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Fusarium diseases in cereals – potential impact from sustainable cropping systems

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Contents

Preface 4

Molecular detection of *Fusarium* species and prediction of mycotoxin levels in food and feed
R. Kristensen et al. 5

Population dynamics of *Fusarium* spp. causing *Fusarium* head blight *J. Köhl et al.* 6

FusaProg: a tool to forecast *Fusarium* head blight and deoxynivalenol contamination in wheat
H.R. Forrer et al. 11

Impact of agronomy on mycotoxin contamination of wheat and oats *S.G. Edwards* 12

The effect of cultivation practices on *Fusarium langsethiae* infection of oats and barley
P. Parikka et al 15

Fusarium head blight and mycotoxins in cereals – potential strategies to control contamination under conservation tillage *S. Vogelgsang et al.*. 19

Screening for resistance to *Fusarium* head blight in organic wheat production *O.E. Scholten et al* 20

Kernel resistance against *Fusarium* head blight as selection criterion in wheat breeding
F. Mascher et al. 24

Breeding efforts to develop resistant cultivars to *Fusarium* head blight and associated mycotoxins in wheat for Romanian sustainable cropping systems *M. Ittu et al* 25

A comparative assessment of potential components of partial disease resistance to *Fusarium* head blight using a detached leaf assay of wheat, barley and oats *R.A. Browne and B.M. Cooke* 31

European *Fusarium* Ringtest- a valuable vehicle for sharing germplasm and screening methods to develop resistance to *Fusarium* head blight across Europe *M. Ittu* 36

Fusarium infection of heads and stems under different cultivation practices *M. Jalli and P. Parikka* 38

Fusarium head blight resistance of old Hungarian wheat genotypes *G. Vida et al* 41

Fungal diversity of winter wheat ears and seeds in Slovakia *M. Pastirčák* 45

Differences between spring wheat cultivars for emergence and early development after seed infection with *Fusarium culmorum B. Timmermans and A. Osman* 49

VAT - a new software for consistent analysis of plant pathogen populations and their hosts
E. Kosman et al. 51

List of participants 52

Preface

This proceeding has been produced as the outcome of a workshop held in the COST860 SUSVAR network from 1 to 2 June, 2007.

SUSVAR stands for 'Sustainable low-input cereal production: required varietal characteristics and crop diversity' and COST is an intergovernmental framework for European co-operation in the field of scientific and technical research. The SUSVAR network, initiated in spring 2004, now includes researchers from more than 100 institutions in 28 European countries. The main aims of the SUSVAR network are to ensure stable and acceptable yields of good quality for low-input, especially organic, cereal production in Europe. This will be achieved by developing ways to increase and make use of crop diversity, by establishing methods for selecting varieties, lines and populations taking into account genotype-environment interactions and by establishing common methodology for variety testing where appropriate.

The Working Group 5 on Plant-pathogen interactions (subgroup "*Fusarium*") organised this workshop as a satellite event to the SUSVAR workshop on "Varietal characteristics of cereals in different growing systems with special emphasis on below ground traits" (29 to 31 May, 2007).

Fusarium pathogens occur in several crop plants. In cereals, *Fusarium* head blight (FHB) is one of the most noxious diseases caused by a complex of *Fusarium* species. Epidemics of FHB often lead to yield losses, a decline in quality, and contamination of cereals with mycotoxins that threaten human and animal health. The aspects covered during this workshop were diverse and ranged from detection, epidemiology, breeding efforts, diversity (both on the pathogen and on the host side), disease forecasting and control, as well as particular aspects of FHB in low-input cereal production.

Five invited speakers and 11 COST members participated by contributing either with oral presentations or posters. An enriching discussion between *Fusarium* researchers with various backgrounds and specialisations took place. This proceedings book contains abstracts and short papers from all contributions.

Funding of this workshop by COST is greatly appreciated.

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Molecular detection of *Fusarium* species and prediction of mycotoxin levels in food and feed

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Key Words: Molecular detection, microarray, Fusarium, mycotoxin prediction.

Members of the genus *Fusarium* are among the most potent plant pathogens worldwide, and members of this genus produce a range of mycotoxins which may be harmful for humans and animals. The current morphology based taxonomical system for *Fusarium* is inadequate, and detection and identification procedures are both time consuming and error-prone. In the last decade molecular detection methods have greatly enhanced the study of *Fusarium*. Molecular detection methods have greatly evolved from diagnostic PCR of undefined loci to real-time PCR and multiplex assays of characterised regions. An overview of molecular detection of *Fusarium* species will be presented with special attention to the *Fusarium* microarray (Kristensen *et al.* 2007). The *Fusarium* microarray has the ability to simultaneously detect and identify 14 *Fusarium* species. The microarray was designed by a phylogenetic approach which makes it possible to detect and identify new or introduced species. The *Fusarium* microarray may prove to be a very valuable tool for screening of cereal products in the food and feed production chain. The correlation between molecular methods and mycotoxins produced will be emphasized, where this correlation have been reported.

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Population dynamics of *Fusarium* spp. causing *Fusarium* head blight

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Key Words: Fusarium, wheat, mycotoxin, organic farming

Abstract

Fusarium head blight (FHB) causes yield losses in cereals. Mycotoxin production by the different *Fusarium* spp. infecting grain results in quality losses. Main risk factors for the disease are the use of susceptible cultivars, the presence of crop residues colonised by FHB pathogens, and favourable weather conditions during flowering which is the most important infection period for the pathogens. Preventative measures such as crop rotation and soil cultivation are often directed against crop residues as inoculum sources of the disease. Studies on the population dynamics of the different FHB pathogens using quantitative TaqMan-PCR showed that the crop residues can be heavily colonised by the pathogens. Population dynamics and species composition in the crop residues depend on type of plant tissue and also can differ between season and location. A better understanding on population dynamics in crop residues can be used for optimising preventative measures, but also for detecting risk factors when cropping systems are modified.

Introduction/Problem

Fusarium head blight (FHB) of cereals can be caused by various *Fusarium* spp. and *Microdochium nivale* (Parry et *al*., 1995). In Northwestern Europe the main FHB pathogens are *F. culmorum*, *F. graminearum*, *F. avenaceum* and *F. poae*. Infections can result in yield losses but more important in contamination of the grain by mycotoxins produced by the pathogens. The most prevailing mycotoxins deoxynivalenol, nivalenol, zearalenon, fumonisin and T2/HT2 are produced by different *Fusarium* spp. Moreover, different genotypes occur within *F. culmorum* and *F. graminearum* producing DON or NIV. FHB can also affect seed quality and several pathogens may also infect seedlings from contaminated seeds. Such effects of FHB on seed quality are a thread especially in organic farming because often efficient seed treatments are lacking (Timmermans & Osman, 2007). The main pathogen causing seedling disease is *M. nivale*, closely related to *Fusarium* spp.

The main infections of ears of wheat and other cereals occur during flowering. After infection, the pathogens spread through the ears and infect the grains. After harvest, pathogen growth and mycotoxin formation may continue if the water content of the grain is too high. Pathogen populations also colonise other plant parts which remain in the field. Such colonised crop residues on the field soil are considered as the main inoculum sources of FHB in subsequent susceptible crops. Conidia produced on the crop residues are transported by wind and rain to the ears. Flight distances of conidia are short (centimetres to meters) so that locally produced inoculum can be considered as the driving force of epidemics. An exception is *F. graminearum* of which the perfect stage *Gibberella zeae* can also produce ascospores. Such ascospores can be transported by wind for longer distances (hundreds of meters) so that inoculum sources in neighbouring fields can also be important for the initiation of epidemics.

Risk factors for damage by FHB and occurrence of mycotoxins are (1) cropping of susceptible cultivars instead of more resistant cultivars; (2) presence of inoculum sources in field during the susceptible flowering period; and (3) climatic conditions favouring infections during flowering or delaying harvest. Important preventative measures are aimed at a reduction of crop residues of infected cereal crops. In the crop rotation, cropping of susceptible cereals including maize should be limited, especially cropping of wheat after maize should be avoided (Khonga & Sutton, 1988). After harvest, crop residues including stubbles should not be left on the soil surface but decomposition should be enhanced by incorporation into the soil.

The objective of our studies was to follow the development of populations of FHB pathogens in crops and crop residues. A better understanding of population dynamics including possible interactions

between the populations of the different FHB pathogens will lead to improved measures aimed at decomposition of colonised crop residues or may also detect unknown risk factors in rotation schemes. Different FHB pathogens may be minor pathogens of other crops or certain weeds or may be favoured by their crop residues. Such studies on population dynamics using isolation techniques are very laborious. After quantitative species-specific PCR techniques became available during the last years (Waalwijk et *al.*, 2004) more detailed studies on population dynamics under field conditions can be carried out. In this paper we report first results obtained by using TaqMan-PCR for analysis of wheat crops and residues present in wheat crops.

Methodology

Samples of wheat crops or crop residues were collected in three field experiments. Two experiments (experiment 1 and 2) were conducted to study the colonisation of different plant parts of wheat by FHB pathogens and to follow such populations in the crop residues of the wheat crop. The experiments were conducted with winter wheat *var*. Vivant at two locations in the Netherlands from June 2003 until June 2004. Twenty stems were collected at flowering and maturation from each of four replicate plots per experiment and stems were separated into stem base, nodes, internodes, ear, and, at maturation, grain and ear residues. After harvest, stubble and straw were left on the field surface and samples were collected at intervals of 2 months until the following June. Further experimental details can be found in Köhl et *al*. (in press).

Experiment 3 was conducted by J.P. Blok, experimental farm Ebelsheerd, from 2003 until 2006 to assess the effect of soil cultivation on various parameters in winter wheat production grown in subsequent crops. Soil treatments were ploughing followed by harrowing, rigid-tine cultivation followed by harrowing and direct drilling. From the different plots, 20 stems were collected at flowering, mid-dough stage and maturation in 2004 and 2005. Stems were dissected into different parts as in experiments 1 and 2. Crop residues were collected at regular intervals from the soil and the top 2.5-cm soil layer by sampling six surface soil samples of an area of 38 cm^2 and a depth of 2.5 cm with a pot corer. All residues of crops and weeds as well as green parts of volunteer plants or weeds growing in the sample area were included in the sample. Samples from each plot were mixed and further processed by elutriation. From each sample a thoroughly washed fraction of organic material was collected on a sieve with a mesh of 1.6 mm.

Field samples were freeze dried, milled to fine powders and sub-samples of approximately 15 mg were taken for DNA extraction using plant DNeasy kit (Qiagen, Germany). Extracts were analysed by separate quantitative TaqMan-PCRs for contents of DNA of *F. avenaveum*, *F. culmorum*, *F. graminearum*, *F. poae*, and *M. nivale* (Waalwijk et *al*., 2004; Köhl et *al*., in press). An internal control was used to detect possible amplification inhibition. In case of inhibition, extracts were diluted and analysed again. The concentrations of species-specific DNA of the pathogens in the various samples were expressed as pg DNA per mg dry weight of plant tissues. DON concentrations in samples of the various plant tissues obtained from three plots of experiment 3 in 2004 and two plots in 2005 were analysed by HPLC.

Results and brief discussion

Colonization of grain in experiments 1 and 2 by FHB pathogens was generally low (Köhl et *al*., in press). The dominating pathogens were *F. avenaceum* and *F culmorum*. Interestingly, colonisation of stem parts by FHB pathogens was significantly higher than of grain. In stem tissues left on the soil after harvest, population sizes of FHB pathogens peaked at harvest but substantially decreased during the following months in residues of nodes and internodes. A different pattern was found in stem base tissue. FHB populations fluctuated during time, but there was no trend of decreasing populations. It could be estimated that at maturation of the winter wheat crop, the majority of FHB populations were present in nodes and internodes. However, at the period of flowering of a subsequent crop in June of the following year, approximately 90% of the populations of the FHB pathogens were present in residues of stem base tissue. In this situation, stem bases of a wheat crop may be the major inoculum source. Preventative measure should thus be aimed at careful stubble treatment to enhance decomposition whereas presence of straw cannot be considered as additional risk factor.

A strong effect of soil cultivation on the amount of crop residues present in the winter wheat crops at flowering was found in experiment 3 in 2004. In ploughed plots, 1.4 g (dry weight) of crop residues were found, whereas after rigid-tining 5.4 g and after direct drilling 10.3 g of crop residues were present in the crop (Fig. 1A). *F. graminearum* was the dominating pathogen in the crop residues from all treatments (Fig. 1B). Crop residues from plots with rigid-tining were colonised stronger by *Fusarium* spp. than those from the other treatments (data not shown). This resulted in 7-times higher amounts of *Fusarium*, especially of *F. avenaceum*, present in these potential inoculum sources within plots with rigid-tining compared to plots with ploughing. For plots with direct drilling 12-times higher amounts were found compared to ploughed plots. It can thus be assumed that in crops grown on ploughed plots, the risk of flower infections were much lower than in crops grown in plots with different soil cultivation. Furthermore, mainly infections by *F. avenaceum* were expected.

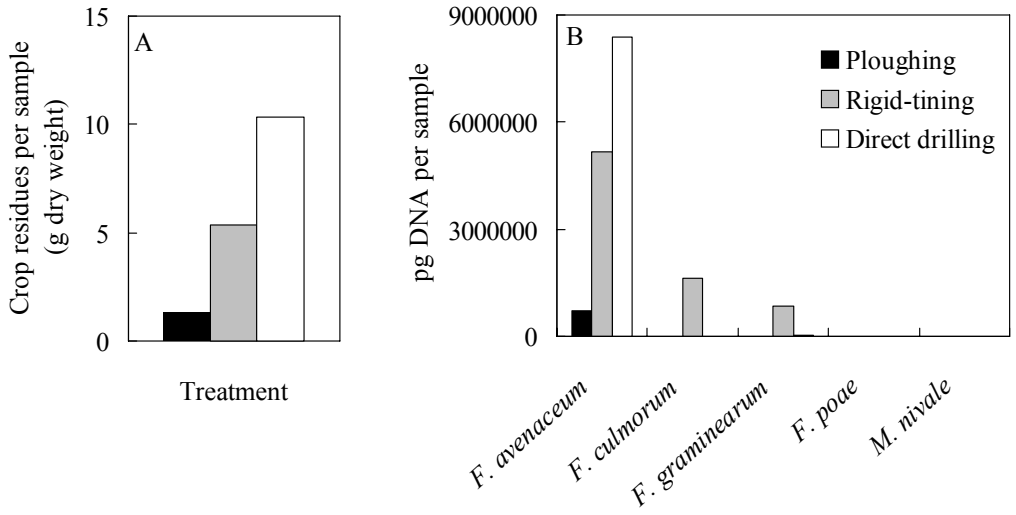

Figure 1. Effect of soil cultivation on the amount of crop residues present in a winter wheat crop at flowering (A) and the amount of DNA of *Fusarium* spp. and *M. nivale* present in the crop residues (B). Preceding crop was winter wheat. Samples consisted of crop residues, volunteer plants and weeds present in six surface soil samples of 28 cm^2 surface and 2.5 cm depth. Means of four replicates. Experiment 3, 2004.

However, colonization of grain by FHB pathogens at harvest was low, possible due to dry weather conditions during the flowering period and there were no effects of soil cultivation. As found in experiments 1 and 2, colonisation of stem parts including the stem base by FHB pathogens was much stronger than of grain (Fig. 2). The dominating pathogen was *F. graminearum*. This pathogen had been almost absent on the crop residues. Possibly the inoculum of *F. graminearum* consisted of ascospores which had been produced in neighbouring fields and became airborne. After harvest in 2004, the amount of crop residues and their colonisation by FHB pathogens was followed until June 2005. Crop residues were colonised mainly by *F. graminearum* and *F. avenaceum*. As found in experiments 1 and 2, a general decrease of *Fusarium* populations in the crop residues was observed. Interestingly, a strong reduction in colonisation occurred in November and December 2004 and no *Fusarium* spp. could be detected in crop residues after that period except *F. avenaceum* of which only traces were found. At flowering of the subsequent winter wheat crops in 2005, the amount of crop residues present in the surface soil samples was comparable to the amounts in 2004. However, only traces of *F. avenaceum* and no other pathogen could be detected in such residues. It can be expected that under these circumstances, risks for FHB were low. Indeed, amounts of FHB pathogens found in grains in 2005 were generally low. However, these low levels of infestation could also be attributed to the dry weather conditions during flowering in 2005.

