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CHEMICAL, CULTURAL AND BIOLIGICAL CONTROL

Chairperson: Marcia McMullen

EAR SPRAY TARGETING FOR IMPROVED EAR BLIGHT AND MYCOTOXIN CONTROL

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OBJECTIVE

There has been interest in improving the targeting of fungicide applications to ripening ears during the crucial anthesis period to provide more effective control of Fusarium head blight development and prevention of mycotoxin contamination.

This study was conducted to examine different spray application techniques for more effective coverage of ripening ears.

INTRODUCTION

Fusarium infection is of concern because of impacts on crop yield and quality and the concomitant contamination with trichothecene mycotoxins, particularly deoxynivalenol (DON) which are produced under conducive environmental conditions during ripening of cereals. Suppression of FHB is partially achieved by the application of fungicides. However, detailed examination of the most effective spray systems for targeting of applications on the ear have not been examined in detailed. Thus this study examined a range of different spray systems for examining efficacy for better control of FHB in cereals in the UK.

MATERIAL AND METHODS

A series of experiments were done to examine deposits of fungicide and trace dyes on coverage of ears of ripening wheat plants in a wind tunnel. Conventional flat fan, 10o angled air induction nozzle, two conventional flat fan nozzles at 45°, one forward and one back (twin cap), wide angle hollow cone coarse spray, conventional flat fan nozzle 45° backword fine spray, conventional flat fan 45° medium spray, wide angle hollow cone fine spray, pre-orifice flat fan and 10° angled air induction nozzle were examined. All were used at 150 l/ha except the final treatment which was at 100 l/ha.

A field trial had been conducted for two years with 4 spray treatments (Conventional flat fan, 10° angled air induction nozzle, pre-orifice flat fan nozzle and a fine hollow cone nozzle. Three fungicides were used: 0.3 l Amistart + 0.3 l/ha folicur; 1.2 l/ha UK187; 0.6 l/ha UK 187. There were all applied at the beginning of anthesis. A full factorial experiment was carried out and the treatment plots were sprayed with a spore suspension of *F. culmorum* three days prior to fungicide application. 100 ears per plot were collected two weeks after spraying and immediately prior to harvest for analysis of DON and nivalenol (NIV). These were analysed using the method described by Ramirez et al. (2004).

RESULTS

The final grain yield was best in the full UK 187 application. There was a gradation of effect with this treatment being the best. There was a trend for the three test-application systems to be better than the conventional flat fan nozzle. There was a marked effect of fungicide treatment on FHB assessed two weeks after fungicide application. However, the effect was not directly related to type of spray system used. Analysis of DON and NIV using HPLC showed that most treatments had < 750 ng/g. In 2003 there was a significant natural contamination with *F. poae* and thus NIV levels were slightly higher than for DON concentrations found in the ear samples.

In 2004 the experiment showed that there was less FHB immediately after anthesis than in 2003. Thus FHB symptoms and DON/NIV levels were very low, with a high isolation of *F. graminearum* than *F. culmorum*. The efficacy of spray treatments are still being analysed.

DISCUSSION

This study is still in progress and the results obtained so far does not indicate that the four different types of spray systems employed have a significant effect on FHB or on mycotoxin levels. However, the droplet size impacting on the ears and the coverage may be critical parameters which will influence the level of control of FHB achieved.

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INTERACTIONS BETWEEN WHEAT APHIDS AND FUSARIUM HEAD BLIGHT AND THEIR INTEGRATED MANAGEMENT IN DURUM WHEAT IN INDIA

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OBJECTIVES

To investigate the role of wheat aphids in Fusarium Head Blight development and their integrated management in durum wheat.

INTRODUCTION

Fusarium Head Blight (FHB) in wheat (*F.graminearum, F. nivale*) has become a disease of serious concern, since the unusual epidemics in Punjab, North West India, in the early 1990s which had apparently resulted from the higher frequency of rains during flowering and the susceptible cultivars grown. Since the disease is of limited importance in India, resistance breeding is presently not conducted against FHB in the region. It has been observed that a large proportion of wheat heads affected by FHB were also heavily infested with aphids which suggests that the wheat aphids may have a role in FHB development. Wheat is affected by several species of aphids worldwide. In addition to direct feeding damage, many of the wheat aphids serve as vectors of serious virus diseases such as Barley Yellow Dwarf Virus (BYDV) (Wiese, 1977). Mites, thrips and aphids are reported as suspected vectors for *Fusarium poae* infection in Swedish cereals (Peterson and Ovlang,1997). Diehl and Fehrmann (1989) reported that mechanical injury or aphid attack led to a significant increase in the number of leaf lesions caused by *Gerlachia nivalis* in wheat. Our objective in this study was to find out the role of wheat aphids in FHB development, both in-vitro and field trials, and their integrated management through fungicides and insecticides.

MATERIALS AND METHODS

Reaction of wheat cultivars to aphids and FHB in the field - Reactions of seven commercial cultivars of wheat, four bread wheats (PBW 343, PBW 154, WH 542, HD 2687) and three durum wheats (PDW 274, PDW 233, PBW 34) to aphid infestation and FHB were studied under natural field infection. Four replicated plots (4.0*1.25 sq.m) of each cv. were sown in the field. The incidence of wheat aphids and FHB was sufficiently high to differentiate the reaction of different wheat cultivars. Observations on mean number of aphids /leaf and head and number of FHB infected heads /plot and number of infected spikelets/spike were recorded.

In-vitro* interactions between wheat aphids and *Fusarium - Spikes of durum wheat cv. PDW 274 (highly susceptible to FHB) were chosen at the boot stage, surface sterilized with sodium hypochlorite solution and placed on the surface of 0.5% water agar containing 10 mg l-1 benzimidazole as a senescence retarder in Petri plates (2 spikes/plate and four replications). There were six treatments viz; T1- Spikes infested with aphid alone, T2- Spikes inoculated with Fusarium sp. alone, T3- Simultaneous inoculation/infestation of Fusarium sp. and aphids, T4- Aphid infestation followed 72 h later by Fusarium sp. inoculation, T5- Inoculation of Fusarium sp. followed 72 h later by aphid infestation, T6-Control. Spikes were infested in the center with a mixture of 10 aphid nymphs /spike and a 5 mm mycelial disc of Fusarium sp. grown on PDA and incubated at 25ºC. Observations on % spike area bleached and % FHB infected spikelets /spike were recorded after 10-15 days.

Effect of Monocrotophos on aphid control and FHB- A field experiment was conducted with a durum wheat cv. PDW 274 (FHB susceptible), planted into rice stubble. Four replicated plots (4*1.25 sq.m) per treatment were sprayed at heading with Monocrotophos insecticide. There were two treatments viz; T1- Monocrotophos @ 0.1%- single spray, T2 –Monocrotophos @ 0.1%-two sprays. Data on aphid infestation /leaf and head and FHB were recorded at soft dough stage of grain development.

Integrated control of Aphids and FHB in the field- Field experiments were conducted with a durum wheat cv. PDW 274 (highly susceptible to FHB), under natural field infection in 2002-03. There were six treatments viz; T1- Propiconazole 0.1%, T2- Monocrotophos 0.1%, T3- Propiconazole 0.1% + Monocrotophos 0.1%, T4- Propiconazole 0.1% followed 72 h later by Monocrotophos 0.1%, T5- Monocrotophos 0.1% followed 72 h later by Propiconazole 0.1%, T6 – Control treatment. Four replicated plots (4.0*1.25 sq. m) per treatment were treated at Feekes growth stage 10.51(flowering). Both the aphid infestation and FHB development were sufficiently high which facilitated evaluation of these treatments. Data on mean number of aphids/leaf and head, number of FHB infected heads/plot and % infected spikelets/spike, grain yield (kg/plot) and thousand grain weight (TGW) were recorded. Data obtained in all the experiments were analyzed statistically and coefficient of correlation (r) between aphids and FHB were determined.

RESULTS AND DISCUSSION

Cultivar reaction- Both the bread wheats and the durum wheats were affected by aphids but aphid infestation was found to be more on heads than on leaves (Table 1). Among the bread wheats, the FHB susceptible cv. PBW 154 which had the highest aphid infestation on heads, also had the highest FHB infection where as the FHB moderately resistant cvs. PBW 343, WH 542 and HD 2687 had much lower aphid infestation and FHB infection. However in durum wheats, there was no apparent relationship between aphid infestation and FHB infection. Significant positive correlations (r = 0.97) were found between the mean number of aphids/head and the number of infected spikelets/spike in wheat cultivars.

In the Laboratory- In-vitro interactions between wheat aphids and FHB, conducted on water agar in Petri dishes(Table 2), showed that Fusarium sp. and aphids when inoculated /infested simultaneously, or when aphid infestation followed 72 h later by *Fusarium* species resulted in higher % infected spikelets/spike (11.0% and 6.2 %, respectively) and % spike area bleached, in comparison with *Fusarium spp*. inoculated alone (5.5%) or when *Fusarium spp.* inoculation followed 72 h later by aphids (4.0 %).

Effect of Monocrotophos on aphid control and FHB- Application of a single spray of Monocrotophos @ 0.1% at heading (Table 3) significantly reduced the mean number of aphids/head and simultaneously reduced the mean number of FHB infected spikes/plot (25.2%) and number of infected spikelets/spike (25.2%), resulting in significant increase in grain yield (20.0%) and thousand grain weight, in comparison with the control treatment, in a susceptible durum wheat cv. PDW 274. Significant positive correlation between aphids and number of infected spikes/plot (r = 0.99) and grain yield (r = 1.0) were found in this trial.

Integrated management- In another field trial on integrated management of wheat aphids and FHB (Table 4), application of Propiconazole and Monocrotophos alone @ 0.1% each, significantly reduced the mean number of infected spikes/plot by 60 % and 42 %, respectively whereas their combined application further reduced the number of infected spikes/plot by 66.7%, improved grain yield by 37 % and thousand grain weight. However, the treatment in which application of Propiconazole was followed 72 h later by Monocrotophos, was the most effective treatment and significantly reduced the number of infected spikes /plot by 76.9% and the number of infected spikelets/spike, resulting in significant increase in grain yield (32%) and thousand grain weight. Significant positive correlations were found between the number of aphids and % infected spikes/plot.

The results suggest that wheat aphids are important in FHB development and that controlling aphids in the field with Monocrotophos, can significantly reduce FHB disease and increase grain yields. Wheat cultivars that are poor hosts for aphids offer the most promise in reducing FHB.

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Table 1. Reaction of wheat cultivars to Aphid infestation and Fusarium Head Blight under natural field infection, 2002-03.

Cultivar	Heading Date	FHB Reaction	Number of Aphids		FLB (%)	Fusarium Head Blight	
			Leaf	Head		No.infected spikes/plot	No.infected spikelets/spike
Bread wheat PBW 343	4/3	MR	0.85	3.27	15	5.2	1.97
PBW 154	25/2	S	1.50	8.75	25	51.0	3.23
WH 542	6/3	MR	2.12	5.12	05	9.5	2.40
HD 2687	6/3	MR	1.37	2.87	15	14.2	1.67
Durum wheat PDW 274	10/3	S	1.25	1.95	10	86.7	4.42
PDW 233	13/3	MR	1.57	2.90	05	17.2	3.54
PBW 34	10/3	S	1.22	4.57	05	36.2	3.50
CD at 5%			NS	1.99	1.78	6.41	0.88
Correlation coefficient (r) between number of aphids and FHB						- 0.03	0 .97**

Table 2. In-vitro interactions between Aphids and *Fusarium spp.* on water agar in Petri dishes.

Treatments	% Spike area bleached	% FHB infected spikelets/spike
T1- Aphid alone	27.50	-
T2- Fusarium sp. alone	39.27	5.5
T3- Aphid +Fusarium sp.	60.71	11.0
T4- Aphid followed 72h later by Fusarium sp.	35.00	6.2
T5- Fusarium sp.followed 72h later by Aphid.	25.00	4.0
T6- Control	0.0	0.0
CD 5%	22.0	1.93

Table 3. Effect of Monocrotophos on Aphids and FHB in durum wheat cv. PDW 274 under natural field infection, 2002-03.

Treatments (%)	No.of Aphids Leaf	Head	Fusarium Head Blight No.infected spikes/plot	No.infected spikelets/spike	Grain Yield (Kg/plot)	TGW (g)
T1- Monocrotophos,0.1 Single spray	0.30	0.60	95.75(25%)	4.12(25%)	3.55(20%)	45.93
T2- Monocrotophos,0.1 Two Sprays	0.07	0.02	96.50	4.15	3.78	46.10
Control	1.7	3.17	128.25	5.55	2.95	40.14
CD 5%	0.81	0.99	NS	NS	0.28	4.2
Correlation coefficient (r) between Aphid and FHB			0.99**	-0.275	1.0*	

Table 4. Integrated management of Aphids and FHB in durum wheat cv. PDW 274, under natural field infection, 2002-03.

Treatment (%)	No. of Aphids Leaf	Head	Fusarium Head Blight No. Infected Spikes/plot	% Infected Spikes	No.Infected Spikelets/spike	Grain Yield (Kg/plot)	TGW (g)
T1- Propiconazole,0.1	5.77	4.30	45.5 (60.0%)	1.98	3.64	3.350	45.47
T2- Monocrotophos,0.1	0.02	0.12	66.7 (42.0%)	2.91	4.57	4.087	48.76
T3- Propiconazole + Monocrotophos	0.07	0.10	38.2 (66.7%)	1.66	3.76	4.300	51.12
T4- Propiconazole followed 72h later by Monocrotophos	0.07	0.52	26.5 (76.9%)	1.15	2.77	4.137	50.92
T5- Monocrotophos followed 72h later by Propiconazole	0.20	0.15	34.7 (69.8%)	1.51	2.65	4.037	51.16
T6- Control	8.0	11.6	115.0	5.02	5.73	3.125	40.79
CD 5%	3.33	3.68	22.5	1.64	1.49	0.25	
Coefficient of correlation (r) between Aphids and FHB				0.68**	0.38	-0.09	

THE PROMISE AND CHALLENGE OF EMPLOYING BIOLOGICAL CONTROL IN THE INTEGRATED MANAGEMENT OF FUSARIUM HEAD BLIGHT

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ABSTRACT

Since cultural measures, fungicides, and resistant cereal genotypes have provided only partial control of Fusarium head blight to date, biological control is being explored as an additional tool in the integrated management of this disease. Microbial antagonists or their metabolites are potentially useful in the disruption of spike infection, fungal spread within the spike, seedling blight development, saprophytic survival of the fungus in crop residues, and fungal sporulation on crop residues. Initial efforts have been focused on bioprospecting for microbial antagonists of *Gibberella zeae*. Bioprotectants have been selected primarily based on their antibiosis and competition abilities, though mycoparasitism, induced resistance, and metabolic inhibition of mycotoxin synthesis are also useful mechanisms of biological control. Numerous isolates of yeast, spore-forming bacteria, and other bacteria have been identified that reduced FHB and/or mycotoxin contamination in laboratory, greenhouse, or small-scale field tests, sometimes with a magnitude of control equivalent to fungicide applications. But a lack of consistency in FHB control, especially in variable field environments, has hampered the transition from the laboratory to meaningful field-testing of biological control agents. Almost nothing is known about the ecology, survival, and antimicrobial activity of biological control agents following their application to plant surfaces in field environments – a fundamental knowledge gap. Some research is underway and much more is needed to define controlled culture systems for producing biological control products of consistently high quality that could then be tested in uniform regional field tests against FHB. Product formulation and application technologies are as or more critical to biocontrol efficacy as they are to fungicide efficacy, yet little research has been conducted in the context of biological control agents. Bioprotectants, especially isolates of spore-forming bacteria, may be combined with foliar fungicides to further reduce FHB mycotoxin contamination in cereal cultivars with partial resistance to FHB. Antifungal metabolites of biological control agents should also be considered as direct tools in the integrated management of FHB. These substances might be applied as foliar sprays or the genes encoding their synthesis might be genetically engineered into cereal crops along with appropriate tissue-specific promoters. While there are significant challenges to overcome, biological control strategies hold considerable promise for contributing to the long-term management of FHB.

