PATHOGENESIS, EPIDEMIOLOGY AND DISEASE FORECASTING

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EFFECT OF INOCULUM LEVELS ON HEAD SCAB OF WHEAT UNDER FIELD CONDITIONS IN NORTH DAKOTA

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ABSTRACT

Epidemiology researchers based at PA, OH, IN, ND, and SD land grant universities are collaborating to provide growers and agricultural industry with timely and reliable disease forecasts for Fusarium head blight (FHB). Knowledge about sources of inoculum, inoculum levels, and weather conditions favorable for FHB development is crucial in devising a handy and reliable disease forecaster. The effect of inoculum levels was studied in ND in 2003 and 2004. Two FHB susceptible hard red spring wheat cultivars, Oxen (an early flowering cultivar) and Granite (a late flowering cultivar) were sown on May 1 and 10 in 2003, and Argent hard white spring wheat (early flowering cultivar) and Granite were sown on April 30 and May 7 in 2004, in a field plot located at the NDSU Agricultural Experiment Station, Fargo. The previous years' crops were dry bean in 2003 and crambe in 2004. Three inoculum levels (zero inoculum; low = 38g/m^2; high =100g/m^2) were applied, using *G. zeae* infested corn kernels, at the 6-leaf stage in all treatments. The experimental design was split plot, randomized complete block. Main plots were inoculum levels, sub-plots were planting date, and sub-subplots were cultivar. Strips, 30 ft wide, of Alsen wheat (moderately resistant to FHB) were planted between subplots and main plots, to serve as buffers. The strips of Alsen were free of inoculum. In both years, the *G. zeae* population from each inoculum treatment was monitored daily from Feekes growth stage 8 (early flag leaf emergence) to Feekes 11.2 (soft dough) for air sampling, and from Feekes stage 10 (boot stage) to Feekes 11.2 for head washings. One hundred-fifty wheat heads from each inoculum treatment were monitored 3x a week for growth synchrony. The disease incidence (number of infected head/total number of heads examined) and head severity (% of individual infected head) data were recorded in all treatments. FHB disease incidence was significantly different among the inoculum levels in both years. The disease incidence range was 5 to 9 % in 2003 and 19 to 40 % in 2004. The majority (>95%) of the plants began and ended flowering in 4-5 days on both planting dates and in all four flowering dates. In both years, high inoculum levels generally resulted in increased number of *G. zeae* colony units (CFU) recovered from both head washings and air sampling. The results indicate that, under favorable weather for FHB, inoculum levels of *G. zeae* may have a significant role in disease development. Also, the fungus has a small window of opportunity to infect wheat heads, as the majority of the plants completed flowering, a crucial stage for infection, in 4 to 5 days. It appears that incorporating information about local sources and levels of pathogen inoculum may increase disease forecasting model performance.

ACKNOWLEDGEMENT

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DEVELOPMENTS IN MODELLING *FUSARIUM VERTICILLIOIDES* IN MAIZE EARS

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OBJECTIVES

To develop a conceptual model for the dynamic simulation of the life cycle of *Fusarium verticillioides* in maize and the consequent production of fumonisin B_1 in kernels.

INTRODUCTION

F. verticillioides is the fungus most frequently isolated from maize; it is associated with disease at all stages of plant development, infecting roots, stalk and kernels, but it is also present in symptomless maize plants. In association with *F. proliferatum* and *F. subglutinans* this fungus causes pink fusariosis prevalent in the dry and warm climates of southern Europe (Logrieco *et al*., 2002). Yield losses caused by this disease are not relevant, but fumonisin production, mainly fumonisin B_1 (FB_1), in grains before harvesting, was documented under many environmental conditions and stimulated the interest in this disease (Shelby *et al*., 1994; Visconti *et al*., 1994; Bottalico *et al*., 1995; Doko *et al*., 1995; Chulze *et al*., 1996; Kedera *et al*., 1999; Orsi *et al*., 2000; Reid *et al*., 2000).

The *F. verticillioides*-maize pathosystem is complex, and the relative importance of its components is still under debate (Munkvold and Desjardins, 1997).

In a previous work, information from literature and from specific experiments was used to elaborate a conceptual model for simulating *F. verticillioides* infection in maize and FB_1 production in kernels (Battilani et al., 2003). The information on the life cycle of *F. verticillioides* in maize was organized in a relational diagram according to the principles of "systems analysis" (Leffelaar and Ferrari, 1989). The same approach had been previously followed when a model simulating *Fusarium* head blight on wheat, and the accumulation of deoxynivalenol and zearalenone, was successfully elaborated (Rossi et al., 2003a, 2003b).

To produce an operative model, some aspects of the disease cycle have to be further investigated.

A weak point of the model regards the dynamic of inoculum during the season. The effect of water activity (a_w) and time of incubation have been investigated, while the role of temperature (T) is not sufficiently known, as well as the effect of a_w fluctuations on maize residues during the season. Information on spore dispersal should also be increased; particularly, more detailed data on the relative importance of wind- and splash-dispersal under field conditions, and on environmental conditions favouring peaks of dispersed conidia, are necessary.

Regarding FB_1, it is known that its synthesis depends on T and on the chemical composition of substrate. Warfield and Gilchrist (1999) showed that the FB_1 production was strongly affected by the ripening stage of kernels: no measurable production occurred until 15 days after silking at 25°C, then FB_1 increased exponentially until the dent stage (35-40 days). These data are of great interest but they should be confirmed in different epidemiological conditions.

MATERIALS AND METHODS

The relational diagram - The relational diagram of the pathosystem is shown in Fig. 1, following the steps of disease cycle. State variables are defined as the status of the pathogen at a given moment, and a flow from one state variable to another is determined. Rate variables are defined as the rate of change of the state

variables in time as a function of some driving variables, which are constants or parameters influencing the rate variables. Wherever possible, rates are expressed as mathematical equations accounting for their relationship with influencing meteorological or host parameters.

SPO – Sporulation rate

In vitro experiments - role of temperature and incubation time - Petri dishes with a semolina based medium were inoculated with *F. verticillioides* and maintained at different temperatures, between 5 and 45°C, for 7 different incubation times, between 3 and 41 days. Spore concentration was measured by an hematocytometer at the end of each incubation time.

In field experiments - role of environmental conditions - Pieces of maize stalks, previously inoculated with *F. verticillioides* and incubated under optimal conditions for sporulation, were placed in an experimental field in wire boxes between maize rows at flowering, in the years 2003 and 2004. Stalks were sampled at 3-day intervals for about one month and spores per weight unit of stalk were counted.

DIS – Dispersal rate - To study dispersal and deposition of *F. verticillioides* inoculum, Petri dishes with a selective medium were exposed for 30 days at the ear level in the experimental fields previously cited, in the years 2003 and 2004. Dishes were exposed to natural spore deposition for 24 hours; afterwards, they were incubated at 30°C, 100% relative humidity, and the number of *F. verticillioides* colonies were identified and counted. Some dishes were changed every 3 hours to determine the diurnal pattern of spore deposition.

TOX – Toxin production rate - Primary ears were collected in 2002 and 2003 at different development stages, included between 2 and 52 days after pollination. They were milled and the semolina obtained was used to prepare media to be inoculated with *F. verticillioides*. Fungal growth and FB1 production were measured respectively after 7 and 14 days of incubation at 30°C.

RESULTS AND DISCUSSION

SPO – Sporulation rate

In vitro experiments - role of temperature and incubation time: *F. verticillioides* produced microconidia in all the conditions tested, with a peak after 12 days of incubation at 30°C (Fig. 2).

In field experiments - role of environmental conditions: The 2 years considered were significantly different in meteorological conditions. Mean temperature was 27°C and 24°C, while summation of rain was 3.1 and 60 mm, respectively in 2003 and 2004.

Nevertheless, abundant microconidia were produced on infected debris during the whole period of exposition under field conditions, in both the years considered,. Data suggest that conidia are abundantly produced on infected debris in a wide range of ecological conditions that take place in maize crops.

DIS – Dispersal rate - *F. verticillioides* inoculum was deposited on Petri dishes exposed at the ear height under different environmental conditions, with a marked diurnal periodicity showing the highest deposition during the night.

Results from both sporulation and dispersal experiments suggested that the inoculum for ear infection is always available within the maize canopy, being produced abundantly, dispersed and deposited in a wide range of conditions.

TOX – Toxin production rate - The growth stage of ears influenced FB_1 production, while no effect was noticed on fungal growth. The rate of FB_1 production increased on media prepared using kernels collected up to 30-40 days after pollination, as shown by Warfield and Gilchrist (1999), whereas it decreased on kernels collected at full ripening.

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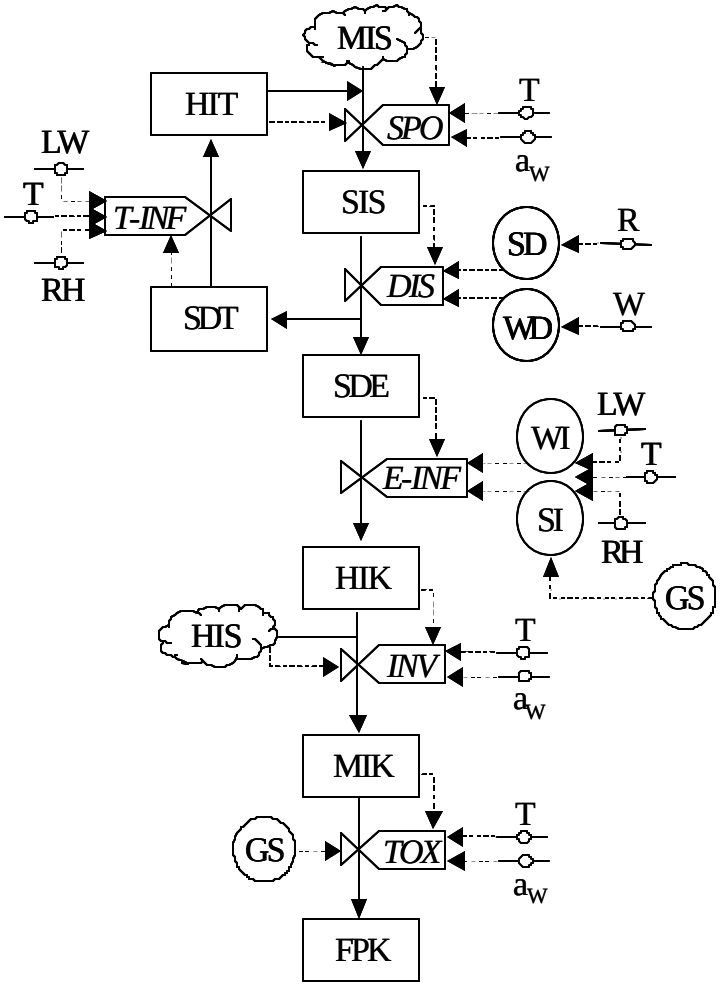

Figure 1. – Relational diagram of a dynamic model simulating the life cycle of *F. verticillioides* on maize.

Legend: MIS = Mycelium Invading Straw; HIT = Hyphae Invading Tassels; SIS = Spores on Inoculum Sources; SDT = Spores Deposited on Tassels; SDE = Spores Deposited on Ears; HIK = Hyphae Infecting Kernels; HIS = Hyphae Invading Stalks; MIK = Mycelium Invading Kernels; FPK = Fumonisin Production in Kernels; SPO = Sporulation rate; DIS = Dispersal rate; T-INF = Tassel Infection rate ; E-INF = Ear Infection rate; INV = Invasion rate; TOX = Toxin production rate; SD = Splash Dispersal; WD = Wind Dispersal; WI = Wound Infection; SI = Silk Infection; GS = Growth Stage of corn plant; T = air Temperature; RH =

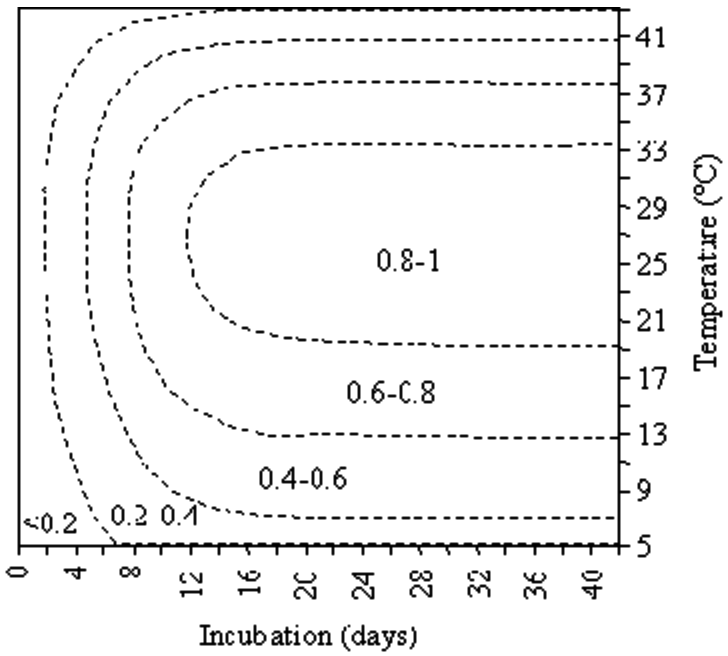

Figure 2. – Sporulation rate of *F. verticillioides* on a semolina based medium under different temperature regimes.

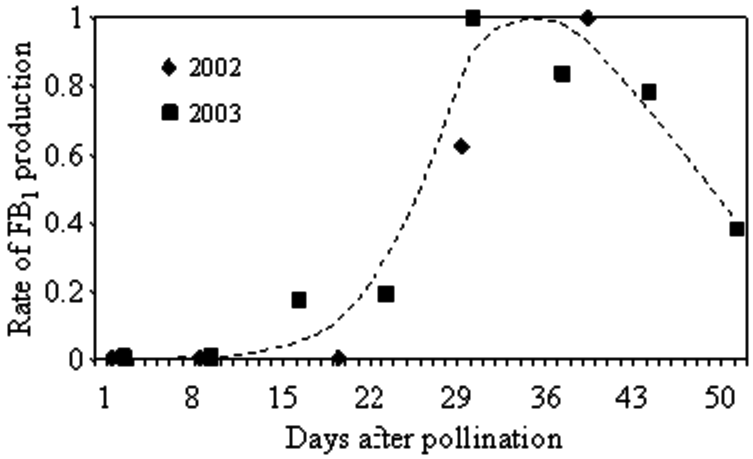

Figure 3. – Rate of fumonisin production on artificial media prepared with maize ears collected on different days after pollination.FC

ULTRASTRUCTURAL STUDIES ON INFECTION PROCESS OF FUSARIUM HEAD BLIGHT IN SUSCEPTIBLE AND RESISTANT WHEAT GENOTYPES

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ABSTRACT

The infection process of *Fusarium culmorum* and spread of fungal hyphae in the spike tissues were studied by scanning and transmission electron microscopy after single spikelet inoculation of the susceptible winter wheat cv. Agent. While hyphal growth on outer surfaces of the spike was scanty and no successful penetration was observed, the fungus developed a dense mycelium on the inner surfaces and effectively invaded the lemma, glume, palea and ovary by penetration pegs. During the inter- and intracellular spreading of the fungus, marked alterations in the host tissues were observed, including degeneration of cytoplasm and cell organelles, and depositions of electron dense material between cell wall and plasma lemma. Ultrastructural studies revealed that host cell walls in proximity of the penetration peg and in contact with hyphae were less dense, which suggested that cell wall degrading enzymes were involved in colonization of host tissues by fungal hyphae. Enzyme- and immunogold-labelling investigations confirmed involvement of extracellular enzymes, that is cellulases, xylanases and pectinases, in degradation of cell wall components. Infection process and spreading of *F. graminearum*, *F. avenaceum* and *Microdochium nivale* (*F. nivale*) in wheat spikes was similar to that of *F. culmorum*. Cytological studies showed that *Fusarium* spp. colonized spike tissue of resistant wheat cvs. Frontana and Arina more slowly than that of the susceptible cv. Agent. Plant structural defense reactions such as formation of thick layered appositions and large papillae were essentially more pronounced in the infected host tissues of the resistant cvs. than in those of the susceptible cvs. There were no differences in lignin contents of wheat spikes between susceptible and resistant cvs. of the uninoculated healthy tissue. While lignin content in cell walls of the infected tissues of the susceptible wheat cv. only slightly increased, lignin accumulated intensely in host cell walls of the infected wheat spikes of the resistant cvs. Immunocytochemical localization of [2]À-1,3-glucanase and chitinase demonstrated distinct accumulation of both enzymes in *F. culmorum*-infested wheat spikes of resistant wheat cvs., whereas in the susceptible cv. both enzymes were hardly increased. The subcellular localization of thionin and hydroxyproline-rich glycoproteins (HRGPs) was studied by means of immunogold-labelling technique. Compared with healthy tissues, labelling densities for the two types of proteins in cell walls of the infected spike tissues was only slightly enhanced in the susceptible cv., while in cell walls of infected tissues of the resistant cv. Arina labelling densities of thionins and HRGPs increased markedly. Localization studies of trichothecenes indicated that toxins could be detected in host tissues at an early stage of infection. However, labelling densities for DON in the resistant cv. were significantly lower than those in the susceptible cv. The studies indicated that FHB-resistant wheat cvs. are able to develop active defense reactions during infection and spreading of *Fusarium spp*. in the spike tissues. It is suggested that the lower accumulation of the toxin DON in infected resistant spike tissue may not essentially interfere in defense responses to the pathogen by the host tissue.

INVESTIGATION ON SPECIES OF *FUSARIUM* ON WHEAT AND RYE IN BAVARIA (GERMANY) IN 2003

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ABSTRACT

In the year 2003 a monitoring program in Bavaria has started. 190 samples of wheat and 80 samples of rye were examined for infections of *Fusarium* species and desoxynivalenol (DON) content. For identification of the species 200 surface disinfected kernels per sample were plated on different media. After 10 days of incubation at 22°C under black light the number of kernels infected with *Fusarium* spp. was determined. Identification of the species was carried out by microscopy.With an infection rate of 52 % *F. graminearum* is the most dominant DON producing fungus on wheat. In the case of rye *F. graminearum* and *F. culmorum* occurred on nearly the same level of 50 %. Percentage of kernels infected with *F. graminearum* ranged from 0 % - 18,5 % among the samples of wheat and 0 % - 1,5 % among the samples of rye. Other predominant species isolated from grain were *F. poae, F. avenaceum, F. equiseti, F. tricinctum* and *Microdochium nivale*. Studies in the 1990‘s support our results that *F. graminearum* is the most important DON producing species concerning of Fusarium head blight (FHB) on wheat in Bavaria. One reason for this observation is that crop rotations of wheat and maize which is also an excellent host for *F. graminearum* are widely practiced in Bavaria favouring the propagation of this fungus.

EVALUATION OF PREDICTION MODELS FOR WHEAT FUSARIUM HEAD BLIGHT IN THE U.S., 2004

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ABSTRACT

Disease prediction models for Fusarium head blight of wheat were revised based on weather, crop growth stage and disease observations from seven states located in both spring and winter wheat production areas. The final models used hourly temperature, humidity and rainfall to predict the risk of disease severity greater than 10%. The model deployed in 2004 also contained variables that allowed users to specify type of wheat (winter vs. spring) and whether winter wheat was planted into corn residue. Model accuracy was estimated to be near 80% based on data used to validate the model. The model was deployed for 23 states in 2004 as part of the National Fusarium Head Blight Prediction Center (www.wheatscab.psu.edu). Weather variables used drive the model predictions came from two sources. Input from the Rapid Update Cycle (RUC) environmental prediction model produced maps of risk level throughout the 23 states with a 20 km resolution. Weather stations maintained by the National Weather Service produced the second source of weather data, and provided users with station-specific predictions within the map. Model evaluation included comparison of weather variables provided by RUC with independent sources weather data. Preliminary results using weather for Fargo, ND indicate that mean absolute error for the RUC estimates of temperature and dew point temperature were 1.6 C and 1.5 C respectively. Observations of rainfall at Fargo were within 2.5 mm in all but 15 of the 1450 observations. Model predictions were evaluated based on 2004 disease survey results and observations of weather, crop growth stage and disease from replicated plots. Model evaluation based on disease survey results will be presented in the form of case studies and based on data collected in replicated plots from six states. Forecasting error appears to be associated with weather conditions during the flowering and grain filling periods of growth that are not considered by the model.

AN EXTRACELLULAR LIPASE, FGLIP1, IS A PATHOGENICITY FACTOR FOR *FUSARIUM GRAMINEARUM*

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ABSTRACT

An extracellular lipase gene, designated FgLip1, was amplified from *Fusarium graminearum* strain PH-1 using the polymerase chain reaction (PCR). To test the potential function of this gene on the pathogenicity of *F. graminearum*, a 4.5KB genomic sequence flanking FgLip1 was cloned into a backbone vector and the coding region of FgLip1 was replaced by a fungal expression cassette governing *Escherichia coli* hygromycin B phosphotransferase gene expression. This construct was introduced into PH-1 through PEG-mediated protoplast transformation. Using this approach we knocked out the wild type FgLip1 gene by homologous replacement. More than 50 transformants resistant to hygromycin were obtained. Strains with positive gene replacement were confirmed by PCR and Southern blotting and the pathogenicity of these strains were tested on a susceptible wheat cultivar CDC Teal. All strains tested showed retarded disease development on wheat head and the function of FgLip1 was to promote the spread of the fungus from inoculated spikelet to others.

MODELING FUSARIUM HEAD BLIGHT IN WHEAT UNDER CLIMATE CHANGE USING LINKED PROCESS-BASED MODELS

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OBJECTIVES

To assess the potential impact of climate change in the epidemics of Fusarium Head Blight in wheat growing regions in southern Brazil, Uruguay and Argentina.

INTRODUCTION

For diseases which a large amount of information is available, predominantly due to their economic importance, a process-based modeling approach offers the most flexible option to assess the potential impact of climate change. This approach offers the added benefit of linking host growth to pathogen life cycles through severity functions and the model is then available for evaluation of management strategies. In this work we have used Fusarium head blight of wheat as a model system to explore this approach.

Fusarium Head Blight (FHB) (*Triticum aestivum* L.), is an important disease throughout much of the world's wheat-growing areas. Several *Fusarium* species can cause head blight, although *Gibberella zeae* Schwain (Petch.) (anamorph *Fusarium graminearum* Schwabe) is the predominant pathogen in most of the regions. Contamination of wheat with the mycotoxin Desoxinivalenol (DON) at levels exceeding the permitted levels results in rejection of sale or severe price dockage in countries that have adopted DON regulation.

FHB is best known as a disease of flowering being the anthers reported as the primary infection site where spores of fungus may land and then grow into the kernels, glumes, or other head parts. Some evidences suggest that wheat may be susceptible in a period up through the soft dough stage of kernel development.

MATERIALS AND METHODS

The wheat model - The CROPSIM-CERES 2002 model included in DSSAT 4.0 (Decision Support System for Agrotechnology Transfer) was used here to simulate growth and development of spring wheat under historical and scenario weather data and soil properties at Passo Fundo, (Brazil), La Estanzuela (Uruguay) and Pergamino (Argentina), respectively. The model simulates crop growth with a daily time step from sowing to maturity, based on physiological processes that describe the crop response to soil, environmental, and management conditions. Phasic development is quantified according to the plant's physiological age. Potential growth is determined from the crop interception of photosynthetically active radiation, and actual biomass production on any day is limited by sub-optimal temperatures, soil-water deficits, and nitrogen stresses. The soil sub-models for water and nitrogen balance operate on the basis of soil layers. The Wheat model has been evaluated and successfully used across sites throughout the world including Brazil, Argentina and Uruguay.

The FHB model – The model used in the present study is a modified version of a model previously developed (Del Ponte et al. – unpublished). The original model starts by the time of emergence of the first group of heads, which is simulated in the wheat model. The daily proportion of heads emerged is a function of the heading rate. Anther's extrusion rate calculates the daily proportion of extruding anthers in a cohort of heads.

The coupling of both heading and flowering models results in the daily proportion of exposed anthers in the field . Empirical rules define anther longevity. Inoculum is assumed to be present on the residues. The density of an airborne *G. zeae* spore cloud is a function of the dispersal rate. Infections take place during an infection event which is defined by means of a combination of daily records of rainfall and mean relative humidity in a two-day window. Infection rate is a function of mean temperature during each infection event. Empirical rules were defined to take into account potential infections up to 14 days after flowering. The daily risk index is the product of the proportion of susceptible tissue, infection rate and spore cloud density. Final risk is calculated by the summation of partial indices. Rates and rules in the models are influenced by weather variables as daily mean temperature, daily mean relative humidity, daily solar radiation, precipitation, and consecutive rainy days. The model evaluation with disease data from 5 years of epidemics in Passo Fundo, Brazil, showed that risk estimated by model explained over 95% of variation in disease field severity (unpublished).

In the present study adjustments were made in the original model in order to use weather dataset without information of relative humidity. Hence, infections events are defined by means of observations of rainfall (>0,5mm) in a two-day window. Hence, daily risk index is the product of the proportion of susceptible tissue and infection rate.

Climatic Data - In each one of the selected sites (Pergamino in Argentina, La Estanzuela in Uruguay and Passo Fundo in Brazil) historical weather data included daily values of maximum and minimum temperature, precipitation and solar radiation from 1970 to 2000.

Climate change scenarios were obtained based on LARS-WG and HadleyCM3 projections. LARS-WG is a stochastic weather generator which can be used for the simulation of weather data at a single site, under both current and future climate conditions. In this paper LARS-WG was used to obtain synthetic weather series taking into account the changes occurred in climate during the last century (comparing the periods 1930-1960 to 1970-2000). By means of HadleyCM3, under A2 emissions scenario centered in 2020, we obtained the second climate change scenario. For this purpose the rate of change of each variable (from the comparison between GCM projections and the baseline period (1960-1990) was applied to the daily climate record in each site.

Epidemics risks were investigated using nine planting dates for each year from 1970 to 2000 and from a 30 year scenario, respectively. Climate change scenarios were originated from trends observed in the daily climate records from Passo Fundo, La Estanzuela and Pergamino for the 1970-2000 period.

RESULTS AND DISCUSSION

The results showed that Fusarium head blight risk index in Passo Fundo, Brazil was higher than in La Estanzuela and Pergamino. Except for Pergamino Fusarium head blight was greater under the climate change scenario than in the historical weather. The results are shown in Figure 1. The highest risk index of FHB was probably due to the presence of more rainy days during September-November period in the climate change scenario. If confirmed, this would have a significant impact on wheat production and mycotoxin contamination for this part of the world.

We have successfully used a linked process based modeling approach to explain FHB epidemics development at three sites in South America. The next step, is to further expand the climatic-dependency of the model to explore the potential impact of climate change and variability on other diseases and wheat yield across different sites in South America. The yield should result from the interaction between the change in climate, the phenology of the cultivar and the impact of disease. To make these results more generally applicable, further work is needed to compile important phenological attributes for the current suite of cultivars in the South American wheat growing regions to extend the linked models. Climate change scenarios are complex and updated regularly.

ACKNOWLEDGEMENT

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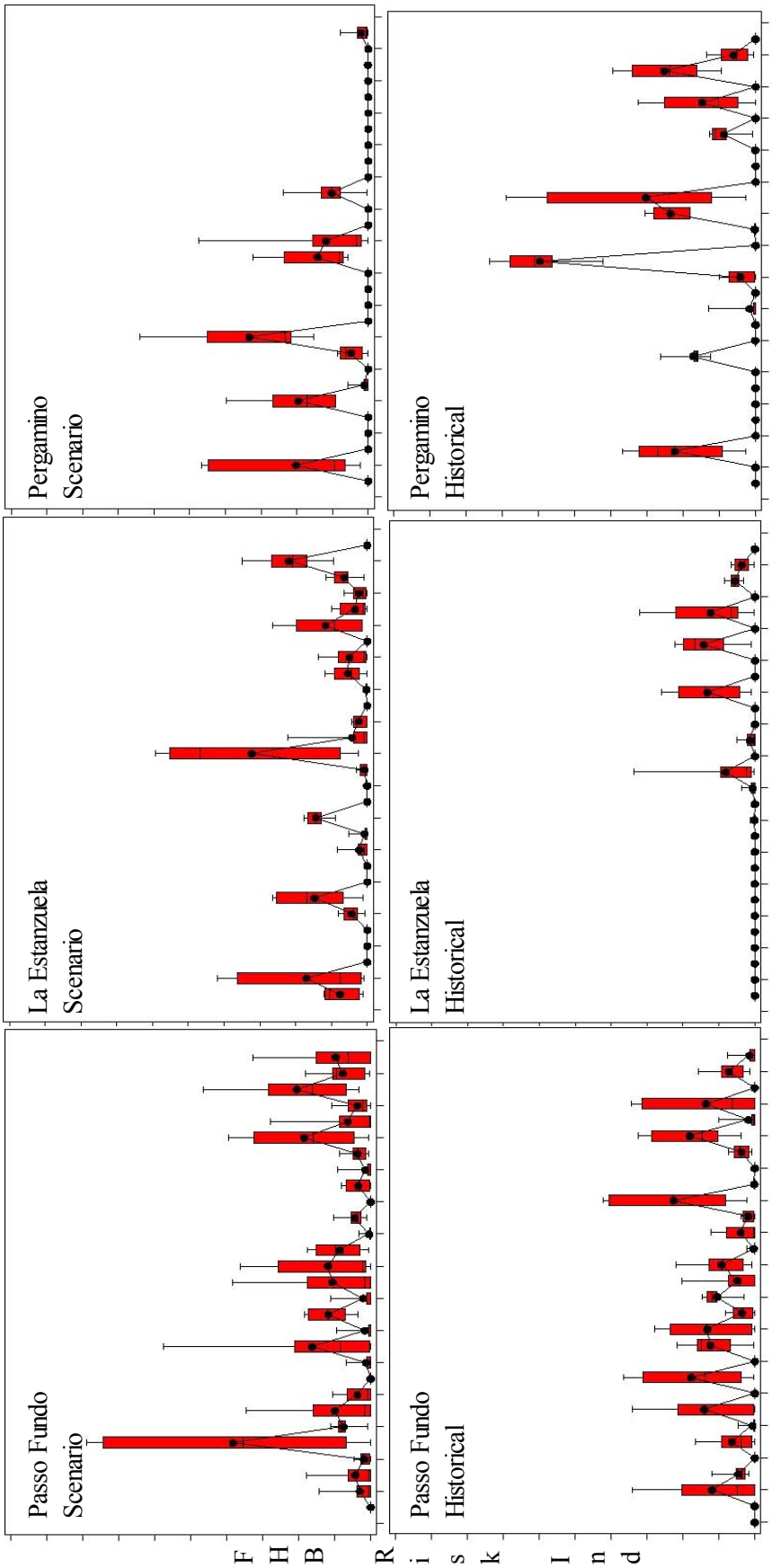

Figure 1. Simulated Fusarium Head Blight risk index under a scenario and historical weather data (1970-2000) from different locations. The shades strips from bottom to top correspond to non epidemic, light, moderate and severe, respectively.

FUSARIUM SPECIES IN ROOTS OF CANOLA, FLAX, LENTIL AND PEA CROPS GROWN IN WESTERN CANADA

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ABSTRACT

In western Canada, wheat and barley are increasingly being grown in rotation with noncereal crops. Many of the *Fusarium* spp. that cause fusarium head blight (FHB) in this region have also been found to cause root/ crown rot of wheat and barley. Because infected ground level and underground plant tissue might be a source of inoculum for cereal head infections, it would be of interest to determine if FHB pathogens could also be present in root tissue of noncereal crops. A total of 80 canola, 33 flax, 13 lentil and 35 pea crops were sampled in 2000 and 2001 in eastern Saskatchewan for fungal populations in roots. Surface-disinfested pieces of discolored roots were plated on nutrient agar for fungal identification. The *Fusarium* species most frequently isolated from discolored roots of these crops was *F. avenaceum*, a pathogen with a wide host range and one of the most important FHB pathogens in Saskatchewan. This fungus was present at the highest levels in pulses. Other common FHB pathogens, such as *F. culmorum*, *F. graminearum*, and *F. sporotrichioides*, were also isolated from roots of noncereal crops, although at lower levels. The same *Fusarium* spp. found in this study had also been isolated from discolored subcrown internodes of wheat and barley sampled in the same area. Comparison of *Fusarium* populations in noncereal roots with those in roots of wheat and barley suggests that levels of *F. avenaceum* were increased while those of other *Fusarium* spp., including the cereal pathogens *F. culmorum* and *F. graminearum*, were maintained in underground tissue of oilseed and pulse crops. Based on these observations, it is suggested that growing the noncereal crops tested in this study might result in increased root rot caused by *F. avenaceum*, and contribute to the development of FHB in subsequently-grown cereal crops. This is the first report of isolation of *F. graminearum* from roots of field-grown pulse and oilseed crops in western Canada.