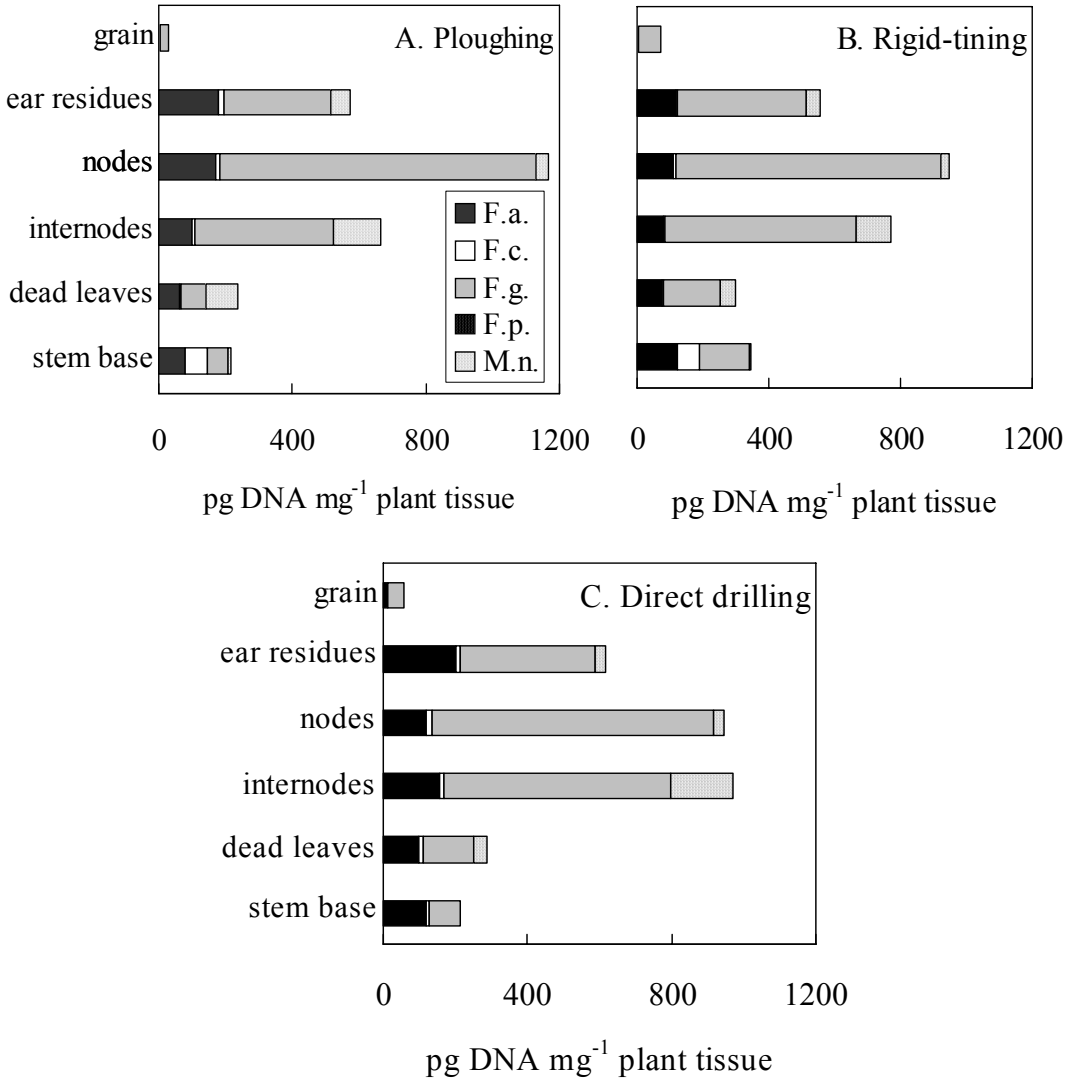

Figure 2. Colonisation of plant tissues of winter wheat at maturation by *F. avenaveum* (F.a.), *F. culmorum* (F.c.), *F. graminearum* (F.g.), *F. poae* (F.p.), and *M. nivale* (M.n.). Wheat was sampled from plots with different soil cultivation (A-C). Means of four replicates. Experiment 3, 2004.

High amounts of DON with up to 27,000 µg kg^{-1} were found (Fig. 3). In grain, nodes and internodes, a linear relationship was found between the colonisation of the different plant tissues by the DON-producing *F. culmorum* and *F. graminearum* (measured as DNA concentration of the pathogens) and the DON concentration. There was a trend that the pathogens produced less DON per unit biomass in nodes than in grain or internodes. In ear residues, a huge variation in DON production was observed and there was no clear relationship of DON production with DNA concentration of the pathogens. The presence of DON and possibly other mycotoxins in straw and other crop residues may have impact on micro-organisms involved in microbial decomposition of organic matter. Furthermore, the high amounts of DON should be considered when straw is harvested and used for various purposes in agriculture.

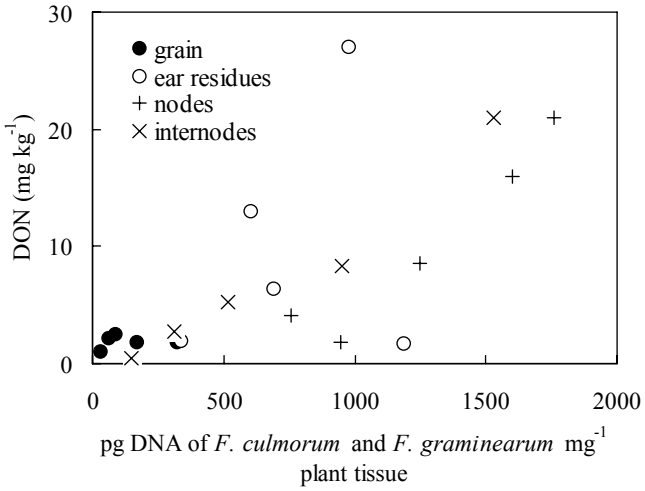

Figure 3. Relationship between the concentration of DNA of *F. culmorum* and *F. graminearum* and the concentration of DON in wheat tissues at maturation (field experiment 3, 2004 and 2005).

The results of our studies demonstrate that it is possible to quantify by TaqMan-PCR the population sizes of FHB populations in various substrates including crop residues extracted from soil which are at different stages of decomposition. Consistent results were obtained showing that FHB pathogens were present in crop residues of wheat. Pathogen populations in crop residues fluctuated in time. The decrease of populations in most plant tissues after harvest differed substantially between seasons so that it can be expected that different amounts of inoculum were present in the subsequent crops, e.g. more DNA of FHB pathogens was present in the crop residues in the wheat crop of 2004 than of 2005 (experiment 3). Differences in species composition in crop residues were also found between years and locations.

In organic farming under Dutch conditions, risks of damage by FHB, especially of mycotoxin contamination of grain is limited by crop rotation schemes with a low percentage of cereal crops, soil cultivation and soil conditions favouring decomposition of crop residues. New agronomical trends in organic farming may lead to more risks. An increased feed production may lead to an increase of cereals, especially of maize and winter wheat, in rotation schemes. Another risk factor is the production of maize as energy crop also in organic systems. The effects of such possible modifications of cropping systems on populations of FHB and the resulting risks should carefully be studied to avoid any increase of risks of mycotoxin contamination of organic food and feed, as well as to prevent yield losses and reductions in seed quality.

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FusaProg: a tool to forecast *Fusarium* head blight and deoxynivalenol contamination in wheat

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Key Words: conservation tillage, Fusarium, survey, DON, FHB

Agriculture, based on intensive soil tillage techniques with heavy machinery, is a real threat for maintaining soil fertility. To improve the situation and to avoid increasing problems with soil compaction, erosion, and nitrate leaching, several Swiss cantons started between 2000 and 2003 to subsidise conservation or no-tillage. No-tillage, a plant production system without any tillage from previous harvest to direct seeding offers the best condition to conserve and improve the soil structure. The soil cover consisting of straw and plant residues is an essential factor to protect the soil and to maintain a high biological activity. However, it could also be an important source for infections of cereals with fungal species causing *Fusarium* head blight (FHB). Therefore and to examine the impact of no-tillage on the prevalence of FHB fungi and mycotoxin contamination in wheat, the canton of Aargau sponsored a FHB survey between 2001 and 2004.

To quantify the effects of soil tillage, rotational and varietal effects on FHB fungi and mycotoxin contamination, we collected wheat samples from ploughed or no-tillage fields, each with or without maize as the previous crop, as well as with the less susceptible varieties Arina and Titlis or any other variety. All samples were collected at harvest by farmers and sent to ART Zürich-Reckenholz. With a seed health test, we screened for the incidence of FHB fungi including all toxigenic *Fusarium* spp. as well as the non-toxigenic species *Microdochium nivale*. In addition, we quantified the deoxynivalenol (DON) content of ground grain samples with a Ridascreen® DON immunoassay.

M. nivale (MN), *F. graminearum* (FG), *F. poae* (FP), and *F. avenaceum* (FA) were the most important FHB fungi. From the toxigenic FHB fungi, FG was the most frequent species. The FG incidence was highly correlated with the DON content in the grain samples (r=0.84). Disease incidence and DON contamination were highest in samples from fields with maize as the previous crop, no-tillage, and varieties with medium to high FG susceptibility. With FP and FA, no strong effects of the previous crop or the tillage on disease incidence were observed. In contrary to FG, no-tillage did not promote the incidence of MN but even significantly reduced its prevalence compared with samples from ploughed fields.

Subsequently, FG incidence and DON data were used to develop the DON forecasting system FusaProg, www.fusaprog.ch. In order to predict a DON content, we allocate a wheat field to one of the four groups pre-crop maize or other pre-crops combined with minimal tillage or plough and take the corresponding value from our survey as the primary input. For a plot-specific DON forecast, the value is corrected with factors appraising the effect of the inoculum on the disease, namely: pre-crop, previous pre-crop, straw management, and seedbed tillage. To consider the host, the DON-value is corrected with the susceptibility of the variety and the current growth stage of the wheat crop. The resulting DON-content is further corrected using the weather parameters temperature, rain fall, and relative humidity in a number of different models to estimate the infection risk.

On-farm trials were used to investigate the influence of common and newly developed straw management systems (see also contribution by Vogelgsang et al.). In our first validation trials in 2004 and 2005, our model correctly predicted in about 80% of all cases a DON content below or above 0.5 ppm. Together with components to display local and regional FG infection risk, the plot-specific prediction model for DON-contamination is part of our FG information and decision support system (DSS) FusaProg. 2007 is the first year with system access for farmers.

Impact of agronomy on mycotoxin contamination of wheat and oats

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Key Words: Fusarium head blight, deoxynivalenol, HT2, T2, organic

Introduction/Problem

Prior to the introduction of fusarium mycotoxin legislation within the European Union in 2006 the UK Food Standards Agency and UK cereal levy board, the Home-Grown Cereals Authority, funded a five year project to identify the level of fusarium mycotoxins in UK cereals and the impact of UK cereal agronomy on these mycotoxins. Legal limits for DON and zearalenone were introduced in 2006. The legal limits for DON in unprocessed wheat and barley is 1250 ppb and 1750 ppb in unprocessed oats. The legal limit for zearalenone is 100 ppb in wheat, barley and oats. Legal limits for HT2 and T2 are under discussion. Previous combined limits used for discussion within the European Commission were 100 ppb HT2+T2 for wheat and barley and 500 ppb for oats.

The aims of the project were to identify if the legislation would be an issue to the UK cereal industry and if it was, to identify what farmers could do to modify their agronomic practices to reduce the risk of exceeding legal limits.

Methodology

Each year from 2001 to 2005, crop consultants and growers collected three hundred wheat and one hundred oat and barley samples and related agronomic data. Samples were collected at harvest from specific fields either from the combine or from trailers leaving the field. Ten approx. 300 g samples were taken from arbitrary points around the field and combined to provide a 3 kg aggregate sample. On receipt, samples they were milled with a 1 mm screen, and mixed in a tumbler mixer before laboratory samples were collected. Samples were analysed for ten trichothecenes using GC-MS (Limit of Quantification [LoQ] = 10 ppb) and zearalenone (LoQ = 5 ppb) using HPLC by UKAS accredited analytical laboratories (RHM Technology and Central Science Laboratory).

For the statistical modelling samples with less than the LoQ were given a value of ½(LoQ) i.e. 5 ppb for trichothecenes and all samples $\log_{10}$ transformed to stabilise the variance. Significant agronomic factors were selected for the model using a stepwise selection ANOVA on Genstat (v8, Lawes Agricultural Trust). Temporal (year) and spatial (region) factors were forced into the model. Other agronomic factors were ordered based on the order in which they occur within a growing season. Interactions between factors were entered into the model where there was a biological reason to expect one to occur.

Results and brief discussion

The fusarium mycotoxin content of all UK cereals were generally low with many samples below or close to the limit of quantification for the majority of mycotoxins.

For barley, all fusarium mycotoxin contents were low, with no samples exceeding the legal limits for DON or zearalenone, and results of modelling of agronomy are not reported here. Results would indicate that UK varieties appear to have a high inherent resistance to fusarium head blight (FHB) compared to the worldwide breeding stock.

For wheat, the predominant mycotoxin detected was DON. DON was detected (>10 ppb) in 86% of samples and the mean was 230 ppb; 2.4% of samples exceeded 1250 ppb. Three percent of samples exceeded 100 ppb zearalenone. Modelling of agronomy identified significant effects of year*region, previous crop*cultivation, varietal resistance and fungicide application at flowering on

DON concentration. Although there was a significant ($p<0.001$) interaction between year and region, there was a consistent trend of DON contamination decreasing northwards. This difference was probably due to differences in weather (some *Fusarium* pathogens prefer warmer conditions). The relative difference in DON contamination in the South and East was probably a result of regional differences in weather conditions between the years.

Wheat grown after maize and minimum tillage had the highest DON concentration (ca. five times higher than other samples) (Fig. 1). Ploughing after maize, wheat, potatoes and brassicas reduced DON contamination of wheat significantly. The difference was greatest for maize and least for brassicas. There was a consistent trend of ploughing reducing DON content after all crops except set-aside.

Results showed an inverse relationship between the fusarium head blight resistance (FHB) rating from national variety trials and the DON content of grain samples for winter wheat cultivars. Although, as UK varieties would all be classed as susceptible compared to the worldwide breeding stock, there was little difference between each resistance rating.

Wheat receiving an azole fungicide at flowering had a significantly lower DON content compared to wheat, which received no fungicides at flowering. The reduction achieved (ca. 30%) is not as good as would be expected for some azoles, this is probably due to the low number of samples which received azoles recommended against FHB at optimum rates and timings. There was no significant difference between wheat samples from conventional and organic farms.

For oats, the predominant mycotoxins detected were HT2 and T2. HT2 was detected (>10 ppb) in 92% of samples, the mean was 570 ppb and 30% of samples exceeded a combined concentration of 500 ppb HT2+T2. Modelling of agronomy identified a significant effect of year*region, previous crop*cultivation, variety and practice on HT2+T2 concentration. There was a highly significant ($p<0.001$) interaction between year and region with no apparent trend for differences between regions. Therefore, high levels could occur in any region across the UK. There appears to be a trend for increasing amounts of HT2 and T2 in England during the project. As there is no previous data for fusarium mycotoxins in UK oats then it cannot be determined if high levels of HT2 and T2 are a recent occurrence.

Cultivation alone did not have a significant effect on HT2+T2 concentration ($p=0.876$), there was however a significant interaction between previous crop and cultivation ($p=0.015$). There was no significant difference between oats following a cereal but HT2+T2 concentration was significantly lower following grass or another non-cereal crop if ploughed. Oats following non-cereal crops, which were not ploughed had a significantly higher HT2+T2 concentration than those that were ploughed.

Of the 28 oat varieties sampled within the project only five were present in high enough numbers (>10 samples) to allow valid statistical analysis. Of these five varieties, Gerald was the most common variety, composing 43% of total samples. Gerald had significantly higher HT2+T2 than any other variety.

There was a highly significant ($p<0.001$) difference between oat samples from conventional and organic farms (Fig. 2). The concentration of HT2+T2 in conventional samples was five times higher than in organic samples. There was some multicolinearity within the dataset as conventional and organic growers favoured different previous crops and varieties. Consequently it is difficult to identify a cause and effect relationship, and to identify the importance for the agronomic factors practice, previous crop and variety. What can be identified by moving practice to the end of the model is that organic practice is still a highly significant factor ($p<0.001$) when previous crop and variety have already been taken into consideration by the model indicating that other differences between the two practices not identified in the statistical model also have a significant influence on HT2+T2 concentrations.

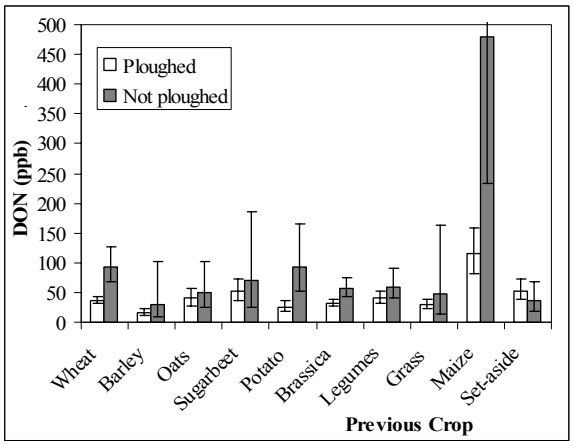

Fig 1. Predicted mean DON concentration for UK wheat samples after different previous crops and methods of cultivation (2001-2005). Bars indicate 95% confidence interval for predictions.

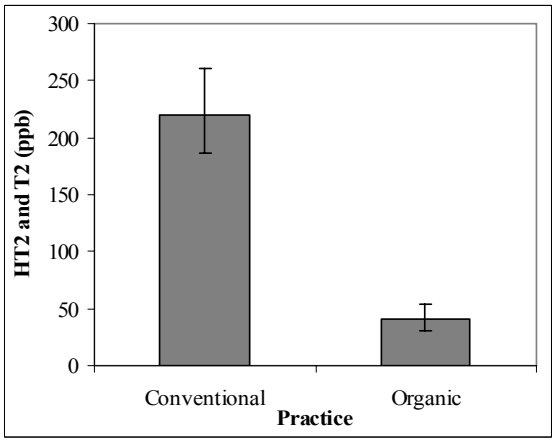

Fig 2. Predicted mean combined concentration of HT2 and T2 in organic and conventional UK oat samples (2002-2005). Bars indicate 95% confidence interval for predictions.

