Ann. Occup. Hyg., Vol. 51, No. 7, pp. 581–592, 2007

doi:10.1093/annhyg/mem038

Determinants of Microbial Exposure in Grain Farming

A. S. HALSTENSEN[1,2]*, K. C. NORDBY[1], I. M. WOUTERS[3] and W. EDUARD[1]

[1]Department of Chemical and Biological Working Environment, National Institute of Occupational Health, PO Box 8149 Dep., N-0033 Oslo, Norway; [2]Department of Plant and Environmental Sciences, Norwegian University of Life Sciences, N-1432 Ås, Norway; [3]Institute of Risk Assessment Sciences, University of Utrecht, PO Box 80.176, NL-3508 Utrecht, the Netherlands

Received 30 May 2006; in final form 9 July 2007; published online 27 September 2007

Objectives: Exposure to organic dust containing high concentrations of microorganisms is common in grain farming, although the farmers have practices to counteract microbial growth to obtain optimal grain yields. We investigated the influence of weather and production practices on personal microbial exposure during grain work.

Methods: Airborne dust was collected by personal sampling during threshing and storage work on 92 Norwegian farms. The personal exposure for bacteria, endotoxin, fungal spores and hyphae, β-(1→3)-glucans and actinomycetes was quantified and compared with climatic data expressed as fungal forecasts from the grain growth season and production practices as reported by farmers.

Results: Farmers were exposed to a geometrical mean of 4.4 mg m^{-3} inhalable dust [geometrical standard deviation (GSD) = 4.0], 4×10^6 m^{-3} bacteria and fungal spores (GSD = 5.2 and 5.9, respectively), 5.9×10^3 EU m^{-3} of endotoxins (GSD = 8.6), 2×10^5 m^{-3} actinomycetes (GSD = 15.3), 120 μg m^{-3} β-(1→3)-glucans (GSD = 4.7) and 5×10^5 AU m^{-3} of hyphae (GSD = 4.4). Univariate associations were found between one or several of these microbial factors and work operation, visible fungal damage, grain species, lodging of grain, storage technology or harvester type. As assessed by general linear models, storage work was the main predictive determinant for microbial exposure, although grain species and visible fungal damage also were also important. Wet and warm weather throughout the grain growth season were associated with elevated exposure for inhalable dust, β-(1→3)-glucans, endotoxins and hyphae during threshing. The β-(1→3)-glucan exposure could biologically be explained by the fungal spore and hyphal exposure, both variables contributing equally. However, spores were most important during storage work, whereas only hyphae were predictive during threshing.

Conclusions: Farmers were exposed to high levels of microorganisms and their components during dusty grain work. Dust prevention and protection may reduce microbial exposure, and may be particularly important in areas with frequent fungal forecasts, when fungal damage has been observed, during storage work or when handling barley.

Keywords: β-(1→3)-glucan; endotoxin; fungal spores; grain work; hyphae; inhalable dust; microbial exposure determinants; personal exposure

INTRODUCTION

Farmers may be exposed to large quantities of grain dust during threshing and grain storage work. Grain dust inhalation may induce respiratory diseases including chronic bronchitis, granulomatous pneumonitis (extrinsic allergic alveolitis, hypersensitivity pneumonitis) and toxic pneumonitis (organic dust toxic syndrome/grain fever), which finally may lead to decreased lung function (Melbostad *et al.*, 1997; Swan and Crook, 1998). Microbial components and individual susceptibility appears crucial for the immunopathology, although the exact mechanisms are still unknown (Melbostad and Eduard, 2001; Sigsgaard *et al.*, 2005). Grain dust is a heterogeneous mixture of inorganic soil particles, plant fragments, insect and mite parts, viable and non-viable microorganisms and their biological active components

*Author to whom correspondence should be addressed.
Tel: +47-23-19-53-38; fax: +47-23-19-52-06;
e-mail: anne.s.halstensen@stami.no

such as endotoxins, glucans, allergens or mycotoxins (Smith, 1989), which all represent health hazards upon inhalation (Dutkiewicz *et al.*, 1985; Lacey and Crook, 1988; Bohn and BeMiller, 1995; Ulmer *et al.*, 1997; Rylander, 2002; Douwes, 2005; Young and Castranova, 2005).

The microbial exposure levels during grain work depend on the microbial contamination of the grain and to which degree the bioactive components become airborne and dispersed in the working environment. The microbial contamination is affected by fungal growth factors, such as moisture and temperature (Lacey, 1980), inoculum from soil particles, plant debris, and residues from harvesting equipment, crop rotation, ploughing, use of fungicides (Hill and Lacey, 1983) and growth regulators, lodging of the grain in the field (Langseth and Stabbetorp, 1996), rapidity of drying during storage, rewetting, ambient humidity (Farant and Moore, 1980; Langseth *et al.*, 1993) and mechanical injury. These factors consequently affect the farmers' microbial exposure during grain handling. Farmers seek to counteract microbial contamination by adapting their practices to the climatic conditions and to the micro-ecological properties of individual fields to obtain optimal grain crop yields. Exposure to airborne dust, microorganisms and their components are nevertheless common during grain work. The assessment of exposure determinants may help to improve grain farmers' protection possibilities, identify specific control measures and allow evaluation of exposure risks without the need for personal exposure measurements.

Increased levels of the mycotoxin deoxynivalenol (DON) in settled grain dust was in a previous study associated with a number of production factors, such as storage work, spring wheat, crop rotation, autumn ploughing, grain dryer with heated air and elevator/air driven grain mixing (Nordby *et al.*, 2004). The concentration of *Aspergillus* spp. was higher in the settled grain dust from storage work than from threshing, but lower after autumn ploughing than after spring ploughing. Other production practices had less influence (Halstensen *et al.*, 2004). In an epidemiological study, meteorological conditions favouring fungal growth in grain were associated with hormone-dependent adverse outcomes among female farmers, interpreted as possible hormonal effects of inhaled mycotoxins during pregnancy (Kristensen *et al.*, 2000). Similar meteorological conditions expressed as potato late blight forecasts were subsequently shown to predict trichothecene mycotoxin contamination of settled grain dust (Nordby *et al.*, 2004). Furthermore, a warm summer and a wet July was associated with increased level of cultivable *Penicillium* spp. and ochratoxin-A in settled grain dust (Halstensen *et al.*, 2004).

