

Comparison of visual head blight ratings, seed infection levels, and deoxynivalenol production for assessment of resistance in cereals inoculated with *Fusarium culmorum*

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Accepted 24 April 1997

Key words: barley, freezing blotter test, *Fusarium* head blight, mycotoxin, oats, wheat

Abstract

Seven spring wheat, 13 barley, 14 oats and 20 winter wheat genotypes were inoculated at flowering in 1993 and 1994 with mixed conidial suspensions of 8 isolates of *Fusarium culmorum*. Four weeks after inoculation, head blight was recorded in the field. After harvest, seed infection was assessed by a Freezing Blotter Test in the laboratory. Seed samples were also analyzed for deoxynivalenol (DON) content. Differences were found in head blight rating, the levels of infected seeds and the DON content between wheat, barley, and oats and between cultivars. Highly significant correlations were found between the percentage of heavily infected seed and the DON content. The weighted mean value of infected seeds and DON content were also significantly correlated. No significant correlation was found between head blight rating in the field and DON content. The level of infected seeds, as determined by the Freezing Blotter Test, was a better indication of the DON content in the seeds than the head blight rating. This visual assessment of levels of infected seeds gives a reliable estimate of resistance to *Fusarium*.

Introduction

In the cooler climates of Northern Europe, the most common species of *Fusarium* responsible for head blight in cereals are *F. culmorum* (W. G. Smith) Sacc and *F. graminearum* Schwabe. *F. culmorum* is the dominant species (Snijders, 1990a, 1990b). In isolations made from seeds in Norway by Haave (1985), *F. poae* and *F. avenaceum* were dominating. A recent survey of *Fusarium* spp. in Norwegian harvested grain indicated that the most frequently isolated *Fusarium* species were *F. avenaceum*, *F. tricinctum*, *F. poae*, *F. culmorum* and *F. graminearum* (Kosiak and Torp, 1995). Various *Fusarium* species, including *F. culmorum*, *F. avenaceum*, *F. poae* and *F. graminearum*, are capable of producing mycotoxins in crops (Marasas et al., 1984). In Norway contamination of homegrown cereals with deoxynivalenol (DON) and 3-acetyl-DON

is a common phenomenon. Their levels may present health risks to humans and domestic animals (Sundheim et al., 1988, Langseth et al., 1995, Langseth and Elen, 1996). Besides a decreasing effect on quality, low infection levels may also lead to significant yield losses (Snijders, 1990a, 1990b).

Breeding of cereal varieties resistant to *Fusarium* head blight has recently been given a high priority in Norway. In wheat, genetic variation in resistance to head blight caused by *F. culmorum* appears to be significant (Snijders, 1990b). However, resistance to *Fusarium* is quantitative, and complete resistance has not been observed. In wheat, *Fusarium* head blight resistance is mainly based on resistance to colonization, while resistance to establishment of the initial infection plays only a minor role (Snijders and Krechting, 1992). Ergosterol analyses to determine the amount of fungal biomass may be used for selection of resis-

tance. A high correlation coefficient has been found between ergosterol content and DON content in the wheat kernels artificially inoculated with *F. culmorum* ($r = 0.85$, $df = 19$) (Snijders and Krechting, 1992). This indicates that the DON concentration in the kernels mainly depends on the amount of fungal biomass in the kernel. Ergosterol to DON ratios were higher in the resistant cultivars than in the susceptible cultivars. This suggests that the resistant cultivars could prevent synthesis and/or promote degradation of DON (Miller et al., 1985). Furthermore, a comparison of two sets of data, from a tissue tolerance test *in vitro*, and a resistance response to FHB determined in the field, shows that the resistant wheat cultivars can tolerate higher concentrations of the trichothecenes tested than susceptible cultivars because there is no effect on growth *in vitro* (Wang and Miller, 1988). Head blight susceptible cultivars of wheat contain much higher concentrations of DON in the kernels than resistant cultivars (Miller et al., 1985). For barley and oats, reports on the relationship between *Fusarium* head blight, the level of infected seeds and the toxin content are scarce. The purpose of this study was: to establish the relationships between head blight caused by *F. culmorum*, and the level of infected seeds and DON content in barley and oats compared to spring and winter wheat. We also wanted to study assessment for resistance to *Fusarium* by comparing head blight rating, the level of infected seeds and the DON content in kernels.