Conclusions

Generally low levels of fusarium mycotoxins were detect on UK cereals. For barley, there were no samples above legal limits. For wheat, a low percentage of samples exceeded the legal limit for DON and zearalenone. Modelling of DON concentration against agronomy identified the importance of various agronomic factors. This data has been compiled in a UK Code of Good Agricultural Practice to Reduce Fusarium Mycotoxins in Cereals (FSA, 2007). For oats the concentraion of the fusarium mycotoxins were low except for HT2 and T2. The combined concentration of these type A trichothecenes exceeded 500 ppb in 30% of samples. Modelling of HT2+T2 concentration against agronomy of oats identified the importance of various agronomic factors. Organic samples had a markedly lower HT2+T2 content compared to conventional samples.

Of major significance is that the models for DON in wheat and HT2+T2 in oats are very different. This would indicate that the *Fusarium* species responsible for the DON in wheat and HT2+T2 in oats have major differences in their epidemiology and ecophysiology. The implication for growers is that the agronomy that is most appropriate to reduce DON in wheat is not appropriate to reduce HT2 and T2 in oats. The lower levels of HT2 and T2 in organic oats maybe have been a result of the long, less cereal intense rotations used by organic growers resulting in a reduction of inoculum compared to conventional rotations in the UK which are generally short and cereal intense. Preliminary studies have indicated that a high proportion (>90%) of HT2 and T2 on oats is present in the outer hulls which are removed during processing for human consumption (Scudamore et al., 2007). However, the high concentration of HT2 and T2 in unprocessed oats could still have a major impact on the oat industry depending on the legislative limits set.

Acknowledgements

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The effect of cultivation practices on *Fusarium langsethiae* infection of oats and barley

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Key Words: oats, barley, Fusarium langsethiae, direct drilling, tillage

Abstract

Direct drilling and conventional soil tillage with autumn ploughing were compared in a field trial in 2004-2006. Oats and barley cultivars were sown with both methods and development of *Fusarium* infection in ears and panicles was studied during the kernel development, from ear emergence until harvested and dried grain. *Fusarium langsethiae* was the first *Fusarium* species observed after ear/panicle emergence. The species was most abundant on oats in early stages of kernel development. Direct drilling increased *F. langsethiae* infection in warm and dry conditions in 2006, especially on oats. Higher T2/HT-2 toxin contents were detected in the grain harvested from direct drilled than traditionally tilled plots.

Introduction

The practice of direct drilling without tillage has increased in Finnish cereal production during recent years. The need to save labour, as well as economic and environmental aspects, have raised interest in this cultivation practice. The results published from other countries indicate, however, an increase in *Fusarium* infection and mycotoxin contents in cereal grain with reduced tillage (Bailey & Duczek, 1996, Yi *et al*, 2001). Crop debris is the main source of *Fusarium* inocula and inoculum production is dependent on rainfall and temperature. Warm and moist conditions favour infection during ear emergence and anthesis of wheat and barley (Xu, 2003), but little is known about infections on oats (Langseth & Elen, 1996). In Finland, oats and barley are the most important cereal crops, but the effect of tillage and *Fusarium* infection on these crops has not been studied. The distribution of *Fusarium* species as ear blight pathogens varies in cereal production in Europe because of their different requirements. In Norway, *F. langsethiae*, which forms T-2/HT-2 toxins, has been the most important mycotoxin producer during recent years (Kosiak *et al*, 2003).

Field trial and sampling

A field trial to compare traditional autumn ploughing and direct drilling without tillage was established on sandy clay soil at MTT Agrifood Research Finland, Jokioinen, in spring 2003. Autumn ploughed and direct drilled areas were kept in the same places in both years.

In 2004-2006, both cultivation practices were applied to grow four cultivars of malting barley and food oats. The cultivars were 'Roope', 'Freja', 'Veli' and 'Belinda' for oats and 'Saana', 'Scarlett', 'Barke' and 'Annabell' for barley. The seed was treated with carboxin+imazalil (Täyssato). For barley plots pre-crop was oats and oats was grown every year following barley.

To study *Fusarium* infection in the developing grain, samples were taken from ear and panicle emergence every two weeks from all plots until harvested, dried grain. Randomly chosen twenty ears or panicles per plot were sampled for investigation. To isolate Fusarium fungi, two kernels of each ear (panicle) were taken for incubation. The harvested grain was dried and samples were taken from both raw and cleaned grain (2-mm sieve) for Fusarium and mycotoxin analyses

The kernels and grains were incubated on agar medium containing pentachloronitrobenzene (PCNB) (Nash & Snyder medium, Nelson *et al*, 1983) at room temperature (22ºC) and the growing hyphae were isolated on potato dextrose (PDA) medium for identification. The Fusarium cultures were

identified microscopically**.** Trichothecenes were analysed from cleaned grain in 2003, in 2004 from both raw and cleaned grain with GC-MS**.**

Results and brief discussion

The first *Fusarium* species, detected at panicle emergence of oats, was *F. langsethiae*. It was found also on barley at ear emergence, but the amount of infected kernels was not as high as on oats (Figures 1 and 2). *F. langsethiae* was the most common *Fusarium* species on oats during the early development of kernels.

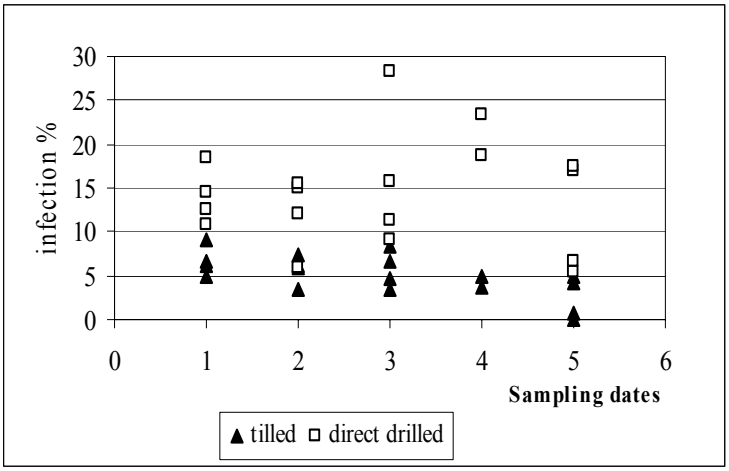

Figure 1. *Fusarium langsethiae* on oats 2006- infection in kernels at sampling dates from panicle emergence to harvest (sampling on weeks 1=27, 2=29, 3=31, 4=33, 5=35, harvest)

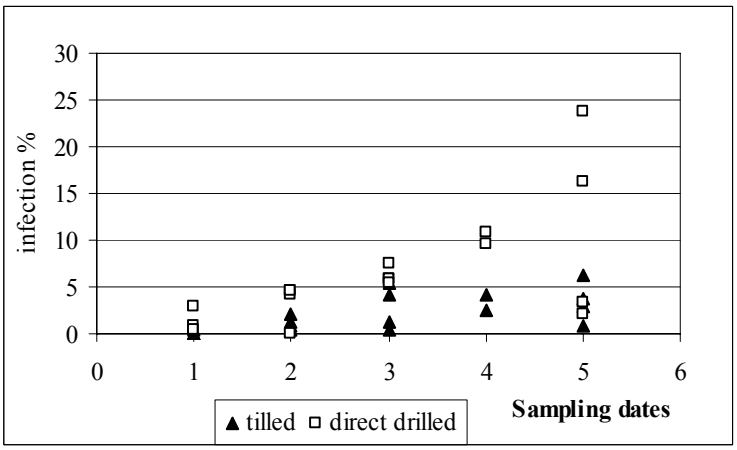

Figure 2. *Fusarium langsethiae* on barley 2006- infection in kernels at sampling dates from ear emergence to harvest (sampling on weeks 1=27, 2=29, 3=31, 4=33, 5=35, harvest)

The other species detected in early stages in flowers and kernels of oats was *F. poae*. Later in the season, other *Fusarium* species infected kernels. *F. avenaceum* and *F. sporotrichioides* infected kernels during July- early August while *F. culmorum* and *F. graminearum* infections became more prevalent in August if the weather was rainy (Parikka *et al* , 2005). Normally *F. langsethiae* was rarely detected in harvested grain. In 2006, however, infection by species like *F. avenaceum* and *F. culmorum* was inhibited in dry conditions and *F. langsethiae* was fairly abundant in harvested, dried grain. It was present both in oats and barley grown in autumn ploughed and direct drilled areas.

There were differences between cultivars in *F. langsethiae* infection levels. As a whole, more infection was detected on oats than on barley and more in late cultivars than in early ones. In 2004, *F. langsethiae* was slightly more prevalent in tilled than in direct drilled areas. In 2005, the situation was opposite on some cultivars and in 2006 in dry conditions direct drilling seemed to produce more infected kernels and grain than tillage (Figures 1 and 2). During the 3-year trial, although a short period, the prevalence of *F. langsethiae* seemed to increase in direct drilled plots. Cleaning the grain with 2 mm sieve did not reduce the amount of *F. langsethiae* infected kernels (Figures 3 and 4). In 2004-2005 the species was not detected on harvested barley grain.

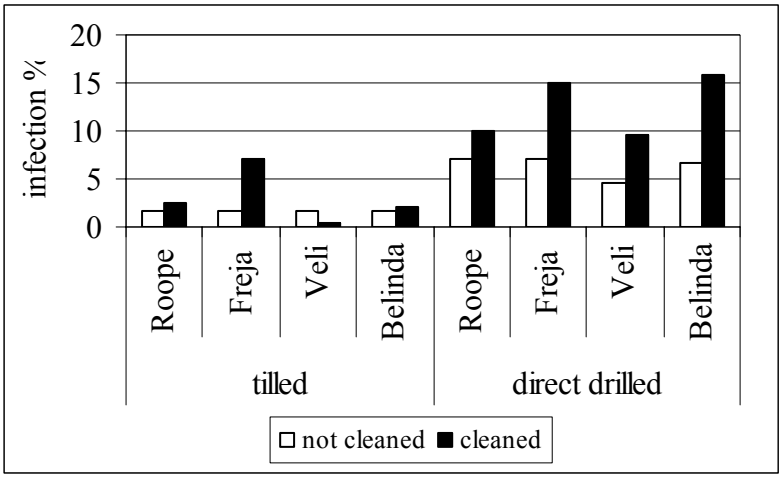

Figure 3. *Fusarium langsethiae* in dried, cleaned and not cleaned oat grain in 2006

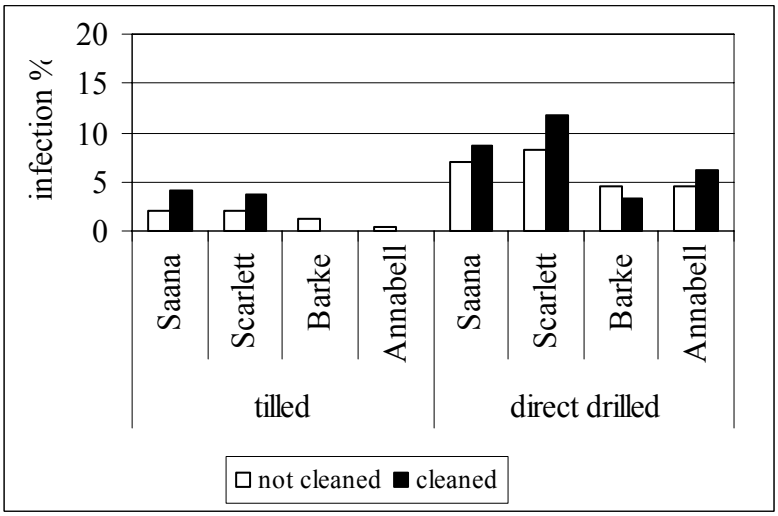

Figure 4. *Fusarium langsethiae* in dried, cleaned and not cleaned barley grain in 2006

F. langsethiae and *F. sporotrichioides* produce A-type trichothecenes T2 and HT-2 (Thrane et al 2004). The contents of these mycotoxins were higher on oats than on barley. *F. sporotrichioides* was present in the investigated crops, although not abundantly. The species was favoured by direct drilling of barley, but was not affected by cultivation practice of oats. *F. langsethiae* is an early colonizer of flowers and kernels, while *F. sporotrichioides* infection seems to increase during later stages of grain development. As a trichothecene producer *F. langsethiae* may be more important than *F. sporotrichioides* in Finnish conditions as it is already in Norway (Kosiak *et al.* 2003). In 2006, the T2/HT-2 contents of oats were higher in direct drilled than in tilled plots and HT-2 was detected in samples taken from direct drilled plots 2-3 weeks before harvest. Cleaning the dried grain with 2-mm sieve reduced T2/HT-2 contamination of oats.

Conclusion

These results, although from a short period of time, indicate that *F. langsethiae* may become a serious problem in cereal, especially oat cultivation where no tillage and crop rotation are used. The species does not seem to be sensitive to weather conditions at panicle emergence but drought enhances its growth during kernel development. However, more information is needed about survival of *F. langsethiae* in the field.

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Fusarium head blight and mycotoxins in cereals – potential strategies to control contamination under conservation tillage

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Key Words: conservation tillage, Fusarium, mulching quality, residue management

The occurrence of *Fusarium* head blight (FHB) in cereals is strongly influenced by cultivation practices such as crop rotation, tillage, and choice of varieties. FHB caused by *Fusarium graminearum* (FG) and contamination of wheat with deoxynivalenol (DON) is more prevalent when wheat is grown after maize and especially with maize residues remaining on the soil surface (i.e. conservation or zero tillage). If these two risk factors co-occur with weather conditions favourable to infection, serious mycotoxin contamination can occur even with the most resistant wheat varieties presently grown. The current regulations on DON (EU: maximum value of 0.75 ppm in cereal flours; Switzerland: tolerance value of 1 ppm in milling products) underline the need for controlling FHB caused by FG. In order to avoid FHB and mycotoxins while protecting the soil with conservation tillage, combinations of several measures have to be developed (see also contribution by Forrer et al.).

We assume that the risk for infection of the wheat crop could be reduced by accelerating the decomposition of maize residues, the main source of FG inoculum. Since 2003, we have been conducting on-farm trials with winter wheat grown after maize and with management of maize residues under conservation tillage. On 4 sites, we are examining the effect of fine mulching with or without surface incorporation of the residues on the occurrence of FHB. Mulching is being performed using a multipurpose shredder equipped with forged hammer knives and counter blades whereas a rototiller is being used for residue incorporation. Wheat varieties are according to the choice of the local farmer. Collected data include visual disease assessment in the field, yield, incidence of different *Fusarium* species on wheat grains (whole seed agar plate method), as well as the amount of DON in grains and straw.

The results show that with fine chopping of maize residues and less susceptible wheat varieties such as Arina or Titlis it is possible to produce no-till wheat with low DON contents in both grains and straw. For example, in 2004 on a site with Arina, the mean DON content in grains from plots with no residue treatment was 1.8 ppm whereas grains from plots with fine mulching showed only 0.5 ppm. Nevertheless, inconsistent results between different trial locations and from one year to the next demonstrate the need for further research. We suppose that the differing results are primarily due to variations in mulch quality such as the size of mulched debris and the homogeneity of dispersal, but also to soil activity, climatic conditions, or the given wheat variety.

For current on-farm trials, we are focusing on further improving the mulching procedure as well as on evaluating the mulching quality and the subsequent decomposition of maize residues. The results of this research project on residue management will be important for both no-till and conservation tillage systems. Furthermore, it will contribute towards safe food and feed while respecting the environment.

Screening for resistance to *Fusarium* head blight in organic wheat production

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Key Words: Organic Farming, spring wheat, Fusarium head blight, DON

Abstract

Organic growers mainly grow spring wheat in the Netherlands. In wet periods during flowering, these cultivars may become infected by *Fusarium* fungi causing *Fusarium* head blight of wheat. This disease is a problem that occurs both in organic and conventional farming systems. *Fusarium* fungi cause problems due to the production of mycotoxins in wheat kernels which are a threat to human and animal health. In addition, seed harvest of infected crops is lower and *Fusarium* fungi cause seedling rot as a result of contaminated seed. Breeding for disease resistance is the only way to prevent or reduce the occurrence of the disease. The aim of the current research project is to identify different mechanisms of resistance in cultivars to be used in further breeding programmes.

Introduction

Fusarium head blight or scab is a disease of wheat caused by a number of *Fusarium* fungi, such as *F. culmorum*, *F. graminearum*, *F. poae, F. avenaceum*, and *Microdochium nivale* (Parry et al., 1995). In the Netherlands, in the 1980s and early 1990s, *F. culmorum* was reported as the predominant species (Snijders, 1990). Surveys carried out by Waalwijk et al. (2003) in 2000 and 2001 demonstrated a drift in the populations from *F. culmorum* to *F. graminearum*. These two pathogens are closely related and it is thought that resistance in wheat to *F. culmorum* is correlated with resistance to *F. graminearum* (Mesterhazy, 1987).

