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# TRICHOTHECENE MYCOTOXINS AND THEIR DETERMINANTS IN SETTLED DUST RELATED TO GRAIN PRODUCTION

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**Abstract:** We hypothesise that inhalant exposure to mycotoxins causes developmental outcomes and certain hormone-related cancers that are associated with grain farming in an epidemiological study. The aim of the present study was to identify and validate determinants of measured trichothecene mycotoxins in grain dust as work environmental trichothecene exposure indicators. Settled grain dust was collected in 92 Norwegian farms during seasons of 1999 and 2000. Production characteristics and climatic data were studied as determinants of trichothecenes in settled dust samples obtained during the production of barley (N = 59), oats (N = 32), and spring wheat (N = 13). Median concentrations of trichothecenes in grain dust were <20, 54, and <50 mg/kg (ranges <20–340, <30–2400, and <50–1200) for deoxynivalenol (DON), HT-2 toxin (HT-2) and T-2 toxin (T-2) respectively. Late blight potato rot (fungal) forecasts have been broadcast in Norway to help prevent this potato disease. Fungal forecasts representing wet, temperate, and humid meteorological conditions were identified as strong determinants of trichothecene mycotoxins in settled grain dust in this study. Differences in cereal species, production properties and districts contributed less to explain mycotoxin concentrations. Fungal forecasts are validated as indicators of mycotoxin exposure of grain farmers and their use in epidemiological studies may be warranted.

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**Key words: cereals, determinant, farming, grain dust, mycotoxin, trichothecene, work environment.**

## INTRODUCTION

In an epidemiological study, mothers engaged in grain handling had a twofold risk of preterm births or late abortions compared to non-grain farmers [21]. The association was strongest for grain farmers pregnant in rainy summers and wet and temperate growing seasons, conditions known to favour fungal growth. Humid climate and grain production were also associated with some hormone-dependent cancers and adverse reproductive outcomes in farmers' families [22]. A hypothesis was therefore put forward that hormonal or immunological effects of mycotoxin exposure may cause adverse reproductive outcomes and cancer in farmers' families.

Mycotoxins are located in spores and mycelium of fungi, and can be excreted in the substrate, such as grain and straw [39]. Ingestion of contaminated food and feed may impose health hazards to man and animals [23, 8, 19,

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1, 7]. Immunosuppression due to chronic exposure to mycotoxins modulates a number of infectious diseases important to human health, especially in developing countries [37]. Trichothecene mycotoxins have been implicated in livestock reproductive disorders such as abortions and ovarian malfunctions [8]. Trichothecene mycotoxins, e.g. T-2 toxin (T-2) and deoxynivalenol (DON) are strongly immunosuppressive compounds [49, 6] produced by *Fusarium* moulds during growth and storage of cereal grain [48, 2, 25, 28] and often found in Norwegian grain [45, 27]. In a pooled study of trichothecenes, in 13,900 samples of small grains from Europe, North America and South America, DON was found in 58% of the samples, while T-2 and its precursor HT-2 toxin (HT-2) was found in 14% and 11%, respectively, of 8,900 samples predominantly from Europe [50]. In a study from Poland [24], DON was identified in 40% of samples of settled grain dust and in the same percentage of wheat grain samples. The incidence of *Fusarium* infection in wheat depends on optimal timing of rainfall, moisture, and the temperature conditions during susceptible periods of cereal development [36]. Wet and temperate weather conditions during cereal pre- and post-heading development were associated with trichothecene concentrations in Norwegian [15, 27] and Canadian grain [18].

In a gestational study, the feeding of dams with 2 ppm of T-2 transplacentally induced immune impairment in the newborn pups [26]. Inhalation exposure to T-2 induced high lethality in mice [49], and toxicity by inhalation of T-2 in mice and rats was 10–20 times higher than by oral exposure [4, 5]. T-2 inhalation induced lesions of the lymphatic tissue in mice [4] and decreased lymphocyte counts in swine [40]. We therefore suspect that maternal inhalation of trichothecenes could be toxic to the developing human foetus at very low exposure levels.

Melbostad and Eduard [35] found a total airborne dust during grain production of 5 mg/m$^3$ (geometric mean, 95% CI 0.5 to 45 mg/m$^3$). Given a hypothetical exposure to 45 mg/m$^3$ total dust containing 2000 µg/kg HT-2, the farmer would inhale a dose of 1.3 µg HT-2 during 8 hrs work of moderate physical exertion (ventilation 30 l/min). This is less than the proposed alimentary maximum tolerable daily intake of HT-2 in Scandinavian countries of 0.2 µg/kg body weight [12], but the toxicity by inhalation is possibly higher than by oral exposure [4,5]. In a study using high volume sampling during handling of grain in Finnish barns, DON was detected in 3 out of 20 samples with a maximum concentration of 20 ng/m$^3$ [31].