Materials and methods

Fusarium inoculum. Cultures of *F. culmorum*, identified according to the manual of Nelson et al. (1983), were obtained from cereal seeds collected in various parts of Norway during 1990 and 1992 (Table 1). Stocks of *F. culmorum* were maintained on moist, autoclaved soil in 100 ml glass bottles. The bottles were inoculated with cultures actively growing on Potato Dextrose Agar (PDA), and incubated at 25 °C for 7 days under alternating fluorescent light (12 h) and darkness (12 h) and then stored at 2 °C for subsequent experiment.

The inoculum used for field experiments comprised a mixture of 8 strains of *F. culmorum* mentioned in Table 1. All strains were found to be pathogenic in greenhouse tests (data not shown). Conidia for inoculation were produced on PDA. The cultures were incubated at 25 °C for 30 days under alternating fluorescent light (12 h) and darkness (12 h). To prepare spore sus-

Table 1. Sources, hosts and pathogenicity of 8 isolates of *Fusarium culmorum*

Isolates	Hosts	Sources	Pathogenicity
F27	Barley	Eidsvoll, Akershus County	+
F28	Barley	Eidsvoll, Akershus County	+
F30	Barley	Eidsvoll, Akershus County	+
F40	Oats	Hordaland County	+
F41	Barley	Aas, Akershus County	+
F43	Oats	Statkorn, Norway	+
F44	Barley	Aas, Akershus County	+
F45	Oats	Statkorn, Norway	+

+ Positive infection.

pensions, conidia were washed from PDA with 0.1% gelatin solution, and filtered through sterile gauze. The spore concentration was adjusted to 10^5 conidia ml⁻¹.

Plant materials and inoculations. Seven spring wheat, 20 winter wheat, 13 barley and 14 oats genotypes from the official trials administrated by The Norwegian Crop Research Institute, Apelsvoll Research Centre, Kapp, Oppland County, Norway, were sown at a standard density of 350 seeds m⁻² in rows 0.2 m apart on 0.75 × 4.5 m plots. There were 3 replicates for each genotype in a randomized block design. The field experiments were run with the same design in both 1993 and 1994.

Artificial inoculations were made at the flowering stage. A spore suspension as described above was used at 0.1 L m⁻². The spore suspension was sprayed from 0.25 m above the crop canopy. The field was mist-irrigated by sprinkling from 8 to 10 AM, 1 to 3 PM and 7 to 8 PM each day in 1993, in 1994 three minutes mist irrigation with 25 minute intervals was run throughout the day, over a period of two weeks after inoculation.

Fusarium head blight rating (FHB) was determined as the product of the percentage of infected heads and the proportion of bleached spikelets per infected head. After harvest, seed samples were collected for the Freezing Blotter Test and mycotoxin analysis.

Freezing Blotter Test. From each replicate, seed samples of 34 cereal genotypes in 1993, and seed samples of 54 cereal genotypes in 1994 were selected for the Freezing Blotter Test and mycotoxin analysis. All seed samples were analyzed at the same time, when the seeds had been dried after harvest.

One hundred seeds of each sample were plated on blotter paper in the bottom of sterile, transparent, plastic boxes, 23 cm × 23 cm × 3 cm in size. The blotter

Table 2. The average^a of *Fusarium* head blight ratings (FHB), heavily infected seeds (HIS), medium infected seeds (MIS), total infected seeds (TOT), weighted mean value of infected seeds (WMV) and DON content in kernels of 7 spring wheat cultivars inoculated with *F. culmorum* in 1993 and 1994

Genotype ^b	FHB ^c (%)	HIS (%)	MIS (%)	TOT (%)	WMV (%)	DON (mg kg ⁻¹)
Bastian	17	28	26	68	27	11.4
Avans	31	27	21	78	25	13.7
Runar	15	31	21	72	27	14.0
Hanno	30	34	28	82	32	17.7
Tjalve	28	45	13	76	32	19.2
Polkka	20	41	21	82	33	21.3
Brakar	33	43	19	80	33	21.3
LSD (5%)	21.2	17.0	11.7	13.2	7.5	12.2

^a Values are means over two years of three replicates from each year.

^b The genotypes are ranked for DON content in increasing order.

^c *Fusarium* head blight ratings were the product of the percentage of infected heads and the proportion of bleached spikelets per infected head.