Problems with *Fusarium* fungi in wheat occur both in organic and conventional farming systems. Infection of seeds by *Fusarium* fungi results in a decrease of yield and seed quality. This is caused by the production of shrunken kernels that poorly germinate and are contaminated with fungi, causing root rot of seedlings. In addition, *Fusarium* fungi are known for the problems they cause with respect to food safety due to the production of mycotoxins in the kernels, such as deoxynivalenol or DON. Infection occurs during the growing season of the plants during flowering. In conventional farming systems spraying against *Fusarium* fungi is being applied, but this does not allways result in lower levels of DON. Several cultivation measures have been proposed to reduce levels of infection. The best way to prevent or reduce *Fusarium* infection is the growth of cultivars with a high level of disease resistance. As levels of resistance in currently available cultivars are insufficient, breeding for improved levels of resistance has a high priority.

Resistance to *Fusarium* fungi has been described as the result of a number of resistance mechanisms: 1. resistance against initial infection, 2. resistance against spread in the ear, 3. resistance to kernel infection, 4. tolerance (no symptoms, but the fungus is present) and 5. resistance against mycotoxin accumulation (Mesterhazy, 1995). Also a number of escape or passive mechanisms have been described, which are based on plant morphology and growth components like plant height, compactness of the ear and flowering time.

The aim of the present research was to study the level of resistance in a number of spring wheat cultivars and to obtain more knowledge on mechanisms of resistance involved in these materials. In case different mechanisms of resistance are present in wheat cultivars, breeders can make use of these mechanisms and combine them in order to obtain cultivars with a higher level of resistance.

Methodology

Plant materials and Fusarium field trials

A collection of spring wheat cultivars and breeding lines were obtained from various breeding companies and individuals (see Figure 1). In 2005 and in 2006, field trials were performed at the organic experimental farm the Broekemahoeve in Lelystad, the Netherlands. The trials consisted of two plots, a control plot and an artifical inoculated plot each consisting of three replicates in a randomized block design. Lines were sown in 1.5 x 4 m^2 sub-plots, in rows 0.25 m apart at a density of 375 seeds m^{-1}.

Cultures of a pathogenic *F. culmorum* strain IPO-39 were multiplied as described by Snijders and Van Eeuwijk (1991). Since wheat is only susceptible at flowering time, artificial inoculations were made at this stage. As not all wheat plants flowered at the same time, inoculation was repeated for several times: four times in 2005 (nine days of flowering) and six times in 2006 (fifteen days of flowering). Plants were kept wet once a day by spraying water over the plants in the evening.

Evaluations

In 2005, four weeks after first inoculation *Fusarium* head blight ratings (FHB-index) were determined as the product of the percentage of infected heads and the proportion of infected spikelets per infected head. As a result of the longer flowering period in 2006, two groups of cultivars were determined: the early flowering and the late flowering cultivars. For both groups FHB ratings were scored four weeks after inoculation.

At harvest, ears and kernels were collected for investigating amounts of mycotoxin and fungal DNA. Plant materials were freeze dried and milled and send for analysis of DON content to the Technical Research Laboratory in Rotterdam, the Netherlands. TaqMan was applied to the same samples to estimate the amount of fungal DNA in the samples (Waalwijk et al, 2004).

In the field the following morphological traits were observed: plant length, distance ear-flag-leaf, openness florets, anther extrusion and compactness of the ear.

Results and discussion

Fusarium head blight ratings

In both years clear differences were found for the level of *Fusarium* infection between spring wheat cultivars (Fig. 1). In 2005, the level of infection varied between 7 and 62 %. Four weeks after inoculation, only in Pasteur we found that less than 50% of the ears were infected. This cultivar also had a relative low average percentage of infected spikelets per ear. In 2006, the average level of infection was more severe than in 2005 and varied from 12 to 90%. Perhaps, this is due to the environmental conditions during ripening of the crop when it was very warm. Some cultivars, like Pasteur and Lavett were more severely infected in 2006 than in 2005, whereas Thasos and Minaret were more stable. This might be an indication of the presence of different mechanisms present in these cultivars. The genetic background of this resistance is unknown, but it is interesting to note that Minaret is one of the parents of Thasos and so Thasos may have inherited its resistance from this parent.

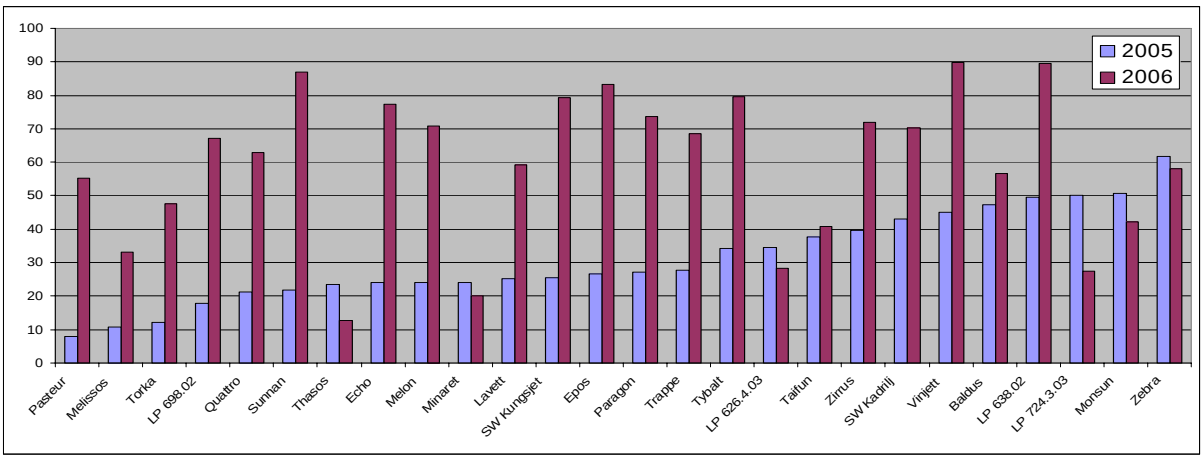

Figure 1. *Fusarium* head blight ratings observed in a set of spring wheat cultivars four weeks after artificial infection with *F. culmorum*.

When comparing 2005 and 2006, differences were observed in the flowering period in 2005. Flowering period is defined as the interval between the first flowering of a certain cultivar and the last flowering of all cultivars and depends strongly on the temperature during the day, which was above 25°C in 2005 and varied strongly between 15 and 27°C, with an average of about 20°C, in 2006. As *Fusarium* infects flowering plants only, more inoculations had to be carried out in 2006 compared to 2005. When we grouped plants in groups of early and late flowering types, we noticed that within the early flowering types hardly any difference was found between 2005 and 2006 (Figure 2), except for cultivar SW Kungsjet. Perhaps, cultivars that flowered late have more variation in their flowering period, and the variation found between the two years is due to the experimental situation that is strongly influenced by the weather conditions, especially in 2006.

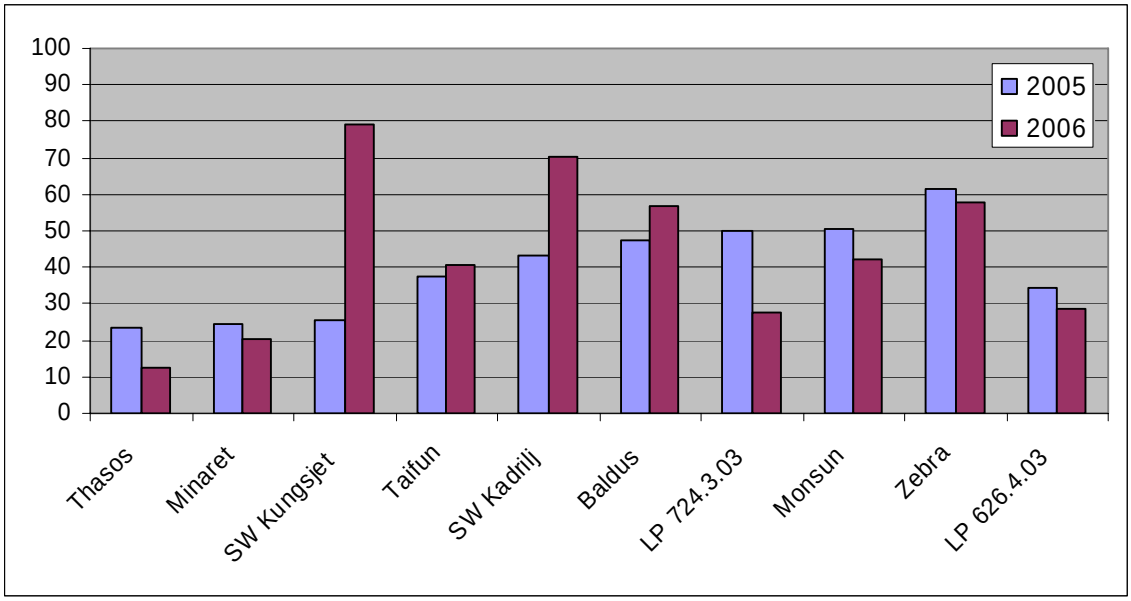

Figure 2. *Fusarium* head blight ratings observed in the early group of spring wheat cultivars four weeks after artificial infection with *F. culmorum*.

Levels of DON and fungal DNA in the ears at harvest time and in the kernels

A subset of cultivars was analysed for the amount of DON in the ears. There was variation between the two years with in general somewhat higher levels of DON in 2005 than in 2006. Some cultivars had DON levels that were 4 times higher in 2005 compared to 2006. This result is unexpected as the levels of infection were equal or higher in 2006 than in 2005. The amount of fungal DNA was in almost all cases higher in 2006 than in 2005.

Only in 2005, kernels could be used for DON and fungal DNA analysis. Due to the severe rainfalls in August 2006, harvested kernels were of bad quality and some already germinated directly after harvest. We found moderate to high correlations between the FHB-index and the level of DON (r^2 = 0.40) and between the FHB-index and the total amount of DNA (r^2 = 0.71). Among the more resistant cultivars, some cultivars produced more or less toxin than expected on the amount of fungal DNA. This could be an indication for resistance against the fungus or against the toxin.

Effect of Fusarium infection on yield

In 2005, seeds were harvested from all plots of both blocks (*Fusarium* trial and control block) to determine the decrease in yield as a result of *Fusarium* infection. We found a high correlation between the level of *Fusarium* infection and the decrease in yield (r^2 = 0.70). In the *Fusarium* trial field yields were between 30-50% less than in the control plots. Cultivars with highest yields in the Fusarium trial field were: Lavett and Pasteur (6-7% less than control) and Melissos, Thasos and Trappe (13-15% less than control). In 2006, harvesting of the plots became problematic as a result of severe rain falls, which lasted the whole month of August. Comparing yields of control plots with inoculated plots became unreliable and was not carried out.

Relationship between FHB and morphological traits

In 2005, we found that among the several morphological traits that were studied, in our cultivar set only compactness of the ear was correlated with higher levels of susceptibility to Fusarium head blight (r^2 =0,46). In 2006, we confirmed those results that cultivars with very compact ears appeared to be very susceptible for FHB. In contrast, cultivars with less compact ears did not always have low levels of *Fusarium* infection. This resulted in less correlation between compactness of the ear and resistance against *Fusarium*. Our results clearly show that compactness of the ear is a factor of influence on resistance to *Fusarium*.

Conclusions

Large differences found for the level of *Fusarium* infection between the various cultivars studied in this research clearly indicate variation between cultivars and probably also for resistance mechanisms that are involved. Unfortunately, differences between years complicate the full understanding of these underlying mechanisms. However, it is clear that some cultivars are more resistant to *Fusarium* head blight than others and that most of these cultivars also produce less DON. From the results of 2006, a preliminary conclusion might be drawn that resistance in some cultivars is more stable than in other cultivars.

We also identified some cultivars that produced more or less toxin than expected on the basis of the amount of DNA. This may be an indication for resistance to toxin production or fungal accumulation. In our cultivar set, morphological characteristics of the ear, also seemed to be a factor of importance. The more compactness of the ear, the more susceptible the cultivar was. We will repeat this experiment in 2007 in order to study if we are able to identify the same cultivars again so that breeders can use these materials in their breeding programmes. The identification of a useful morphological trait is important as it can be used as an easy tool for selection by breeders.

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Kernel resistance against *Fusarium* head blight as selection criterion in wheat breeding

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Fusarium head blight is perceived as a threat to wheat production all over the world. Besides the reduction of the yield potential in infected plants, the fungus contaminates the kernels with different mycotoxins. The most prominent mycotoxin found in kernels is deoxynivalenol (DON).

The control strategies against this disease aim at avoiding the infection by adequate cultural techniques (i.e. avoiding maize before wheat), the use of fungicides and the use of resistant varieties. Only these latter are also able to contain accumulation of mycotoxins once the infection has happened.

Different resistance mechanisms have been described. The most known mechanisms, that are also largely used in the different resistance breeding programmes world wide, are the resistance to primary infection of the spikelets (type 1) and the reduction of spreading of the infection in other parts of the ear (type 2). In the last years, the ability of the kernels to prevent infection of the fungus and the accumulation of mycotoxin has received increasing attention. Yet, the detection of kernel resistance for breeding purposes is rather difficult, as the corresponding resistance mechanisms are not fully understood. In the present work, different aspect of kernel resistance, such as DON accumulation, kernel deformation and the reduction of baking quality traits after FHB infection are presented. The possible use of these components of kernel resistance as criteria in a wheat breeding programme are discussed.

Breeding efforts to develop resistant cultivars to *Fusarium* head blight and associated mycotoxins in wheat for Romanian sustainable cropping systems

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Key Words: Fusarium head blight (FHB), scab, deoxynivalenol, DON, breeding of resistance

Abstract

Fusarium head blight (FHB or scab), has reached in the past decades worldwide damaging proportions in wheat crops and other small grains, in conditions of wet weather during flowering and grain filling.

This disease may also produce significant economic losses in terms of food safety. Contamination of grains with several *Fusarium* secondary toxic metabolites (mycotoxins), that are harmful for the health of humans and animals, drastically reduces their use for processing and consumption. Such effects are not entirely predictable or easy to be controlled.

Host resistance remains the most economical and effective method to reduce losses caused by this disease, including resistance to contamination with mycotoxins. As a consequence, in many wheat breeding programs from the world, development of resistance to FHB became a major objective during the past decades. However, real progress is hindered by the complexity of quantitative resistance, a lack of effective sources of resistance, as well as the high importance of genotype × environment interactions. Application of Marker Assisted Selection (MAS) to enhance the effectiveness of breeding for FHB resistance is generally agreed as a valuable alternative, but the capacity to implement this on a broad scale has still not been optimised.

Results of our breeding efforts, using both conventional and molecular tools, to optimise the evaluation methods, as well as to develop germplasm with improved resistance to FHB and to accumulation of DON are reviewed. Genotypes that combine a low percentage of Fusarium diseased kernels and DON content were identified. Marker-assisted introgression of the donor-QTL alleles 3B (Sumai 3) and 3A (F 201R) into Romanian winter wheat germplasm combined with phenotypic selection, is a promising component of the strategy to reduce the vulnerability to FHB epidemics of the new cultivars in conventional and low input systems in Romania.

Introduction

Fusarium head blight (FHB or scab), caused primarily by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch) and *F. culmorum* (Wm.G.Sm.) Sacc., has reached worldwide damaging proportions in wheat crops and other small grains. The main factors responsible for the current destructive influence of this facultative pathogen are continuous spread of wheat/maize rotation, low or no tillage crop technologies, a limited number of resistant commercial cultivars, and the lack of consistent alternative measures to control this disease. FHB may produce significant economic losses also in terms of food safety (Leonard and Busnell, 2003). Contamination of grains with several *Fusarium* mycotoxins, such as deoxynivalenol (DON) or nivalenol (NIV), that are harmful for the health of humans and animals, drastically reduces their use for processing and consumption.

In this context, host resistance is considered a cost-efficient and environmentally sound strategy to combat FHB **(**Miedaner, 1997). The employment of FHB-resistant cultivars, carrying one or both known resistance types (I - resistance to initial infection and/or penetration and, respectively II - resistance to spread of disease within the spike), remains the most economical and effective method to reduce losses caused by this disease in wheat, including resistance to contamination with mycotoxins, in both conventional and organic cropping systems. As a consequence, in many wheat breeding programs from the world, development of resistance to FHB became in the past decades a major

objective. At NARDI-Fundulea our breeding program focuses on searching new sources of resistance to FHB and DON contamination, pyramidation of resistance from various origins, and combination of resistance to FHB with other desirable agronomic traits. However, progress in developing FHB-resistant wheat cultivars is hindered by the complexity of quantitative resistance (more components were described), a lack of effective sources of resistance, as well as the high importance of genotype x environment interactions.