FUSARIUM SPP. IN RESIDUES OF CEREAL AND NONCEREAL CROPS GROWN IN EASTERN SASKATCHEWAN, CANADA

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ABSTRACT

Fusarium head blight (FHB) is well established in eastern regions of the Canadian Prairies and has been spreading further west in the last few years. Cereal crop residues are considered the most important source of inoculum for the development of this disease; hence crop rotation has long been recommended as a disease management tool. However, surveys conducted in eastern Saskatchewan indicate that growing wheat and barley in rotation with noncereal crops is not effective in reducing FHB levels. It was of interest to determine if residues of noncereal crops commonly grown in rotation with wheat and barley can also be colonized by FHB pathogens, and thus also be a source of inoculum for FHB development. In July of 2000 and 2001, residues of cereal (wheat, barley and oat) and noncereal (canola, flax, lentil and pea) crops grown the previous season were sampled from over 300 fields in eastern Saskatchewan. The noncereal crops had been preceded by a cereal crop. Residues were surface-disinfested and plated on nutrient agar for fungal identification. *Fusarium* most often constituted the largest genus isolated from residues of all crop samples, and ranged from pathogenic to weakly pathogenic on cereals. The most commonly isolated species was *F. avenaceum*, which was in general present at the highest levels in pulse and flax residues. Among those found at lower levels in both cereal and noncereal residues were *F. equiseti*, *F. acuminatum*, *F. culmorum*, and *F. graminearum*, although in most cases the percent isolation of these species was higher in one or more of the cereal crops than in the noncereal crops. All *Fusarium* spp. found in residues were also isolated from wheat and barley heads affected by FHB in Saskatchewan. One of the most important FHB pathogens in the province was *F. avenaceum*. Colonization of canola, flax, lentil and pea residues by fungi commonly isolated from cereal crops affected by FHB suggests that growing those crops in rotation with wheat or barley would not be expected to result in a significant reduction or eradication of *Fusarium* spp. pathogenic to cereals. This could be attributed to the wide host range of the fungi or their ability to colonize nonhost plant residue. Based on these observations, we conclude that the alternative crops tested might be a source of inoculum for head infections of subsequently-grown cereal crops, especially in areas where environmental conditions are more conducive to FHB than where the present study was conducted. This is the first report of isolation of *F. graminearum* from residues of noncereal crops in western Canada.

IMPACT OF GLYPHOSATE APPLICATION, CROP SUSCEPTIBILITY AND PREVIOUSLY-GROWN CROP ON DEVELOPMENT OF FUSARIUM HEAD BLIGHT IN SPRING WHEAT UNDER MINIMUM-TILL MANAGEMENT

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ABSTRACT

Because of the increasing significance of Fusarium head blight (FHB) in western Canada, it is important to identify crop production factors (CPF) that may be associated with the spread and development of this disease. From 2000 to 2002, 312 spring wheat fields under minimum-till management were sampled for FHB in eastern Saskatchewan. *Fusarium*-damaged kernels (FDK) were also evaluated in 2000 and 2001. Environment was the most important factor determining disease development. There were fewer effects of the various CPFs on FHB in a year with high (2001) and low (2002) disease pressure, compared to a year with moderate (2000) disease pressure for this region. The CPFs that most affected FHB in crops under minimum-till management were previous application of a glyphosate formulation (GF), crop susceptibility, and previously-grown crop. The use of herbicide Groups 1, 2 or 4, N fertilizer use, seeding rate, and seeding date did not have a significant effect on disease development in any year. GF application in the previous 18 months or 3 years was the only CPF significantly associated with a higher FHB index every year, indicating that its effect was not influenced by environmental conditions as much as that of the other CPFs. In 2000 and 2001, crop susceptibility and previous GF application were the only CPFs that were associated with a significant change in percent FDK. Compared to untreated fields, wheat crops grown in fields previously treated with GF had an increase in the mean FHB index from 1.9% to 4.3% in 2000, and from 5.0% to 11.5% in 2001, and an increase in the mean percent FDK from 0.3% to 0.8% in 2000, and 0.4% to 0.8% in 2001. The higher percent FDK in crops grown in GF-treated fields would have resulted in further loss of market value. It is not known if a similar association of previous GF application with FHB and FDK would occur in environments different from the ones encountered in this study, or more conducive to disease development. Because of the nature of this study, it was not possible to establish a cause-effect relationship between previous GF application and disease development. Based on the significant and consistent association between previous GF application and FHB, further research to elucidate the underlying mechanisms is warranted.

THE EFFECT OF PREVIOUSLY-GROWN CROP AND TILLAGE SYSTEM ON *FUSARIUM* SPP. IN UNDERGROUND TISSUE OF WHEAT AND BARLEY CROPS GROWN IN WESTERN CANADA

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ABSTRACT

Fusarium head blight (FHB) has been causing significant damage to the wheat and barley industries in eastern regions of western Canada, and appears to be spreading west. A comprehensive strategy to stop or reduce the spread of this disease is necessary. Most of the same *Fusarium* spp. responsible for FHB are also associated with root/crown rot in cereal crops. Controlling *Fusarium* spp. in underground plant tissue might help to control the spread of FHB and reduce the damage it has been causing in areas where it is already established. To this end, the effect of agronomic practices on fungal populations in underground tissue was examined in a total of 400 wheat and 138 barley fields surveyed in eastern Saskatchewan between 1999 and 2001. Crops sampled had been preceded by a pulse or oilseed crop, or by summerfallow, and were under conventional-till (7 or more tillage passes in last 3 years, and an average of less than 1 glyphosate application in previous 18 months), minimum-till (one to six tillage passes in last 3 years, and average of 1 glyphosate application in previous 18 months) or zero-till (no mechanical tillage in last 3 years, and an average of 2 glyphosate applications in previous 18 months) management. Many of the *Fusarium* spp. isolated from discolored subcrown internodes had also been previously isolated from cereal heads affected by FHB in Saskatchewan, including *F. avenaceum*, *F. culmorum* and *F. graminearum*. There was a negative correlation between percent isolation of *Fusarium* spp. and that of *Cochliobolus sativus*, the fungus most commonly isolated from subcrown internodes. Analysis of fungal populations in crops under minimum-till management according to crop history revealed that percent isolation of *Fusarium* spp. was not consistently affected by the previously-grown crop. However, crops preceded by summerfallow had higher levels of *C. sativus* than those preceded by a crop. Analysis of fungal populations by tillage system revealed that in general there was a lower percent isolation of *C. sativus*, and a higher percent isolation of *Fusarium* spp., especially *F. avenaceum*, with a decrease in the intensity of tillage and an increase in the use of glyphposate formulations. We conclude that whereas *Fusarium* populations in underground tissue of wheat and barley will not be affected by the preceding crop, the isolation frequency of some of these species will increase as soil disturbance decreases and glyphosate use increases. Whether the increase in *Fusarium* populations in reduced tillage systems is due to the absence of competition from *C. sativus*, or a direct growth stimulation, is not known and requires further investigation as an increasing number of producers adopts conservation tillage practices.

THE GPMK1 MAP-KINASE REGULATES THE SECRETED LIPASE FGL1, A NOVEL VIRULENCE FACTOR OF *FUSARIUM GRAMINEARUM*

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ABSTRACT

Mitogen activated protein (MAP) kinases play important roles during different developmental processes including pathogenic stages of many filamentous fungi. It has been reported that Gpmk1 MAP kinase disruption mutants of *Fusarium graminearum* are apathogenic and cannot infect wheat spikes. At this time it is not possible to explain the complete apathogenicity of the MAP kinase deletion mutants, because the Gpmk1 MAP kinase affects several different processes in the cells.

An effective fungal pathogen must overcome physical and chemical barriers made up by the host to block infection. The actual mode of penetration and invasion of *F. graminearum* is still not fully elucidated. However, the formation of appressoria has been excluded, as such structures were never found. Instead, the fungus probable enters the host through natural openings, such as the glume stomata, or penetrates the epidermal cell walls directly with short infection hyphae. *F. graminearum* secretes cell wall degrading enzymes during colonization of its host. Jenczmionka and Schäfer (2004) could show that the regulation of various cell wall degrading enzymes, like endoglucanases, proteolytic and lipolytic enzymes is mediated by the map-kinase pathway. *Gpmk1* MAP kinase disruption mutants of *F. graminearum* show *in vitro* a reduced lipolytic activity in comparison to the wild type strain. We have cloned, characterized, and disrupted a secreted lipase (FGL1) of *F. graminearum* and found it to be a novel virulence factor. Here we show the regulation of FGL1 gene in dependence of MAP kinase Gpmk1.

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POLYKETIDE FUNCTION IN *GIBBERELLA ZEAE*

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ABSTRACT

Gibberella zeae is the causal agent of Fusarium Head Blight on cereal. Since the disease affects the grain, yield losses can be as high as 40% when infection is severe. During host colonization, the fungus produces mycotoxins, including deoxynivalenol, zearalenone and aurofusarin, which make the grain unfit for human and animal consumption. Zearalenone and aurofusarin belong to the family of compounds called *polyketides*. Polyketides are produced by Polyketide Synthases (PKS) using acetyl or malonyl precursors. In fungi, PKSs are large multidomain enzymes and have an iterative function. All Polyketide Synthases have Ketosynthase, Acyl Transferase and Acyl Carrier Protein domains. In addition to this they may have one or more functional domains such as Ketoreductase, Dehydratase, Enoylreductase which give rise to the immense structural diversity of these compounds. We used the recently released genomic sequence of *Gibberella zeae* to identify all the PKS genes in the genome. We then disrupted each gene individually and analyzed the mutants phenotypically. We were able to assign function to five of 15 identified PKS genes. We continue to explore their role in the life cycle of this important pathogen.

LONGEVITY OF ASCOSPORES OF *GIBBERELLA ZEAE* EXPOSED TO VARIOUS DEGREES OF RELATIVE HUMIDITY AND TEMPERATURE

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ABSTRACT

Fusarium head blight (FHB) is the most important cereal disease in the province of Manitoba, Canada, and has caused major losses to all sectors of the grain industry since 1993. The principal pathogen causing FHB is *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* [Schwein.] Petch). Disease forecasting systems are being developed to aid producers in fungicide application decisions. One area that is poorly understood is the duration of survival and conditions under which ascospores remain viable once released from perithecia. The objective of this study was to determine the longevity of ascospores under various levels of relative humidity (RH). Ascospores were recovered from lids of Petri dishes, where they adhered in condensation droplets, within 24 h of being released from perithecia. Viability was tested with the vital stain, trypan blue, and by germination on water agar. Humidity chambers were created using the salts $MgCl_2$ Að6H_2O, NaBrAð2H_2O^5, and KNO_3^{11} to provide RH levels of 33%, 59% and 93.5%, respectively at 20^0 C for periods of time extending for 2, 4, 6 and 8 hours. Preliminary results indicate that trypan blue was taken up by only a small percentage (10%) of ascospores indicating high viability, but this was not always confirmed by equally high levels of germination on water agar. Under RH of 30%, viability and germinability rapidly dropped to approximately 30%, while ascospores suspended in water for as long as 7 days remained viable and germinated on water agar.

THE ROLE OF CROP STUBBLE IN PRODUCTION OF INOCULUM OF *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium head blight (FHB), caused principally by *Fusarium graminearum* Schwabe, is the most important cereal disease in the province of Manitoba, Canada, and has caused major losses to all sectors of the grain industry since 1993. As part of a long-term crop rotation study, residues from foundation crops and two year rotations were examined for presence of *Fusarium* spp. under conditions of natural inoculum. Foundation plots (10 x 60 m) of wheat, oat, pea, and canola were established in 2001. In 2002, residue was retrieved from each of these plots on 2 or 3 occasions during the growing season. Also in 2002, into each foundation plot, each of the 4 crops was planted in 10 x 15 m plots. In 2003, residue was retrieved from the 10 x 15 m plots of each crop and stored at 4^0 C. Residue was cut into 2.5 cm sections and surface sterilized. Four sections per Petri dish/ replicate were plated on potato dextrose agar amended with streptomycin, and incubated under white light at room temperature for 5 to 7 days. There were 4 replicates. In 2002, the two predominant *Fusarium* spp. isolated from residue from foundation plots were *F. sporotrichioides* Scherb.(mostly from oat and pea residue) and *F. acuminatum* Ell. & Ev.(from all residue types). *Fusarium graminearum*, and *F. equiseti* (Corda) Sacc. were the second most abundant. Other *Fusarium* spp., including *F. culmorum* (W.G. Smith), *F. poae* (Peck) Wollenw., and *F. sambucinum* Fuckel were found at low levels. In both years, more *F. graminearum* was found on wheat and oat residue, than on canola and pea residue, and levels were higher in 2003 than in 2002. In 2003 there was more *F. graminearum* isolated from residue of wheat grown on oat stubble, and from wheat and oat grown on pea stubble. Pea residue appeared to be a favourable substrate for *F. graminearum*, as the highest levels were isolated from plots that had been planted into pea stubble. *Fusarium sporotrichioides* was the most abundant species isolated in 2003, especially from residue of oat and wheat planted into canola, oat and pea stubble. There were consistently lower levels of *F. sporotrichioides* from oat, pea and wheat that was planted into wheat stubble.

FUSARIUM HEAD BLIGHT IN MICHIGAN IN 2004

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INTRODUCTION

Widespread and severe epidemics of Fusarium head blight (FHB) occur when rain periods coincide with flowering and grain fill in wheat, and other small grains (Hart, et al, 1984; McMullen, et al, 1997). In 2004, favorable pre-flowering infection periods occurred during the weeks of May 11-18, 22-27 and June 10-19 according to the Model 1 FHB predictor (DeWolf, et al, 2000). The two earlier favorable events coincided with flowering in the many parts of the state. Rainfall during these periods would have resulted in the extensive release of spores that initiate the disease on the wheat heads. Based on the Model II post-flowering infection FHB model (DeWolf, et al, 2000) the latter dates were also favorable for post-flowering infection. Therefore, it is not surprising that FHB was a common occurrence in Michigan in 2004, and resulted in the contamination of much of the wheat in the state with DON.

METHODS AND MATERIALS

FHB Survey. Ninety-three wheat fields were surveyed in thirteen counties extending from central Michigan, through the thumb, and south into southeast Michigan. Fields were randomly chosen for sampling two to three weeks prior to harvest. Heads were collected from a minimum of ten locations within each field, and the number of heads collected per field ranged from a low of 48 to a high of 403. Fewer heads were collected when the incidence of disease was high (Table 1). The percent of infected head was determined by rating each head as infected or non-infected, and the severity of disease on each of the infected heads was determined following the guidelines established by Stack, et al (1996). A disease severity index for each field was determined by multiplying the average percent of infected heads by the average disease severity. After counting, the grain was threshed from the heads, and assayed for DON. A few days prior to harvest heads were collected from eleven of the previously sampled fields, and the grain again assayed for DON.

Wheat Variety Trial. A Michigan State University wheat variety trial in southeast Michigan was uniformly diseased with FHB. Each of the four replications was evaluated for disease incidence and severity, and one replication was harvested for DON analysis. Additional information on the wheat variety trial can be found at http://www.msue.msu.edu/msuwheat/index.html.

Fungicide Trials. Two wheat varieties, Freedom and Harus, were planted in the fall of 2003. Michigan State University wheat management recommendations were followed. Environmental conditions for FHB were very favorable in Michigan in 2004, and these plots were neither irrigated nor inoculated. Fungicides were applied as described previously (Hart, et al, 1999). Fungicide applications (Table 2) were delayed until eight days after the start of anthesis due to excessively wet conditions.

RESULTS AND DISCUSSION

FHB Survey. The average number of infected heads in these fields was 77.6%, and ranged from a low of 12% to high of 100% (Table 1). The average disease severity of infection was 29% (determined from both infected and non-infected heads), and ranged from a low of 1% to a high of 64%. DON values ranged from less than 1 ppm to 22 ppm in grain collected 1-2 weeks prior to harvest, with an average of 8.3 ppm. DON values two taken two weeks prior to harvest were similar to values taken within a few days of harvest in eleven common. Stagonospora leaf blotch was common and relatively severe throughout the state.

Wheat Variety Trial. Wheat variety trial results are posted at http://www.msue.msu.edu/msuwheat/index.html. The average percent FHB infection was 97.2, the average disease severity as a percentage of the head infected was 51.4 (infected and uninfected heads), and average disease severity index was 50.5, across four replications. The DON average from one of the four replications was 5.9 ppm. Overall, the largest differences among varieties were in the amount of infection on heads, and in DON levels in the grain.

Fungicide Trial. Fungicides were applied eight days after flowering which is too late to be as effective as applications at the beginning of flowering. Weather conditions did not permit an earlier application. Differences between treatments were not significant (Table 2). There was a non-significant reduction of 0.6 to 2.5 ppm of DON with experimental, non-labeled fungicides. However, the disease incidence and severity was not affected. Stagonospora leaf blotch was severe and widespread in Michigan (http://www.msue.msu.edu/msuwheat/index.html). Differences between Harus and Freedom were significant (P=0.05) for the percentage of infected heads, percent disease severity, disease severity index DON and yield.

The results from the wheat variety trial, and the fungicide trial, suggest that differences in susceptibility, or resistance, are primarily in the spread of *F. graminearum* within the head, and in the production of DON. The latter was especially evident in the fungicide trial where the variety Freedom was significantly more affected by FHB in disease indices, but the DON level were less than a one-third the level in Harus. These data also support the necessity for DON analysis of grain from research projects within the initiative to select treatments and wheat varieties that are capable of reducing DON levels in the absence of a reduction in disease.

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Table 1. Survey results for Fusarium head blight in Michigan in 2004. Number of heads indicates the number of wheat heads collected in each field and taken back to the laboratory to determine levels of infection, and for analysis of the grain for DON. Disease severity is the average disease severity for both infected and uninfected heads. Severity index is the percent infection times the disease severity.

Field #	Location	Total # Heads	# Heads Infected	% Infection	% disease severity	Severity Index	DON ppm	2nd DON
1	N42 42.726 W84 39.810	195	185	94.9%	28.2%	26.8	3.4	
2	N42 39.438 W84 44.348	304	213	70.1%	25.9%	18.2	11.0	
3	N42 38.498 W84 44.342	169	146	86.4%	42.3%	36.5	7.0	
4	N42 34.751 W84 44.336	178	171	96.1%	74.3%	71.4	8.0	
5	N42 34.051 W84 43.827	309	302	97.7%	43.3%	42.3	6.0	
6	N42 34.059 W84 43.370	227	164	72.2%	30.9%	22.3	11.0	
7	N42 32.029 W84 43.144	254	191	75.2%	26.3%	19.8		2.8
8	N42 31.763 W84 42.505	209	98	46.9%	23.5%	11.0	10.0	2.2
9	N42 24.124 W84 36.146	190	149	78.4%	31.7%	24.9	4.0	3.4
10	N42 23.388 W84 34.835	272	230	84.6%	34.5%	29.2	4.2	
11	N42 35.812 W84 26.064	216	192	88.9%	39.3%	35.0	3.8	3.8
12	N42 36.662 W84 25.846	244	237	97.1%	42.1%	40.9	5.0	2.8
13	N42 37.070 W84 25.978	221	209	94.6%	56.4%	53.3	4.4	4.4
14	N42 38.076 W84 25.977	193	166	86.0%	22.1%	19.0	2.6	2.2
15	N42 47.001 W84 44.369	114	89	78.1%	13.2%	10.3	4.4	4.2
16	N42 54.032 W84 44.565	128	43	33.6%	13.4%	4.5	>50.0	6.0
17	N42 56.611 W84 44.222	123	59	48.0%	12.0%	5.7		9.0
18	N43 00.097 W84 41.635	124	54	43.5%	9.3%	4.1	14.0	13.0
19	N42 59.490 W84 34.943	131	25	19.1%	5.7%	1.1	4.4	6.0
20	N42 55.260 W84 14.656	163	103	63.2%	9.3%	5.9	10.0	6.0
21	N42 53.902 W84 20.164	131	125	95.4%	33.4%	31.9		1.0
22	N42 52.694 W84 27.480	148	27	18.2%	6.0%	1.1	22.0	1.4
23	N42 50.634 W84 29.038	153	39	25.5%	3.6%	0.9	8.0	1.1
26	N41 56.458 W83 48.607	167	148	88.6%	24.1%	21.4	18.0	
27	N41 56.097 W83 53.150	139	138	99.3%	62.6%	62.1	2.8	
28	N41 55.182 W83 53.149	126	124	98.4%	59.5%	58.6	4.8	
29	N41 51.860 W83 50.711	133	124	93.2%	34.3%	32.0	2.2	
30	N41 48.547 W83 50.366	154	148	96.1%	36.0%	34.6	3.0	
31	N41 49.194 W83 49.054	160	144	90.0%	34.6%	31.2	0.8	
32	N41 48.368 W83 47.322	210	209	99.5%	45.2%	45.0	3.0	
33	N41 51.354 W83 42.555	137	125	91.2%	44.0%	40.2	7.0	
34	N43 13.889 W83 06.518	81	81	100.0%	26.0%	26.0	<0.5	
35a	N43 12.745 W83 01.111	76	76	100.0%	38.4%	38.4	<0.5	
35b	N43 12.745 W83 01.111	133	130	97.7%	25.6%	25.0	1.8	
36	N43 12.789 W82 56.494	87	86	98.9%	38.7%	38.2	0.8	
37	N43 14.463 W82 48.362	136	136	100.0%	48.3%	48.3	0.0	
38	N43 19.336 W82 49.294	55	55	100.0%	30.4%	30.4	1.2	
40	N43 24.421 W82 48.827	141	141	100.0%	48.5%	48.5	2.2	
41a	N43 24.421 W82 48.827	97	95	97.9%	47.0%	46.0	4.4	
41b	N43 22.892 W82 48.481	66	66	100.0%	43.2%	43.2	2.4	
42	N43 28.732 W82 49.079	133	132	99.2%	29.3%	29.1	0.0	
43	N43 24.526 W85 06.197	164	22	13.4%	6.1%	0.8	21.0	
44	N43 23.111 W85 05.114	135	20	14.8%	1.2%	0.2	29.0	
45	N43 21.617 W85 05.182	173	31	17.9%	3.2%	0.6	8.0	
46	N43 18.080 W85 05.017	274	64	23.4%	15.1%	3.5	15.0	
47	N43 09.926 W85 04.379	384	384	100.0%	64.3%	64.3	19.0	

Table 1. cont.

Field #	Location	Total # Heads	# Heads Infected	% Infection	% disease severity	Severity Index	DON ppm
48	N43 09.536 W85 04.389	135	68	50.4%	14.6%	7.3	15.0
49	N43 03.464 W85 04.523	180	73	40.6%	12.1%	4.9	>50.0
50	N42 52.023 W85 15.515	142	92	64.8%	20.7%	13.4	20.0
51	N42 59.205 W85 05.060	143	133	93.0%	37.3%	34.7	14.0
52	N42 09.536 W85 41.376	166	149	89.8%	38.5%	34.6	11.0
53	N43 44.831 W84 03.429	52	52	100.0%	20.2%	20.2	<0.5
54	N43 50.556 W83 57.798	60	53	88.3%	37.7%	33.3	23.0
55	N43 39.323 W84 05.964	56	56	100.0%	62.2%	62.2	>50.0
56	N43 48.188 W84 06.971	59	58	98.3%	50.9%	50.0	23.0
57	N43 51.953 W84 03.111	67	67	100.0%	28.8%	28.8	0.6
58	N43 34.405 W83 44.732	48	48	100.0%	23.0%	23.0	<0.5
59	N43 38.594 W84 00.174	51	23	45.1%	3.2%	1.4	<0.5
60	N42 53.298 W84 34.931	117	116	99.1%	44.1%	43.7	12.0
61	N42 53.116 W84 42.414	100	86	86.0%	15.4%	13.2	7.0
62	N43 00.029 W84 46.987	121	91	75.2%	15.2%	11.4	2.2
63	N43 04.592 W84 46.488	110	107	97.3%	30.1%	29.3	6.0
64	N43 06.535 W84 38.257	106	106	100.0%	32.7%	32.7	17.0
65	N43 06.110 W84 29.609	110	110	100.0%	37.7%	37.7	5.0
66	N43 06.049 W84 23.872	83	40	48.2%	16.8%	8.1	5.0
67	N43 01.556 W84 24.620	87	83	95.4%	17.6%	16.8	11.0
68	N42 56.152 W84 24.703	132	132	100.0%	41.2%	41.2	3.0
69	N43 14.124 W84 40.384	81	62	76.5%	27.3%	20.9	
70	N43 09.996 W84 48.275	95	73	76.8%	25.1%	19.3	
71	N43 14.671 W84 48.108	66	27	40.9%	15.4%	6.3	
72	N43 20.136 W84 43.441	87	52	59.8%	14.2%	8.5	
73	N43 21.472 W84 46.024	103	95	92.2%	36.8%	33.9	
74	N43 21.289 W84 31.855	130	52	40.0%	15.1%	6.0	
75	N43 23.840 W84 25.938	117	109	93.2%	59.2%	55.2	
76	N43 18.800 W84 26.688	128	110	85.9%	44.5%	38.2	
77	N43 09.267 W84 25.771	403	81	20.1%	4.6%	0.9	17.0
78	N42 59.591 W83 03.948	92	82	89.1%	29.5%	26.3	<0.5
79	N42 03.603 W83 37.033	113	52	46.0%	17.9%	8.2	6.0
80	N41 48.970 W83 42.840	128	126	98.4%	40.4%	39.7	4.6
81	N41 50.245 W83 27.383	168	150	89.3%	22.1%	19.7	3.0
82	N43 15.702 W85 04.308	105	94	89.5%	37.8%	33.9	22.0
83	N43 22.928 W85 23.722	121	34	28.1%	13.8%	3.9	13.0
84	N43 23.232 W84 57.726	113	52	46.0%	17.9%	8.2	
85	N43 16.586 W83 32.360	75	62	82.7%	18.4%	15.2	12.0
86	N43 17.320 W83 31.317	57	57	100.0%	41.1%	41.1	7.0
	M66 & Romes	141	18	12.8%	1.3%	0.2	
	M66 S of Ionia	153	100	65.4%	32.9%	21.5	20.0
	Stamps - Folicur	170	168	98.8%	25.2%	24.9	0.6
	Stamps - Headline	109	106	97.2%	22.2%	21.6	0.8
	Stamps - Headline & Folic	163	162	99.4%	28.2%	28.0	1.8
	Stamps - No Fungicide	156	155	99.4%	25.3%	25.1	2.0
				77.1%	29.1%	25.7	8.3

Table 2. Results of MSU Fungicide Variety Trials – 2004. The disease severity index was obtained by multiplying the mean of head infection by the mean of infection of individual heads (includes infected and uninfected). Means are the average of four replications. Treatments with different letters (a, b) are significantly different from the untreated controls at p=0.05. Significant differences between varieties are indicated by c, d.

Variety	Treatment	Mean % of Heads Infected	Mean % Infection of Individual Heads	Mean Severity Index	Mean DON ppm	Mean Yield bu/acre
Freedom	Control	96	43	41	3.3	45.3
Freedom	Folicur 432SC 4 fl. oz + 0.125% Induce	97	42	41	2.9	51.2
Freedom	Tilt 3.6EC 4.0 fl oz	97	49	48	2.8	48.5
Freedom	JAU6476 480SC 5.0 fl. oz +0.125% Induce	94	41	39	3.3	47.1
Freedom	JAU6476 480SC 2.85 fl. oz + Folicur 3.17 fl. Oz + 0.125% Induce	98	41	40	2.7	50.1
Freedom	V-10116 1.81 FL @ 6 fl oz/A + 0.125% Induce	98	43	42	3.3	49.3
Freedom	V-10116 1.81 FL @ 4 fl oz/A + 0.125% Induce	93	45	42	2.7	46.9
Freedom	Biocontrol strain OH 182.9	96	43	42	3.1	46.8
	Freedom Means	96 c	43 c	42 c	3.0	48.2
Harus	Control	82	32	27	10.8	51.8
Harus	Folicur 432SC 4 fl. oz + 0.125% Induce	96	35	34	9.5	51.1
Harus	Tilt 3.6EC 4.0 fl oz	97	35	35	10.8	49.9
Harus	JAU6476 480SC 5.0 fl. oz +0.125% Induce	89	35	33	8.3	53.0
Harus	JAU6476 480SC 2.85 fl. oz + Folicur 3.17 fl. Oz + 0.125% Induce	86	37	34	9.5	54.2
Harus	V-10116 1.81 FL @ 6 fl oz/A + 0.125% Induce	99	36	35	8.8	56.4
Harus	V-10116 1.81 FL @ 4 fl oz/A + 0.125% Induce	83	29	26	9.3	50.4
Harus	Biocontrol strain OH 182.9	86	33	29	10.0	49.7
	Harus Means	90	34	32	9.6	52.1

THE DONCAST MODEL: PREDICTING DEOXYNIVALENOL (DON) IN WHEAT

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ABSTRACT

DONcast is an empirical model used mainly to predict deoxynivalenol (DON) in mature wheat grain at heading. We have used DONcast in Ontario, Canada, primarily as a tool for making informed decisions on whether or not a fungicide should be applied at heading on a regional scale. Validation analysis shows greater than 80% accuracy for determining whether a fungicide application is warranted to reduce DON. We have posted predictions on the web on a regional scale in Ontario since 2000. DONcast was also adapted for use in Uruguay, South America, in 2002 and 2003, where DON was found in baked goods ranging from 1 to 5 ppm, resulting from a severe *Fusarium* epidemic in 2001. In Uruguay, DONcast has been employed as a pre-harvest alert to DON contamination for targeting regulatory and marketing action on fields within various regions for markets destined for food. In total, the present DONcast has been developed using field-specific weather and agronomic variables from over 700 farm fields across two countries since 1996.

Three critical periods of weather remain important in DONcast around wheat heading, including daily temperature, rainfall, and relative humidity. Agronomic variables are also important, including wheat variety susceptibility to DON accumulation, tillage systems, and crops grown before wheat. Overall, predictions have explained 76% of the variability in DON using all fields from the database from 1996 to 2003. For the first time in 2004, a web-based interactive model was developed for the industry in Ontario, which allowed input of field-specific weather and agronomic variables for more accurate predictions. Details of the interactive model will be presented. The robustness and applicability of the DONcast model will also be presented, using data invited from several countries, including the United States, United Kingdom, Uruguay, and others.

CYTOLOGICAL ANALYSIS OF THE INFECTION COURSE OF *FUSARIUM GRAMINEARUM* ON BARLEY CARYOPSES

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ABSTRACT

In a co-transformation assay (Jenczmionka et al. 2003), we disrupted the *Tri5* gene of *Fusarium graminearum* by homologous recombination, and integrated the gene for the green fluorescent protein (*gfp*) under the control of a constitutive promoter at the disrupted *Tri5* locus. The resultant transformants (*DTri5*) were shown to be deoxynivalenol deficient and expressed constitutively GFP. Additionally, the wild type (WT) strain 8/1 (Miedaner et al. 2000) was also transformed to express GFP constitutively. We used (*DTri5*) and wild type (WT) strains of *F. graminearum* to investigate the infection course on barley caryopses of cv. Chevron, and the highly susceptible cultivars Triumph and Golden Promise. We also implied the NIL Pallas, BCPallas-*mlo5* (P22) and Ingrid, BCIngrid-*mlo5* (I22) to determine whether the mutation of *Mlo* has an impact on the susceptibility of barley to FHB.

We found marked differences in the susceptibility to *F. graminearum* of the various barley lines. Chevron and Pallas were less susceptible than Triumph and Golden Promise. P22 and I22 were clearly the most susceptible barley lines in this investigation, indicated by a faster spread and development of the fungus and the huge lesions in the hypodermis. Both observations might be explained by the mutation in the *Mlo* locus, which leads to a loss of cell death control and, consequently, to more frequent cell death in the plant tissue. This might be favourable for the spread of necrotrophic pathogens like *F. graminearum*, as already shown for the hemibiotrophic fungi *Magnaporthe grisea* (Jarosch et al. 1999) and *Bipolaris sorokiniana* (Kumar et al. 2001).

In our study no differences in the course or strength of the infection by WT or *DTri5* strains of *F. graminearum* were detectable. Thus, the early pathogenesis of the DTri5 strains was not affected by the *knock-out* of the trichothecene biosynthesis.

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ANALYSIS OF THE OCCURRENCE OF *FUSARIUM* SPECIES IN SPANISH CEREALS BY PCR ASSAYS

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OBJECTIVES

To know the occurrence of the main tricothecene and fumonisin producing *Fusarium* species in several wheat and maize cultivars from a region in the South West of Spain using a rapid PCR-based method.

INTRODUCTION

Fungal contamination of cereals represents a serious risk for human and animal health, because the ability of many of the fungal species to produce toxins in the grain. These species also produce diseases which result in yield loss. The genus *Fusarium* is one of the most relevant fungus affecting agronomical important crops with a cosmopolitan distribution. This genus includes diverse species differing in geographical, climatic and host distribution as well as in the array of toxins the different species are able to produce, some of them responsible of serious chronic and acute diseases, among which trichothecenes and fumonisins are the most relevant. The correct and early identification of the critical *Fusarium* species or populations permits the prediction of occurrence of a particular toxin and the control of the fungus in order to prevent toxin entering the food chain.

In the case of cereals, particularly wheat and maize, trichothecene-producing species frequently occur in wheat while fumonisin-producing species are more often associated to maize. However, geographic and climatic factors are playing a crucial role in the distribution of *Fusarium* species as well as some farming practices such crop rotation and host genotype (Doohan et al., 2003, Bottalico and Perrone, 2002). In the case of Spain, there are few data regarding the distribution of *Fusarium* species in cereals. The diseases produced by *Fusarium*, particularly in wheat, are not considered of special concern although the occurrence of trichothecenes and fumonisins have been reported in cereals and in food and feed products (Sanchis et al., 1994; Jiménez and Mateo, 2001; Sanchis et al., 2001). Our objective was to identify the main *Fusarium* species in several wheat and maize cultivars responsible for the synthesis of those toxins using an identification method rapid and precise based on PCR.

MATERIAL AND METHODS

The sampling of hard wheat was carried out in April 2003 in six locations in a South West region of Spain, latitude of 37º 11' and longitude of -5º 45' (Utrera). Maize fields were sampled in August 2004 in four locations in the same region. At least one field was sampled in each location.