Personal measurements of exposure to trichothecenes are not feasible. Therefore indirect estimates of exposures are sought. Exposure determinants may be exploited to improve exposure assessment in epidemiological studies [44]. Factors related to the cultivation, growth, harvest and storage of grain are expected to influence trichothecene contents in grain dust. Such determinants, if identified, can serve as surrogate measures of exposure to mycotoxins in epidemiological studies. The aims of this study were to assess determinants of measured trichothecenes in grain dust and to validate these determinants as indicators of trichothecene exposure for their use in epidemiological studies.

**Table 1.** Number of settled grain dust samples; by season, district and *à priori* selected grain production characteristics (N = 104).

| Determinants | N | % |
|---|---|---|
| Cereal species | | |
| Barley | 59 | 57 |
| Oats | 32 | 31 |
| Spring wheat | 13 | 12 |
| District | | |
| River Glomma | 36 | 35 |
| Lake Mjøsa | 21 | 20 |
| Trondheim Fjord | 47 | 45 |
| Growth season | | |
| 1999 | 20 | 19 |
| 2000 | 84 | 81 |
| Ploughing | | |
| In autumn only | 54 | 52 |
| In spring only | 28 | 27 |
| Grain batch composed from autumn ploughed and spring ploughed plots | 14 | 13 |
| No ploughing | 8 | 8 |
| Production last year | | |
| Same grain cultivar in preceding season | 49 | 47 |
| Cereals, but other cultivar in preceding season | 35 | 34 |
| Potato, oil seed, cabbage, or no crop | 20 | 19 |
| Field fungicide or growth regulator application | | |
| Fungicides, all types | 45 | 43 |
| Growth regulators | 18 | 17 |
| Lodging of grain (horizontal straws) | | |
| Lodged grain on >10% of crop | 62 | 60 |
| Farmers observation | | |
| Visible mould damage to grain observed by the farmer | 35 | 34 |
| Work operation | | |
| Threshing | 34 | 33 |
| Storage (grain mixing, ventilation, or bin emptying) | 70 | 67 |
| Grain storage technology[a] | | |
| Cold air grain dryer | 41 | 59 |
| Heated air dryer | 29 | 41 |
| Grain elevator or air driven mixing of grain during drying | 21 | 30 |
| Manual mixing of grain during drying | 12 | 17 |

[a]Fraction of storage samples only (N = 70).

**Table 2.** Climatic covariates, arithmetric mean (AM), standard deviation (SD) and range.

| Determinants | AM | SD | Range |
|---|---|---|---|
| Climatic conditions[a] in growth season (N = 104) | | | |
| Fungal forecasts | | | |
| A forecasts June[b] | 2.8 | 1.3 | 1–5 |
| A forecasts July | 5.9 | 3.4 | 1–11 |
| A forecasts August | 4.6 | 1.7 | 2–9 |
| Seasonal A forecasts | 14 | 5.0 | 6–26 |
| B forecasts June[c] | 0.9 | 0.7 | 0–3 |
| B forecasts July | 0.4 | 0.8 | 0–3 |
| B forecasts August | 0.3 | 0,7 | 0–2 |
| Seasonal B forecasts | 1.6 | 1.6 | 0–6 |
| Rainfall (mm precipitation) | | | |
| May | 55 | 21 | 26–110 |
| June | 100 | 23 | 45–160 |
| July | 91 | 43 | 25–180 |
| August | 79 | 30 | 25–120 |
| September | 45 | 29 | 21–140 |

[a]Data obtained from automated meteorological stations of the Norwegian Crop Research Institute; [b]A fungal A forecast (number of days) is issued when the following conditions are met during a 24 hr period: $T_{max} \geq 17°C$, $T_{min} \geq 10°C$, Relative humidity at noon $\geq 75\%$, Rainfall $\geq$ 1mm; [c]A fungal B forecast is issued when fungal forecasts criteria are met for 2 consecutive days.

**Table 3.** Trichothecene mycotoxins in settled grain dust from barley, oats and spring wheat production.

| | Samples | | Detection limit (DL) | Mycotoxin concentration (µg/kg) | | |
|---|---|---|---|---|---|---|
| Mycotoxin | N | %>DL | µg/kg | Median | Mean[a] | Maximum |
| DON | 104 | 43 | 20 | *15*[b] | 31 | 340 |
| T-2 | 104 | 23 | 50–100[c] | n.f.[b] | 62 | 1200 |
| HT-2 | 104 | 88 | 30 | 54 | 130 | 2400 |
| NIV | 104 | 1.9 | 50 | n.f. | | 67 |
| MAS | 104 | 0.0 | 20 | n.f. | | n.f. |
| DAS | 104 | 5.8 | 10 | n.f. | | 37 |
| 3-A-DON | 104 | 0.0 | 20 | n.f. | | n.f. |
| Fusarenon-X | 104 | 0.0 | 40 | n.f. | | n.f. |

[a]For mycotoxins present above DL in more than 20% of samples, a mean concentration was calculated. Values below DL were treated as follows in calculation of the means: if a signal corresponding to less than DL was detected, the concentration obtained from the read signal was used. If no signal was read, the lowest obtained concentration divided by √2 was substituted. These values were 4.2 for DON and 24 for T-2; [b]Readable values below DL are shown in italics, n.f. (not found) is shown if there was no readable value below DL; [c]For T-2 toxin, DL was 100 µg/kg in 7 negative samples; and 50 µg/kg in the rest of negatives and all positives.

