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## Statistical analysis of agronomical factors and weather conditions influencing deoxynivalenol levels in oats in Scandinavia

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**Statistical analysis of agronomical factors and weather conditions influencing deoxynivalenol levels in oats in Scandinavia**

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Methods/Techniques:	Statistical analysis, Mycology
Additives/Contaminants:	Mycotoxins - trichothecenes
Food Types:	Cereals and grain
Abstract:	<p>The relation between weather data and agronomical factors and deoxynivalenol (DON) levels in oats was examined with the aim to develop a predictive model. Data were collected from totally 674 fields during periods of up to ten years in Finland, Norway, and Sweden, and included DON levels in the harvested oats crop, agronomical factors and weather data. The results show that there was a large regional variation in DON levels, with higher levels in one region in Norway compared to other regions in Norway, Finland and Sweden. In this region, the median DON level was 1 000 ng g<sup>-1</sup> and the regulatory limit of for human consumption (1750 ng g<sup>-1</sup>) was exceeded in 28 % of the samples. In other regions the median DON levels ranged from 75 to 270 ng g<sup>-1</sup>, and DON levels exceeded 1750 ng g<sup>-1</sup> in 3 to 8 % of the samples. Including more variables than region in a multiple regression model only increased the adjusted coefficient of determination from 0.17 to 0.24, indicating that very little of the variation in DON levels could be explained by weather data or agronomical factors. Thus, it was not possible to predict DON levels based on the variables included in this study. Further studies are needed to solve this problem. Apparently the infection and/or growth of DON producing <i>Fusarium</i> species are promoted in certain regions. One possibility may be to study the species distribution of fungal communities and their changes during the oats cultivation period in more detail.</p>

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3 **1 Statistical analysis of agronomical factors and weather conditions**  
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5 **2 influencing deoxynivalenol levels in oats in Scandinavia**  
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15 Running head: DON in oats  
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3 **Abstract**

4 The relationship between weather data and agronomical factors and deoxynivalenol  
5 (DON) levels in oats was examined with the aim of developing a predictive model.  
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7 Data were collected from a total of 674 fields during periods of up to ten years in  
8 Finland, Norway, and Sweden, and included DON levels in the harvested oats crop,  
9 agronomical factors and weather data. The results show that there was a large regional  
10 variation in DON levels, with higher levels in one region in Norway compared to  
11 other regions in Norway, Finland and Sweden. In this region, the median DON level  
12 was 1000 ng g<sup>-1</sup> and the regulatory limit of for human consumption (1750 ng g<sup>-1</sup>) was  
13 exceeded in 28 % of the samples. In other regions the median DON levels ranged  
14 from 75 to 270 ng g<sup>-1</sup>, and DON levels exceeded 1750 ng g<sup>-1</sup> in 3 to 8 % of the  
15 samples. Including more variables than region in a multiple regression model only  
16 increased the adjusted coefficient of determination from 0.17 to 0.24, indicating that  
17 very little of the variation in DON levels could be explained by weather data or  
18 agronomical factors. Thus, it was not possible to predict DON levels based on the  
19 variables included in this study. Further studies are needed to solve this problem.  
20 Apparently the infection and/or growth of DON producing *Fusarium* species are  
21 promoted in certain regions. One possibility may be to study the species distribution  
22 of fungal communities and their changes during the oats cultivation period in more  
23 detail.

24 **Key words:** mycotoxins, trichothecenes; *Fusarium*; DON; small grain cereals

## 1 Introduction

2 Contamination of cereals with deoxynivalenol (DON) causes problems for the food  
3 and feed industry. *Fusarium graminearum* and *F. culmorum* are important producers  
4 of DON in cereals (Magan and Olsen 2004). The occurrence of *F. graminearum* has  
5 increased in North Western Europe compared to the 1980s and 1990s, when *F.*  
6 *culmorum* was the dominant species (Waalwijk et al. 2003; Goswami and Kistler  
7 2004). The maximum level of DON set by the European Commission (EC) in  
8 unprocessed durum wheat, oats and maize for human consumption is 1750 ng g<sup>-1</sup> (EC  
9 2006a). For cereals intended for animal feeding the maximum recommended level of  
10 DON is set to 8000 ng g<sup>-1</sup> (EC 2006b).