Five spikes, preferentially showing symptoms compatible with *Fusarium* head blight disease (FHB), were collected in five different places within each of the fourteen wheat fields studied. Seeds with the glumes were separated and pooled. In the case of maize, one kernel was collected at the five places in each of the five fields sampled. Four grams of the pool of seeds were incubated in 20 mL Sabouraud liquid medium supplemented with chloramphenicol (0.5%) at 25 ºC during five days. The liquid medium was discarded and the seeds were grounded in liquid nitrogen and genomic DNA extracted with Genomix (Talent, Italy) according the manufacturer's instructions. Subsequently standard PCR were carried out using specific primer pairs for *F. graminearum, F. culmorum, F. equiseti, F. poae, F. sporotrichiodes, F.*

verticillioides and *F. proliferatum*, as well as, a primer set for *Fusarium spp*. These primers were developed in our laboratory and were based on the multycopy IGS region (Intergenic spacer of rDNA units) (Jurado et al., submitted).The PCR assay for detection of *F. verticillioides* is described elsewhere (Patiño et al., 2004). Maize samples were tested for *F. graminearum, F. culmorum, F. equiseti, F. verticillioides* and *F. proliferatum.*

Alternatively, in the case of wheat, ten to twenty five seeds (five seeds per plate) from the fourteen fields were plated on PDA supplemented with chloramphenicol (0.5%) and incubated at 25ºC during three to five days in order to isolate *Fusarium* strains. The isolates obtained were analyzed using the direct PCR assay. When the *Fusarium* isolate was none of the critical species, a partial sequence of the elongation factor (EF-1a) was obtained by PCR using the primers and the PCR protocol described by O'Donnell et al.(2000). The sequences obtained were sent to the FUSARIUM-ID v 1.0 data base (Geiser et al., 2004) to find out the *Fusarium* species showing the most similar sequence. Four *F. graminearum* and eleven *F. culmorum* isolates were further analyzed using PCR protocols based on the partial sequences of *tri7* and *tri13* genes (Chandler et al., 2003) to determine the ability of a particular isolate to produce tricothecene DON (deoxynivalenol) or NIV (nivalenol).

RESULTS AND DISCUSSION

Presence of *Fusarium* spp. has been detected in all the locations although the number of sampled places differed (Table 1). This result basically agreed with the places where visible symptoms of FHB were observed. *F. graminearum, F. culmorum* and *F. equiseti* occurred either together or separately in 50 % of the fields. *F. sporotrichioides, F. poae* and *F. verticillioides* were not detected in any of the fields tested. The conventional approach alternatively used basically confirmed the presence of those species detected by direct PCR assay and, additionally, *F. sambucinum, F. avenaceum, F. udum* and *F. robustum* were identified by means of their partial sequence of the EF-1a (Table 2).

The occurrence of *F. graminearum, F. culmorum* and *F. avenaceum* in the region analyzed in this study represents a similar situation to Sourthern Italy, where also the incidence of FHB is low (Bottalico and Perrone 2002, Logrieco et al. 2003). However, the occurrence of *F. equiseti* seemed to be more important in our region. These data contradict in some extent the general consideration of certain species being more prevalent in either northern (*F. culmorum* or *F. avenaceum*) or southern regions, indicating on one hand, the relative influence of fluctuations in climatic conditions which can temporally displace one species in favor of another. On the other hand, the intraspecific diversity of the species considered, still insufficiently known, could be responsible of the existence of lineages or strains with different features regarding environmental requirements. Intraspecific variability has been reported in *F. graminearum* (O'Donnell, 2000), *F. verticillioides* (Mirete et al., 2004, Moretti et al., 2004) or *F. avenaceum* (Yli-Mattila et al., 2002).

The analysis of the potential ability to produce DON/ NIV of a subset of *F. graminearum* and *F. culmorum* isolates predicted the potential occurrence of wheat contaminated with DON, but not NIV, and, as reported in literature, it is expected the synthesis of zearalenone produced by *F. equiseti, F. culmorum* and *F. graminearum,* zearalenols produced by *F. equiseti* and *F. culmorum,* type A trichothecenes due to the occurrence of *F. sambucinum* and moniliformin, enniantins and beauvericin produced by *F. avenaceum*. Although the incidence of *F. proliferatum* was low, it can be indicative of a possible spread of this species in wheat, a situation previously reported in wheat and rye in diverse geographical locations (reviewed in Bottalico and Perrone, 2002).

In the case of maize, the results showed a prevalence of *F. verticillioides* and *F. proliferatum* (Table 3) which apparently have completely displaced other *Fusarium* species commonly associated to maize, such as *F. graminearum* or other occasionally found such as *F. culmorum* and *F. equiseti,* also present in the wheat analyzed in this region (Table 1). However, the more susceptible period of wheat and maize plants to fungal infection occurs in different months (April

and August, respectively) and the climatic conditions are probably different enough to condition the distribution of species observed. In the case of Utrera, the average temperature and pluviometry are 15ºC and 56 mm, respectively, in April and 25.9ºC and 16 mm, respectively, in August. Anyhow, the prevalence of these two species in maize seems not to be restricted to this region. A recent survey of maize collected in the central part of Spain (Madrid), also showed the presence of *F. verticillioides* and *F. proliferatum* and the absence of *F. graminearum* (Jurado et al., unpublished). In other Mediterranean areas the co-occurrence of both *F. verticillioides* and *F. proliferatum* has been also reported and a more relevant role for *F. proliferatum* has been suggested in fusariotoxicoses of maize grown in that region (Logrieco et al., 2003). We can conclude that in the case of maize, fumonisins and/or beauvericin and moniliformin will be the main, and probable unique, source of toxins in maize grown in this region.

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Table 1. Occurrence of *Fusarium* ssp. (Fspp), *F. graminearum* (Fg), *F. culmorum* (Fc), *F. sporotrichioides* (Fsp), *F. poae* (Fp), *F. equiseti* (Feq), *F. verticillioides* (Fv) and *F. proliferatum* (Fpr) in wheat fields.

Location	Field	*Symptoms	*Fspp	*Fg	*Fc	*Fsp	*Fp	*Feq	*Fv	*Fpr
Utrera	U1	0	2	-	-	-	-	-	-	1
Utrera	U2	1	1	-	-	-	-	-	-	-
Utrera	U3	5	5	-	-	-	-	-	-	-
Utrera	U4	2	2	-	-	-	-	-	-	-
Utrera	U5	2	2	-	-	-	-	-	-	-
Utrera	U6	5	4	-	-	-	-	3	-	-
Lebrija	L1	5	5	1	2	-	-	1	-	-
Lebrija	L2	5	3	-	-	-	-	-	-	-
Lebrija	L3	5	4	2	-	-	-	1	-	-
Écija	E1	5	4	-	1	-	-	-	-	-
Écija	E2	4	2	-	-	-	-	-	-	-
Beas	BE1	5	3	-	-	-	-	2	-	-
Bonares	BO1	4	3	1	-	-	-	-	-	-
Niebla	N1	3	1	1	2	-	-	2	-	-

* Numbers indicate the positive sampled places within each field (up to five) showing visible symptoms compatible with FHB (Symptoms) and the presence of the *Fusarium* species tested.

Table 2. *Fusarium* strains isolated from wheat seeds by conventional method.

Field	Species (nº isolates)
U1	*F. sambucinum* (3)
U3	*F. avenaceum* (2)
U6	*F. equiseti* (1), *F. udum* (1)
L1	*F. culmorum* (8), *F. equiseti* (1), *F. graminearum* (7)
L3	*F. graminearum* (1), *F. equiseti* (1), *F. robustum* (2), *F. sambucinum* (2)
BO1	*F. sambucinum* (1)
N1	*F. culmorum* (9), *F. equiseti* (1),
BE1	*F. avenaceum* (1)

Table 3. Occurrence of *F. graminearum* (Fg), *F. culmorum* (Fc), *F. equiseti* (Feq), *F. verticillioides* (Fv) and *F. proliferatum* (Fpr) in maize fields.

Location	Field	*Symptoms	*Fg	*Fc	*Feq	*Fv	*Fpr
Utrera	UM-1	2	-	-	-	5	5
Utrera	**UM-2	1	-	-	-	1	1
Morón	MM-1	0	-	-	-	5	5
Las Cabezas	C1	1	-	-	-	5	5
Trajano	**TM-1	1	-	-	-	1	1

* Numbers indicate the positive sampled places within each field (up to five) showing visible symptoms compatible with Pink-ear rot (Symptoms) and the presence of the *Fusarium* species considered.

**Only one place sampled within the field

CORRELATION OF SEED SIZE TO DON ACCUMULATION IN SPRING WHEAT GRAIN

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ABSTRACT

Fusarium head blight (FHB) reduces grain yield, test weight, grade, and may also contaminate kernels with mycotoxins. The most common mycotoxin associated with FHB infected grain in the Northern Great Plains is deoxynivalenol (DON or vomitoxin). DON reduces the marketability of grain as food flavor, baking quality, and also animal health may be impacted after consumption of contaminated feed. In this study we explored the relationship between DON accumulation within, and seed size of, thirty six spring wheat varieties and advanced breeding lines, where seed size represents an indirect estimate of the glume: endosperm ratio. Test entries were selected from South Dakota State University (SDSU) and North Dakota State University (NDSU) spring wheat breeding programs and represented a sample of germplasm which resulted from FHB resistance breeding efforts conducted from 1998 to 2003. Field tests were carried out as a Randomized Complete Block Design with three replications. Tests were conducted at Brookings, SD and Prosper, ND during the 2004 growing season. Seed weight was obtained by weighing a 1000 seed sample of each entry, while DON concentrations were collected on ground wheat samples by NDSU Veterinary Diagnostic Services. Seed size and DON concentration data were analyzed by ANOVA both within and over locations. Location entry mean values were calculated for correlation analysis. Pearson's product moment correlation coefficients were computed to evaluate seed size and DON concentration relationships at each location and over locations. Results from Brookings reveal a negative association between seed size and DON accumulations. When all thirty six entries were included in the analysis, the correlation coefficient ($r=-0.35906$; $p=0.0315$) remained similar to after the removal of a potential outlier ($r=-0.32233$; $p=0.0501$). We will explore whether an outlier is present in this dataset and also intend to include results from the North Dakota and combined locations analysis.

APPLICATION OF REAL-TIME PCR IN THE EPIDEMIOLOGY OF FUSARIUM HEAD BLIGHT

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ABSTRACT

In epidemiological studies on FHB, fungal biomass needs to be determined in different matrices. The assays have to be species-specific, quantitative and capable of processing a high number of samples in a short time. Real-time PCR fulfills all these requirements, replacing ELISA, which has a drawback of not being species-specific. Major breakthrough in the development of diagnostic PCR applications was the invention of real-time detection systems, which monitor the amount of PCR product after each cycle rather than at the end of the reaction. Species-specific PCR primers are available for all FHB-relevant *Fusarium* species. Real-time PCR can be used in three detection modes. Firstly PCR products can be detected by means of the fluorescence of intercalating cyanine dyes. The second detection mode uses an oligonucleotide labeled with a fluorescent dye and a quencher as a probe which hybridizes with the PCR product. Finally, the melting curve analysis feature of real-time thermocyclers can be used for multiplexing when a fast, cost-effective, qualitative assay for a high number of samples is required. We adopted published species-specific PCR primers for FHB-relevant Fusarium species to all three detection modes of real-time PCR, including a new melting curve-based qualitative duplex assay for *F. culmorum* and *F. graminearum*.

The goal of our major current project on FHB is to supply quantitative data for the development of a prediction model for FHB in Germany. The model designated "FUS-OPT" consists of modules which simulate biological processes relevant to FHB, including build-up of inoculum in plant residues, sporulation of the fungus, discharge of ascospores and infection of glumes and other flower parts. The model needs to be fitted with experimental parameters first. After verification, it will be accessible by growers and plant protection services via a web interface. The purpose of the model is to help growers minimize the risk of FHB and contamination of wheat grains with mycotoxins and to give them guidance for decisions on the application of fungicides.

The effects of environmental conditions, soil management and agronomical practices (precrop, tillage, cultivar...) on key variables of the life cycle of the fungus are represented by model parameters, which need to be determined experimentally. We use three sources of samples for analysis: plant material from field trials (both artificial inoculation and natural infection), ear and grain samples collected in global FHB monitoring, and material from experiments with fungal inoculum in incubators under defined temperature and humidity. Real-time PCR with different detection modes has been used for different tasks in this project. For example, melting point-based multiplexing serves to determine which Fusarium species colonized wheat rachises in a large number of samples collected all over Germany, and quantitative species-specific assays for *F. graminearum* and *F. culmorum* are used for the estimation of *Fusarium* biomass in kernels from monitoring samples and field trials.

CYTOLOGICAL ANALYSIS OF THE INFECTION COURSE OF *FUSARIUM GRAMINEARUM* ON BARLEY CARYOPSES

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OBJECTIVES

Comparison of the infection course of trichothecene-producing and non-producing GFP-marked strains of *F. graminearum* on caryopses of different barley lines.

INTRODUCTION

The trichothecenes are mycotoxins produced by *Fusarium* spp. They are major virulence factors of *F. graminearum* infecting wheat heads (Bai et al 2001). In a co-transformation assay, we disrupted by homologous recombination the *Tri5* gene of *F. graminearum*, which encodes the trichodiene synthase catalysing the first step in trichothecene biosynthesis. Simultaneously, we integrated the gene for the green fluorescent protein (gfp) under the control of a constitutive promoter at the disrupted *Tri5* locus. The resultant transformants (*DTri5*) were shown to be deoxynivalenon deficient and expressed constitutively gfp. Additionally, the wild type (WT) strain 8/1 (Miedaner et al. 2000) was also transformed to express constitutively gfp. We used (*DTri5*)and wild type (WT) strains of *F. graminearum* to investigate the infection course on barley caryopses of cv. Chevron, a six-rowed barley with partial resistance to *Fusarium* (de la PeHa et al. 1999) and the highly susceptible cultivars Triumph and Golden Promise. Moreover, since trichothecenes are known to lead to cell death in plant tissues due to damaging the plasma membrane (Pavlovkin et al. 1986), we were especially interested in analysing the spread of the different fungal strains inside the plant tissue of barley lines differentially expressing the cell death regulator MLO.

MATERIALS AND METHODS

Fungi - All *F. graminearum* strains were maintained at 20°C on SNA-medium (Nirenberg, 1981) or stored as an aqueous conidia suspension at –70°C. For spore production the fungi were grown on SNA-medium at 20°C under near-UV-light. The Tri5 gene of *F. graminearum* isolate 8.1, a highly virulent deoxynivalenol producer, was cloned, sequenced, and disrupted by transformation mediated homologous recombination. To facilitate fluorescence and confocal microscopy WT and *DTri5* strains were additionally transformed with GFP. In general, transformations were performed according to Jenczmionka et al. (2003).

Plant Material - The barley cultivars Chevron, Triumph and Pallas and the near-isogenic backcross line BCPallas-*mlo5* (P22) were grown in the greenhouse (14-18°C, 60 % rel. humidity, 16 h light period) and daylight was supplemented if required with light from high pressure sodium lamps to maintain a constant light intensity of 15 klux. Spikes were harvested at anthesis and caryopses were placed separately in petridishes filled with 0.5 % (v/v) phytagar after removal of the glumes. Each caryopsis was covered with 10 µl of a 0.05 % Tween 20 solution containing 1,500 conidia ml^{-1} of *F. graminearum* WT or *DTri5*. After inoculation, the caryopses were incubated in a cabinet at 22°C, 16 h light period, 100% rel. humidity for three to four days.

Tissue processing for microscopic analysis - The inoculated caryopses were taken 24, 48, 72, and 96

hours after inoculation (hai). By 24 and 48 hai, the infection by *F. graminearum* was examined in stripped epicarps. To analyse the infection of inner tissues, the caryopses were frozen in tissue freezing medium at -29°C (72 and 96 hai). Sections of 50 µm thickness were cut by using a cryotome (HM 500 OM, Microm, Heidelberg, Germany). The sections were embedded in Eukitt® after thawing.

Microscopy - Fluorescence microscopy was performed as described by Hückelhoven and Kogel (1998). Confocal microscopy was performed with a CLSM (Leica TCS SP2, Leica Microsystems, Bensheim, Germany). The GFP was excitated with a 488 nm laser-line and detected at 505-530 nm.

RESULTS AND DISCUSSION

In the first 48 hours after inoculation growth of *F. graminearum* was restricted to the epicarp, the outmost layer of the barley caryopses. Infection of the plant cells took place by direct penetration through cuticles and cell walls of epicarp cells by the fungus. The cell to cell movement of *F. graminearum* in this layer showed two notable features: 1) Appressoria like swellings of the hyphae were often observed before they passed through the cell wall of an infected cell (Fig.1A). 2) The passage through the cell wall occurred often in regular intervals after branching of the hyphae which indicates the use of pits for cell-to-cell movement (Fig. 1B).

From 72 hai on, hyphae of *F. graminearum* were detected in the honeycomb shaped cells of the hypodermis (outer pericarp, Fig. 1C), and also in the chlorenchyma (inner pericarp, Fig. 1D). In cv. Chevron and Pallas the growth of the fungus was restricted to the outer three layers of the hypodermis. Only in the near-isogenic line P22 (BCPallas-*mlo5*) we also found fungal hyphae in the testa, the thin cell layer below the chlorenchyma, at 72 hai (Fig. 1F).

At 96 hai *F. graminearum* had entered the testa and aleuron layer in all investigated cultivars (Fig. 1G). Sporulation of the fungus occurred at the surface of the caryopses whereby most spores were found on caryopses of P22. Specially cv. Triumph and P22 showed vigorous collapse of the hypodermis 96 hai, whereas only in P22 large lesions were observed inside this tissue (Fig. 1H).

We found marked differences in the susceptibility to *F. graminearum* of the various barley lines. Chevron and Pallas were less susceptible than Triumph and Golden Promise.

P22 was clearly the most susceptible barley line in this investigation, indicated by a faster spread and development of the fungus and the huge lesions in the hypodermis. Both observations might be explained by the mutation in the *Mlo* locus, which leads to a loss of cell death control and, consequently, to more frequent cell death in the plant tissue. This might be favourable for the spread of necrotrophic pathogens like *F. graminearum*, as already shown for the hemibiotrophic fungi *Magnaporthe grisea* (Jarosch et al. 1999) and *Bipolaris sorokiniana* (Kumar et al. 2001).

In our study no differences in the course or strength of the infection by WT or *DTri5* strains of *F. graminearum* were detectable. Thus, the early pathogenesis of the *DTri5* strains was not affected by the *knock-out* of the trichothecene biosynthesis. These findings point up that DON is not a pathogenicity factor of *F. graminearum*, i.e. a prerequisite for infection, as already shown by Bai et al. (2001). Instead, DON seems to be important for the spread of FHB in infected spikes and therefore poses as a virulence factor of *F. graminearum*.

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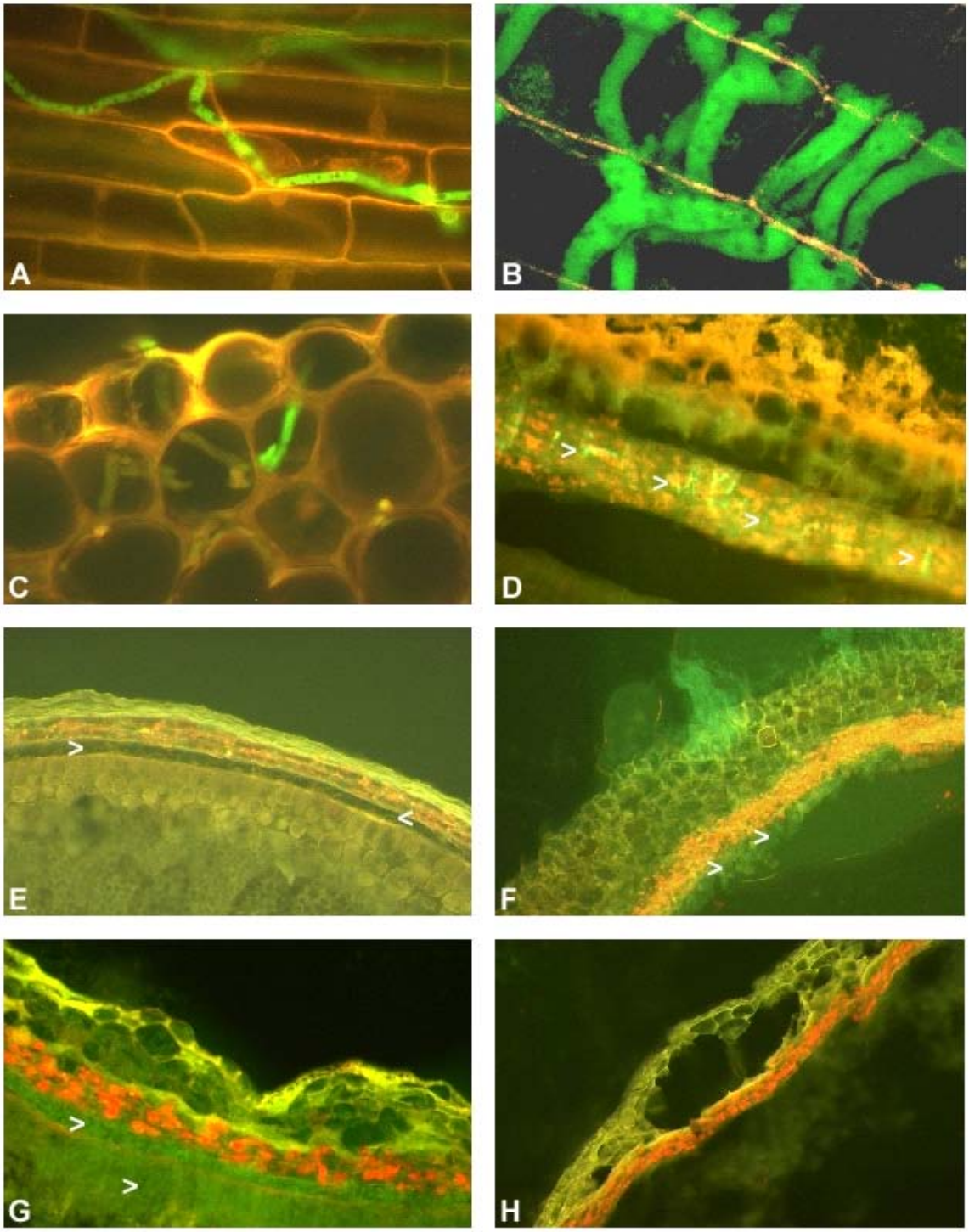

Figure 1. Microscopic pictures of barley caryopses inoculated with GFP-marked strains of *F. graminearum*. A: Fungal hypha of ΔTri5 strain in epicarp cells. B: Hyphae of WT strain passing trough cell walls of epicarp cells (A, B: cv. Golden Promise, 48 hai). C: Hyphae of ΔTri5 strain in hypodermis (cv. Chevron, 72 hai). D: Hyphae of ΔTri5 strain in chlorenchyma (arrowheads, cv. Pallas, 96 hai). E: Testa (arrowheads) free of WT hyphae (cv. Pallas, 72 hai). F: Testa (arrowheads) filled with WT hyphae (P22, 72 hai).G: Massive collapse of hypodermis and spreading of ΔTri5 strain in testa and aleuron (arrowheads, cv. Triumph, 96 hai). H: Lesion in hypodermis (P22, WT strain, 96 hai).

DEVELOPING FORECASTING SYSTEMS FOR FUSARIUM HEAD BLIGHT

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ABSTRACT

A forecasting or predictive system for Fusarium head blight of wheat was developed by a cooperative research effort among scientists at five universities in the U.S. Using historical records of weather and observations of disease severity in field plots, logistic regression models were developed to predict the probability of mean disease severity exceeding 10%. Several empirical models were developed that used weather data for the: 7 days prior to flowering; 7 or 10 days starting at flowering; or both pre- and post-flowering time windows. The currently used models, which were 80% accurate in predicting the data used in model development, utilize only pre-flowering environmental data. The implicit assumption underlying the models is that scab epidemics are determined (at least in part) by inoculum availability at flowering, and that weather immediately preceding flowering determines the magnitude of available inoculum.

In 2004, Penn State and Ohio State Universities deployed the predictive system in a web-based format for evaluation in 23 states. Separate models were used for: winter wheat with low level of corn residue (i.e., relatively low inoculum density in the region); winter wheat with high corn residue (i.e., relatively high inoculum density in the region); and spring wheat production systems. Scab risk maps at a 20-km resolution were produced using temperature and relative humidity data obtained from the National Weather Service, Rapid Update Cycle (RUC) system, and also rainfall data obtained based on Doppler radar estimates. Data from specific weather stations also were made available to the users in the region, to be utilized as a secondary way of obtaining predictions.

Current research concerns the validation of the system, and the development of more accurate models for scab risk prediction, based on additional scab observations, weather data for different time windows, and the integration of empirical observations of epidemics with results from field and laboratory studies on scab. Consideration is being given to predicting the risk of other severities of disease or of DON level in grain. The current system was generally accurate in field testing, but improvements in accuracy are needed. The concept of model validation for such a large-scale warning system will be discussed in some detail, and presented in the context of Bayesian decision theory.

ENVIRONMENT AND INTERACTIONS WITH OTHER FUNGI ON GROWTH AND DON PRODUCTION BY *F. CULMORUM* AND *F. GRAMINEARUM*

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OBJECTIVE

Fusarium species do not exist alone in the phyllosphere of ripening ears. It is thus inevitable that interactions between these ear blight pathogens and other species occurs. Environment and fungicides all impact on these interactions and on mycotoxin production. Very few studies have examined these interactions especially in relation to other phyllosphere genera such as *Alternaria, Microdochium, Aureobasidium, Cladosporium* and *Penicillium*. These interactions need identifying to enable interpretation of the mycotoxin contamination obtained.

INTRODUCTION

Fusarium infection is of concern because of impacts on crop yield and quality and the concomitant contamination with trichothecene mycotoxins, particularly deoxynivalenol (DON) and nivalenol (NIV) which are produced under conducive environmental conditions during ripening of cereals. While suppression of FHB is partially achieved by the application of fungicides this also affects the balance of mycoflora colonising the ripening ear and flag leaves which can impact on colonisation of ears and mycotoxin production. Very few studies have examined the impact that such interactions may have on colonisation potential of *F.culmorum* and *F.graminearum* and on DON production (Magan & Lacey, 1984). The competitiveness of these species is influenced also by the competitiveness of the other species, especially *Microdochium* which is more effectively control by foliar fungicides and by weakly parasitic genera such as *Alternaria* and *Cladosporium*, the so called sooty mould species (Magan & Lacey, 1986; Magan et al., 2004). In the UK *F.graminearum* is becoming more common as fodder maize is used more often in the southern regions as a break crop. This may act as a reservoir for F.graminearum for wheat infection.

MATERIAL AND METHODS

Growth and interactions between Fusarium species and other mycoflora: Studies were conducted on wheat grain of different water availabilities containing 0.5 ug/g of fungicide (azoxystrobin, propiconazole, epiconazole) at 15 and 25oC. The interactions with *Alternaria tenuissima, Cladosporium herbarum, Microdochium nivale var majous and Pencillium verrucosum*. Interactions were scored and growth rates measured. DON levels were quantified (Magan et al., 2002).

Niche overlap indices: Studies were conducted to compare the capacity of *Fusarium* pathogens and other competing mycoflora for utilisation of key nutritional compounds present in wheat grain to determine whether co-existence or niche exclusion occurs between these species under different environmental conditions (Magan et al., 2003).

Field interactions: *F.culmorum* was applied to ripening ears in 2003 and 2004 to examine the population development and relative impact on community structure on the ripening ears. This was done by serial washings and isolations from ripening grain.

RESULTS

Growth and interactions between Fusarium species and other mycoflora:

Growth of *F.culmorum* and *F.graminearum* and competitiveness against other species was influenced by temperature and water availability. *F.graminearum* was more competitive that *F.culmorum* against all other species at 25°C. However, at 15°C they were mutually antagonistic to each other with no dominance. The presence of a triazole or strobilurin fungicide did not modify this significantly. Generally, at 15°C and freely available water interactions with other fungi or fungicide resulted in a reduction of DON. Howver, *C.herbarum* and *M.nivale* and *P.verrucosum* resulted in a stimulation of DON especially at reduced water availability.

Niche overlap indices: Niche size for *F.culmorum* and *F.graminearum* was similar under freely available water conditions. However, under drier conditions the niche size for the former species was bigger (18 vs 7). The NOI for these two species suggests that they do not share there niche with other species under environmental stress, but do so when water is available.

Field interactions: Inoculation with *F.culmorum* was effective and resulted in population establishement over a two week period after inouculation. The populations of other species varied with whether fungicides had been applied. The presence of high populations of *Fusarium* species always coincided with low populations of *Alternaria*. There were no significant differences between DON levels sothat interpretation of outcome of interactions on the ripening ears were difficult. Again, NIV levels were higher than those for DON as *F.poae* was common in 2003.

DISCUSSION

This study was focussed on understanding the relative competitiveness of *F.culmorum* and *F.graminearum* in vitro and in situ. Generally, *F.graminearum* is more competitive under slight warmer conditions, and is less so in cooler climatic regimes. Fungicides do influence growth and competitiveness in the presence of other mycoflora species which colonise ripening ears and sometimes stimulate DON or NIV production.

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THE TRICHOTHCENES ARE MAJOR VIRULENCE FACTORS OF *FUSARIUM GRAMINEARUM* TO WHEAT, BUT DISPENSIBLE FOR INFECTION OF MAIZE AND BARLEY

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ABSTRACT

In a comprehensive study we investigated whether virulence of *Fusarium graminearum* is only determined by the presence of the trichothecenes or is a quantitative character that is heterogeneously determined by several factors differing from one isolate to the other. Three isolates of *F. graminearum*, well characterized in field experiments were selected: FG06, a medium aggressive isolate producing mainly nivalenol (NIV chemotype), FG25, a medium aggressive isolate of the deoxynivalenol (DON) chemotype, producing medium levels of DON, FG2311, a highly virulent isolate of the DON chemotype, producing high levels of DON.

The Tri5 genes of these three isolates were cloned, sequenced, and disrupted by transformation mediated homologous recombination. Disruption mutants were found to grow in vitro like the respective wild type but were unable to produce trichothecenes. The mutants in comparison to the respective wild types were tested on wheat and barley as well as on maize for their ability to develop FHB or cob rot.

In wheat, despite the initial aggressiveness of the three different wild type isolates, all the disruption mutants showed a basal infectivity to the inoculated spikelet but were unable to spread into the entire head.

In contrast to that, in barley and maize, the mutants showed no visible difference from the wild types, all were fully aggressive.

Hence, for the first time it could clearly be shown that both trichothecenes, DON and NIV contribute in the same amount to aggressiveness of *F. graminearum* to wheat. Furthermore, it could be demonstrated that the trichothecenes belong to the host specific toxins with a visible effect only to wheat and not to barley and maize.

COMPARISON OF METHODS FOR DEVELOPING FUSARIUM HEAD BLIGHT FORECASTING MODELS

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ABSTRACT

Fusarium head blight (FHB) is an important disease of wheat and barley east of the Rocky Mountains. A forecasting system with 70% accuracy has been deployed; however, models with improved accuracy could help producers manage the disease, as well as the associated mycotoxins. Our objective was to produce a more accurate model for the prediction of FHB epidemics in wheat. We defined epidemics with a threshold of 10% of severity. New prediction models were developed using 124 cases that included hourly weather, crop growth stage, disease level, and a variable for corn residue and wheat type (winter/spring). The cases came from the years 1982-2003 from 7 different states. The cases were divided into data sets used for model development (n=86) and validation (n=38). Logistic regression, non-parametric discriminant analysis, decision tree models and neural networks were compared for accuracy and other diagnostic criteria using both model development and validation data. Identified models used temperature, relative humidity, rain, and corn residue to distinguish epidemics from non-epidemics. We identified models that had the greatest accuracy without sacrificing sensitivity (percentage of correctly classified epidemics) and specificity (percentage of correctly classified non-epidemics). Accuracy of all four modeling approaches was greater than 80% for both model development and validation data sets. Sensitivity and specificity of the selected models was also close and, in several cases, greater than 80%. Classification trees and logistic regression models performed better than the other methods evaluated. Models with a higher level of complexity (with interactions) performed 2-5 percentage points better than models composed of single terms.

AWNS REDUCE FHB INFECTION IN NEAR ISOGENIC BOWMAN BARLEY WITH DIFFERENT AWN LENGTHS

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ABSTRACT

Both 6-rowed and 2-rowed barley cultivars used for malting in the upper mid-west of the US have long awns about twice as long as the spike and extending beyond the tip of the spike up to 1.5 times the spike length. In addition, depending on cultivar, the awns can have a rough surface and vary from appressed to slightly flared around the spike. The awns can potentially filter spores and applied fungicides from the air and reduce the quantity of spores and fungicides alighting on the kernel if rain or dew does not later wash them down the awn. Hyphae from spores that land on awns are unlikely to directly penetrate the very thick walled cells of the awn and growth down the awn toward the kernel and the developing embryo is likely to be inhibited by diurnal cycles in heat, light and humidity. The awned character is simply inherited in barley and near-isogenic lines of bowman barley that were fully awned, partially awned and awnless were supplied by Dr J Franckowiak at NDSU. A factorial design experiment of the three near- isogenic barley lines and 3 fungicide treatments (Folicur 290 ml ha^{-1}, JAU 6476 415 ml ha^{-1}, water control) was conducted in an inoculated and mist irrigated field site in Fargo in 2004. The length of awns significantly affected the percent fusarium head blight (FHB) infection in the spikes ($P<0.000$) with the awnless line having 14.7 % infection compared to partially awned 5.0% and fully awned 4.4% lines. This result was consistent with preliminary experiments where a similar trend was seen when the partially awned and awnless lines were compared. There was a significant effect of fungicides on percent FHB infection in the spikes ($P<0.02$) with the fungicide JAU 6476 significantly reducing disease compared to the water control. None of the fungicides significantly reduced deoxynivalenol (DON) concentration. There was no statistically significant awned character x fungicide interaction which suggests that under the conditions of this field experiment, awns did not influence the effectiveness of the fungicides.