Table 3. The average^a of *Fusarium* head blight rating (FHB), heavily infected seeds (HIS), medium infected seeds (MIS), total infected seeds (TOT), weighted mean value of infected seeds (WMV) and DON content in kernels of 13 barley cultivars inoculated with *F. culmorum* in 1993 and 1994

Genotype ^b	FHB ^c (%)	HIS (%)	MIS (%)	TOT (%)	WMV (%)	DON (mg kg ⁻¹)
Flare	18	15	21	68	17	8.8
Pernilla	31	14	33	72	22	10.0
Sunnita	30	18	31	75	23	10.4
Bamse	49	20	32	78	25	11.4
Arve	48	20	26	80	22	11.4
Olsok	54	20	31	76	24	11.6
Thule	58	23	39	87	29	15.4
Tea	29	26	35	83	30	16.5
Gunilla	29	28	34	83	30	17.5
Olve	42	27	33	77	29	19.9
Tyra	39	29	28	80	29	20.9
Tore	31	25	30	80	27	21.8
Kinnan	16	28	32	83	30	21.9
LSD (5%)	19.8	8.3	10.1	11.7	5.7	7.2

^{a,b,c} As in Table 2.

paper was then saturated with distilled water. The boxes were incubated for 24 h at room temperature, and they were then kept for 24 h at -20 °C, followed by 7 days at 22 °C in cycles of 12 h darkness and 12 h fluorescent light. The deep freezing treatment killed

Table 4. The average^a of heavily infected seeds (HIS), medium infected seeds (MIS), total infected seeds (TOT), weighted mean value of infected seeds (WMV), and DON content in kernels of 14 oats cultivars inoculated with *F. culmorum* in 1993 and 1994

Genotype ^b	HIS (%)	MIS (%)	TOT (%)	WMV (%)	DON (mg kg ⁻¹)
Oloram	6	14	33	9	1.8
Moholt	4	8	29	6	2.3
Freja	5	10	29	7	2.4
Kolbu	4	13	29	8	2.5
Grane	9	10	37	9	3.4
Svea	8	11	33	9	3.6
Mustang	8	13	34	10	3.8
Sv 88608	9	9	36	9	3.8
Lena	6	10	28	8	4.0
Martin	7	13	37	9	4.5
Frigg	8	12	37	9	4.8
Kapp	9	15	39	11	4.8
Ramiro	9	13	37	11	5.0
Celsia	13	15	45	14	5.0
LSD (5%)	5.3	4.0	9.1	3.8	2.6

^{a,b} As in Table 2.

the seeds to make examination easier without reducing *Fusarium* spp. levels (Limonard, 1966; Tempe, 1968). The seeds were examined under a stereoscopic microscope at 10× to 50× magnification and separated into three classes: Class 1, heavily infected seeds (HIS) (>80% of a seed was covered with *Fusarium* mycelium); Class 2, medium infected seeds (MIS) (20–80% of a seed was covered with *Fusarium* mycelium); and Class 3, lightly infected seeds (LIS) (<20% of a seed was covered with *Fusarium* mycelium (Uninfected seeds were not included). To determine which level of infected seeds is responsible for the DON content, we also used total infected seeds (TOT) and weighted mean value of infected seeds (WMV) (mean of 3 times percentage heavily infected seeds and 2 times percentage medium infected seeds) in the assessment.

Chemical analysis. The mycotoxin analysis was performed on whole grains with a water content of about 15%. DON, 3-acetyl-DON, and nivalenol were determined according to Langseth and Clasen (1992). In this method, the grain sample is extracted with acetonitrile-water (84 + 16, v/v) for one hour, and this is followed by an automated clean-up procedure on a column packed with charcoal/alumina/celite. The samples were derivatized with 1-(trimethylsilyl)imidazole and analyzed by capillary gas chromatography with

Table 5. The percentage^a of *Fusarium* head blight rating (FHB), heavily infected seeds (HIS), medium infected seeds (MIS), total infected seeds (TOT), weighted mean value of infected seeds (WMV), and DON content in kernels of 20 winter wheat cultivars inoculated with *F. culmorum* in 1994

