0	33,206	47	2,235	±	823	0	8,363	91
Bacteria	2,454	±	1,598	0	25,507	68	19,053	±	8,305	61	86,114	100
Others	923	±	838	0	13,475	41	539	±	172	54	1,660	100
Total	8,848	±	5,085				27,712	±	9,632			

a - Includes other species not listed in table.

Table IVd. Concentrations and frequencies of viable flora in respirable dust samples when grouped by elevators

	Elevator 7 (n = 30)						Elevator 8 (n = 4)					
				Range		%				Range		%
Organism (spores/m^3)	Mean	±	SEM	Min.	Max.	Freq.	Mean	±	SEM	Min.	Max.	Freq.
Aspergillus (total)[a]	2,785	±	937	0	20,636	94	2,775	±	880	1,306	5,290	100
versicolor	219	±	88	0	2,135	50	452	±	322	0	1,364	50
nidulans	28	±	11	0	228	19	0	±	0	0	0	0
glaucus	1,851	±	673	0	14,468	78	3,388	±	2,564	525	11,073	100
fumigatus	351	±	132	0	3,558	62	0	±	7	0	27	25
flavus	24	±	2	0	171	25	89	±	58	0	246	50
Penicillium (total)[a]	4,672	±	1,972	0	45,843	78	4,382	±	1,266	1,028	6,890	100
cyclopium	2,560	±	1,064	0	23,060	44	2,117	±	715	278	3,771	100
viridicatum	564	±	280	0	7,306	38	875	±	406	128	1,784	100
urticae	28	±	11	0	228	25	172	±	107	0	444	50
chrysogenum	65	±	46	0	1,370	25	696	±	511	0	2,215	75
brevi-compactum	2	±	2	0	60	3	61	±	61	0	246	25
Cladosporium	555	±	139	0	2,792	78	3,359	±	1,507	530	7,382	100
Mucorales	44	±	18	0	455	34	50	±	30	0	105	50
Alternaria	35	±	14	0	262	25	271	±	238	0	984	50
Yeasts	10,968	±	3,307	0	91,323	97	6,756	±	5,958	0	24,606	75
Bacteria	14,175	±	5,065	0	147,299	97	14,401	±	6,724	1,784	32,727	100
Others	523	±	179	0	4,032	72	1,902	±	1,418	334	6,152	100
Total	52,327	±	15,202				33,894	±	16,901			

a - Includes other species not listed in table.

Table IVe. Concentrations and frequencies of viable flora in respirable dust samples when grouped by elevators

Organism (spores/m^3)	Elevator 9 (n = 2)				
	Mean	SEM	Range Min.	Range Max.	% Freq.
Aspergillus (total)[a]	574 ±	31	543	606	100
versicolor	0 ±	0	0	0	0
nidulans	0 ±	0	0	0	0
glaucus	371 ±	235	136	606	100
fumigatus	0 ±	0	0	0	0
flavus	0 ±	0	0	0	0
Penicillium (total)[a]	1,167 ±	598	569	1,765	100
cyclopium	322 ±	17	304	339	100
viridicatum	140 ±	64	75	203	100
urticae	34 ±	2	32	36	100
chrysogenum	136 ±	136	0	272	50
brevi-compactum	34 ±	34	0	68	50
Cladosporium	163 ±	27	136	191	100
Mucorales	0 ±	0	0	0	0
Alternaria	0 ±	0	0	0	0
Yeasts	1,097 ±	418	679	1,514	100
Bacteria	2,440 ±	1,082	1,358	3,522	100
Others	156 ±	116	39	272	100
Total	5,594 ±	844			

a - Includes other species not listed in table.

Table Va. Concentrations and frequencies of direct (non-viable) counts in respirable dust samples when grouped by elevators (spores/m^3)

Organism	Mean ±	SEM	Range Min.	Range Max.	% Freq.	Mean ±	SEM	Range Min.	Range Max.	% Freq.
	Elevator 1 (n = 44)					Elevator 2 (n = 26)				
Aspergillus-type	99,035 ±	69,470	0	3,055,956	97	10,492 ±	1,360	0	24,656	96
Ustilago	9,284 ±	1,958	0	47,553	93	4,497 ±	1,771	0	39,508	76
Mucorales	1,107 ±	272	0	7,157	45	37 ±	37	0	965	4
Fusarium	443 ±	118	0	3,037	31	308 ±	123	0	1,991	24
Cladosporium	3,358 ±	1,262	0	47,268	70	2,234 ±	1,940	0	7,471	80
Alternaria	184 ±	87	0	3,224	13	30 ±	30	0	774	4
Other	2,104 ±	712	0	25,858	39	613 ±	245	0	5,435	36
Total	115,514 ±	70,678				18,210 ±	3,034			
	Elevator 3 (n = 6)					Elevator 4 (n = 59)				
Aspergillus-type	13,605 ±	11,051	0	68,447	67	13,339 ±	3,644	0	210,538	96
Ustilago	22,029 ±	12,530	1,480	81,929	100	19,890 ±	6,083	0	250,717	93
Mucorales	1,210 ±	1,210	0	7,260	17	519 ±	138	0	4,821	32
Fusarium	173 ±	173	0	1,037	17	218 ±	67	0	2,496	19
Cladosporium	1,279 ±	1,017	0	6,222	33	2,065 ±	340	0	11,651	72
Alternaria	0 ±	0	0	0	0	96 ±	83	0	4,821	4
Other	761 ±	527	0	3,111	33	674 ±	308	0	16,748	26
Total	39,056 ±	26,042				36,800 ±	9,466			

Table Vb. Concentrations and frequencies of direct (non-viable) counts in respirable dust samples when grouped by elevators (spores/m^3)

Organism	Mean ±	SEM	Range Min.	Range Max.	% Freq.	Mean ±	SEM	Range Min.	Range Max.	% Freq.
		Elevator 5 (n - 21)					Elevator 6 (n = 16)			
Aspergillus-type	6,541 ±	1,696	0	23,446	89	13,174 ±	2,991	0	42,151	94
Ustilago	1,672 ±	695	0	11,503	42	19,411 ±	9,424	1,674	155,994	100
Mucorales	232 ±	130	0	2,512	21	865 ±	437	0	6,131	31
Fusarium	142 ±	106	0	2,081	11	150 ±	81	0	859	20
Cladosporium	970 ±	315	0	5,201	42	2,275 ±	707	0	10,781	69
Alternaria	255 ±	209	0	4,314	10	874 ±	774	0	12,441	19
Other	1,176 ±	587	0	10,065	26	2,265 ±	1,962	0	31,582	27
Total	10,987 ±	2,670				39,012 ±	12,188			
		Elevator 7 (n = 33)					Elevator 8 (n = 34)			
Aspergillus-type	123,994 ±	107,638	0	3,565,959	96	9,108 ±	2,001	0	59,571	100
Ustilago	18,340 ±	4,139	0	95,896	96	4,403 ±	1,100	0	31,704	87
Mucorales	656 ±	198	0	4,211	44	299 ±	122	0	2,882	22
Fusarium	192 ±	69	0	1,510	23	64 ±	47	0	1,441	6
Cladosporium	2,806 ±	625	0	12,038	85	1,407 ±	311	0	5,752	64
Alternaria	34 ±	34	0	1,118	4	42 ±	42	0	1,438	3
Other	3,200 ±	2,343	0	77,000	33	640 ±	172	0	4,323	45
Total	149,222 ±	107,159				15,962 ±	2,782			

Table Vc. Concentrations and frequencies of direct (non-viable) counts in respirable dust samples when grouped by elevators (spores/m^3)

	Elevator 9 (n = 7)				
				Range	
Organism	Mean ±	SEM	Min.	Max.	% Freq.
Aspergillus-type	5,430 ±	1,122	2,564	10,669	100
Ustilago	1,090 ±	608	0	4,238	43
Mucorales	369 ±	254	0	1,695	28
Fusarium	129 ±	129	0	904	14
Cladosporium	621 ±	409	0	2,543	28
Alternaria	139 ±	139	0	975	14
Other	486 ±	314	0	1,709	28
Total	8,265 ±	1,610			