11  
12 Data from Norway from 1988 to 1994 shows that DON levels were higher in oats than  
13 in wheat and barley (Langseth and Elen 1996). In Finland, almost all grain and feed  
14 sampled in 1987 and 1988 contained DON at low levels. In addition, six lots of oats  
15 containing 300 to 2600 ng g<sup>-1</sup> DON were found (Hietaniemi and Kumpulainen 1991).  
16 According to a Finnish quality monitoring programme carried out since 1999, DON  
17 levels exceeded the regulatory limit of 1750 ng g<sup>-1</sup> in 6% of the oats samples from  
18 1999 to 2009 (Hietaniemi et al. 2010). Oats is a major crop in Finland, Norway, and  
19 Sweden, ranking second to fifth in produced quantity among vegetable crops in 2008  
20 (FAOSTAT 2011).

21  
22 In recent years, DON levels in Norwegian oats have increased rapidly and in 2009 and  
23 2010 the mean levels in certain regions were above the legislative limit for oats  
24 intended for human consumption (Stokke, Felleskjöpet, personal communication). In  
25 Sweden, a similar trend has been observed although data has not been collected as

1 extensively as in Norway (Minsér, Lantmännen, personal communication). The cost  
2 for returning a boat load of oats that at delivery has DON levels above the legislative  
3 limit can be very high, in the vicinity of 1 million Euros. The cost for putting up a  
4 scheme for analyzing all incoming oats is probably lower, but for practical reasons  
5 difficult. Even if the quickest way of analyzing DON contents of oats at delivery is  
6 used (lateral flow ELISA), the time of 20 minutes (Matthew and Pratt 2007) is too  
7 long to analyze all incoming loads (Karlsson, Lantmännen, personal communication).  
8 Therefore, a model predicting the risk of encountering highly contaminated loads  
9 based on weather data and other factors would be valuable. Such a model will support  
10 risk based monitoring of DON.

11  
12 In Norway, studies on the relationship between weather conditions and DON  
13 contamination have indicated that weather parameters during different periods of the  
14 plant growth can influence the DON contents. In one study, the highest DON levels in  
15 oats were found in years characterized by early summer drought (Langseth et al.  
16 1995). The lowest DON levels were, however, found in years with very different  
17 weather. In a later study, a positive correlation between yearly mean DON levels of  
18 oats, barley and wheat together and the amount of precipitation in July August was  
19 shown (the average flowering date for oats in Norway is about Juli 10<sup>th</sup>). Grains  
20 exposed to wet weather in the period after mealy ripe were, in some cases, heavily  
21 contaminated with DON even with temperatures approaching 0 °C (Langseth and Elen  
22 1997). Elen et al. (1997) showed that DON levels in oats in field trials in Norway  
23 (district Solør) were higher in 1995 than in 1994. The July precipitation in 1995 was  
24 147 mm and the temperature 14.8°C, whereas the precipitation and temperature in  
25 1994 were 39 mm and 18.6°C, respectively. Correlations between weather conditions

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3 and DON contents in oats have also been studied in Finland where DON levels were  
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5 higher in two years with warm and dry summers than in a year with a rainy and cold  
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7 summer (Hietaniemi et al. 2004). The authors did not find any correlation between  
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9 regional variation in DON levels and weather parameters such as precipitation and  
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11 temperature sums.  
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16 Studies in wheat show that DON production is affected by agronomical practices.  
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18 Variety is the most important factor, followed by previous crop (Schaafsma and  
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20 Hooker 2007). Average DON levels are higher if the crop in the previous year is  
21  
22 maize, compared to other pre-crops. Choice of tillage system is generally of minor  
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24 importance, but ploughing before planting wheat after maize reduces the risk of high  
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26 DON levels (Teich and Hamilton 1985; Schaafsma and Hooker 2007). There is a  
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28 paucity in studies on the effect of agronomical factors on DON in oats, but Hietaniemi  
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30 et al. (2004) showed that mean DON levels differed little among oat varieties and that  
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32 there was no significant effect of soil type.  
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38 Several models predicting DON contamination in wheat based on weather data and  
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40 agronomical factors have been developed (Schaafsma and Hooker 2007; Franz et al.  
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42 2009; Van der Fels-Klerx et al. 2010). In oats, however, few models are available  
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44 except for some preliminary DON prediction models in Norway based on multiple  
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46 regression analysis.  
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52 In this study, the relation between weather data and agronomical factors and DON  
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54 levels was examined with the aim to develop a predictive model for oats. The analysis  
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1 is based on field and weather data collected during time periods of up to ten years in  
2 Finland, Norway, and Sweden.