The effect of weather and production practices on other microbial contaminants of grain dust has to our knowledge not yet been investigated. The aim of this study was therefore to characterize farmers' personal exposure to airborne grain dust, fungal spores, hyphae, β-(1→3)-glucans, bacteria and endotoxins during grain threshing and storage work in three Norwegian districts and to identify exposure determinants associated with weather and production practices.

METHODS

Sampling strategy

Eleven of the most important grain producing municipalities in Norway, each with >340 grain farms, were identified according to the Census of Agriculture and Forestry of 1989 (Central Bureau of Statistics, 1992) and were grouped into three geographically and climatically different districts according to their vicinity to the River Glomma (eastern Norway), the Lake Mjøsa (eastern inland) or the Trondheim Fjord (mid-Norway). All districts are located north of 59 degrees northern latitude. A list of active cereal farmers in these districts was obtained from the Norwegian Grain Corporation and farmers were contacted by telephone and invited to participate in the study. All the contacted farmers who had available grain for threshing or storage work agreed to participate. A total of 85 farms were visited; 40 were by the Trondheim Fjord, 17 by the Lake Mjøsa and 28 were by the River Glomma. Two work categories were used, threshing and storage work. Threshing involved operating a combine harvester during threshing of the grain and sometimes the process of transferring the grain from the harvester to a trailer used for transportation to the storage. Farmers using harvesters with cabin often worked with open door or window. Storage work included various solutions for grain drying, ventilation and rotation, and bin emptying. Each task was carried out with variable frequency and in variable time span depending on farm size and storage technology. The farmers' personal exposure to grain dust was measured once for each task. In cases where farmers did several subsequent tasks, either in different work categories or in the same work category but with different grain species, the exposure during work in each category and with each grain species was measured separately and was treated individually in the subsequent analyses. For tasks lasting <60 min, the measurement continued as long as the task lasted and for tasks with longer duration the sampling time was maximum 60 min.

Personal sampling of airborne dust

Personal airborne grain dust samples (n = 106) were collected from the farmers' breathing zone during work with the 1999 and 2000 grain crops.

Samples were collected in parallel on pre-weighed 25-mm polycarbonate (PC) filters with 0.8-μm pore size (Poretics, Osmonics, Livermore, MN) and 25-mm glass fibre filters (Whatman GF/A, Whatman, Maidstone, MA) mounted in PAS-6 personal aerosol sampler cassettes (Van der Wal, 1983) at a flow rate of 2 l min^{-1} using portable pumps (PS101; National Institute of Occupational Health, Oslo, Norway) for 10–60 min, resulting in dust loads ranging from 0.1 to 1.9 mg.

Sample preparation and analysis of microbial content

PC filters were weighed in a preconditioned room of 19–21°C and 38–42% relative humidity using a Sartorius Supermicro S4 analytical balance (Sartorius Ltd., Dublin, Ireland) and transferred to plastic containers (Nunc AS, Roskilde, Denmark) for storage at 4°C until analysis, whereas glass fibre filters were stored at −20°C in glass Petri dishes (Anumbra, Simax, Liberec, Czech Republic). Airborne dust collected on the PC filters were suspended in 5 ml of 0.01% Tween-80 by sonication for 3 min. The dust suspensions (2.5 ml) were added 20% formaldehyde solution (resulting in 2% formaldehyde in the suspensions), separated in two aliquots and stored at −20°C.

One aliquot was filtered on a PC filter with 0.4-μm pore size (Poretics) for quantification of fungal and actinomycetes spores by scanning electron microscopy (SEM). Specimens of approximately 10 × 10 mm were cut from the filters, mounted with carbon tabs on carbon stubs with diameter 15 mm and coated with platinum under vacuum (<0.4 mbar) using a Bal-Tec SCD 050 sputter coater (Bal-Tec AG, Balzers, Principality of Liechtenstein). The specimens were examined with a Jeol JSM-6400 (Jeol Ltd., Tokyo, Japan) operated at 12 keV at 8 mm working distance. In each sample, spores were counted in 100 fields at ×2000 magnification. Fungal spores were separated from actinomycetes spores by a size cut-off of 1.5 μm spore diameter (Eduard *et al.*, 1988). The concentration of fungal and actinomycetes spores was calculated from the number of spores counted, the area observed by SEM, the area of the exposed filter and the volume of the air sample. A substantial amount of fungal hyphae was observed during the microscopic analysis, and the amount of hyphae was semi-quantitatively determined by scoring the presence in each microscopic field independent of hyphal length and quantity. The percentage of microscopic fields that contained fungal hyphae was adjusted for uptake volume of the sample, divided by air volume, and expressed as arbitrary units (AU) m^{-3} as a measure of airborne hyphal exposure.

The second aliquot was stained with acridine orange for quantification of bacteria by epifluorescence microscopy as previously described (Heldal *et al.*, 1996).

Endotoxins and β-(1→3)-glucans were extracted from the glass fibre filters and analysed as previously described (Douwes *et al.*, 1995, 1996). The laminarin affinity purified anti-glucan rabbit antibody specifically binds both linear and branched β-(1→3) glucans, including yeast and plant glucans (Douwes *et al.*, 1996).

Grain production routines and weather conditions

The farmers provided information on grain species, cultivation and production details predefined as possible determinants of microbial growth (Table 1). Meteorological data (temperature, humidity and rainfall) broadcasted as potato late blight (fungal) forecasts from June to August of each growth season were obtained from eight regional meteorological stations of The Norwegian Crop Research Institute located in the vicinity of the study farms and allocated to the respective farms. A fungal forecast is a prognosis for fungal disease based on disease observation and meteorological conditions favouring fungal growth and is issued whenever the following criteria are met during a 24-h period: T_{max} 17°C or higher, T_{min} 10 °C or higher, relative humidity at noon 75% or higher and precipitation of rain 1 mm or more (Førsund, 1983). Fungal forecast frequencies by each month of the grain growth season are given in Table 2. Seasonal fungal forecasts were defined as the number of forecasts from 1 June to 30 August in each cultivation season.