## MATERIALS AND METHODS

**Study population.** Tasks generating high grain dust exposure levels and production factors expected to influence growth of trichothecene producing fungi were identified during work in which grain farmers participated. Eleven Norwegian municipalities in climatically different grain producing districts were identified according to the Census of Agriculture and Forestry of 1989 [3]. Active cereal farmers in these municipalities were selected randomly from a list of grain producers supplied by the Norwegian Grain Corporation and were contacted by telephone and invited to participate in the study.

**Sampling.** Grain threshing, drying and delivery were selected for sampling. Sampling was carried out from the surfaces of the grain container, elevator, combine harvester or grain trailer where grain dust settled during work. Whenever possible, we avoided surfaces that were visibly contaminated before work started, i.e. during threshing. During grain storage sampling the pre-work cleaning status of surfaces could not be verified. Settled dust was collected on cellulose filters covered with a stainless steel wire of 400 µm mesh, using a filter cassette mounted on a dust collector nozzle (ALK Abelló; Horsholm, Denmark), which was fitted to a Black & Decker HC431 dust buster (Dustbuster Turbo, Berkshire, UK). We obtained sample masses between 0.1–3.0 g. Samples were frozen at -20ºC until analysis.

**Determinants.** Sample characteristics and information provided by the farmer on *á priori* defined production characteristics are given in Table 1. We arbitrarily used

1 September instead of the actual date of harvesting in calculations of storage time, because stored grain batches were often composed of grain harvested on different days. Information on temperature, relative humidity, and rainfall from May–September of each growth season was obtained from 8 regional meteorological stations of the Norwegian Crop Research Institute. Meteorological data from the station situated nearest to each farm was allocated to each sample. Late blight potato rot (fungal) forecasts (number of days) were calculated for each sample (Tab. 2). A fungal forecast is issued whenever the following criteria are met during a 24 hr period: $T_{max}$ 17ºC or higher, $T_{min}$ 10ºC or higher, relative humidity at noon 75% or higher, and precipitation 1 mm or more, modified after Førsund [14]. Each fungal forecast issued was defined as an A-forecast event. Two fungal forecasts issued on consecutive days was defined as a B-forecast event. The sum of fungal forecast events from 1 June–30 September in each season was defined as seasonal fungal forecasts. To account for regional differences not specified by our covariates, the municipalities were grouped in districts according to their vicinity to the River Glomma, Lake Mjøsa, or the Trondheim Fjord.

**Quantification of trichothecenes.** Grain dust extracts of acetonitrile-water were purified and derivatized, and trichothecenes were determined by gas chromatography-mass spectrometry (GC-MS) according to a method previously described [29]. 15-acetyl-DON (never found in Norwegian cereals) served as the internal standard, while an external standard calibration curve was used for quantification. The detection limit (DL) obtained for the different toxins are given in Table 3. The mean recovery was 102, 101, 96, 84, 84, 84, 83, and 81%, for diacetoxyscirpenol (DAS), T-2 toxin, HT-2 toxin, nivalenol (NIV), fusarenon-X, monoacetoxyscirpenol (MAS), 3-acetyl-DON, and DON, respectively. A mean value of 780 µg/kg of DON (certified value 670 µg/kg) with a relative standard deviation of 16% was obtained for the reference material (BCR Wheat RM 379).

**Statistical methods.** Univariate associations were explored using non-parametric methods. Multivariate modelling of associations was performed in parametric regression after log-transformation of trichothecene concentrations. A value of $p < 0.05$ was considered significant. Measurement values below DL were treated as follows: a readable value above the background noise level was applied in calculations and modelling, while a non-readable value was arbitrarily substituted with the value of the lowest readable value divided by the square root of 2 [11]. Models were built by forward stepwise regression including significant covariates (evaluated by partial F-tests) and covariates which changed any coefficients of covariates in the model by 15% or more when introduced into the model. The adjusted explained variance ($R^2_{adj}$) and the influence of outliers were used to choose between models. Categorical covariates with more than 2 values were dummy-coded.

As fungi may differentially affect cereal species [28] and trichothecenes may be produced during grain storage [30], we accordingly analysed the material in appropriate subsets and reported subset results if they deviated from other subsets. SPSS 11.0 and Sigma Plot 2001 were used for analysis and presentation.