#### 4 **Material and Methods**

##### 5 *Field data*

6 Data was obtained from 470 oats fields in Finland between 2000 and 2009, from 171  
7 oats fields in Norway between 2004 and 2008, and from 33 oats field trials in Sweden  
8 between 2006 and 2009. The Finnish data set was divided into 257 fields from the  
9 southern region (FI<sub>ab</sub>, the CAP subsidy areas A and B) and 213 fields from the  
10 northern region (FI<sub>c</sub>, the CAP subsidy area C)(Mavi, 2009). The Norwegian data set  
11 was divided into 93 fields from Solør (NO<sub>s</sub>), a district within the province of Hedmark  
12 in Southeastern Norway, and 78 fields from other parts of Norway (NO<sub>ns</sub>). The oats  
13 field trials in Sweden (SE) were located in the southern and central part of the  
14 country, but were not divided into geographical regions due to the limited number of  
15 observations.

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17 Field data included DON levels in the oats crop, agronomical factors and weather  
18 data. All samples were taken from unprocessed oats grain after harvest. DON levels in  
19 samples from Finland were analyzed by a GC-MS method at MTT Agrifood Research  
20 Finland (Hietaniemi et al. 2004). Samples from Norway were analyzed by a  
21 multitoxin LC-MS/MS analysis at Evira, Finland, according to the method described  
22 by Kokkonen and Jestoi (2009). DON levels in samples from Sweden were analyzed  
23 by GC at the Swedish University of Agricultural Sciences (Pettersson, 1998)

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3 1 The agronomical factors were flowering date and harvest date (ordinal date), time  
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5 2 period between flowering and harvest (days), tillage system (ploughed or not),  
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7 3 precrop (oats as precrop or not) and soil type (silty or not) . A silty soil is defined as  $\geq$   
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9 4 80% silt and  $< 12\%$  clay. Flowering and harvest was earliest in SE and latest in FI<sub>c</sub>.  
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11 5 (Table 1). Unploughed fields were less common in the Finnish regions and in SE than  
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13 6 in the Norwegian regions, where about one third of the fields were unploughed. Oats  
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15 7 as precrop was most common in Finland. Silty soils were most common in NO<sub>s</sub>, and  
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17 8 least common in SE and NO<sub>ns</sub>.  
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23 10 Weather data were collected from the nearest weather station, most often within 20  
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25 11 km from each field. Weekly weather variables were calculated for the time period  
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27 12 from four weeks before the week of flowering to four weeks after the week of  
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29 13 flowering (totally nine weeks). The weekly weather variables were mean temperature  
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31 14 ( $^{\circ}\text{C}$ ), number of days with a relative humidity exceeding RH 80%, and rainfall (mm).  
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33 15 The selection of weather variables were based on results from work with preliminary  
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35 16 DON prediction models in Norway.  
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41 18 Samples that had a DON level below the limit of quantification (LOQ) were assigned  
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43 19 the LOQ value, or, in the case of Norway, the limit of detection (LOD) value if below  
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45 20 the LOD. The LOQ for data from Finland was  $25 \text{ ng g}^{-1}$ , and for data from Norway  
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47 21 and Sweden  $100 \text{ ng g}^{-1}$ . The LOD for data from Norway was  $50 \text{ ng g}^{-1}$ . To stabilize  
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49 22 the variance, DON levels were  $\log_{10}$  transformed before statistical analysis.  
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*Statistical analyses*

1 An initial correlation analysis showed that all pair-wise combinations of weekly  
2 relative humidity data were highly correlated ( $r > 0.7$ ). Therefore, a new variable  
3 comprising the number of days with a relative humidity exceeding RH 80% during the  
4 whole nine week time period before and after flowering was calculated and used in  
5 the statistical analysis. Date of harvest and the number of days between flowering and  
6 harvest were also highly correlated ( $r = 0.8$ ) and only the latter variable was used in  
7 the analysis.