2004 FHB MONITORING FOR SPRING WHEAT IN SOUTH DAKOTA

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INTRODUCTION AND OBJECTIVES

Fusarium head blight (FHB) of wheat and barley, caused by numerous *Fusarium* species, but primarily by *Fusarium graminearum* (teleomorph: *Gibberella zeae*) continues to occur at epidemic levels in some regions of the U.S. and Canada and at sub-epidemic levels in many wheat growing areas in the central and eastern U.S. Recent advancements in the forecasting of FHB have been put forth for evaluation by the public, and by researchers in key FHB states. Collaborators in South Dakota, North Dakota and Minnesota (spring wheat region) and also in Ohio, Indiana, Pennsylvania, and other winter wheat growing areas have agreed to evaluate the forecasting tools arising from several years of research under the U.S. Wheat and Barley Scab Initiative (USWBSI). In South Dakota, FHB, or scab, has been a chronic threat to wheat production in the northeastern part of the state, though it has also been found to occur at high levels throughout the eastern half of the state under favorable conditions. Producers and crop advisors in South Dakota are very interested in a FHB-predictive system for scheduling fungicide treatment, and making economic decisions during the growing season. In 2002 and 2003, South Dakota State University (SDSU) delivered a web-based risk advisory system for FHB in the northeastern part of SD based on predictive models released by Ohio State University (De Wolf et al. 2003). In 2004, a web-based forecasting system was placed in service by Pennsylvania State University (PSU) researchers in collaboration with the USWBSI. The system was intended to incorporate most of the FHB-affected areas in the US through the use of a broad-based meteorological system and newer FHB-forecasting models. Daily forecast information could be retrieved by accessing the internet site (www.wheatscab.psu.edu). The system provides risk information, weather data, and multiple models (spring wheat, winter wheat, and winter wheat over corn residue) in a graphical interface. Weather data is modeled over a 20km grid, resulting in risk maps for any of the participating states.

The objective of this research was to closely monitor wheat and FHB development throughout the northeastern part of South Dakota historically most affected by FHB. The information collected by SDSU researchers would then serve to assess the accuracy and precision of the broad-based PSU system for predicting FHB on spring wheat in South Dakota. This is also part of a larger effort underway in several states to further define the epidemiology of Fusarium head blight and validate FHB-forecasting systems for spring wheat, winter wheat, and barley.

MATERIALS AND METHODS

Field plots of spring wheat (*Triticum aestivum* L.) were established near Brookings, Redfield, Groton, and Watertown in northeastern South Dakota. Plots of 'Norm', a FHB-susceptible spring wheat, were at planted into spring wheat variety trials in large blocks measuring 6.1m by 15.2m. Seven-day recording spore samplers were placed at each location along with temperature and humidity recording devices (dataloggers). Each site was located in close proximity to SD Automated Weather Data Network (SDAWN) weather stations. Plots were planted into tilled fields previously planted with soybeans (2003). At each location, wheat phenology was monitored to assess flowering date and maturity. Near crop maturity, FHB incidence (% of plants affected) and severity (% area of spikes affected, on average, based on Stack and McMullin, 1995) was noted . At harvest, several samples of grain were collected at each location for enumeration of *Fusarium*-damaged kernels (FDK) and for mycotoxin analysis to assess DON-contamination. DON analysis was performed at North Dakota State University Veterinary Diagnostics Labora-

tory using Gas Chromatography – Electron Capture techniques.

RESULTS AND DISCUSSION

Disease levels varied widely across the region from minor amounts (1-2% severity) to major epidemic levels in local areas. The 2004 Brookings plots showed the greatest level of disease, with 60% field severity (for highly susceptible variety 'Norm'). Development of the disease was later than in previous years. Weather was very cool throughout much of the anthesis period season, with warm weather coming later as seeds developed (Fig. 1). Scab was especially severe at the Brookings, Watertown, and Groton locations (Table 1). Redfield had high incidence though overall severity was not high. Flowering dates, and disease data are shown in Table 1. Inoculum data, as well as DON data were not available at the time this report was written, and will be added at a later time.

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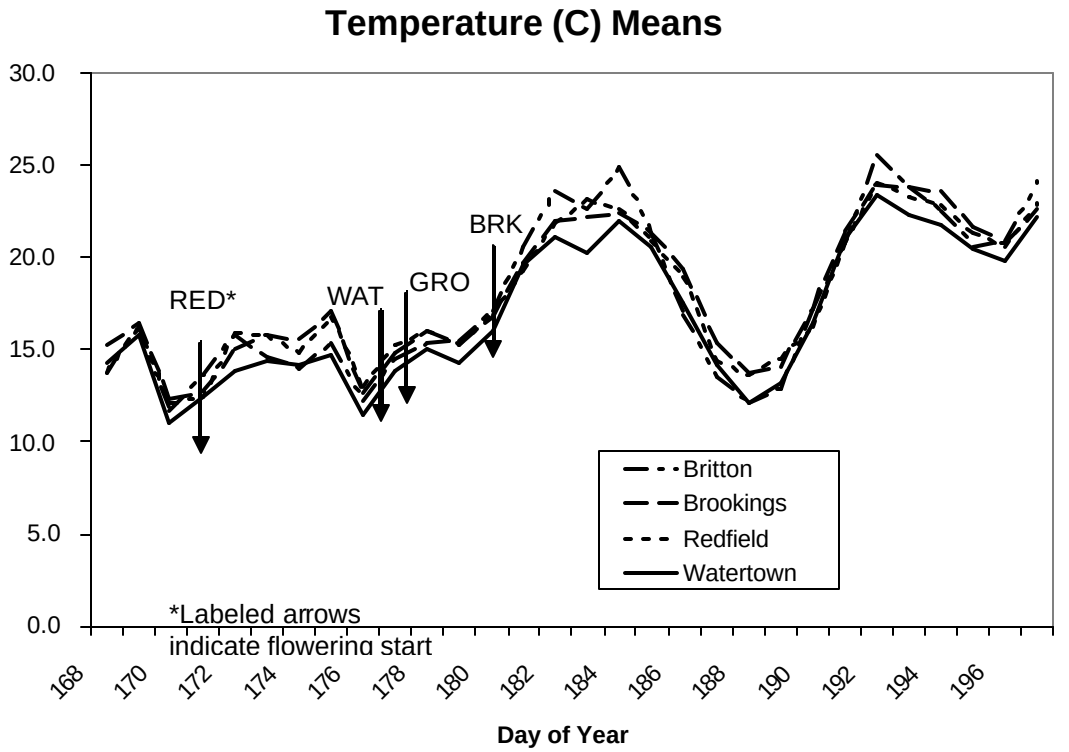
Table 1. Field Locations and Disease Data

Location	Variety	Anthesis Date (DOY)	Incidence (%)	Plot Severity
Watertown	Norm	June 25 (177)	70	10%
Groton	Norm	June 26 (178)	60	10%
Redfield	Norm	June 19 (171)	40	5%
Brookings	Norm	June 28 (180)	80	60%



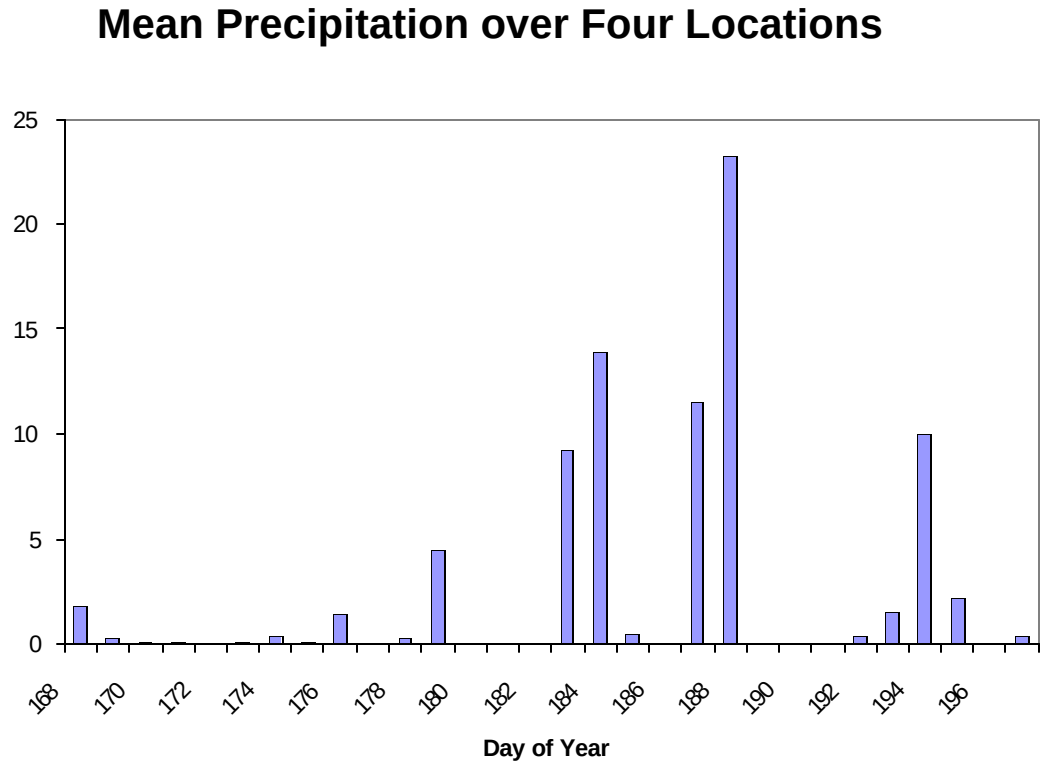


Figure 1. Temperature and Precipitation for FHB Monitoring Locations, 2004.

INOCULUM DISTRIBUTION AND TEMPORAL DYNAMICS WITHIN THE SPRING WHEAT CANOPY

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OBJECTIVES

The objective of this research is to quantify the amount of *Fusarium* head blight inoculum on leaves and head tissues within the wheat canopy in order to define the vertical spatial variability and temporal dynamics of FHB inoculum.

INTRODUCTION

Fusarium head blight (FHB) of wheat and barley can be caused by several species of *Fusarium* but is primarily caused by *F. graminearum* (teleomorph: *Gibberella zeae*). The disease is thought to be primarily initiated by ascospores of the sexual form of the fungus. Wheat is most susceptible to infection during anthesis. Perithecia of the fungus develop near the soil surface on infected crop residues such as corn stalk tissues and small grains straw. The ascospores are forcibly ejected from perithecia, but their fate is not certain. It is believed that most infection takes place during a relatively short period of time when the wheat crop is flowering, and only if environmental conditions are adequate for fungal development. It is not well understood how ascospores are deposited on susceptible spikes. In other words, the presence or absence of *Fusarium*-colonized residues beneath the wheat canopy may be less important if there are other major inoculum sources within the vicinity (and upwind) of the susceptible crop, and conditions are highly favorable for inoculum development and infection. Also, FHB can be initiated by asexual propagules (conidia) as well as ascospores. Observations indicate that epiphytic production of conidia is possible on wheat leaves (S. Ali, pers. comm.). Previous research shows that both ascospores and conidia are found on wheat leaves throughout the canopy, and that a bimodal distribution of ascospores on wheat leaves within the canopy was present (i.e. higher concentrations at the uppermost, and lowermost healthy leaves) (Osborne et al., 2002) suggesting the importance of both lower canopy (residue and leaves) and airborne propagules (from distant or local sources). This study expands upon previous findings to look at changes in inoculum density on leaves and spikes over time as well as to further examine the bimodal aspect of ascospore deposition.

MATERIALS AND METHODS

Field plots of spring wheat (*Triticum aestivum* L.) were established near Aurora, Redfield, Groton, and Watertown in northeastern South Dakota. Plots of 'Norm', a FHB-susceptible spring wheat, were planted into spring wheat variety trials in large blocks measuring 6.1m by 15.2m. Seven-day recording spore samplers were placed at each location along with temperature and humidity recording devices (dataloggers). Each site was located in close proximity to SD Automated Weather Data Network (SDAWN) weather stations. Plots were planted into tilled fields previously planted with soybeans (2003).

Spore enumeration

Each location was sample four times, at 6 to 8 day intervals. For each sampling event, six samples were collected consisting of five primary tiller stems with leaves. In the laboratory, each sample was dissected into the following components (subsamples): spike (if emerged), flag leaf, second leaf from the top (flag-1), and the fourth leaf from the top, (flag-3). All corresponding components from a sample were combined and placed into a 250ml Erlenmeyer flask, with 50ml of deionized water + Tween80 nonionic surfactant (0.05% v/v). Subsamples were shaken at 300 rpm for five minutes to dislodge spores, then the leaves were discarded, and the resulting suspension was cen-

trifuged for seven minutes at full speed in a bench-top clinical centrifuge. The supernatant was discarded, and the pellet re-suspended into 15ml of deionized water, and centrifuged again for five minutes at full speed. The supernatant was decanted to leave 3.0ml remaining in the tube. The pellet was then re-suspended and two 1.5ml aliquots were placed into Eppendorf tubes. Glycerin was added to one of the aliquots to make up 10% v/v. The tube was then kept at –20°C until microscopic evaluation could be performed. The second aliquot was plated onto each of three plates of Komada's *Fusarium*-selective agar (500ul per plate). Plates were placed into 23°C incubators with 12hr dark/12hr light cycles and allowed to develop for 5-6 days. Plates were examined for *Fusarium graminearum* type colonies, which were counted and recorded. The second (frozen) aliquot was examined microscopically. *Fusarium graminearum* conidia and the ascospores of the teleomorph *G. zeae* were counted under 400x magnification, using a hemacytometer/cell counting chamber. Analysis of variance was performed on total inoculum counts for each location across sampling dates and plant components.

RESULTS AND DISCUSSION

Across all locations, inoculum levels were generally highest on lower leaves (up to over 4000 CFU per leaf), and no bimodal distribution was obvious with respect to colony forming units (CFU) on leaves, which is contrary to earlier results. Figures 1-4 represent the mean levels of inoculum density on plant tissues within the canopy for four locations in 2004. There were greater numbers of CFU on spikes than on flag leaves at the later samplings which is likely a result of *Fusarium* colonization and reproduction, often evident on spikes in the form of sporodochia. The colonization of the flag leaf is interesting in that this may serve as a source of inoculum for spikes during anthesis. Spring wheat often flowers at or soon after spike emergence, prior to elongation of the culm tissue. This positions the susceptible spike tissue in close proximity to spore-laden flag leaves. If heavy dew or rain-splash conditions were present, it is quite likely that conidial or ascospore inoculum is transferred to the spike. If such inoculation occurs frequently, then spore survival and distribution information would be critical to fully understanding the epidemiology of FHB on wheat and other grains.

Microscopic counts of conidia and ascospores were incomplete at the time of manuscript preparation therefore they are not addressed at this time.

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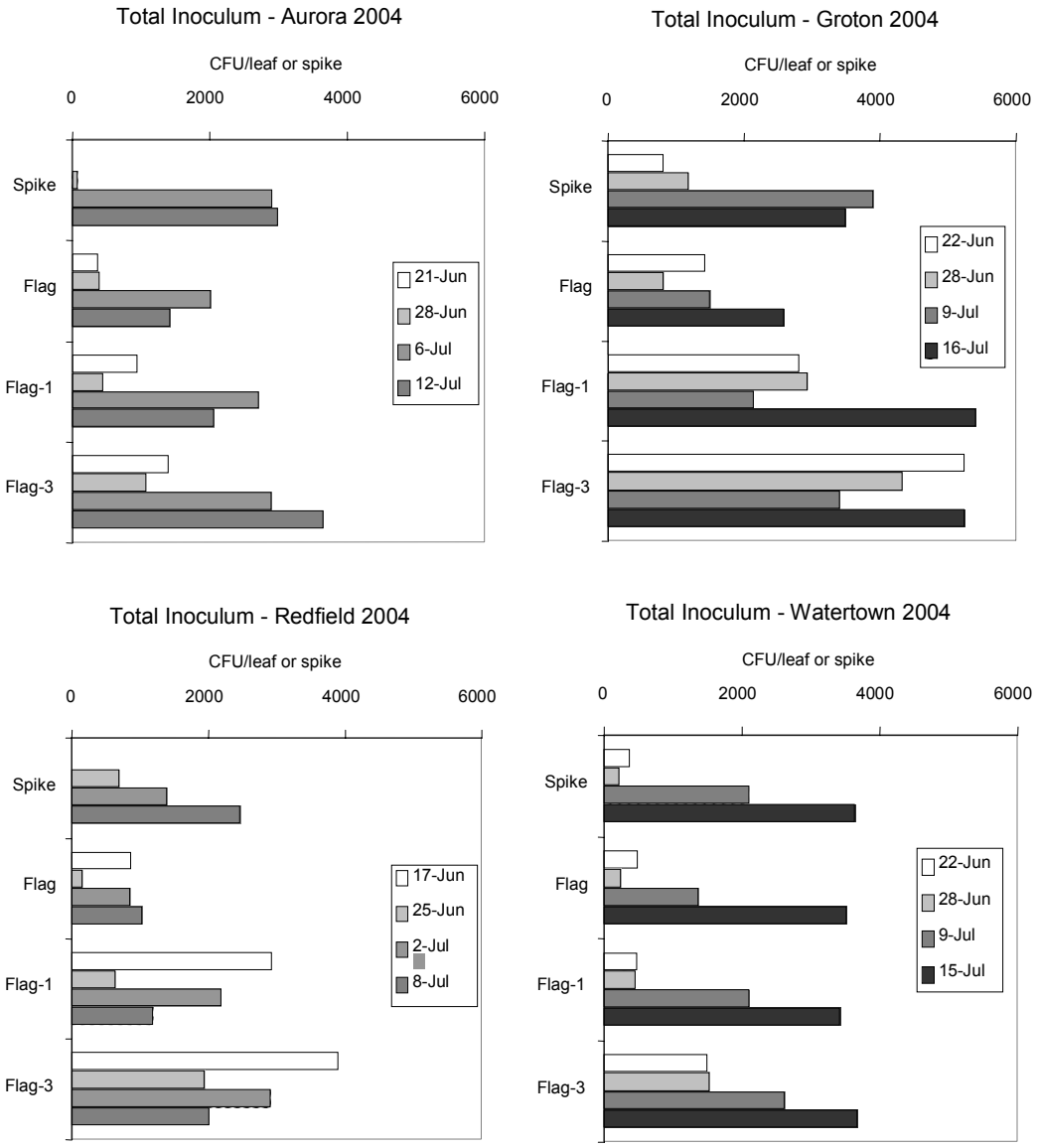

Figure 1. Inoculum estimates for four locations in 2004.
Each graph represents four sampling times, and four plant components (spike, flag leaf, flag leaf-1, and flag leaf-3 (usually the lowermost healthy leaf). Values represent all *Fusarium graminearum* type colonies on Komada's medium.

INOCULUM GRADIENT OF *GIBBERELLA ZEAE* FROM SMALL AREA SOURCES WITHIN WHEAT CANOPIES IN OHIO

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ABSTRACT

This study was designed to document the spread of propagules of *Gibberella zeae*, causal agent of Fusarium head blight of wheat, from an area source of inoculum located at the corner of wheat plots (58 m^2) during the 2004 growing season. Maize kernels were infested with a mixture of several isolates of *G. zeae* and places on the soil surface in a cleared area (1.5 x 1.5 m) at the southwestern corner (downwind) of each plot. Samples of wheat spikes and rain splash were collected at 1.5-m intervals at four points in three directions (north, northeast, and east) from the source of inoculum. Rain splash was collected using sheltered rain gauges placed at 30 and 100 cm above the soil surface. Wheat spikes were collected and washed in sterile distilled water to remove spores. Aliquots of splashed rain and spike wash solutions were transferred to petri plates containing Komada's selective medium, and *G. zeae* was identified based on colony and spore morphology. Colony forming units (CFUs) per milliliter of splashed rain and CFUs per spike were used as a measure of spore dispersal. Based on the results of this study, spores were recovered from rain splash and wheat spikes at every distance and direction from the source of inoculum, however, their abundance decreased with increasing distance from the source. The mean number of CFU per spike decreased from 42 to 28 as distance increased from 0.30 to 4.80 m. Similarly, averaging across sampler height, direction, and rain event, the mean number of CFUs per milliliter of splashed rain decreased from 8.8 to 1.5 as distance increased from 0.30 to 4.80 m. Log transformed distance (LD), height, and the interaction between LD and height significantly affected the number of spores recovered from splash samplers. Log-transformed distance from the source of inoculum also had a significant effect on the mean number of spores recovered from wheat spikes and mean disease severity. Mean disease severity decreased from 17 to 11% as distance increased from 0.30 to 4.80 m. Direction had no significant effect on spore dispersal and disease intensity. Further investigation of these relationships would help us to determine how important a within-field source of inoculum is in an area with background inoculum density and would reveal how influential rainfall intensity is on the amount of inoculum dispersed and the distance traveled from the source.

RELATIONSHIP BETWEEN THE ENVIRONMENT AND THE NUMBER OF *GIBBERELLA ZEAE* PROPAGULES RECOVERED FROM WHEAT SPIKES

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OBJECTIVES

The objectives of this study were to determine the relationship between weather variables and the abundance of inoculum of *Gibberella zeae* on wheat spikes and to develop models describing this relationship. Ultimately, we hope to use inoculum density as an indicator of potential risk of FHB epidemics and incorporate this information into an existing FHB risk assessment model.

INTRODUCTION

Fusarium head blight (FHB) of wheat is caused predominantly by *Gibberella zeae* (Schwein.) Petch (anamorph: *Fusarium graminearum* Schwabe) in North America. The increased intensity of FHB has been attributed to the widespread adoption of minimum tillage, a practice which favors the survival of *G. zeae* between growing seasons. Spores produced in the residue are disseminated to the spikes, causing severe blighting under favorable weather conditions. Knowledge of the relationship between the amount of inoculum of *G. zeae* on wheat spikes and FHB intensity is important for understanding the contribution of different sources of inoculum to the development of this disease. The results of several inoculation studies conducted under controlled conditions demonstrated that there is a direct relationship between inoculum dose and FHB development (Andersen, 1948, Bai, 1995, Fauzi and Paulitz, 1994). According to Bai (1995), as the number of spores of *G. zeae* per floret increased, incubation period decreased, and FHB incidence increased. Fauzi and Paulitz (1994) reported that log spore concentration explained 91% of the variation in arcsine-transformed percentage of spikelet diseased. For spores to reach the spikes, they first have to be produced, released, transported, and deposited. Several weather variables influence these processes.

MATERIALS AND METHODS

Sample collection and species identification. Samples of wheat spikes were collected daily between Feekes GS 10 and 11.2 during the 2000, 2001, 2002 and 2003 wheat growing seasons in Indiana, Manitoba, North Dakota, Ohio, and South Dakota. Each sample consisted of 5 spikes collected at 11:00 am. Spikes were washed in 50 ml sterile distilled water and an aliquot was transferred to replicate plates of Komada's selective medium. Colonies were grown at room temperature for 1 wk under a 12-h photoperiod. Colony forming units (CFUs) per plate were counted and categorized based on colony morphology. Sample colonies of each type were transferred to potato dextrose agar (PDA), carrot agar, and/or carnation leaf agar for identification of *Fusarium* species (Nelson et al. 1983).

Weather monitoring and data organization. An automated weather station (Campbell Scientific Inc., Model CR10X, Provo, UT) was deployed to record temperature (degree Celsius), rainfall (mm), surface wetness (kilo-ohms of electrical resistance, converted to a nominal scale [dry or wet]), relative humidity (percentage), wind speed ($m\ s^{-1}$), wind direction (degrees), and solar radiation ($KJ\ m^2 s^{-1}$) at 30-min intervals. Rainfall amounts were recorded using a Tipping Bucket Rain Gauge (Model TE525WS, Campbell Scientific Inc., Provo, UT) with a 20-cm-diameter collector and a 0.25-mm resolution. The data were edited to generate average, maximum, minimum, and cumulative hours for each variable for 1-, 5-, and 7-day periods prior

to the dates samples were collected. To avoid discontinuity in high-RH and surface wetness periods, a day was defined as a 24-h period beginning and ending at 1200 h (noon). For each period, the following variables were generated: i). hours of temperature > 9°C, ii). hours of temperature between 15 and 30°C, iii). hours of RH between 75 and 90%, iv). hours of RH > 90%, v) hours of RH >95%, and vi.) hours of coincidence of various combinations of temperature and RH within the aforementioned ranges. These variables were generated for 24-h, daily (600 to 1800h), and nightly (1800 to 600h) periods.

Data analysis. A total of 287 observations were collected. Head wash spore count was expressed as CFU/head and log transformed (log[CFU+1]) to stabilize variances. Correlation analysis was performed using PROC CORR of SAS (SAS, Cary, NC). For each sample date, rainfall intensity and duration from the previous day were used.

RESULTS AND DISCUSSION

Variables summarized for consecutive 5- and 7-day periods prior to the day samples were collected were more strongly correlated with the log of CFU/head than variables summarized for the 24 h prior to sampling. Average nighttime canopy temperature (°C) for the last 7 days prior to sampling (AVNCT7), average nighttime relative humidity (%) of the air for the last 7 days prior to sampling (AVNARH7), surface wetness duration (h) for the last 5 days prior to sampling (WD5), average wind speed (m/s) for the last 7 days prior to sampling (AVWS7), number of hours with both air temperature > 9°C and relative humidity > 95% for the last 7 days prior to sampling (AT9RH957), and rainfall intensity for the day prior to sampling had the highest correlations with the log of CFU/head (Fig 1). This probably reflects the fact that extended periods of high moisture conditions (Dufault et al., 2002) and temperatures between 15 and 28°C (Tschanz et al., 1976) are necessary for the production of ascospores of *G. zeae*. Ascospore discharge occurs during periods of high relative humidity (Ayers et al., 1975, Paulitz, 1996) and temperatures between 11 and 30°C (Paulitz, 1996). Reported associations between rainfall events 1-4 days before spore sampling and inoculum density (De Wolf et al. 2001, Francl, et al. 1999, Rossi, 2002), and the results of splash dispersal studies (Paul et al., 2004) suggest that rainfall plays a key role in the dispersal of inoculum of *G. zeae*. Although spore morphology and spore sampling studies indicate that spores are also wind-disseminated (Paulitz, 1996), the results of this study showed that wind speed was negatively correlated with the log of CFUs/head (Fig. 1). Even though spores are wind-blown, they may not be deposited on wheat spikes if the conditions are not favorable. High wind speeds are probably unfavorable for spore deposition. Only a small fraction of the spores in the air may actually reach the infection court. Burkard spore counts (airborne spores) are not always correlated with head wash spore counts (De Wolf et al. 2001, Osborne, 2000, Paul et al. 2003, Shaner and Buechley 2000).

ACKNOWLEDGEMENTS

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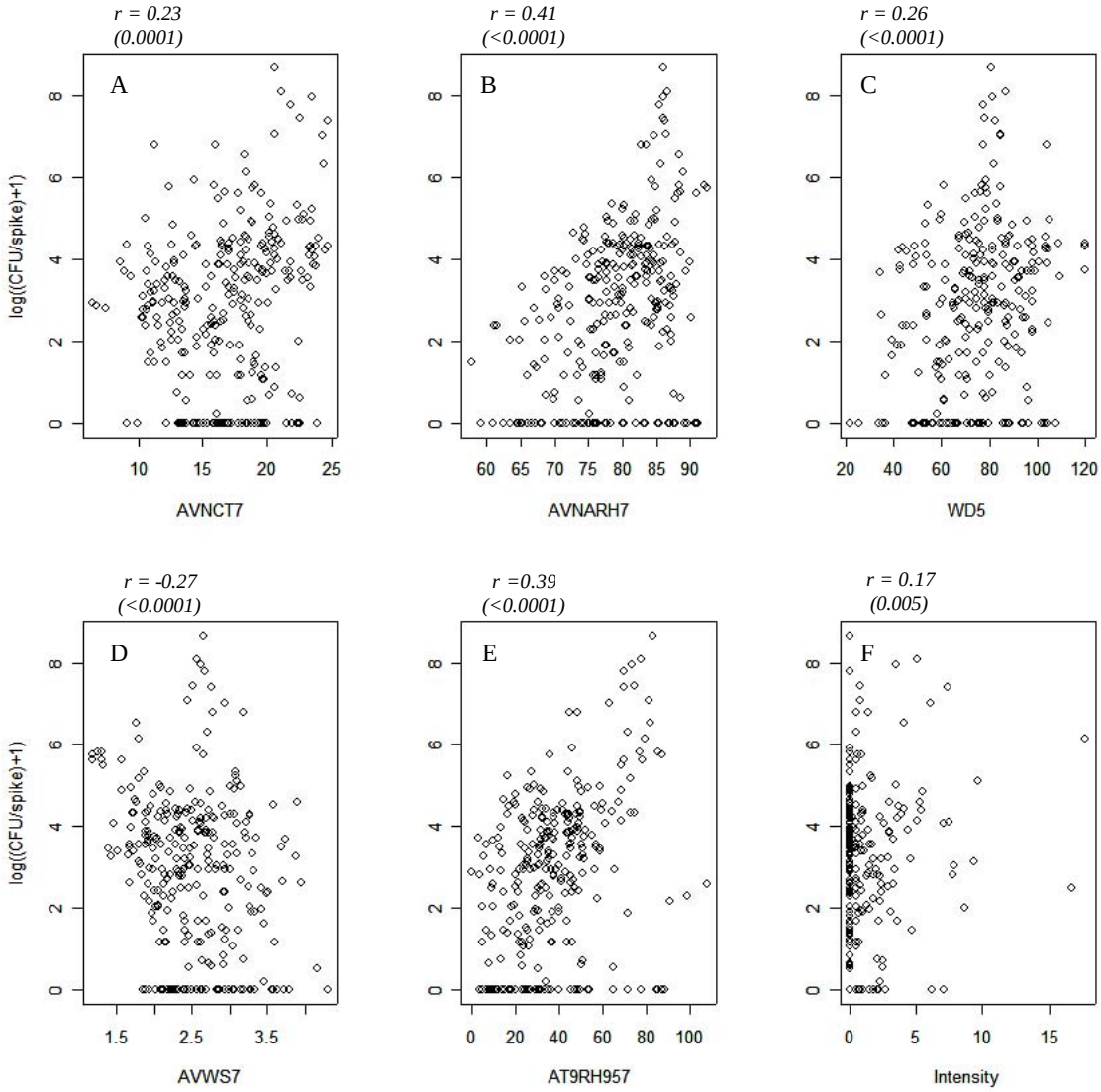

Figure 1. Scatter plots depicting the relationship between log-transformed CFU/spike and AVNCT = average nighttime wheat canopy temperature (^{o}C) (A); AVNARH = average nighttime air relative humidity (%) (B); WD = surface wetness duration (h) (C); average wind speed (m/s) (D); number of hours with both air temperature > 9^{o}C and relative humidity > 95% (AT9RH95) (E); and rainfall intensity (mm/h) (F) for the day prior to the date samples were collected. Temperature, relative humidity, and wetness variables are for 5 or 7 day periods prior to the date samples were collected. The correlation coefficients (*r*) and their corresponding probability values are indicated above each plot.

FUSARIUM SPECIES PRESENT IN WHEAT AND BARLEY GRAINS IN URUGUAY

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ABSTRACT

Fusarium head blight (FHB) of wheat and barley has been a sporadic disease in Uruguay in the past. However, its occurrence has increased over the last decade, with epidemics occurring in 2001 and 2002. *Fusarium graminearum* is the main species causing FHB in both crops. However, there has not been a systematic survey of *Fusarium* species present in wheat and barley grains in different cultivars, locations, and years. Grain samples (0.2 kg) were collected in 2001 and 2002 from regional cultivar evaluation trials. Five barley and four wheat cultivars that account for the bulk of the commercial production in different environments (locations and planting dates) were tested each year. Mean percentage of wheat grains colonization by *Fusarium* spp. was 32% in 2001 and 51% in 2002. Colonization of barley grain was 31% and 18% for 2001 and 2002, respectively. The greatest percentage of *Fusarium*-colonized grains and highest deoxynivalenol (DON) content occurred in the more FHB-susceptible cultivars. *F graminearum* was the most frequently recovered species both years in both crops from all cultivars and all environments. *F. graminearum* represented 77% and 60% of all *Fusarium* species isolated in wheat grains in 2001 and 2002, respectively and 65% and 51% in barley grains in 2001 and 2002, respectively. Other species recovered in wheat grains were: *F. avenaceum* (10.8%, 2001; 16.6%, 2002), *F. culmorum* (5.7%; 4.2%), *F. poae* (3.9%; 9.8%), and *F. equiseti* (2.8%; 0.9%). *F. acuminatum* (2.1%) and *F. trincictum* (5.9%) were only recovered from wheat in 2002. *Fusarium* species recovered in barley grains included *F. poae* (17.2%, 2001; 29.4%, 2002), *F. avenaceum* (2.7%; 5%), *F. equiseti* (11.6%; 5.8%), *F. sambucinum* (1%;1.2%), and *F. trincictum* (1.2%;1.8%). *F. semitectum* (1.2%), *F. chlamydosporum* (2.2%) were only recovered from barley in 2002. All species were pathogenic on wheat and barley in inoculation tests in the greenhouse, except *F. semitectum* on wheat. Greater levels of FHB severity and FHB incidence on wheat and barley spikes were obtained with the *F. graminearum* isolates, followed by *F. avenaceum* and *F. poae*. Data from this study raises the concern of presence of other mycotoxins different from DON in wheat and barley grains.