## RESULTS

A total of 109 grain dust samples were analyzed for trichothecene mycotoxins. The concentrations of trichothecenes in 104 samples of barley (N = 59), oats (N = 32) and spring wheat (N = 13) that were selected for statistical analysis are given in Table 3. Five samples from other grain species were omitted from statistical analysis due to their small numbers. In the municipality of Larvik no storage samples were obtained due to routine field-to-mill grain delivery. The highest single value of DON was found in an oats storage sample, and the highest single values of HT-2 and T-2 were found in an oats threshing sample.

**Determinants of trichothecenes in grain dust.** T-2 and HT-2 concentrations were correlated with a Spearman

**Table 4.** Associations between grain dust DON or HT2 and categorical variables. Categories were compared by Mann-Whitney tests (2 categories) or Kruskal-Wallis tests (>2 categories).

| Determinant | N | Mycotoxin concentration (µg/kg) | | | |
|---|---|---|---|---|---|
| | | DON | | HT-2 | |
| | | median[a] | p[b] | median | p |
| **All samples (N = 104)** | | | | | |
| Cereal species | | | *** | | NS |
| barley | 59 | n.f. | | 64 | |
| oats | 32 | 23 | | 35 | |
| spring wheat | 13 | 34 | | 35 | |
| District | | | *** | | ** |
| River Glomma | 36 | 37 | | 100 | |
| Lake Mjosa | 21 | *15* | | 60 | |
| Trondheim Fjord | 47 | n.f. | | 35 | |
| Growth season | | | * | | NS |
| 1999 | 20 | 27 | | 54 | |
| 2000 | 84 | *11* | | 54 | |
| Autumn ploughing | | | * | | NS |
| yes (area related to the complete grain batch) | 54 | 22 | | 44 | |
| no | 50 | *7* | | 58 | |
| Same grain cultivar in preceding season | | | * | | NS |
| yes | 49 | n.f. | | 51 | |
| no | 55 | 23 | | 56 | |
| Field fungicide application | | | NS | | NS |
| yes | 45 | *15* | | 41 | |
| no | 59 | *15* | | 72 | |
| Growth regulator field application | | | NS | | NS |
| yes | 18 | 21 | | 56 | |
| no | 86 | *13* | | 54 | |
| Lodging of grain (horizontal straws) | | | NS | | NS |
| yes | 62 | *11* | | 38 | |
| no | 42 | *19* | | 70 | |
| Visible mould damage observed by farmer | | | NS | | NS |
| yes | 35 | *19* | | 58 | |
| no | 69 | *6* | | 47 | |
| Task | | | * | | NS |
| threshing | 34 | n.f. | | 73 | |
| storage | 70 | 18 | | 50 | |
| **Barley samples (N = 59)** | | | | | |
| Barley subspecies | | | * | | NS |
| 2 row | 37 | n.f. | | 74 | |
| 6 row | 22 | *18* | | 57 | |
| Lodging | | | NS | | (*) |
| yes | 38 | n.f. | | 54 | |
| no | 21 | n.f. | | 81 | |
| **Threshing samples (N = 34)** | | | | | |
| Growth regulator field application | | | NS | | NS |
| yes | 4 | 25 | | 320 | |
| no | 30 | n.f. | | 64 | |
| **Storage samples (N = 70)** | | | | | |
| Grain dryer | | | ** | | NS |
| cold air | 41 | *11* | | 41 | |
| heated air | 29 | 33 | | 65 | |
| Manual grain mixing | | | * | | (*) |
| yes | 11 | *11* | | 35 | |
| no (air/elevator driven mixing) | 59 | 20 | | 58 | |

[a]Values below DL were treated as follows: if a signal corresponding to less than DL was detected, the concentration obtained from the read signal is shown (*in italics*). If no reading value was obtained, not found (n.f.) is shown; [b]NS (not significant) $p > 0.1$, (*)$p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

**Table 5.** Correlations between grain dust DON or HT2 and continuous variables.

| Climatic variables | N | Spearman rank correlations[a] | | |
|---|---|---|---|---|
| | | DON | T-2 | HT-2 |
| June fungal A forecasts[b] | 104 | 0.28 ** | 0.20 * | 0.25 * |
| July fungal A forecasts | 104 | 0.52 ** | 0.28 ** | 0.32 ** |
| August fungal A forecasts | 104 | 0.27 ** | 0.19 | 0.07 |
| Seasonal fungal A forecasts | 104 | 0.46 ** | 0.32 ** | 0.31 ** |
| June fungal B forecasts[c] | 104 | -0.03 | 0.14 | 0.08 |
| July fungal B forecasts | 104 | 0.25 * | 0.03 | 0.15 |
| August fungal B forecasts | 104 | 0.05 | -0.02 | 0.00 |
| Seasonal fungal B forecasts | 104 | 0.08 | 0.06 | 0.05 |
| May rainfall (mm precipitation) | 104 | 0.41 ** | 0.25 * | 0.29 ** |
| June rainfall (mm precipitation) | 104 | -0.05 | -0.17 | -0.24 * |
| July rainfall (mm precipitation) | 104 | 0.38 ** | 0.14 | 0.25 * |
| August rainfall (mm precipitation) | 104 | -0.31 ** | -0.24 * | -0.27 ** |
| Storage samples only | | | | |
| Storage time | 70 | -0.21 | -0.32 ** | -0.25 * |