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9 The relationship between DON levels and geographical, agronomical and weather  
10 variables was examined by using the General Linear Model (GLM). First, differences  
11 in mean log DON levels between regions were analyzed. Second, the proportion of  
12 variation in log DON levels explained by year to year variation within each region  
13 was estimated, hypothesizing that large between year differences indicate that weather  
14 is of significant importance for variability in DON levels (Schaafsma and Hooker  
15 2007). Third, univariate analyses were performed to estimate the effect of each  
16 agronomical variable on DON levels. In these analyses, region was added as a  
17 blocking factor to adjust for any regional differences in the distribution of  
18 agronomical factors. Fourth, backward stepwise regression was performed including  
19 all significant agronomical variables from the univariate analysis and all weather  
20 variables as independent factors.

## 21 22 **Results**

23 DON levels in harvested oat crops ranged from below the limit of quantification to  
24 22000 ng g<sup>-1</sup>. Mean log DON levels differed significantly between regions (GLM,  $P <$   
25 0.001). The highest levels occurred in region NO<sub>s</sub>, where the median DON level was

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3 1000 ng g<sup>-1</sup> and the regulatory limit of for human consumption (1750 ng g<sup>-1</sup>) was  
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5 exceeded in 28 % of the samples. Differences between DON levels in the other  
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7 regions were smaller. The median DON levels ranged from 75 to 270 ng g<sup>-1</sup>, and  
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9 DON levels exceeded 1750 ng g<sup>-1</sup> in 3 to 8 % of the samples (Table 1).  
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14 Mean regional DON levels varied little between years. In most regions yearly mean  
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16 log DON levels ranged from about 2 to 2.7 log ng g<sup>-1</sup>. The exception was region NO<sub>s</sub>,  
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18 where mean log DON levels exceeding 3.0 log ng g<sup>-1</sup> were observed in 2007 and 2008  
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20 (Figure 1). Accordingly, variation between years only explained a small part of the  
21  
22 total variation in DON levels within each region. The adjusted coefficient of variation  
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24 ranged from 0.01 to 0.15 in the different regions, except for in NO<sub>s</sub> where it was  
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26 slightly higher, 0.34.  
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32 The univariate analyses (with region as a blocking factor) of the effect of agronomical  
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34 variables showed that log DON levels were significantly related to tillage system and  
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36 the number of days between harvest and flowering date (GLM,  $P < 0.05$ ), but not to  
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38 the flowering date, soil type or precrop (GLM,  $P > 0.05$ ). DON levels were higher in  
39  
40 ploughed fields than in non-ploughed fields (2.4 and 2.2 mean log ng g<sup>-1</sup>,  
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42 respectively), and increased with increasing number of days between harvest and  
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44 flowering date. The effect of region remained significant (GLM,  $P < 0.05$ ) in all  
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46 analyses, indicating that none of the agronomical variables explained the regional  
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48 differences in DON levels.  
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54 In the stepwise regression analysis region and six other variables remained significant  
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56 ( $P < 0.05$ ). These were tillage system, number of days with a relative humidity  
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3 1 exceeding RH 80% during the nine week period, rainfall three weeks before  
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5 2 flowering, and mean temperature during the week of flowering, one week before and  
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7 3 two weeks after the week of flowering. However, the inclusion of more variables than  
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9 4 region in the multiple regression model only increased the adjusted coefficient of  
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11 5 determination from 0.17 to 0.24, indicating that very little of the variation in DON  
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13 6 levels could be explained by the additional variables.  
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## 19 8 **Discussion**