Statistical analyses

Standard measures of the central tendency and distribution (arithmetic mean and range, geometric mean and geometric standard deviation) were calculated. As the exposure data were best described by lognormal distribution, the data were log transformed before statistical analysis. Endotoxin and β-(1→3)-glucan values below the detection limit were substituted by the lowest determined value divided by the square root of 2 (Eduard, 2002), whereas zero values of bacteria, fungal spores and hyphae were replaced by 0.5 bacterium, 0.5 spores or 0.5 hypha observed in 100 microscopic fields before calculation of the concentration and subsequent log transformation. Differences in exposure levels due to various categories of production practices were tested by one-way ANOVA assuming equal variances between groups, if warranted according to Levene's test; otherwise a set of *t*-tests for equality of means not requiring equal variances was done. Linear regression models were used to explore associations between fungal forecasts and microbial exposure as well as the biological association between bacterial and endotoxin exposure and between fungal (spores and hyphae) and glucan exposure. General linear models with the microbial exposure variables as univariate dependent variables and production practice categories that were significantly associated with the dependent variable in one-way ANOVA or linear regression models were built to find grain

Table 1. Grain production details predefined as possible fungal growth determinants related to $n = 106$ airborne dust samples

Variables	Categories	*n*
Growth season	1999	24
	2000	82
District	River Glomma	38
	Lake Mjøsa	21
	Trondheim Fjord	47
Cereal species	Barley	57
	Oats	35
	Spring wheat	14
Barley subspecies	Two row	37
	Six row	20
Ploughing	In autumn only	55
	In spring only	28
	Both spring and autumn	15
Production last year	Same species as preceding season	51
	Cereals, but other species preceding season	38
	Potato, oil seed, cabbage or no crop	8
Field fungicide or growth regulator	Fungicides, all types	46
	Growth regulators	20
Farmers observation	Lodged grain on >10% of crop	57
	Visible fungal damage	36
	Problems with drying[a]	20
Work operation	Threshing	31
	Storage (grain ventilation and bin emptying)	75
Grain dryer technology[a]	Cold air grain dryer	44
	Heated air grain dryer	31
Grain storage technology[a]	Manual	29
	Air/Elevator driven	46
Harvester type[b]	With cabin	25
	Without cabin	5

[a] Storage samples only ($n = 75$).
[b] Threshing samples only ($n=31$).

production-related determinants of microbial exposure. The statistical analyses were performed using the software package SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS

Personal airborne exposure levels of dust and microbial components during grain work

Fungal spores, bacteria, endotoxins, β-(1→3)-glucans and hyphae were detected in almost all samples, but actinomycetes were found in only 45% of the samples (Table 3). The exposure levels were highly variable. Although sampling time was short and task based, 50% of the samples were higher than the Norwegian 8-h time weighted average (TWA) organic dust occupational exposure limit (OEL) of 5 mg m^{-3} (Norwegian Labour Inspection Authority, 2003) and 20% exceeded three times this OEL, which is the short-term OEL. Ninety-one percent of the samples contained >200 endotoxin Units m^{-3}.

Table 2. Fungal forecasts[a] issued in three grain producing districts in 1999 and 2000

Time period	No. of days[a]
June	3 (1–5)
July	6 (1–11)
August	4 (2–9)
June–August (seasonal)	13 (5–24)

[a]Median number of days with fungal forecasts (range).

Microbial particles as sources of β-(1→3)-glucan and endotoxin exposure

Fungal spores and hyphae together explained 58% of the β-(1→3)-glucan exposure and were equally strong determinants of exposure, as assessed by linear regression (Table 4). Whereas both fungal spores and hyphae could explain the β-(1→3)-glucan

exposure during storage, only hyphae explained the exposure during threshing. Only 28% of the endotoxin exposure was explained by the bacteria exposure when all samples were considered, but as much as 68% was explained by bacteria in threshing samples (Table 4).

Microbial exposure determinants related to grain production practices

Personal airborne microbial exposure was differentially associated with various grain production practices as shown in Table 5. Univariate associations were found particularly with work operation and visible fungal damage; however, the increased exposure due to observed fungal damage on the field was restricted to storage samples (Fig. 1). Associations were furthermore found with grain species, lodging of grain, storage technology and harvester type (Table 5).

Storage work was the main predictive determinant for microbial exposure when the significant variables were adjusted for each other in general linear models (Table 6). However, work with barley or spring wheat also contributed to the glucan and endotoxin exposure variance and visible fungal damage contributed to explain the variance in inhalable dust exposure. Inhalable dust exposure was the best explained variable. Tests of between-subjects effects showed no interactions between factors in models with more than one factor.

Table 3. Microbial exposure from airborne grain dust

Component	Analytical method	*n*	% Positive samples	Exposure level			
				AM	Range	GM	GSD
Inhalable dust (mg m^{-3})	Gravimetry	104	100	11.0	110	4.4	4.0
Fungal spores ($\times 10^6$ m^{-3})	SEM	105	95	62	5200	4.0	5.9
Actinomycetes ($\times 10^6$ m^{-3})	SEM	105	45	43	3000	0.2	15.8
Hyphae ($\times 10^6$ AU m^{-3})	SEM	105	76	3.0	200	0.5	4.4
Glucans (μg m^{-3})	EIA	103	89	350	6200	120	4.7
Bacteria ($\times 10^6$ m^{-3})	FM	105	93	15	420	4.0	5.2
Endotoxins ($\times 10^3$ EU m^{-3})	LAL	104	99	30	700	5.9	8.6

AM, arithmetical mean; GM, geometrical mean; GSD, geometrical standard deviation, SEM, scanning electron microscopy; EIA, enzyme immunoassay; FM, fluorescence microscopy; AU, arbitrary units; EU, endotoxin units.