Application of MAS to enhance the effectiveness of breeding for FHB resistance is generally agreed as a valuable alternative. Several FHB resistance loci have been found mainly in Asian (Shen et al., 2003, Zhou et al., 2003) and Brazilian spring wheats (Steiner at al., 2004) and additionally in several wild species such as *Triticum macha* (Mentewab et al., 2000**)**, *T. dicoccoides* (Otto et al., 2002, Stack et al., 2002), and *Lophopyrum elongatum* (Shen et al., 2004). In the Chinese source Sumai 3 (spring wheat), a major quantitative trait loci (QTL) on chromosome 3BS (*Qfhs.ndsu-3BS,* re-designated as *Fhb 1),* primarly associated with Type II resistance to FHB that explained up to 50% of the phenotypic variation, has been identified (Bai et al., 1999, Waldron et al, 1999, Anderson et al., 2001). In comparison with spring wheat, only a few resistant winter wheat cultivars have been genetically analysed for FHB resistance to date. The Romanian winter bread line *Fundulea 201R* was reported as having FHB resistance genes derived from cultivars NS 732 and Amigo, having no relation to any of the previously described sources of resistance (Ittu et al., 1998). Regional QTL mapping of population derived from crosses of *Fundulea 201R /Patterson* (susceptible parent) with simple sequence repeat (SSR) analysis suggested four interval regions located on chromosomes 1B, 3A, 3D and 5A that confer FHB resistance. The four QTLs together accounted for 33% of the phenotypic variation, or 43% of the genotypic variation (Shen et al., 2003). Additional QTLs associated with FHB resistance localised in different genomic regions were identified in populations *Renan/Recital* (Gervais et al., 2003), *Arina/Forno* (Paillard et al., 2004) and *Dream/Lynx* (Schmolke et al., 2005). In spite of these results**,** the capacity to implement MAS on a broad scale has still not been optimised and effective for FHB resistance. Hence, further research is needed to elucidate the genetic relationship of the resistance in germplasm to be improved with identified FHB resistance QTLs.

The latest results of our breeding efforts to optimise the evaluation methods as well as to develop germplasm with improved resistance to FHB and to accumulation of DON are reviewed. The identification of genotypes with low DON content and the effect of QTLs for FHB resistance on phenotypic resistance traits that expressed resistance Type II (FHB severity and progress) and DON content in Romanian winter wheat breeding germplasm are reported.

Methodology

Plant material. Fifty-three advanced bread winter wheat lines from NARDI were analysed for DON content (ppm) and the percentage of *Fusarium* diseased kernels (FDK, %) was determined. Effects of single QTLs for FHB resistance on phenotypic resistance traits were evaluated in 36 lines selected from crosses involving *F201R* (QTL class 3A), a winter wheat type with improved agronomic traits, and *Sumai 3* (QTL class 3B+3A+6A), a less adapted spring wheat. These genotypes showed various levels of FHB resistance in field tests. The parents and their derivative lines were previously genotyped with specific SSR markers *Xgwm* and *Xbarc* from regions of the genome where QTLs for FHB resistance have been identified and the presence/absence of corresponding QTLs was documented (Ciuca, 2006). Among the *F201 R* derivatives, 18 lines were QTLs carriers, while 7 were non-carriers. Lines derived from Sumai 3 were also QTLs carriers and non carriers (5:6) (Ittu et al., 2006).

Fusarium isolates. Single-spore isolates of *F. graminearum* (FG 96) and *F. culmorum* (FC 46), originally isolated from winter wheat in Romania and in The Netherlands, respectively, were separately used for inoculation. FC 46 was kindly provided by Dr. T. Miedaner from the University of Hohenheim, State Plant Breeding Institute Stuttgart, Germany. Inoculum of both isolates was produced on Mung bean liquid medium, continuously aerated for seven days under continuous exposure to black UV lamps (Philips HPL-N 400W E40) at room temperature (approximately 24°C).

Artificial inoculation. In 2005 and 2006, wheat genotypes were grown at NARDI-Fundulea and artificially field point inoculated. For point inoculation, investigating resistance Type II to FHB, approximately 10 µl were injected with a syringe directly through the glumes in a central floret of each side of 20 arbitrarily chosen, marked heads per plot. Each genotype was inoculated at mid-flowering.

Resistance traits. Recording of FHB ratings started 10 days post inoculation (pdi) and were repeated 20 dpi in terms of infected spikelets/entry/isolate The arithmetic mean of the individual successive ratings was used for further calculation of FHB severity (damaged spikelets, % of control at the onset of symptom development, i.e. 20 dpi), and disease progress (area under disease progress curve -AUDPC). Heading date was recorded on a time scale starting at January 1st. At full ripening, inoculated and random main-tiller spikes/entry/isolate were both harvested and threshed by hand, to save highly infected, shrivelled and degenerated kernels. From these samples, the percentage of FDK was determined and calculated per entry/isolate. Regression between FDK and content of DON was calculated.

DON immunoassay analysis. Grain samples from heads inoculated separately with isolates of *F. graminearum* (FG 96) and *F. culmorum* (FC 46) within each entry were bulked, ground, and analysed for DON content at the Institute of Food Bioresources, Bucharest, Romania. The concentration of DON was quantified with an ELISA kit according to the manufacturer's description (Ridascreen®FAST, R- Biopharm GmbH, Darmstadt, Germany).

Results and discussion

Identification of genotypes with low DON content. The response of wheat genotypes to FHB induced by artificial inoculation with two *Fusarium* isolates varied for both traits, the percentage of Fusarium diseased kernels and the DON content. LSD values *≥5%* were 10.3 for FDK and 2.6 for DON content. Averaged across results within each data set/trait x *Fusarium* isolate, the minimum and maximum values were observed for FDK (0-53.4, FC 46) and the content of DON (0.3-56.5 ppm, FG 96), respectively (Table 1).

Table 1. Range of variation for *Fusarium* diseased kernels and DON content in 53 wheat genotypes following artificial field inoculation with *F. culmorum* and *F. graminearum*.

Trait	*Fusarium culmorum* (FC 46)			*Fusarium graminearum* (FG 96)			Mean from both isolates
	Minimum	Maximum	*Average*	Minimum	Maximum	*Average*	
Fusarium diseased kernels (FDK, %)	0	53.4	*11.4*	0	41.0	*7.6*	*9.5*
DON (ppm)	1.8	53.0	*11.3*	0.3	56.5	*8.3*	*9.8*

Evaluation of regression between DON concentration in grains from artificially inoculated heads demonstrated that this trait was highly correlated with *Fusarium* diseased kernels (r=0.86***, n=53) (Fig. 1). These findings suggest that in our breeding germplasm, selection of genotypes that combine low FHB with corresponding DON reduction is possible.

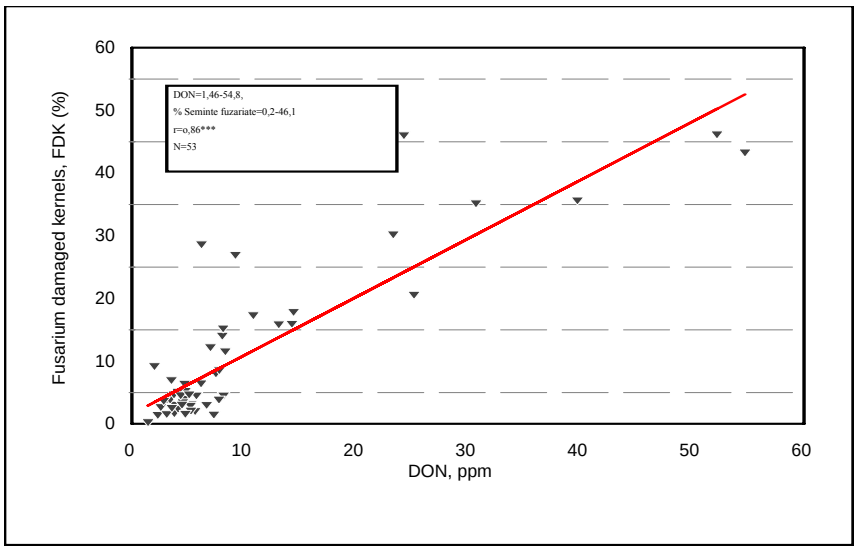

Figure 1. Regression between DON content in grains and *Fusarium* diseased kernels from 53 winter wheat genotypes artificially inoculated with isolates *Fusarium graminearum 96 and F. culmorum* 46 (mean values).

Effect of QTLs for FHB resistance. Field evaluation of FHB resistance revealed differences between donors and their derivatives for most of the resistance traits analysed. Sumai 3, carrier of the major QTL *Fhb 1*, that explains up to 50% of resistance to FHB Type II, confirmed in this experiment across combinations environment/isolate its high potential of resistance expressed in terms of FHB severity (16 % of damaged spikelets at 20 days post-inoculation), FHB progress (AUDPC= 174), Fusarium diseased kernels 17 %, and DON content (4.5 ppm) (Table 2). Fundulea 201R recorded lower values for these parameters, respectively, FHB severity=29 %, AUDPC=258, FDK=29 % and 6.2 ppm (Table 3). Differences regarding the mean values and range of variation for the resistance traits among QTLs carriers and non-carriers were observed for derivatives groups of both donors. FHB severity, disease progress, diseased kernels were reduced in QTL carriers derived from crosses with both donors of resistance, as compared with the corresponding non-carrier lines(Tables 2&3).

Table 2. Means for heading date; FHB severity; FHB progress; FDK, and DON of Sumai 3 and corresponding derivatives breeding lines

Donors/derivatives	Heading date	FHB			DON content
		Severity	Progress	FDK (%)	(ppm)
Sumai 3	**141**	**16**	**174**	**17**	**3.8**
Carriers of Sumai QTLs alleles (*fhb1*-3 BS)					
Average	141	21	215	20	4.5
Range	138-144	9-44	138-362	3-27	
Effect (%)*	*100*	*131*	*124*	*118*	*118*
Non-carriers of Sumai QTLs alleles					
Average	143	27	294	24	7.8
Range	143-144	18-46	216-528	10-58	
Effect (%)	*101*	*169*	*169*	*141*	*205*
LSD, P≥5%		*12*	*74.5*	*24.4*	

*) Difference to the donor

Table 3. Means for heading date; FHB severity; FHB progress; FDK, and DON of F 201R and corresponding derivatives breeding lines

Donors/derivatives	Heading date	FHB			DON content
		Severity	*Progress*	FDK (%)	(ppm)
F 201R	141	29	258	29	6.2
Carriers of F 201R QTLs alleles (3A)					
Average	142	26	240	23	6.0
Range	138-145	15-79	176-598	14-34	3.4-8.4
Effect (%)	*101*	*90*	*93*	*79*	97
Non-carriers of of F 201R QTLs alleles					
Average	144	29	344	18	6.2
Range	140-147	17-62	198-732	2-38	
Effect (%)	*102*	*100*	*133*	*62*	*100*
LSD, P≥5%		*17.0*	*168.3*	*14.2*	

*) Difference to the donor

These differences cannot be explained by differences in heading date, as average earliness of carrier and non-carrier lines was not significantly different. There was considerable overlapping of distributions for carriers and non-carriers of single QTLs, for all measured traits. These results suggest a variable effect of the analysed QTLs on each trait.

The validation of QTLs is a general prerequisite condition before their use in MAS breeding programs.

As expected, our results prove that selecting for only one or even a major QTL cannot guarantee a satisfying level of FHB resistance. However, data on the presence of single FHB resistance QTLs can be useful for choosing parents to increase the level of resistance, by cumulating various QTLs.

Conclusions

The close significant correlation found between DON concentration in grains from artificially inoculated heads and Fusarium diseased kernels suggests the possibility to select concomitantly for these two FHB traits.

Marker-assisted introgression of the donor-QTLs alleles 3B (*Sumai 3*) and 3A (*F 201R*) into Romanian winter wheat germplasm combined with phenotypic selection, is a promising component of the strategy to reduce the vulnerability to FHB epidemics of the new cultivars in conventional and low inputs systems in Romania.

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A comparative assessment of potential components of partial disease resistance to *Fusarium* head blight using a detached leaf assay of wheat, barley and oats

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Key Words: components of partial disease resistance, incubation period, latent period, Microdochium nivale

Abstract

The relative resistance of 15 winter barley, three winter wheat and three winter oat cultivars on the UK recommended list 2003 and two spring wheat cultivars on the Irish 2003 recommended list were evaluated using *Microdochium nivale* in detached leaf assays to further understand components of partial disease resistance (PDR) and *Fusarium* head blight (FHB) resistance across cereal species. Barley cultivars showed incubation periods comparable to, and latent periods longer than, the most FHB resistant Irish and UK wheat cultivars evaluated. In addition, lesions on barley differed from those on wheat as they were not visibly chlorotic until sporulation occurred, in contrast to wheat cultivars where chlorosis of the infected area occurred when lesions first developed. The pattern of delayed chlorosis of the infected leaf tissue and longer latent periods indicate that resistances are expressed in barley after the incubation period is observed, and that these temporarily arrest the development of mycelium and sporulation. Incubation periods were longer for oats compared to barley or wheat cultivars. However, oat cultivars differed from both wheat and barley in that mycelial growth was observed before obvious tissue damage, indicating tolerance of infection rather than inhibition of pathogen development, and morphology of sporodochia differed, appearing less well developed and being much less abundant. Longer latent periods have previously been related to greater FHB resistance in wheat. The present results suggest the longer latent periods of barley and oat cultivars, than wheat, are likely to play a role in overall FHB resistance if under the same genetic control as PDR components expressed in the head. However, the limited range of incubation and latent periods observed within barley and oat cultivars evaluated was in contrast to wheat where incubation and latent periods were shorter and more variable among genotypes.

Introduction

Fusarium head blight (FHB) is one of the most serious fungal diseases of cereals; most research has focused on wheat and barley with oats receiving less attention. There is no complete resistance to FHB although wheat and barley genotypes have been identified with partial resistance. There is no strong evidence for species-specific resistance to FHB, associated with at least 17 causal organisms, in wheat (Parry et al., 1995) or barley (Steffenson, 2003).

Despite this lack of strong evidence for species-specific resistance, differences in host preference have been reported for *M. nivale* (Diamond & Cooke, 1997a; Simpson et al., 2000), which is differentiated into var. *majus* and var. *nivale* based on conidial morphology (Wollenweber, 1931; Gains & Muller, 1980). The majority of isolates from wheat and barley seed have been found to be *M. nivale* var. *majus* (Parry et al., 1995; Diamond & Cooke, 1997a) although a higher proportion of *M. nivale* var. *nivale* isolates were obtained from barley than from wheat (Diamond & Cooke, 1997a). *Microdochium nivale* var. *nivale* was predominantly isolated from oats (Diamond & Cooke, 1997a). However, *M. nivale* var. *majus* and var. *nivale* are able to cross-infect between different cereal hosts (wheat, barley and oats) irrespective of their original host (Diamond & Cooke, 1997a). It is unclear as to why differences in host preference are found although *M. nivale* var. *majus* is more pathogenic to wheat than var. *nivale* in detached leaf assays (Diamond & Cooke, 1997a, 1999; Browne & Cooke 2004b) and in a seed germination assay (Browne & Cooke, 2005) while *M. nivale* var. *nivale* has been reported to cause greater disease to the stem-base of oat seedlings than var. *majus* (Simpson et al., 2000).

In European wheat, FHB resistance was most strongly correlated to the PDR component latent period (time from inoculation to sporulation) in a detached leaf assay and to a lesser extent incubation period (time from inoculation to first symptoms of damage to the leaf surface) (Diamond & Cooke, 1999; Browne & Cooke 2004b); this pattern of relatively long incubation and latent periods was also

found in moderately FHB resistant US cultivars (Browne et al., 2005).

The relative susceptibility of wheat, barley and oats to FHB is unclear and resistance mechanisms and potential susceptibility factors across these crops are poorly understood. Nevertheless a number of authors have used comparative assessments between cereal crops in order to facilitate improving the limited understanding of FHB resistance among cereal species (Liu et al., 1997; Langevin et al., 2004). The aims of the research reported here were to comparatively assess the PDR components detectable in a range of commercial cultivars of wheat, barley and oats using the *M. nivale* detached leaf assay.

Methodology

Cultivars of wheat, barley and oats used in this study were selected from the 2003 UK and Irish recommended lists; the FHB-resistant wheat genotype Frontana (Browne & Cooke, 2004b) was also included. The cultivars were grown in a controlled environment chamber and the first leaf harvested on day 14; 4 cm sections were placed on 0.5% water agar (4 leaves per Petri dish) containing 10 mg l^{-1} kinetin as a senescence retarder (Browne & Cooke, 2004b). Single-spore isolates of *M. nivale* var. *majus* isolated from wheat seed from the Irish 2001 harvest, pre-screened for pathogenicity to detached leaves of wheat, were used. In addition, a further isolate of *M. nivale* var. *majus* and three isolates of *M. nivale* var. *nivale* from wheat (obtained courtesy of Josephine Brennan, University College Dublin, Ireland and Simon Edwards, Harper Adams University College, UK, respectively) were used. Mycelium-free conidial inoculum of *M. nivale* was produced on potato dextrose agar coated in cellophane (CPDA) (Browne & Cooke, 2004a) and incubated on cool plates (Cooke, 1980) for 7 days under a diurnal cycle of near-ultraviolet (NUV) and white light. Leaf segments were inoculated at the centre of the adaxial surface with a 10 ul droplet of *M. nivale* spore suspension adjusted to 1 x 10^6 conidia ml^{-1}. The detached leaves were then incubated at either 10 or 15°C under a 24 h diurnal cycle of NUV and white light.