Table VIa. Concentrations and frequencies of viable flora in respirable dust samples when grouped by jobs

	Weighers (n = 15)					Annex workers (n = 5)				
			Range		%			Range		%
Organism (spores/m3)	Mean ±	SEM	Min.	Max.	Freq.	Mean ±	SEM	Min.	Max.	Freq.
Aspergillus (total)[a]	2,313 ±	855	126	13,219	100	15,227 ±	10,490	262	54,204	100
versicolor	131 ±	42	0	444	60	3,536 ±	2,216	0	11,046	80
nidulans	524 ±	296	0	5,971	53	140 ±	100	0	513	40
glaucus	654 ±	190	0	2,259	87	10,113 ±	7,139	65	36,735	100
fumigatus	462 ±	228	0	3,137	67	1,350 ±	1,093	0	5,652	60
flavus	111 ±	44	0	506	47	224 ±	152	0	771	40
Penicillium (total)[a]	808 ±	389	0	5,621	60	9,869 ±	5,995	0	32,368	60
cyclopium	535 ±	280	0	3,771	47	6,677 ±	4,357	0	23,377	60
viridicatum	119 ±	88	0	1,331	33	1,265 ±	582	0	2,826	60
urticae	51 ±	31	0	444	27	173 ±	73	0	351	20
chrysogenum	15 ±	15	0	221	7	70 ±	70	0	351	20
brevi-compactum	42 ±	38	0	560	13	23 ±	23	0	117	20
Cladosporium	652 ±	254	0	3,846	80	2,869 ±	1,248	0	6,213	60
Mucorales	47 ±	17	0	188	40	304 ±	151	0	771	60
Alternaria	61 ±	33	0	501	40	103 ±	63	0	259	40
Yeasts	2,294 ±	903	63	12,220	100	8,574 ±	5,510	0	27,700	60
Bacteria	3,255 ±	991	63	12,654	100	82,635 ±	50,203	0	272,338	80
Others	319 ±	127	0	1,877	73	1,155 ±	701	0	3,624	60
Total	9,747 ±	2,413				120,786 ±	63,332			

a - Includes other species not listed in table.

Table VIb. Concentrations and frequencies of viable flora in respirable dust samples when grouped by jobs

	Laborers (n = 18)					Transport within elevators (n = 6)				
Organism (spores/m^3)	Mean ±	SEM	Range Min.	Range Max.	% Freq.	Mean ±	SEM	Range Min.	Range Max.	% Freq.
Aspergillus (total)[a]	2,265 ±	1,339	0	24,440	85	3,658 ±	2,798	53	17,545	100
versicolor	66 ±	33	0	569	45	257 ±	223	0	1,364	33
nidulans	838 ±	777	0	14,035	35	241 ±	241	0	1,445	17
glaucus	613 ±	240	0	4,356	75	2,448 ±	2,003	0	12,384	83
fumigatus	425 ±	218	0	2,974	55	400 ±	336	0	2,064	50
flavus	157 ±	107	0	1,936	35	83 ±	66	0	413	50
Penicillium (total)[a]	1,393 ±	533	0	7,700	80	2,652 ±	1,527	0	9,701	83
cyclopium	654 ±	324	0	4,379	45	1,671 ±	1,164	0	7,224	50
viridicatum	210 ±	117	0	1,692	30	583 ±	360	0	1,784	50
urticae	20 ±	14	0	242	20	69 ±	69	0	413	17
chrysogenum	13 ±	7	0	114	20	0 ±	0	0	0	0
brevi-compactum	21 ±	15	0	242	10	0 ±	0	0	0	0
Cladosporium	350 ±	115	0	1,820	80	1,053 ±	304	53	2,064	100
Mucorales	55 ±	28	0	455	30	91 ±	66	0	413	50
Alternaria	16 ±	13	0	227	16	0 ±	0	0	0	0
Yeasts	10,781 ±	5,235	0	91,323	90	1,614 ±	935	0	5,779	67
Bacteria	6,143 ±	2,382	36	37,923	100	4,660 ±	2,397	0	13,664	83
Others	1,062 ±	745	0	13,475	80	361 ±	103	27	659	100
Total	52,978 ±	22,218				14,087 ±	7,127			

a - Includes other species not listed in table.

Table VIc. Concentrations and frequencies of viable flora in respirable dust samples when grouped by jobs

Organism (spores/m^3)	Maintenance (n = 24) Mean ±	SEM	Range Min.	Max.	% Freq.	Grain inspectors (n = 23) Mean ±	SEM	Range Min.	Max.	% Freq.
Aspergillus (total)[a]	2,474 ±	946	0	19,197	83	2,710 ±	892	34	20,636	100
versicolor	210 ±	95	0	2,095	54	230 ±	113	0	2,135	35
nidulans	439 ±	257	0	5,848	46	19 ±	10	0	179	17
glaucus	982 ±	394	0	9,281	79	2,047 ±	747	0	14,469	87
fumigatus	558 ±	339	0	7,974	62	226 ±	153	0	3,558	52
flavus	137 ±	63	0	1,398	46	28 ±	13	0	246	22
Penicillium (total)[a]	866 ±	304	0	6,879	88	2,278 ±	744	0	13,046	87
cyclopium	475 ±	223	0	5,099	54	956 ±	464	0	10,674	61
viridicatum	176 ±	84	0	1,939	50	170 ±	62	0	1,186	56
urticae	18 ±	7	0	127	29	34 ±	13	0	246	39
chrysogenum	0 ±	0	0	0	0	137 ±	96	0	2,215	30
brevi-compactum	10 ±	6	0	138	12	29 ±	14	0	246	22
Cladosporium	738 ±	237	0	5,721	71	1,084 ±	438	0	7,798	87
Mucorales	19 ±	11	0	239	25	43 ±	16	0	262	35
Alternaria	5 ±	2	0	61	12	80 ±	44	0	984	26
Yeasts	4,635 ±	2,335	0	49,966	67	5,471 ±	2,190	0	44,830	91
Bacteria	6,767 ±	2,866	0	50,447	91	13,741 ±	6,460	0	147,299	87
Others	426 ±	204	0	4,789	67	743 ±	303	0	6,152	87
Total	15,928 ±	5,037				26,148 ±	10,314			

a - Includes other species not listed in table.

Table VId. Concentrations and frequencies of viable flora in respirable dust samples when grouped by jobs

Organism (spores/m^3)	Equipment operators (n = 13)					Supervisors (n = 5)				
	Mean ±	SEM	Range Min.	Range Max.	% Freq.	Mean ±	SEM	Range Min.	Range Max.	% Freq.
Aspergillus (total)[a]	5,455 ±	1,945	30	19,863	100	2,145 ±	1,778	60	9,235	100
versicolor	1,188 ±	898	0	11,792	46	97 ±	52	0	230	60
nidulans	57 ±	35	0	419	31	11 ±	11	0	57	20
glaucus	4,880 ±	2,117	0	25,835	92	1,947 ±	1,717	0	8,806	80
fumigatus	372 ±	154	0	2,055	77	49 ±	42	0	215	40
flavus	357 ±	318	0	4,158	23	6 ±	6	0	29	20
Penicillium (total)[a]	8,343 ±	4,257	0	45,843	69	3,673 ±	3,485	30	17,611	100
cyclopium	4,726 ±	2,239	0	23,060	62	2,591 ±	2,520	0	12,672	80
viridicatum	918 ±	561	0	7,306	61	876 ±	855	0	4,295	40
urticae	82 ±	49	0	559	23	54 ±	42	0	215	40
chrysogenum	122 ±	105	0	1,370	15	66 ±	43	0	215	40
brevi-compactum	44 ±	26	0	316	23	8 ±	8	0	39	20
Cladosporium	2,300 ±	1,201	0	15,225	85	767 ±	515	31	2,792	100
Mucorales	29 ±	13	0	140	31	52 ±	42	0	215	40
Alternaria	13 ±	11	0	140	15	0 ±	0	0	0	0
Yeasts	1,295 ±	751	0	9,361	54	3,993 ±	2,551	172	13,172	100
Bacteria	11,238 ±	5,970	0	76,346	85	13,658 ±	9,240	1440	49,613	100
Others	732 ±	492	0	5,867	38	351 ±	162	0	859	80
Total	29,403 ±	10,488				24,636 ±	16,045			

a - Includes other species not listed in table.