USE OF HPLC IN EXAMINING CULTURE SUPERNATANTS OF BACTERIA USED IN BIOLOGICAL CONTROL OF FHB FOR THE PRESENCE OF ITURIN

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ABSTRACT

Selected strains of bacteria in the genus *Bacillus* can antagonize *Fusarium graminearum* in laboratory, greenhouse, and field-plot studies. In some field plot studies where *Bacillus spp*. have been sprayed on to wheat or barley, symptoms of FHB have been reduced, and/or DON levels in grain have declined. The mechanism of the antagonism is not understood, but may depend in part on bacterial antibiotics, such as cyclic lipopeptides in the iturin family. We have cultured *Bacillus sp. s*train 1BA in a variety of defined (synthetic) and semi-defined broth media that lack glucose (which can suppress iturin production). The three broth media that were studied were: (1) a basal defined medium (BDM) containing mannitol, glutamic acid and inorganic salts; (2) a defined medium (DM) similar to BDM but containing increased amounts of mannitol and glutamic acid; and (3) a defined medium with the same composition as (2) but with increased concentrations of calcium and manganese, two elements which are known to be important in regulating different aspects of *Bacillus* metabolism. Broth cultures of *Bacillus* strain 1BA were grown for different time periods in these three media. At selected time intervals culture samples were aseptically removed for measurement of optical density at 600 nm, and for iturin analysis using HPLC, to see if different phases of bacterial growth resulted in differences in iturin production. Standard curves of iturin A (Sigma) were linear in a range from 50 ug/ml up to 250 ug/ml, with absorption maxima for iturin occurring at 214 nm and 275 nm for each iturin peak. In the BDM broth, maximum OD_{600} of 1.7 was reached after 5 to 6 days of growth, and maximum iturin production occurred at this time (about 720 ug/ml). After this time, iturin levels declined greatly. In the DM broth having increased levels of mannitol and glutamic acid, maximum OD_{600} of 2.5 was not reached until 14 days incubation. However, maximum iturin production was reached around 5 days of growth (OD_{600} of 1.3; iturin production of 400 ug/ml). After 5 to 6 days of growth, iturin production sharply declined in this DM broth. In broth medium (3) containing the same components as (2) but with increased levels of Ca and Mn, maximum OD_{600} of 3.6 was reached after 8 days of growth, giving the highest cell yield of any broth medium. Iturin production in this medium had a much different pattern than the other two, with greatest iturin levels found within the first 24-48 hours of growth (132 ug/ml)., and then declining sharply. Production of iturin in medium (3) was greatest during exponential growth, not stationary phase, and increased levels of Ca and Mn allowed iturin to be produced sooner, during early log to mid log phase of growth. Different growth media will result in different amounts of iturin; and the time of incubation will also affect iturin levels. This has implications for growing these bacteria for field application, in determining medium composition and incubation time.

MICROBIOLOGICAL AND CHEMICAL COMBINATION FOR PROTECTION AGAINST FUSARIUM HEAD BLIGHT OF WHEAT

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ABSTRACT

Combinations of tactics for disease management offer a modern means for plant protection and may provide remarkable advantages over each method isolately, resulting in a series of benefits, including control efficacy, consistence, expansion of modes of action, and reduction of pesticide applications. The objetctive of this work was to determine the effect of the combination of bioprotectants with fungicide to control Fusarium Head Blight (FHB) of wheat, induced by *Fusarium graminearum*, under field conditions. Treatments and dosages of active ingredient per hectare were: Tebuconazole (Folicur 200 CE) (150 g); *Pantoea agglomerans* (Embr. 1494) (60 g); *P. agglomerans* (Embr. 1494) (60 g) + Tebuconazole (150 g); *Bacillus megaterium* (Embr. 9790) (60 g); *B. megaterium* (Embr. 9790) (60 g) + Tebuconazole (150 g); *Curtobacterium pusillum* (Embr. 9769) (60 g); *C. pusillum* (Embr. 9769) (60 g) + Tebuconazole (150 g); *Bacillus subtilis* (Trigocor 114) (60 g) and *B. subtilis* (Trigocor 114) (60 g) + Tebuconazole (150 g). A nontreated check was mantained as control. The experimental design was a randomized block with four replications. None of the biological or chemical treatments significantly controlled the disease intensity nor increased grain yield in 2002 and 2003. However the combinations of the biologicals with the chemical treatment significantly reduced the intensity of FHB and provided significant increase in grain yield over the nontreated control in both years of study. Combinations may have an important impact on disease management.

OBJECTIVE

To determine the effect of the interactions of bioprotectants with fungicide to control FHB under field conditions in Brazil.

INTRODUCTION

Fusarium head blight (FHB), induced by *Fusarium graminearum* is an economically significant disease in the south of Brazil. It has been controlled by fungicide treatement applied at the stage of complete anthesis. Biological control has been studied using several bioprotectants (Perondi et al., 1996; Luz 2000; Luz et al., 2003). This study included both biological and chemical products alone or in combination to control FHB.

MATERIAL AND METHODS

Treatments and dosages of active ingredient per hectare were: Tebuconazole (Folicur 200 CE) (150 g); *Pantoea agglomerans* (Embr. 1494) (60 g); *P. agglomerans* (Embr. 1494) (60 g) + Tebuconazole (150 g); *Bacillus megaterium* (Embr. 9790) (60 g); *B. megaterium* (Embr. 9790) (60 g) + Tebuconazole (150 g); *Curtobacterium pusillum* (Embr. 9769) (60 g); *C. pusillum* (Embr. 9769) (60 g) + Tebuconazole (150 g); *Bacillus subtilis* (Trigocor 114) (60 g) and *B. subtilis* (Trigocor 114) (60 g) + Tebuconazole (150 g).

The experiment, were carried out in the field in Passo Fundo, RS, Brazil in a randomized complete block design with four replications. The sowing date was

22nd of june, 2002 and on 20th at june 2003, respectively. Each plot consisted of 12 rows of 3m, spaced by 20cm apart. Treatments were applied at early anthesis. Each plot was rated for disease incidence and severity 21 days after applications scoring by the percent of heads showing disease symptoms. Mature grains were harvested and yield was recorded.

RESULTS

None of the biological or chemical treatments significantly controlled the disease intensity nor increased grain yield in 2002 and 2003 (Table 1). However the combinations of the biologicals with the chemical treatment significantly reduced the intensity of FHB and provided significant increase in grain yield over the nontreated control in both years of study. Combining fungicide and biological agents may have an important impact on disease management for wheat in Brazil.

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Table 1. Microbiological and Chemical Protection of FHB of wheat in the field. Passo Fundo, Brazil. 2002 and 2003.

Treatment	Disease Intensity		Yield/Kg/ha	
	2002	2003	2002	2003
Testemunha	22 b *	23 b *	2788 b *	3012 b *
Tebuconazole	11 b	19 b	2969 b	3141 b
Pantoea agglomerans (Embr. 1494)	17 b	18 b	2883 b	3191 b
P. agglomerans + Tebuconazole	6 a	13 a	3199 a	3463 a
Bacillus megaterium (Embr. 9790)	15 b	17 b	2858 b	3179 b
B. megaterium + Tebuconazole	4 a	10 a	3196 a	3577 a
Curtobacterium pusillum (Embr. 9769)	18 b	19 b	2889 b	3112 b
C. pusillum + Tebuconazole	5 a	12 a	3188 a	3559 a
Bacillus subtilis (Trigocor 114)	16 b	19 b	2871 b	3113 b
B. subtilis + Tebuconazole	3 a	14 a	3189 a	3380 a
CV %	14.5	15.1	9.7	14.7

* Treatment means followed by different letters differ significantly at P= 0.05 according to Fisher's least significant difference (LSD) test.

CULTURAL CONTROL OPTIONS FOR THE MANAGEMENT OF FUSARIUM HEAD BLIGHT IN WHEAT AND BARLEY

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ABSTRACT

Fusarium head blight (FHB or scab) is a serious disease of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). Fusarium head blight reemerged in the Upper Midwest of the U.S. in 1993 and has caused numerous, widespread and severe epidemics throughout much of the U.S. small grains production area in subsequent years. Breeding for disease resistance is a long term solution that will reduce the risk of FHB in both wheat and barley. However, the lack of immunity to FHB in wheat and barley germplasm means that even cultivars with improved FHB resistance are likely to see some disease development, and incur yield losses and quality reductions, when inoculum pressure is high and environmental conditions favorable for FHB development. Chemical and biological control options may be able to reduce the development of the disease but may not reduce the pathogen population or lower the inoculum pressure. Fusarium head blight is caused by *Fusarium graminearum* (Shcwabe) [teleomorph: *Gibberella zeae* Schw. (Petch)], the principal pathogen in the U.S., and several other species in the genus *Fusarium*. The residues of host crops, such as wheat, barley and corn, are considered the principal reservoir of these fungi, providing the inoculum that generate FHB epidemics. Given the incomplete nature of available disease control options, it seems most likely that the successful long term management of FHB, especially in 'at risk' production areas, will rely on an integrated approach to disease management. Disease management employing cultural control options would be a key component in such a management strategy. Cultural control options for FHB management principally focus on crop residues. Crop rotations to avoid planting wheat and barley on *Fusarium*-infested residues has been suggested in the management of FHB since researchers first recognized residues as the principal source of inoculum. Planting wheat directly after wheat or corn should be avoided and where residue decomposition is slow rotations could be extended to allow greater residue decomposition between host crops. The inoculum in residues can be neutralized if buried, which both prevents perithecia formation and the release of spores into the air. Tillage practices can be used to bury residues and/or promote residue decomposition. Similarly fertilizer applications or green manures may increase residue decomposition or reduce the population of *Fusarium* by increasing microbial competition. The removal of residues from field (e.g. baling straw), the infield destruction of residues at the soil surface (e.g. burning) may also aid in reducing inoculum. Cultural control practices are likely to be most effectively used when integrated with disease forecasting models so that the benefit to a future crop can be weighed against the cost of implementing a control option. The success of cultural control practices will ultimately depend on the understanding of the biology of the pathogen, the development of effective practices and the adoption of these practices by wheat and barley producers.

2004 UNIFORM FUNGICIDE PERFORMANCE TRIALS FOR THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA

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ABSTRACT

Fusarium head blight (FHB – scab) has been a serious concern for wheat and barley producers in South Dakota for ten years. The objective of this study was to continue to evaluate the efficacy of various fungicides and fungicide combinations for the suppression of Fusarium head blight and other wheat diseases. Two hard red spring wheat cultivars, Oxen and Ingot, were planted at three South Dakota locations (Brookings, Groton, and South Shore/Watertown) and Robust barley was planted at Brookings. Data were collected from the barley trial and two of three spring wheat study sites, South Shore/Watertown and Brookings, SD. Little FHB developed at the third site and DON levels were similarly low. A winter wheat study site South Shore/Watertown, was lost due to poor stand associated with dry conditions at seeding. Trial treatments were from the Uniform Fungicide Trial treatments list for the suppression of FHB and included an untreated check, Folicur (tebuconazole) applied at 4.0 fl oz/A, Tilt (propiconazole) applied at 4.0 fl oz/A, JAU6476 (prothioconazole) applied at 5.0 fl oz/A, a premix of JAU6476 (2.85 fl oz/A) + Folicur (3.17 fl oz/A), and V-10116 applied at 4 or 6 fl oz/A. All treatments included Induce, a non-ionic surfactant, applied at 0.125% v/v. Trials were planted in a factorial randomized complete block design with six replications. Trial treatments were applied at anthesis. Plots were inoculated by spreading *Fusarium graminearum*(Fg4) inoculated corn (*Zea mays*) grain throughout the field and providing overhead mist irrigation on a 16 hr/8 hr on/off schedule (overnight mist) throughout anthesis at Brookings. Other sites had natural inoculum from corn stalk residue and natural moisture conditions. Twenty-one days following treatment, plots were evaluated for leaf diseases, FHB incidence, FHB head severity, and FHB field severity. Samples were collected for Fusarium damaged kernels (FDK), deoxynivalenol (DON), grain yield, and test weight. Under dryland conditions at South Shore/Watertown FHB was not severe, with only 4.3% total disease on the untreated. Only Folicur and the V-10116 (6 fl oz) reduced FHB incidence. The same treatments as well as JAU 6476 + Folicur and the low rate of V-10116 significantly reduced total FHB. All treatments but the low rate of V-10116 significantly increased yield while only Tilt increased test weight. Either rate of V-10116 or the JAU 6476 + Folicur mix significantly decreased FDK. All products significantly reduced DON, but none were decreased to levels acceptable to the market. At the mist irrigated site at Brookings, FHB plot severity was greater than 30% on the untreated. All products except Tilt significantly reduced head severity of FHB while plot severity was reduced by JAU 6476, JAU 6476 + Folicur, or either rate of V-10116. While numeric reductions in disease and DON were observed on 'Robust' barley, neither disease nor DON was significantly reduced under mist irrigation conditions.

2004 UNIFORM TRIALS FOR THE PERFORMANCE OF BIOLOGICAL CONTROL AGENTS IN THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA

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ABSTRACT

Fusarium head blight (FHB – scab) has been a serious concern for wheat and barley producers in South Dakota for ten years. The objective of this study was to continue to evaluate the efficacy of various fungicides and fungicide combinations for the suppression of Fusarium head blight and other wheat diseases. Ingot hard red spring wheat and Robust barley were planted at Brookings, South Dakota. Trial treatments included an untreated check; Folicur (tebuconazole) applied at 4.0 fl oz/A; TrigoCor 1448 (*Bacillus* sp.) from Cornell University, Ithaca, NY; AS 54.6 (gram positive bacterium) from USDA-ARS, Peoria, IL; C3R5 (*Lysobacter enzymogenes*) from University of Nebraska, Lincoln, NE, and; 1BC (*Bacillus subtilus*) from South Dakota State University, Brookings, SD. Treatments were grown on site according to specifications from their originating labs. Trial treatments were applied at anthesis. Plots were inoculated by spreading *Fusarium graminearum*(Fg4) inoculated corn (*Zea mays*) grain throughout the field at least ten days prior to flowering (wheat) or head emergence (barley) and providing overhead mist irrigation on a 16 hr/8 hr on/off schedule (overnight mist) throughout anthesis at Brookings. Twenty-one days following treatment, plots were evaluated for FHB incidence, FHB head severity, and FHB field severity. Samples were collected for Fusarium damaged kernels (FDK) and deoxynivalenol (DON). Yields were not measured in the barley trial due to significant depredation by birds. Under the mist augmented environment of 2004, FHB was severe at this location. FHB incidence was as high as 60% on wheat and 100% on barley. FHB plot severity ranged from about 14-21% in spring wheat and 15 to 24% in barley. No significant improvements on the untreated were observed among the biological treatments. Also, the Folicur treatment did not provide the level of disease and DON suppression expected from past experiences. Beyond the uniform trial treatments, 1BA (*Bacillus* sp., SDSU, Brookings, SD) was also included in the trial as well as a combined application of 1BC + C3R5. The combined treatment gave no greater response that each component separately. 1BC and 1BA were also grown in a defined medium supplemented with casamino acids. These two isolates responded differently to the casamino acids. 1BA performed numerically better that the same isolate in a standard undefined culture medium, while 1BC performed numerically worse than when grown in a standard medium. Strikingly, the only significant differences recorded in the trial were between 1BA and 1BC grown in the casamino acid culture. 1BA was significantly superior to 1BC from that medium as measured by FDK reduction.

EFFECTIVENESS OF CHEMICAL SEED TREATMENTS IN LIMITING THE SPREAD OF *FUSARIUM GRAMINEARUM* THROUGH INFECTED SPRING WHEAT SEED

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ABSTRACT

The potential spread of Fusarium head blight (FHB) to western regions of the Canadian Prairies is of major concern to wheat and barley growers. Planting *Fusarium*-infected seed may introduce *F. graminearum* into areas that for the most part are still free of FHB. It is therefore of interest to determine the effectiveness of seed treatments in preventing the spread of this pathogen which could result from planting an infected seed lot. In 2003 and 2004, *F. graminearum*-infected seed of common and durum wheat treated with fungicides currently registered in Canada, or untreated, were planted in replicated trials at two locations in eastern Saskatchewan. At stem elongation, 50-75 plants from one row in each plot were removed, and subcrown internodes collected and rated for incidence and severity of discoloration. Pieces of discolored tissue were then surface-disinfested and plated on nutrient agar for fungal identification. In some cases, there was a lower severity of subcrown internode discoloration in the seed-treated than in the untreated control; however, these differences were not consistent and no seed treatment resulted in a lower level of subcrown internode discoloration at both locations and years. *Fusarium graminearum* was recovered from discolored subcrown internodes in all treatments. In addition, percent isolation of *F. graminearum*, and other *Fusarium* spp., from discoloured subcrown internodes in seed-treated plots was in general not significantly different from the untreated control. Based on the observation that none of the products tested appeared to prevent or consistently reduce the growth of *F. graminearum* from infected seed into underground plant tissue, we conclude that treating infected seed with currently registered fungicides will not likely prevent the spread of this pathogen to areas that are still relatively free of this pathogen.