Genotype ^b	FHB ^c (%)	HIS (%)	MIS (%)	TOT (%)	WMV (%)	DON (mg Kg ⁻¹)
Portal	23	5	7	33	6	3.7
WW37017	10	6	15	49	10	5.8
VDH 1022-87	22	6	7	36	6	6.1
V9001	57	7	17	50	11	7.0
V1004	58	14	11	48	13	7.6
WW38322	42	18	13	57	16	8.4
Nova	76	10	13	56	12	8.9
WW35327	47	12	12	54	12	8.9
SW39103	22	10	14	52	12	9.6
Folke	40	13	7	46	11	9.9
Rudolf	38	18	15	55	17	10.0
Urban	66	12	9	53	11	10.1
SW39323	39	10	13	45	11	10.2
Kalle	41	8	11	50	9	10.4
WW37078	26	10	7	50	9	10.9
Terra (PF)	73	12	8	46	10	11.0
V9005	59	16	20	58	17	12.2
Tryggve	55	12	12	54	12	12.7
SVB9054	85	20	15	66	18	16.2
SJD897120	74	18	20	70	19	16.4
LSD (5%)	26.2	11.3	8.8	18.4	11.7	6.2

^a Values are means of three replicates.

^{b,c} As in Table 2.

electron-capture detection. The detection limits for DON and 3-acetyl-DON were 30 $\mu\text{g kg}^{-1}$ and for nivalenol 50 $\mu\text{g kg}^{-1}$. A sample of BCR wheat RM 379 reference material was included in each series of samples. The mean value of DON was 726 $\mu\text{g kg}^{-1}$ with a relative standard deviation of 10.9% for 118 determination of the reference material in the period of measurement (certified value 670 $\mu\text{g kg}^{-1}$).

Statistical analysis. Analysis of variance and correlation analysis were done by the MSTAT program (Nissen et al., 1995).

Results

In Tables 2, 3, 4 and 5, the *Fusarium* head blight rating (FHB), the levels of heavily infected seeds (HIS), medium infected seeds (MIS), total infected

seeds (TOT), weighted mean value of infected seeds (WMV) and kernel DON concentration (DON) in spring wheat, barley, oats, and winter wheat, respectively, are compared. Significant differences were found between spring wheat, barley, oats and winter wheat and between cultivars of barley and winter wheat for the variables FHB, level of infected seeds and DON content (Tables 2, 3, 4 and 5). The ratio between the average DON level in the most susceptible cultivars and that in the least susceptible cultivars was 2.0, 2.5, 2.5, and 4.5 in spring wheat, barley, oats and winter wheat, respectively.

High values of FHB were observed in winter wheat, barley and spring wheat. FHB was not observable in oats. The level of infected seeds and DON content differed between cereal species. For spring wheat, the level of HIS was 5, 3, and 1.6 times higher than in oats, winter wheat, and barley, respectively. The average DON level in the spring wheat was 4.9, 1.9, and 1.3 times higher than in oats, winter wheat, and barley, respectively (Figure 1).

For the spring wheat, barley, winter wheat and oats, the correlation coefficients using FHB and the different levels of infected seeds versus the DON content based on the data from 1993 and 1994 are given in Table 6. The correlations between HIS, or WMV and the DON content were significant for all crops (Figure 1). The variable TOT was also significantly correlated to DON with one exception in barley in 1993. A significant correlation between MIS and DON content was only found in oats in both years. There were no significant correlations between FHB and DON content in spring wheat, winter wheat and barley (Table 6). Regression analyses showed that about 55–92% of the variation of DON content can be explained by HIS or WMV in different cereals.

When varieties were compared, it appeared that the barley cultivars Bamse, Arve and Olsok, and the winter wheat cultivars V9001 and Nova, had relatively high values of head blight and low values of heavily infected seeds and DON content. On the contrary, the barley variety Kinnan had the lowest value of head blight and the highest values of heavily infected seeds and DON content (Tables 3 and 5).

Discussion

Snijders and Krechting (1992) concluded that the percentage of infected spikelets of wheat is a better indication of the amount of fungal biomass present,