Table 4. Linear regression models explaining β-(1→3)-glucan and endotoxin in airborne grain dust

Dependent variable	Models	Explaining variables	Regression coefficients (*B*)	*p*
β-(1→3)-glucans	All samples ($n = 103$)			
	$R^2_{adj} = 0.58, p < 0.001$	Constant	1.99	<0.001
		Log fungal spores	0.40	<0.001
		Log hyphae	0.42	<0.001
	Storage work ($n = 72$)			
	$R^2_{adj} = 0.44, p < 0.001$	Constant	2.02	<0.001
		Log fungal spores	0.39	<0.001
		Log hyphae	0.23	0.03
	Threshing ($n = 31$)			
	$R^2_{adj} = 0.72, p < 0.001$	Constant	2.25	<0.001
		Log hyphae	0.99	<0.001
Endotoxins	All samples ($n = 103$)			
	$R^2_{adj} = 0.28, p < 0.001$	Constant	3.35	<0.001
		Log bacteria	0.69	<0.001
	Storage work ($n = 72$)			
	$R^2_{adj} = 0.21, p < 0.001$	Constant	3.76	<0.001
		Log bacteria	0.44	<0.001
	Threshing ($n = 31$)			
	$R^2_{adj} = 0.65, p < 0.001$	Constant	2.41	<0.001
		Log bacteria	1.38	<0.001

Table 5. Associations between airborne exposure and categorical grain production variables tested by one-way ANOVA

Variables	n[a]	Inhalable dust (mg m^{-3})			β-(1→3)-glucan (μg m^{-3})			Fungal spores (counts × 10^6 m^{-3})			Hyphae (AU × 10^6 m^{-3})			Actinomycetes (counts × 10^6 m^{-3})			Bacteria (counts × 10^6 m^{-3})			Endotoxin (×10^3 EU m^{-3})		
		GM	GSD	p	GM	GSD	p	GM	GSD	p	GM	GSD	p	GM	GSD	p	GM	GSD	p	GM	GSD	p
Growth season																						
1999	24	6.3	3.4	0.2	133	3.8	0.7	6.2	7.6	0.2	0.5	6.1	0.8	0.1	18.8	0.6	5.7	6.5	0.2	5.9	7.4	1.0
2000	80	4.0	4.2		118	5.0		3.6	5.4		0.5	4.0		0.2	15.2		3.6	4.8		5.9	9.0	
District																						
Trondheim Fjord	46	4.2	4.9	0.7	94	5.9	0.1	3.9	5.4	0.7	0.4	4.4	0.6	0.2	16.6	0.9	3.9	4.8	0.5	5.9	11.3	0.1
Lake Mjøsa	20	5.5	3.2		227	3.9		5.6	4.8		0.6	3.6		0.1	8.7		5.8	4.4		13	6.8	
River Glomma	38	4.2	3.6		114	3.5		3.6	7.5		0.4	5.1		0.1	20.6		3.4	6.2		3.9	6.1	
Grain species																						
Barley	56	4.7	4.5	0.9	159	5.3	**0.02**	4.0	5.4	1.0	0.4	4.1	0.2	0.1	12.5	0.6	4.9	5.1	0.4	9.2	9.9	**0.02**
Oats	35	4.0	3.9		65	3.4		4.2	8.3		0.5	5.6		0.2	27.3		2.9	6.6		2.7	6.1	
Spring wheat	14	4.3	2.8		174	4.2		4.0	3.4		0.9	2.9		0.1	9.2		3.8	2.6		7.7	5.7	
Barley subspecies[b]																						
Two row	35	4.5	4.3	0.8	148	5.1	0.7	3.6	5.7	0.6	0.4	4.1	0.9	0.1	13.9	0.5	4.6	5.5	0.7	8.2	9.7	0.6
Six row	20	5.0	5.3		179	5.8		4.8	5.1		0.4	4.2		0.2	10.5		5.6	4.5		11.5	10.5	
Autumn ploughing																						
Yes	69	4.7	3.5	0.6	146	4.7	0.1	4.3	7.1	0.8	0.5	4.6	0.7	0.2	19.4	0.6	3.7	5.3	0.5	6.8	8.6	0.4
No	35	4.1	5.1		88	4.6		3.8	4.0		0.4	4.3		0.1	10.7		4.8	5.2		4.8	8.3	
Same species as preceding season																						
Yes	50	5.2	4.4	0.3	156	5.2	0.1	4.0	6.1	1.0	0.5	5.1	0.6	0.1	12.0	0.6	5.3	5.6	0.1	7.3	11.3	0.3
No	55	3.8	3.7		96	4.3		4.1	5.9		0.4	4.0		0.2	20.2		3.1	4.8		4.9	6.5	
Field fungicide																						
Yes	46	5.1	3.8	0.4	155	4.8	0.2	4.2	4.7	0.5	0.5	3.5	0.3	0.1	9.7	0.2	4.1	4.7	0.6	8.2	9.8	0.4
No	44	4.0	4.1		105	4.3		3.4	5.5		0.4	4.4		0.2	17.8		3.4	5.2		5.6	6.5	
Growth regulator																						
Yes	20	6.4	3.7	0.3	178	4.6	0.3	6.8	6.2	0.2	0.4	4.2	0.8	0.5	16.0	0.06	3.3	8.7	0.5	8.4	6.8	0.5
No	80	4.4	4.1		122	4.6		3.7	6.1		0.5	4.6		0.1	15.9		4.3	4.6		6.1	9.0	
Lodging																						
Yes	59	3.7	3.9	0.09	101	4.5	0.1	3.0	5.5	**0.04**	0.4	3.7	0.2	0.2	17.5	0.8	3.4	4.9	0.2	5.2	9.5	0.4
No	45	5.9	4.1		165	4.7		6.3	6.2		0.6	5.5		0.2	14.7		5.2	5.5		7.3	7.4	