SURVIVAL OF *GIBBERELLA ZEAE* AND INOCULUM CONTRIBUTION OF DIVERSE PLANT SPECIES IN PREVALENT CROP ROTATIONS IN URUGUAY

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OBJECTIVES

To quantify *Gibberella zeae* survival and inoculum production on some gramineous and non-gramineous residues commonly present in the Uruguayan production systems

INTRODUCTION

Fusarium head blight (FHB) has become one of the most devastating diseases of wheat and barley in the southern cone of South America. Particularly, in Uruguay its occurrence has increased in the last decade with moderate to severe outbreaks every two years (Pereyra and Díaz de Ackermann, 2003). The main pathogen associated with FHB of wheat and barley in Uruguay is *Fusarium graminearum* Schwabe [perfect stage *Gibberella zeae* (Schwein.) Petch] (Boasso, 1961; Pritsch, 1995; Pereyra and Stewart, 2001). *Fusarium graminearum* is capable of surviving on host residues, including corn, wheat, barley, numerous other grasses (Sutton, 1982; Reis, 1988; Pereyra *et al.*, 2004), and non-gramineous species (Fernandez and Fernandes, 1990; Fernandez, 1991; Baird *et al.*, 1997).

Wheat, barley, and corn residues have long been regarded as the major source of primary inoculum (Sutton, 1982; Shaner, 2003). Since FHB has primarily been regarded as a monocyclic disease, the quantity of primary inoculum is related to the amount of crop residue on the soil surface (Dill-Macky and Jones, 2000). The increased use of soil conservation practices in Uruguay since 1992 (MGAP-DIEA, 2000) and the unknown contribution of some pasture species, common weeds, and crop residues as sources of inoculum, raise questions about the ecology and epidemiology of *F. graminearum* in the prevalent crop rotations and tillage practices.

No effort has focused on the cultural management, especially as it pertains to managing infected residue from the previous crop. Establishing effective strategies to manage *F. graminearum* infected residue requires an understanding of the role of crop residues and the presence of gramineous species in the build up, survival, and production of *F. graminearum* inoculum. Further studies are needed to identify those agronomic practices that may aid in reducing inoculum pressure in the Uruguayan production systems.

MATERIALS AND METHODS

A field site was selected at INIA La Estanzuela (INIA, National Institute for Agricultural Research, Uruguay) on a clay-loamy, horizon B textural soil. Experimental plots were established in 1996. Treatments consisted of combinations of two tillage treatments (reduced tillage and no-till) and two crop rotations (continuous agriculture: oats-corn/barley-sunflower/wheat, and crop-pasture rotation: oats-corn/barley-sunflower/wheat/alfalfa for three years) in a randomized complete block design (RCBD) with three replicates in time. Reduced tillage consisted of vertical tillage with chisel plow or eccentric (off-set) disc harrow. No-till plots were seeded with a direct drill.

Residue was sampled every three months from February 2001 to March 2003 for determination of amount of each type of residue on the soil surface and colonization of residue by *G. zeae*. Every six months, G. zeae and other *Fusarium* species were identified and

the inoculum production of *G. zeae* was assessed. Residue was collected from five arbitrarily selected quadrats (0.50 X 0.50 m) in each plot. Collected residue from each quadrat was air dried at 25-30°C for 24 hrs. Residue was separated visually into previous crops and each type of residue was weighted separately.

Each type of residue collected in each quadrat was evaluated for *G. zeae* colonization and inoculum production. *G. zeae* colonization was assessed on stem pieces, each 1.5-2 cm long, including a node in the case of gramineous species. Stem pieces from each residue type was assessed with a maximum of 30 pieces assessed. Stem pieces were surface disinfected, placed on pentachloronitrobenzene (PCNB) agar medium, and incubated at 20-22°C with 12 hr light per day for seven days. Colonies growing with salmon to pink-white mycelium were recorded as *Fusarium* spp. *G. zeae* colonies were determined by transferring 10 arbitrarily selected *Fusarium* colonies per sample to carnation-leaf piece agar (CLA) and PDA. Cultures were incubated at 20-22°C with 12 hr light per day for 10 days. Perithecia formation indicated the presence of *G. zeae* isolates. *Fusarium* colonies not forming perithecia were identified to species every other sampling date, according to the procedures of Nelson *et al*. (1983) and Burgess *et al*. (1994).

Ascospores production of *G. zeae* was determined on a uniform weight of residue pieces that comprised nodes for wheat, barley, corn, and gramineous weeds residues, stem pieces for sunflower and corn residue. Residue pieces were surface disinfected and placed on sterile sand moistened with distilled water in plastic containers. To facilitate perithecia development, residue was kept moist and incubated at 20-22°C with 12 hr light per day for 21 days. Following incubation, residue pieces with mature perithecia were placed in a sterile distilled water solution (dilution 1:20) and a drop of Tween 20. Nodes were left in solution for 12 hours to allow ascospore discharge and then vigorously shaken for ten minutes. Three aliquots of 0.02 ml were obtained from each treatment and used to determine ascospore concentration, expressed as ascospores number per gram of residue. The number of ascospores per square meter was calculated based on the number of ascospores produced per gram of residue and the amount of each residue type per square meter expressed as a mean of five quadrats.

Residue colonization and ascospore production data was subjected to analysis by generalized linear models (SAS procedure GENMOD) (SAS Institute Inc., Cary, NC). Results are presented as the likelihood ratio statistics of chi-square distribution.

RESULTS AND DISCUSSION

Recovery of *G. zeae* from all cereal crop residues decreased with residue age (Figure 1). Wheat and barley residues had significantly (*P=0.0001*) higher levels of *G. zeae* colonization than corn residue, gramineous weeds (*Digitaria sanguinalis*, *Cynodon dactylon*, *Lolium multiflorum*, and *Setaria* sp.), fescue (gramineous pasture component: *Festuca arundinacea*), and sunflower residue (Figure 2). Forage legumes residues (legume pasture components: Birdsfoot trefoil *Lotus corniculatus*, white clover *Trifolium repens*) were not colonized by *G. zeae*.

Corn residue could be recovered until four years after harvest and was still colonized by *G. zeae* indicating that it remains a host for a longer period of time compared to wheat and barley residue, probably associated to its slower decomposition rate. The lower levels of colonization in corn residue (Figure 2) could be explained by the fact that it is a summer crop and the most susceptible period for corn infection usually occurs under unfavorable environmental conditions (moisture deficiency).

Sunflower residue can be a substrate for *G. zeae* survival. To our knowledge this is the first report indicating that sunflower residue may host *G. zeae*. However, *G. zeae* colonized sunflower residue at significantly lower levels compared to cereal crop residues (<10% of residue pieces colonized by *G. zeae*) and it could only be recovered in 1-yr old residue (Figure2).

No-till barley and gramineous weeds residues had significantly higher *G. zeae* colonization *(P=0.0001)* than residue in reduced tillage plots (Figure 2). No signifi-

cant differences were found on the other residues, except for specific sampling dates.

Gibberella zeae was the main *Fusarium* species recovered from wheat and barley residues during the first months after harvest (*data not presented*). Other *Fusarium* species with higher saprophytic competitive ability (in order of importance, *F. avenaceum, F. equiseti, F. acuminatum, F. sambucinum, F. culmorum, F. trincictum, F. sporotrichioides, F. oxysporum*, and *F. solani*) increased as *G. zeae* decreased as a proportion of the population over time. The main species recovered in corn residue was *F. verticillioides*. *Gibberella zeae* was rarely recovered *from* sunflower residue. The *Fusarium* species commonly recovered from gramineous weeds were *F. equiseti, F. avenaceum, F. poae, F. oxysporum,* and *F. solani*.

Cereal residues significantly (*P=0.0001*) contributed more inoculum per unit area than gramineous weeds. Inoculum contribution per unit area was greater in 1-yr old residues compared to >1yr old residues. When comparisons were performed among cereal residues, winter cereals residues (wheat and barley) significantly (*P= 0.0001*) produced more ascospores per square meter compared to corn residue (2.44 x 10^6 and 0.33 x 10^6, respectively).

Even when at low levels, gramineous weeds had continuous ascospore production. Weeds with summer growth habits (*D. sanguinalis, C. dactylon, and Setaria* sp.) that remain dry during winter and early spring when wheat and barley flowering/heading occurs could have an epidemiological role in the Uruguayan production systems. Although *G. zeae* can colonize sunflower residue, no ascospore production was recorded on this substrate.

Information from this study may aid growers when deciding the crop sequences in their production systems.

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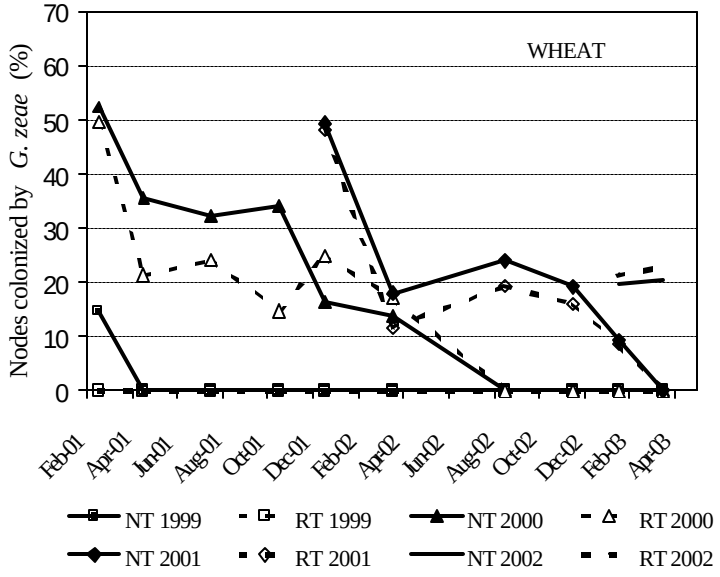

Figure 1. Percentage of wheat nodes from which *G. zeae* was recovered in different residue age and tillage systems from February 2001 to March 2003 (RT: reduced tillage; NT: no till). Values are mean percentages of all residue pieces sampled.

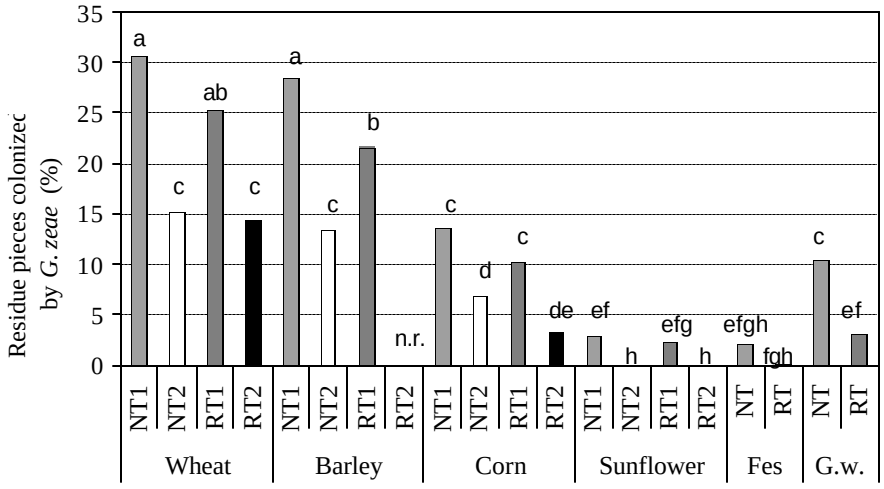

Figure 2. Mean *Gibberella zeae* colonization of different residue types, residue age, and tillage systems from February 2001 to March 2003.Values are mean percentages of all residue pieces sampled. Values with different letters are significantly different at *P=0.0001* based on likelihood ratio statistics. Fes.: Fescue, G.w.: gramineous weeds, n.r.: No residue recovered. NT1: residue under no-tillage and 365 days old or less; NT2: residue under no-tillage and 365 days old or more; RT1: residue under reduced tillage and 365 days old or less; RT2: residue under reduced tillage and 365 days old or more. Wheat and barley crops harvested in December each year. Sunflower and corn crops harvested in March/April each year.

MONITORING THE INFECTION PROCESS OF *GFP*-EXPRESSING *FUSARIUM GRAMINEARUM* IN BARLEY (*HORDEUM VULGARE*) SPIKE TISSUES

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ABSTRACT

Our goal is to further characterize infection pathways in barley spikes and to analyze whether and how infection patterns are affected in both widely used wild types and transgenic resistance sources. Here, we present evidence of infection pathways in cv Conlon (susceptible) using the previously reported GFP-expressing GZT501 *F. graminearum* strain and visualizing its green fluorescence with a stereoscope, an epifluoresence and a confocal microscope. Localized inoculations with very low concentration conidial suspensions were performed both *ex planta* and *in planta*. Detached palea, lemma and kernels placed on 0.7% water agar plates were inoculated with 2 µl drops containing 5-20 conidia on either adaxial or abaxial faces (palea, lemma), and dorsal or ventral faces (kernels). After 48 h at 21°C, the presence/absence of fungal colonies was assessed. *In planta* inoculation was performed by depositing 2 µl drops containing ca. 5 conidia on the tip of individual florets, within the space defined by the brush hair region of the kernel and the adaxial faces of both palea and lemma tips at early dough stages. Spikes were then covered for two days with a plastic bag. Inoculated spikelets were sampled at 6 days after inoculation (dai). All experiments included a water-inoculation treatment as a control. Fungal colonies were more frequent on adaxial inoculated than abaxial inoculated paleas (9/9 vs. 4/10) and lemmas (7/10 vs. 3/10). In all cases, colonies consisted of sparse superficial hyphae. At 2 dai *ex planta*, no evidence of fungal penetration and invasion was observed in cross-sectioned tissues. Conversely, fungal colonies were frequent in both ventral (12/15) and dorsal (10/15) inoculated detached kernels. Abundant intercellular hyphae were readily observed within pericarp cells of cross sectioned kernels. At 6 dai *in planta*, fluorescence was localized at the upper two thirds of the inoculated spikelet and closely associated with discoloration of palea, lemma and kernel. Very few superficial hyphae were observed on the abaxial faces of lemmas and paleas. Little or no discoloration or fluorescent hyphae were observed in associated glumes and adjacent empty spikelets and rachilla. However, several fluorescent hyphae emerging from the infected floret tip started colonizing the spikelet immediately above as well as adjacent green tissues, including glumes and empty spikelets. Most of the macroscopically observed fluorescence resulted from dense superficial mycelia growing in the space between the pericarp and the adaxial surface of the palea and lemma. Fungal penetration and intra and intercellular invasion was observed in both palea and lemma cross-sections from the adaxial thin cell wall epidermis and parenchyma towards the abaxial thick cell wall parenchyma. Kernel cross-sections showed massive intercellular hyphae along pericarp epidermis and parenchyma and cross-cell layers. Our preliminary results show that invasion of pericarp occurred earlier and more abundantly than lemmas and paleas, and that adaxial surfaces of palea and lemma are the candidate primary sites of invasion in these tissues. These findings support a model in which early fungal development occurs towards the inside of the kernel, then towards the kernel surface generating a dense mass of mycelia, which in close contact with adaxial surfaces of the paleas and lemmas may start second wave of invasion. A third wave may occur when running hyphae start exploring adjacent green tissues in the spikes. In accordance with these results, we will target the monitoring of early invasion at the upper pericarp tissue and at the adaxial faces of the lemmas and paleas.

DYNAMIC SIMULATION OF FUSARIUM HEAD BLIGHT EPIDEMICS

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OBJECTIVES

To develop a dynamic model simulating the risk for both FHB infection and mycotoxin accumulation in wheat kernels.

INTRODUCTION

Fusarium head blight (FHB) is caused by several fungal species (Parry *et al.*, 1995); in Italy, the most common species are *F. graminearum* Schwabe, *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W.G. Smith) Sacc, and *Microdochium nivale* (Fr.) Samuels *et* Hallet. Though FHB is a potentially destructive disease, its severity varies greatly in different years and locations, being strictly dependent on the epidemiological conditions. Accumulation of mycotoxins in kernels produced by the infected heads is one of the main problem caused by FHB. Both type and quantity of mycotoxin depends on the prevalence of fungi involved in the disease, on the time at which spike infection occurs, and on the environmental conditions between infection and harvesting. Because of the dependence of FHB epidemics on a wet and warm growing season, and the relatively short period of susceptibility of heads to infection, it would appear that FHB could be forecast (Parry *et al.*, 1995).

McMullen et al. (1997) stressed the need of a disease prediction system for improving crop protection against FHB. Models have been developed to predict disease incidence (Moschini and Fortugno, 1996) or the deoxynivalenol content in kernels (Hooker et al., 2002), based on the regression analysis of field collected data. Since advantages of dynamic mechanistic models versus empirical models in simulating or predicting the development of plant disease epidemics have been demonstrated, a research work aimed at collecting information about the relationships between environmental conditions, host growth and the infection stages of FHB epidemics was carried out, and a dynamic simulation model was developed using this information.

MATERIALS AND METHODS

Model development - The "systems analysis" (Leffelaar & Ferrari, 1989) was used to drawn a relational diagram for FHB epidemics as a first step in model development (Rossi et al., 1993). State variables were defined as the status of the pathogen at a given moment; a flow from one state variable to another was also determined. Rate variables were defined as the rate of change of the state variables in time as a function of some driving variables, as constants or parameters influencing the rate variables. Rates variables were then expressed as mathematical equations accounting for their relationships with influencing meteorological or host parameters.

Equations were developed using data collected in both environment controlled experiments or in experimental fields. The following aspects were investigated: i) influence of temperature and humidity on spore yield; ii) influence of weather on spore dispersal and deposition; iii) influence of temperature, wetness duration, relative humidity and host growth stage on spore germination and infection; iv) influence of temperature, available water and ripe stage of kernels on fungal growth and mycotoxin (deoxynivalenol and zearalenon) production. Details of these experiments were published elsewhere (Rossi et al., 2000a, 2000, 2002a, 2002b, 2003). The model was then developed by combining rates according to the relational diagram.

Model validation - Validation was performed by using field data not used for model development. FHB epidemics (expressed as disease intensity, incidence of infected kernels and mycotoxin content of kernels) developed under different epidemiological conditions

were compared with the model outputs; weather data collected by standard meteorological stations were used as driving variables for the model. Validations were performed for a three-year period (2002 to 2004) in different wheat-growing areas of Italy, using different cultivars of bread (*Triticum aestivum*) and durum (*T. durum*) wheat.

RESULTS AND DISCUSSION

Model development - The relational diagram of the model is shown in Fig. 1.

The inoculum source is the mycelium inside basal wheat organs or in cereal straw (MIS, Mycelium in Inoculum Sources); the model assumes that MIS is always present for all fungi (FS, Fungal Species), in equal dose. Inoculum produced on sources (SIS, Spores on Inoculum Sources) depends on a sporulation rate (SPO), while the amount of spores reaching the head tissues (SHS, Spores on Head Surface) is regulated by a dispersal rate (DIS). An infection rate (INF) accounts for the proportion of the head tissue affected (HTI, Head Tissue Infected). At the end of incubation (INC), FHB symptoms appear on spikes (SHT, Scab on Head Tissue); fungal invasion of head tissues (HIH, Hyphae Invading Head tissue) and mycotoxin production (MAH, Mycotoxin Accumulation on Heads) are regulated by invasion (INV) and mycotoxin accumulation (MAC) rates. Rates are regulated by air temperature (T), relative humidity (RH), rainfall (R), sequences of rainy days (DAR), wetness duration (W), and free water inside the host tissue (a_w); fungal species (FS) and the host growth stage (GS) are also considered. Equations relating these variables to SPO, DIS, INF, INV and MAC were published elsewhere (Rossi et al., 2000a, 2000, 2002a, 2002b, 2003).

Indexes for head infection mycotoxin production in affected kernels, named FHB-risk and TOX-risk, are calculated daily for four pathogenic species and for two mycotoxin producing species (*F. graminearum* and *F. culmorum*), and accumulated over the growing season until harvesting (Fig. 2).

Model validation - Validation showed a general agreement between model simulations and actual FHB epidemics grown under different epidemiological conditions. The FHB-risk index was significantly correlated with both disease intensity and incidence of infected kernels.

In Fig. 3 the FHB-risk index for *F. graminearum* was compared with the proportion of kernel infected by the fungus: when the index was lower than 0.4 no measurable infection occurred, while kernel infection increased linearly as the index increased.

In Fig. 4 the TOX-risk for *F. graminearum* and *F. culmorum* was compared with the actual DON accumulated in kernels, showing a close relationship between simulated and actual mycotoxins.

This work demonstrates that the "systems analysis" is an useful tool for developing mechanistic dynamic models for mycotoxin producing *Fusaria* on wheat; this approach can improve simulations of the epidemics caused by these fungi compared to an empirical approach based on the regression analysis of field collected data.

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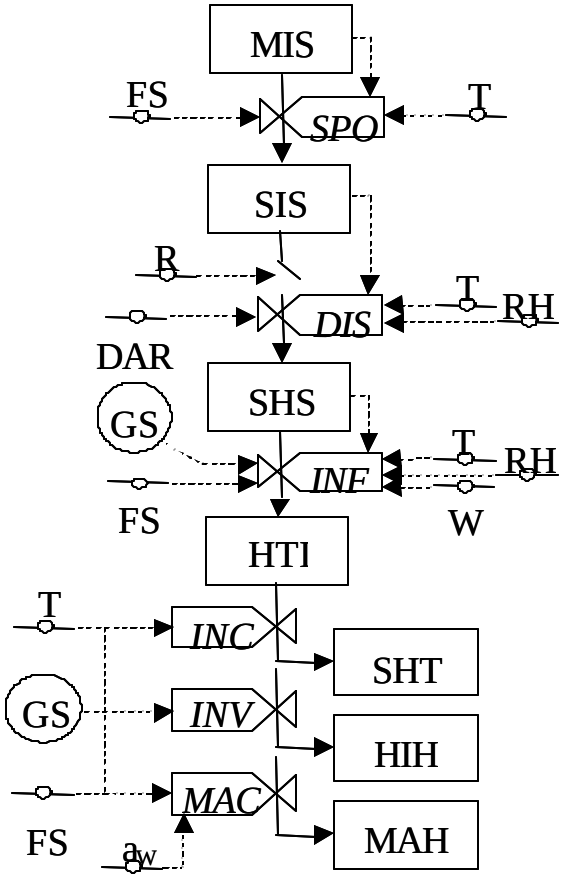

Figure 1. Relational diagram of the model.

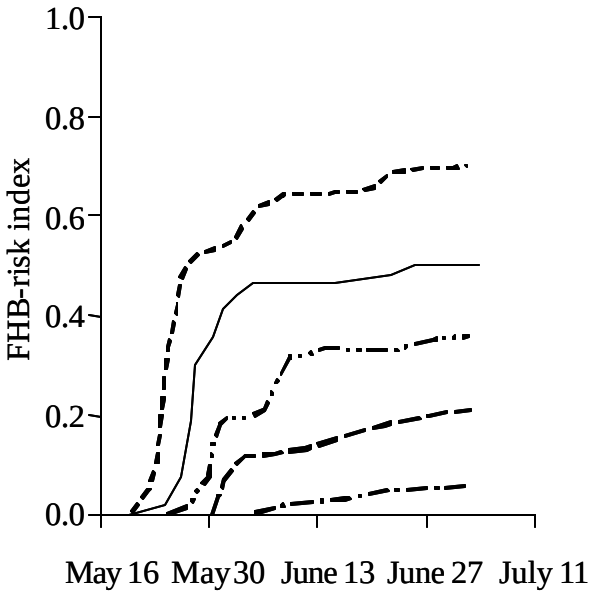

Figure 2. Dynamics of the FHB-risk index under different epidemiological conditions (Po valley, Italy, 2002).

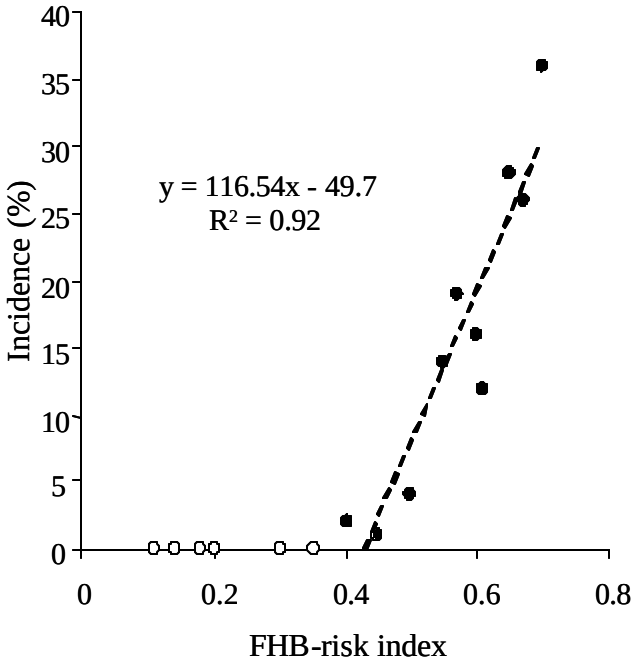

Figure 3. Relationship between the FHB-risk index for *F. graminearum* and the % of wheat kernels affected by the fungus (white points not used to calculate the regressione line).

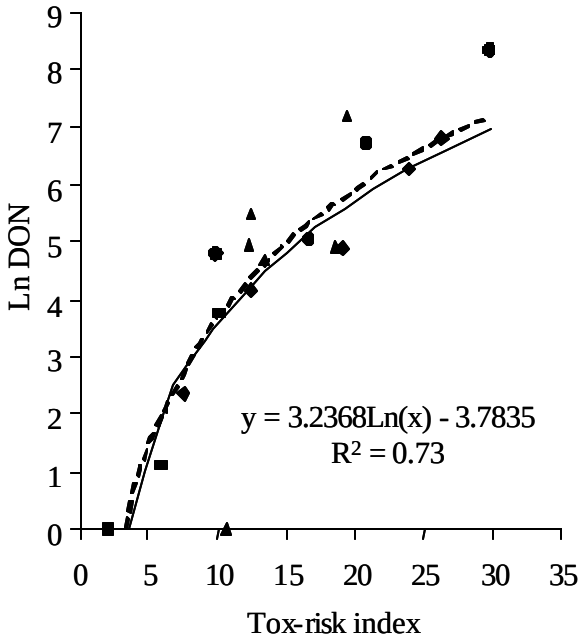

Figure 4. Relationship between the Tox-risk index for *F. graminearum* and *F. culmorum* and the content of DON in wheat kernels (points are actual data from different experiments; --- is the regression fitting these data; — is the estimate made by the model).

INFLUENCE OF THE CROPPING SYSTEM ON *FUSARIUM* MYCOTOXINS IN WHEAT KERNELS

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OBJECTIVES

To determine the weight of cropping practices on the presence of *Fusarium* mycotoxins (deoxynivalenol, DON, and zearalenon, ZEN) in wheat kernels under farm conditions.

INTRODUCTION

Fusarium head blight (FHB) is a serious disease of cereals in many areas of the world, caused by several fungal species (Parry *et al.*, 1995). Under favourable conditions, they cause severe epidemics, heavy yield losses and reduce grain quality due to the presence of mycotoxins McMullen et al., 1997). Mycotoxin contamination occured mainly in the field (Snijders, 1990; Bottalico and Perrone, 2002). Field contamination depends on environmental factors and cultural practices favouring the development of toxigenic *Fusaria*, as well as the level of resistance of the host variety. Rotation intervals between host crops, land preparation, use of fertilizers, irrigation, and weed control have been listed as influencing factors (Parry et al., 1995).

To determine the weight of the cultural practices in the wheat cropping regimes used in the PoValley (northern Italy), data on the content of DON and ZEN in harvested kernels were collected in 907 crops, in 2002 and 2003, and analysed on the basis of the cultural information collected for each crop using multivariate statistical techniques.

MATERIALS AND METHODS

In 2002 and 2003, representative farms were selected in several districts of the Po Valley and winter-sown commercial fields of bread wheat, durum wheat and barley were monitored during the wheat-growing season to collect data on cultural practices, wheat phenology, and incidence of Fusarium head blight. Kernel samples were taken from the mass discharged by the threshing machine, following the rule 98/53 published by the European Community. Sub-samples of 100 g of kernels were selected and ground; 5 g of flour were analysed for the presence of DON and ZEN using the RIDASCREENâ (R-Biopharm, Darmstadt, D) kits R5906 and R1401, respectively; these kits are based on a competitive enzyme immunoassay.

To each farm was delivered an agronomic sheet to record information about: variety, crop rotation, soil preparation, fertilizer inputs (times and rates), weed control, fungicide applications on seeds and on leaves (times of application, active ingredients, rates). Each sampled crop was identified by spatial coordinates in order to make possible a cartographic representation of data. Information about the proportion of cultivated land planted to susceptible crops was collected for each district.

Data about the cropping regimes were grouped in categories, based on the hypothetic role on biology and epidemiology of the FHB causing fungi. For instance, the previous crops were grouped in five categories: cereals (wheat, barley, rice, corn, sorghum), grasses, renewal crops (beet, sunflower, soybean, tomatoes, etc.), vegetables and others (flowers, fruit orchards, vineyards, etc.); the land preparations were grouped in five categories: no tillage, minimum tillage, ploughing, ploughing and ripping, milling and other preparations with no clod upsetting. The same analysis was performed on the phenological growth of wheat,

grouping crops on the basis of the period of flowering. The wheat-growing-areas were also grouped based on the proportion of arable land area cropped to cereals and on the geographical location.

DON and ZEN values were log-transformed, and mean values were calculated for each category of the cropping regimes. A cluster analysis was applied for grouping categories in homogeneous groups of muycotoxin content; groups were sorted from the lowest to the highest value of mycotoxins and then codified (1 to n).

A stepwise discriminant analysis (DA) was applied to the codified data-set. DA is based on data whose group membership is known (in this case, the class of mycotoxin content) and is able to identify the variables that are important for distinguishing among the groups and to develop linear equations, as combinations of the independent variables (i.e. the cropping practices) for predicting group membership for new cases whose mycotoxin content is unknown. Usually, the first two discriminant functions (DF) accounting for the highest percentage of total variability are considered. DA produces also a graphic output called 'territorial map' which represents the boundaries of the groups and in each group area are included the combinations of DF values that result in the classification of new cases into the groups. DA results are summarized in a table where the diagonal elements are the number of cases correctly classified into groups, other cells contain the number of misclassified cases. This table represents an evaluation of the degree of reliability of the DF in classifying cases into groups.

The discriminant functions and the territorial map obtained for bread wheat were used to classify cases of durum wheat and barley into the three groups of mycotoxin content.

RESULTS AND DISCUSSION

In 2002 and 2003, 742 samples of bread wheat were collected in aggregate (476 in 2002 and 266 in 2003), 65 of durum wheat (49 in 2002 and 16 in 2003) and 100 of barley (73 in 2002 and 27 in 2003); a few cases were discarded because of an incomplete information set. The collected samples came from 9 districts of the Po Valley and belonged to 18 different varieties. DON ranged between 0 and 13000 ppb in bread wheat, between 0 and 6200 ppb in durum wheat and between 0 and 5400 ppb in barley. Highest values were detected in 2002 which was wet and warm during the period of host susceptibility, while in 2003 the season was particularly dry. ZEN was low in 2002 and absent in 2003; for this reason, only DON was considered in the multivariate analysis of data. Three classes of DON were considered to classify crops in: 1) no measurable DON in kernels (detection limit 18.5 ppb, recovery rate > 80%); 2) low DON (>0 and £500 ppb), 3), high DON (>500 ppb).

The stepwise DA selected the best set of independent variables for the separation of cases (crops) into the three groups of DON (no, low, high) for bread wheat and provided two functions, FD1 accounted for 97.6% of the explained variability:

$$DF_1 = -11.194+3.160 \cdot Year+1.044 \cdot WGA+0.279 \cdot CV+0.331 \cdot PC+0.354 \cdot ST$$
$$DF_2 = -5.703-0.880 \cdot Year-0.212 \cdot WGA+0.556 \cdot CV+0.740 \cdot PC+1.481 \cdot ST$$

where: Year = year; WGA = wheat-growing area; CV = cultivar; PC = previous crop; ST = soil tillage. All the other variables were excluded from the analysis, including nitrogen fertilization, fungicides and herbicides and period of wheat flowering.

Based on these two functions it was possible to correctly classify 72% of total cases (439 out of 613) (Tab. 2). In 78 cases out of 613 (13%) the DON was underestimated and in the 16% of cases it was overestimated. The most important variable for the separation of crops into DON groups was the year, with a coefficient of +3.160 in DF_1; this effect was undoubtedly associated with weather conditions favouring FHB epidemics. The second important factor was WGA, with a coefficient of +1.044 in DF_1: both differences in climatic conditions, due to the geography of the Po Valley that determines thermal gradients from East to West and changes in humidity around the Po river, and in the proportion of land area cropped to cereals (between 14 and 46%) can explain the role of WGA. The third factor was ST (soil tillage), with coefficients

of +0.354 in DF_1 and +1.481 in DF_2, no-tillage and minimum tillage being more conducive than ploughing or milling, and than ploughing and ripping. The fourth factor was PC (previous crop), with coefficients of +0.331 in DF_1 and +0.74 in DF_2, where cereals were the most conducive and vegetables the less ones. The less important factor was the cultivar sown, probably because the varieties actually in use have a low to intermediate level of resistance to FHB.

The two functions make possible to assign new cases to groups of DON content calculating DF_1 and DF_2 and placing the coordinates on the territorial map to identify the group (Fig. 1). The map and the two DF calculated for the bread wheat data were used to assign cases of durum wheat and barley to the three DON content groups. For durum wheat, 33 cases out of 52 (63%) were correctly classified, 17 cases were underestimated and 2 overestimated. For barley, the 81% (48 out of 59) of cases was correctly assigned to the DON groups, 5 cases were underestimated and 6 overestimated. Therefore, the relationship between DON content and the influencing variables did not change for the three cereals.