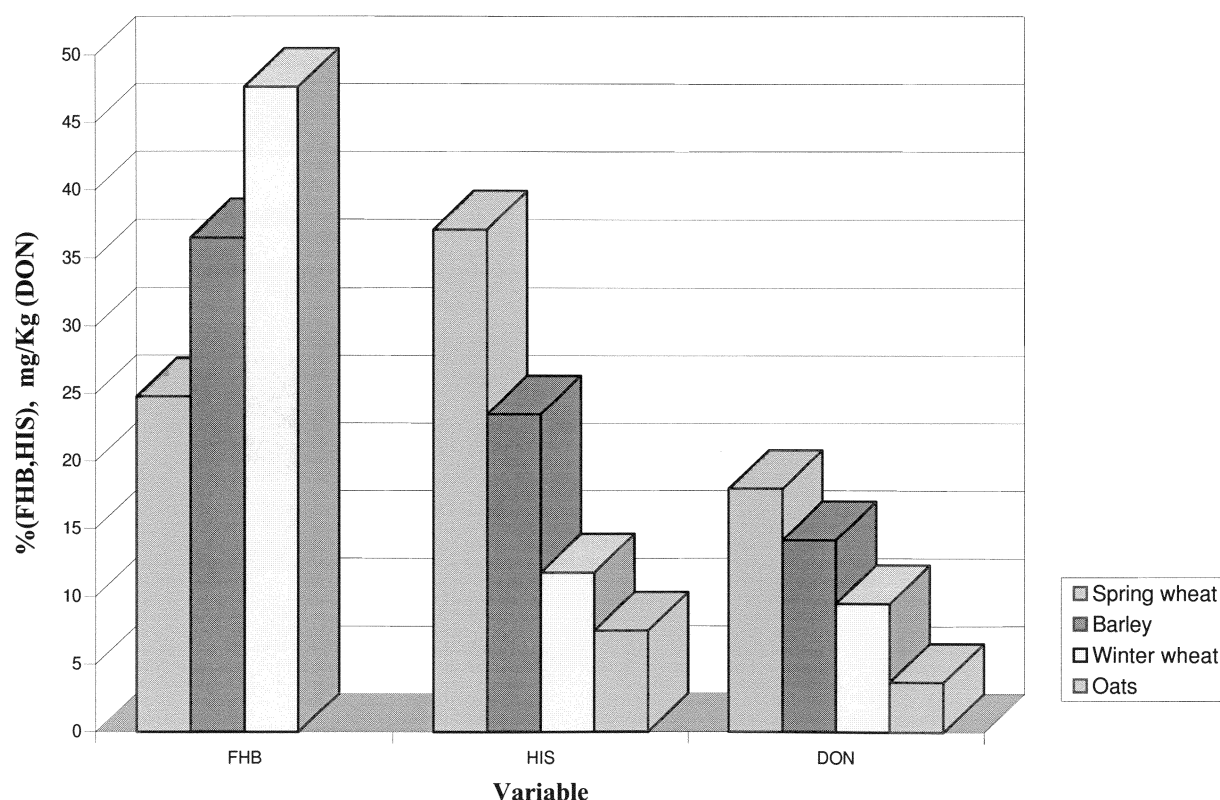


Figure 1. The average of *Fusarium* head blight rating (FHB, %), heavily infected seeds (HIS, %) and DON content (mg kg^{-1}) in spring wheat, barley, winter wheat and oat.

Table 6. Correlation coefficients of percentage of *Fusarium* head blight rating (FHB), heavily infected seeds (HIS), medium infected seeds (MIS), lightly infected seeds (LIS), weighted mean value of infected seeds (WMV) (by Classes 1 and 2), and total infected seeds (TOT) versus deoxynivalenol (DON) content (mg kg^{-1}) in the kernels of 7 spring wheat, 13 barley, 14 oats and 20 winter wheat genotypes after inoculation with *F. culmorum*

Correlation	Spring wheat (df=6)		Barley (df=12)		Oats (df=13)		Winter wheat (df=19)
	93	94	93	94	93	94	94
FHB vs. DON	0.68	0.56	-0.05	-0.04	–	–	0.64
LIS vs. DON	0.01	0.43	-0.04	-0.38	-0.08	0.16	0.44
MIS vs. DON	-0.67	0.11	0.39	0.07	0.63*	0.68*	0.43
HIS vs. DON	0.91**	0.93**	0.86**	0.86**	0.87**	0.71**	0.74**
WMV vs. DON	0.90**	0.96**	0.75**	0.82**	0.94**	0.78**	0.71**
TOT vs. DON	0.69*	0.88**	0.18	0.72**	0.79**	0.71**	0.80**

Note: Correlation coefficients are based on the means presented in Tables 2, 3, 4 and 5.

* Significant at $P = 0.05$.

** Significant at $P = 0.01$.

– Not detected.

than the percentage of infected head. In our experiment, DON analyses showed that a visual assessment of the level of infected seeds from a Freezing Blot-

ter Test was a better indication of the DON content than the *Fusarium* head blight rating. There were high correlations between heavily infected seeds and DON

content, weighted mean value of infected seeds (by classes 1 and 2) and DON content in all barley, oats, spring wheat and winter wheat, and no significant correlations between lightly infected seeds and DON content. Thirteen percent of heavily infected seeds and a DON content of 5 mg kg^{-1} were found in oats where symptoms could not be observed. Although the percentage of total infected seeds correlated to DON content, the percentage of heavily infected seeds was the most important component associated with the final concentrations of DON in the kernels (Table 6). Also, the weighted mean value of infected seed was significantly correlated with the DON content in our investigation. Both HIS and WMW are useful parameters for assessment of resistance to *F. culmorum* and DON contamination.