Table 5. *Continued*

Variables	n[a]	Inhalable dust (mg m^{-3})			β-(1→3)-glucan (μg m^{-3})			Fungal spores (counts × 10^6 m^{-3})			Hyphae (AU × 10^6 m^{-3})			Actinomycetes (counts × 10^6 m^{-3})			Bacteria (counts × 10^6 m^{-3})			Endotoxin (×10^3 EU m^{-3})		
		GM	GSD	p	GM	GSD	p	GM	GSD	p	GM	GSD	p	GM	GSD	p	GM	GSD	p	GM	GSD	p
Visible fungal damage																						
Yes	35	7.7	4.2	**0.003**	214	4.8	**0.007**	6.0	5.6	0.1	0.7	3.6	0.07	0.1	9.1	0.5	6.5	3.1	**0.01**	14.2	6.7	**0.003**
No	70	3.3	3.7		90	4.3		3.3	6.0		0.4	4.7		0.2	20.2		3.1	6.1		3.8	8.5	
Work operation																						
Threshing	31	1.2	3.3	**<0.001**	39	4.8	**<0.001**	1.3	4.9	**<0.001**	0.2	3.9	**0.001**	0.1	5.3	**0.009**	3.2	4.0	0.3	1.2	10.3	**<0.001**
Storage	74	7.6	3.0		197	3.6		6.5	5.2		0.6	4.2		0.2	21.0		4.4	5.8		11.5	5.4	
Grain dryer technology[c]																						
Ambient air dryer	43	7.2	2.8	0.7	210	3.3	0.6	6.3	3.7	0.9	0.5	3.8	0.1	0.2	15.0	0.3	5.7	4.2	0.2	11.2	5.2	0.9
Heated air dryer	31	7.9	3.2		180	4.0		6.8	7.9		0.8	4.6		0.4	31.3		3.2	8.1		11.9	5.8	
Grain storage technology[d]																						
Manual	29	6.9	3.4	0.6	173	4.2	0.5	5.3	5.4	0.4	0.5	4.3	0.2	0.1	13.8	**0.05**	3.8	6.5	0.5	9.1	6.9	0.4
Air/elevator driven	45	8.0	2.8		213	3.2		7.5	5.1		0.8	4.1		0.4	24.3		4.9	5.4		13.3	4.5	
Harvester type[c]																						
With cabin	25	0.9	2.8	**0.03**	30	4.3	0.2	1.0	4.6	0.07	0.2	3.5	0.2	0.1	5.4	0.7	2.3	3.3	**0.02**	0.8	9.3	**0.05**
Without cabin	5	3.1	3.5		85	5.6		3.9	4.2		0.5	6.2		0.1	5.4		10.3	3.7		6.6	4.1	

GM, geometrical mean; GSD, geometrical standard deviation.
[a]For most variables n (dust) and n (endotoxin) are one less than shown and n (glucan) is two less than shown.
[b]Barley samples only.
[c]Threshing samples only (n=31).
[d]Storage samples only (n=75).
p values indicate significance level of the difference between groups as tested by one-way ANOVA assuming equal variances between groups. The homogeneity of variances between groups was tested by Levene's statistic and in cases where p values were <0.05, a t-test for equality of means was done with equal variances not assumed (independent sample t-test). p-values given in bold are ≤0.05.

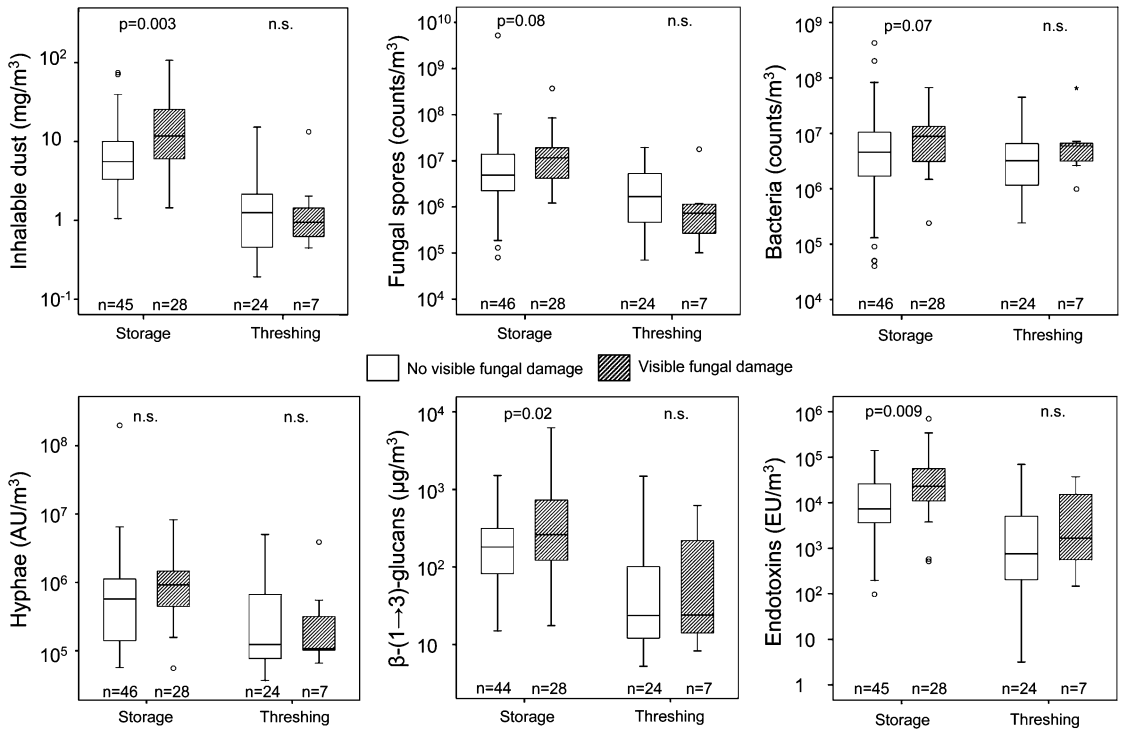

Fig. 1. Microbial exposure during threshing and storage work related to visible fungal damage observed on the field. Significant differences between groups according to independent sample *t*-tests are indicated by *P* values or n.s. (not significant).

Meteorological impact on microbial exposure

Inhalable dust, β-(1→3)-glucan, endotoxin and hyphal exposure during threshing were positively correlated with the frequency of fungal forecasts throughout the grain growth season (results not shown). Bacterial exposure was correlated with July fungal forecasts. Seasonal fungal forecasts explained 31% ($p = 0.001$) of the β-(1→3)-glucan exposure, 22% ($p = 0.005$) of the hyphal exposure, 12% ($p = 0.04$) of the endotoxin exposure and 23% ($p = 0.04$) of the dust exposure, as assessed by linear regression analysis. Exposure levels during storage were negatively correlated to seasonal fungal forecasts for hyphae ($r_s = -0.27$, $p = 0.02$), and inhalable dust ($r_s = -0.26$, $p = 0.03$). Closer examination revealed that this applied to grain stored in ambient air dryers (results not shown). When both types of grain work were evaluated together, no correlation between fungal forecasts and microbial exposure was revealed.