[a]Non-parametric correlation. Two-tailed significance at the 0.05 level marked (*), at the 0.01 level marked (**); [b]Fungal A forecasts = Days of meteorological forecasts against late blight potato rot (a potato mould disease); [c]A Fungal B forecast is issued when fungal A forecast conditions are met for 2 consecutive days.

**Table 6.** Determinants of mycotoxins in settled grain dust. Regression models.

| Mycotoxin | Determinant | Regression coefficient (B) | Standard error of B | p |
|---|---|---|---|---|
| DON, all samples (N = 104) | | | | |
| | Constant | 1.4 | 0.31 | <0.001 |
| $R_{adj}^2$ = 0.30 | Seasonal fungal A forecasts | 0.12 | 0.02 | <0.001 |
| | Threshing *vs.* storage | -0.66 | 0.21 | 0.003 |
| | Visible mould damage | -0.47 | 0.21 | 0.03 |
| DON, barley (N = 59) | | | | |
| | Constant | 1.1 | 0.28 | <0.001 |
| $R_{adj}^2$ = 0.27 | July fungal A forecasts | 0.15 | 0.036 | <0.001 |
| | 6 row barley *vs.* 2 row barley | 0.52 | 0.26 | 0.05 |
| | Storage weeks[a] after Sept 1st | 0.026 | 0.012 | 0.03 |
| HT-2, all samples except one outlier (N = 103)[b] | | | | |
| | Constant | 3.4 | 0.25 | <0.001 |
| $R_{adj}^2$=0.13 | Seasonal fungal A forecasts | 0.07 | 0.018 | <0.001 |
| | Oats[c] *vs.* barley | -0.44 | 0.20 | 0.03 |
| | Wheat[c] *vs.* barley | -0.45 | 0.27 | 0.10 |
| | Barley[c] | 0.00 | - | - |
| HT-2, storage (N=70) | | | | |
| | Constant | 3.8 | 0.44 | <0.001 |
| $R_{adj}^2$=0.10 | Seasonal fungal A forecasts | 0.053 | 0.026 | 0.04 |
| | Storage weeks after Sept 1st | -0.024 | 0.012 | 0.06 |

[a]The storage time was set to zero for all threshing samples; [b]In modelling with all 104 HT-2 data points, the residual of one outlier exceeded 3SD, that data point was consequently removed. The model including this outlier was significant for seasonal fungal A forecasts only; [c]The variables "Oats" and "Wheat" are dummy variables for cereal species (barley: both are zero).

rank correlation coefficient ($r_S$) of 0.69. As T-2 associations with determinants generally followed those of HT-2, we will only show the associations of HT-2. Associations between categorical covariates and DON or HT-2 are shown in Table 4. Several categorical covariates including cereal species and geographical district were associated with DON, while only weak associations were seen with HT-2, except for the district which was strongly correlated with both mycotoxins. Associations between DON or HT-2 and climatic variables are shown in Table 5. Both DON and T-2 were strongly associated with seasonal, June, and July fungal A forecasts and with May and July rainfall. DON was also associated with July fungal B forecasts. Modelling of determinants of trichothecenes other than DON and HT-2 was not meaningful, as mycotoxins were detected in too few samples.

Multivariate model building started with seasonal fungal A forecasts or July fungal A forecasts which showed the strongest univariate associations with mycotoxins. July rainfall and July fungal A forecasts were strongly correlated ($r_S$ = 0.78). Thus, concomitant use of these covariates in the same model was avoided. Additional covariates shown in Tables 4 and 5 were then included in the models by forward selection. The variable specifying district was finally selected after all other covariates had been considered, to evaluate if district specific factors were not accounted for by the other covariates in the model. Multiple regression models of associations between determinants and DON or HT-2 are shown in Table 6.

DON was associated with fungal A-forecasts, threshing and visible mould damage to the grain in a model including all samples. In the barley samples, subset DON was associated with storage time and 6-row *versus* 2-row barley. HT-2 was associated with fungal A-forecasts and cereal species, the latter being of borderline statistical significance in the model with all samples (not shown) and significant in a model made after exclusion of an outlier. Subset analyses showed a decay of HT-2 by time in stored grain and an increase of DON by time in stored barley (Tab. 6). Univariate associations of the covariates in the final models of DON and HT-2 are shown in Figure 1 for categorical covariates and in Figure 2 for continuous covariates.