ALTERNATIVE AGENTS AND TARGETS FOR BIOLOGICAL CONTROL OF *FUSARIUM GRAMINEARUM/GIBBERELLA ZEAE*

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ABSTRACT

Studies are underway investigating biological control of *Fusarium graminearum* Schwabe (teleomorph = *Gibberella zeae* (Schwein.) Petch). Several potential fungal and bacterial biocontrol agents are being assessed *in vitro* and under field conditions. The long term objective of all three projects will be to identify the active compounds and mechanisms of control. The first study is examining the biocontrol potential of *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur. *Fusarium graminearum* and *C. sativus* were applied to spikes of CWRS wheat cv. 'McKenzie, during anthesis. FHB severity (FHB Index), levels of *Fusarium* spp. on harvested seed, and plot yields were determined. Substantial reduction of FHB severity and an increase in yield was observed on spikes treated first with *C. sativus* at mid-anthesis followed by *F. graminearum* 2-3 days later. Percent FDK, TKW, Hectoliter weights and DON will be determined for all treatments. The role of antifungal compounds produced by *C. sativus* in the suppression of FHB will be investigated. In a second study, bacterial antagonists, *Pseudomonas chlororaphis* (PA23), *P. chlororaphis* (63), *P. chlororaphis* (314), *Bacillus amyloliquefaciens* (BS6) and *B. subtilis* (H-08-02) were evaluated *in vitro* for their antagonistic action against *F. graminearum*. All antagonists except isolate 314 inhibited pathogen growth. Isolate H-08-02 inhibited mycelial growth of *F. graminearum* by 50.59%. Isolates PA23, BS6 and 63 inhibited the mycelial growth of *F. graminearum* up to 47.36, 43.71 and 36.43% respectively. Culture filtrate of antagonists H-08-02, PA23, BS6 and 63 reduced the germination of macroconidia of *F. graminearum*. Antifungal compounds produced by the antagonists might be responsible for the suppression of pathogen *in vitro*. Evaluation of the efficacy of these isolates to manage FHB under controlled conditions is in progress. A third project is investigating the biocontrol potential of *Trichoderma harzianum* (Rifai). Eleven *T. harzianum* isolates were evaluated by confrontation plate assays. *Trichoderma harzianum* isolates were paired with *F. graminearum* in Petri plates containing PDA. All but one isolate showed some ability to overgrow *F. graminearum*. Isolates T83, T51, T30, and T183 overgrew *F. graminearum* by 20 mm or more. To determine the effect of *T. harzianum* on the production of perithecia and ascospores of *G. zeae* on wheat residue, spore suspensions, or cell-free filtrates of *T. harzianum* isolates, were applied to wheat residues either 24 h before, co-inoculated, or 24 h after, inoculation with *G. zeae*. Plates containing the treated residues were placed under UV light in a randomized complete block design with 4 replicates per treatment. On residues that were inoculated with either spore suspensions or cell-free filtrates of *T. harzianum,* 24 h before *G. zeae*, perithecia and ascospore development were substantially reduced. Residues that were co-inoculated showed moderate reduction. No control was achieved when the residues were inoculated first with *G. zeae*. The effect of spore concentration and mechanisms of control are currently being investigated.

AERIAL FUNGICIDE APPLICATION TECHNOLOGY TO ENHANCE FOLICUR EFFICACY FOR FUSARIUM HEAD BLIGHT (FHB)

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OBJECTIVES

To evaluate the aerial application technology parameters drop size and travel direction to improve efficacy of fungicide for control of Fusarium head blight through improved hard red spring wheat spike coverage.

INTRODUCTION

The purpose of spray application technology research is to enhance fungicide efficacy for control of Fusarium head blight (FHB). Research efforts to control FHB have been accelerated with the initiation of the United States Wheat and Barley Scab Initiative funding program and the severe epidemics that devastated the northern Great Plains region of the United States and Canada in the early 90s. Much of the early trials with fungicide application technology to date have focused on ground application equipment. The reasons for this focus are aerial application technology research is complicated by the cost of the equipment, the necessary skills to operate the equipment, the large area needed to conduct the research, and the limited practical range of adjustment parameters available to aircraft currently being utilized for spray applications. However, aerial spray units currently spray approximately 50% of the acreage treated with fungicides for FHB control in North Dakota. Research to identify efficient aerial spray application technology to enhance fungicide needs to be addressed.

MATERIAL AND METHODS

Trials were conducted with randomized complete block design arranged as a 2 x 2 (drop sizes x spray directions) factorial with four replicates at Hunter and five replicates at St. Thomas in 2003. Hunter is located in east central North Dakota and St. Thomas in northeast North Dakota. An untreated plot was included in trial but not included in statistical analysis. Prior to trial initiation the spray planes were pattern tested with the use of the WRK pattern test system to determine the appropriate orifices and speed needed to obtain the required spray volumes. The pattern test system determines the uniformity of the spray pattern across the swath width which was adjusted to produce the optimum spray pattern for the spray width. The pattern test is useful to identify uneven pattern deposition. A preferred spray pattern should slope out on the edges with the center of the pattern as horizontal or uniform as possible. Adjustments to the pattern can be made by adding or moving nozzles and identifying equipment leaks and taking corrective action. The pattern is adjusted by moving the nozzles and orifices along the boom. Additional orifices can be added or closed to increase or decrease spray volume. The planes used CP nozzles. The drop sizes are adjusted by changing the angle of deflection relative to the travel direction and speed. A straight back delivery has larger drops than deliveries at angles oriented downward. Increased speed decreases drop size. Drop diameters were measured using the WRK DropletScan system. The DropletScan system uses water sensitive paper to determine the drop sizes produced by the spray nozzles. This paper has calculated spread factors which are used to determine drop sizes. The measured values are shown in Table 3.

Both locations were selected for their field uniformity, the skill of the associated cooperator, and the proximity to the aerial applicators that participated in this effort. Collaborators for the trials were farmers Mark Richtsmeier and Pete Carson from Hunter and St. Thomas, respectively and aerial applicators Tim McPherson and Don Hutson from Page, and Grafton, respectively. Two spray planes were used in this study.

The spray planes and application parameters are listed in Table 1.

Water sensitive cards were placed on three stakes at grain head height in the sprayed strips. On each stake, four cards were mounted vertical back to back, two cards oriented parallel with flying direction and 2 cards placed perpendicular to flying direction. An additional card was placed horizontal face up. The results are shown in Table 3. The column titled "area" is an estimate of the area of the paper covered with spray drops. The number is a relative value to compare the vertical front against the vertical back side and the horizontal card. The column titled "VMD" is the volume median diameter of the spray drops deposited on the cards. The column titled "GPA" is the estimated gallons per acre applied as determined from the spray drops deposited on the water sensitive cards. The value is only relative as the GPA applied to the field is determined by the spray applicator and the calibration of the sprayer.

Spike coverage was determined by placing a food grade fluorescent dye (Day Glo) in the airplane spray tank with the fungicide and adjuvant. The dye was added to a tank mix of Folicur fungicide (4 oz/ acre) + Induce adjuvant (0.125% v/v) at 1.75% v/v at St. Thomas and 3% v/v at Hunter. The St. Thomas rate was reduced after consultation with the system developer, Suranjan Panigrahi, North Dakota State University. After the plots were sprayed, grain head samples, 5 per plot, were collected. The samples were placed under an incandescent light followed by an ultraviolet light and photographed. The ultraviolet light delineates the area covered by the fluorescent dye. By subtracting the total area of the spike, determined by the photograph under incandescent light, from the area determined by the fluorescent dye photograph, spike coverage can be computed. Coverage was measured on both sides of the spike to compute mean percent spray coverage of the spike.

A strip, representing one airplane boom width was sprayed for each treatment area. An untreated area between each plot was not treated to minimize drift to the adjacent treatment areas. Treatment areas were 120 ft. on center at Hunter and 90 ft on center at St. Thomas which allowed for five treatments and the subsequent replicates at each location. The field headlands were excluded from all data collection. Large colored flags were placed centrally in each plot at each end of the plot to designate data collection areas after the crop had emerged and all non fungicide pesticide applications had been completed. General hard red spring wheat crop production practices recommended by NDSU Extension were followed by the respective cooperators.

The treatments at both locations included spray solution applications at 2.5 and 5 GPA obtained by making either one or two spray passes. Spray applications were made from east to west for one application treatment and both directions for two application treatments. Twenty grain spikes were evaluated determine incidence and field severity of Fusarium head blight and leaf disease from each of two transects across the treatment areas, one near each end of the respective strip. A grain sub sample from each replicate was retained after combining from the weigh wagon to determine deoxynivalenol concentration, percent protein, and test weight. Data was analyzed with the general linear model (GLM) in SAS. Least significant differences were used to compare means at the 5% probability level.

The Hunter location was previously cropped soybean. The trial was located on the east half of the quarter directly south of a field previously cropped corn. Winds in North Dakota blow predominately from the NW enhancing the potential for Fusarium head blight. The cultivar 'Walworth' hard red spring wheat, susceptible to Fusarium head blight, was planted in early May. Treated plots were 1000 feet long lying in an east-west direction. Fungicide applications were made on 8 July from at 10:00 to 11:00 a.m. about three days after Feekes growth stage 10.51, the optimum time for fungicide application. Fusarium head blight incidence and field severity counts were taken on 21 July. The plots

were harvested on 13 August by threshing the center 30 feet of the spray area with a John Deere model 9600 combine and measuring the sample in a weigh wagon provided by Pioneer and operated by David Strand.

The St. Thomas location was previously cropped sugarbeet. The cultivar 'Oxen' hard red spring wheat, also susceptible to Fusarium head blight, was planted on May 7. Treated plots were 850 feet long lying in an east-west direction. Fungicide applications were made on 7 July from at 9:00 to 11:00 a.m. at Feekes growth stage 10.51 Fusarium head blight incidence and field severity counts were taken on 26 July. The plots were harvested on 18 August by threshing the center 25 feet of the spray area with an AGCO Gleaner model R65 combine and measuring the samples in a weigh wagon.

RESULTS AND DISCUSSION

Levels of FHB disease were small at both locations. No significant differences in FHB incidence or field severity, foliar disease, yield, test weight, and protein were measured at the St. Thomas site. Deoxynivalenol (DON) was not present. No differences in spike coverage were determined between treatments at St. Thomas. Average FHB incidence was less when large drop treatments were compared to small drop treatments at Hunter, 21.3% to 16.9% respectively Table 2. Two spray applications with small drops had smaller yields than two applications with large drops and one application with small drops. The yield data did not correlate with the spike coverage data. The coverage data indicated significantly less backside coverage on the two greater yielding treatments and significantly greater front coverage on the one pass large drop treatment compared to the greater yielding treatments. This seems to infer that the yield increase was not a result of increased fungicide coverage. The data from the water sensitive cards (Table 3) showed almost no coverage on the backside of the papers with one spray application and a 5 to 10 fold increase in backside coverage with a second spray application from the opposite direction. This increase was likely not a significant amount of area covered and enough spray volume deposition compared to front side or to the horizontal card coverage to affect yield. The volume median diameter (VMD) deposition data did contrast drop size between the front and backside of the cards, small drop range 235-299 VMD and large drop range of 340-439 VMD on front side and small drop range 162-182 VMD and large drop range 109-165 VMD on backside. The cards also showed that only relatively small drops deposit on the backside regardless of travel direction indicating the wind likely was a contributing factor to deposition side. The volume medium diameter range, 235-439, also indicates a comparatively large drop selection range can be achieved by aerial spray units with minor adjustments.

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Table 1. Aerial application equipment type and spray application technology parameters by location, 2003.

Equipment or Parameter Description	Treatment Parameter	St. Thomas	Hunter
Aircraft Type		**Airtractor**	**502Agtruck**
Nozzle Type & Orifice Size	Small Drops	CP 0.078	CP 0.078
	Large Drops	CP 0.125	CP 0.125
Nozzle Deflection Angle from Horizontal in Degrees	Small Drops	90	30
	Large Drops	0	0
Nozzle Number		34	44
Operating Pressure		30	35
Spray Volume (gpa)	One Pass	5	5
	Two Passes	2.5	2.5
Swath Width (ft.)		50	64
Flying Speed (mph)		118	125
Flying Height (ft. above canopy)		8	8

Table 2. FHB incidence and field severity, leaf disease, yield, test weight, percent protein, DON, and spike coverage by number of spray applications and droplet size at Hunter and St. Thomas, 2003.

# of Spray Applications	Droplet Size	FHB Incidence	FHB Severity	Leaf Disease	Yield	Test Weight	Protein	*DON[a]	**Spike Coverage[b] Back	Front	Mean
		%	%	%	bu/ac	lb/bu	%	Ppm	%	%	%
Hunter											
Untreated	na	22.5	0.9	8.0	68.1	60.6	13.8	0.9	0.1	0.3	0.2
One	large	21.3	1.3	4.1	66.7	miss.	13.7	0.9	1.1	8.4	4.7
Two	large	21.3	1.4	3.4	68.6	61.0	13.6	0.6	0.7	3.5	2.1
One	small	14.4	0.6	2.2	69.0	60.9	13.6	0.7	0.6	2.0	1.3
Two	small	19.4	0.7	7.0	65.8	60.6	13.9	0.9	1.5	5.7	3.6
Applications[c]		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Droplet Size		4.0*	NS	NS	NS	NS	NS	NS	NS	NS	NS
Appl.*Drop.		NS	NS	NS	**2.8****	**NS**	NS	0.3*	**0.3***	**4.6****	**2.7****
% CV		19	78	69	3	1	5	23	53	59	57
St. Thomas											
Untreated	na	25.0	1.3	31.5	78.7	61.2	14.0	<0.05	0.82	2.20	1.51
One		19.5	0.7	19.1	82.5	61.4	14.1		0.5	3.3	1.9
Two		19.8	0.8	23.4	81.7	61.4	14.0		0.3	1.5	0.9
	large	19.3	0.7	18.0	82.0	61.3	14.0		0.4	3.0	1.7
	small	20.0	0.8	24.6	82.2	61.5	14.1		0.4	1.9	1.1
Applications		NS	NS	NS	NS	NS	NS		NS	NS	NS
Droplet Size		NS	NS	NS	NS	NS	NS		NS	NS	NS
Appl.*Drop.		NS	NS	NS	NS	NS	NS		NS	NS	NS
% CV		41	45	49	2	1	1		56	87	79

[a] Deoxynivalenol

[b] Hunter dye rate 3.0 % v/v, St. Thomas dye rate 1.75% v/v

[c] *,**, significant at 0.1 and .05 % level, respectively.

Table 3. Coverage Area, VMD, and GPA by Front and Backside of Water and Oil Sensitive Paper on Cards Oriented Vertically and Horizontally by Spray Applications and Droplet Size at Hunter and St. Thomas, 2003.

Spray Applications	Droplet Size	Water and Oil Sensitive Paper								
		Vertical Cards						Horizontal Cards		
		Front side Mean			Backside Mean					
		Area	VMD	GPA	Area	VMD	GPA	Area	VMD	GPA
Hunter										
Untreated [a]		0.1	153	0.04				0.2	138	0.1
Two Sprays	large	6.1	340	2.40	0.07	165	0.04	7.1	373	2.8
One Spray [b]	large									
One Spray	small	7.3	299	2.90	0.12	181	0.04	7.3	292	2.9
Two Sprays	small	4.1	235	1.50	0.64	203	0.20	8.0	240	3.0
St. Thomas										
Untreated [a]		0.1	172	0.04				0.5	170	0.2
Two Sprays	large	10.5	439	3.60	0.02	109	<0.01	[c]	[c]	[c]
One Spray	large	6.7	340	2.50	[c]	[c]	[c]	[c]	[c]	[c]
One Spray	small	2.6	267	1.00	0.03	177	0.01	2.1	214	0.8
Two Sprays	small	[c]	[c]	[c]	0.16	162	0.49	[c]	[c]	[c]

[a]Mean coverage of both sides.
[b]No data due to spray error.
[c]Excessive moisture damaged the cards so no data was included.

