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

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Running head: DON in oats

Abstract

The relationship between weather data and agronomical factors and deoxynivalenol (DON) levels in oats was examined with the aim of developing a predictive model. Data were collected from a total of 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 1000 ng g⁻¹ and the regulatory limit of for human consumption (1750 ng g⁻¹) was exceeded in 28 % of the samples. In other regions the median DON levels ranged from 75 to 270 ng g⁻¹, and DON levels exceeded 1750 ng g⁻¹ 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 *Fusarium* 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.

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

1 Introduction

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

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

In recent years, DON levels in Norwegian oats have increased rapidly and in 2009 and 2010 the mean levels in certain regions were above the legislative limit for oats intended for human consumption (Stokke, Felleskjöpet, personal communication). In 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 10th). 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

1 and DON contents in oats have also been studied in Finland where DON levels were
2 higher in two years with warm and dry summers than in a year with a rainy and cold
3 summer (Hietaniemi et al. 2004). The authors did not find any correlation between
4 regional variation in DON levels and weather parameters such as precipitation and
5 temperature sums.

6
7 Studies in wheat show that DON production is affected by agronomical practices.
8 Variety is the most important factor, followed by previous crop (Schaafsma and
9 Hooker 2007). Average DON levels are higher if the crop in the previous year is
10 maize, compared to other pre-crops. Choice of tillage system is generally of minor
11 importance, but ploughing before planting wheat after maize reduces the risk of high
12 DON levels (Teich and Hamilton 1985; Schaafsma and Hooker 2007). There is a
13 paucity in studies on the effect of agronomical factors on DON in oats, but Hietaniemi
14 et al. (2004) showed that mean DON levels differed little among oat varieties and that
15 there was no significant effect of soil type.

16
17 Several models predicting DON contamination in wheat based on weather data and
18 agronomical factors have been developed (Schaafsma and Hooker 2007; Franz et al.
19 2009; Van der Fels-Klerx et al. 2010). In oats, however, few models are available
20 except for some preliminary DON prediction models in Norway based on multiple
21 regression analysis.

22
23 In this study, the relation between weather data and agronomical factors and DON
24 levels was examined with the aim to develop a predictive model for oats. The analysis

1 is based on field and weather data collected during time periods of up to ten years in
2 Finland, Norway, and Sweden.

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10 **Material and Methods**

11 *Field data*

12 Data was obtained from 470 oats fields in Finland between 2000 and 2009, from 171
13 oats fields in Norway between 2004 and 2008, and from 33 oats field trials in Sweden
14 between 2006 and 2009. The Finnish data set was divided into 257 fields from the
15 southern region (FI_{ab}, the CAP subsidy areas A and B) and 213 fields from the
16 northern region (FI_c, the CAP subsidy area C)(Mavi, 2009). The Norwegian data set
17 was divided into 93 fields from Solør (NO_s), a district within the province of Hedmark
18 in Southeastern Norway, and 78 fields from other parts of Norway (NO_{ns}). The oats
19 field trials in Sweden (SE) were located in the southern and central part of the
20 country, but were not divided into geographical regions due to the limited number of
21 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)
24

1 The agronomical factors were flowering date and harvest date (ordinal date), time
2 period between flowering and harvest (days), tillage system (ploughed or not),
3 precrop (oats as precrop or not) and soil type (silty or not) . A silty soil is defined as \geq
4 80% silt and $< 12\%$ clay. Flowering and harvest was earliest in SE and latest in FI_c.
5 (Table 1). Unploughed fields were less common in the Finnish regions and in SE than
6 in the Norwegian regions, where about one third of the fields were unploughed. Oats
7 as precrop was most common in Finland. Silty soils were most common in NO_s, and
8 least common in SE and NO_{ns}.

9
10 Weather data were collected from the nearest weather station, most often within 20
11 km from each field. Weekly weather variables were calculated for the time period
12 from four weeks before the week of flowering to four weeks after the week of
13 flowering (totally nine weeks). The weekly weather variables were mean temperature
14 ($^{\circ}\text{C}$), number of days with a relative humidity exceeding RH 80%, and rainfall (mm).
15 The selection of weather variables were based on results from work with preliminary
16 DON prediction models in Norway.