DISCUSSION

Norwegian grain farmers seek to maximize quality and crop yields by counteracting plant diseases by using resistant grain species, fungicides or growth regulators and artificial drying of damp grain when appropriate. Nevertheless, the current study indicates that they are exposed to substantial amounts of microorganisms and their components during grain work. The high bacterial levels and low actinomycete prevalence in grain dust were similar to previous reports (Darke *et al.*, 1976; Dutkiewicz, 1978; Eduard *et al.*, 2001). Fungal spore exposure levels during storage work were not only higher than the lowest observed effect level (10^5–10^6 spores m^{-3}) for respiratory symptoms (Eduard *et al.*, 2001) but also three times higher than previously reported (Melbostad and Eduard, 2001) for Norwegian farmers.

Airborne hyphae have not previously been considered a significant part of occupational fungal exposure, although fungal fragments derived from spores and mycelium have been described in a few studies (Sorensen *et al.*, 1987; Li and Kendrick, 1995; Robertson, 1997). These studies reported that indoor and outdoor air contained an average concentration of 29–146 particles m^{-3}. This is considerably lower than the concentration of hyphae on the grain farmers' work place in the present study, which revealed that hyphae constituted a considerable part of the fungal exposure [geometrical mean (GM) = 0.5×10^6 m^{-3}, Table 3]. This suggests that the fungal exposure may in some situations be higher than previously estimated, although a reliable method for hyphal quantification is needed to evaluate this in general. Other hyphal exposure studies have primarily focused on the allergenic potential (Green *et al.*, 2005a,b) or on hyphae as inoculants in clinical fungal

Table 6. General linear models describing determinants of microbial exposure in grain farming[a]

Variables	Factors	Categories	n	GM	GSE	p[b]
Inhalable dust						
R^2_{adj}= 0.39, $p < 0.001$	Work operation	Threshing	31	1.5	1.2	<0.001
		Storage	73	8.1	1.1	
	Fungal damage of grain	Visible fungal damage	35	4.6	1.2	0.01
		No visible fungal damage	69	2.5	1.1	
Fungal spores						
R^2_{adj}= 0.16, $p < 0.001$	Work operation	Threshing	31	1.3	1.3	<0.001
		Storage	74	6.5	1.2	
Hyphae						
R^2_{adj}= 0.10, $p = 0.001$	Work operation	Threshing	31	0.2	1.3	0.001
		Storage	74	0.6	1.2	
Glucans						
R^2_{adj}= 0.31, $p < 0.001$	Work operation	Threshing	31	36	1.8	<0.001
		Storage	74	200	1.2	
	Grain species	Barley	56	121	1.2	0.001
		Oats	35	42	1.3	
		Spring wheat	14	121	1.4	
Bacteria						
R^2_{adj}= 0.03, $p = 0.03$	Fungal damage of grain	Visible fungal damage	35	0.7	1.3	0.03
		No visible fungal damage	70	3.1	1.2	
Endotoxins						
R^2_{adj}= 0.31, $p < 0.001$	Work operation	Threshing	31	1.1	1.4	<0.001
		Storage	74	11.0	1.3	
	Grain species	Barley	56	6.2	1.3	0.001
		Oats	35	1.3	1.4	
		Spring wheat	14	4.7	1.6	

[a]Univariate general linear models. GM, geometric mean; GSE, geometric standard error.
[b]p, significance level of the difference between categories for each factor as given by two-way ANOVA. Tests of between-subject effects showed no interaction between factors in models with more than one factor.

infections studies (Lowman *et al.*, 2003). Hyphae, like fungal spores, contain biological active components such as β-(1→3) glucans (Bohn and BeMiller, 1995), mycotoxins (Palmgren and Lee, 1986) and allergens (Green *et al.*, 2005b) and should therefore be included in microbial exposure assessments. The fact that the hyphal exposure explained 72% of the β-(1→3) glucan exposure during threshing and contributed equally as fungal spores to explain the β-(1→3) glucan exposure in all samples emphasizes the importance of hyphae exposure assessments.

The β-(1→3)-glucan exposure levels (GM = 120 μg m^{-3}) were considerably higher than previously reported for short-term, task-based exposure measurements in Norwegian farming using the same analytical method and laboratory (GM = 0.8 μg m^{-3}; Eduard *et al.*, 2001). Linear regression models of the β-(1→3)-glucan exposure during threshing and storage work suggested a shift from hyphal-derived to predominantly spore-derived fungal β-(1→3)-glucan source during the time from threshing until storage work. This is biologically plausible since the fungi are likely to terminate hyphal growth and rather sporulate during drying and storage.

Airborne β-(1→3)-glucan levels have often been used as markers of fungal exposure (Douwes, 2005), but this may overestimate the fungal exposure since only 58% of the β-(1→3)-glucan exposure could be explained by fungal (spores and hyphae) exposure in the present study. Plants are likely to be additional β-(1→3)-glucans sources during grain handling and other agricultural work. Thus far the biological activity of plant-derived glucan has hardly been studied, but if grain- and fungal-derived β-(1→3)-glucans have similar biological activity, it seems important to assess exposure to β-(1→3)-glucans as such, and not only as markers of fungal exposure. However, an OEL for β-(1→3) glucan exposure has not yet been established because of inconclusive scientific evidence for health effects, partly due to glucan source variability in different environments and analytical differences (Douwes, 2005).

The Gram-negative soil bacterium *Enterobacter agglomerans* is frequently found in airborne dust

from freshly harvested grain (Dutkiewicz, 1976, 1978). The low, although significant predictive value of bacteria for explaining endotoxin exposure during storage work compared to threshing, may reflect dehydration-induced bacterial death in the storage environment. Since endotoxins are more sustainable than bacterial cells, endotoxin levels would remain high even if bacteria numbers would diminish. Breakdown of the bacterial cell wall may also increase the solubility and thereby the proportion of endotoxins detectable by the limulus amoebocyte lysate (LAL) assay. However, in the threshing samples, this may not have occurred to the same degree due to higher water content, resulting in a better correlation between bacteria and endotoxins. Thus, LAL analysis is necessary for endotoxin exposure assessment, since bacterial exposure only in some situations correlates with the endotoxin exposure. The fact that almost all measurements exceeded 200 EU m^{-3}, suggested so high endotoxin exposure levels that it might affect the farmers' health (ICOH Committee on Organic Dust, 1997). Although endotoxin exposure levels were lower in the present study than previously observed in Norwegian farmers (Melbostad and Eduard, 2001), it was similar to a recent Dutch report based on approximately 8 h TWA (Spaan *et al.*, 2006).