EFFECT OF APPLICATION TECHNOLOGY PARAMETERS SPRAY VOLUME AND DROP SIZE ON FUNGICIDE EFFICACY FOR CONTROL OF FUSARIUM HEAD BLIGHT

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OBJECTIVES

The study had two principle objectives: 1) Determine if increasing spray volume can improve fungicide performance for control of Fusarium head blight (FHB). 2) Determine the effects of drop size on fungicide efficacy for control of FHB.

INTRODUCTION

Fungicide applications to small grains for control of FHB have often give results that are inconsistent. Studies have been completed that define application timing parameters that improve on previous results. Studies on application volume and pressure in greenhouse and field environments have show improved coverage of spikes with increasing application volume but still having inconsistent fungicide efficacy. Droplet size is different with changes in volume and this interaction. Possible effects on fungicide efficacy have not been thoroughly examined. This report is a summary of 2004 field application studies completed at Langdon.

MATERIALS AND METHODS

A series of studies using ground application equipment were initiated in 2004 to determine if spray solution volume and drop size can improve the performance of fungicide for the control of Fusarium head blight (FHB). An interdisciplinary team involving agriculture engineering and plant pathology researchers was used to address the application problems. Four trials were established at the Langdon Research Extension Center in spring 2004. Barley, durum and hard red spring wheat (HRSW) were planted to evaluate spray solution volume for improved efficacy of fungicide for FHB control. A fourth study was established on HRSW to measure differences among drop sizes in the category fine, fine, and medium.

The durum and barley spray volume studies were established on an area previously cropped with small grains. The HRSW spray volume and spray drop size studies were established on an area previously cropped soybean. The trials were designed as randomized complete blocks with five replicates on the barley and durum trials and six replicates on the HRSW trials. The soil type was a Barnes-Svea complex. Approximately three weeks prior to heading all trials received an inoculum of 330 grams of barley grains colonized by *F. graminearum*. The inoculum was hand broadcast on individual plots to increase chances of FHB infection. The durum study also received a spray application of *F. graminearum* macroconidia. The macroconidia, 250,000 spores/ml, were applied by CO_2 backpack sprayer in 18.4 GPA water. Five oz/acre of the Bayer experimental fungicide (prothioconazole), JAU 6476 with 0.125% v/v Induce surfactant, was applied to the barley at growth stage Feekes 10.3, and wheat at Feekes 10.51. The spray volumes, 5, 10 or 20 gpa/acre, were applied by CO_2 pressurized tractor sprayer through Spraying Systems XR8001 nozzles on either one or two parallel boom configurations depending on desired volume for the three spray volume studies. The nozzles were mounted on a double swivel and angled 30 degrees downward from horizontal and oriented to spray forward and backward or forward to maximize spike coverage. The spray boom was configured with 5 sets of nozzles on 20-inch nozzle spacing. For the drop

size study Spraying Systems XR8001, XR8002, and XR8003 nozzles were used to attain the fine, fine, and medium drop sizes and applied at 10 gallons water/ acre. The pressure was 40 psi for all the treatments except for the XR8003 nozzles which was 20 psi. The tractor traveled at 6 mph for the studies. North Dakota State University Extension recommended crop production practices for Northeast North Dakota were followed.

Differences between treatments were measured by using water sensitive cards and spike photography. Water sensitive cards were placed back to back on stands and oriented vertically both in the direction of travel and perpendicular to the direction of travel and horizontally. The WRK DropletScan system was used to determine the drop size from deposits on the water sensitive paper. Day-Glo orange dye, mixed with water at 1.75% v/v and Induce adjuvant at 0.125%v/v, was sprayed on additional plots to characterize spike coverage. Grain heads were removed and imaged with a low light CCD camera under incandescent lighting and under ultraviolet lighting to determine total spike area and spray coverage on both the front and back sides of the spike. Twenty-one spikes were photographed from each treatment. Additionally, from the barley spray volume study and the HRSW spray volume and drop size studies, approximately 200 grams of heads were sampled from each of four plots and combined to form two replicates of treatments. The samples were immediately frozen and shipped to Bayer Crop Science to determine fungicide and metabolite residue (Data not reported in this paper). A visual disease estimation was made from 20 samples per plot 20 to 30 days after fungicide application to estimate the FHB incidence (number of spikes infected) and FHB field severity (number of FHB infected kernels per head divided by total kernels per individual spike) of each plot. Each plot was harvested with a Hege plot combine and the grain sample cleaned and processed for yield, protein, and test weight determination and plump on barley. A sub sample was ground and analyzed for the toxin deoxynivalenol (DON) by North Dakota State University. Data was analyzed with the general linear model (GLM) in SAS. Least significant differences (LSD) were used to compare means at the 5% probability level.

RESULTS AND DISCUSSION

The Langdon area mean summer temperatures were colder than the previous low by over 1.5 degrees making this the coldest summer in over 100 years. The amount of disease development in the studies was small and was a reflection of the summer growing environment. Additionally, the fungicides were required to provide protection for much longer periods of time than the normal 20-30 days it takes for the plants to mature in this region. Disease development on the barley study was minimal so only the untreated plots were rated for disease levels. The fungicide treatments with nozzles oriented forward and backward and the five gallon treatment increased the % plump significantly over the untreated (Data not included). No differences were measured in yield or test weight. In the HRSW spray volume study the 5 gpa treatment had the same yield as the untreated (Data not included). The 10 gpa nozzles forward had less yield than the either the untreated or the 5 gpa treatment as did the 10 gpa F+B and the 20 gpa treatments. No differences were measured in test weight or percent protein. The durum spray volume study's disease level was influenced by the crop's susceptibility to FHB, the planting date, and the application of additional inoculum and had the greatest FHB of the studies. Although there were no differences in yield and test weights, all the fungicide treatments reduced FHB incidence and field severity and deoxynivalenol levels significantly (Table 1).

In the drop size study all the fungicide treatments decreased FHB incidence and field severity over the untreated (Table 2). No differences in yield, test weight, and protein were determined. Spray coverage (Table 3) generally was linear by spray volume increasing as gpa increased. The backside of the spike had up to 1/ 5 less coverage than the front side. Forward orientations had less coverage than forward and back orientations. Straight down orientation front on barley were less at 10 gpa than F + B and not different at 5 and 20 gpa. Forward orientation was not different from F + B orientation on barley at 5 or 10 gpa but much less on HRSW at 10 gpa. The durum data was excluded because the fluorescing flowers on tip of the kernels cannot be removed physically or by means of filtering

without affecting the actual spray droplets. Because these flowers occupied relatively larger areas than spray droplets, we can not make conclusions on spray coverage for durum in this case.

From the data on the water sensitive cards several conclusions can be drawn (Table 4). At spray volumes greater than 5 gpa, drop sizes are the same due to sequential deposition on areas previously receiving spray deposits. Smallest drop sizes are associated with cards with very few total deposits. Most of these were on the card side opposite the direction of the prevailing wind gust during application. The cards with the most coverage reflected the direction of the prevailing wind or wind gusts. Fewer spray deposits were found on cards in barley compared to the less dense HRSW canopy. Mean coverage was greater on forward oriented nozzles at 5 gpa than straight down oriented nozzles. F + B nozzle orientation at 20 gpa had greater coverage than all other spray volumes and most orientations. Any benefits of small drop size diminished with spray volumes greater than 5 gpa. There were no differences in coverage by nozzle type.

ACKNOWLEDGEMENT

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Table 1. FHB incidence, field severity, yield, test weight, and DON in durum by spray volume, Langdon 2004.

Spray volume (gallons/acre)	Boom number and nozzle orientation	Fusarium head blight Incidence (%)	Field severity (%)	Yield (bu/ac)	Test weight (lb/bu)	DON[1] (ppm)
untreated		46	4.6	72.7	60.7	3.5
5	1 F	19	1.1	79.0	60.2	0.8
10	2 F	15	0.9	70.7	60.3	0.7
10	1 F+B	16	0.7	80.8	60.5	0.7
20	2 F+B	17	1.3	80.8	60.0	0.9
LSD[2]		19	1.6	NS	NS	1.1
% C.V.		61	68	12	2	59

[1] Deoxynivalenol

[2] Significant at 0.05 probability level for mean comparison

Table 2. FHB incidence, field severity, yield, test weight, and protein in HRSW by spray drop size, Langdon 2004.

Nozzle (gallons/acre)	Drop size category	Fusarium Head Blight Incidence (%)	Field severity (%)	Yield (bu/ac)	Test weight (lb/bu)	Protein (%)
Untreated		60.8	3.1	54.0	56.9	12.7
XR8001	Fine	26.7	0.9	52.0	56.3	12.8
XR8002	Fine	33.3	1.4	54.0	57.0	12.6
XR8003	Medium	25.8	0.9	57.9	56.7	12.6
LSD[1]		18.5	1.3	NS	NS	NS
% C.V.		41	65	20	2	3

[1]Significant at 0.05 probability level for mean comparison.

Table 3. Spike spray coverage by crop, spray volume and nozzle orientation, Langdon 2004.

Crop or spray volume	Nozzle orientation	Area of spray deposition Back (%)	Front (%)	Mean (%)
Barley				
Untreated		0.06	0.04	0.06
5	Straight Down	0.48	2.36	1.42
5	Forward	0.47	1.20	0.83
10	Straight Down	0.15	0.87	0.51
10	Forward	1.66	3.16	2.41
10	F + B[1]	1.31	5.00	3.15
20	Straight Down	1.22	6.83	4.02
20	F + B	1.83	9.50	5.66
LSD		0.8	3.01	1.87
% C.V.		51	48	47
HRSW				
5	Forward	1.1	2.6	1.8
10	Forward	6.1	10.4	8.2
10	F + B	9.2	23.1	16.2
20	F + B	14.9	26.2	20.6
LSD[2]		4.7	8.7	5.5
% C.V.		30	28	23

[1] Nozzle orientation forward and backward
[2] Significant at 0.05 probability level for mean comparison

Table 4. Drop Size and coverage from water and oil sensitive cards, Langdon 2004.

Spray Volume	Nozzle or Orientation[2]	Drop Size $VMD_{0.5}$[1] Hori.	Front	Back	Left	Right	% Coverage Hori.	Front	Back	Left	Right	Mean
Barley												
5	SD	342	247	244	348	297	12.1	2.4	6.1	4.8	1.3	3.6
5	Forward	450	331	298	388	370	28.5	23.1	15.5	11.3	9.5	14.8
10	SD	611	351	387	518	461	48.9	13.2	23.8	12.3	16.1	16.1
10	Forward	559	*	357	460	390	49.4	69.2	1.2	15.1	9.5	23.7
10	F + B	489	343	546	399	408	28.5	6.9	39.7	10.4	20.1	19.3
20	Forward	513	256	474	379	*	53.2	2.4	35.6	7.0	70.2	28.8
20	F + B	545	469	*	484	568	51.5	22.3	66.7	20.3	41.1	37.6
HRSW												
5	Forward	273	341	369	333	298	5.3	1.3	20.4	1.1	9.2	8.0
10	Forward	495	*	122	652	256	26.1	77.5	5.0	63.1	7.5	38.3
10	F + B	510	401	*	653	395	30.9	14.3	66.5	63.7	16.7	40.3
20	F + B	649	552	*	561	918	59.2	30.9	87.5	34.7	75.4	57.2
untreated		243	156	20	15	112	0.1	0.1	0	0	0	0
10	XR8001	607	*	101	574	453	43.1	69.8	0.1	35.5	15.9	30.3
10	XR8002	550	*	142	557	367	39.4	70.9	1.2	25.8	3.5	25.3
10	XR8003	693	770	84	517	369	33.0	66.1	8.0	17.7	15.1	26.7
LSD[3]												9.8
% C.V.												20

[1] Volume median diameter, * represents missing data due to card overload.
[2] SD=straight down, F + B=forward and backward.
[3] Significant at 0.05 probability level for mean comparison.

EFFECT OF APPLICATION OF A SUBLETHAL DOSAGE OF GLYPHOSATE ON FHB SEVERITY IN SPRING WHEAT AND DURUM

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OBJECTIVES

To determine whether glyphosate drift onto growing spring wheat and durum plants at a sublethal dosage affects their subsequent susceptibility to FHB.

INTRODUCTION

The 2004 Monsanto decision to withdraw development of "Roundup Ready" wheat for the foreseeable future has somewhat lessened urgency of concern over effects of glyphosate on FHB. Despite that, there have been recent reports that wheat grown on land where glyphosate had been used on a previous crop might have a greater risk of FHB (Fernandez et al., 2004); and reports of tests showing that low levels of glyphosate stimulated growth of *Fusarium* in culture (Hanson and Fernandez, 2003). In wheat country, the use of herbicide resistant cultivars in crops such as soybean and canola has grown dramatically in the past decade. In North Dakota, for example, 74% of the 1.4 million ha of soybean grown were herbicide resistant cultivars as were about 80% of the 0.5 million ha of canola. Together that acreage is equivalent to about one-third of the area planted to spring wheat and durum, making it very likely that a field with a herbicide resistant row crop may be adjacent to a wheat field or in a rotation with a wheat crop.

MATERIALS AND METHODS

In North Dakota, the spring wheat and durum breeding programs maintain nurseries on a university farm at Prosper, ND, some 40 km northwest of Fargo. One of those nurseries is the FHB testing nursery, equipped with overhead mist irrigation and inoculated with *Fusarium graminearum*-infested corn kernels spread on the ground thoughout the nursery. The trials in this nursery use hill plots planted at a spacing of 30 x 45 cm; each hill is one genotype. In replicated trials the hill plots are grouped into blocks based on configuration of the planter but each replicate is in a different block.

In 2003 a plot of "Roundup-Ready" soybeans was located immediately adjacent to the wheat and durum breeding nurseries. The spring wheat and durum FHB nursery was located along the border with the soybean plot. The soybean plot was sprayed with glyphosate during a period when the wind was blowing directly from the soybean plot toward the wheat nursery. The wheat was in the late tillering stage at this time. The wheat border strips (ca. 3 m wide) were killed outright or severely stunted and never recovered. Within the FHB nursery several trials were planted in a randomized complete block design. Four of these replicated trials were located in such a way that some blocks were on the side of the nursery closest to the misapplication and others were on the side farthest away. The closest plots were approx. 3 to 10 m downwind from the directly sprayed area; presumably they were subject to highest exposure and showed visible height reduction at time of flowering. Plants in plots on the side of the nursery farthest away (approx. 40 m) from the spray drift area showed no visual symptoms and grain harvested from these plants appeared normal. Within the exposed plots some individual genotypes appeared to be more affected than others by the drift but all showed some symptoms. The distance from the spray drift source and the most distant blocks was beyond the range considered appropriate for buffer zones for protection of very sensitive vegetation from glyphosate drift (Yates et al., 1978).

FHB scores were taken visually at 3.5 weeks post anthesis. At least 20 individual spikes from each genotype and replicate were scored for FHB symptoms (Stack and McMullen, 1995). Hills were marked individually at anthesis so that FHB scoring was done at the same number of days post anthesis, regardless of the flowering date for the genotype. FHB severity scores were taken on plants in all replicate plots.

In durum, replicate blocks of the Uniform Regional Durum Nursery (URDN) and the Elite Durum Nursery (EDA) were distributed throughout the nursery site. While each trial had different sets of lines, we were able to identify ten genotypes that were present in every replicate block of both of these trials. Two replicate blocks containing those ten lines were adjacent to the glyphosate drift, two most distant, and two were in between.

In spring wheat there were four replicated trials available; these were the Uniform Regional Nursery (URN), the Variety and Advanced Lines Trial (VAL), the Advanced Yield Trial lines (AYT) and the Uniform Regional Scab Nursery (URSN). Because each trial had replicates distributed across the positions in the nursery, we did not attempt to identify a common set of lines but report the means for the entire sets of genotypes in each trial: URN 39 entries; VAL 41 entries; AYT 71 entries; URSN 39 entries.

RESULTS AND DISCUSSION

The FHB severity was significantly lower in the glyphosate affected plants in both spring wheat and in durum. There was no observed relationship between the amount each genotype was visually affected by the glyphosate exposure and its FHB susceptibility.