In experiment 1, barley and wheat cultivars were inoculated separately with five wheat isolates of *M. nivale* var. *majus* using two replicates and incubated at 10°C. In experiment 2, barley, wheat and oat cultivars were inoculated with a *M. nivale* var. *majus* wheat isolate, known to have high pathogenicity to detached wheat leaves, using five replicates and incubated at 10°C. In experiment 3, barley cvs Angela, Antonia, Haka, Pearl, Regina and Siberia, wheat cvs Biscay and Claire and oat cvs Gerald, Jalna and Millenium were inoculated with a *M. nivale* var. *majus* isolate and three isolates of *M. nivale* var. *nivale* using five replicates and incubated at 15°C. In each experiment, each value was the mean of four observations for each replicate. Assessments of symptom appearance and sporulation were carried out daily. The PDR components measured were: incubation period (days from inoculation to symptom development) and latent period (days from inoculation to sporulation) (Browne & Cooke (2004b).

Results and discussion

There were significant differences for incubation period among barley and wheat cultivars *(*$P < 0.001$) in experiment 1 (Figure 1). All barley cultivars showed incubation periods comparable to wheat cvs Solstice, Biscay and Claire, and Frontana, and had significantly longer incubation periods than susceptible cvs Raffles and Alexandria. While all isolates sporulated on detached leaves of wheat cultivars within 14 days, no sporulation was observed on barley cultivars reflecting the large differences in latent period between cereal species.

In experiment 2, (Figure 2) incubation periods were shorter than in experiment 1, consistent with a more pathogenic isolate. Differences between oat, barley and wheat cultivars were highly significant *(*$P < 0.001$). Again incubation periods of all barley cultivars were comparable to those for wheat cvs Solstice, Biscay and Claire and Frontana. The incubation periods on oat cvs Gerald, Jalna and Millenium were significantly longer than barley or wheat. There were also marked differences in the first appearance of damage to the leaf surface. On wheat, dull-grey green water-soaked lesions were present extending outside the initial inoculum droplet. On barley, symptoms were similar, but less extensive. On oats mycelial growth was observed on the leaf surface outside the inoculum droplet, but without apparent damage to the leaf, indicating a tolerance to rather than inhibition of pathogen development. By day 14, extensive sporulation occurred on all wheat cultivars; however only sporadic sporulation occurred on barley cultivars and no sporulation was observed on oats, reflecting marked differences in latent period between the three cereal species. Lesions observed on barley differed from those on wheat; although these were necrotic, chlorosis of the underlying leaf tissue did not occur until sporulation.

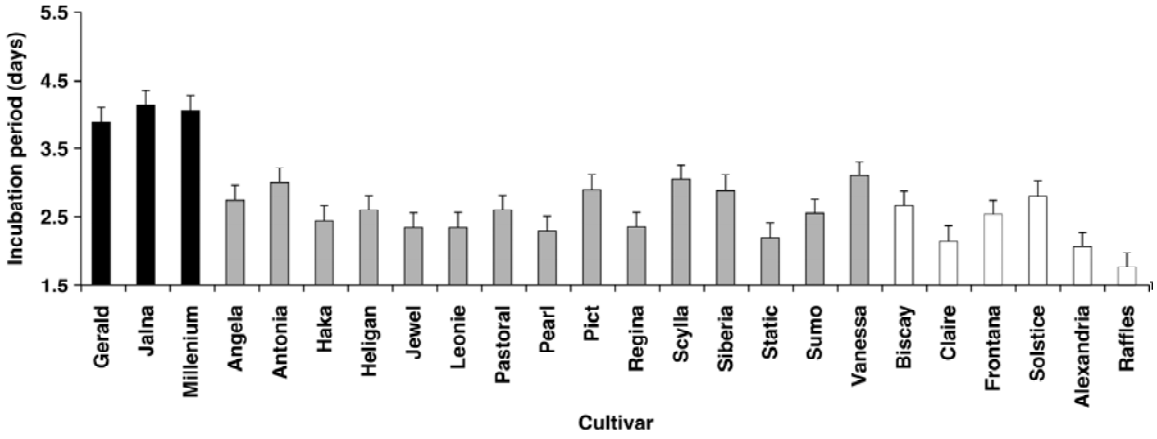

Figure 2. Experiment 2. Incubation periods of oat ■, barley ▫ and wheat □ cultivars inoculated with *M. nivale* var. *majus* isolate Dard1/M on detached leaves incubated at 10ºC. Bars represent standard error of the mean.

A higher incubation temperature of 15°C was used in experiment 3 (Figure 3). Incubation periods were longer for *M. nivale* var. *nivale* isolates than for the var. *majus* isolate across all cereal species *(P* < 0.001). As in experiments 1 and 2 all barley cultivars had incubation periods comparable to or longer than the wheat cvs Biscay and Claire *(P* < 0.001) (Figure 3a); oat cvs Gerald, Jalna and Millenium showed the longest incubation periods. By day 4 at 15°C sporulation occurred on most wheat leaves with sporodochia forming a pattern along the rows of stomata on the adaxial leaf surface in and around the inoculum droplet and mycelium was observed growing over the leaf surface. In barley, lesions extended beyond the inoculum droplet but were less extensive than on wheat, and growth of mycelium was not evident. Symptom expression was not as extensive on oats as in wheat and barley, although as at 10°C, mycelial growth was observed without obvious damage to the underlying leaf tissue. Wheat differed from barley and oats in that lesions were consistently accompanied by chlorosis of the leaf tissue; this was not observed in barley until sporulation occurred. By day 10 extensive mycelial growth was observed in wheat, incubated at 15°C. Mycelial growth was observed less frequently in barley and was less extensive where it did occur, as was leaf chlorosis and necrosis. In oats necrotic lesions first occurred in sporadic isolated spots rather than in consolidated lesions, as occurred in wheat and barley, although mycelial growth from the infected leaf was quite extensive.

Differences in latent periods across oat, barley and wheat were highly significant *(P* < 0.001). Again oat cultivars had the longest latent periods; those of barley and oats were much longer than on wheat cultivars (Figure 3b). *Microdochium nivale* var. *nivale* isolates caused longer latent periods (as for incubation period) than var. *majus* on wheat cvs Biscay and Claire. However this was not observed on oats and barley. In wheat and barley, sporodochia were observed in lines between the veins above the stomata on the leaf surface. However in oats, the appearance and distribution of sporodochia differed; they were much less abundant after the onset of sporulation. Diamond & Cooke (1997b), in scanning electron microscope studies, observed that sporodochia on detached leaves of oats had a less regular and compact structure than those of wheat and barley.

On wheat leaves, sporulation occurred in close proximity to the initial inoculum droplet, at the mid-point of the leaf; however, in barley sporulation was observed at the cut ends of the detached leaves where necrosis also occurred, although the leaf tissue between the inoculum droplet and the leaf ends appeared healthy. In oats more general chlorosis was observed where mycelium was present, and necrosis and sporulation were restricted to the cut ends of the leaves.

Evaluation of PDR components revealed marked differences between cereal species in incubation period, latent period and the symptoms associated with the evaluation of PDR components. Barley showed longer latent periods than the most resistant Irish and UK commercial wheat cultivars with similar or longer incubation periods. This observation suggests that in barley the development of the pathogen was slowed or arrested, due to resistance mechanisms expressed after the incubation period was completed. These findings are consistent with Perry (1986) who reported that in the field the outer leaves of the stem-base in barley were frequently necrotic and brown, and although *M. nivale* could be isolated, there was no evidence that the fungus caused the symptoms but rather persists in the tissue producing sporodochia when senescence occurs. The necrotic lesions in barley may therefore reflect a defence response after initial penetration of the leaf tissue (incubation period) rather than be solely an indicator of susceptibility. Oats had longer incubation and latent periods than wheat or barley; however, the reaction of oat cultivars differed as quite extensive mycelial growth occurred on

the leaf surface before obvious symptoms of infection, indicating tolerance to rather than inhibition of pathogen development.

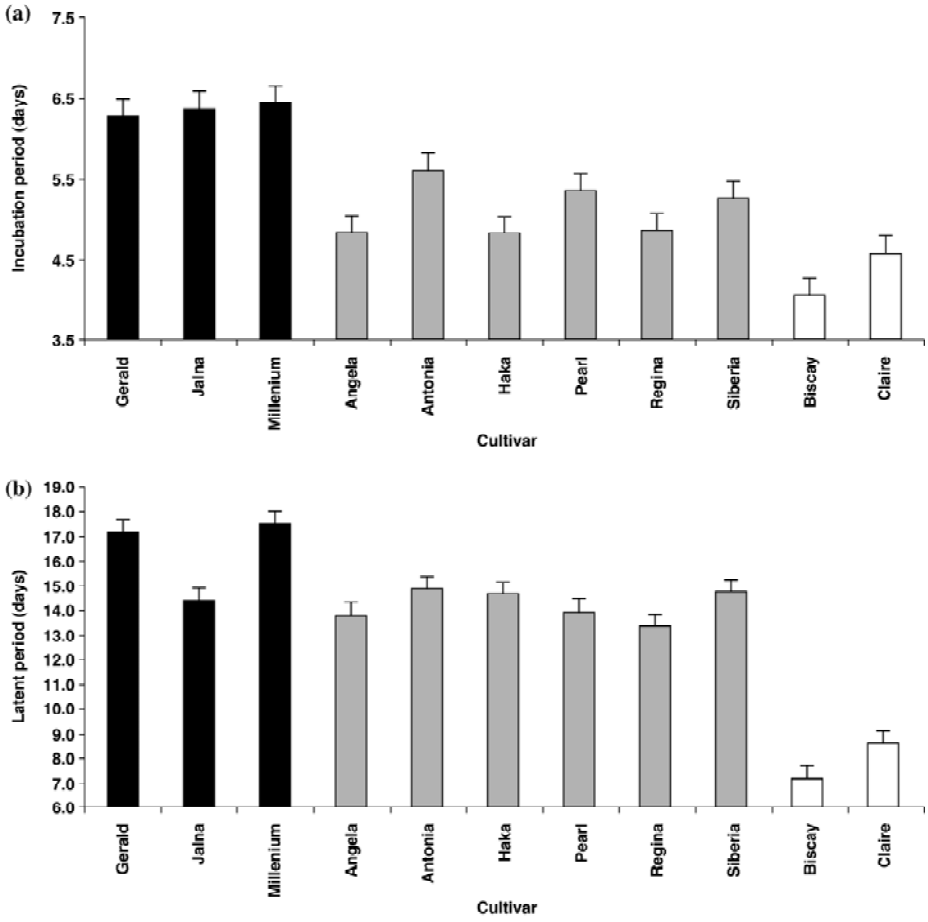

Figure 3. Experiment 3. Incubation period (a) and latent period (b) of oat ■, barley ▒ and wheat □ cultivars inoculated with *M. nivale* var. *majus* isolate Dard1/M and *M. nivale* var. *nivale* isolates 44/S/N, SO28/2/N and SO48/1/N on detached leaves incubated at 15ºC. Bars represent standard error of the mean.

The present results comparing infection by both fungal varieties of *M. nivale,* while preliminary, suggest that while *M. nivale* var. *majus* has higher pathogenicity to detached leaves in wheat (Diamond & Cooke, 1997a, 1999; Browne & Cooke, 2004b) this may not be the case in barley and oats where incubation periods were longer for *M. nivale* var. *nivale* but where latent periods occurred at a similar time post-inoculation. The longer incubation periods for *M. nivale* var. *nivale* in barley and oats may be a strategy whereby the fungus is not exposed to resistances expressed after initial infection as rapidly as var. *majus* allowing the pathogen to colonise the leaf surface using extracellular enzymes. The longer latent periods of barley and oats than wheat in the current study may therefore explain the greater frequency of isolation (host preference) of *M. nivale* var. *nivale* than var. *majus* in barley and oats (Diamond & Cooke 1997a). Further investigations into the infection of both *M. nivale* var. *majus* and var. *nivale* in wheat, barley and oats are desirable to further understand possible implications of the host preference of both fungal varieties particularly at the early stages of infection during the incubation period. This work provides a basis on which investigations into the relationship between PDR components detected in the detached leaf assay and whole plant resistance in barley and oats can begin.

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European *Fusarium* Ringtest- a valuable vehicle for sharing germplasm and screening methods to develop resistance to *Fusarium* head blight across Europe

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Key Words: European Fusarium Ringtest (EFR), Fusarium head blight (FHB, scab), wheat, resistance, ring test

Introduction

In the past decades, Fusarium head blight (FHB, scab**),** caused by *Gibberella zeae* (Schwein.) Petch. (anamorph *Fusarium graminearum*) has become a major constrain of winter wheat production and quality in many regions of the world. Wet weather during flowering and grain filling as well as introduction of new cropping systems (maize-wheat rotation and minimum tillage) has favored disease development.
Due to possible grain contamination with several mycotoxins (Arseniuk et al., 1999, Leonard and Bushnell, 2003), among which the trichotecene deoxynivalenol (DON) is the most prevalent (Placinta et al., 1999), the impact of this disease on food safety could be detrimental. Storage of cereals under warm and humid conditions may further increase the mycotoxin content, even when field infections were only moderate (Homdork et al., 2000).

Crop management measures are not always effective for disease control. In addition, chemical treatments are less recommended or totally avoided (organic cropping system). Hence, host resistance remains the main cost efficient and environmentally sound strategy to combat FHB (Miedaner, 1997). However, progress in developing FHB resistant wheat cultivars has been hindered by the complexity of quantitative resistance (more components involved), a lack of effective sources of resistance, and the high importance of genotype x environment interaction.

In order to align disease quantification across environments, a multi-environment approach and assessment of resistance with artificial inoculations are pre-requisites to accelerate the development of resistance to FHB in wheat. This emphasises the need for a large cooperation focused on the search of new sources of resistance, better adapted to the local environment and current agronomic management systems.

Consequently, Romania and the Czech Republic initiated several years ago a mutual cooperation for a reciprocal evaluation of responses to FHB in their wheat breeding germplasm. Germany, France, and Switzerland have joined to this ring test entitled European *Fusarium* Ringtest (EFR).

Goals

The main goals of the EFR are to develop a reliable background for sharing germplasm and effective methods of screening for resistance to FHB in bread winter wheat across Europe and to accelerate the selection of promising entries that could minimise the impact of FHB.

In this respect a multi-location assessment of FHB resistance and DON content in wheat following artificial field inoculation is performed in each cycle.

Breeders or scientists involved in *Fusarium* research confirm the level of resistance to FHB in their wheat entries in various environments, including diverse conditions in terms of climate, soil, *Fusarium* species, and agronomic practices.
In the season of 2006/2007, the EFR partnership included cooperators from nine countries/institutes:
Hermann Buerstmayr - Austria (Univ. Natural Resources and Applied Life Sciences Vienna, Department IFA-Tulln, Hermann.Buerstmayr@boku.ac.at);
Vaklav Sip & Janna Chrpova - Czech Republic (Research Institute of Crop Production, Prague, Ruzyne, sip@vurv.cz/chrpova@vurv.cz); Marie-Noël Mistou - France (GEVES La Minière 78285 Guyancourt Cedex, marie-noel.mistou@geves.fr), Lorenz Hartl - Germany (Bavarian State Research

Center for Agriculture, Freising, lorenz.hartl@LfL.bayern.de), Mariana Ittu - Romania (National Agricultural Research-Development Institute-Fundulea, ittum@ricic.ro/gittu@pcnet.ro);
Fabio Mascher-Frutschi - Switzerland (Research Station Agroscope Changins-Wädenswil ACW, Nyon, fabio.mascher@acw.admin.ch); Akos Mesterhazy - Hungary (Cereal Research non-Profit Company, Szeged, Hungary, akos.mestehazy@gk-szeged.hu); Julie Nicol - Turkey (CIMMYT, Ankara, j.nicol@cgiar.org) and Olga Babayants - Ukraine (Plant Breeding and Genetics Institute, Odessa, fungi@ukr.net).

Most EFR participants are also members of the COST Action 860 SUSVAR. This facilitates direct contacts and exchange of information. Furthermore, the EFR participants contribute to the activity of *Fusarium* SUSVAR subgroup.

Materials and methods

In each cycle, the EFR entry list is composed of contributions from each participant with five local entries sent in time each to another. Terms of germplasm exchange and handling are regulated by a Material transfer agreement (MTA)

For artificial inoculation in the field and screening of resistance to FHB are not imposed but rather according to the particular case of each participant. Spore suspensions are sprayed on the heads at anthesis, followed by overhead irrigation or point (head) inoculation. Criteria of scoring include pre-harvest (severity, disease index etc) and post-harvest (relative weight of grain, *Fusarium* diseased kernels etc) components of resistance to FHB and DON analyses, if available.

Committments

share results on responses to FHB, DON content, and other results with the collaborating partners from the team, as is stipulated in the Memorandum of Understanding (MoU), upgraded when necessary and signed by all participants.

Further development

Based on the potential benefits of such a cooperation across Europe, a continuous development seems necessary. Contacts with other FHB nurseries from the USA, Canada, and the Fusarium Global Initiative, initiated by CIMMYT (2006), are planned.

A real current constrain is the lack of an EFR web page. Thanks to the kind invitation of CIMMYT, information on EFR will probably be available in the future on their website (http://www.fusarium-net.org).