Microbial growth is influenced by meteorological conditions during the grain growth season as suggested by the increased exposure levels of β-(1→3)-glucans, hyphae, endotoxins and inhalable dust during threshing in areas with frequent seasonal fungal forecasts. Similar wet and warm grain growth seasons may also predict *Fusarium* fungal infection on grain (Langseth and Elen, 1997; Hooker *et al.*, 2002) and *Fusarium* mycotoxin contamination in settled grain dust (Nordby *et al.*, 2004). However, the association between meteorological conditions and *Fusarium* mycotoxins in settled dust were stronger and consistent across tasks (Nordby *et al.*, 2004). Thus, meteorological conditions may predict *Fusarium* grain infection and presence of mycotoxins in the dust potentially better than personal exposure to the microbial components investigated in the present study.

Storage work was the main predictive qualitative determinant for microbial exposure, probably because this work operation generated the highest concentrations of dust containing microbial components. Storage work, together with visible fungal damage, was also a stronger predictor of inhalable dust than of the microbial components in the dust. Work operation-dependent variations in dust levels were consequently the most important predictive factor for microbial exposure under the conditions for limited microbial contamination given by the farmers' preventive practices. Thus, any dustiness prevention or protection will decrease microbial exposure. The amount of dust generated by grain conveyors or grain transfer operations varies widely and depends on harvesting methods, type, source and amount of grain handled. It is also influenced by the number of previous handlings and conveying equipment characteristics, especially the extent of enclosure provided at transfer points where grain falls freely (Farant and Moore, 1980). Having a cabin on the harvester reduced dust exposure considerably (Thorpe *et al.*, 1997; Swan and Crook, 1998; this study), but the protective value will depend on the air intake filter efficiency and the need to open the cabin door during threshing.

Although cabin on the harvester, choked unloading from trucks and hoppers, aspiration and ventilation to dust collection systems and aspiration systems used for cleaning all have been identified as dust reducing interventions (Thorpe *et al.*, 1997; Halter, 1980), personal respiratory protection matched to the exposure level may be the simplest. However, the individual acceptance of such respiratory protection is generally low due to breathing resistance and discomfort of mal-fitting, hot and sweaty equipment (Mpofu *et al.*, 2002).

CONCLUSIONS

Despite farmers' practices to prevent fungal growth, grain and grain dust were contaminated with microorganisms and their components resulting in high, sometimes putative hazardous exposure levels during grain work, irrespective of the production practices. Fungal hyphae constituted a considerable part of the fungal exposure and were important biological predictors of β-(1→3)-glucan exposure. However, a substantial amount of β-(1→3)-glucan might be grain derived rather than fungal derived and should be included in health effect considerations. Warm and wet meteorological conditions, indicated by seasonal fungal forecasts, visible fungal damage of the grain, storage work and barley, were the main determinants of exposure to inhalable dust and its microbial components. Thus, preventive or protective measures against dust exposure would generally reduce microbial exposure levels and may be particularly important during storage work, work with barley and grain visibly damaged by fungi.

FUNDING

Norwegian Research Council (grant no. 159263/V50).

Acknowledgements—Thanks are due to Elianne Seberg at the National Institute of Occupational Health in Oslo for microscopic counting of bacteria.

REFERENCES

Bohn JA, BeMiller JN. (1995) (1→3)-β-*D*-glucans as biological response modifiers: a review of structure-functional activity relationships. Carbohydrate Polymers; 28: 3–14.

Central Bureau of Statistics. (1992) Census of Agriculture and Forestry 1989. Vol. 4. Agriculture Area Utilization, Oslo, Kongsvinger: NOS (Norwegian Official Statistics) C24.

Darke CS, Knowelden J, Lacey J *et al*. (1976) Respiratory-disease of workers harvesting grain. Thorax; 31: 294–302.

Douwes J. (2005) (1→3)-β-*D*-glucans and respiratory health: a review of the scientific evidence. Indoor Air; 15: 160–9.

Douwes J, Doekes G, Montijn R *et al*. (1996) Measurement of β (1→3)-glucans in occupational and home environments with an inhibition enzyme assay. Appl Environ Microbiol; 62: 3176–82.

Douwes J, Versloot P, Hollander A *et al*. (1995) Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. Appl Environ Microbiol; 61: 1763–9.

Dutkiewicz J. (1976) Studies on endotoxins of Erwinia herbicola and their biological activity. Zbl Bakt Hyg I Abt Orig A; 236: 487–508.

Dutkiewicz J. (1978) Exposure to dust-borne bacteria in agriculture. I. Environmental studies. Arch Environ Health; 33: 250–9.

Dutkiewicz J, Kus L, Dutkiewicz E *et al*. (1985) Hypersensitivity pneumonitis in grain farmers due to sensitization to *Erwinia herbicola*. Ann Allergy; 54: 65–8.

Eduard W. (2002) Estimation of mean and standard deviation. Letter to the editor. AIHA J; 63: 1–4.

Eduard W, Douwes J, Mehl R *et al*. (2001) Short term exposure to airborne microbial agents during farm work: exposure-response relations with eye and respiratory symptoms. Occup Environ Med; 58: 113–8.

Eduard W, Lacey J, Karlsson K *et al.* (1988) Evaluation of methods for enumerating microorganisms in filter samples from highly contaminated environments associated with non-infectious occupational lung disease. In: Nordic Council of Ministers. Harmonization of sampling and analysis of mould spores. Copenhagen: Nordic Council of Ministers; 88: 36–70.

Farant JP, Moore CF. (1980) Dust exposure in the Canadian grain industry. In: Dosman JA and Cotton DJ, editors. Occupational pulmonary disease: focus on grain dust and health. New York: Academic Press; pp. 477–506.

Førsund E. (1983) Late blight forecasting in Norway 1957–1980. Bull OEPP; 13: 255–8.