In durum (Table 1.) the mean FHB severity of the 10 selected lines in the plots closest to the glyphosate drift was 62%, significantly lower than that in the plots midway or most distant from the drift which did not differ from each other (74.9%, 73.7%, respectively).

In spring wheat (Table 2) entire replicates of 39 to 71 genotypes were present in positions adjacent to or distant from the glyphosate source. In each of the four trials represented, the FHB scores of the lines in the blocks closest to the glyphosate were lower than those in the more distant blocks. Differences in location were statistically significant in each of the four trials present.

Given the number of genotypes and number of hill plots involved and the large number of individually scored spikes (over 7,000 in spring wheat, 1,200 in durum), it seems unlikely that the observed systematic differences are simply due to random variation among plots.

Some reports have implied that glyphosate may increase FHB in wheat. Such reports have been seized upon by anti-GMO activists who have interpreted far more into them than the authors themselves. The reports published so far, however, have been based on crops growing on land treated the previous season (Fernandez et al., 2004), or on culture studies with the fungi (Hanson and Fernandez, 2003) - - both are indirect evidence at best. The results we present here are based on crops exposed to glyphosate during the growing season as any possible "Roundup-Ready" wheat would have been. This is quite a different situation than that reported by Fernandez et al.

The opportunity for this study was fortuitous; the misapplication and subsequent spray drift was not planned. The layout of the nurseries with replicate blocks on opposite sides of the nursery was also fortuitous; had the drift come from east or west instead of south to north, it would not have been possible to match up exposed and non-exposed groups of the same genotypes.

The dosage of glyphosate to these plants was not determined but was likely very low since wheat is very sensitive and most plants survived. In a dosage response study of simulated glyphosate drift on wheat, a height reduction of the order seen here was associated with exposure levels in the range of about 3% to 10% of the normal field application rate (Deeds et al., 2005).

Correlations do not prove causation. We examined possible factors which might have resulted in such a pattern of disease. Malfunction of the mist irrigation

or improper distribution of inoculum in this nursery were not found. The soil on which the nursery was located was uniform and had a uniform cropping history over the previous several years.

To guide FHB scoring, every hill in each replicate block was individually marked at flowering time o that each could be scored at the same number of days post anthesis. If the crop were retarded or advanced by the glyphosate exposure, that effect would have been compensated for by the marking procedure and any differences in flowering time in the glyphosate exposed plots were, therefore, unlikely to account for the observed FHB difference. Other factors not investigated might be found to account for these observations although every effort was made to examine such.

The results of Fernandez et al. (2004) are also only correlations, although over several years and locations. In controlled field and greenhouse studies in North Dakota where low doses of glyphosate were applied to wheat, no consistent effect of those treatments to either increase or decrease FHB was found (G. Bresnahan and S. Neate, 2004, Personal communication). Controlled studies under different environmental conditions using proper experimental design are needed to sort out these effects or the lack thereof.

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Table 1. Comparison of FHB severity in ten durum wheat lines present in plots adjacent to or distant from a sublethal glyphosate spray drift occurring at late tillering stage.

	FHB Severity (%) *		
	Location §		
Line†	Distant from drift	Midway	Adjacent to drift
D901155	50	65	41
D91103	55	61	48
RUGBY	58	62	52
BELZER	56	67	75
PIERCE	88	77	52
LEBSOCK	91	77	55
RENVILLE	82	70	76
D87450	80	93	73
MAIER	88	86	76
D88541	89	91	72
Average	73.7 a	74.9 a	62.0 b

* FHB severity scored on minimum of 20 spikes per replicate.
§ Location: Distant plots were 25 - 40 m from spray application; Adjacent plots were 3 - 10 m from sprayed area.
† Lines are listed in order of overall mean FHB severity. D901155 and D91103 are MR checks, D87450 and D88541 are susceptible checks.
a, b: Means followed by different letters are significantly different at p=0.05.

Table 2. Comparison of FHB severity in spring wheat in plots adjacent to or distant from a sublethal glyphosate spray drift occurring at late tillering stage.

% FHB Severity *				
	Trial § (Number of lines)			
Position of plots †	URN (39)	VAR (41)	AYT (71)	URSN (39)
Distant from drift	47.0 b	49.8 b	39.7 b	45.5 b
Adjacent to drift.	27.8 a	18.2 a	17.9 a	19.6 a

* FHB Severity value is mean of all entries. (Twenty spikes of each genotype in each block were individually scored for FHB).
§ Trials (all had 4 replicate blocks): URN = Uniform Regional Nursery for Spring Wheat; VAR = Varieties and Elite Lines Test; AYT = Advanced Yield Trial lines; URSN = Uniform Regional Scab Nursery for Spring Wheat.
† Location: Distant plots were 25 - 40 m from spray application; Adjacent plots were 3 - 10 m from sprayed area.
a, b: comparisons within columns only; values followed by different letters are significantly different at p=0.05.

GUSHING AND "FUSARIUM HEAD BLIGHT"(FHB)

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ABSTRACT

The quality of barley and consequently of malt is of basic importance for attaining the good beer quality. Gushing of beer is a very negative phenomenon so far not completely investigated.

The term gushing is common both in the English and in German literature and it usually indicates spontaneous over-foaming of beer from a bottle or tin. It expresses itself mainly in beer but it can occur in non-alcoholic beverages as well. Basically, immediate release of carbon dioxide (CO_2) upon bottle opening, is regarded here [1].

Causes for gushing creation can be various. So called "primary gushing" is probably associated with formation of compounds produced in barley after it is attacked by *Fusarium* spp. The above mentioned fungal disease occurs most often in wheat and barley and it is called "Fusarium head blight"(FHB) altogether

The presence of FHB in a barley caryopsis and malt is also connected with other side effects influencing the beer quality such as off-flavor or premature flocculation of yeasts leading to precocious termination of fermentation [2].

Occurrence of so called primary gushing is connected with a barley caryopsis attack by microscopic fibrous fungi not only by *Fusarium* spp. but also by e.g. *Aspergillus*, *Rhizopus, Penicillium*, and *Nigrospora.* The actual compounds that cause gushing are unknown. These compounds are probably a product of a plant - pathogen interaction, result of a preceding stress of an organism.

We followed the occurrence of *Fusarium* spp. and amount of over-foamed beer – gushing in the selected spring barley varieties that were grown after different forecrops, i.e. sugar beet, maize, rape and cereal.

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EFFECT OF PREVIOUS CROP RESIDUES AND TILLAGE ON FUSARIUM HEAD BLIGHT OF WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a major disease in all European wheat producing regions. The principle pathogen associated with FHB in Europe is *Fusarium graminearum* and its teleomorph *Gibberella zeae*, but also other species occur. Monitoring results in Europe indicate that FHB epidemics occur preferably in maize-wheat rotations. These results were confirmed by a 10-year monitoring of natural FHB incidence in Bavaria with annually 400-700 samples. Maize as a pre-crop is especially worse in combination with conservation tillage systems, which are favoured for economic reasons and by governmental support over the last years (Beck & Lepschy, 2000). Important factors determining infection of FHB are: quantity, inoculum potential, incorporation and decomposition of pre-crop residues interacting with climate conditions and wheat cultivar resistance. The present study was conducted in order to evaluate the influence of maize residues differing in Gibberella stalk rot infection and residue management practices on FHB incidence of winter wheat. In a 2-y (2003, 2004) factorial field trial at two locations (Ihinger Hof 480 m a. s. l., 8°C, 690 mm; Oberer Lindenhof 700 m a. s. l., 7°C, 930 mm) maize residues collected of cv. Arsenal (stalk rot infested) and cv. Helix (non-infested) were spread onto winter wheat plots (cv. Darwin – susceptible to FHB, cv. Petrus – rather resistant to FHB) to simulate no-till winter wheat after maize. In another experiment different tillage practices were simulated by dispersal of maize residues on winter wheat plots (cv. Darwin). Experimental factors were: (a) simulated mulch tillage (maize residues incorporated with rotary tiller), (b) simulated no-till (application of maize residues after sowing without incorporation), (c) chopped maize residues (5-10 mm length, application after sowing), (d) application of compensating fertilization (30 kg N ha^{-1}) on maize residues after sowing. To avoid plot-to-plot dispersal of ascospores, test plots were spaced 14 m apart with winter wheat crops in between. FHB incidence was substantially affected by the interaction of year and stalk rot infection of maize residues. Significant effects of different maize residues were observed in the susceptible winter wheat cv. Darwin in 2004, when wet weather conditions favoured FHB infection. Stalk rot infested maize residues (cv. Arsenal) caused on average a higher disease incidence in following wheat (cv. Darwin) than non-infested maize residues (cv. Helix). FHB incidence was mainly affected by winter wheat cultivar, whereas FHB incidence of the rather resistant cv. Petrus was 96% less than that of the susceptible cv. Darwin. Incorporation and partial burial of maize residues did not significantly affect FHB disease incidence. When maize residues were chopped fine, FHB incidence was lower than in corresponding plots with residues cut to pieces of 250-300 mm. In contrast, application of nitrogen to enhance decomposition of maize residues did not affect FHB incidence. The results indicated that small amounts of infested residue can provide sufficient inoculum for FHB epidemics in susceptible wheat varieties under favourable conditions for infection. Cultural practices in conjunction with Gibberella stalk rot resistance of the previous crop maize can help to reduce inoculum potential and subsequent FHB infection in epidemic years. Additionally, high resistance of wheat varieties to FHB is a precondition to meet existing guidelines on mycotoxin levels at high risk for FHB infection.

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ANALYSIS OF 2004 UNIFORM WHEAT FUNGICIDE TRIALS ACROSS LOCATIONS AND WHEAT CLASSES

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OBJECTIVES

To evaluate a common set of foliar fungicide treatments, across a range of environments and wheat classes, for effectiveness in managing Fusarium head blight (FHB) symptoms and deoxynivalenol (DON) accumulation in wheat.

INTRODUCTION

FHB is a potentially devastating disease that can result in serious economic losses for wheat producers, millers, and end-users of wheat products. Grain contaminated with DON, a mycotoxin usually associated with FHB, can cause health problems in both humans and livestock. As a result, grain exceeding 2 ppm DON is often discounted at sale and grain with higher DON accumulation may be rejected by the buyer. Thus, identifying fungicides that significantly reduce FHB symptoms in the field, and DON accumulation in harvested grain, would have widespread benefits to growers and end-users of all market classes of wheat. The Uniform FHB Fungicide Trials were established in 1998 as a means of evaluating promising, manufacturer-supported, fungicides that may be useful in FHB management programs nationwide.

MATERIALS AND METHODS

Uniform Test - Scientists from 12 states conducted 27 trials across a range of environments and wheat classes in 2004 (Table 1). Six fungicide treatments and a non-treated check where evaluated in each trial. Disease pressure was enhanced in about one-half of the trials by inoculating with *Fusarium graminearum* and mist-irrigating. Twelve of 27 trials were conducted in fields with 20% or greater surface residue (barley, corn, or wheat). Fungicides were applied at early flowering (Feeke's stage 10.51) using a CO^2-pressurized sprayer, equipped with Twinjet XR8001 nozzles mounted at a 60^o angle backward and forward. The experimental design was a randomized complete block. Plot size, crop husbandry, spray volume and pressure, sprayer type, and number of treatment replications varied by location. Consult individual state trial reports for details. For all trials, percent FHB incidence, severity, index (i.e., plot severity), and *Fusarium*-damaged kernels (FDK) were measured as previously described (McMullen, et al., 1999). DON accumulation was measured at one of the two USWBSI-funded DON Testing Laboratories.

Summarization of Results - In several instances, more than one wheat class or variety was grown at the same location. These were treated as separate experiments for the purposes of this summary. Data were grouped and statistically analyzed according to whether they involved spring or winter wheat. The experimental design was a randomized complete block using locations as blocks. Data were subjected to analysis of variance (ANOVA). Percentage data were arcsine-transformed prior to being statistically analyzed. When ANOVA results indicated a significant (P= 0.05) treatment effect, means were subjected to a means separation test (Student-Newman-Keuls, P=0.05). Percent control by each treatment (an industry standard measure of fungicide efficacy) was calculated to give the reader an additional means of comparing treatment efficacy. Tests with very low disease incidence or severity (<10%) and low DON (<2ppm) were not included in statistical analyses.

RESULTS AND DISCUSSION

Winter Wheat - Data from winter wheat trials are summarized in Table 2. FHB pressure was heavy in most trials. All treatments except Folicur and Tilt significantly reduced FHB incidence compared to the check. FHB severity, index, and FDK were significantly reduced by all treatments. Both treatments involving JAU6476 and the high rate of V-10116 significantly reduced DON compared to the check. Treatments involving Folicur, Tilt, and the low rate of V-10116 (4 fl oz/A) had DON levels comparable to the check. FHB and DON suppression associated with treatments were within the ranges previously reported for fungicides in the United States (Hershman and Milus, 2003a, 2003b). No treatment provided better than average control of FHB or DON. Generally, the high rate of JAU6476 (5 fl oz/A) was the best performing treatment, followed by JAU6476 (2.85 fl oz/A) + Folicur (3.17 fl oz/A) and both rates of V-10116 (4 or 6 fl oz/A).

Spring Wheat - Data from spring wheat trials are summarized in Table 3. FHB pressure was highly variable across locations. All treatments except Tilt significantly reduced FHB incidence, while all treatments significantly lowered FHB severity and index compared to the check. FDK and DON were significantly reduced by all treatments except Folicur and Tilt. As in the winter wheat trials, no treatment provided better than average control of FHB or DON. In contrast to winter wheat trials, there was little difference between treatments involving JAU6476 or V-10116.

Spring and Winter Wheat Comparison- When data were averaged across fungicide treatments, fungicide efficacy (expressed as percent control) was statistically similar for winter and spring wheat (**Table 4**). This is in contrast to 2003 Trials where spring wheat had an overall greater response to fungicides than winter wheat (Hershman and Milus, 2003b)

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Table 1. 2004 Uniform Wheat Fungicide Trials.

State and Principal investigator	Test and location	Wheat class	Test ID	Inoculated	Water applied?	20% or more barley, corn, or wheat residue?
AR / Milus	Fayetteville	SRWW	AR	Yes	Yes	No
IN / Shaner	West Lafayette	SRWW	IN1	No	Yes	Yes
	North Vernon	SRWW	IN2	No	No	Yes
IL / Malvick	Urbana	SRWW	IL1	No	No	Yes
/ Adee	Monmouth	SRWW	IL2	No	No	Yes
/ Adee	Carbondale	SRWW	IL3	No	No	Yes
LA / Padgett	Baton Rouge	SRWW	LA1	Yes	Yes	No
	Winnsboro	SRWW	LA2	Yes	Yes	No
MD / Grybauskas	Queenstown	SRWW	MD1	Yes	Yes	No
MI / Hart	East Lansing 1	SRWW	MI1	Yes	Yes	No
	East Lansing 2	SRWW	MI2	Yes	Yes	No
MO / Sweets	Columbia 1	SRWW	MO1	No	No	No
	Columbia 2	SRWW	MO2	No	No	No
MN / Hollingsworth	Crookston	HRSW	MN	No	No	Yes
ND / McMullen	Carrington	HRSW	ND1	No	Yes	No
	Fargo	HRSW	ND2	Yes	Yes	Yes
	Langdon 1	Duram	ND3	Yes	Yes	No
	Langdon 2	HRSW	ND4	Yes	Yes	No
	Minot	HRSW	ND5	No	No	No
OH / Lipps	Wooster	SRWW	OH	Yes	Yes	No
SD / Draper	Brookings 1	HRSW	SD1	Yes	Yes	Yes
	Brookings 2	HRSW	SD2	Yes	Yes	Yes
	Watertown 1	HRSW	SD3	No	No	Yes
	Watertown 2	HRSW	SD4	No	No	Yes
	Groton 1	HRSW	SD5	No	No	No
	Groton 2	HRSW	SD6	No	No	No
VA / Stromberg	Warsaw	SRWW	VA	No	No	Yes
12 States	27 Tests	15 SRRW 11 HRWW 1 Durum		12 yes 15 no	14 yes 13 no	12 yes 15 no

Table 2. Winter wheat results: FHB incidence, head severity, index (plot severity), Fusarium damaged kernels(FDK) and deoxynivalenol (DON) accumulation in harvested grain.