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21 9 The results show that there was a large regional variation in DON levels, with much  
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23 10 higher levels in one region in Norway compared to other regions in Norway, Finland  
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25 11 and Sweden. Agronomical factors or weekly weather data did not, or only to a limited  
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27 12 degree, explain the variation in DON levels. Thus, it does not seem to be possible to  
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29 13 predict DON levels in oats based on the variables included in this study.  
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34 15 The fact that the annual variation in regional DON levels was limited indicates that  
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36 16 between year variation in weather conditions had little influence on DON levels. This  
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38 17 is in contrast with DON levels in wheat, where variation between years have been  
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40 18 shown to explain a large part of the total variation (Schaafsma and Hooker 2007). The  
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42 19 results of earlier studies in oats vary. Whereas Langseth and Elen (1997) found a  
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44 20 correlation between DON levels and precipitation in July, Hietaniemi et al. (2004)  
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46 21 could not explain regional differences in DON levels based on weather conditions  
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48 22 around flowering. Since variables such as temperature and humidity indeed are  
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50 23 important factors in the epidemiology of *Fusarium* species (Osborne and Stein 2007)  
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52 24 it is possible that access to field specific weather data, instead of data from weather  
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54 25 stations within a certain distance from the field, could improve possibilities to predict  
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3 1 DON levels in oats based on weather data. One possibility may also be to include data  
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5 2 on weather conditions during the time period just before the harvest time, or to use  
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7 3 weather data with a more detailed time scale than weekly.  
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11 5 *F. graminearum* and *F. culmorum* hibernate on plant residues from small grain or  
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13 6 maize. The survival and potential for infestation of the following crop of these  
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15 7 pathogens are enhanced in non-tilled fields, while tillage reduces survival and  
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17 8 reproduction (Osborne and Stein 2007). In spite of this, the results of the present study  
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19 9 did not indicate that DON levels were higher when oats was planted after oats than in  
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21 10 other fields, or in fields which were not ploughed. On the contrary, DON levels were  
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23 11 slightly higher in ploughed than in non-ploughed fields. That finding that DON levels  
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25 12 were not higher in non- ploughed fields is not surprising since maize, which is the  
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27 13 pre-crop that has the greatest potential to serve as a source of infection, is not  
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29 14 commonly cultivated in Norway, Finland or Sweden and hardly used as a precrop for  
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31 15 oats. The results are also in agreement with previous studies in wheat, where tillage  
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33 16 system was found to be a factor of minor importance for DON levels (Schaafsma and  
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35 17 Hooker 2007). On the other hand, if reduced tillage systems are established over wide  
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37 18 areas the effect of ploughing local plots may be lost due to inoculum pressure from  
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39 19 the established system.  
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21 Studies of wheat and maize have shown that cultivar resistance is an agronomical  
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23 22 factor with significant influence on DON levels in these crops (Schaafsma and  
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25 23 Hooker 2007; Van der Fels-Klerx et al. 2010). However, because variation in DON  
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27 24 resistance among oats cultivars is not well described, this factor could not be included  
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29 25 as an explanatory variable in the present study.

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5 2 The reasons for the differences in DON levels between region NO<sub>s</sub> and other regions  
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7 3 remain unknown. Weather conditions in NO<sub>s</sub> during the years when most  
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9 4 observations were made (2006 through 2008) were comparable with the conditions in  
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11 5 other regions in Norway. For example, during the week around flowering the mean  
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13 6 temperature was 15.6 and 16.0 °C in NO<sub>s</sub>, and NO<sub>ns</sub>, respectively, and the mean  
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15 7 precipitation 23 and 28 mm. Compared to other areas in Norway silty soils are more  
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17 8 common in NO<sub>s</sub>, and it has been hypothesized that this may be a factor affecting  
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19 9 regional DON levels. However, the results showed that there was no significant effect  
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21 10 of soil on DON levels if the factor regions was included in the statistical model.  
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23 11 Additionally, no significant differences were detected between silty and non-silty soils  
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25 12 within each region (data not shown). This means that there has to be other, unknown,  
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27 13 reasons for the variation of DON levels among regions. Further research is needed to  
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29 14 characterize differences between regions with high DON levels, such NO<sub>s</sub>, and other  
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31 15 regions.  
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17 The present study shows that DON in oats is a significant problem, especially in the  
18 region NO<sub>s</sub>, but also to some extent in other regions in Finland, Norway and Sweden.  
19 The data also shows that the DON levels in Finland were more stable over years than  
20 in Norway (Figure 1). This could indicate that the increase of the incidence of *F.*  
21 *graminearum* that has occurred in Norway in the recent years, still is absent in  
22 Finland. Recent unpublished trials in Sweden show that there are obvious differences  
23 in fungal communities between regions, indicating that this may the reason for  
24 regional differences in contents of mycotoxins in grains. Further monitoring of  
25 regional differences in species distribution of fungal communities and their changes

1 during the oats cultivation period in more detail is needed, as well as efforts to  
2 understand which factors that favors infection, growth and/or mycotoxin production  
3 of DON producing *Fusarium* in certain regions.  
4

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19 oats.