Schroeder and Christensen (1963) assumed that wheat plants have two types of resistance to *Fusarium* head blight: (i) resistance to the establishment of the initial infection (Type 1) and (ii) resistance to hyphal invasion of plant tissue (Type 2). These two types of resistance have also been observed by Snijders and Krechting (1992). They concluded that *Fusarium* head blight resistance is mainly based on the resistance to colonization (i.e. to hyphal invasion), and that resistance to establishment of the initial infection plays only a minor role. Wilcoxson et al. (1988) observed that spikes of Era wheat inoculated with *F. culmorum* became necrotic, and that the necrosis began in the inoculated spikelets and spread into more than half of the other spikelets of the spikes in susceptible cultivars. This phenomenon supports the hypothesis that susceptible cultivars have lower resistance to hyphal invasion (Type 2 resistance). Miller et al. (1985) found that ergosterol to DON ratios were much higher in the resistant cultivars than in the susceptible cultivars and suggested that a third type of resistance exists in resistant cultivars. These have either the ability to prevent DON synthesis or to promote degradation of DON or both. The degradation phenomena were also observed in artificial infections of winter wheat (Miller et al., 1983, Miller and Young, 1985,) and in natural infections (Scott et al., 1984). Tolerance to *Fusarium* mycotoxins by resistant cultivars has been suggested as another type of resistance (Wang and Miller, 1988). Mesterhazy (1995) recently summarized all resistant types to *Fusarium* head blight of wheat as five active mechanisms: 1. resistance against initial infection; 2. resistance to pathogen spreading in the head to uninfected tissue; 3. resistance to kernel infection; 4. tolerance (high yield with high infection); 5. resistance

by decomposing toxins. According to the evaluation system summarized by Mesterhazy, comparison of the FHB between the different cultivars in our experiment suggests that in barley type 1 or type 2 resistance (resistance to initial infection) is present at a higher level in Kinnan than in Thule. The winter wheat variety WW37017 has higher type 1 resistance than SVB9054. The lack of significant correlation between FHB and DON in Table 6 shows that FHB was independent of DON or vice versa. This indicates that FHB could be used to assess Type 1 and Type 2 resistance. The levels of infected seeds and DON content in Tables 2, 3, 4 and 5 confirm that besides the first type of resistance which determines the head blight severity, there may be a second or the third type of resistance which influences the final amount of fungus and the DON content. The available evidence from the results achieved in the experiments described here suggests that the final DON content depended mainly on heavily infected seeds or the weighted mean value of infected seeds. The barley cultivars Bamse, Arve and Olsok, and the winter wheat cultivars V9001 and Nova showed a high percentage of head blight, but a low level of infected seeds and DON content, while the cultivar Kinnan presented the lowest percentage of head blight and the highest level of infected seeds and DON content in barley. This means that Kinnan does not possess Type 3 and type 5 resistance. Comparing all four cereals, winter wheat showed a higher percentage of head blight but a lower level of infected seeds and DON content than spring wheat and barley. Relatively higher WMV to DON ratios were found in the oats cultivars Olram and Kolbu. They probably possess the fifth type of resistance which is more common in oats than in other cereals.

Effect on yield and percentage *Fusarium*-infected kernels (FHB) have been used as the visual selection criteria for resistance to *Fusarium* head blight (Snijders, 1990b, Miedaner et al., 1993). Even though these may be reliable estimates for resistance to *Fusarium* head blight on wheat, the methods can not be used on oats due to the fact that FHB generally is unobservable on oats.

We conclude that the Freezing Blotter Test has provided an effective visual assessment for resistance to *Fusarium*. The levels of infected seeds indicate that *Fusarium* head blight estimated from a Freezing Blotter Test in the laboratory gives a reliable estimate of the amount of DON content. Therefore, it can be used as a selection criterion for resistance to *Fusarium* head blight.

Acknowledgements

We thank The Norwegian Research Council, Norwegian Grain, Norwegian Co-operative Feed Organization, Norkorn and Stormøllen AS for economic support.

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