	Incidence		Severity		Index		FDK		DON	
Treatment and rate/A	(%)	% Ctrl[1]	(%)	% Ctrl	(%)	% Ctrl	(%)	% Ctrl	(ppm)	% Ctrl
1. Non-treated	56.3a[2]		36.9a		21.8a		22.2a		7.4a	
2. Folicur 432SC 4.0 fl oz + 0.125% Induce	50.9ab	9.6	29.0b	21.4	16.8b	22.9	15.9b	28.4	6.0ab	18.9
3. Tilt 3.6EC 4.0 fl oz/A	50.2ab	10.8	29.0b	21.4	16.8b	22.9	15.9b	28.4	7.6a	0.0
4. JAU6476 480SC 5.0 fl oz + 0.125% induce	38.0c	32.5	26.3b	28.7	11.4c	47.7	11.2b	49.6	4.3b	41.9
5. JAU6476 480SC 2.85 fl oz + Folicur 432SC 3.17 fl oz + 0.125% Induce	42.4bc	24.7	27.4b	25.7	13.4bc	38.5	13.7b	38.3	4.4b	40.5
6. V-10116 1.81FL 6.0 fl oz + 0.125% Induce	46.4bc	17.6	27.1b	26.6	14.0bc	35.8	13.3b	40.1	4.9b	33.8
7. V-10116 1.81FL 4 fl oz + 0.125% Induce	45.5bc	19.2	26.9b	27.1	13.5bc	38.1	15.0b	32.4	5.9ab	20.3
[1] % control relative to check CV	14.2%		12.2%		17.3%		15.0%		29.4%	
[2]Student-Newman-Keuls, P=0.05 N	12		13		12		10		9	

Table 3. Spring wheat results: FHB incidence, head severity, index (plot severity), *Fusarium* damaged kernels(FDK) and deoxynivalenol (DON) accumulation in harvested grain.

	Incidence		Severity		Index		FDK		DON	
Treatment and rate/A	(%)	% Ctrl[1]	(%)	% Ctrl	(%)	% Ctrl	(%)	% Ctrl	(ppm)	% Ctrl
1. Non-treated	56.5a[1]		26.4a		17.8a	-	13.5a	-	11.4a	
2. Folicur 432SC 4.0 fl oz + 0.125% Induce	47.4bc	16.1	19.7b	25.4	12.3bc	30.9	11.8ab	12.6	10.3ab	9.6
3. Tilt 3.6EC 4.0 fl oz/A	51.5ab	8.8	20.9b	20.8	14.0b	21.3	12.9a	4.4	10.8ab	5.3
4. JAU6476 480SC 5.0 fl oz + 0.125% induce	43.3c	23.4	18.5bc	29.9	9.9cd	44.4	8.4b	37.8	8.0bc	29.8
5. JAU6476 480SC 2.85 fl oz + Folicur 432SC 3.17 fl oz + 0.125% Induce	41.7c	26.2	15.7d	40.5	9.6cd	46.1	8.6b	36.3	7.2c	36.8
6. V-10116 1.81FL 6.0 fl oz + 0.125% Induce	41.6c	26.4	16.3cd	38.3	9.0d	49.4	8.5b	37.0	6.7c	41.2
7. V-10116 1.81FL 4 fl oz + 0.125% Induce	45.6bc	19.3	19.0bc	28.0	11.6b-d	34.8	9.3b	31.1	8.0bc	29.8
[1] % control relative to check CV	10.4%		17.6%		7.6%		12.1%		19.6%	
[2]Student-Newman-Keuls, P=0.05 N	9		8		8		7		8	

Table 4. Winter versus spring wheat comparison; % FHB/DON control across all fungicide treatments in relation to the control.

	% Control				
Wheat class	**Incidence**	**Severity**	**Index**	**FDK**	**DON**
Spring Wheat	20.0	30.5	37.8	26.5	25.4
Winter Wheat	19.1	25.2	34.4	36.2	25.9
Difference	0.9%	5.3%	3.4%	9.7%	0.5%
$P>F$	0.79	0.12	0.38	0.17	0.92

PLANT NUTRIENT SUPPLEMENT WITH BIOSTIMULANTS REDUCED DEVELOPMENT OF FUSARIUM HEAD BLIGHT IN WINTER WHEAT

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ABSTRACT

We are studying resistance to Fusarium head blight (FHB) in winter wheat plants pre-treated with chemical defense activators. Several potential defense-inducing compounds including chitosans, acibenzolar-S-methyl, DL-3-aminobutyric acid, Milsana, Trehalose, Resistim have been pre-screened for their defense inducing capacity by using a detached leaves test (Browne and Cooke 2004). Promising candidates were further tested for their FHB-resistance inducing capacity on mature winter wheat plants in a controlled environment and in a field experiment during the summer of 2004.

Symptom development in heads of winter wheat after *Fusarium culmorum* inoculation was reduced in plants pre-treated with Resistim one week prior to inoculation. The reduced disease development in Resistim treated compared to water-treated winter wheat plants, was found after both point inoculation and spray inoculation of the heads with *F. culmorum* in greenhouse and field studies, respectively. Further studies on the percentage infected kernels and mycotoxin content will be performed on grains from the different treatments in the field experiment.

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UNIFORM FUNGICIDE TRIAL ON FHB OF HARD RED SPRING WHEAT IN MINNESOTA

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OBJECTIVE

Evaluate and compare the fusarium head blight (FHB) control efficacy of experimental chemical products when applied to hard red spring wheat in Minnesota. Cooperatively, the multi-state uniform fungicide trial effort will indicate which fungicide compounds are most effective in reducing disease severity on wheat across diverse environments and under various disease pressures.

INTRODUCTION

Fusarium head blight was originally described more than a century ago (Stack, 2000). Since that time the disease has caused severe and repeated epidemics on small grain crops (Sutton, 1982; McMullen et al., 1997; Steffenson, 1998; Windels, 2000) resulting in billions of dollars in crop losses (McMullen et al., 1997; Wood, 2002). More specifically, Nganje et al. (2004) estimated the recent 1993-2001 FHB epidemics caused economic losses of greater than $5.2 billion in Minnesota and North Dakota alone. The disease remains a constant threat to the economic stability of small grain growers in production areas with rain, humidity, or heavy dews during critical fungal infection periods (McMullen, 1997).

Successful infection of *Fusaria* pathogens is largely dependent on environmental conditions prior to, and during the period when the crop is in a susceptible growth stage. Cultural disease management strategies (i.e.: crop rotation, tillage, and field sanitation) have offered producers partial suppression. Likewise, moderate disease suppression has also been achieved from application of select fungicide products at Feekes 10.51 (early flowering stage). Ongoing research on disease control efficacy of experimental fungicides is needed to preserve small grain yield and quality losses in regions most at risk for catastrophic crop losses.

MATERIALS AND METHODS

Hard red spring wheat cultivar 'Oxen' was planted 4 May 2004 into wheat stubble at 1.25 million live seed/acre in a randomized complete block design with four replicates. Each plot was inoculated with 112 kg ha^{-1} of *Fusarium graminearum* infested corn grain five weeks after planting. Night-cycle mist irrigation was initiated after inoculation and continued until 3 August; growth stage Feekes 11.2 (soft dough stage). Misting was discontinued temporarily during the growing season when weather events caused standing water at the testing site. Puma, Harmony GT, MCPA, and Tilt were applied to the test site on 8 June to control weeds and early season leaf disease. Afterward, weeds were managed by hand as needed.

Ten weeks after planting (14 July), fungicide treatments were applied to wheat in the Feekes 10.51 growth stage (early flowering). Treatment applications were made with a CO_2 backpack-type sprayer adjusted to 40 psi at 18-20 gpa with forward and backward positioned 'XR' Teejet flat fan 8001 VS nozzles. On 26 July, leaf spotting disease severities were recorded. The same day spikes were collected and frozen until FHB symptoms could be rated. The test was harvested 17 weeks after planting on 31 August.

Fusarium head blight severities were estimated according to the visual scale published by Stack and McMullen (1995), while percent visually scabby kernels (VSK) was estimated using a set of grain standards provided by R. Jones and based on Jones and Mirocha (1999). Percent leaf disease was estimated using James (1971). Grain sample deoxynivalenol (DON) levels were determined by the University of Minnesota Toxi-

cology Lab in St. Paul utilizing the gas chromatography/mass spectrometry (GC/MS) method. ANOVAs were performed with SAS using PROC GLM. Fisher's protected least significant difference (LSD) mean comparisons were used to identify statistically different treatments.

RESULTS AND DISCUSSION

The nontreated control had significantly more severe disease than the fungicide treatment with the best disease control (Table 1). The mixed-product treatment ('JAU6476' + 'Folicur') significantly reduced FHB and leaf disease symptoms, preserving crop yield and grain quality across all categories tested. The 'JAU-6476' (5 fl oz) treatment significantly controlled fusarium head blight incidence and visually scabby kernels, while increasing yield. DON levels, as well as fusarium head blight and leaf disease severities were controlled with the 'V-10116' (6 fl oz.) treatment while test weights were improved over the nontreated control. Application of 'Tilt' resulted in the least disease control of all products with results in five of eight categories not significantly different from the nontreated control (e.g.: FHB incidence, visually scabby kernels, DON, leaf disease severity, and kernel test weight). 'Folicur' offered the greatest level of disease control of those products commercially available to small grain producers. Compared with the nontreated control, it produced significantly better results in six of eight categories. While not significantly different from the nontreated control, 'Quadris' caused an increase in grain DON levels over the control. This phenomenon has been noted in the past resulting from head applications of strobilurin-based fungicides.

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Table 1. Fusarium head blight and leaf spot disease responses from 'Oxen' hard red spring wheat in Crookston, Minnesota during 2004.

	Fusarium Head Blight							
Treatment[1]	*HS (%)*	*I (%)*	*FS (%)*	*VSK (%)*	*DON (ppm)*	*LD*[2] *(%)*	*Test Wt. (lb/bu)*	*Yield (bu/A)*
1. Nontreated control.....	41.9a	98.5a	41.2a	27.5a	15.2a	7.2a	51.8a	33.3a
2. Folicur 432SC 4 fl oz	32.7bc	86.0bcd	28.1bc	22.5ab	12.9a	4.2bc	53.9bc	42.7b
3. Tilt 3.6EC 4 fl oz	34.8b	92.5ab	32.2b	27.5a	12.2ab	6.9a	52.4ab	41.3b
4. JAU6476 480SC 5 fl oz ..	26.3de	78.5d	20.6cd	10.3d	7.4bc	4.8bc	57.2d	55.6d
5. JAU6476 480SC 2.85 fl oz + Folicur 3.17 fl oz	22.9e	81.5d	18.7d	9.8d	4.9c	4.1bc	57.2d	56.6d
6. V-10116 1.81FL 6 fl oz ..	24.1e	84.0cd	20.3cd	12.8cd	5.0c	3.4c	56.5d	50.9cd
7. V-10116 1.81FL 4 fl oz ..	31.1bc	90.7bc	28.2bc	14.5bcd	7.5bc	4.2bc	53.7bc	51.5cd
8. Quadris 2.08F 9 fl oz	29.7cd	86.0bcd	25.6bcd	20.8abc	16.0a	5.6ab	52.6ab	39.6b
9. Headline 2.09EC 9 fl oz ..	32.2bc	93.0ab	29.9b	20.0abc	11.9ab	3.2c	54.7c	48.9c
$LSD_{0.05}$	3.90	7.81	8.81	9.26	5.19	2.09	1.82	5.94
CV	60.0	6.1	22.2	34.5	34.4	98.2	2.3	8.7

[1]Each fungicide treatment included 0.125% Induce. Treatment abbreviations are HS, head severity; I, incidence; FS, field severity (field index); VSK, visually scabby kernels; LDS, leaf disease severity.
[2]Fungal foliar diseases consisted of Septoria/Stagonospora blotch complex (*Septoria tritici* and *Stagonospora nodorum*) and tan spot (*Pyrenophora tritici-repentis*).

UNIFORM FUNGICIDE TRIAL ON FHB OF SPRING BARLEY IN MINNESOTA

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OBJECTIVE

Evaluate and compare fusarium head blight (FHB) suppression resulting from application of fungicide products on spring barley in northwest Minnesota. Cooperatively, the multi-state uniform fungicide trial effort will indicate which fungicide compounds are most effective in reducing disease severity on barley across diverse environments and under various disease pressures.

INTRODUCTION

Fusarium head blight was originally described more than a century ago (Stack, 2000). Since that time the disease has caused severe and repeated epidemics on small grain crops (Sutton, 1982; McMullen et al., 1997; Steffenson, 1998; Windels, 2000) resulting in billions of dollars in crop losses (McMullen et al., 1997; Wood, 2002). More specifically, Nganje et al. (2004) estimated the recent 1993-2001 FHB epidemics on small grains resulting in economic losses of greater than $5.2 billion in Minnesota and North Dakota alone. The disease remains a constant threat to the economic stability of small grain producers in areas with rain, humidity, or heavy dews during critical fungal infection periods (McMullen, 1997).

Successful infection of *Fusaria* pathogens is largely dependent on environmental conditions prior to, and during periods when crops are susceptible. Cultural disease management strategies (i.e.: crop rotation, tillage, and field sanitation) have offered barley producers partial suppression, and barley varieties with resistance to FHB are not yet available. Disease suppression has been achieved from application of select fungicide products at Feekes 10.50 (early-heading stage). Ongoing research on disease control efficacy of experimental fungicides is needed to preserve malting quality barley grain in areas where the crop has been grown historically.

MATERIALS AND METHODS

Spring barley cultivar 'Robust was planted 4 May 2004 into wheat stubble at 1.375 million live seed/acre in a randomized complete block design with four replicates. Each plot was inoculated with 112 kg ha^{-1} of *Fusarium graminearum* infested corn grain five weeks after planting. Night-cycle mist irrigation was initiated after inoculation and continued until 3 August; growth stage Feekes 11.2 (soft dough stage). Misting was discontinued temporarily during the growing season when weather events caused standing water at the testing site. Puma, Harmony GT, MCPA and Tilt were applied to the test site on 8 June to control weeds and early season leaf disease. Afterward, weeds were managed by hand as needed.

Nine weeks after planting (7 July), fungicide treatments were applied to barley in the Feekes 10.5 growth stage (early-heading). Treatment applications were made with a CO_2 backpack-type sprayer adjusted to 40 psi at 18-20 gpa with forward and backward positioned 'XR' Teejet flat fan 8001 VS nozzles. On 26 July, leaf spotting disease severities were recorded. On 28 July, spikes were collected and frozen until FHB symptoms could be rated. The test was harvested 15 weeks after planting on 17 August.

Fusarium head blight severities were determined by counting the number of symptomatic glumes on each head and dividing diseased glumes by the total glumes per head. Percent leaf disease was estimated using James (1971). Grain sample deoxynivalenol (DON) levels were determined by the University of Minnesota Toxicology Lab in St. Paul utilizing the gas chromatography/mass spectrometry (GC/MS) method.

ANOVAs were performed with SAS using PROC GLM. Fisher's protected least significant difference (LSD) mean comparisons were used to identify statistically different treatments.

RESULTS AND DISCUSSION

The cool growing season provided an optimum environment for barley production in the Red River Valley while frequent rainfall increased disease pressures. Four of eight disease response categories (FHB incidence, FHB field severity, DON, and yield) did not have significantly different results (Table 1). Of the categories with significantly different results, the nontreated control had the largest ratings for FHB head severity, visually scabby kernels, and leaf disease severity, but was not different from 'Folicur' (4 fl oz/A) for most reduced 1000-kernel weights. 'Headline' resulted in significant control of FHB head and leaf disease severities and fewer visually scabby kernels were noted. Two treatments ('JAU6476' 5 fl oz and 'JAU6476' + 'Folicur') resulted in increased 1000-kernel weights.