Table VIIa. Concentrations and frequencies of direct counts in respirable dust samples when grouped by jobs (spores/m^3)

Organism	Mean ±	SEM	Range Min.	Range Max.	% Freq.	Mean ±	SEM	Range Min.	Range Max.	% Freq.
	Weighers (n = 26)					Annex workers (n = 11)				
Aspergillus-type	267,262 ±	176,316	732	3,565,959	100	23,555 ±	6,338	762	68,447	100
Ustilago	6,162 ±	1,895	0	41,674	73	23,181 ±	7,977	0	81,929	91
Mucorales	1,112 ±	370	0	5,647	38	1,302 ±	812	0	7,260	27
Fusarium	251 ±	102	0	1,801	23	375 ±	132	0	1,037	45
Cladosporium	2,976 ±	1,798	0	47,268	58	3,191 ±	950	0	11,021	91
Alternaria	120 ±	74	0	1,700	12	0 ±	0	0	0	0
Other	2,395 ±	1,108	0	25,858	42	1,100 ±	579	0	5,878	40
Total	280,276 ±	177,301				52,702 ±	14,531			
	Laborers (n = 40)					Transport within elevators (n = 15)				
Aspergillus-type	16,086 ±	5,677	0	210,538	97	13,010 ±	3,971	0	54,801	93
Ustilago	20,852 ±	7,362	0	250,717	88	32,925 ±	16,102	0	216,917	92
Mucorales	562 ±	175	0	4,821	34	708 ±	339	0	3,850	38
Fusarium	273 ±	110	0	3,037	20	205 ±	117	0	1,510	23
Cladosporium	2,123 ±	450	0	12,039	80	3,122 ±	1,016	0	11,651	77
Alternaria	451 ±	331	0	12,441	8	58 ±	58	0	872	8
Other	1,129 ±	800	0	31,582	23	6,690 ±	5,142	0	77,000	46
Total	41,473 ±	13,076				56,716 ±	18,184			

Table VIIb. Concentrations and frequencies of direct counts in respirable dust samples when grouped by jobs (spores/m^3)

Organism	Mean ±	SEM	Range Min.	Range Max.	% Freq.	Mean ±	SEM	Range Min.	Range Max.	% Freq.
	Maintenance (n = 39)					Inspectors (n = 80)				
Aspergillus-type	22,235 ±	10,472	0	414,272	95	10,483 ±	1,506	0	86,572	96
Ustilago	8,312 ±	2,084	0	59,173	82	7,130 ±	1,675	0	95,896	87
Mucorales	406 ±	108	0	2,813	34	368 ±	109	0	7,157	25
Fusarium	284 ±	90	0	2,418	26	189 ±	58	0	2,436	14
Cladosporium	2,517 ±	842	0	30,627	63	1,537 ±	219	0	8,737	63
Alternaria	140 ±	91	0	3,224	8	99 ±	58	0	4,314	6
Other	849 ±	344	0	12,210	32	863 ±	220	0	10,065	33
Total	34,797 ±	12,616				20,668 ±	2,825			
	Equipment operators (n = 25)					Supervisors (n = 10)				
Aspergillus-type	17,069 ±	4,193	0	101,493	96	10,761 ±	5,418	0	57,092	89
Ustilago	10,459 ±	3,460	0	78,104	92	4,013 ±	1,331	0	9,987	89
Mucorales	594 ±	254	0	4,489	25	422 ±	333	0	3,310	22
Fusarium	184 ±	97	0	2,489	17	70 ±	70	0	703	11
Cladosporium	2,349 ±	528	0	11,112	79	1,073 ±	490	0	4,921	56
Alternaria	78 ±	55	0	1,040	8	0 ±	0	0	0	0
Other	784 ±	353	0	7,282	37	297 ±	216	0	2,069	22
Total	31,518 ±	7,343				16,635 ±	6,031			

Table VIII. Insect specimens isolated from 1977 bulk grain samples

Date	Sample No.	Grain	Description*
		Elevator #4	
12-10-77	1	Barley	Head (Acrididae) and elytral fragments (Coleoptera)
"	3	"	Head (Carabidae); femur and misc. leg fragments (Orthoptera)
"	4	Spring wheat	Head (Coleoptera)
"	5	" "	Unknown beetle head & possible non-insect fragments
13-10-77	9	Oats	Tibia (Acrididae) and an unknown insect fragment
"	10	"	Insect leg and cuticle fragments
"	11	"	" " " " "
"	12	"	Insect abdomen; body fragment (Orthoptera; insect antennal fragment
"	13	Sunflower	Elytra & forewing (Coleoptera); Hemipteran (Miridae?) adult; and insect thoracic setment
"	15	"	Adult beetle (Anthicidae)
"	16	"	Head (Diptera) and unknown fragment
"	17	Spring wheat	Lepidopteran larvae
"	18	Durum	Head (Hemipteran) and insect femur
"	19	Corn	Insect cuticle fragment
"	20	Sunflower	Insect leg fragments
"	22	Barley	Beetle head and thorax (Elateridae), and 3 insect leg fragments
"	23	Oats	Beetle (Carabidae); elytra (Coleoptera); insect abdomen fragment
"	24	"	Weevil (headless) Curculionidae)
17-10-77	25	Spring wheat	Beetle (headless); Elytra (Anthicidae); 3 insect cuticle fragments

* Specimens labeled "insect" could not be further identified

Table VIII. (Continued)

Date	Sample No.	Grain	Description*
17-10-77	28	Spring wheat	Mandible (Arthropoda); Head (Orthoptera)
"	30	" "	Elytral fragment (Coccinelidae); elytral fragment; insect leg fragment
"	31	Oats	2 Insect fragments
"	32	Spring wheat	Insect fragments (Orthoptera)
18-10-77	40	Durum wheat	Unknown insect fragments
"	41	Barley	Head (Curculionidae); elytra; 1 insect mandible
"	42	"	Insect leg fragment and misc. insect fragments
"	43	Durum	Head (Orthoptera); insect leg & cuticle fragment
"	44	Spring wheat	2 Beetle heads (Carabidae: Harpalus sp.); 4 insect leg fragments; 1 non-insect fragment
		Elevator #2	
18-10-77	33	Durum wheat	2 Insect leg fragments & 1 abdominal fragment
"	36	" "	Leg fragment (Orthoptera); insect femur; unknown insect leg fragment
"	39	" "	Insect leg fragment
19-10-77	50	" "	3 Rice weevils (Sitophilus oryzae) and 1 lesser grain borer (Rhizopertha dominica)
"	52	" "	Elytron (Coccinelidae); insect head; insect leg fragment; 1 millipede
"	54	Spring wheat	Large insect fragment
"	55	" "	Abdominal segment (Orthoptera); insect cuticle fragment
20-10-77	60	" "	Head fragment (Orthoptera:Acrididae)
"	61	" "	Larvae (Silphidae); 1 adult beetle (Coccinelidae:Hippodamia tridecimpuctata)

* **Specimens labeled "insect" could not be further identified**

Table VIII. (Continued)