Multivariate analysis discriminated the most important factors influencing the DON content in wheat and barley kernels and assigned a weight to each of them; this analysis was sufficiently accurate since classified correctly 72% of cereal crops (520 out of 724 in aggregate). The analysis showed that weather and geographical factors prevail over the cropping practices in determining the level of DON contamination. Type of land preparation and crop rotation were the only cultural techniques able of influencing significantly the DON content in kernels from commercial crops; cereal varieties used in the Po Valley had a lower effect.

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Table 1. Variables used in the multivariate statistical analysis, and average DON (ln-transformed) content in bread wheat kernels in the different classes.

Parameter	***Class***				
	1	**2**	**3**	**4**	**5**
Year	2003 0.55	2002 4.90			
Wheat-growing area	1st 0.97	2nd 4.08	3rd 5.73		
Cultivar	n=1* 1.12	n=4 2.53	n=2 3.42	n=8 3.82	n=3 4.53
Previous crop	Vegetables 1.45	Others 3.17	Renewals or grasses 4.82	Cereals 5.86	
Soil tillage	Ploughing and ripping 3.12	Ploughing or milling 5.01	No or minimum tillage 6.64		

*number of wheat cultivars in each class.

Table 2. Comparison between the actual classification of bread wheat crops on the basis of DON and the classification estimated by the stepwise discriminate analysis (DA) calculated using the variables listed in Table 1. Underlined values were classified correctly.

DON	**DA estimated group**			
Actual group	**1**	**2**	**3**	**Total**
1 No	222	40	11	273
2 Low	21	<u>90</u>	45	156
3 High	3	54	<u>127</u>	184
Total	246	184	183	613

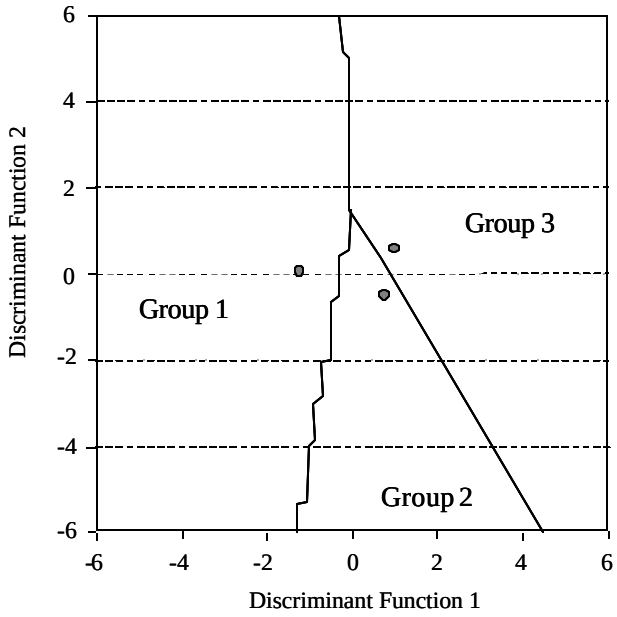

Figure 1. Territorial map resulted from the stepwise discriminate analysis: Group 1, no DON in kernels; Group 2, low DON; Group 3, high DON. Points on the map represent centroids.

INCIDENCE OF *FUSARIUM GRAMINEARUM* IN PRE-HARVEST AND OVERWINTERED RESIDUES OF WHEAT CULTIVARS DIFFERING IN FUSARIUM HEAD BLIGHT-RESISTANCE

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ABSTRACT

Cereal residues are the principal reservoir of the inoculum of *Fusarium graminearum* (Schwabe) [teleomorph: *Gibberella zeae* Schw. (Petch)], the fungal organism that incites of Fusarium head blight (FHB or scab). Despite the importance of crop residues in the epidemiology of FHB, there is little information on the levels of colonization of wheat by *F. graminearum* and other pathogenic Fusaria, either of plant tissues immediately prior to the harvest or following the overwintering of crop residues in the field. In this study the colonization of six wheat cultivars immediately prior to harvest and of crop residues eight months later were examined. Six hard red spring wheat cultivars (Wheaton, Norm, P2375, Ingot, BacUp, and Alsen), which differ in their FHB susceptibility, were planted in Rosemount, MN in May 2003. The experimental design was a randomized complete block with four replications. The plots were naturally infected by *F. graminearum*. Twenty plants were collected arbitrarily from each plot immediately prior to harvest (physiological maturity) in August 2003. Plots were not harvested, rather the plants were left in situ over the winter. Thirty plants which had overwintered (spring residue) were arbitrarily collected in early April 2004. Plants were stored at –20 C till processed. The crowns, nodes, and kernels were excised from each plant with the identity of position of the node within the canopy being preserved. Crowns and nodes segments were split longitudinally into two pieces. Tissue pieces were surface-sterilized, plated onto petri-plates containing Komada media (selective for *Fusarium* species) and incubated for 14 days. *F. graminearum* isolates were identified based on perithecia formation on carnation leaf piece agar (CLA). Other Fusaria were identified based on morphological characteristics on potato dextrose agar and CLA. Overall, *F. graminearum* was the Fusaria most frequently isolated from both pre-harvest (11.6%) and over-wintered residues (25.9%). Other pathogenic Fusaria such as *F. sporotrichioides*, *F. poae*, and *F. avenaceum* were isolated less frequently (range 0.3-6.7%). There was a significant effect (P=0.01) of cultivar, position of the tissue within the canopy, and the interaction of cultivar by canopy position on the recovery of *F. graminearum*. In general, there was a greater level of *F. graminearum* in spring residues (range 41.8 – 72.2%) as compared to mature plants. The relative distribution of *F. graminearum* within the plant was comparable irrespective of the level of *F. graminearum* recovered. The level of *F. graminearum* in mature plants was highest in Wheaton (16.4%) and Norm (18.1%), and least in Alsen (6.6%). The recovery of the pathogen from the spring sampled residue was highest from Wheaton (38.9%) and least from Ingot (19.3%) and BacUp (19.3%). In mature plants, *F. graminearum* was highest from the third node from the base of the plant (18.5%) and least from crowns (5%). In the overwinter residues, *F. graminearum* was abundant in the third node (35.5%), forth node (39.2%) and kernels (38.7%) as compared to crowns (7.5%). Our data confirms that wheat nodes are a good source of Fusaria inoculum for the development of FHB. This study also demonstrated that *F. graminearum* colonization varies with cultivar and within the canopies of individual plants. The results of this research suggest that farmers may benefit from cropping resistant cultivars and by discarding the straw of susceptible cultivars.

ACKNOWLEDGEMENT

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STRATIFIED COLONIZATION OF WHEAT PLANTS BY *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium head blight (FHB or scab) is a devastating disease of wheat. Epidemics in the Upper Midwest of the United States are considered to be the most important limitation to wheat production. The development of resistant cultivars to control FHB is a priority for breeding programs in the region, and several wheat genotypes with improved FHB resistance have been released recently. Breeding programs generally evaluate material for resistance based on head symptoms and grain quality. The colonization of plant parts other than heads by *F. graminearum*, the principle pathogen causing FHB, is largely unknown, although tissues such as nodes are known to contribute to the inoculum that generates FHB epidemics. This study examined the incidence of colonization of subcrown internodes (SCI), nodes and kernels of sixteen wheat cultivars grown in non-inoculated trials at Strathcona and Humboldt, Minnesota. At each location wheat entries were arranged in a randomized complete block design with two replications. At hard dough, 20 plants were arbitrarily sampled from each plot. SCI, crowns, node pieces and kernels were dissected from the plants. Crowns and nodes were split longitudinally into two pieces, surface-sterilized, and tissues pieces were plated on Petri plates containing Komada medium which is selective for *Fusarium*. Plates were incubated for 14 days. The recovered *Fusarium* isolates were identified to species using standard taxonomic procedures. The incidence of colonization by *F. graminearum* was determined as the percentage of each plant part (SCI, crowns, nodes, kernels) from which *Fusarium* spp. were recovered.. Among the species of *Fusarium* pathogenic to wheat; *F. graminearum* (7%) was most frequently isolated, followed by *F. avenaceum* (6%), *F. sporotrichioides* (1%), and *F. poae* (1%). *F. culmorum* was recovered only from the Strathcona site(1%). The non-pathogenic species of *Fusarium* recovered included; *F. acuminatum* (5%), *F. equiseti* (3%) and *F. oxysporum* (2%). The cultivars Oxen (12%) and Reeder (13%) were the two cultivars most frequently colonized by *F. graminearum*. Verde (5%), Knudson (5%), Hanna (4%), Alsen (4%) and Granite (4%) were among those less frequently colonized by *F. graminearum*. The relative frequency of colonization was directly correlated with the FHB resistance of the wheat cultivars tested. Recovery of *F. graminearum* was higher from kernels (11%), the first node (8%) and second node (8%) than from nodes higher on the plant (e.g. node three, node four), crowns or sub-crown internodes. Surprisingly, *F. avenaceum* was recovered at high levels from the plants grown at Strathcona. The frequencies of colonization of the wheat cultivars by *F. avenaceum*, e.g. Walworth (12%), Oxen (11%), Oklee (10%), Parshall (10%), Ingot (10%), Alsen (9%), Granite (9%), Hanna (8%), Mercury (7%) and Briggs (7%), suggests that this pathogenic species may occasionally contribute to FHB in Minnesota. Our data indicates that wheat cultivars are differentially colonized by *F. graminearum*, as are the individual tissues of a given plant. Differential colonization of plants by *Fusarium* suggests that host resistance, in addition to providing disease protection to the crop, may provide the additional benefit of reducing inoculum in subsequent growing seasons.

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APPROACHES TO USING EPIDEMIOLOGICAL KNOWLEDGE FOR THE MANAGEMENT OF FUSARIUM HEAD BLIGHT IN WHEAT

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INTRODUCTION

The economic impact of Fusarium head blight (FHB) epidemics is tightly linked to the contamination of grain by mycotoxins, of which deoxynivalenol (DON) is the most common and important worldwide. Among the several species of *Fusarium* that cause FHB, *F. graminearum* and *F. culmorum* have the highest incidence and produce the bulk of DON in wheat grain. Several important agronomic and environmental (climatological) factors have been associated with *Fusarium* epidemics and elevated DON concentrations in wheat; knowledge of these factors provides the basis for developing prediction models for improved management of FHB in wheat.

FACTORS ASSOCIATED WITH EPIDEMICS

Among agronomic and environmental factors, those associated with environment plays the major role in epidemics (Schaafsma et al., 2001; Champeil et al 2004; Shaner 2003). Agronomic factors modify the severity of epidemics, which include wheat variety, crop, tillage, fertilizer, and herbicide histories. Of these, the use of resistant varieties is the most desirable but challenging, and will be discussed at length elsewhere. When we look at the remaining agronomic factors, crop history (i.e., crop residues that provide the substrate for inoculum), and tillage (i.e., placement of crop residues on the soil surface) are significant agronomic contributors to FHB epidemics, while fertilizer (Lemmens et al., 2004) and herbicide histories are relatively minor factors (M. Fernandez, unpublished).

Three scenarios have emerged across Europe and North America with respect to FHB epidemics. The most common scenario of an epidemic occurs in regions where maize is widely produced, conservation tillage is practiced, and *F. graminearum* dominates. Tillage systems that maintain crop residue on the soil surface are encouraged for environmental reasons. Even though better crop rotations and burying host residue may reduce the risk of FHB in these regions, the likelihood is limited by the vastness of regions involved and the emerging problem of the saprophytic nature of *F. graminearum* on non-host crop residues. Therefore, when environmental factors are favorable for infection, only a minimum amount of inoculum is necessary for an epidemic. In maize producing regions, emphasis should be placed on avoiding planting wheat directly into host stubble, developing better host resistance, and well-timed fungicide applications.

The second scenario occurs in the northern regions of Europe where the climate is cooler, maize productionis scarce, and *F. culmorum* predominates. This region appears to be shrinking because of increased maize production and perhaps a warming climate (pers. communication, Naresh Magan, Institute of BioScience and Technology, Cranfield University, Silsoe, Bedfordshire,

UK); the result is that *F. graminearum* is battling for dominance. In this region, tillage that buries infected residue, restricting maize production, and rotation with non-cereal crops are more effective strategies to restrict inoculum production than in the region of more continental climate in North America.

The third scenario occurs mainly north of the maize producing areas of North America, where *F. graminearum* is expanding into regions of small grain production. *F. culmorum* is more or less absent in this system. Here, the temporal increase of *F. graminearum* appears associated saprophytically with non-host crops like canola (J. Gilbert, unpublished), with conservation tillage, and perhaps the repeated use of glyphosate (M. Fernandez, unpublished) may even favor inoculum production. A shift in climate to warmer and wetter conditions during flowering may also increase the prevalence of *F. graminearum*. In this third region, due to its vastness and wide adoption of conservation tillage, crop rotation may be marginally effective for reducing the risk of epidemics; the reliance on host plant resistance and timed fungicides are likely better management alternatives.

PREDICTION MODELS

The understanding of the relationship between environment and FHB caused by *F. graminearum* has grown rapidly in recent years. A disease forecast model for FHB first appeared in South America (Moschini et al., 2001). More recently, researchers from Ohio State University, Purdue University, North Dakota State University, South Dakota State University and Penn State University in North America have initiated a cooperative effort to forecast the risk of head scab epidemics in the U.S. (De Wolf et al., 2003). This effort uses data representing many wheat production systems common in that country. These models focus on predicting epidemics, which is useful for identifying conditions suitable for infection and to determine whether a fungicide should be applied, but it is less useful for predicting DON contamination at harvest. The model uses weather observed 7 d before wheat flowering for predicting disease epidemics (>10% severity). Model accuracy for predicting epidemics is >80% based on data used to validate model performance. These models are currently being evaluated in 23 states in the eastern U.S. via a Fusarium Head Blight Prediction Center (www.wheatscab.psu.edu).

In Belgium, a similar approach is being taken to forecast both FHB and DON. The objectives are advisory to fungicide applications and pre-harvest contamination by DON. Data on leaf wetness and crop history (presence of maize residue) are layered into the model in the critical period between 8 d before flowering and 7 days after flowering. The model classifies values of DON content compared to the actual DON recommendation in force for bread flour in Belgium (0.75 mg kg^{-1}). Of the173 samples of wheat grain collected before harvest in 2003, 69% of them were determined correctly by the model, detection was 61%, and the percentage of false detections was 29%. This model depends on real-time data collected every 20 min, which results in a huge amount of data that sometimes saturates the calculation module. Typical of all FHB models, observed *Fusarium* and DON in the flour is poorly correlated. The lack of correlation could be explained by the presence of different *Fusarium* chemotypes, different wheat varieties collected, and the maturity of the wheat kernels when they were infected (pers. communication, P. Detrixhe).

In Ontario Canada, DONcast (http://www.ontarioweathernetwork.ca/lib/fusarium.cfm) is an empirical prediction model that focuses on relationships among weather during critical periods of wheat development, agronomic variables, and DON measured at harvest (Hooker et al., 2002). Since 2000, it has been used mainly for aiding in decisions for fungicide spraying at flowering. Recently however, it was adapted to Uruguay, in South America, to help target pre-harvest regulatory and marketing actions for reducing DON in wheat destined for food markets. The weather inputs of DONcast at heading are simple, including daily maximum-minimum air temperatures, relative humidity at 11 am and daily precipitation. Agronomic inputs include crop history, tillage, and wheat variety. Using these different layers of input data from over 700 farm fields during 9 years, 76% of the variability of DON can be explained, with an average accuracy of spray decisions (i.e., >1.0 mg kg^{-1}) of approximately 80%. Interestingly, this model identifies

similar critical periods and important weather variables as the FHB-based models, and is equally useful in predicting the risk of infection. Unlike the FHB risk models from the United States and Belgium however, DONcast has the advantage of using additional weather to harvest for more accurate forecasts of DON accumulation before the wheat is harvested.

One of the serious limitations of all the models is their dependence on the events of flowering or heading date. The coincident timing of flowering and favorable environmental conditions for infection is critical for FHB severity and subsequent DON accumulation. Variability of flowering dates in the same field and within the canopy (i.e., primary vs tillers) may challenge accurate predictions. This perhaps is a more serious problem in maritime Europe and North America, where cool temperatures may extend the window of flowering and susceptibility for infection by as much as 2 to 3 weeks. In more continental regions, the window of flowering may be much shorter, resulting in potentially more accurate predictions for FHB and DON, and perhaps a more efficacious fungicide application.

Another challenge for these models is the need for site-specific data. Critical site-specific inputs important for all models include precipitation, heading or flowering date, crop history and tillage regime. Some of these challenges are being overcome by interpolation of data through GIS software, or measuring precipitation by proxy using a combination of weather radar and GIS software. These types of inputs are useful for generalized forecast maps. A more recent development is the use of interactive web-based forecasting where users can enter or modify their own inputs (http://www.ontarioweathernetwork.ca/lib/ssd_demo.cfm).

As far as we are aware, the only model that uses forecasted data, in addition to near real time weather data, is DONcast. This approach has been met with its own challenges, mainly due to the uncertainty inherent in weather forecasts. These challenges can only partially be addressed by probabilities (of precipitation) given in the forecast, or in conjunction with the likelihood of weather from 30-year normals when forecasted data is not available.

SUMMARY

Models used to forecast *Fusarium* epidemics and DON have been developed using knowledge of *Fusarium* epidemiology. These models serve as tools for decisions of whether or not to apply fungicides, and as an early warning of the mycotoxin risk associated with an epidemic before the crop is harvested. During the last decade, much has been learned and invested in the process of modeling disease severity or mycotoxin accumulation, and of systems to process data and deliver predictions at the field level. It is clear that a more collaborative effort is needed to increase the database of field-specific information for further development, validation, and for testing the applicability of existing models.

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PATHOGENICITY DETERMINANTS OF *FUSARIUM GRAMINEARUM*

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OBJECTIVE

Defining virulence and pathogenicity traits of *Fusarium graminearum* on a molecular level.

INTRODUCTION

The molecular basis of fungal pathogenicity is poorly understood. Understanding the fungal mechanisms of pathogenicity will allow the design of specifically improved defense reactions of the infected plant or the development of new fungicides. Some steps may be crucial to the establishment of fungal pathogenicity: 1. attachment to the host surface; 2. germination on the host surface and formation of infection structures; 3. penetration of the host surface; 4. colonization of the host tissue. To get a detailed unterstanding of the involvement of a specific fungal gene in the infection process this gene may either be overexpressed or destroyed by transformation mediated gene disruption. The resultant mutants are tested for altered pathogenic behavior. Furthermore, the infection processes can be studied microscopically facilitated by the constitutive expression of marker genes like the green fluorescent protein (GFP). The expression of different genes can be monitored during different phases of the infection process by qunatitative PCR and by fusing them to a maker gene (i.e. GFP). Despite this broad arsenal of methods, and in spite of the economical importance of *F. graminearum*, surprisingly little is known about the molecular basis of *F. graminearum* infections.
Our objective is to identify different virulence and/or pathogenicity genes of this fungus by applying the above mentioned methods and to characterize these traits in the context of the physiology of this important pathogen.

MATERIALS AND METHODS

Strains, transformation and enzymatic procedures - *Fusarium graminearum* wild type strains 8/1, FG06, FG25, and FG 2311 were kindly provided by T. Miedaner, Hohenheim, Germany. Genetically defined mutants were produced by targeted disruption of a given gene via integration of a transformation vector through homologous recombination (Jenczmionka et al., 2003). Integration of the gfp marker gene was targeted to the analysed gene to avoid mutagenic ectopic integrations into the genome.

To evalutate the ability of wild type and different mutant isolates to produce secreted enzymes fungal mycelium was grown for 48 hrs in complete medium and transfered into minimal medium with a defined substrate as the sole carbon source.

Pathogenicity tests - Wheat and barley plants were grown in 11-cm pots at 20 °C with a 16-h photoperiod (8,000 lx) and 70% relative humidity. Spikes at anthesis were point-inoculated with the *F. graminearum* wild type and independent mutants by placing a conidia suspension in one spikelet in the middle of the wheat spike tested (modified after Pritsch et al., 2001). The inoculated spikes were enclosed in small plastic bags during the first 3 days to ensure a high humidity. Plants were evaluated 3 weeks after inoculation.

Maize cultivars were grown in greenhouse. Approximately four months old plants were inoculated 6 d after silks emerged by injecting conidia suspensions into the silk channel (Reid et al., 1995). Before inoculation, silks were manually pollinated to ensure optimal pollination. The inoculated ears were enclosed in plastic bags during the first 3 days to ensure a high humid-

ity for infection. Disease severity of maize cobs was monitored five weeks post inoculation.

RESULTS

Deoxynivalenol (DON) is a host specific virulence factor - Deoxynivalenol (DON) are the first known virulence factors of *F. graminearum*. Genetic disruption of the gene Tri 5 leads to trichothecene negative mutants (Proctor et al., 1995) with a dramatic reduction in their ability to colonize wheat (Bai et al., 2001).

To further substantiate and expand this result, we investigated whether virulence is only determined by the presence of the trichothecenes or is a quantitative character that is heterogeneously determined by several factors differing from one isolate to the other. Three isolates of *F. graminearum*, well characterized in field experiments (Miedaner et al., 2000), were selected: FG06, a medium aggressive isolate producing mainly nivalenol (NIV chemotype), FG25, a medium aggressive isolate of the deoxynivalenol (DON) chemotype, FG2311, a highly virulent isolate of the DON chemotype, producing high levels of DON.

The Tri5 genes of these three isolates were cloned, sequenced, and disrupted by transformation mediated homologous recombination. Disruption mutants were found to grow *in vitro* like the respective wild type but are unable to produce trichothecenes. The mutants in comparison to the respective wild types were tested on wheat and barley as well as on maize for their ability to develop FHB or cob rot.

All mutant strains, irrespective whether the corresponding wild type strains produced NIV or DON, were equally reduced in their ability to colonizing the spike. They showed a basal infectivity to the inoculated spikelet but were unable to spread throughout the entire head, indicated by the very low percentage of diseased spikelets (Fig. 1).

In sharp contrast to these results, inoculation of barley and maize displayed no difference in virulence beween wild types and trichothecene negative mutants, all were fully aggressive.

Mitogen-Activated Protein Kinases - Mitogen-activated protein kinases are central regulators within different signal transduction pathways. Recent examinations of two of these genes revealed their importance in general fitness and pathogenicity in particular of *F. graminearum* (Hou et al., 2002, Jenczmionka et al., 2003; Urban et al., 2003). Transformation-mediated gene disruption of the Fus3 / Pmk1 MAP kinase homologue Gpmk1 of *F. graminearum* results in mutants that are reduced in conidia production and are sexually sterile. Furthermore, the mutants were shown to be fully apathogenic to wheat and strongly reduced to maize, even though they still produce trichothecenes. This leads to the conclusion that gpmk1 is responsible for signal transduction processes taking place during the most important developmental stages in the life cycle of this fungal pathogen, including pathogenicity. However, it is yet not known how much and which genes are involved up and down stream from the MAP kinases. We analyzed the Gpmk1 MAP kinase disruption mutants of *F. graminearum* for their ability to produce cell wall degrading enzymes *in vitro* in comparison to the wild type strain. The gpmk1 disruption had no effect on the production of pectinolytic or amylolytic enzymes. However, it could be shown that Gpmk1 regulates the early induction of an endoglucanase, a xylanolytic, a proteolytic, and a lipolytic activity. (Jenczmionka and Schäfer, 2004).

Extracellular Lipase - In general, fungal pathogens secrete various enzymes which might be involved in virulence (Wanjiru et al., 2002). Among the secreted enzymes, triacyl-glycerol lipases (EC 3.1.1.3) form an extensive family of enzymes which catalyze both the hydrolysis and the synthesis of ester bonds. The biological function of lipases is the hydrolytic decomposition of triacyl-glycerols into glycerol and free fatty acids. So far, no direct evidence concerning the involvement of a lipase in fungal virulence has been provided.

We cloned and characterized the first secreted lipase of *F. graminearum*. The functional identity of the lipase gene was established by heterologous gene expression in the *P. pastoris* expression system. *In planta*, lipase transcripts were already detectable one day post inoculation of wheat spikes and during all

later examined stages of infection. Ebelactone B, a known lipase inhibitor, represses the lipolytic activity of the enzyme *in vitro*. After complementing the inoculum with ebelactoneB *F. graminearum* infected the inoculated spikelet but was unable to colonize the spike.

F. graminearum mutants with a disrupted lipase gene were constructed and showed a greatly diminished secreted lipolytic activity *in vitro*.

Consequently, wheat, barley, and maize plants were inoculated with wild type and lipase deficient mutants. All mutants displayed a strongly reduced virulence towards all different host plants. Infected wheat and barley spikes developed normally, only the inoculated spikelets showed signs of infection. Maize ears inoculated with conidia from wild type or ectopic strains revealed strong symptoms of *F. graminearum* ear rot e.g. up to 100 % infected cobs (rating 7, Reid et al., 1995). In contrast to this, lipase-mutants infected cobs showed only minor infection areas of 4-10 % (rating around 3) and normal kernel development in uninfected cob parts (Fig. 2).

DISCUSSION

Pathogenicity is defined as the capability of a fungus to cause disease. In molecular terms, a fungal pathogenicity gene is directly and essentially involved in pathogenicity but is not necessary for completion of the life cycle. Following this context, a gene that modulates the degree of pathogenicity is a virulence gene. The disruption of a pathogenicity gene will result in a total loss of pathogenicity, whereas the disruption of a virulence gene leads only to a reduction in the fungal ability to cause disease. For both types of genes, pathogenicity and virulence genes, examples were given in this article. The map-kinase gpmk1 is a pathogenicity gene. It is a central signal transduction component, which regulates most likely several traits. The disruption of each of these individual traits may lead only to reduced virulence, the disruption of the central pathway however, leads to a total loss of pathogenicity, even though trichothecenes are still produced. Trichothecenes are host specific virulence factors. DON and NIV contribute in the same amount to virulence of *F. graminearum* towards wheat, but fungal virulence is unchanged to barley and maize whether the fungus produces the toxins or not.

The secreted lipase is a novel and general virulence factor, equally important to wheat, barley, and maize.

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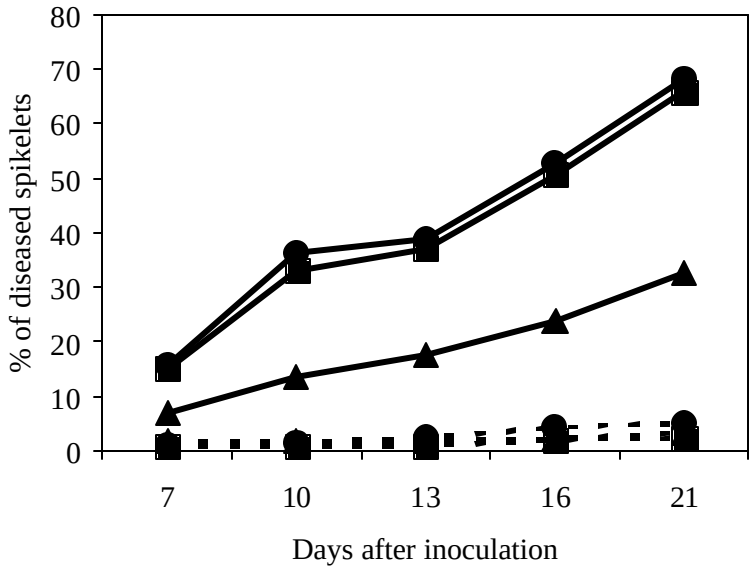

Figure 1. Wheat FHB severity depending on time post inoculation of the *F. graminearum* wild type strains (continuous lines) FG06 (?), FG25 (?), and FG2311 (¦) in comparison to respective *tri5* deletion mutants (dashed lines). Each fungus was tested at least on 20 spikes.

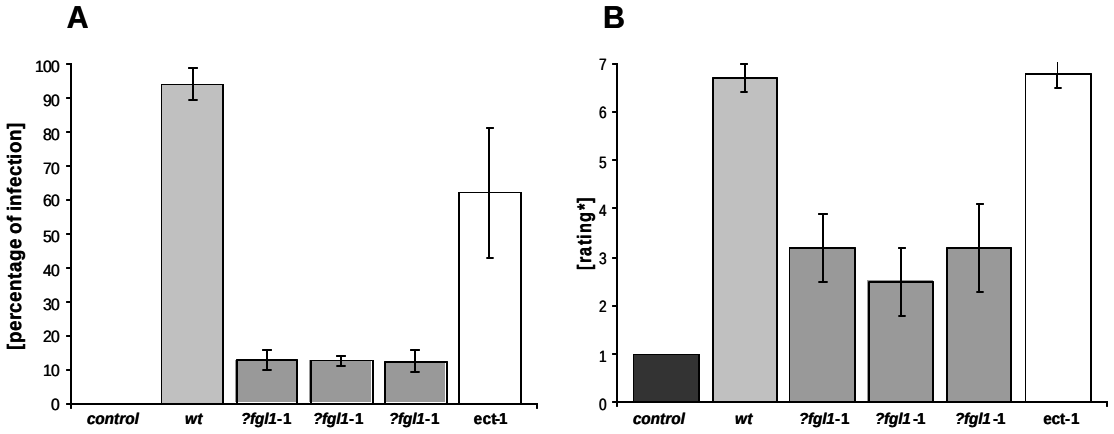

Figure 2. Infection of cereal flowers with *F. graminearum* wild type and ?fgl1strains.

A) Virulence of *F. graminearum* to wheat. Infection referred to partially or completely bleached spikelets observed 3 weeks post inoculation. Results are the average of 15 inoculated wheat heads (14-22 spikelets per head).B) Virulence of *F. graminearum* isolates to maize. Disease severity referred to visual rating scales after Reid et al., (1995) observed 5 weeks post inoculation. Results are the average of 10 maize ears. (control: inoculated with water, wt: wild type, ? fgl1-1/-2/-3: lipase deficient strains, ect-1: mutant with ectopic integration of the disruption construct. Error bars: confidence interval a = 0.05).

SPATIAL PATTERNS OF VIABLE SPORE DEPOSITION OF *GIBBERELLA ZEAE* IN WHEAT AND CORN FIELDS

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ABSTRACT

An increased understanding of the epidemiology of *Gibberella zeae* will contribute to a rational and informed approach to the management of Fusarium head blight (FHB) and Gibberella ear rot (GER). An integral phase of the FHB and GER cycle is the deposition of airborne spores, yet there is no information available on the spatial pattern of viable spore deposition of *G. zeae* above and within wheat and corn canopies. We examined spatial patterns of viable spore deposition of *G. zeae* over multiple years inside rotational (lacking cereal debris) wheat and corn fields in Aurora, New York, USA. Viable airborne spores of *G. zeae* were collected above and within wheat and corn canopies on Petri plates containing selective medium. Spores were collected over a total of 80 day and night sample periods in all of the fields over all of the years. Contour plots of spore counts over entire fields showed that the spatial pattern of spore deposition was unique for each sample period. Spatial Analysis by Distance IndicEs (SADIE) statistics and Mantel tests were used to classify spore deposition patterns during individual sample periods. The majority (93%) of the spore deposition patterns was random; a lesser proportion (7%) was aggregated. All of the aggregated patterns in both the wheat and corn fields were observed at night. In all but one year, the spatial patterns for cumulative spore deposition became aggregated over time. Spatial patterns of spore deposition should be considered when assessing the cumulative exposure of wheat spikes and corn silks to inocula of *G. zeae*.

THE FORCIBLE DISCHARGE DISTANCE OF ASCOSPORES OF *GIBBERELLA ZEAE*

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ABSTRACT

In order to become airborne, ascospores of *G. zeae* must be discharged with enough force to surpass the boundary layer of air surrounding the surface of the substrate bearing perithecia. We measured the forcible discharge distance of ascospores of *G. zeae* inside small glass chambers, and related this distance to the mechanical forces acting on the ascospores. Ascospores were discharged away from culture surfaces at distances ranging from < 1 mm to nearly 10 mm. Six-day-old cultures had discharge distances of 4.6 mm on average, while twelve-day-old cultures had discharge distances that were 3.9 mm on average. A large percentage of spores were discharged at a sufficient distance to surpass the boundary layer of air. Spores that pass the boundary layer have a high probability of being transported away from their source in air currents.

PATHOGENIC VARIABILITY OF FUSARIUM HEAD BLIGHT PATHOGENS IN BARLEY

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ABSTRACT

Fusarium Head Blight (FHB) threatens the barley (*Hordeum vulgare*) production in Austria under humid and warm weather conditions and has the potential of reduced food and feed safety for barley products. Sources of resistance to *Fusarium graminearum* have been identified in spring barley collections. However, very little is known about the reaction of barley to other *Fusarium* species to effectively manage FHB resistance. Two *F. graminearum* susceptible (Stander, ICB 111809) and two resistant (Chevron, CIho 4196) six- and two-rowed spring barley cultivars were investigated for their reaction towards Austrian isolates of *F. graminearum*, *F. culmorum*, *F. poae*, *F. avenaceum* and *F. sporotrichioides* in pot and field experiments under moderate and severe disease pressure, respectively. At the late-milk to early-dough stage, the spikes were spray-inoculated at dusk with a macroconidial suspension of *Fusarium* spp., (10,000 macroconidia/ml). For disease evaluation, the average percentage of infected kernels/spike (on 5-10 randomly chosen spikes) was assessed on each accession 14 and 21 days after inoculation to record possible changes in the infection level. In both experiments, the six-rowed spring barley variety Chevron was resistant against all *Fusarium* species, while both susceptible lines, the two-rowed barley line ICB 111809 and six-rowed barley variety Stander were highly susceptible for all Austrian *Fusarium* species. Overall, *F. graminearum* presented high aggressiveness at moderate and high disease pressure, while *F. poae* exhibited higher aggressiveness at lower humidity and *F. sporotrichioides* and *F. culmorum* were more adapted to more humid screening conditions. The ranking of *Fusarium* species severity on Stander and Chevron 14 and 21 days after inoculation was very similar between moderate and severe disease pressure. Based on observations for both two-rowed barley lines, a potential species ´ genotype interaction requires further investigation.