## DISCUSSION

Trichothecenes in settled dust related to Norwegian grain production in this study were mainly DON, HT-2 and T-2. These were associated with local fungal forecasts (climate), cereal species, visible mould damage and storage. Mycotoxin concentrations in grain dust in this study were somewhat higher than published results in Norwegian grain for HT-2 and T-2, while the concentration of DON compared to grain levels 1994–1998 [43].

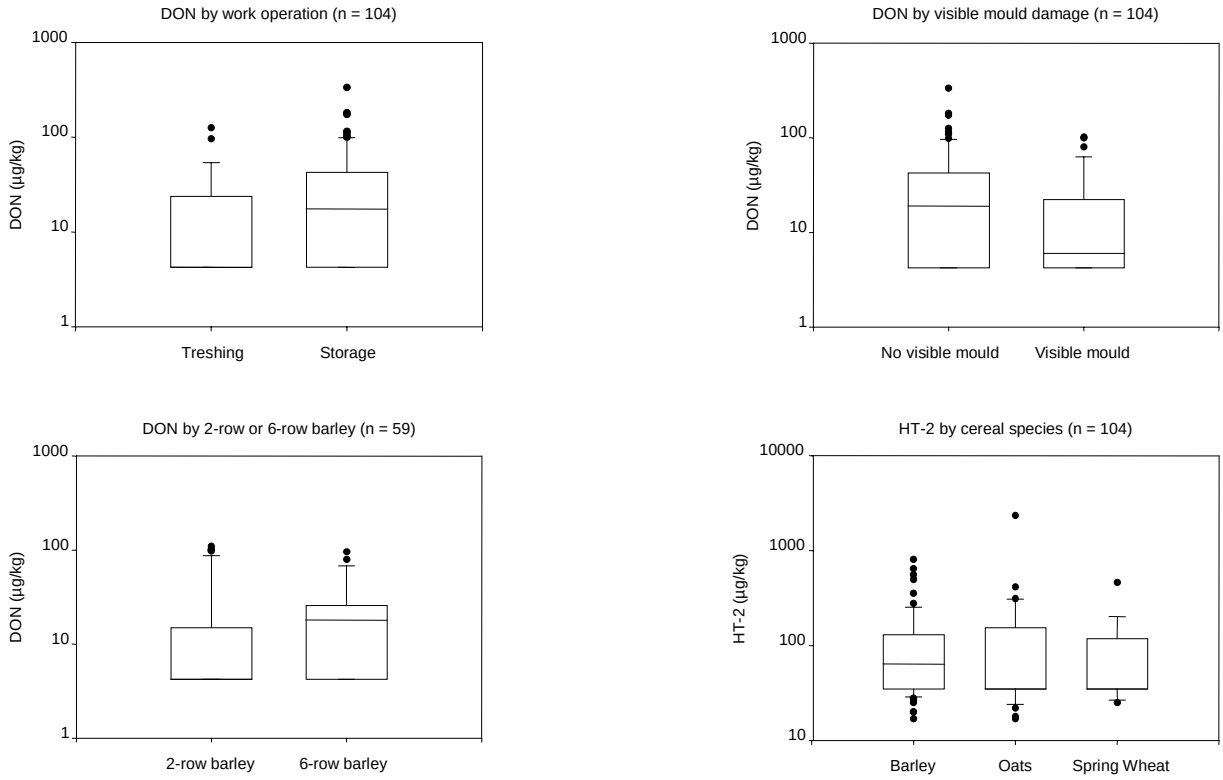


**Figure 1.** Box-and-whisker plots of trichothecene mycotoxin concentrations in strata of covariates included in final models. Boxes range from the 25th–75th percentile; a horizontal line inside box represents the median. In plots without a horizontal line inside box, the median is equal to the 25th percentile. Whiskers represent the 10th and 90th percentiles and dots represent data points above the 90th or below the 10th percentile.

**Validity.** As contacts were made by home telephone, full-time farmers were more likely to be selected than part-time farmers. Threshing samples were taken from surfaces not visibly contaminated before grain work started, but storage samples could not be evaluated for pre-work contamination and contamination from earlier grain batches may have occurred. The difference between storage and threshing samples was small. Therefore such contamination probably did not substantially disturb the results. Several confounding or interacting factors could affect our estimates of associations, such as differential use of fertilizers, application of fungicides or growth regulators, and other actions taken to combat plant disease and secure crop quality (e.g. the use of heated air drying of moist grain). Cultivation practices (e.g. autumn ploughing) have also been restricted by public environmental regulations. Therefore, it will be difficult to assess whether associations that we report are true or confounded. Misclassification of the exposure, if non-dependent of the outcome variable, will commonly attenuate the true estimate of the exposure-effect associations [42] and these will accordingly be underestimated. Hence, if associations are found they should be taken seriously.