Date	Sample No.	Grain	Description*
		Elevator #8	
24-10-77	65	Spring wheat	Head (Acrididae)
"	67	" "	Head and abdomen (Acrididae); unknown insect fragment
"	68	" "	Head and thorax (Acrididae)
"	70	" "	Abdomen (Acrididae)
"	71	" "	2 Heads and 1 abdomen (Acrididae); unknown insect fragment
15-10-77	83	Wheat ?	Head (Orthoptera)
26-10-77	89	Wheat ?	Head (Orthoptera:Acrididae)
27-10-77	99	Durum wheat	Head (Orthoptera:Acrididae)
		Elevator #6	
31-10-77	117	Spring wheat & sunflower	2 Unknown larvae (Coleoptera)
1-11-77	122	Oats	Head and thorax (Acrididae)
"	124	"	Head (Acrididae)
		Elevator #7	
8-11-77	162	Spring wheat	Insect abdomen, head, and femur
"	165	" "	Head and tibia (Orthoptera)
10-11-77	182	Barley	Femur (Acrididae)
"	184	"	Granary weevil (Sitophilus granarius)
"	185	"	Insect head
		Elevator #1	
15-11-77	194	Durum wheat	Head (Acrididae)
"	195	" "	Mouthpart (Orthoptera); unknown fragment
"	196	" "	1/2 Head (Orthoptera)

* Speciments labeled "insect" could not be further identified

Table VIII. (Continued)

Date	Sample No.	Grain	Description*
		Elevator #1	
15-11-77	197	Durum wheat	Granary weevil (Sitophilus granarius)
"	200	" "	Abdomen (Orthoptera)
16-11-77	202	Spring wheat	Femur (Acrididae)
"	204	" "	Abdomen (Acrididae)
17-11-77	211	" "	Weevil (possibly Anthonomus disjuncta)
18-11-77	221	Barley	Insect fragment
"	224	Rye	Head (Acrididae)
"	225	"	Head, femur, mandible (Acrididae)
29-11-77	241	Oats	Insect head & body fragment
"	243	"	Head, tibia, and 1/2 abdomen (acrididae)

* Specimens labeled "insect" could not be further identified

Table IX. Insect specimens isolated from 1977 settled dust samples

Date	Box No./ Sample No.	Description
		Elevator #2
18-10-77	Box I/#11	Tenebrio molitor adult & larva
"	Box I/#13	Cast coleopteran larval integuments (Dermestidae & Tenebrionidae)
		Elevator #8
24-10-77	Box II/#11	1 Pseudoscorpion* & 1 dermestid larva
"	Box II/#13	1 Attagenus megatoma canadensis*
		Elevator #1
31-10-77	Box III/#2	1 Tenebrio molitor larva*
"	Box III/#3	3 Lepidopteran larvae* (Pyralis farinalis) & 1 mite*
"	Box III/#4	2 T. molitor larvae*
"	Box III/#9	1 T. molitor larva
		Elevator #4
11-10-77	Box IV/#9	2 T. molitor larvae*
		Elevator #5
15-11-77	Box V/#1	3 T. molitor larvae
"	Box V/#6	1 T. molitor larva

* Live when isolated

Table X. INVENTORY OF SCANNING ELECTRON MICROGRAPHS

Photo #	Worker #	Sample #	Magnification	Photo #	Worker #	Sample #	Magnification
1	138	(S-270)	1×10^4	37	252	(S-66)	1×10^4
2	38	(S-135)	3×10^3	38	252	(S-66)	1×10^4
3	38	(S-135)	1×10^4	39	252	(S-66)	1×10^4
4	38	(S-135)	1×10^4	40	252	(S-66)	1×10^4
5	135	(S-51)	3×10^2	41	252	(S-66)	1×10^4
6	135	(S-51)	1×10^3	42	230	(S-102)	1×10^3
7	135	(S-51)	1×10^3	43	230	(S-102)	1×10^3
8	135	(S-51)	3×10^3	44	230	(S-102)	1×10^3
9	135	(S-51)	3×10^3	45	230	(S-102)	1×10^3
10	135	(S-51)	3×10^3	46	230	(S-102)	1×10^3
11	300	(S-111)	1×10^3	47	230	(S-102)	1×10^3
12	300	(S-111)	3×10^3	48	230	(S-102)	3×10^3
13	252	(S-66)	3×10^2	49	230	(S-102)	3×10^3
14	252	(S-66)	1×10^3	50	230	(S-102)	3×10^3
15	252	(S-66)	1×10^3	51	230	(S-102)	3×10^3
16	252	(S-66)	1×10^3	52	230	(S-102)	3×10^3
17	252	(S-66)	1×10^3	53	230	(S-102)	3×10^3
18	252	(S-66)	3×10^3	54	230	(S-102)	3×10^3
19	252	(S-66)	3×10^3	55	230	(S-102)	3×10^3
20	252	(S-66)	3×10^3	56	230	(S-102)	1×10^4
21	252	(S-66)	3×10^3	57	168	(S-110)	1×10^3
22	252	(S-66)	3×10^3	58	168	(S-110)	1×10^3
23	252	(S-66)	3×10^3	59	168	(S-110)	1×10^3
24	252	(S-66)	3×10^3	60	168	(S-110)	1×10^3
25	252	(S-66)	3×10^3	61	168	(S-110)	1×10^3
26	252	(S-66)	3×10^3	62	168	(S-110)	1×10^3
27	252	(S-66)	3×10^3	63	168	(S-110)	1×10^3
28	252	(S-66)	3×10^3	64	168	(S-110)	1×10^3
29	252	(S-66)	3×10^3	65	168	(S-110)	3×10^3
30	252	(S-66)	3×10^3	66	168	(S-110)	3×10^3
31	252	(S-66)	3×10^3	67	168	(S-110)	3×10^3
32	252	(S-66)	1×10^4	68	264	(S-132)	1×10^3
33	252	(S-66)	1×10^4	69	264	(S-132)	3×10^3
34	252	(S-66)	1×10^4	70	264	(S-132)	3×10^3
35	252	(S-66)	1×10^4	71	94	(S-46)	3×10^3
36	252	(S-66)	1×10^4	72	94	(S-46)	3×10^3

INVENTORY OF SCANNING ELECTRON MICROGRAPHS

Photo #	Worker #	Sample #	Magnification	Photo #	Worker #	Sample #	Magnification
73	94	(S-46)	3×10^3	80	204	(S-136)	3×10^3
74	94	(S-46)	3×10^3	81	204	(S-136)	3×10^3
75 (Neg. destroyed)	94	(S-46)	3×10^3	82	204	(S-136)	3×10^3
76	94	(S-46)	3×10^3	83	204	(S-136)	3×10^3
77	165	(S-124)	3×10^3	84	204	(S-489)	3×10^3
78	165	(S-124)	3×10^3	85	204	(S-500)	3×10^3
79	280	(S-58)	3×10^3				

LEGEND FOR FIGURES

Fig. 1. Clean portion of the filter from worker #138 (1 x 10^4).
Fig. 2. Grain dust debris and spores from worker #300 (1 x 10^3).
Fig. 3. Pestalotia sp. spores and hyphal fragment from worker #300 (3 x 10^3).
Fig. 4. Densely covered filter of worker #135 (1 x 10^3).
Fig. 5. Grain dust debris and spores from worker #135 (3 x 10^3).
Fig. 6. Grain dust debris and spores from worker #135 (1 x 10^4).
Fig. 7. Aspergillus sp. spores from worker #252 (1 x 10^3).
Fig. 8. Aspergillus sp. spores from worker #252 (3 x 10^3).
Fig. 9. Cladsporium sp. spores from worker #252 (3 x 10^3).
Fig. 10. Unidentified irregular particle from worker #252 (3 x 10^3).
Fig. 11. Aspergillus sp. spore from worker #252 (1 x 10^4).
Fig. 12. Cladosporium sp. and Ustilago sp. spores from worker #252 (1 X 10^4).
Fig. 13. Grain dust debris and spores from worker #230 (1 x 10^3).
Fig. 14. Ustilago sp. and Penicillium sp. spores and large debris particle from grain worker #230 (3 x 10^3).
Fig. 15. Large debris particles from grain worker #230 (3 x 10^3).
Fig. 16. Debris and mycelial fragments found on the total dust sample from grain worker #168 (1 x 10^3).
Fig. 17. Large debris particles on the total dust sample from grain worker #264 (1 x 10^3).
Fig. 18. Aspergillus (or Penicillium) spores and fine particles from worker #94 (3 x 10^3).
Fig. 19. Enlarged view of fine particles on the filter of worker #94 (3 x 10^3).
Fig. 20. Unidentified spiny particle from worker #165 (3 x 10^3).
Fig. 21. Fine particulate dust from worker #38 (3 x 10^3).
Fig. 22. Aspergillus sp. spore from worker #38 (1 x 10^4).
Fig. 23. Spores from pure cultures of Penicillium cyclopium (1 x 10^4).
Fig. 24. Spores from pure cultures of Penicillium viridicatum.