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	Fusarium Head Blight							
Treatment[1]	*HS (%)*	*I (%)*	*FS (%)*	*VSK (%)*	*DON (ppm)*	*LD*[2] *(%)*	*1000 Kernel*	*Yield (bu/ac)*
1. Nontreated control.....	37.9a	98.5	37.4	73.8a	18.6	0.93a	34.1a	88.2
2. Folicur 432SC 4 fl oz	29.4bcd	99.5	29.4	42.5bc	13.8	0.50bcd	34.1a	93.0
3. Tilt 3.6EC 4 fl oz	31.8b	99.3	31.6	56.3ab	15.0	0.73ab	35.8bc	99.1
4. JAU6476 480SC 5 fl oz ..	24.2ef	94.5	22.9	26.3c	11.6	0.25d	36.8c	96.6
5. JAU6476 480SC 2.85 fl oz + Folicur 3.17 fl oz	30.2bc	97.0	29.3	43.8bc	11.1	0.33cd	36.7c	92.9
6. V-10116 1.81FL 6 fl oz ..	25.8def	99.0	25.5	43.8bc	9.9	0.60bc	36.0bc	99.2
7. V-10116 1.81FL 4 fl oz ..	27.7def	99.0	27.4	55.0ab	11.6	0.55bc	34.9ab	99.4
8. Quadris 2.08F 9 fl oz	32.4b	99.5	32.2	31.3bc	14.8	0.33cd	36.4bc	104.3
9. Headline 2.09EC 9 fl oz ..	23.5f	97.0	22.8	20.0c	15.8	0.23d	35.8bc	94.4
$LSD_{0.05}$	*3.94*	*NS*	*NS*	*27.8*	*NS*	*0.30*	*1.56*	*NS*
CV	*67.0*	*33.0*	*24.1*	*43.6*	*30.5*	*41.8*	*3.0*	*9.8*

[1]Each fungicide treatment included 0.125% Induce. Treatment abbreviations are as follows: HS, fusarium head severity; I, FHB incidence; FS, field severity (field index); VSK, visually scabby kernels; LD, leaf disease severity.
[2]Foliar diseases consisted of Speckled leaf blotch (*Septoria passerinii* and *Stagonospora avenae* f. sp. *triticea*), net blotch (*Pyrenophora teres*) and spot blotch (*Cochliobolus sativus*).

EFFECTIVE APPLICATION OF FUNGICIDES ON WHEAT HEADS: WHAT'S THE BEST?

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ABSTRACT

Uniform coverage of Folicur® (tebuconazole) on wheat heads is critical for overall protection from Fusarium head blight. Spray coverage on wheat heads using a conventional sprayer with single-spaced nozzles on a boom has not been satisfactory. Wheat producers and custom chemical applicators need more options to achieve the highest possible effectiveness of the fungicide (total and uniform coverage), simple and inexpensive spray-boom configurations, and the ability to spray at high travel speeds. This has led to an investigation of various spray delivery systems and nozzle configurations for overall coverage — and uniformity of coverage — of spray solution on wheat heads. In 2001, UV dye in various sprayers showed coverage on wheat heads from various spray configurations. In 2002, 2003, and 2004, water sensitive papers (Spraying Systems Co., Wheaton, IL) were used to evaluate spray coverage, which were transformed into cylinders to mimic wheat heads before spraying. After each spray treatment, the papers were unfolded, scanned, and analyzed for coverage on each "side" of the "heads" using SigmaScan Pro Version 5.0 software. In addition to the spray coverage data, copper was used in the spray solution in all years, except 2001, to assess the amount of chemical applied on each "side" of the paper cylinders. The ground sprayer nozzle configurations included the use of Turbo TeeJet® nozzles in a forward-back configuration, TwinJet® nozzles, air induction nozzles, Turbo FloodJet® (single nozzles alternating forward and backward along the boom), FullJet nozzles, and the use of Twin Caps; all nozzle configurations on ground sprayers were compared at forward speeds of 10 and 19 kph (6 and 12 mph) and sprayed at the same water volumes. Ground sprayer configurations were compared with the airplane and helicopter in 2002. The backward-forward nozzle configuration and the FloodJet configuration produced the highest coverage and apparent distribution of chemical on the simulated wheat heads when compared to all other spray applicators. In these two sprayer configurations, a forward speed of 19 kph was equal in total coverage and uniformity of coverage compared to 10 kph at the same water volumes. All other spray nozzle configurations, however, had either lower total coverage, higher variability, or both, when spraying at 19 kph compared to 10 kph. TwinJet nozzles at 9 kph produced half the coverage of the backward-forward nozzles, but coverage was relatively uniform compared to the Twin Cap and flat fan configurations. Although the spray coverage from the airplane and helicopter was relatively low (<3%), the amount of chemical that reached the "heads" was comparable to most of the other ground applicator systems, but less than the backward-forward and FloodJet configurations. These data will be presented, along with a ranking of sprayer systems for effective application of fungicides for controlling Fusarium head blight.

THE EVALUATION OF *TRICHODERMA HARZIANUM* AS A BIOLOGICAL CONTROL AGENT OF *GIBBERELLA ZEAE*

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ABSTRACT

Fusarium head blight (FHB) is currently the most important disease of wheat and other small grains in Canada. In Manitoba, the principal pathogen associated with FHB is *Gibberella zeae* (Schwein.) (anamorph = *Fusarium graminearum* Schwabe). Perithecia and ascospores of *G. zeae* develop on residue in the spring and are the primary source of inoculum. Presently, there are no registered resistant wheat varieties, and no reliable chemicals or biological agents to control FHB. The objectives of this study were to investigate the biocontrol potential of *Trichoderma harzianum* (Rifai) and to determine the mechanisms in which control of the disease is achieved. Eleven *T. harzianum* isolates were evaluated by confrontation plate assays for their antagonistic action against *F. graminearum*. *Trichoderma harzianum* isolates were paired with *F. graminearum* in Petri plates containing potato dextrose agar (PDA). All but one isolate showed some ability to overgrow *F. graminearum*. Isolates T83, T51, T30, and T183 overgrew *F. graminearum* by 20 mm or more. Isolates of *T. harzianum*, which reduced mycelial growth of *F. graminearum,* were further tested to determine their effects on the production of perithecia and ascospores of *G. zeae* on wheat residue. Spore suspensions, or cell-free filtrates of *T. harzianum* isolates, were applied to wheat residues either 24 h before, co-inoculated, or 24 h after, inoculation with *G. zeae*. Plates containing the treated residues were placed under UV light in a randomized complete block design with 4 replicates per treatment. On residues that were inoculated with either spore suspensions or cell-free filtrates of *T. harzianum*, 24 h before *G. zeae*, perithecia and ascospore development were substantially reduced. Residues that were co-inoculated showed moderate reduction. No control was achieved when the residues were inoculated first with *G. zeae*. The effect of spore concentration and mechanisms of control are currently being investigated.

EFFECT OF FUNGICIDE TIMING AND APPLICATION RATE ON CONTROL OF FUSARIUM HEAD BLIGHT

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ABSTRACT

In the UK, Fusarium head blight (FHB) is generally associated with a complex of five different pathogens: *Fusarium culmorum*, *F. avenaceum*, *F. poae*, *Microdochium nivale* and more recently *F. graminearum*. The increase in *F. graminearum* combined with the imminent introduction of EU legislation, setting limits for mycotoxin contamination of grain, means that the effective control of FHB is becoming increasingly important in the UK.

There are several approaches to the control of FHB infection these include use of resistant cultivars, fungicides, biological control and cultural practices. The use of resistant cultivars is potentially the most effective approach to FHB control; however, in the absence of effective resistant cultivars, the main approach for FHB control is likely to remain the use of fungicide. The effectiveness of fungicides against FHB in the field has been questioned due to inconsistent results, in many instances the inconsistency may be attributed to incorrect fungicide application, especially through wrong product choice or miss-timing of application. However, even when applied optimally the best products currently available are still likely to be only 60-70 % effective.

To achieve optimal control of FHB pathogens and their associated mycotoxins there are several areas where choices have to be made, these include the product used, application rate and application timing. In the UK it is not uncommon to find several FHB pathogens infecting the ear at the same time. This can complicate disease control, particularly as different products can be differentially active against the different pathogens involved in the disease complex e.g. triazole fungicides such as tebuconazole have consistently shown good efficacy against *Fusarium* species but not *M. nivale*, whereas the reverse is true for strobilurin fungicides such as azoxystrobin. It has also been shown that depending on the species present on the ear, product choice can adversely influence the levels of mycotoxin found in grain. The optimum time for FHB infection is during crop flowering. Fungicides currently on the market are most effective when applied as fusarium spores arrive at the ear. The efficacy of all fungicides reduce as the timing between fungicide application and inoculum arrival increases, until eventually all efficacy is lost. In general, this occurs when the difference between spore arrival and fungicide application is greater than five days. The level of control achieved by a fungicide can also be greatly affected by the rate at which it is applied with, not surprisingly, a higher rate of application giving greater control. This paper will focus on how timing and rate of fungicide application affect the control of FHB pathogens and associated mycotoxins.

THE LONGEVITY OF FUNGICIDES CONTROLLING FHB IN WHEAT

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OBJECTIVES

Longevityor duration of fungicide activity is an importantfeature of practical fungicide technology. Several fungicides were tested with artificial inoculation up to 28 day inoculation following spraying at flowering

INTRODUCTION

The durability of the fungicides is a long issue in plant protection. For the leaf diseases it is relative easy to do as spraying technology is good or excellent and the natural infection is normally enough to secure infection severity. The extinction of the fungicide effect can be seen by the newly developing symptoms, so in this respect it was never a hard to gain data. For FHB the situation is more problematic. In many years no or sporadic natural infection is present, the spraying technology is not good to cover heads and artificial inoculation has also the problem of not complete coverage of heads. For this reason the information is less reliable. Our microplot method (Mesterházy et al. 2003) corrects most of these setbacks of the methodology and with the precise timing of the spraying and inoculation the problem can be tested much better than by any earlier methods.

MATERIALS AND METHODS

The tests were made in 1999, 2000 and 2001. Three cultivars with differing resistance were used (Zugoly, Samson, Bence), three plot replicates (5 m^2) for a cultivar were used for a fungicide treatment. Within each plot four isolates of *Fusarium* were used in three replicates as head of groups consisting of 15-20 heads (Mesterházy et al. 2003). Spraying terming: full flowering. Inoculation: 1, 5, 10 and 15 days after fungicide treatments in 1999, 1, 7, 14, and 21 days in 2000, and 1, 14, 21 and 28 days in 2002 by gradually expanding the duration test. Evaluation: FHB, FDK, yield loss and DON contamination. Fungicides: Kolfugo S 1.5 l/ha, 20 % carbendazime, Caramba 1.0 (2000) and 1.2 L/ha (2001), metconazole 60 g/L, Falcon 250 g spiroxamine, 167 g tebuconazole and 43 g triadimenole in one liter. In 2001 AMS 21619 and Prosaro (125 g prothioconazole and 125 g tebuconazole/L) were additionally tested.

RESULTS AND DISCUSSION

Table 1 shows the FDK values for 1999. The Fusarium control data show that later inoculation leads to reduced infection severity. Two weeks after flowering only 10 % infection severity remained as mean across all cultivars and isolates. When data are expressed as a % of the Fusarium check, we see that efficacy for Falcon 0.6 L/ha increased from 79 % to 37 % in two weeks. For Falcon 0.8 no change was observed, Kolfugo and Caramba remained nearly unchanged. The DON data (Fig. 1) show a somewhat different picture. The Falcon 0.6 L/ha had decreasing efficacy, the Falcon 0.8 had only slight worsening during the two week... Kolfugo was stable for 10 days, thereafter rapid decrease of efficacy followed.

Of the 2000 tests only the DON data will be shown (Fig. 2.).Up to two weeks the data correspond to the results in 1999. Up to the 21st day all lost efficacy, but Falcon 1.0 L/ha had the smallest decrease. Caramba showed the least stability. Kolfugo performed well two weeks, thereafter lost rapidly efficacy.

The 2001 results agreed so far with the previous results than up to 14 days an acceptable stability was found. Thereafter Caramba and Kolfugo lost all efficacies, Falcon 0.8 proved better than the two fungicides mentioned. The best performance was registered at AMS2619 and Prosaro having after one month only 20 % of the check value.

CONCLUSIONS

The three years study showed that durability of the fungicide protection differs strongly between fungicides. Earlier we tested a number a fungicides (Mesterházy 2003, Mesterházy et al. 2003), in this tests only the bests were tested, Kolfugo was kept only as less effective check. It seems that tebuconazole and prothioconazole are the most durable products among the tested fungicides. Metconazole was also often mentioned as powerful fungicide, but in these tests it ranked only third after prothipoconazole and tebuconazole.

The best fungicides have now about one month protective time. In warmer traditional wheat production areas this secures a good control up to the ripening. In the northern regions where vegetation period is 2-3 weeks longer than in Hungary or Fargo, another spraying can be necessary to combat late infection.

We found that the susceptible phase of wheat is not only the flowering, but may take at least about 10-12 days. In 1992 we made a similar test, repeated the inoculation 10 days later and there was no difference between the infection severity of the first and second inoculation. The reason was that after the second inoculation we received 50 mm rain and this humidity could enhance a significant infection.

ACKNOWLEDGEMENTS

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Table 1. Fungicide durable effect on FHB in wheat, grain infection data (%), 1999.

Inoculation days after fungicide application	Fungicides				
	Kolfugo S 1.5	Falcon 0.6	Falcon 0.8	Caramba 1.0	Fus.contr.
1	42.78	49.72	28.69	33.42	62.17
5	17.11	19.64	8.19	12.67	29.67
10	14.83	16.83	13.53	13.42	30.61
15	5.28	3.47	4.47	3.81	9.22
Mean	20.00	22.42	13.72	15.83	32.92

Data expressed as % of the Fusarium check

	Fus.contr.	Falcon 0.6	Kolfugo S 1.5	Caramba 1.0	Falcon 0.8
1	100.00	79.98	68.81	53.75	46.15
5	100.00	66.19	57.67	42.69	27.62
10	100.00	55.54	48.94	44.26	44.63
15	100.00	37.66	57.24	41.28	48.51
Mean	100.00	59.84	58.17	45.50	41.73

Figure 1.

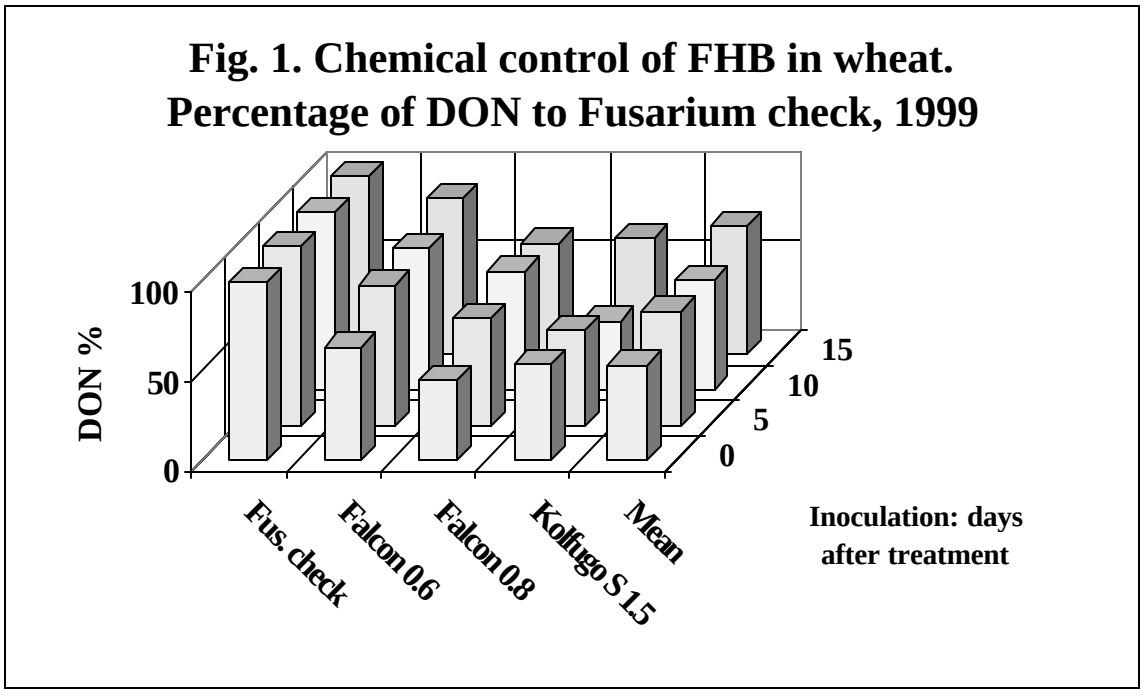


Figure 2.