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Fusarium infection of heads and stems under different cultivation practices

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Key Words: direct drilling, plant protection, root diseases, oat, barley

Introduction

Changes in cropping techniques result in new challenges in plant protection. Previous crop residues and tillage practices in cereal production might affect the occurrence of *Fusarium* head blight as well as *Fusarium* stem diseases. The aim of our project 'Plant protection in direct drilling - need and solutions' is to study the role of plant diseases, pests, and weeds under direct drilling cultivation which is becoming more widespread in Finland (8 % of the total area of cereal and oilseed crops in 2006). The purpose of this research was to study the correlation of *Fusarium* incidence in heads and on stems in ploughed and no-till sowing practices on four spring barley and on four oat cultivars.

Methodology

Research was conducted in Jokioinen in Finland in a field trial established in 2003. The results presented in the poster are from the year 2005. The barley cultivars studied were two-rowed malting barleys Annabell, Barke, Saana, and Scarlett. The studied oat varieties were Belinda, Freja, Roope, and Veli. All the cultivars were sown both with and without tillage.

For the *Fusarium* root rot assessment, a sample of 60 plants was collected from each treatment at the milk ripening stage (BBCH 75). Sub-samples of 10 plants were taken from each plot. Stems and roots of the plants were rinsed with water and the symptoms were assessed. The plants were divided in five groups according to the severity of the symptoms and a disease index was calculated from the number of the plants in different groups. The results are presented also as percentage of healthy plants and plants with different severity of symptoms. It was assumed that most of the symptoms were caused by *Fusarium* spp. Later, the stems were incubated on potato dextrose agar and the *Fusarium* cultures were identified.

DISEASE INDEX = ((B+2*C+3*D+4*E)*100) / (4*(A+B+C+D+E))
Group A = no symptoms
Group B = small spot on coleoptiles
Group C = more attack on coleoptiles and some on roots, healthy plants
Group D = severe attack on coleoptiles and roots, plants depressed
Group E = dead plants

Fusarium species were analysed from the cleaned harvested yield. Fifty seeds per plot were incubated on agar medium containing pentachloronitrobenzene (PCNB) at room temperature (22 ºC) and the growing hyphae were isolated on potato dextrose (PDA) medium for identification. The *Fusarium* cultures were identified microscopically.

Preliminary results and conclusions

The beginning of the growing season 2005 was rather dry and cool. In July it was very warm and there were heavy rains in the end of July and in August. The weather in 2005 favoured the leaf spot diseases as well as *Fusarium* head blight on cereals.

Results from the year 2005 indicate that barley cultivar explains more the disease index on stems than the tillage method. In oats, the disease index on stems was higher with all four varieties when the plant was grown in a no-tillage environment (Figure 1, 2).

The incidence of *Fusarium culmorum* was lower in no-tillage environment compared to the field with ploughing, both on stems and on seed. On the contrary, the incidence of *Fusarium avenaceum* was higher in the low-tillage system, both on stems and on seed (Figure 3, 4).

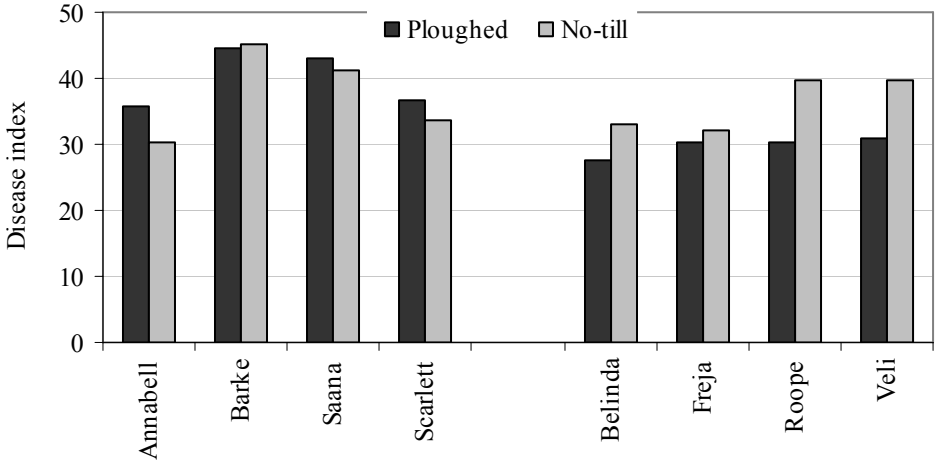

Figure 1. Stem disease index on four barley and four oat varieties in ploughed and no-till environments at early milk ripening stage.

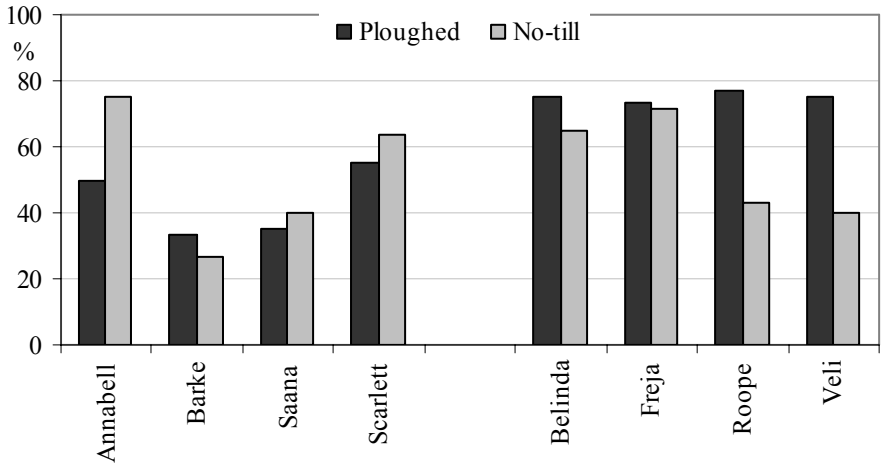

Figure 2. Percentage of healthy stems (groups A+B) on four barley and on four oat varieties in ploughed and no-till environments at early milk ripening stage.

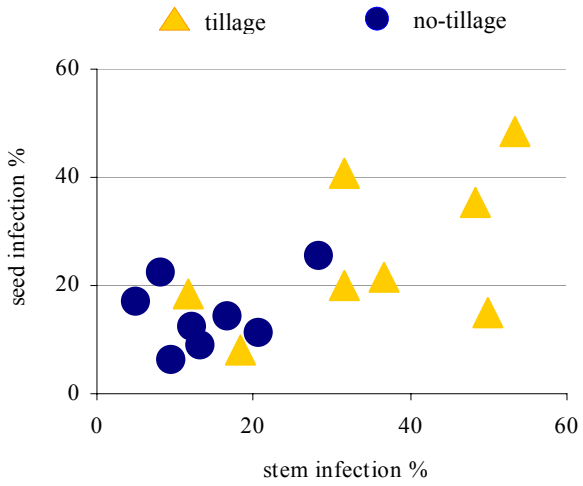

Figure 3. Incidence of *Fusarium culmorum* on seed and stems under tillage and no-tillage practices.

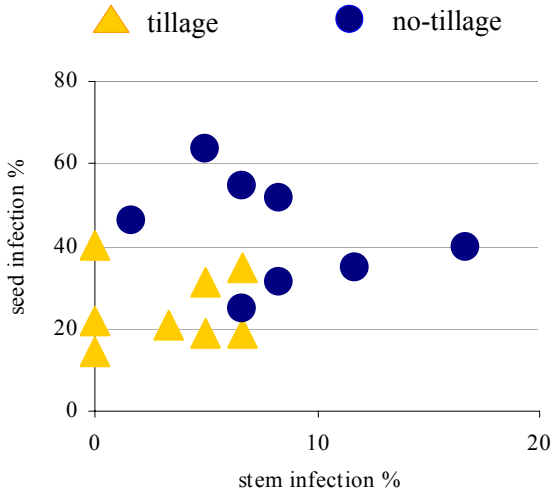

Figure 4. Incidence of *Fusarium avenaceum* on seed and stems under tillage and no-tillage practices.

The research continues in 2007. The preliminary results indicate a clear correlation between the stem and seed infection as well as the effect of tillage method on the occurrence of the two common *Fusarium* species in Finland; *Fusarium culmorum* and *Fusarium avenaceum*.

Due to important effect of *Fusarium* incidence in plant debris on *Fusarium* head blight occurrence, our results indicate the need to further study the effect of different crop rotation systems on the incidence of *Fusarium* infection in cereals.

Fusarium head blight resistance of old Hungarian wheat genotypes

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Key Words: Fusarium head blight, resistance, wheat

Abstract

Since the *Fusarium* head blight (FHB) occurred only sporadically in Hungary until the early '70s, it thus seemed worthwhile investigating whether the wheat varieties bred from the twenties to the fifties carried genetically determined FHB resistance that contributed to the lack of serious economic loss. Earlier observations have already indicated that the old Hungarian wheat variety Bánkúti 1201 had outstanding resistance to FHB in experiments artificially inoculated with *Fusarium* species. In field experiments, 98 old Hungarian wheat populations and lines were investigated under artificially inoculated conditions. Above-average FHB resistance were observed for 17 lines developed from Bánkúti 1201, Bánkúti 5, Fertődi 293, Székács 1055, and Béta Bánkúti. The head blight severity of these lines did not differ significantly from that of the resistant control variety. Although none of the lines selected from the old Hungarian wheat varieties was completely resistant in all three years, but a level of 10–20% was an excellent result, given the great pathogen pressure created in the artificially inoculated nursery.

Introduction

The genetic resources preserved in gene banks may form valuable basic material for resistance breeding (Tyriskin et al. 2006). Wild relatives of wheat, landraces, and wheat varieties bred several decades ago often contain previously unidentified resistance genes, or chromosome regions influencing disease resistance. It was observed by Börner et al. (2006) that the probability of identifying effective resistance declines as the ploidy level increases, though even in hexaploid varieties and lines there is a 10% chance of success.

Investigations on *Fusarium* head blight (FHB) in wheat are gaining importance throughout the world. This can be attributed to the fact that Fusarium species not only cause yield losses, but also produce mycotoxins in infected plant tissues, the accumulation of which makes the grain unsuitable for both human and animal consumption (Hornok et al. 2005).

Efficient protection against Fusarium species could be achieved by growing FHB-resistant wheat varieties. Only a limited number of FHB-resistant varieties are currently available to breeders, so intensive work is in progress worldwide to find new resistance sources. At present spring genotypes of Far-Eastern origin, especially Sumai 3 and its derivatives (e.g. CM82036), are considered to have the best resistance (Bai-Shaner 2004), but the agronomic traits of these genotypes differ greatly from those of the winter wheat varieties cultivated in Hungary. The same is true of spring varieties from Brazil (e.g. Frontana). Many winter wheat varieties bred in Europe and claimed in the literature to be resistant, proved in later experiments to be only moderately resistant (e.g. Arina; Ruckenbauer et al. 2001) or to have Type II resistance (e.g. F201R; Shen et al., 2003). Mesterházy et al. (2004) suggested that genotypes not derived from the known resistance sources should be screened as a possible way of broadening genetic variability. According to Liu and Wang (1991), instead of using Chinese varieties, it would be expedient to use varieties with moderate resistance, but excellent agronomic properties, since genotypes with very good FHB resistance could well be found among the progeny as the result of transgressive segregation.

In Hungary, FHB occurred only sporadically until the early '70s, when intensive production technologies were introduced, together with the respective wheat cultivars (Kükedi 1988). The gene bank maintained in Martonvásár contains populations of several old Hungarian wheat varieties. Analyses on the technological quality of lines developed from these varieties in previous years have proved that these old varieties had a level of genetic heterogeneity similar to that of landraces (Vida *et*

al. 1998, Takács *et al*. 2005). Earlier observations indicated that the variety Bánkúti 1201 had outstanding resistance to FHB in experiments artificially inoculated with *Fusarium* species (Szunics and Szunics 1992).

Methodology

Field experiments artificially inoculated with *Fusarium culmorum* were conducted in three years (2003, 2004, 2006) on 98 populations and lines of old Hungarian varieties, together with two control varieties (Sumai 3, resistant, and GK Zugoly, susceptible). Conidium suspensions were used to spray-inoculate plants at 50% flowering, and the inoculations were repeated two days later. The spore concentration applied was 5×10^4 macroconidia·ml^{-1}. Mist irrigation was applied to provide favourable conditions for infection. As a measure of FHB severity the ratio of *Fusarium*-infected spikelets was determined by visually scoring the inoculated plot on the 26th day after the first inoculation.

The moderately susceptible wheat variety Mv Magvas was crossed with a line of Bánkúti 1201 origin (B9086-95), which had proved resistant in FHB tests. The 219 SSD lines developed from this combination were then tested for Type II resistance (spread of Fusarium within the spike). The *F. culmorum* strain 'IFA-104' was used for the inoculation. The conidia were rinsed off the surface of infected grains and the spore concentration was adjusted to 106·ml-1. A 5 µl quantity of conidium suspension was inoculated into a spikelet located a third of the way down the spike on five plants of each line. The degree of *Fusarium* infection in the spikes (% severity) was scored on the 21st day after inoculation. In addition to the lines, Type II resistance was also monitored for the two parents (B9086-95 and Mv Magvas) and for two control varieties with known levels of FHB resistance (Sumai 3 and GK Zugoly). Statistical analysis was carried out using the "Two-factor ANOVA without replications" program of the Data Analysis Module of Microsoft Excel 2000.

Results and discussion

The results of analysis of variance demonstrated that the mean field spike infection of old Hungarian wheat varieties and lines was significantly influenced by the year. The most severe infection was recorded in 2004 (43.0%), followed by 2003 (36.1%) and 2006 (31.5%, LSD5%=3.5%). Significant differences were also observed between the lines. The FHB infection of the wheat lines and varieties fluctuated over a wide range (7.0–76.7%) averaged over the three years (Fig. 1).

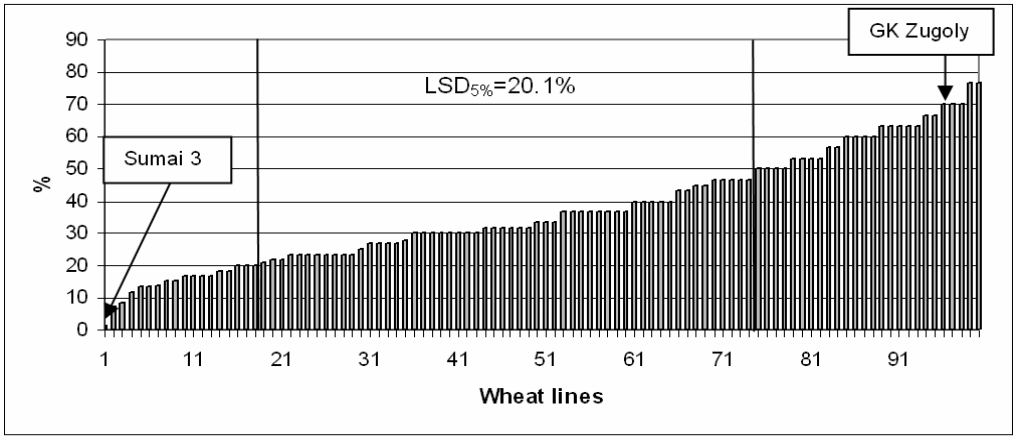

Figure 1. *Fusarium* head blight infection severity of old Hungarian wheat varieties and lines, and of control varieties (Martonvásár, average of three years)

Values of 20% or less were observed for 17 lines, nine of which originated from Bánkúti 1201, three from Bánkúti 5, two each from Fertődi 293 and Székács 1055, and one from Béta Bánkúti. The spike infection severity of these lines did not differ significantly from that of the resistant control variety. The data recorded for a further 25 lines did not differ significantly from the susceptible control, GK Zugoly (70% infection), while the majority of the wheat lines (56) exhibited intermediate values, and could thus be classified as moderately resistant or moderately susceptible. None of the lines selected from the old Hungarian wheat varieties was completely resistant in all three years, but a

level of 10–20% was an excellent result, given the great pathogen pressure created in the artificially inoculated nursery.

When the Type II resistance of the lines originating from the B9086-95×Mv Magvas combination was evaluated, the level of spike cover was 36.7% in 2005 and 31.7% in 2006, averaged over the lines. Averaged over these two years, the infection levels of the lines, parents and control varieties ranged from 5.0 to 72.3%. Based on the mean data for 2005 and 2006 the B9086-95 parent had the lowest rate of infection (5.0%), followed by the resistance source Sumai 3 (6.37%). It should be noted that this difference could be attributed to the number of spikelets per spike, as the spikes of Sumai 3 contained 2–3 fewer spikelets than those of B9086-95 on average. The infection severity of 36 lines did not differ significantly from that of the better parent (LSD5%=16.8). The difference in the rate of infection of Mv Magvas and that of the resistant parent was more than double the significant difference (44.8%). Spike cover significantly greater than that of the susceptible parent was observed for six lines. The distribution of the lines according to categories of FHB infection exhibited a normal distribution pattern.