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Fig. 1 Clean portion of the filter from worker #138 (1 x10^4).

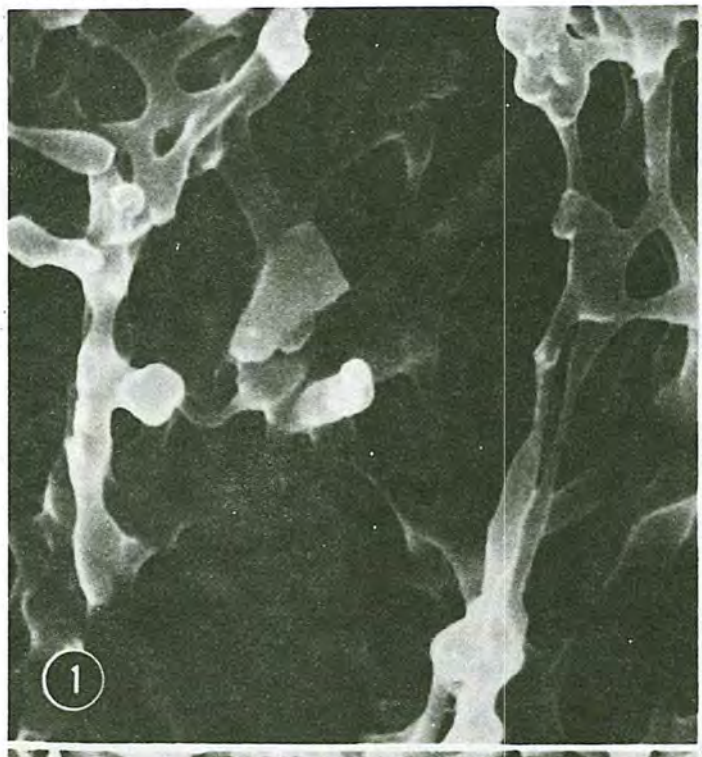


Fig. 2 Grain dust debris and spores from worker #300 (1 x 10^3).

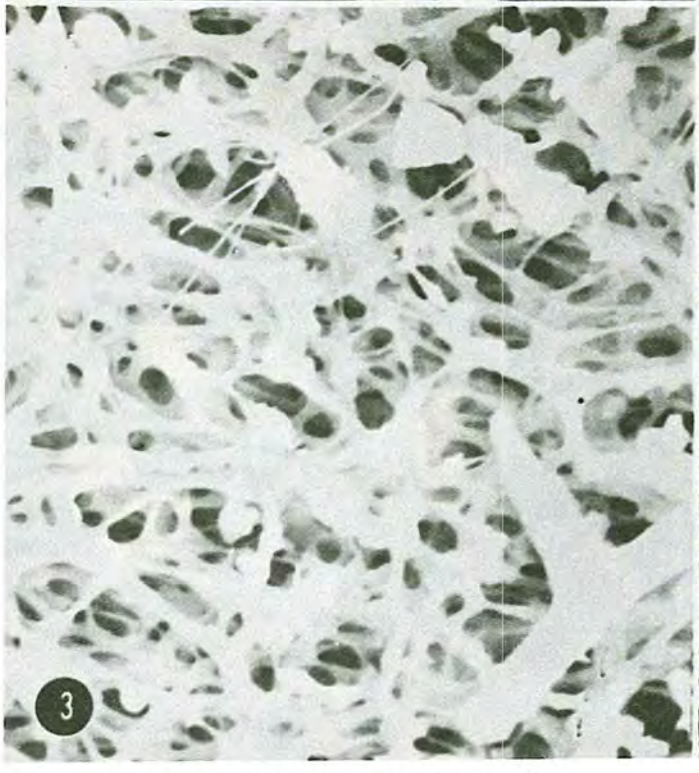


Fig. 3 Pestalotia sp. spores and hyphal fragment from worker #300 (3 x 10^3).

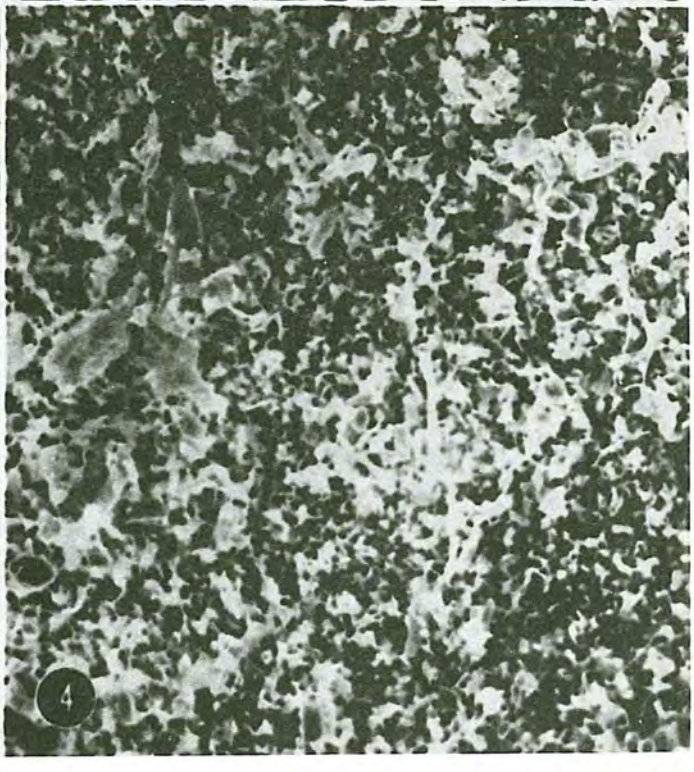


Fig. 4 Densely covered filter of worker #135 (1 x 10^3).

Fig. 5 Grain dust debris and spores from worker #135 (3 x 10^3).

Fig. 6 Grain dust debris and spores from worker #135 (1 x 10^4).