The study was performed in districts with different climatic conditions and sampling was equally divided between 3 main tasks (threshing, grain drying and ventilation, and grain delivery). We could not control the distribution of other independent covariates. Therefore, some covariates of interest were not optimally distributed for comparison between strata, resulting in a lower power to detect true differences. The northern boundary of cereal cultivation runs through mid-Norway. Farmers' use of optimal cereal species, cultivars and cultivation practices with respect to control of plant mould diseases has evolved over time, and thus are expected to be well adapted to local climatic conditions. This could lead to some model specification problems if any exposure factors important to trichothecene formation cannot be included in the model because they are eliminated by cultural practices in some, but not all situations. However, in inferring the results it should be borne in mind that measurements taken during realistic grain cultivation conditions may provide information that reflects actual exposure situations.

**Determinants of trichothecene concentrations.** In laboratory and field studies, several determinants of *Fusarium* growth and trichothecene production in grain have been identified, such as temperature optimum, substrate, and water activity [13, 34]. Wet, temperate and humid weather conditions during grain plant head emergence and delayed harvesting of the grain increase *Fusarium* infection levels and trichothecene concentrations in grain [27, 15]. In agreement with this, we found strong and consistent associations between fungal A-forecasts and trichothecene concentrations in grain dust. However, trichothecene production is dependent on specific *Fusarium*

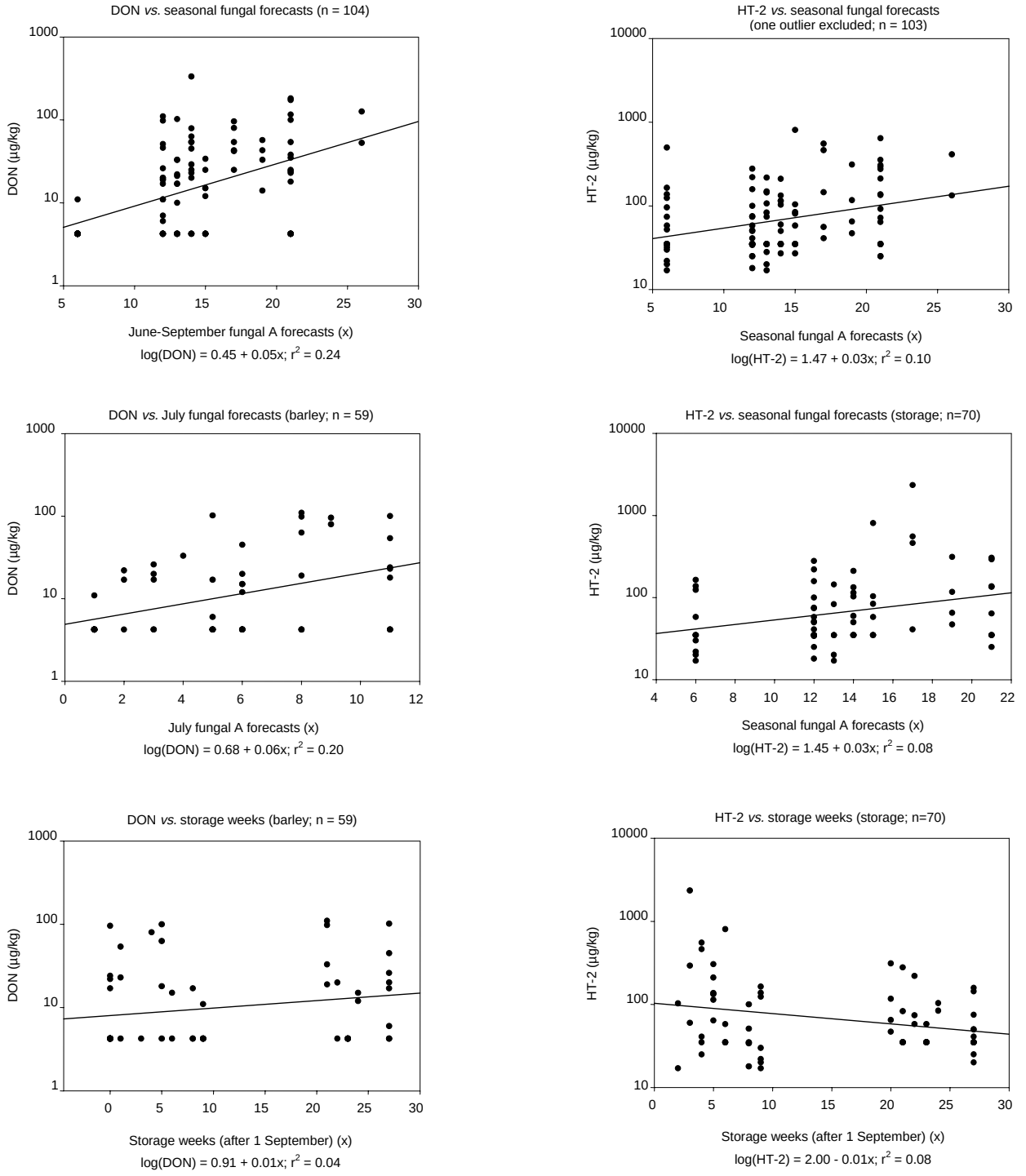


**Figure 2.** Scatter plots of univariate associations of trichothecene mycotoxin concentrations with continuous covariates that are included in final models. For each plot, the least square regression line and Pearson's r squared are shown.