17
18 Samples that had a DON level below the limit of quantification (LOQ) were assigned
19 the LOQ value, or, in the case of Norway, the limit of detection (LOD) value if below
20 the LOD. The LOQ for data from Finland was 25 ng g^{-1} , and for data from Norway
21 and Sweden 100 ng g^{-1} . The LOD for data from Norway was 50 ng g^{-1} . To stabilize
22 the variance, DON levels were \log_{10} transformed before statistical analysis.

23
24 *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.

8
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⁻¹. Mean log DON levels differed significantly between regions (GLM, $P <$
25 0.001). The highest levels occurred in region NO_s, where the median DON level was

1 1000 ng g⁻¹ and the regulatory limit of for human consumption (1750 ng g⁻¹) was
2 exceeded in 28 % of the samples. Differences between DON levels in the other
3 regions were smaller. The median DON levels ranged from 75 to 270 ng g⁻¹, and
4 DON levels exceeded 1750 ng g⁻¹ in 3 to 8 % of the samples (Table 1).

5
6 Mean regional DON levels varied little between years. In most regions yearly mean
7 log DON levels ranged from about 2 to 2.7 log ng g⁻¹. The exception was region NO_s,
8 where mean log DON levels exceeding 3.0 log ng g⁻¹ were observed in 2007 and 2008
9 (Figure 1). Accordingly, variation between years only explained a small part of the
10 total variation in DON levels within each region. The adjusted coefficient of variation
11 ranged from 0.01 to 0.15 in the different regions, except for in NO_s where it was
12 slightly higher, 0.34.

13
14 The univariate analyses (with region as a blocking factor) of the effect of agronomical
15 variables showed that log DON levels were significantly related to tillage system and
16 the number of days between harvest and flowering date (GLM, $P < 0.05$), but not to
17 the flowering date, soil type or precrop (GLM, $P > 0.05$). DON levels were higher in
18 ploughed fields than in non-ploughed fields (2.4 and 2.2 mean log ng g⁻¹,
19 respectively), and increased with increasing number of days between harvest and
20 flowering date. The effect of region remained significant (GLM, $P < 0.05$) in all
21 analyses, indicating that none of the agronomical variables explained the regional
22 differences in DON levels.

23
24 In the stepwise regression analysis region and six other variables remained significant
25 ($P < 0.05$). These were tillage system, number of days with a relative humidity

1 exceeding RH 80% during the nine week period, rainfall three weeks before
2 flowering, and mean temperature during the week of flowering, one week before and
3 two weeks after the week of flowering. However, the inclusion of more variables than
4 region in the multiple regression model only increased the adjusted coefficient of
5 determination from 0.17 to 0.24, indicating that very little of the variation in DON
6 levels could be explained by the additional variables.

7
8 **Discussion**

9 The results show that there was a large regional variation in DON levels, with much
10 higher levels in one region in Norway compared to other regions in Norway, Finland
11 and Sweden. Agronomical factors or weekly weather data did not, or only to a limited
12 degree, explain the variation in DON levels. Thus, it does not seem to be possible to
13 predict DON levels in oats based on the variables included in this study.

14
15 The fact that the annual variation in regional DON levels was limited indicates that
16 between year variation in weather conditions had little influence on DON levels. This
17 is in contrast with DON levels in wheat, where variation between years have been
18 shown to explain a large part of the total variation (Schaafsma and Hooker 2007). The
19 results of earlier studies in oats vary. Whereas Langseth and Elen (1997) found a
20 correlation between DON levels and precipitation in July, Hietaniemi et al. (2004)
21 could not explain regional differences in DON levels based on weather conditions
22 around flowering. Since variables such as temperature and humidity indeed are
23 important factors in the epidemiology of *Fusarium* species (Osborne and Stein 2007)
24 it is possible that access to field specific weather data, instead of data from weather
25 stations within a certain distance from the field, could improve possibilities to predict

DON levels in oats based on weather data. One possibility may also be to include data on weather conditions during the time period just before the harvest time, or to use weather data with a more detailed time scale than weekly.