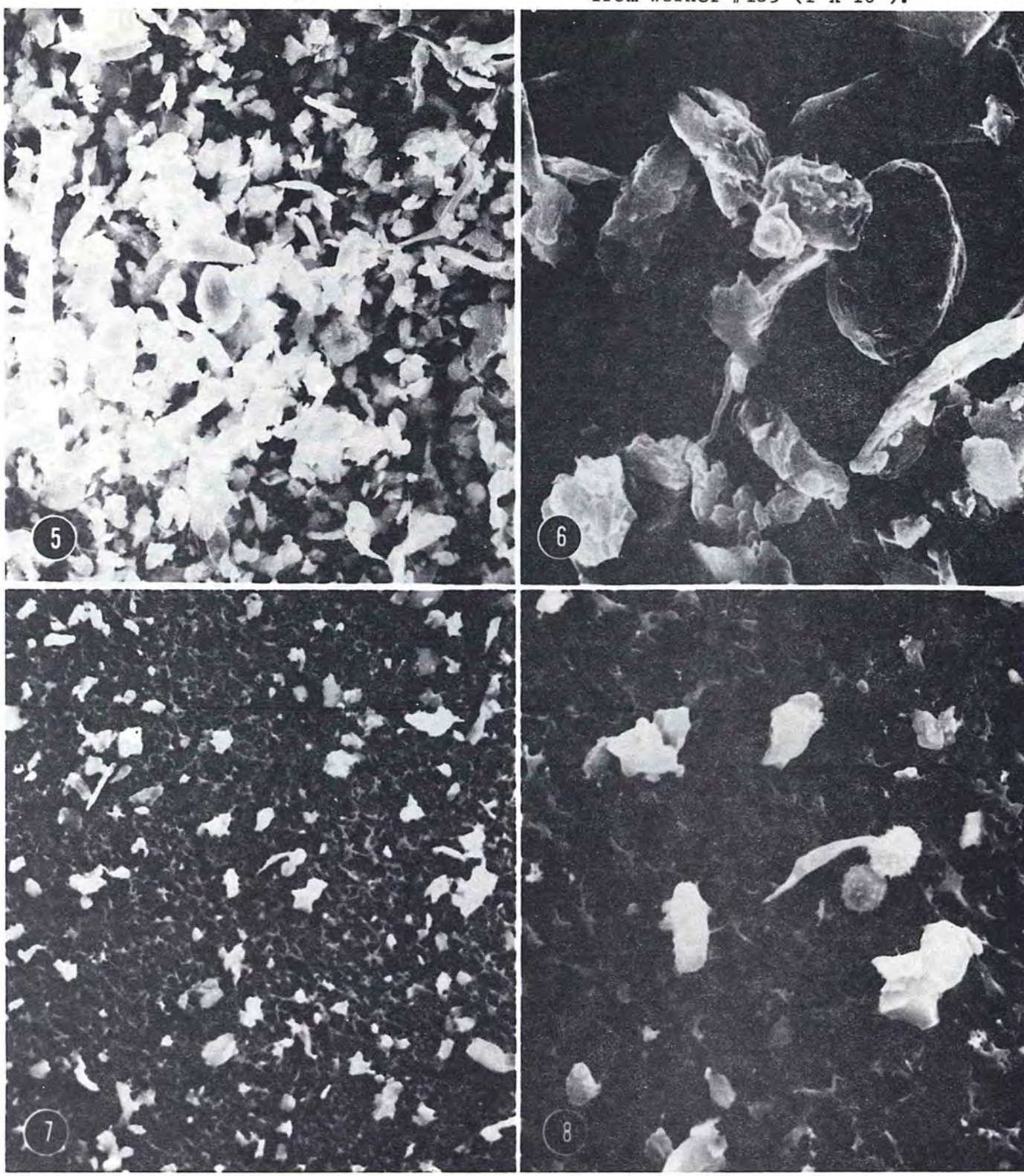


Fig. 7 Aspergillus sp. spores from worker #252 (1 x 10^3).

Fig. 8 Aspergillus sp. spores from worker #252 (3 x 10^3).

Fig. 9 Cladsporium sp. spores from worker #252 (3×10^3).

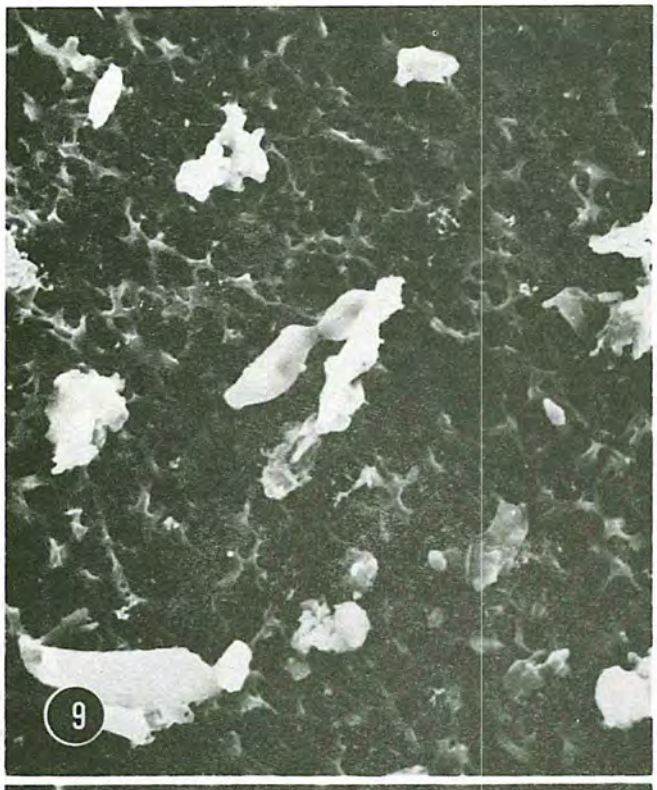


Fig. 10 Unidentified irregular particle from worker #252 (3×10^3).

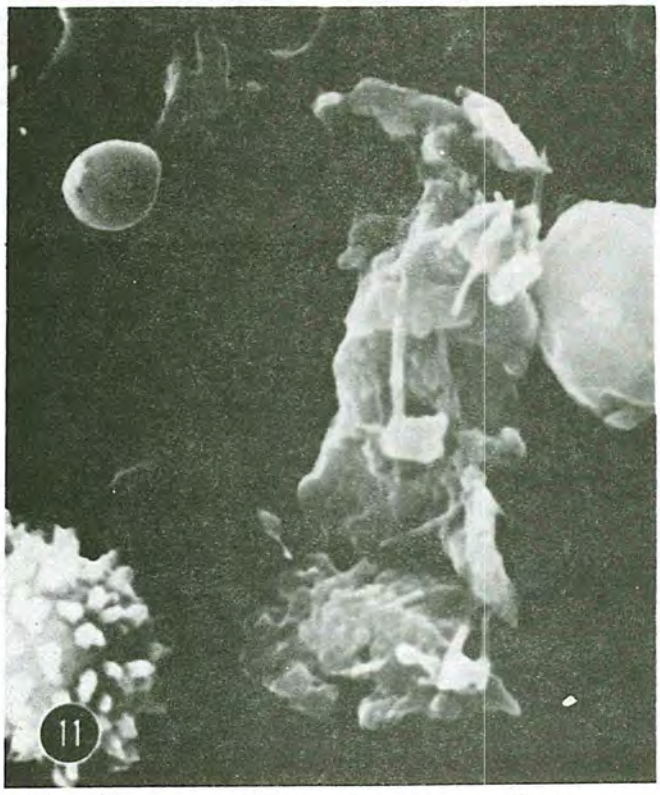


Fig. 11 Aspergillus sp. spore from worker #252 (1×10^4).