THE CRYPTIC PROMOTER ACTIVITY OF THE HMR1 CODING REGION IN *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium graminearum is an important pathogen of small grains and maize in many areas of the world. In North America, the scab disease caused by *F. graminearum* poses a major threat to wheat and barley production. To better understand the molecular mechanisms of *F. graminearum* pathogenesis, we have generated a collection of random insertional mutants. One of the mutants, mutant 222, was significantly attenuated in virulence. Its vegetative growth and the production of DON and zearalenone also were reduced. In mutant 222, the transforming vector was inserted at amino acid 268 of the hydroxymethyl-glutaryl CoA reductase gene (*HMR1*), which encodes a key enzyme involved in sterol and isoprenoid biosynthesis. The integration disrupted the N-terminal transmembrane domains of the *HMR1* gene, but its catalytic domain at the C-terminus was intact. We failed to isolate mutants deleted for the entire *HMR1* gene by gene replacement after screening over 500 transformants, suggesting that *HMR1* is an essential gene in *F. graminearum*. However, mutants deleted for only the N-terminal 269 amino acids of the *HMR1* gene were viable and phenotypically similar to mutant 222. In both mutant 222 and the *hmr1*$^{\Delta 269}$ mutant, a 3-kb truncated *HMR1* transcript was detectable by northern blot analyses. In the wild-type strain, only the 5-kb full length messenger was observed. The initiation site of truncated *HMR1* transcripts was determined by 5'-RACE to be 200 bp upstream from the catalytic subunit, indicating that the entire catalytic subunit of *HMR1* was expressed in these transformants. When a *HMR1* fragment corresponding to the region between the insertion site of pCB1003 and the transcription initiation site in mutant 222 were used to express a promoter-less EGFP construct, green fluorescent signals were detectable in conidia, germlings and vegetative hyphae of the resulting transformants. These data illustrate that this region of *HMR1* ORF had cryptic promoter activities and were able to express the downstream catalytic domain in mutants deleted of its N-terminal portion. Our results also indicate the importance of the *HMR1* gene and the function of its transmembrane domains in *F. graminearum*.

RELATION BETWEEN HEAD BLIGHT SEVERITY AND DON IN NATURAL EPIDEMICS OF FHB

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ABSTRACT

Fusarium head blight was widespread and severe on soft red winter wheat in Indiana in 2004. To investigate the relation between head blight severity and grain quality, we assessed the disease in cultivar trials located at 5 sites around the state: PPAC (northwest), LAF (west-central), DPAC (east-central), SWPAC (southwest), and SEPAC (southeast). At each site the experimental design was a randomized complete block with 4 replications. We measured incidence of FHB by counting the blighted spikes in 5 samples of 20 culms per plot. Severity of head blight was visually estimated as the average percentage of the head blighted on symptomatic heads. From these 2 statistics we calculated a disease index (FHBX = incidence ´ severity/100). Grain quality measurements included the frequency *Fusarium*-damaged kernels (FDK), the frequency of asymptomatic infection (AI), and DON content. Asymptomatic infection was determined by plating visibly sound kernels on Komada medium after surface sterilization. DON analyses were conducted at Michigan State University. At each site differences among cultivars for FHBX and DON were highly significant. Ranges for FHBX at each site were as follows: 3 to 38% at PPAC, 0.1 to 19% at LAF, 1 to 23% at DPAC, 6 to 35% at SWPAC, and 5 to 38% at SEPAC. Ranges for DON were: 1.9 to 8.5 ppm at PPAC, 0.8 to 9.8 ppm at LAF, 0.7 to 3.7 ppm at DPAC, 0.1 to 2.2 ppm at SWPAC, and 0.8 to 9.8 ppm at SEPAC. At each site the correlations between heading date of a line and the various measures of FHB were low, suggesting that differences among cultivars were the result more of genetic differences in susceptibility than to differences in favorability of weather for infection and disease development over the range of flowering dates. There was considerable variation in DON concentrations among sites. For example, the mean for PPAC was 3.4 ppm, whereas the mean for SWPAC was 0.5 ppm, yet the mean values for FHBX at these 2 sites were similar (16.3% at PPAC and 16.6% at SEPAC). Mean FHBX was 7.7% for LAF, but the mean DON value was 3.1 ppm, similar to the value at PPAC, where the mean FHBX value was twice that high. The correlation between FHBX and DON was significant only at PPAC and SEPAC, but was only moderate. For data pooled over all sites, the correlation was significant, but low. DON concentrations of 2 ppm or greater were associated with FHBX values from 1.5% to 38.5%. DON concentrations of less than 2 ppm were associated with FHBX values from 0.6% to 34.8%. As was seen in a similar study in 2003, the severity of head blight in the field was of limited value for predicting DON content in the harvested grain.

ULTRASTRUCTURE OF CELLS OF YOUNG MAIZE PLANTS TREATED BY FUSARIOTOXINS

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OBJECTIVES

Impact of fusariotoxins mixture (moniliformin, fumonisin B_1, fusaproliferin, zearalenone, zearalenol and deoxynivalenol, each at concentration 35 µg-mL^{-1}) on maize plants of resistant (Lucia) and susceptible (Pavla) cultivars was studied.

INTRODUCTION

Simultaneous infection with different *Fusarium* species on maize has been observed and their interaction anticipated. Because certain fungal strains are able to synthesize a number of toxic metabolites (e.g. Bottalico, 1998), it is possible that several different mycotoxins will be present in a single plant. In Slovakia moniliformin, fumonisins, fusaproliferin, zearalenone, and deoxynivalenol were identified in maize kernels (Nadubinská et al., 2002, Šrobárová et al., 2002) and through the seed may be transmitted to seedling. However, to this time their impact and role in plant is not fully understood. Eudes et al. (1997) demonstrated that plant toxicity of several trichothecenes was very different to their toxicity in animals. The aim of our work was to test the effects of mixture of selected fusariotoxins on the cell ultrastructure in maize seedlings.

MATERIAL AND METHODS

Two maize cultivars (Zeainvent, Trnava, Slovakia) were used: Lucia, resistant to *Fusarium* infection, and Pavla, the susceptible one to ear rot (Pastirák et al., 2002). Seeds were surface-sterilized with 1% sodium hypochlorite (commercial bleach) for 2 min and rinsed three times in sterile distilled water for 2 min. The seeds germinated for 4 days on filter paper moistened with distilled water, and then they were selected for uniformity and grew hydroponically in Knopp nutrient solution at the temperature of 21/15°C (day / night), photoperiod 16 / 8. After 10 days of cultivation roots of intact plants were immersed in 10 ml of toxin mixture, or for control treatment in distilled water for 72 hours.

Mixture of toxins included all toxins which were identified in naturally infected maize kernels in concentration according to that one, which was an effective in our experiment with maize seedlings chlorophyll (Nadubinska et al., 2003). Moniliformin (MF), fumonisin B_1 (FB_1), zearalenone (ZEN), zearalenol (ZOH) and deoxynivalenol (DON) were obtained from firm Sigma – Aldrich Chemie GmbH, fusaproliferin (FP) was isolated and purified by RITIENI et al. [1995]. MF and FB_1 were dissolved in deionised distilled water, FP and ZEN in methanol >99% Mikrochem Bratislava and DON in acetone (for UV spectroscopy, Lachema Brno): methanol (2:1) to make stock solution (concentration 0,5mg·mL^{-1} of each toxins was used). To obtain 10 ml of final concentration 35¼g'"mL^{-1}, 1mL of stocks were diluted with distilled water.

For electron microscopy, segments from central part of 3rd leaves and 5mm long root apices were fixed with 3% glutaraldehyde and 1% OsO_4, dehydrated in ethanol and embedded in Spur's medium. Ultrathin sections from five embedded specimens (blocks with pieces of organs) of each variant were prepared and stained with uranyl acetate and Pb-citrate and investigated with the EM Tesla BS 500.

RESULTS AND DISCUSSION

Cells of both cultivars had large central vacuoles with thin peripheral layer of cytoplasm. In the cytoplasm, endoplasmatic reticulum (ER), mitochondria, nucleus and plastids were present. Well organised grana and

stroma thylakoids occupied almost the whole chloroplasts volume in the control sample (Fig. 1A). Cell ultrastructure of the treated plants was not significantly different from that of the non-treated ones.

Sporadically, chloroplasts with disorganized thylakoids and an electron transparent stroma in mesophyll cells were observed in the treated plants of the susceptible cv. Pavla (Fig. 1B).

At certain distance form the root tip, the root cells of both cultivars had a large centrally located vacuole. Plasma membrane and tonoplast were distinct and well preserved. Mitochondria, dictyosomes, plastids, ER and nucleus, were present in the peripheral layer of cytoplasm (Fig. 2A). In the roots of the susceptible cultivar treated by fusariotoxins, higher vacuolation and plasmolysis were found in the young cells of the outer cortex than in the resistant cultivar (Fig. 2B).On the plasma membrane surface sporadically small, darkly stained osmiophilic globules were observed. In the young root cells of the susceptible cultivar vacuolation of cytoplasm besides plasmolysis was observed.

ILarge amount of osmiophilic globules were present within periplasmic space and in the cytoplasm (Fig. 3). The osmiophilic globules were found to be associated with plasmodesmata (Fig. 3A) and plasma membrane and with endomembranes like ER, tonoplast and plastids (Figs 3 A, B). Osmiophilic globules are generally considered of lipid composition. In intact plants they were suggested to be related with the process causing wall loosening during cell elongation (‘amajová et al., 1998). Their presence during cold acclimation (Ristic and Asworth 1993), drought and freezing stresses (iamporová and Mistrík 1993) has been related to the changes in plasma membrane composition. In pathosystem (*Gossypium barbadense* L. infected by *Fusarium oxysporum* f. sp. *vasinfectum)*, osmiophilic droplets may represent defence mechanism against fungus infection (Shi et al,. 1991). Some electron-dense large bodies and lipid granules were observed in infected cells of resistant plants (Ilarsan AND DOLAR 2002). Nonetheless, the complete effects of toxins in our experiment probably involve various biochemical events. Fumonisin B1 has been shown to inhibit cell growth and to cause accumulation of free sphingoid bases and alteration of lipid metabolism. Some of the used toxins are phytotoxic (moniliformin), namely fumonisin damages cell membranes and reduces chlorophyll synthesis (Lamprecht et al., 1994).

Lipids from the affected membranes may be accumulated in a different way in cells. Our results indicate that even a low concentration of fusariotoxins may have an impact on plants ultrastructure of young maize plants which are without macroscopical symptoms. The treatment by fusariotoxins evoke the different degree of cell damage in young hosts plant according to their response to the pathogen.

ACKNOWLEDGEMENTS

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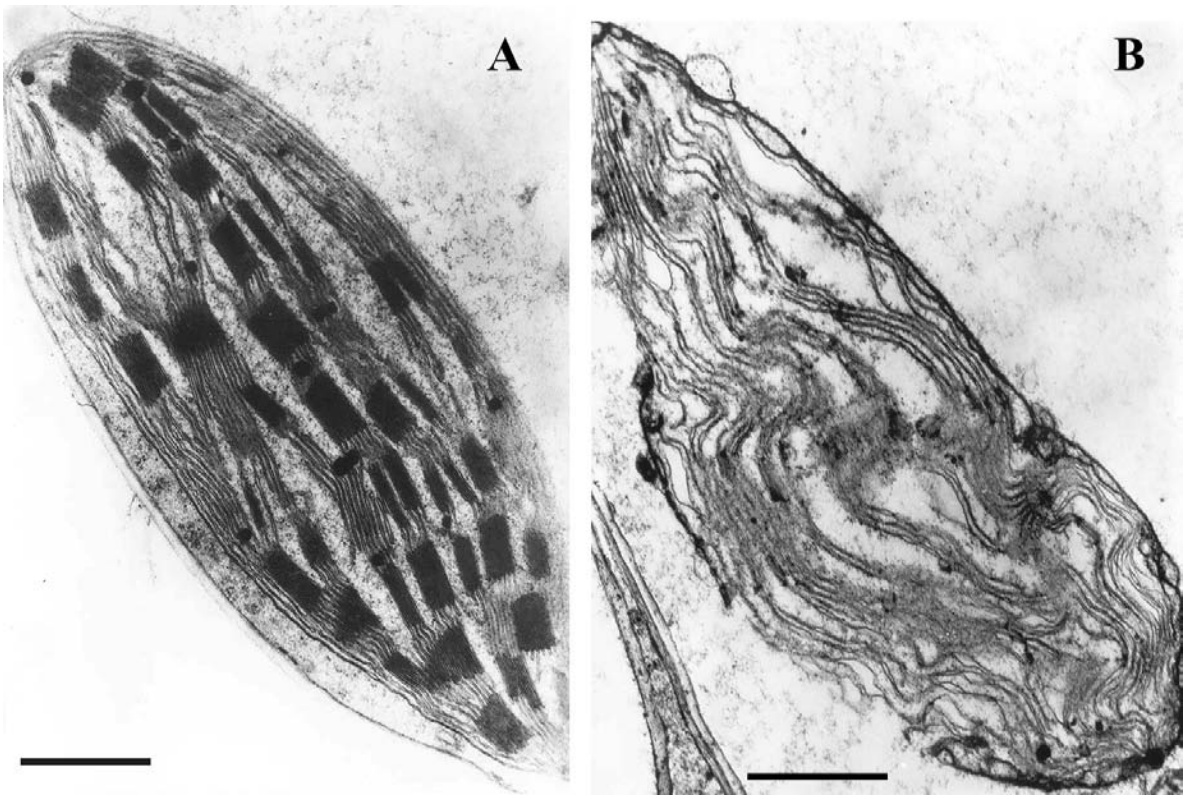

Figure 1. Well-developed inner membrane organization of chloroplasts in leaf mesophyll cells in control plants (A). In the susceptible cv. Pavla (B) treated by fusariotoxins, thylakoids are not organized in grana their arrangement is disturbed. Bars represent 1 µm.

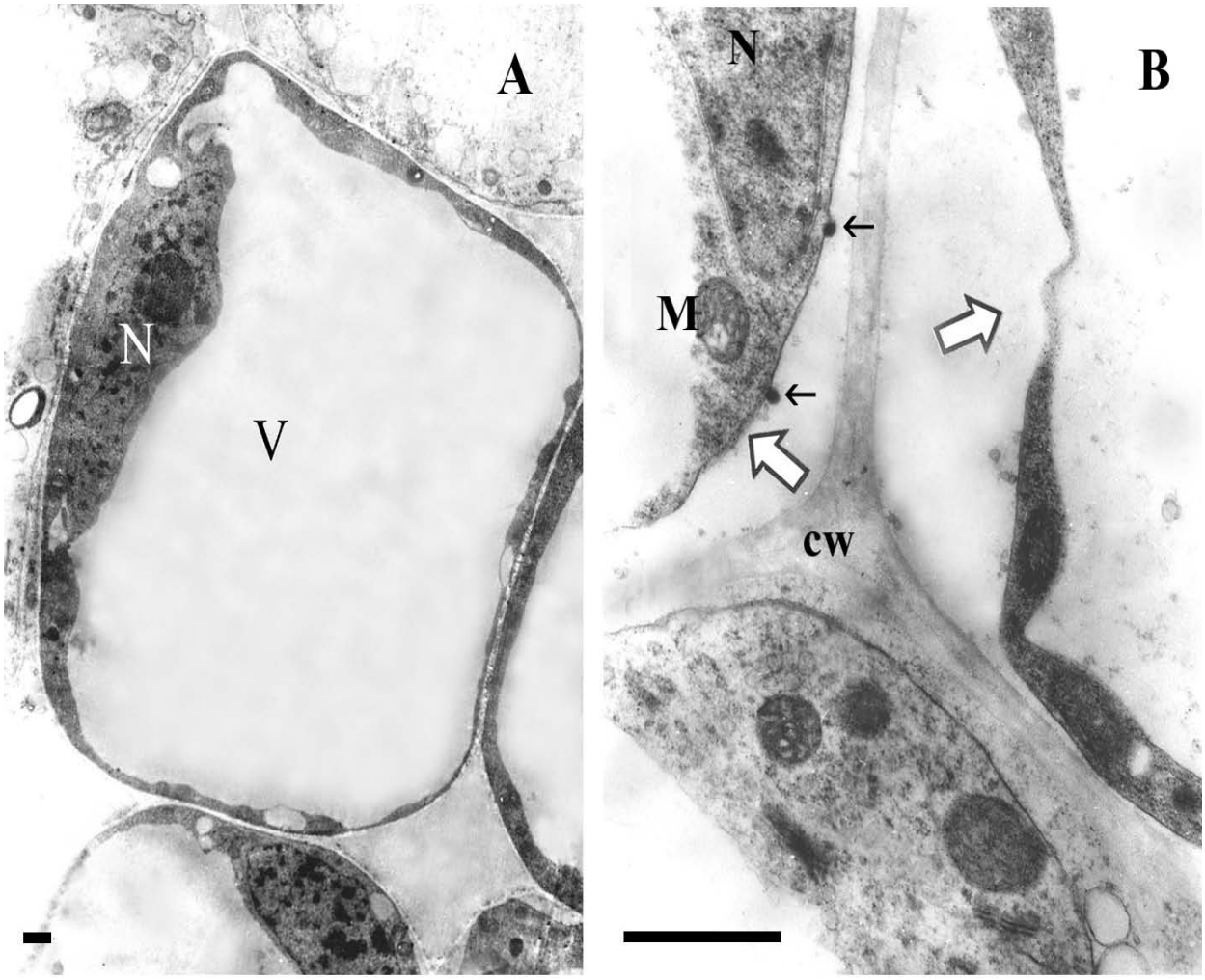

Figure 2. The ultrastructure of control **(A)** and fusariotoxins-treated **(B)** root cells of the susceptible cv. Pavla. Arrows in B indicate protoplast retreat due to plasmolysis, small arrows the osmiophilic globules associated with plasma membrane. M – mitochondria, cw – cell wall, N – nucleus, V – vacuole. Bars represent 1 µm.

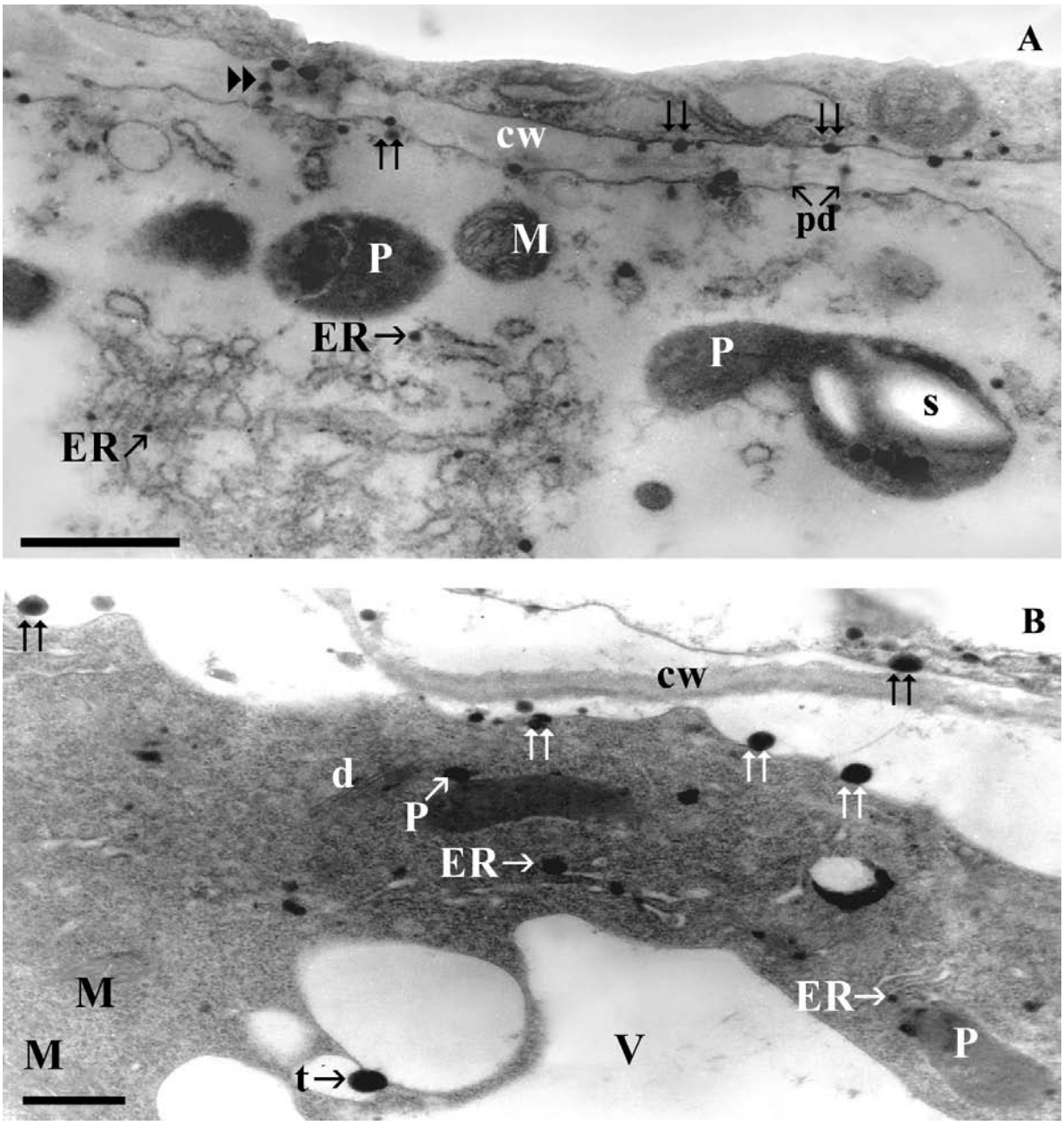

Figure 3. Ultrastructure of cells of young root cortex of the susceptible cv. Pavla treated by fusariotoxins. **A**. Osmiophilic granules were associated with plasmodesmata-pd (A, double arrowheads), plasma membrane (double arrows), endoplasmatic reticulum (ER) (**A, B**), plastids (P), cw – cell wall and tonoplast - t, too **(B), V** – vacuole d - dictyosome, M-mitochondria, s - starch grain. Bars represent 1 µm.

RELATIONSHIPS BETWEEN *FUSARIUM* RESISTANCE IN THE SEEDLING STAGE WITH RESISTANCE IN THE SPIKE STAGE OF WHEAT USING *IN VITRO* AND *IN VIVO* SCREENING TECHNIQUES

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* (Schwabe), is an important wheat disease. The goal of this study was to determine the relationship between resistance of wheat lines to *F. graminearum* in the seedlings and resistance in spikes. The wheat lines were tested *in vitro* in the seedling stage and *in vivo* at the spike stage. Three wheat populations were tested. The first two were derived using the pedigree method ('Frontana'/'Ruby' and 'WEKO60DH4'/ 'Pioneer 2737W') and the last ('Wuhan'/'Maringa') through double haploidy using a corn pollination method. Seedling assays for FHB were conducted individually on slants of Knop agar in glass tubes in the laboratory. A mycelium disk (4.0 mm diameter) of the *F. graminearum* strain (DAOM178148) grown on potato dextrose agar (PDA) medium at room temperature for 1 week, was placed on Knop agar medium in glass tubes near the bottom of the slant. A single germinated seed was placed 2 cm above the mycelium disk in each tube. Controls were fungus-free. Seedlings were grown under artificial light for 14 d, and then evaluated using a scale from 1 to 6. In the field each line was spray inoculated with *F. graminearum* at flowering and mist irrigated. The lines were evaluated for visual symptoms of *Fusarium* using the FHB index (incidence x severity/100), and deoxynivalenol (DON) was estimated using an enzyme-linked immunosorbent assay (ELISA) test. This study shows that *Fusarium* seedling resistance is not completely independent of FHB resistance in spikes.

FUSARIUM HEAD BLIGHT OF OAT: A NEW PROBLEM OR AN OVERLOOKED DISEASE?

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ABSTRACT

Fusarium head blight (FHB) of oat was hardly on the 'radar screen' in the eastern Canadian prairies (MB and eastern SK) or the American upper mid-west (ND, SD, MN) during the 1990s when barley and wheat crops in these regions were being devastated by the disease. Since 2001, when we began to monitor oat more closely, it has become evident that in Manitoba, FHB is a prevalent disease of the crop. FHB usually is not apparent in a field of oats, and unlike the situation in wheat (in particular) and barley, visual in-crop severity is not a valid indicator of the damage to mature seed. In 2001, 15 Canadian oat cultivars tested at three field sites exhibited nil or negligible amounts of mid- to late-season FHB, but had 28.3% and 14.3% average levels of total *Fusarium* and *F. graminearum*, respectively, on the seed; levels of putative fusarium damaged kernels (FDK) ('scabby kernels') and deoxynivalenol (DON), were 8.5% and 5.6 ppm. These amounts, while substantial, were about half those found in the susceptible and partially resistant wheat (DON, 11.1 ppm) and barley checks (DON, 11.2 ppm). By contrast, in 2002, when field conditions were less favourable for disease, the 1.3 ppm average DON level in oats was similar to that determined for wheat (1.1 ppm) and barley (0.8 ppm). A review of the literature indicated that 'FHB' had been reported previously as a disease of oat in Canada, firstly in 1929, but only sporadically and almost exclusively from the Atlantic Provinces and Quebec. Its occurrences have been few and quite localized, and until recently with no verification of the causal pathogen(s) involved. Only a single historic report of 'FHB' in oat from western Canada is available, a 1934 observation that 'slight' disease affected a single Alberta field, with '*Fusarium* spp.' mentioned as a possible cause. It is likely that FHB in oat may at times have been overlooked in western Canada during the previous 50-75 years. If true, and as is better documented in wheat and barley, FHB outbreaks prior to the 1990's would have been relatively modest compared to the devastating episodes since 1993. The continued occurrence of FHB on oat in the region is likely, based on current levels of endemic *Fusarium* inoculum. It also is possible that the *F. graminearum* population, and (or) that of other *Fusarium* spp. has become better adapted to this host, resulting in the higher levels of damage presently observed. FHB must now be regarded as major 'new' disease of oat in western Canada.

EXPRESSION OF TRICHOTHECENE BIOSYNTHESIS GENES (TRI5, TRI6, TRI10, AND TRI12) AND DON PRODUCTION IN *FUSARIUM GRAMINEARUM*

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ABSTRACT

Trichothecenes, such as deoxynivalenol (DON), are sesquiterpenoid mycotoxins that are produced by several genera of filamentous fungi, including *Fusarium*. The trichothecene biosynthetic pathway has been studied in detail. In *Fusarium sporotrichioides*, at least twelve trichothecene pathway genes are localized in a gene cluster. Another pathway gene, TRI101, is unlinked from the cluster. The objectives of this study were to identify the effects of temperature and glucose concentration on expression of the trichothecene biosynthesis genes TRI5 (encodes trichodiene synthase), TRI6 (encodes a transcription factor), TRI10 (encodes an unidentified hypothetical protein), and TRI12 (encodes a toxin efflux pump) using quantitative real time PCR (qRT-PCR) with cDNA targets. Trichothecene concentrations from DON and Acetyl-DON were measured directly from the culture broth by a surface plasmon resonance immunobiosensor over a period of 30 days. *Fusarium graminearum* TMW 4.0185 was used for trichothecene and RNA analysis. Fungal cultures were grown in Erlenmeyer flasks (500 mL) containing 300 mL GYEP medium (0.25 % and 1 % glucose, respectively, 0.1 % yeast extract, 0.1 % peptone). Twenty cultures each were incubated at 15 °C and 28 °C, respectively. All cultures were shaken in the dark at 120 rpm. At days 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30, respectively, one flask from each of the four growth parameters was randomly removed from the incubator for further analysis. To isolate RNA, cells were harvested by filtration. The resulting semi-dried mycelial mats were immediately ground to a fine powder in liquid nitrogen. Total RNA was isolated with the RNeasy® Plant Mini Kit (Qiagen, Hilden, Germany). After digestion of the residual genomic DNA, synthesis of the first cDNA strand from RNA was accomplished with reverse transcriptase. Gene expressions were measured by qRT-PCR using SYBR® Green I and the LightCycler™. We constructed specific PCR primer pairs to amplify fragments from the genes TRI5, TRI6, TRI10, and TRI12, respectively. A beta-tubulin gene specific primer pair was used as an external standard for cDNA quantification. The amount of template cDNA was calculated from a calibration curve set up with a serial dilution of purified genomic DNA of *F. graminearum* TMW 4.0185. Tests with different glucose concentrations in the GYEP medium showed that high sugar concentrations resulted in higher trichothecene yield. In the medium with 0.25 % glucose the optimum temperature of toxin production was found to be 28 °C. Toxin concentrations in media with 1 % glucose were almost unaffected by temperature. Relative expression of the TRI6 gene was low. TRI6 activated the expression of TRI5 during the first days of cultivation. A temperature of 28 °C resulted in a second short expression peak of the TRI6 gene. Relative expression of the TRI5 gene appeared prior to the toxin production. We observed an increase in TRI5 expression at 28 °C. Our data indicate that the relative TRI10 expression remained at a constantly high level during 30 days at all growth conditions. According to our findings, relative TRI10 expression in *F. graminearum* did not positively regulate TRI6 expression, and TRI10 expression was not negatively regulated by TRI6. We also observed a relationship between TRI10 expression and TRI5 expression. qRT-PCR analysis of TRI12 showed no relationship to toxin concentrations found in the cultures.

ENHANCEMENT OF THE AUTOMATED WEATHER DATA NETWORK IN SOUTH DAKOTA

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ABSTRACT

South Dakota has a variety of atmospheric observation systems controlled by many different state and federal agencies for a wide-ranging clientele within the state. State agencies such as South Dakota State University and the Department of Transportation and federal agencies such as the Federal Aviation Administration, United States Geological Service, National Weather Service, and Bureau of Reclamation collect data to serve the general public as well as specific clientele including ranchers, row-crop agriculture, forestry interests, and many others. Data from these networks are often available, but in different formats, in different database locations. Data sharing and coordination among these networks can improve the atmospheric monitoring across the state. Despite the variety of stations, there are large parts of the state that have limited data and many types of data are not gathered and certain locations. Rainfall is a good example of a parameter with extremely high variability across the state, yet the detail in rainfall observations is limited. Soil moisture measurements are non-existent and the density of soil temperature data which are critical to agricultural interests for management decisions such as nitrogen application is lacking. Evapotranspiration estimates require solar radiation measurements, which are few in the state. To address these serious concerns, the state climate office has been approached to help improve the capabilities of our weather data systems in South Dakota, and to aid in bringing about greater access to the current databases.

Disease forecasting for wheat is obviously heavily based on observed environmental conditions. In the state we cannot currently observe the important wheat-growing regions of the state well. The expansion of our network into these areas will improve the capability to depict recent and current weather conditions in greater detail. Such improvements should facilitate development and validation of environmentally based models that are applied to wheat and barley production. Of great importance to *Fusarium* head blight management efforts is the ground truthing of disease forecasting models. Enhancements in the coverage and accuracy of the weather data network in South Dakota will greatly affect the ability of agricultural researchers to produce better results and make stronger inferences about the influence of weather on biosystems.

SEXUAL DEVELOPMENT IN *GIBBERELLA ZEAE*

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ABSTRACT

In *Gibberella zeae*, ascospores are produced in ephemeral perithecia on the surface of field debris and fired into the air. This study was designed to elucidate the process of colonization of wheat tissue, which leads to perithecium production. Stems were systemically and extensively colonized following inoculation of the wheat head. Haploid mycelia moved down the vascular system and pith and then colonized the stem tissue radially. Dikaryotic hyphae developed at two distinct stages: in the xylem, in support of radial hyphal growth and in the chloremchyma, in support of perithecium development. Perithecium formation was initiated in association with stomates and silica cells. Vascular occlusions prevented mycelia from colonizing the stem in 25% of inoculated plants. Vascular occlusions could be an important component of resistance to FHB in wheat varieties.

We have begun to elucidate the gene expression shifts that accompany sexual development *in vitro* using EST-based microarrays. Genes showing highest expression level at earlier development stages were mainly those related to metabolism and cell type differentiation, while genes showed highest expression level at later development stages were mainly those related to cellular transport. These studies provide new targets for control of this devastating pathogen. This research provides information about infection pathways and serves as a basis for these and future investigations into the genetics of host-pathogen interactions.

FUSARIUMSCREEN™, A SENSITIVE, REALTIME AND NON-DESTRUCTIVE MONITORING TOOL FOR *FUSARIUM* INFECTION IN CEREALS

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ABSTRACT

A complex of *Fusarium* spp cause head blight on wheat. Unlike the advances in the area of *Fusarium* genomics, progress in the understanding of the infection process and the possible resistance mechanisms of the host is slow. In wheat five different resistance mechanism to *Fusarium* are currently recognized, including resistance to infection, resistance to colonization, resistance to kernel infection, tolerance to mycotoxins and resistance to mycotoxin accumulation. To exploit these natural types of resistance in wheat breeding programs it is important to monitor the infection process to identify the type and the level of resistance in wheat genotypes. We developed a tool, called FusariumScreen™ to quickly identify different levels and mechanisms of resistance in wheat. FusariumScreen™ is based on high throughput fluorescence imaging (HTFI) and enables fast, detailed, non-destructive studies of the *Fusarium*-wheat interaction. We inoculated wheat heads with a *Fusarium*::GFP transformant after which FusariumScreen™ simultaneously monitored the stress response on the wheat plant by fluorescence of the chlorophyl and the occurrence of *Fusarium* due to the presence of GFP. We checked the optimal spectrum by three dimensional scanning of the absorption and emission spectrum, which enabled the proper adjustments to the filters resulting in a strong improvement of the signal:noise ratio. We used these optimised settings to monitor the infection process in a time lapse series and quantified the disease progress in an automated image analysis pipeline. Preliminary results show that we are able to detect *Fusarium* transformant not only on the surface of florets but also inside the flower tissue. The integration of plant stress response and the increase in fungal biomass enables efficient screening of wheat lines and will generate invaluable information about the infection process and the genetic variation for resistance mechanisms in wheat and barley to *Fusarium* species.