F. graminearum and *F. culmorum* hibernate on plant residues from small grain or maize. The survival and potential for infestation of the following crop of these pathogens are enhanced in non-tilled fields, while tillage reduces survival and reproduction (Osborne and Stein 2007). In spite of this, the results of the present study did not indicate that DON levels were higher when oats was planted after oats than in other fields, or in fields which were not ploughed. On the contrary, DON levels were slightly higher in ploughed than in non-ploughed fields. That finding that DON levels were not higher in non- ploughed fields is not surprising since maize, which is the pre-crop that has the greatest potential to serve as a source of infection, is not commonly cultivated in Norway, Finland or Sweden and hardly used as a precrop for oats. The results are also in agreement with previous studies in wheat, where tillage system was found to be a factor of minor importance for DON levels (Schaafsma and Hooker 2007). On the other hand, if reduced tillage systems are established over wide areas the effect of ploughing local plots may be lost due to inoculum pressure from the established system.

Studies of wheat and maize have shown that cultivar resistance is an agronomical factor with significant influence on DON levels in these crops (Schaafsma and Hooker 2007; Van der Fels-Klerx et al. 2010). However, because variation in DON resistance among oats cultivars is not well described, this factor could not be included 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_s and other regions
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7 3 remain unknown. Weather conditions in NO_s 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_s, and NO_{ns}, 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_s, 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_s, 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_s, 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.

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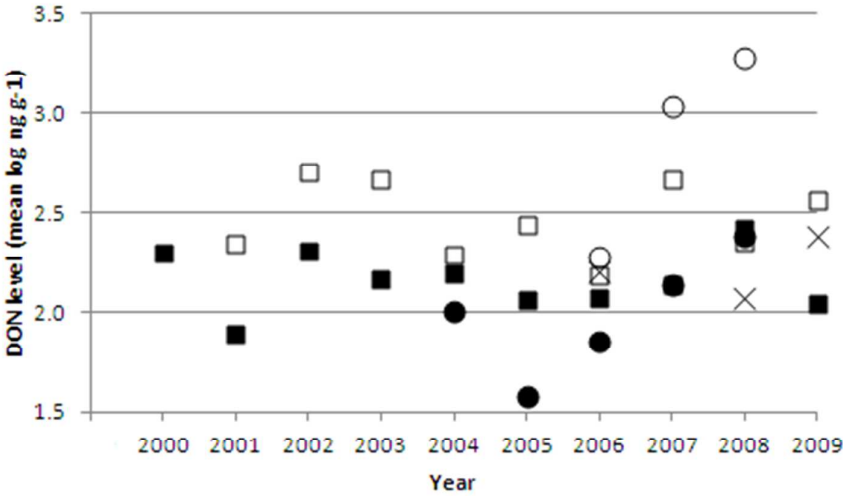
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1 **Figure headings**

2 Figure 1. Regional levels of deoxynivalenol (DON) in oats (mean log ng g⁻¹). The
3 number of observations per region and year ranges from 6 to 39. Symbols indicate
4 different regions: Southern Finland, CAP subsidy areas A and B (■); Northern
5 Finland, CAP subsidy area C (□); Norway, region Solør (○); Norway, other regions
6 (●); Sweden (x).

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Table 1. Regional agronomical practices in Southern Finland (FI_{ab}); Northern Finland (FI_c); Norway, region Solør (NO_s); Norway, other regions (NO_{ns}); 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 _{ab}	79	187	95	227	28	254	188	242	257
FI _c	87	151	98	194	48	211	194	247	213
NO _s	69	93	42	93	60	93	191	243	93
NO _{ns}	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 ⁻¹)	Median DON level (ng g ⁻¹)	Max DON level (ng g ⁻¹)	Percentage samples with DON levels exceeding 1750 ng g ⁻¹
FI _{ab}	257	2.2 ^a	118	5 580	4 %
FI _c	213	2.5 ^b	270	8 800	8 %
NO _{ns}	78	2.2 ^a	75	4 500	4 %
NO _s	93	2.9 ^c	1000	22 000	28 %
SE	33	2.3 ^{ab}	161	2 340	3 %

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