Green BJ, Schmechel D, Sercombe JK *et al*. (2005a) Enumeration and detection of aerosolized *Aspergillus fumigatus* and *Penicillium chrysogenum* conidia and hyphae using a novel double immunostaining technique. J Immunol Methods; 307: 127–34.

Green BJ, Sercombe JK, Tovey ER. (2005b) Fungal fragments and undocumented conidia function as new aeroallergen sources. J Allergy Clin Immunol; 115: 1043–8.

Halstensen AS, Nordby KC, Elen O *et al*. (2004) Ochratoxin A in grain dust—estimated exposure and relations to agricultural practices in grain production. Ann Agric Environ Med; 11: 245–54.

Halter GS. (1980) Dust control in grain elevators. In: Dosman JA and Cotton DJ, editors. Occupational pulmonary disease: focus on grain dust and health. New York: Academic Press; pp. 545–53.

Heldal K, Skogstad A, Eduard W. (1996) Improvements in the quantification of airborne microorganisms in the farm environment by epifluorescence microscopy. Ann. Occup. Hyg; 40: 437–47.

Hill RA, Lacey J. (1983) The microflora of ripening barley grain and the effect of preharvest fungicide application. Ann Appl Biol; 102: 455–65.

Hooker DC, Schaafsma AW, Tamburic-Ilinic L. (2002) Using weather variables pre- and post-heading to predict deoxynivalenol content in winter wheat. Plant Dis; 86: 611–9.

ICOH Committee on Organic Dust. (1997) Endotoxin in the environment: a criteria document. Int J Occup Environ Health; 3: 1–48.

Kristensen P, Andersen A, Irgens LM. (2000) Hormone-dependent cancer and adverse reproduction outcomes in farmers' families—effects of climatic conditions favouring fungal growth in grain. Scand J Work Environ Health; 26: 331–7.

Lacey J. (1980) The microflora of grain dusts. In: Dosman JA and Cotton DJ, editors. Occupational pulmonary disease: focus on grain dust and health. New York: Academic Press; pp. 416–40.

Lacey J, Crook B. (1988) Fungal and actinomycete spores as pollutants of the workplace and occupational allergens. Ann Occup Hyg; 32: 515–33.

Langseth W, Elen O. (1997) The occurrence of deoxynivalenol in Norwegian cereals—differences between years and districts, 1988–1996. Acta Agric Scand Sect B Soil and Plant Dis; 47: 176–84.

Langseth W, Stabbetorp H. (1996) The effect of lodging and time of harvest on deoxynivalenol contamination in barley and oats. J Phytopathol; 144: 241–5.

Langseth W, Stenwig H, Sogn L *et al*. (1993) Growth of moulds and production of mycotoxins in wheat during drying and storage. Acta Agric Scand Sect B Soil and Plant Sci; 43: 32–7.

Li D-W, Kendrick B. (1995) A year-round study of functional relationships of airborne fungi with meteorological factors. Int J Biometeorol; 39: 74–80.

Lowman DW, Ferguson DA, Williams DL. (2003) Structural characterization of (1→3)-β-D-glucans isolated from blastospore and hyphal forms of *Candida albicans*. Carbohydr Res; 338: 1491–6.

Melbostad E, Eduard W. (2001) Organic dust-related respiratory and eye irritation in Norwegian farmers. Am J Ind Med; 39: 209–17.

Melbostad E, Eduard W, Magnus P. (1997) Chronic bronchitis in farmers. Scand J Work Environ Health; 23: 271–80.

Mpofu D, Lockinger L, Bidwell J, McDuffie HH. (2002) Evaluation of a respiratory health program for farmers and their families. J Occup Environ Med; 44: 1064–74.

Nordby KC, Halstensen AS, Elen O *et al*. (2004) Trichothecene mycotoxins and their determinants in settled grain dust related to grain production. Ann Agric Environ Med; 11: 75–83.

Norwegian Labour Inspection Authority. (2003) Occupational limit values [In Norwegian]. Available from http://www.arbeidstilsynet.no/regelverk/veiledninger/pdf/361.pdf

Palmgren MS, Lee LS. (1986) Separation of mycotoxin containing sources in grain and determination of their mycotoxin potential. Environ Health Perspect; 66: 105–8.

Robertson LD. (1997) Monitoring viable fungal and bacterial bioaerosol concentrations to identify acceptable levels for common indoor environments. Indoor Built Environ; 6: 295–300.

Rylander R. (2002) Endotoxin in the environment: exposure and effects. J Endotoxin Res; 8: 241–52.

Sigsgaard T, Bonefeld-Jørgensen EC, Hoffmann HJ *et al*. (2005) Microbial cell wall agents as an occupational hazard (review). Toxicol Appl Pharmacol; 207: 310–9.

Smith JD. (1989) Some biological components of grain and forage dusts. In: Dosman JA and Cockroft DW, editors. Principles of health and safety in agriculture. Florida: CRC Press; pp. 144–7.

Sorensen WG, Frazer DG, Jarvis BB *et al*. (1987) Trichothecene mycotoxins in aerosolized conidia of *Stachybotrys atra*. Appl Environ Microbiol; 53: 1370–5.

Spaan S, Wouters I, Oosting I *et al*. (2006) Exposure to inhalable dust and endotoxins in agricultural industries. J Environ Monit; 8: 63–72.

Swan JRM, Crook B. (1998) Airborne microorganisms associated with grain handling. Ann Agric Environ Med; 5: 7–15.

Thorpe A, Gould JRM, Brown RC *et al*. (1997) Investigation of the performance of agricultural vehicle cab filtration systems. J Agric Engng Res; 66: 135–49.

Ulmer AJ, Schletter J, Heine H *et al*. (1997) Biochemistry and cell biology of endotoxins. Int J Occup Environ Health; 3: s8–s17.

Van der Wal JF. (1983) Comparative measurements of the total dust concentration at the work place with different samplers—part I. Staub Reinhalt Luft; 43: 292–4. [in German].

Young SH, Castranova V. (2005) Animal model of (1→3)-β-glucan-induced pulmonary inflammation in rats. In: Young SH and Castranova V, editors. Toxicology of (1→3)-β-glucans—glucans as a marker for fungal exposure. London: Taylor and Francis; pp. 65–95.