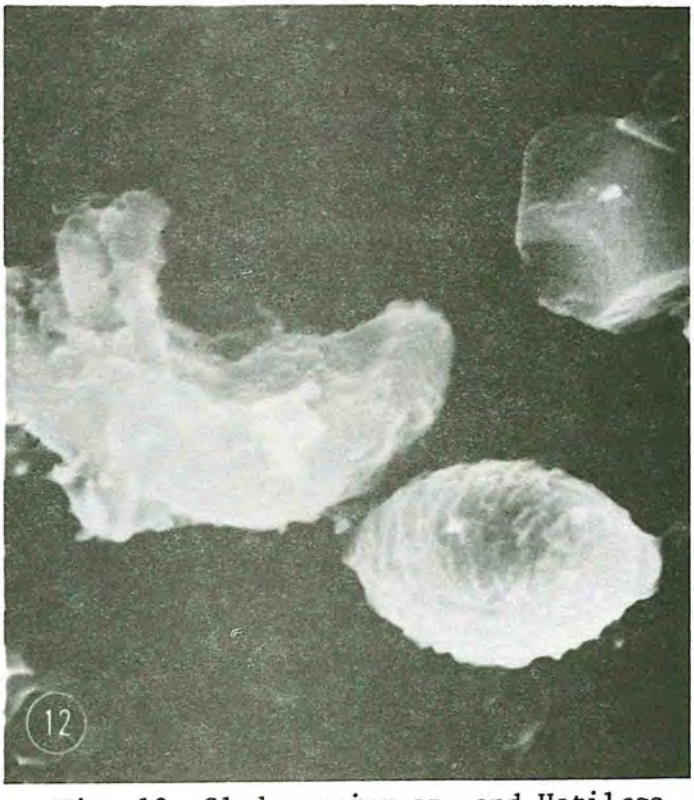
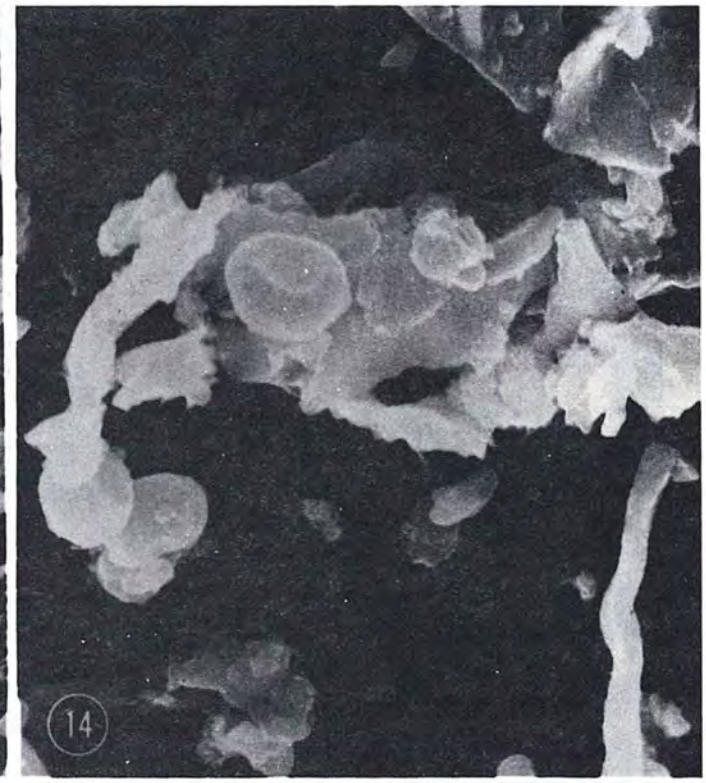


Fig. 12 Cladosporium sp. and Ustilago sp. spores from worker #252 (1×10^4).

Fig. 13 Grain dust debris and spores from worker #230 (1 x 10^3).

Fig. 14 Ustilago sp. and Penicillium sp. spores and large debris particle from grain worker #230 (3 x 10^3).



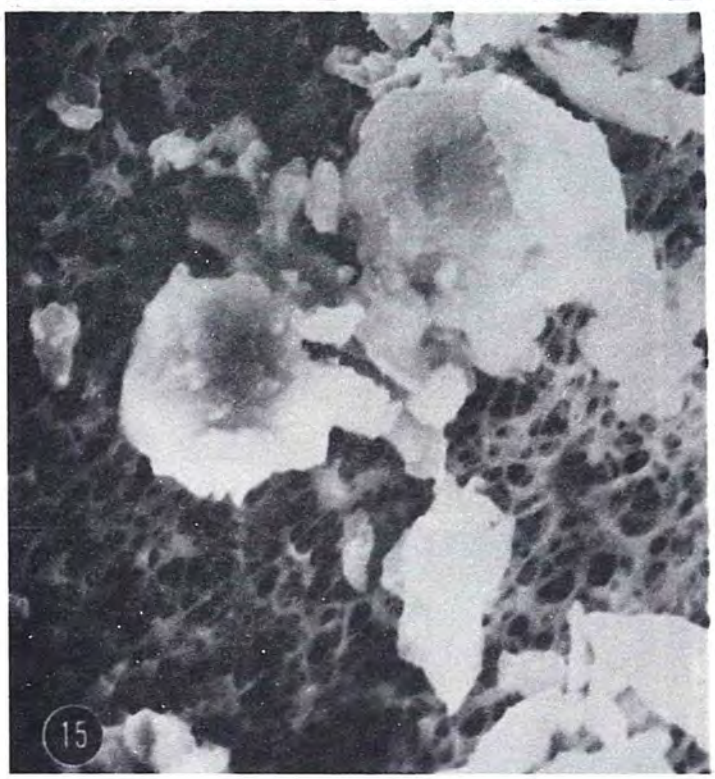
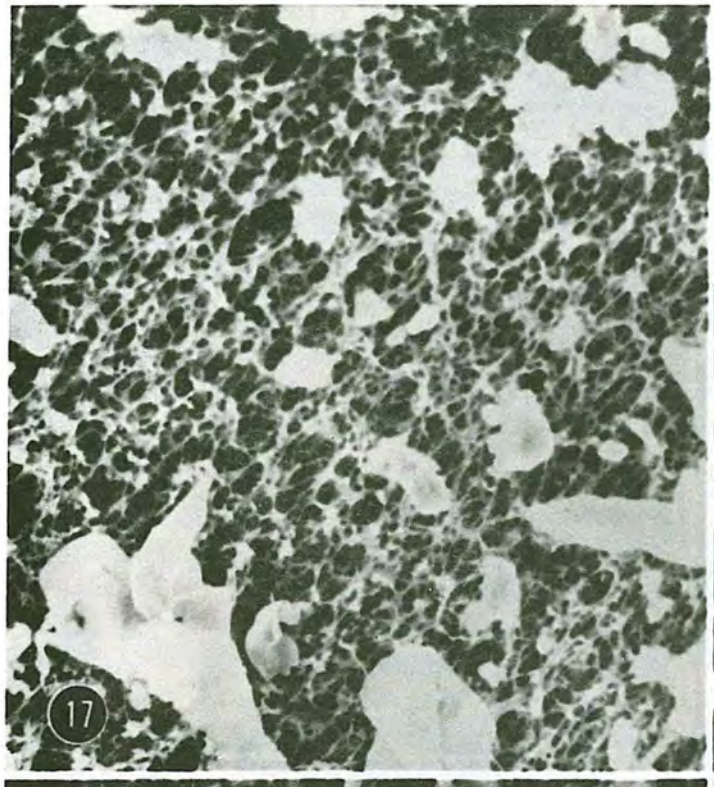


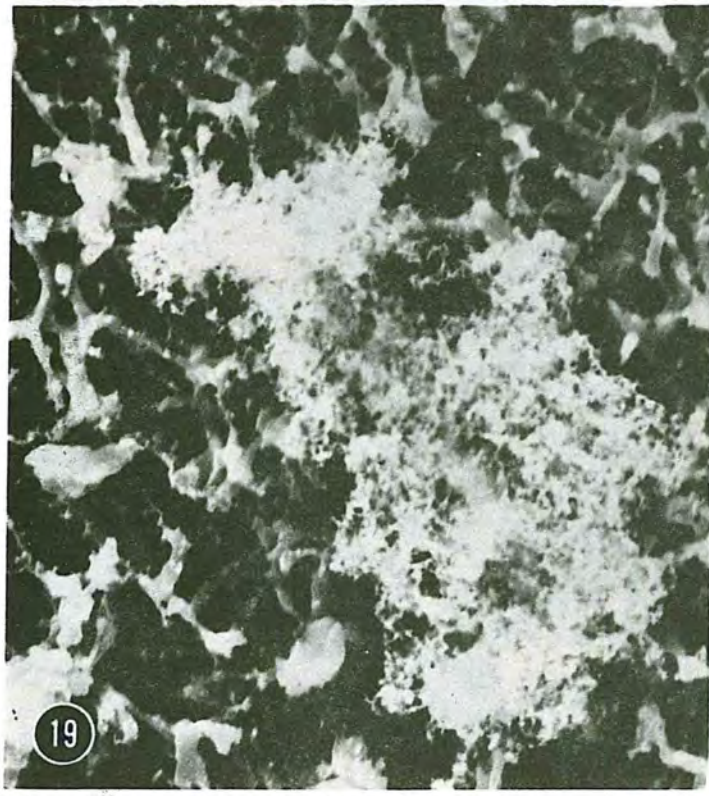
Fig. 15 Large debris particles from grain worker #230 (3 x 10^3).

Fig. 16 Debris and mycelial fragments found on the total dust sample from grain worker #168 (1 x 10^3).