FUNCTION OF ASCI IN *GIBBERELLA ZEAE*

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ABSTRACT

We have been investigating the mechanism of forcible ascospore discharge. For over 100 years, the working hypothesis has been that turgor pressure drives ascospore ejection. For the first time, we show that components of the ascus fluid are crucial to generating turgor within the ascus. The components include mannitol and ions. We are in the process of elucidating the role of each in discharge. In addition, we have identified a DNA binding protein that may be involved in the mechanism of ascospore discharge. These findings shed light on the environmental factors that influence spore discharge in the field.

ALTERED MYCOTOXIN PRODUCTION OF *FUSARIUM GRAMINEARUM* MUTANTS REDUCED IN VIRULENCE

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ABSTRACT

In some cases fungicide application in sublethal concentrations might lead to a reduction of *Fusarium graminearum* biomass but to an increase in trichothecene contamination of grain. The molecular basis of trichothecene regulation is poorly understood. In this study we want to elucidate the mycotoxin production, namely deoxynivalenol (DON) and zearalenone (ZON), in several genetically defined mutants of *F. graminearum*. These mutants are either strongly reduced in virulence or totally apathogenic.

We want to determine the amount of mycotoxin production of virulence mutants of. We cultivated the fungi under controlled conditions on autoclaved grains of different cereals:

FGL1 lipase deficient mutants, which caused only minor disease symptoms after inoculation of wheat, barley, and maize.

Gpmk1 pathogenicity MAP kinase disruption mutants, which are complete apathogenic.

Tri5 deficient mutants were generated out of three isolates of *F. graminearum*, well characterized in field experiments. FG06 (medium aggressive, NIV chemotype), FG25 (medium aggressive, DON chemotype, produces medium levels of DON), FG2311 (highly virulent, DON chemotype, produces high levels of DON). Disruption mutants were found to grow *in vitro* like the respective wild-type but are unable to produce trichothecenes. The mutants were strongly reduced in virulence on wheat, but not on barley and maize.

In the case of FGL1 and Gpmk1 mutants, we want to assess, whether these virulence and pathogenicity genes of *F. graminearum* are signal mediators for toxin production. In the case of the Tri5 disruption mutants we want to assess the influence of the disruption the trichothecene pathway on the zearalenone polyketide pathway. Both pathways primarily use acetyl-CoA starter molecules to synthesize the different toxins.

FGL1, A SECRETED LIPASE OF *FUSARIUM GRAMINEARUM* IS A NOVEL VIRULENCE FACTOR DURING INFECTION OF WHEAT AND MAIZE

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ABSTRACT

Fungal pathogens have evolved a number of different strategies to infect and colonize host plants. A lot of fungal pathogens secrete various extracellular enzymes which are supposed to be involved in host infection. Enzymes like xylanases, pectinases, cutinases, lipases, proteinases, and laccases are capable to degrade structural components of plants.

We could detect, clone, and characterize a secreted lipase (FGL1) of *F. graminearum*. The ORF of the FGL1 gene consists of 1056 bp and is interrupted by two introns. The encoded lipase is composed of 337 amino acids with a calculated molecular weight of 35.7 kDa. The functional identity of the lipase was examined by heterologous gene expression in *Pichia pastoris*. The FGL1 gene shows a high homology to known lipases from *Nectria haematococca* and *F. heterosporum*. Expression analysis of FGL1 indicated that the gene can be induced by suitable substrates and is repressed by catabolites. In planta, FGL1 transcripts were already detected one day after inoculation of wheat spikes. To evaluate the role of FGL1 during the infection process we created lipase deficient mutants by gene disruption and compared them to the wild type strain. Gene disruption of FGL1 resulted in a significantly reduced extracellular lipolytic activity of the mutants. After infection of wheat spikes, the FGL1 deficient strains showed a drastically reduced virulence. In contrast to *F. graminearum* wild type infected wheat spikes, FGL1 deficient strains were unable to colonize the rachis of the spike. Infections of spikes were therefore restricted to the point of inoculation. Additionally, maize ears inoculated with *F. graminearum* wild type conidia are fully infected and develop no kernels. In contrast, the maize ears develop normally and showed minor disease symptoms when inoculated with FGL1 deficient strains.

Our data are the first molecular proof that a secreted lipase is a major virulence factor of a fungal pathogen.

QUANTITATIVE DETECTION OF FUSARIUM SPECIES IN WHEAT USING TAQMAN

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ABSTRACT

Fusarium Head Blight (FHB) of wheat and other small-grain cereals is a disease complex caused by several fungal species. To monitor and quantify the major species in the FHB complex during the growing season, real-time PCR was developed. TaqMan primers and probes were designed that showed high specificity for *Fusarium avenaceum, F. culmorum, F. graminearum, F. poae* and *Microdochium nivale* var. *majus*. Inclusion of an internal PCR control and serial dilutions of pure genomic DNAs allowed accurate determination of the concentration of fungal DNA for each of these species in leaves, ears as well as harvested grains of winter wheat. The DNA concentration of *F. graminearum* in grain samples correlated ($R^2 = 0.7917$) with the incidence of this species on the grain as determined by isolation from individual kernels.

Application of the TaqMan technology on field samples collected in 40 wheat crops in The Netherlands during the growing season of 2001 revealed that *M. nivale* var. *majus* predominated on leaves early in the season (GS 45-65). Ears and harvested grains from the same fields, however, showed *F. graminearum* as the major species. In 2002, grain samples from 40 Dutch fields showed a much wider variety of species, whereas in ears from 29 wheat crops in France *F. graminearum* was the predominant species. The concentration of DON correlated equally well with the incidence of the DON producing species *F. culmorum* and *F. graminearum* in the grain samples ($R^2 = 0.8232$) as well as with total DNA of both these species ($R^2 = 0.8259$). The *Fusarium* TaqMan technology we developed is an important tool to quantify and monitor the dynamics of individual species of the complex causing FHB in cereals during the growing season. This versatile tool has been applied in a comparison of different genotypes, but can be applied to other disease management systems, e.g. fungicide treatments.

STUDY OF GENES IMPORTANT TO SCAB PATHOGENESIS AND RESISTANCE IN WHEAT

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OBJECTIVE

To identify wheat and fungal genes that play important roles in FHB pathogenesis in wheat.

INTRODUCTION

In general, establishment of plant disease results from complex interactions between the host plant and the fungal pathogen, involving the expression of resistance genes of the plant and the pathogenic genes of the fungus. In both organisms, altered gene expressions occur from onset of the attempted fungal invasion. These genes, specifically induced by each other (e.g. host resistance genes by the pathogen and pathogenic genes by the host), would be essential to disease development. In this study, we systematically compared the gene expression profiles from FHB-infected as well as healthy wheat spikes of both the FHB-resistant cultivar 'Sumai 3' and FHB-susceptible cultivar 'Wheaton'. Among the differentially expressed cDNA fragments identified, those that are associated with the FHB-resistance of Sumai 3 were further analyzed, assuming that they should be related to the essential genes for the FHB-resistance of Sumai 3 or pathogenicity of *F. graminearum.*

MATERIALS AND METHODS

Spring wheat *(Triticum aestivum* L.*)* cultivars 'Sumai 3' (FHB-resistant) and 'Wheaton' (FHB-susceptible) were used in this research. The procedures for FHB inoculation, sample collection and preparation, and DDRT-PCR were described by Yen et al. (2000). cDNAs of interest were cut off directly from gels, purified, re-amplified with the corresponding primer set used in the DDRT-PCR, cloned with PCR-TRAP® Cloning System (GenHunter, Nashville, TN USA), and sequenced using ABI 310 automated DNA sequencer with the primers Lseq and Rseq complementary to the vector. The cloned cDNA fragments were used as probes for Southern and Northern analyses. For Northern, 1 µg mRNA per sample, prepared from high-quality, total RNA, was separated on 1.2% denaturing agarose gel and transferred onto nylon membrane using 10XSSC. For Southern blotting, genomic DNA was extracted from young wheat leaves and mycelia of *F. graminearum* Fg4. For each sample, 10 µg of total genomic DNA was digested with EcoRI, loaded on 1% agarose gel and run overnight with TBE buffer at 20 volts. DNA fragments were transferred onto nylon membrane with 0.4N NaOH as the buffer.

RESULTS AND DISCUSSION

Five cDNAs (namely, A7, C4, C7, G12 and G75) that were differentially expressed in the FHB-inoculated 'Sumai 3'at around 32 hai and usually in a short time frame were identified, cloned and sequenced (Fig. 1, Table 1). These differential expressions were consistently observed in repeated experiments. Southern analyses with A7 and G12 as the probes revealed strong hybridizing signals in both Sumai 3 and Wheaton, but not in *F. gramnearum* (Fig. 2). Therefore, A7 and G12 should represent wheat genes. C4 probe revealed two bands in *F. graminearum*, but no signal in either 'Sumai 3' or 'Wheaton', suggesting that the gene represented by C4 is a *F. graminearum* gene. However, With C7 and G75 as probes, homologous DNA fragments were detected in both wheat and *F. graminearum* (Fig. 2). Therefore, Southern analyses failed to determine the organismal origin of C7 and G75.

As shown in Fig. 3, Northern analysis with C7 as the probe revealed hybridizing signals only in *F. graminearum*. Considering the result of our Southern analysis (Fig.2), C7 may represent two different *F. graminearum* mRNA species that may be encoded by the same DNA sequence. Alternatively, the two mRNAs may share a homologous sequence at their 3' ends and the two corresponding coding genes may located closely on the same chromosome, or they may not linked but happen to have the same size. Gene expressions were not detected by Northern blotting in the two *Fusarium*-inoculated wheat cultivars, although the mRNA represented by C7 should be there, at least in the *Fusarium*-inoculated 'Sumai 3' as indicated in Figure 1. A possible explanation is that the expression level of the corresponding genes in the *Fusarium*-inoculated wheat spikes was too low to be detected by Northern blotting. A strong gene expression of about 8.23 kb mRNA was detected in the *Fusarium* inoculated 'Sumai 3' spike by Northern analysis with G75 as the probe (Fig. 3). We could not, however, determine whether the expressed gene represented by G75 belongs to wheat or *F. graminearum*, or both because Southern analysis revealed homologous sequences in both wheat and *F. graminearum* genomes (Fig. 2). We could not rule out the possibility that the cognate fungal gene expresses or greatly increases its expression level only after the pathogen invades wheat. No hybridization signal was detected by A7, G12 and C4 as the probes in the Northern analysis. Again, it might be due to that their expression levels were too low to be detected with Northern blotting.

No sequence in GenBank databases was found significantly similar to any of the five cDNAs when using BLASTX. Using BLASTN, many sequences similar to the cDNAs were revealed. With C4 as the query, no similar sequence was found in NR database while 8 highly similar sequences were found in EST database. Of these sequences, one (BM134483, 4e-83) is from the cDNA library of wheat spikes infected by *F. graminearum* (Kruger et al. 2002), three (BU065460, 1e-83; BU059720, 1e-83; BU059357, 8e-76) from the cDNA library of nitrogen- or carbon-starved mycelia of *G. zeae* (Trail et al. 2003); two (BI200744, 2e-21; BI191958, 1e-06) from the cDNA library of *Tri 10* overexpression strain of *Fusarium sporotrichioides*; one (CD456658, 7e-67) from the mycelium cDNA library of *G.zeae* under trichothecene-production conditions; and one (BI950399, 1e-25) from the spike cDNA library of *Fusarium*-infected *Hordeum vulgare*. Those similar sequences further suggested that C4 represent a *Fusarium* gene, which might be involved in the wheat-*Fusarium* interaction. Among the eight sequences highly similar to C4 revealed by the GenBank searching, three (BI200744, BI191958 and CD456658) were from cDNA libraries related to trichothecene production.

With C7 as the query, searching the NR database revealed hundreds of 18S rRNA sequences with e-values less than e-100, including the partial rRNA sequences of four *Fusarium* species. Also, hundreds of EST sequences were revealed similar to C7 when searching the EST database. C7 might represent a gene that shares homology sequence with the 18S rRNA gene. Actually, sequences complementary to 18S rRNA have been found in a large number of eukaryotic mRNAs. Such mRNA-rRNA complementarity has also been shown to inhibit translation in eukaryotes by stalling the initiation complex (Tranque et al., 1998; Hu et al., 1999; Verrier and Jean-Jean 2000). Our preliminary data suggest that C7 or other similar transcripts that selectively regulate the availability of the target mRNAs for translation.

With G12 as the query, 11 blast hits with e-value less than 1e-87 were found in the NR database. The sequence (X02595.1) with the least e-value (e-120) was a chloroplast gene encoding ATP synthase CF-O subunit I & III from wheat. However, it was the minus strand of this gene that is similar to G12. G12 is also highly similar to the minus strand of the barley chloroplast gene (AJ010573) encoding ATP synthase CF-O Subunit I. Searching the EST data base revealed 100 blast hits with e-values less than 2e-64. The sequence (CA483759) with the least e-value (e-123) is a wheat cDNA from a subtracted library enriched for Russian wheat aphid feeding response. This sequence was, also, highly similar to the minus strand of the wheat chloroplast ATP synthase CF-O subunit I & III gene.

ATP synthase is an important enzyme in both ATP synthesis and hydrolization (Alberts et al. 1994). The change of vacuolar H+-ATP synthase activity has been observed during salt-stress response in iceplant (*Mesembryanthemum crystallinum*) (Low et al. 1996). In tomato cell suspension culture, the activity of plasma membrane H+-ATP synthase increased when the cells were treated with elicitors from the fungal pathogen *Cladosporium fulvum* (Vera-Estrella et al., 1994). Also, the gð-subunit mRNA of F0F1-ATP synthase is moderately up-regulated during a compatible interaction between potato and its fungal pathogen *Phytophthora infestans* (Madrid et al., 1999). Therefore, the cognate gene of G12 might have a broader function in ATP synthase-mediated host defense response against pathogen invaders or abiotic stress.With A7 as the query, one cDNA (BU991324) with e-value 8e-31 in the EST database was found. This cDNA was from *Hordeum vulgar* callus. No similar sequence was found for G75.

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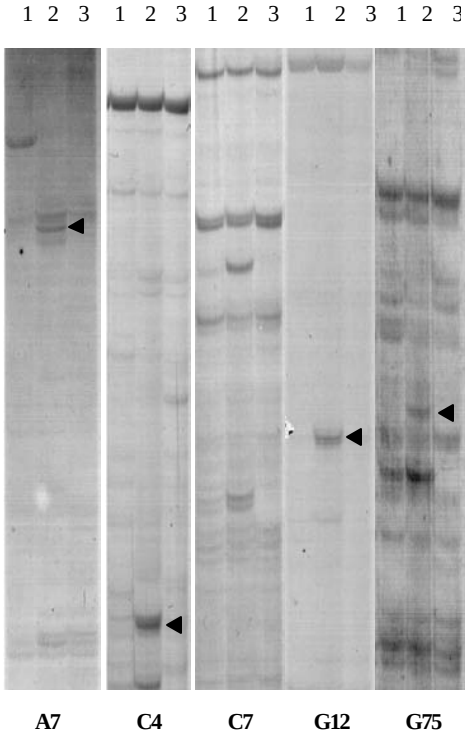

Figure 1. Differential display of mRNAs of wheat cultivars 'Sumai 3' and 'Wheaton' in 32 hai. Lane1, 2, 3 are the FHB-inoculated 'Wheaton', the FHB-inoculated 'Sumai 3', and the water-inoculated 'Sumai 3', respectively. Arrows point to the cDNAs cloned.

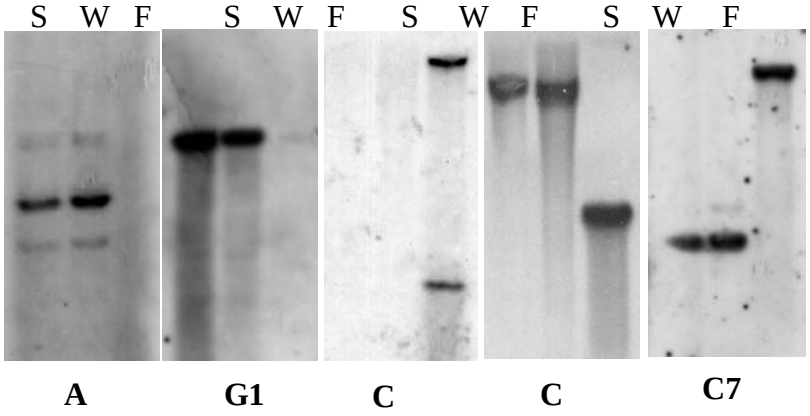

Figure 2. Southern analysis of the five differentially expressed cDNAs. S: 'Sumai 3'; W: 'Wheaton'; F: *F. graminearum. Genomic DNA was* digested with *Eco*RI.

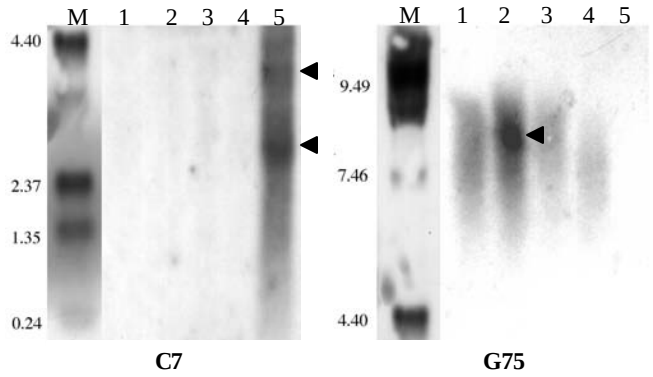

Figure 3. Northern analyses of mRNA with cDNAs C7 and G75 as the probes. Lane 1: the water-inoculated 'Suami3'; Lane 2: the FHB-inoculated 'Sumai 3'; Lane 3: the FHB-inoculated 'Wheaton'; Lane 4: the water-inoculated Wheaton; Lane 5: *F. graminearum*; M: RNA ladders (kb).

Table 1. The sequences of specific ESTs

cDNA	Sequence*	Length(bp)
C7	AAGCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCTAATAGCGTATA TTAAAGTTGTTGTGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCTCACCGCGTGTAC TGGTCCGGCCGGGCCTTTCCCTCTGTGGAACCCCATGCCCTTCACTGGGCGTGGCGGGGAAACAGGACTTTTAC TGTGAAAAAAAAAAAGCTT	241
G75	AAGCTTTTATTCGAAGGGAATGGCGAAGGATTGGAGGATAACCCATGGTCTGCATTGACCAAGAGGAGTAACGT TTGTTTCCTGATGTAAAGTGTATATGTCATTTTTGCTGTGATCAAGGATGAGTAAAGCACTGATGATGGAAGAT GGTGAGGTTGCTTGGTAGATTATATACCCAGTGTTGATGCCCCAAAAAAAAAAAGCTT	206
A7	AAGCTTATACCCGGCCAGGGCAATGTAGCTATCCCAATAAGATATGAAAGCTGGGCCACACCTGAGCAAGGAAC TTATACATAGAAGTAACAGTAAATGTGCAAGATTTGGACCACTGCCATTTCCTTAAACCATCAATCAACAAATT GAAATAGAGATATCGGTATCTTCCAATCTGCCACAAGGATCTAGTCGCCAGTATCTTTGCCTTCTTCCCAACTT ATCCCAGCATTCCTTGTAAGCGTTGCTATCACTACGAAAAAGCAAGCCTACTTACTACGTGTTTTCCTCTATTG GCAAATACGCATATACTGACCTACTCACGTGCATATCTTGCATAAAGCCTTCCCCTAAAAAAAAAAAGCTT	367
C4	AAGCTTCTCAACGATGAAACGAAATTAATTCAAAAGAGTTGGAATGACGAAATACATATGGACCCTACAAATGT CGGACCAAGTTAAATGGGGTGAAGCCCTTTGGGGTTTCCATCCTCCGCTGCCGATAGCTGTACATTATACTTAG CTGAGAAATCATACCCTTTCGTTTTGCGAAAAAAAAAAAGCTT	191
G12	AAGCTTATACCGTGTTAATGGTCTCACATTCTTGGTTTATAGAGAATCAAAGTTGATTTACCAATGAGTCGCGA AATGCTATGGTTCTTCCATATGATTTCTGAATTTATTCAGTAAGTAATTCGTCGAGATCGTGCACCCTTTTCTT ATTTATCCGAAAAATACTAAAAAATATTATAAAGTGCAGCCGGATAGATCCAATCTATTCTTGAAATAGACAAC TCGCACACACTCCCTTTCCAAAAAAAAAAAGCTT	256

*The underlined sequences are the primer (5’ end) or the annealing sequence of primer (3’ end) used to define the ESTs in DDRT-PCR.

SYMPTOMS AND MYCOTOXIN ACCUMULATION IN RICE INOCULATED WITH *FUSARIUM GRAMINEARUM* ISOLATED FROM WHEAT AND BARLEY

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ABSTRACT

There have been several reports of 'scab' on rice caused by *Fusarium graminearum*. In addition, rice is included, along with other cereals, in CODEX's discussion on setting up maximum levels of deoxynivalenol (DON) since DON can be detected in rice. However, knowledge regarding 'scab' on rice has been quite limited. We artificially inoculated rice with *F. graminearum* isolates obtained from wheat and barley: spikes of the potted rice plants were spray-inoculated at the anthesis with macroconidia suspension of the isolates, the plants were placed in a dew chamber at 100% humidity and 25°C for 16 h, and they were finally placed in a greenhouse equipped with a sprinkler system that intermittently produces fine mist in order to keep the inoculated spikes wet. Within a week of inoculation, discoloration was observed on the glumes, and later, some of the severely discolored glumes were somewhat bleached. Salmon-pink sporodochia were observed on some of the severely discolored florets after more than two weeks of inoculation, and such florets were sterile in most cases. In the grains harvested from the inoculated spikes, trichothecene mycotoxins DON and nivalenol (NIV) were detected. Wheat and barley were inoculated with *F. graminearum* isolates obtained from rice, similar to the inoculation of rice with isolates obtained from wheat and barley. Wheat, barley, and rice were inoculated with twenty-five isolates comprising 13 isolates from wheat or barley and 12 from rice; these isolates were used individually. All the isolates were virulent to all the crops. However, their virulence to rice differed from their virulence to wheat and barley. While a good correlation was observed between their virulence to wheat and barley ($r = 0.76$, $p < 0.001$) and between their virulence to two rice cultivars ($r = 0.60$, $p < 0.01$), no positive correlation was observed between their virulence to wheat or barley and their virulence to rice. Additionally, the difference in the isolates' virulence to the different crops appeared to associate with the isolates' chemotype. Among the tested isolates, the DON-chemotype group showed higher virulence than the NIV-chemotype group to wheat and barley, whereas in case of rice, the NIV-chemotype group was more virulent than the DON-chemotype group. Our results suggest that wheat or barley and rice would be the inoculum source of Fusarium head blight (FHB) and scab in double-cropping system of those crops in Japan and that there is a risk of mycotoxin contamination in rice. Moreover, it is possible that prevalence of NIV-chemotype of *F. graminearum* on wheat and barley in the western part of Japan could be attributed to the prevalence of the double-cropping system of rice and wheat or barley in the area.

INVESTIGATION OF KERNEL INFECTION BY *FUSARIUM GRAMINEARUM* IN WHEAT

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OBJECTIVES

This study was to investigate infection of wheat kernels by *Fusarium graminearum* in 14 spring wheat lines, and ~~and~~ correlation of kernel infection with visual scabby kernel (VSK), DON, and kernel discoloration.

INTRODUCTION

Fusarium head blight (FHB) or scab of wheat, primarily caused by *Fusarium graminearium* in the US is a destructive disease. The pathogen primarily infects the kernel and produce mycotoxin, deoxynivalenol (DON). Host resistance to FHB is a complex trait. Shroeder and Christensen (1963) proposed that resistance to scab is of two types: resistance to initial infection and resistance to the spread of the infection within a plant. Later, additional types or components of resistance were proposed (Mesterhazy, 1995). The two principle resistance types proposed by Shroeder and Christensen have been widely used, while the others are not clearly defined and methods of measuring them are not yet standardized (Bushnell, 2002). The proposals and debates on types and components of resistance to FHB reflect the complexity and difficulties to work with this disease.

Currently, screening of FHB resistance of wheat relies heavily on field nurseries. Besides assessment of visual symptoms on the spikes (disease severity and disease incidence), estimates of kernel damage by the pathogen on the basis of percentage of Fusarium-damaged kernels (FDK) or visual scabby kernels (VSK, Jones, 1999), and concentration of deoxynivalenol (DON) have been a common practice in measuring the disease. Symptoms used to determine VSK or FDK include color (pink, chalky white, or pale gray), and size of kernels (thin or shriveled) in comparison with kernels of normal color and size. However color of kernels harvested from FHB nursery could be a trait of continuous variation. In our multiple year spring wheat FHB germplasm screening trials, it has been observed that some lines exhibit a high percentage of kernel discoloration (bleached), while the kernel appear plump and sound. In some materials, high percentage of shriveled kernels with normal color is a distinct reaction type of those lines. Variations of kernel color and size could be due to environmental stress or *F. graminearium* damage, posing difficulties in distinguishing Fusarium damage from environment-induced damage and in interpreting data. This study was initiated to investigate infection by *F. graminearum* of seed harvested from field FHB nursery, to compare seed infection with VSK, DON, and seed infection levels with the visual appearance of the kernel measured by kernel color and size.

MATERIALS AND METHODS

Plant materials and field nursery management. Fourteen spring wheat lines from the FHB germplasm screening nursery were used. ND 2710 and Wheaton were used as the resistant and susceptible checks, respectively. Field management and inoculation procedures were described by Zhang et al. (2000, 2001). A randomized complete block design was used in the experiment with three replicates. Entries were planted in two-row five-foot long plots in 2001 and 2002 in Brookings, SD. The field was inoculated with *F. graminearum* colonized corn kernels of 10 isolates at a weekly interval for four consecutive weeks beginning at the early jointing stage of plant development. The plots were tagged at anthesis and inoculated with

a conidial suspension (50,000-70,000 conidia/ml) of a mixture of the same 10 isolates using a sprayer. A second inoculation was applied five to seven days after the first inoculation. The nursery was mist-irrigated following a schedule of 3-min misting with 30-min recess between 8:00pm and 9:00am during the course of inoculation. At maturity, the plots were hand-harvested, and threshed with a combine then a single plant thresher at minimum wind force.

Data collection and analysis. Percentage of VSK of each sample was estimated. DON concentration was collected from 15g of the grain sample, and data was provided by Beth Tacke, Dept. of Veterinary Diagnostic Services, North Dakota State University, Fargo, ND. Two hundred kernels of each experiment unit were randomly selected from the field grain samples and sorted into four classes, 1) plump normal—the color of the seed appeared normal, the seed was fully-developed; 2) plump bleached—the seed appeared partially to completely discolored, bleached, the seed was of normal size and fully-developed; 3) shriveled normal—color of the seed appeared normal, but the seed was small and shriveled; and 4) shriveled discolored—the seed color was pink, bleached, or gray, the seed was shriveled. The sorted kernels were surface-sterilized in 10% sodium hypochlorite for 45-60 seconds, then transferred to sterile distilled water for 60 seconds. Sterilized seeds were placed in a modified acidic PDA medium, then incubated at 22C for 72h. The number of kernels with recovered *F. graminearum* was recorded.

Analysis of variance was conducted on the percentage of kernel infection, VSK, DON using line, rep, year and line*year as variables. Correlation of the kernel infection, FDK, and DON were calculated using the means of each line over two years. Stepwise regression analysis was used to detect the most important class contributing to the total infection of the sample.

RESULTS AND DISCUSSION

Percentage of seed infection, VSK, and DON concentration of each line were presented in Table 1. The means of infected kernel and VSK were higher in 2002 than 2001, while DON concentration was similar between the two years. Analysis of variance of seed infection, VSK, and DON indicated significant effect ($p<0.01$) of line, year, and year *line of all those three variables. This result agrees with the general consensus that FHB is highly influenced by the environment. Multiple environment test is essential for screening for resistance.

Correlation of seed infection and VSK was significant ($r=0.82$, $p<0.001$). Correlation between seed infection and DON concentration was not significant ($r=0.28$, $p=0.335$). VSK and DON were significantly correlated ($r=0.67$, $p=0.009$). The result might be explained by the fact that some lines (i.e. Sapporo Haru Komugi Jugo) in this study showed high DON but low seed infection and VSK, and some lines (i.e. Tokai 66, Nobeoka Bozu) had low DON but moderately high seed infection.

The fungus was recovered from all classes of wheat kernels (Table 2) with the highest infection occurring in the class of shriveled and discolored kernels (overall mean infection frequency=85.7%). The mean frequency of infected kernel of normal seed was 40.4%, of bleached plump seed was 56.4%, and of shriveled normal color kernel was 57.0%. Infection frequency of normal kernel was significantly correlated with the bleached plump and normal shriveled kernels, whereas kernel infection in the class of shriveled and discolored was not related to that in other classes (Table 3). This result indicates that bleached plump and normal shriveled kernels were mainly due to the environment, i.e. irrigation and high temperature. Stepwise multiple regression of the percentage of seed infection in the four classes to percentage of seed infection indicated that all the four categories of kernels were included in the model, while discolored (including pink and chalky seeds) shriveled kernels explained 92.7% of the variation.

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Zhang, X., Y. Jin, J. Rudd, and H. Bockelman. 2001. Evaluation of USDA spring wheat germplasm for Fusarium head blight resistance. Pages 220-224 *In:* Proc. 2001 National Fusarium Head Blight Forum. Dec. 8-10, 2001, Erlanger, KY.

Table, 1. Percentage of kernel infected by *Fusarium graminearium* , VSK, and DON concentration in 14 spring wheat lines tested in field nurseries in 2001, and 2002 in Brookings, SD.

	Seed infection (%)			VSK (%)			DON (ppm)		
Name	2001	2002	Mean	2001	2002	Mean	2001	2002	Mean
Excelsior	13.7	59.3	36.5	14.0	25.2	33.3	6.9	7.4	7.2
Prodigio I.	22.8	62.1	42.5	16.0	33.3	24.7	4.2	8.6	6.4
Surpresa	33.6	53.9	43.8	23.3	36.7	30.0	8.5	7.4	8.0
Sappro H. K,	42.6	47.9	45.2	17.7	40.0	28.8	12.2	11.8	12.0
ND 2710	26.4	70.4	48.4	19.0	43.3	31.2	5.6	4.6	5.1
Nobeoka B.	31.6	70.4	51.0	15.7	25.3	20.5	2.2	1.7	2.0
Tokai 66	31.9	79.0	55.4	12.0	33.3	22.7	2.4	2.3	2.4
Abura	43.0	70.3	56.6	47.3	56.7	52.0	4.6	3.7	4.2
Nyu Bai	38.8	77.1	58.0	15.3	33.3	24.3	3.8	2.2	3.0
Norin 34	42.0	82.2	62.1	30.0	70.0	50.0	5.9	6.3	6.1
Norin 43	62.1	78.4	70.1	56.7	70.0	63.3	7.5	8.0	7.8
Sin Chunaga	67.9	82.5	80.2	76.8	76.8	76.8	11.8	13.8	12.8
Wheaton	80.3	85.5	82.9	83.3	90.0	86.7	24.3	13.0	18.7
Gogatsu-K.	83.0	84.7	83.9	66.7	73.3	70.0	10.0	7.2	8.6

Table 2. Percentage of kernel infection in four classes of kernels from FHB field nurseries in 2001 and 2002 in Brookings, SD.

Line	Normal color plump kernel (%)	Normal color shriveled kernel (%)	Bleach plump kernel (%)	Shriveled discolored kernel (%)
Surpresa	26.4	54.3	52.2	87.8
Sappro H. K,	27.0	45.0	14.8	85.6
Excelsior	29.5	37.5	41.8	86.0
Norin 43	31.0	10.0	61.3	79.5
Prodigio I.	33.3	27.3	50.4	76.4
Abura	36.4	45.8	52.5	82.7
ND 2710	36.8	67.3	53.3	80.6
Nobeoka B.	39.6	47.1	73.5	84.8
Average	40.1	49.7	56.1	85.7
Nyu Bai	42.7	59.8	67.3	90.4
Sin Chunaga	46.2	40.1	65.6	85.1
Norin 34	46.4	47.4	59.3	83.5
Tokai 66	47.4	64.3	60.2	93.9
Wheaton	49.3	62.4	72.2	91.5
Gogatsu-K.	79.2	79.9	75.2	87.7

Table 3. Correlation coefficients of percentage of kernel infection in four classes of seed samples harvested from the FHB field nursery in 2001 and 2002.

	Normal color plump kernel	Normal color shriveled kernel	Bleached plump kernel	Discolored shriveled kernel
Normal color plump seed		0.4866**	0.2906*	0.0987
Normal color shriveled			0.1506	0.1531
Bleached plump				0.2034

*, ** = significant at 0.05 and 0.01, respectively.