1  
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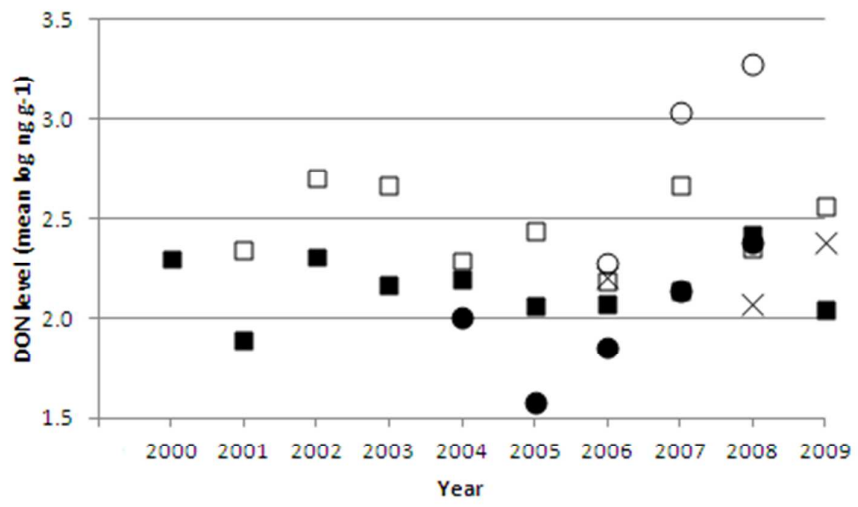
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3 **1 Figure headings**  
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6 2 Figure 1. Regional levels of deoxynivalenol (DON) in oats (mean log ng g<sup>-1</sup>). The  
7  
8 3 number of observations per region and year ranges from 6 to 39. Symbols indicate  
9  
10 4 different regions: Southern Finland, CAP subsidy areas A and B (■); Northern  
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12 5 Finland, CAP subsidy area C (□); Norway, region Solør (○); Norway, other regions  
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14 6 (●); Sweden (x).  
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Table 1. Regional agronomical practices in Southern Finland (FI<sub>ab</sub>); Northern Finland (FI<sub>c</sub>); Norway, region Solør (NO<sub>s</sub>); Norway, other regions (NO<sub>ns</sub>); Sweden (SE)

Region	Ploughing		Precrop		Soil type		Flowering	Harvest	Total no. of fields
	Ploughed (%)	No. of fields*	Oats (%)	No. of fields	Silty (%)	No. of fields	Mean day no.	Mean day no.	
FI <sub>ab</sub>	79	187	95	227	28	254	188	242	257
FI <sub>c</sub>	87	151	98	194	48	211	194	247	213
NO <sub>s</sub>	69	93	42	93	60	93	191	243	93
NO <sub>ns</sub>	60	78	29	78	8	78	186	239	78
SE	94	32	3	32	0	32	183	235	32

\* Number of fields with data recorded on each agronomical factor. Data on flowering and harvest date was available from all fields

Table 2. Regional levels of deoxynivalenol (DON) in oats

Region	No. of fields	Mean log DON level* (log ng g <sup>-1</sup> )	Median DON level (ng g <sup>-1</sup> )	Max DON level (ng g <sup>-1</sup> )	Percentage samples with DON levels exceeding 1750 ng g <sup>-1</sup>
FI <sub>ab</sub>	257	2.2 <sup>a</sup>	118	5 580	4 %
FI <sub>c</sub>	213	2.5 <sup>b</sup>	270	8 800	8 %
NO <sub>ns</sub>	78	2.2 <sup>a</sup>	75	4 500	4 %
NO <sub>s</sub>	93	2.9 <sup>c</sup>	1000	22 000	28 %
SE	33	2.3 <sup>ab</sup>	161	2 340	3 %

\* Mean log DON levels with different letter superscripts are significantly different (Tukey's test,  $P < 0.05$ )