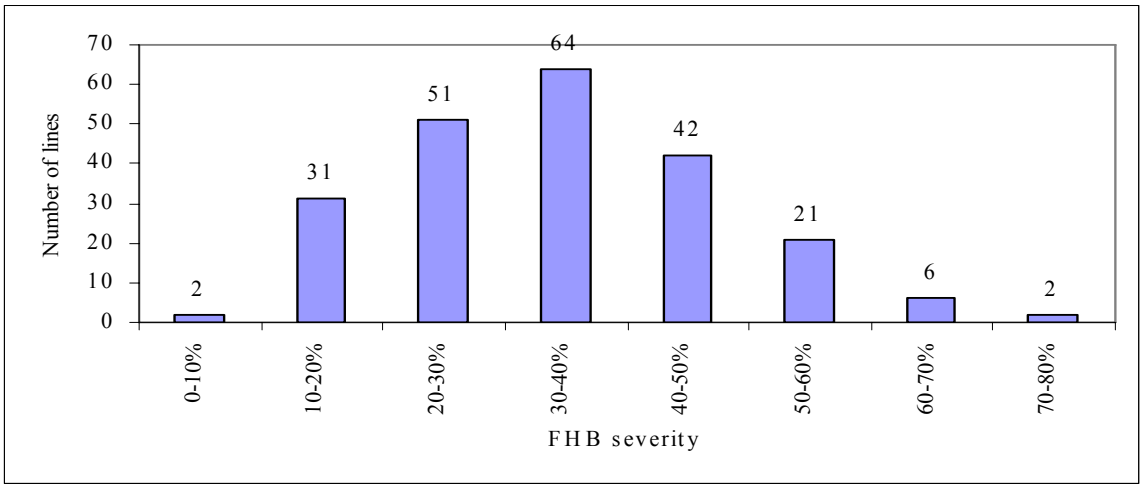

Figure 2. Distribution of FHB infection in B9086-95×Mv Magvas lines (Martonvásár, 2005–2006)

Investigations on the Type II resistance of the lines is continuing in the field and under greenhouse conditions. Molecular analysis will shortly be commenced, aimed at identifying QTL regions responsible for FHB resistance in Bánkúti 1201.

Conclusions

Some of the lines developed from old Hungarian wheat varieties bred prior to 1960 have above-average FHB resistance. As many other characteristics of these varieties (winter habit, winter hardiness, excellent bread making quality) are more favourable under Hungarian conditions than those of the Far Eastern genotypes used worldwide, their use as resistance sources would definitely be beneficial in wheat breeding. The results achieved so far indicate that the phenotypic and genotypic analysis of the lines should be continued in order to obtain a detailed knowledge of the genetic background of FHB resistance. The use of new resistance sources with diverse genetic backgrounds could help to avoid genetic vulnerability. The cultivation of FHB-resistant varieties would lead to a reduction in pesticide application, contributing through lower costs and environment pollution to an improvement in the sustainability of wheat production.

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Fungal diversity of winter wheat ears and seeds in Slovakia

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Key Words: winter wheat, Triticum aestivum, Fusarium, seed-borne fungi, ears mycoflora

Abstract

The fungal microflora of winter wheat (*Triticum aestivum* L.) was determined for 25 samples obtained from Slovakia. The mycoflora of ears and seeds was assessed by using two methods of fungal examination. In total 28 genera of micromycetes were found. The prevalent seed borne fungal genera on winter wheat seeds were *Alternaria*, *Epicoccum*, *Papularia*, *Nigrospora* and *Penicillium*. Seven species from the genus *Fusarium* were observed, namely *Fusarium graminearum*, *F. avenaceum*, *F. poae*, *F. culmorum*, *F. acuminatum, F. merismoides* and *F. oxysporum* . The majority of these species were fungi sporulating on the glume of ears. The saprophytic fungi *Alternaria* sp., *Cladosporium* sp. and parasitic fungi from the genus *Fusarium* and *Septoria* were the most dominant.

Introduction

Cereal seeds, including wheat, are vulnerable to attack by different organisms upon harvest and during storage. About 72% of all organisms which attack wheat seeds belong to microscopic fungi (Richardson, 1996). Many authors (e.g. Tančinová *et* al., 2001; Dawood, 1982) surveyed different species of fungi from fresh harvested wheat seeds. These species belong to the genera *Alternaria*, *Cladosporium*, *Helminthosporium*, *Fusarium*, *Septoria*, *Penicillium*, *Pythium*, *Rhizopus* and *Mucor* (Dawood, 1982). The genus *Fusarium* is comprised of a large, complex group of fungi and contains numerous species that produce noxious secondary metabolites and/or cause serious plant diseases. Fusarium head blight is one of the most devastating and insidious diseases of winter wheat. It is caused by a number of different *Fusarium* species (e.g. *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. sporotrichioides* and *F. poae*) (Parry et al., 1995). In Slovakia, *Fusarium* species have been studied on the wheat ears during 1993-96 by Šrobárová (2001). About 11 *Fusarium* species (mainly *F. verticillioides*, *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. sporotrichioides* and *F. poae*) were identified from wheat ears. There are not many publications about the biology and natural occurrence of seed-borne fungi of winter wheat in Slovakia. Seed-borne mycoflora may cause serious diseases for either seed or the developing crop plant. The aim of this preliminary study were to determine the mycoflora of ears and seeds of winter wheat collected from different parts of Slovakia.

Material and methods

Winter wheat samples (ears and seeds) from investigated sites were collected from different parts of Slovakia of two different farming systems (conventional and ecological farming). The wheat samples were investigated by using two different methods. First method: ears and seeds were surface sterilized by immersion in 5% commercial bleach solution of sodium hypochlorite for 1 minute and rinsed with sterile distilled water. The ears and seeds were blotted dry and plated on 2% (w/v) potato dextrose agar (PDA) in 90 mm Petri dishes. Petri plates were incubated at 22°C and examined after ten days. Mycelial outgrowths from the segments were subcultured for identification. *Fusarium* isolates were identified to species by the criteria of Nelson et al. (1983) and Gerlach and Nirenberg (1982). Growing fungi were stained with lactophenol cotton blue and identified microscopically with reference to standard texts Domsch et al. (1980), Malone and Muskett (1997), Barnet and Hunter (1998), Kiffer and Morelet (2000), Hanlin (1990) and Champion (1997). Second method: wheat ears were examined macroscopically (binocular microscope (60x) and microscopically (JENAMED2, Carl Zeiss Jena) for presence of fungal reproduction structures. The glumes were analysed by mounting them in water and staining with lactophenol-cotton blue. Species identification was done according to fructification structures and measurements of conidia or spores.

Table 1 Composition and frequency of selected genera of microscopic fungi isolated from ears and seeds of winter wheat (1 - ears from ecological farming systems; 2 - ears from conventional farming systems; 3 - seeds from ecological farming systems; 4 - seeds from conventional farming systems)

Isolated species of microscopic fungi	1		2		3		4		Total	
	NCI	%	NCI	%	NCI	%	NCI	%	NCI	%
Acremonium sp.	1	0.2			5	0.8	4	0.4	10	0.39
Alternaria sp.	163	38.6	194	38.7	249	38.2	302	30.1	908	35.23
Aspergillus sp.	4	0.9			4	0.6	30	3.0	38	1.47
Botrytis cinerea					4	0.6	6	0.6	10	0.39
Cladosporium sp.	14	3.3	4	0.8	16	2.5	31	3.1	65	2.52
Curvularia sp.	2	0.5							2	0.08
Circinella sp.							2	0.2	2	0.08
Epicoccum sp.	9	2.1	40	8.0	37	5.7	74	7.4	160	6.21
Eupenicillium sp.	1	0.2	3	0.6			14	1.4	18	0.70
Fusarium sp.	13	3.1	18	3.6	83	12.7	54	5.4	168	6.52
Graphium sp.					4	0.6			4	0.16
Helminthosporium sp.	10	2.4	2	0.4	10	1.5	17	1.7	39	1.51
Chaetomium sp.	4	0.9	7	1.4	5	0.8	15	1.5	31	1.20
Nigrospora sp.	19	4.5	2	0.4	49	7.5	36	3.6	106	4.11
Papularia sp.	24	5.7	11	2.2	33	5.1	59	5.9	127	4.93
Penicillium sp.	33	7.8	23	4.6	25	3.8	142	14.2	223	8.65
Pyrenophora sp.			2	0.4	8	1.2	17	1.7	27	1.05
Pleospora sp.			3	0.6					3	0.12
Phoma sp.			1	0.2	18	2.8			19	0.74
Rhizoctonia sp.							1	0.1	1	0.04
Rhizopus sp.	24	5.7	34	6.8	12	1.8	61	6.1	131	5.08
Scopulariopsis sp.							2	0.2	2	0.08
Septonema sp.					7	1.1			7	0.27
Septoria sp.	7	1.7	41	8.2	12	1.8	10	1.0	70	2.72
Sordaria sp.	49	11.6	5	1.0			9	0.9	63	2.44
Stemphylium sp.	2	0.5			3	0.5	3	0.3	8	0.31
Trichoderma sp.					2	0.3	6	0.6	8	0.31
Ulocladium sp.					2	0.3	15	1.5	17	0.66
sterile mycelium	26	6.2	111	22.2	60	9.2	87	8.7	284	11.02
bacteria	17	4.0			4	0.6	5	0.5	26	1.01

NCI – number of cases of isolation; % - percentage of occurrence.

Results and discussion

The ears of winter wheat were attacked by parasitic fungi during the end of the growing season. On winter wheat ears in both farming systems, 7 genera of fungi were identified, mainly *Fusarium*, *Alternaria*, *Cladosporium*, *Epicoccum*, *Blumeria*, *Septoria*, *Phoma*. *Mycosphaerella graminicola* and *Ascochyta tritici* were found on the glume of ears at low frequency. The species from genera *Alternaria* and *Cladosporium* belong to common saprophytic mycoflora. The species from the genera *Fusarium* and *Septoria* belong to parasitic mycoflora. All three species from the genus *Septoria* were found on the glumes but only *S. nodorum* and *S. avenae* were occurring with high frequency. The species *Epicoccum purpurascens* was sporulating abundantly and identified in the middle or at the margin of the glumes in all collected samples with different percentages of occurrence. The species *Ascochyta tritici* was found on winter wheat ears in all examined samples at low frequency. During 2004-2005, phytopathogenic fungi such as *Gibberella zeae, Leptosphaeria nodorum*, *L. avenae*, *Pyrenophora tritici-repentis* and *Pleospora herbarum* on ears of winter wheat were recorded.

Fungi are the most important spoiling organisms in cereal grains. The mycoflora of winter wheat ears and seeds in both farming systems consisted primarily of Deuteromycetes and some Ascomycetes. As expected, yeasts and Zygomycetes were rarely found. During the study period, 28

genera of fungi were isolated and identified; 20 genera were recorded on winter wheat ears and 26 genera on seeds (Table 1). The most frequently isolated fungi from ears and seeds of wheat were *Alternaria* (35.2%), *Penicillium* (8.7%), *Fusarium* (6.5%), *Epicoccum* (6.2%), *Rhizopus* (5.1%), *Papularia* (4.9%), *Nigrospora* (4.1%), *Septoria* (2.7%), *Cladosporium* (2.5%), and *Sordaria* (2.4%). Most of these fungi were also determined as fungi sporulating on the glume of ears. Mainly *Fusarium* spp. produced survival structures as sporodochia on the glume or on other parts of the ears. Pycnidia from the genera *Septoria*, *Phoma*, and *Ascochyta* were also found on the glumes from some localities with high frequency. The fungal saprophytes *Alternaria*, *Cladosporium cladosporoides* and parasitic fungi from the genera *Fusarium* and *Septoria* were the most dominant in both farming systems. The species *Epicoccum purpurascens* was recorded on ears and seeds of winter wheat in all collected samples with different percentage of occurrence. Bruton *et* al. (1993) reported *Epicoccum purpurascens* as the causal agent of red rot of cantaloupe. The authors described symptoms of red rot as red discoloration. The same symptoms were occurring on glume of wheat with red discoloration and sporulation of fungi. We didn´t observe clear differences between the prevalence of seedborne fungi on winter wheat seeds in both farming systems. The prevalence seedborne fungi on winter wheat seeds in all collected samples were *Alternaria* spp. (33.3%), *Fusarium* spp. (8.3%) and *Epicoccum purpurascens* (6.7%). The fungi *Papularia* sp., *Nigrospora* sp. and *Penicillium* sp. were also isolated with more than 3.5% of relative frequency.

Table 2 Composition and number of isolates of selected *Fusarium* species from 25 winter wheat seed samples ollected from fields in Slovakia (1-17 samples from conventional farming systems; 18-25 samples from ecological farming systems)

	Samples																								
Fusarium	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
graminearum	2[a]	19	4	7			7		4		1	1	34		11		1				5	3	2	10	9
avenaceum		1		2	1	1	15	4	1	26	4		7	11	7						1	2	1		
poae	6					10			3	1		2	6								1	2	1	9	1
culmorum					1						5		1			2						1	3		1
sporotrichioides						1																			
oxysporum		1									1						1				2			1	
sambucinum						1																			
merismoides				10																					
acuminatum					4						1		6												
equisetum											4			2											1
moniliforme										4															
langsethiae													1	1											
tricinctum						1				2															

[a] – number of isolates per sample (1 sample =100 seeds)

Fusarium species were detected in 22 seed samples (Table 2). In the Slovak winter wheat samples, 13 different species of *Fusarium* were recovered. Commonly up to seven different species of *Fusarium* were isolated in a given sample including *F. graminearum*, *F. avenaceum, F. poae*, *F. culmorum*, *F. acuminatum*, *F. merismoides* and *F. oxysporum*. *F. graminearum* was the dominant species in all examined samples.

The isolated genera *Aspergillus*, *Penicillium* and *Fusarium* are considered as the most important producers of mycotoxins (Betina, 1994; Placinta *et* al., 1999).

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Differences between spring wheat cultivars for emergence and early development after seed infection with *Fusarium culmorum*

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Key Words: Spring wheat, Organic Farming, Fusarium culmorum, seed

Introduction

Infection of spring wheat seeds with *Fusarium* (*Fusarium* spp., *Microdochium nivale*) is of greater importance in organic agriculture than in conventional agriculture, because seeds cannot be chemically treated. Use of infected seeds results in lower plant densities (Gilbert *et al.* 1997; Bechtel *et al.* 1985). However, when weather conditions after sowing are favourable for a rapid crop establishment, plant emergence is less affected by seed infection. The current project focuses on detection of differences between cultivars in susceptibility to seedling loss caused by Fusarium on the seed. Also the relationship between cultivar differences in susceptibility and in rapid early development are studied.

Methodology

In 2006 and 2007 seeds of six spring wheat cultivars (Melon, Lavett, SW Kungsjet, Epos, Pasteur, Thasos) containing three infection levels of *Fusarium culmorum* (about 0%, 12% and 25%) were sown in trial in an organic experimental field (Colijnsplaat, The Netherlands) and in a pot experiment.

All seeds were harvested in 2005 from a field experiment on varietal resistance against *Fusarium* head blight with artificial inoculation with *F. culmorum* (Scholten et al., 2007) As the seeds of control (not inoculated) plots showed an average infection level of 12%, for the 0% level these seeds were treated with warm water (Osman et al., 2004). The 25% treatment was obtained by mixing seeds of control and inoculated plots.

Percentage of seedling emergence was measured for cultivars in both the pot and field experiment. For each cultivar, rate of early development was assessed by measuring plant heights, leaf widths, ground cover, and above ground dry matter at three successive times. Data were used to calculate relative growth rates. In pots also root development was assessed.

Preliminary Results

The research is still ongoing. First results show:

- A significant effect of seed infection levels on plant density and a delay in crop canopy closure.
- Differences between varieties for speed of early above and below ground development.
- The preliminary data also indicate that varieties with a more rapid early growth show less seedling loss, despite the infection of seeds with *Fusarium*. The variety Thasos behaved differently, though despite its early rapid development plant loss due to *Fusarium* was also among the highest.

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VAT - a new software for consistent analysis of plant pathogen populations and their hosts

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The analysis of plant pathogen populations is commonly based on experimental data which are organized in large tables with two entries. The *Virulence Analysis Tools* (VAT) is a user friendly software for processing such kind of data. The VAT aims mainly at supporting comprehensive, effective and logically consistent (Kosman and Leonard, 2007) evaluation and presentation of virulence data of pathogen populations and resistance data of host populations. The package can also be applied to molecular marker data (Kosman and Leonard, 2005).
The VAT software includes the following blocks:

(1) Tools supporting the basic routine steps such as data entry and transformation, dichotomization, identification of phenotypes etc. A tool to convert phenotype names from one nomenclature to another (e.g. from binary/octal to binary/hexadecimal) are implemented to make results of different researchers compatible.
(2) Descriptive tools for characterization of isolate and host samples (e.g. by distribution of phenotypes, virulence/resistance frequencies and complexities, associations, diversities, distances etc.), displayed as histograms, frequency tables and indices.
(3) Inference-statistical procedures that estimates various diversity and distance indices and other parameters for sexually and asexually reproducing populations. These estimates are obtained by resampling methods allowing further statistical evaluation (e.g. significance tests and confidence intervals).
(4) Sample size recommendations for reliable estimation in specific experimental situations will be offered.

All package output is suitable for direct input into Excel and other commonly used software (SAS, NTSYS, SPSS etc.) facilitating additional analyses (clustering, dendrograms, PCA etc.).

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