species. The main DON producer in Norway and other cooler maritime areas in the world (e.g. UK) is *F. culmorum*, whereas *F. graminearum* is the predominant species in warmer regions [20]. This may have impact on the interaction between weather and the production of DON because *F. graminearum* is producing ascospores and macroconidia, while *F. culmorum* produces macroconidia only. Most models to predict *Fusarium* head blight and DON content in wheat grain are developed for *F. graminearum* [38, 18, 10], so there may be discordance between models for these regions and models developed for Northern Europe, as in the Netherlands, where a positive correlation was found between cumulative precipitation during the months June, July and August, and winter wheat seeds infected with *Fusarium* spp. and *Microdochium nivale* [9]. The main producer of the trichothecenes T-2 and HT-2 in Norway is *F. langsethiae* [47], and the weather conditions promoting toxin

production by this species may differ from the demands of *F. culmorum*. Thus, the conclusions drawn from the present study may apply to temperate regions of grain cultivation, but not necessarily to warmer regions. The effects of climatic covariates outrange the effects of the other putative determinants of trichothecene contents in grain dust that were addressed in this study (Tab. 1) and their associations hold across districts and cereal species. Trichothecene contents in grain vary across geographic districts and cereal species [27, 30, 16]. These differences in the present study were to a large extent explained by meteorological covariates. Seasonal fungal A forecasts was the best overall trichothecene predictor in this study. We did not know the timing of grain plant development related to meteorological data and the weather observations were also inaccurate due to a varying geographical distance between each measurement station and farm. This may explain why climatic covariates were weaker predictors of DON in this study than in a Canadian study of associations between DON in winter wheat and climatic measurements obtained on the farm during defined susceptible stages of grain plant development [18].

*Fusarium* inoculum can survive from one season to another on crop debris at the soil surface and infect the flowering cereal plant by splash dispersal of spores during heavy rain [41, 15]. Rainfall during July was a strong determinant of DON in grain in the study of Langseth and Elen [27]. In the present study, trichothecene associations of rainfall were weaker than those of fungal forecasts. The reason may be that fungal forecasts include several components in addition to rainfall.

In an experimental study by Langseth *et al.*, DON increased with storage time in moist and suboptimally ventilated grain [30]. In the present study, the influence of storage on DON concentrations was small except in the subset of barley showing an increase of DON with storage time. The negative association between DON and visible mould damage to the grain may be due to prior infection of cereal spikelets by saprophytic fungi, which can prevent or limit infection by *Fusarium* spp. [32].

Most Fusaria are more resistant to fungicide actions than are the plant pathogens for which they are applied. Fungicides were found to increase mycotoxin contents in some field trials [41, 17] and to lower *Fusarium* infection levels, although mycotoxin contents were not significantly affected in other trials [33, 51]. Neither lodging of cereals, growth regulator applications, tillage practices, nor field fungicide use were associated with trichothecene levels in the present study.

**Application of determinants of trichothecenes in grain dust in epidemiological studies.** Trichothecene measurements by personal sampling are currently not available. Furthermore, chemical analysis of personal samples is not feasible in studies of low-prevalent outcomes if a large number of samples need to be analysed by time-consuming and expensive procedures. Therefore, exposure surrogate variables are often needed to perform epidemiological studies of low prevalent outcomes, even if this makes inferences from the results difficult. Information on determinants of exposures may be used in the development of case-control study questionnaires and contribute to a better quantitative exposure assessment than obtained from other indirect exposure assessment techniques [46]. The hypothesis that wet and temperate climatic conditions are determinants of trichothecene exposure during grain handling is supported by this study. The trichothecene variance explained by applied covariates in this study amount from 10–30%, leaving a substantial amount of unexplained variation. Fungal forecasts are expected to be associated also with other microbial agents than trichothecenes (e.g. fungal spores, other mycotoxins, bacteria, and endotoxins) possibly related to health effects that have been attributed to a certain trichothecene exposure. Further, wet climatic conditions are associated with a number of actions taken by the farmer, e.g. fungicide spraying, possibly associated with adverse health effects. We would not be able to specify if pests or pesticides were the causative agents [22]. Inferences drawn from associations based on surrogates of real exposures therefore should be made with caution.

## CONCLUSIONS

The results of this study indicate that late blight fungal forecasts in potato cultivation may possibly serve as an indicator of trichothecene exposure in epidemiological studies related to grain farming in Norway. Before the use of wet and temperate meteorological conditions as an indicator of trichothecene exposure in countries of other climatic zones than the Nordic, they should be validated within that zone.

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