Fig. 17 Large debris particles on the total dust sample from grain worker #264 (1 x 10^3).

Fig. 18 Aspergillus (or Penicillium) spores and fine particles from worker #94 (3 x 10^3).





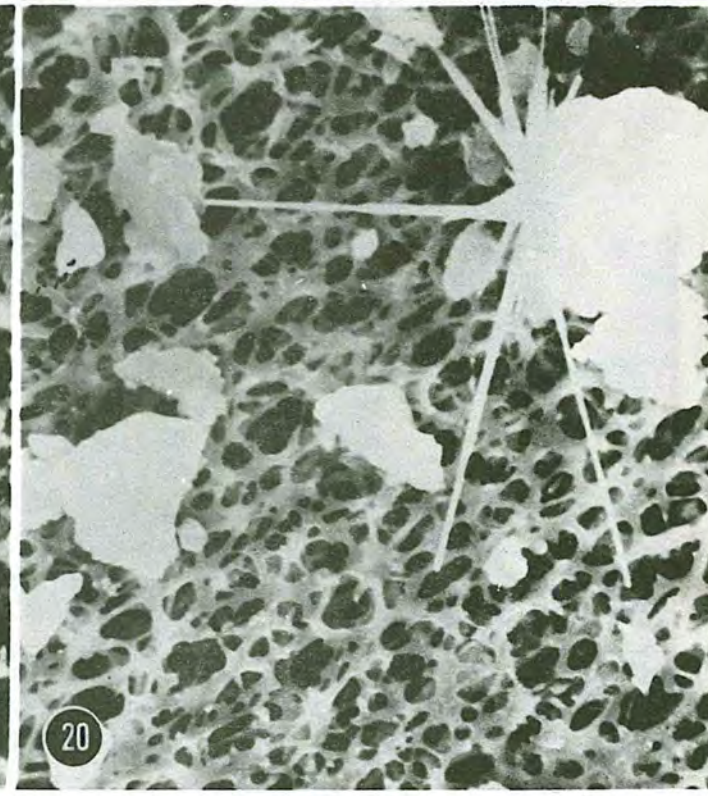


Fig. 19 Enlarged view of fine particles on the filter of worker #94 (3 x 10^3).

Fig. 20 Unidentified spiny particle from worker #165 (3 x 10^3).

Fig. 21 Fine particulate dust from worker #38 (3 x 10^3).

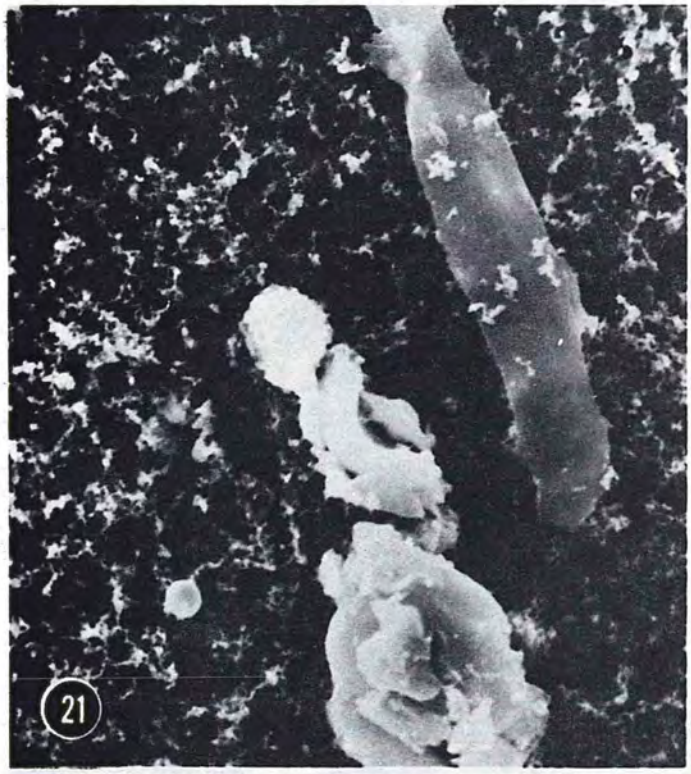


Fig. 22 Aspergillus sp. spore from worker #38 (1 x 10^4).

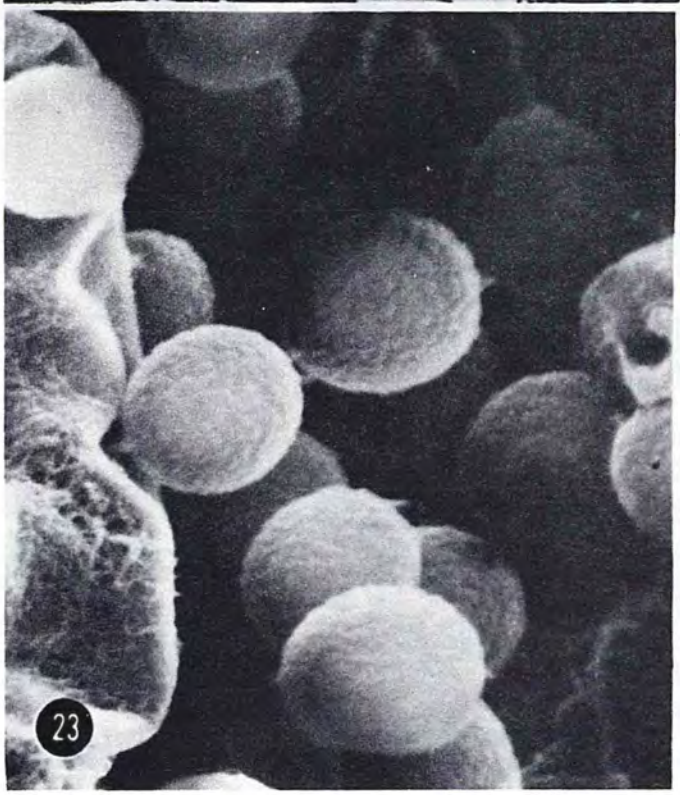


Fig. 23 Spores from pure cultures of Penicillium cyclopium (1 x 10^4).

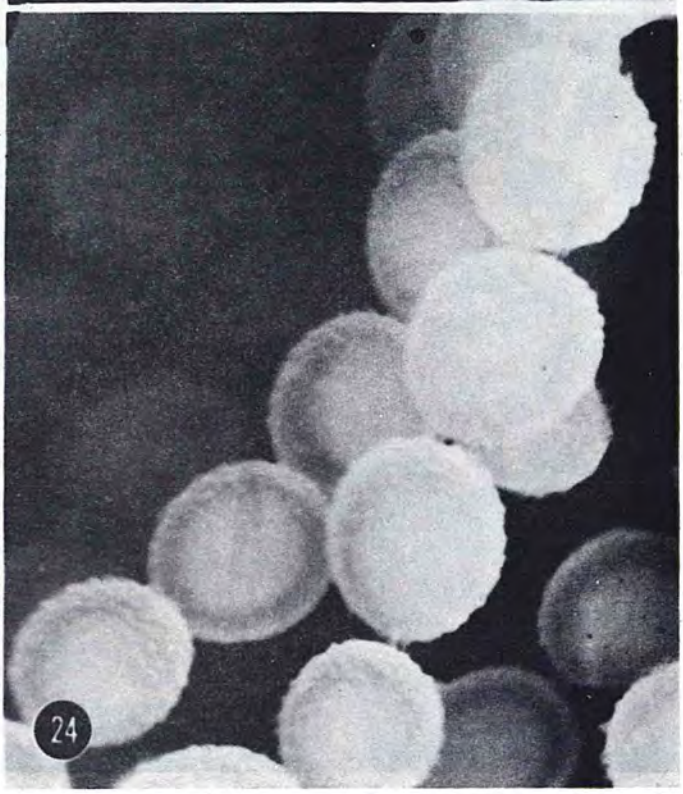


Fig. 24 Spores from pure cultures of Penicillium viridicatum.

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