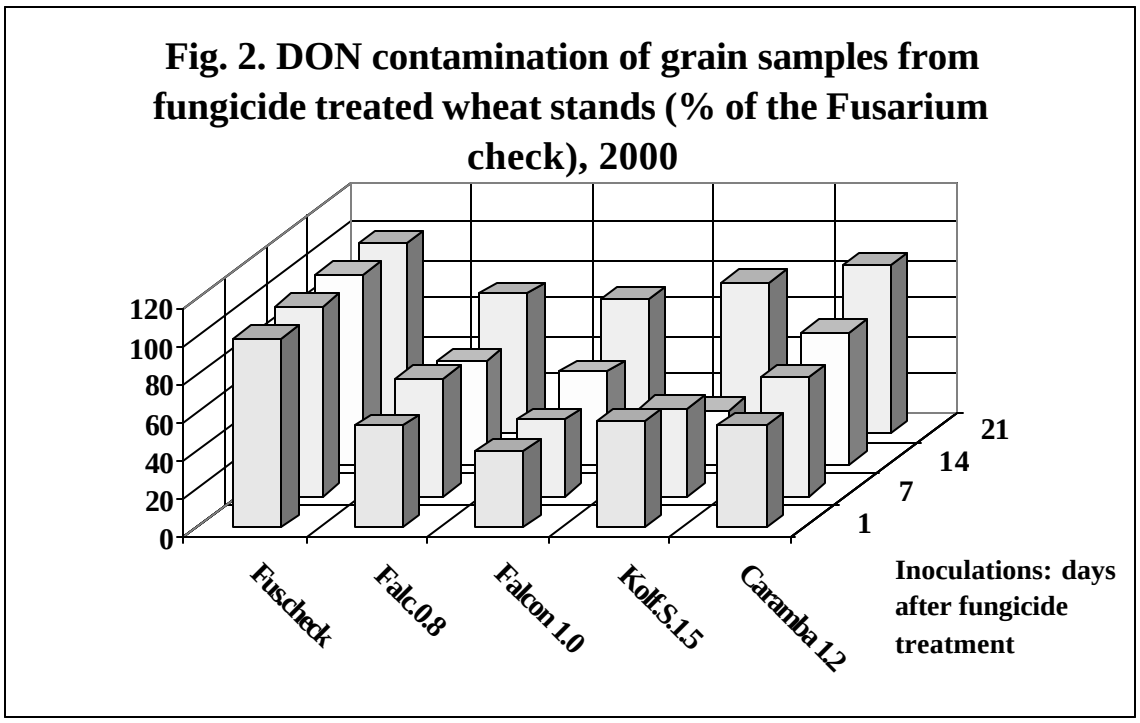
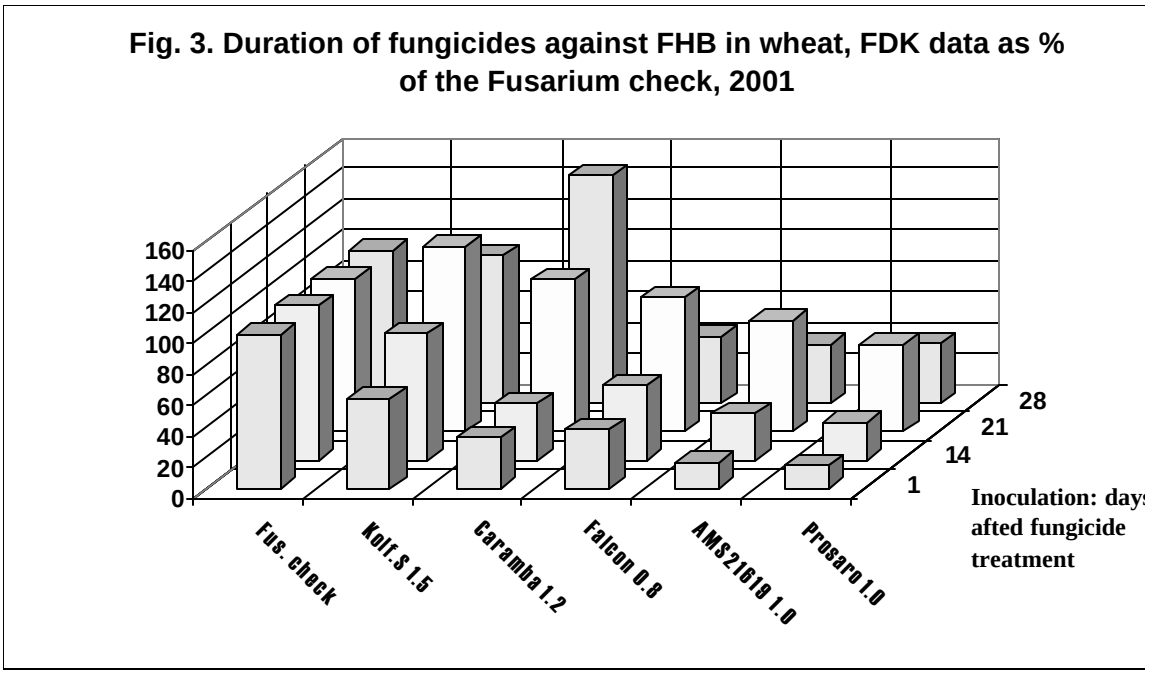


Figure 3.



EXPERIENCE WITH DMI FUNGICIDES FOR THE CONTROL OF FHB - EFFICIENT USE OF EFFICIENT TOOLS

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OBJECTIVES

To describe experience with DMI (demethylation inhibitors)-fungicides for the control of Fusarium Head Blight (FHB).

INTRODUCTION

DMI-fungicides are the largest and most important class of fungicides applied in cereals over the last thirty years. The different generations of azole fungicides have contributed to suppress more and more disease in wheat and barley. Nevertheless Fusarium Head Blight remained the main challenge, cereal growers have to face. Tackling this disease is very difficult because of the presence of several pathogens with different epidemiology. With the introduction of tebuconazole Bayer CropScience offered a tool for the chemical control of FHB. The development of prothioconazole amongst all fungicides sets new standards of *Fusarium* control. It presents an unsurpassed performance against FHB and all major associated mycotoxins, thus contributing to the production of high quality yield. This paper presents an overview on factors influencing FHB infection and its impact on quality wheat production. The possibility of an effective use of tebuconazole and prothioconazole against FHB are extensively discussed.

MATERIAL AND METHODS

Field trial - Field trials were carried out under natural infection conditions in different areas of Western-Europe (Germany, France, UK, The Netherlands) in compliance with approved guidelines from 1997 to 2002 to characterise the efficacy of products containing tebuconazole and prothioconazole in comparison to commercial standards. Treatments were carried out during the flowering growth stage, preferably at the beginning of anthesis (EC 61) one or two days after rain. The level of infection was evaluated on the basis of "percentage of infected spikelets" at EC 85 (wax-ripe stage). At growth stage EC 99, grains from each experimental treatment were harvested for further investigations.

Mycotoxin analysis - Grains sampled from field trials were ground and analysed at IFA Tulln (Austria). Detection was performed on GC with electron-capture detection (Weingärtner, 1997). All samples were analysed in the µg/kg range for contamination with deoxynivalenol. Furthermore, samples were partly analysed for other B-trichothecenes (3 Ac-deoxynivalenol (3 Ac-DON), 15 Ac-deoxynivalenol (15 Ac-DON) and nivalenol (NIV)) and/or zearalenone (ZEA).

Cytological studies - Cytological studies were performed under controlled conditions by Buchenauer at the University of Hohenheim. As described by Kang & Buchenauer (1999, 2000), the wheat plants were sprayed with prothioconazole 1 day before and 1 day after inoculation. *Fusarium graminearum* was inoculated at mid anthesis (EC 65) with a conidia suspension. The conidia suspension was pipetted into the cavity between the lemma and palea of a spikelet in the middle of a spike. One or three days after inoculation the inoculated and uninoculated wheat spikes were analysed by electron microscopy.

RESULTS AND DISCUSSION

Integrated approach to the reduction of Fusarium Head Blight - Tackling Fusarium Head

Blight in wheat is a complicated problem as the degree of severity of infection is a function of the occurrence of various factors favourable to the development of the disease. In addition , epidemiological studies have demonstrated the complex biology of *Fusarium* species and the difficulty of forecasting the disease (Suty & Mauler-Machnik, 1996). Plants are particularly susceptible to Fusarium Head Blight at the flowering growth stage. Nevertheless, depending on the climatic conditions, dominating species may differ from year to year. Moreover, tillage operations or choice of variety are definitely involved in disease severity. As the *Fusarium* fungus is spread by residual plant matter, reduced tillage in form of direct sowing or minimal tillage dramatically increases disease incidence and consequently mycotoxin contamination. The presence of higher inoculum density is encouraged by monocotyledonous previous crop like maize or wheat. Especially maize as previous crop represents a high risk for an increased infection by Fusarium Head Blight and high mycotoxin content. At last, even if no resistant varieties are available, differences in sensitivity to Fusarium Head Blight may also influence the mycotoxin contamination of harvested grains (Obst et al., 2000).

Chemical control - Generally, all applications of *Fusarium* active compounds at the different plant growth stages contribute to maintain the crop healthy and reduce the risk of ear infection (Mauler-Machnik & Zahn, 1994).

In the last 10 years (Suty et al., 1996), tebuconazole containing products applied at flowering proved to clearly reduce the disease severity of Fusarium Head Blight and consequently decrease mycotoxin contamination and increase technological quality of cereal grains (baking and cooking performance, seed quality). Similar results were obtained either after inoculation or under natural infection conditions as described by different authors (Homdork et al., 2000, Matthies & Buchenauer, 2000, Schaffsma et al., 2001).

Depending on application timing and technique variation in efficacy level of tebuconazole containing products have been observed. Studies have shown that tebuconazole should be applied +/- 5 days around infection date. The best results have been obtained when tebuconazole containing products were applied at the beginning of anthesis one or two days after rain. Also the quality of fungicide application plays an important role in efficacy level. Reaching the ear is due to its verticalness a critical issue. Field studies showed that standard application techniques using normal spraying machinery cover only one face of the ear. As only a partial redistribution of the fungicidal compound takes place, particular attention should be paid on the use of appropriate spraying nozzles. For example, the use of double fan nozzles, one spraying forward and the other one backward, improved the efficacy of tebuconazole significantly against Fusarium Head Blight (Courbon, 1995).

Prothioconazole – a new standard to control FHB

Mode of action

Prothioconazole, as a sterol biosynthesis inhibitor, shows no effect on spore germination but inhibits development of germ tubes at very low concentration.

Results of studies using scanning electron microscopy show that prothioconazole, applied in a protective way (1 day before inoculation [I-1d]), inhibits germ tube extension and causes severe morphological alterations of the fungus. In comparison to untreated fungi the germ tubes are swollen and show multiple buds one day after inoculation. The hyphal tip is often extremely swollen and appears in spherical shape (Fig. 1). Consequently, no hyphal network can be formed and no penetration of hyphae in any tissues of the wheat spikes can take place.

Three days after inoculation, *F. graminearum* forms a dense hyphal network in untreated control. After curative application of prothioconazole (1 day after inoculation [I+1d]), one day after inoculation, newly formed hyphae become irregularly swollen and distorted (Fig. 2), whereas hyphae that have been developed before fungicide treatment, show no morphological alteration. The whole hyphal development is less dense compared to the hyphae observed in the control. Furthermore, no hyphal growth can be detected in the rachis.

Efficacy of prothioconazole against *Fusarium* species and yield response

Results on field efficacy of prothioconazole against *Fusarium roseum* (*F. graminearum and F. culmorum*) and *Microdochium nivale* are presented in table 1 in comparison to tebuconazole, the commercial standard for Fusarium Head Blight control to date.

The relatively low amount of results available for *M. nivale* is due to the low incidence of this pathogen the last 5 years. Globally, results show that prothioconazole has a high activity potential against both *F. graminearum* and *M. nivale*. Efficacy level obtained with this molecule is significant higher than that of tebuconazole. Increased efficacy of prothioconazole is also correlated with yield response, in fact yield is improved much more when prothioconazole is applied at anthesis.

Reduction of mycotoxins by application of tebuconazole and prothioconazole

Incidence of prothioconazole on formation of the three main *Fusarium* mycotoxins, deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEAS) in wheat grains, has been investigated in comparison to tebuconazole (Fig. 3).

Results demonstrate that independent of the mycotoxin considered, prothioconazole reduces more significantly the level of mycotoxin in grain than all other commercial standards.

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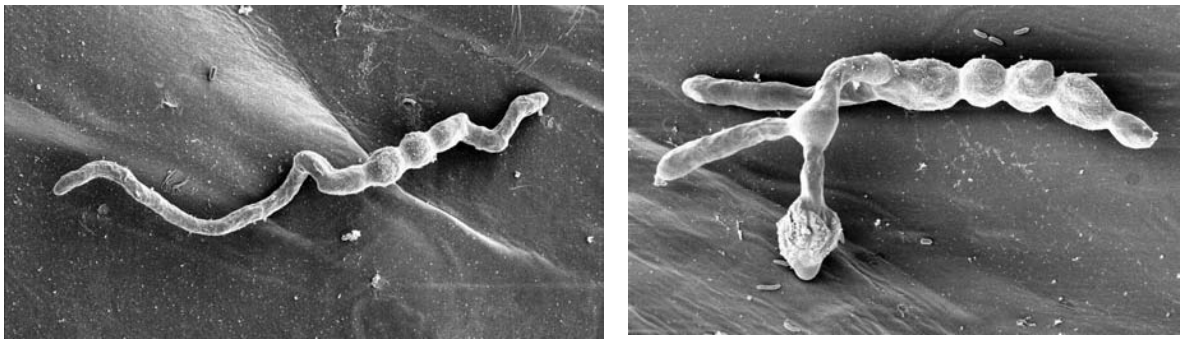
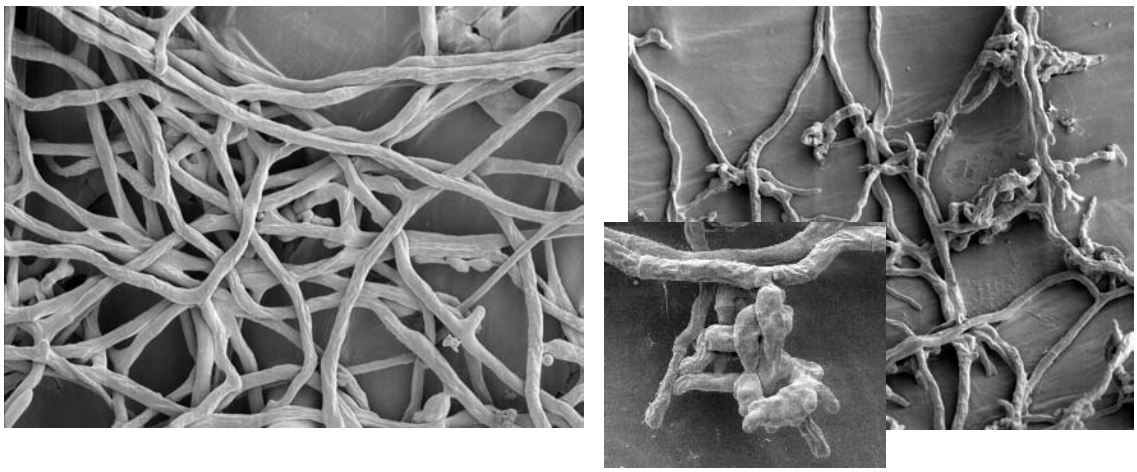


untreated 12 hours after inoculation prothioconazole 1day after inoculation [I-1d]

Figure 1: Electron microscopical study on the effect of prothioconazole on development of *Fusarium graminearum* applied protectively one day before inoculation (pictures: Buchenauer & Kang, University of Hohenheim).



untreated 3 days after inoculation prothioconazole 3 days after inoculation [I+1d]

Figure 2: Electron microscopical study on the effect of prothioconazole on development of *Fusarium graminearum* applied curatively one day after inoculation (pictures: Buchenauer & Kang, University of Hohenheim).

Table 1. Efficacy of prothioconazole against *Fusarium roseum* and *M. nivale* on ear disease severity.

Treatment	Dose rate (g a.i./ha)	efficacy (% untreated) *Fusarium spp.* n* = 35	*M. nivale* n = 3	relative yield (%) n = 33
untreated	-	(31%)	(19%)	(73.3 dt/ha)
Tebuconazole	250	63	48	116
Prothioconazole	**200**	**73**	**76**	**125**

*n = number of trials

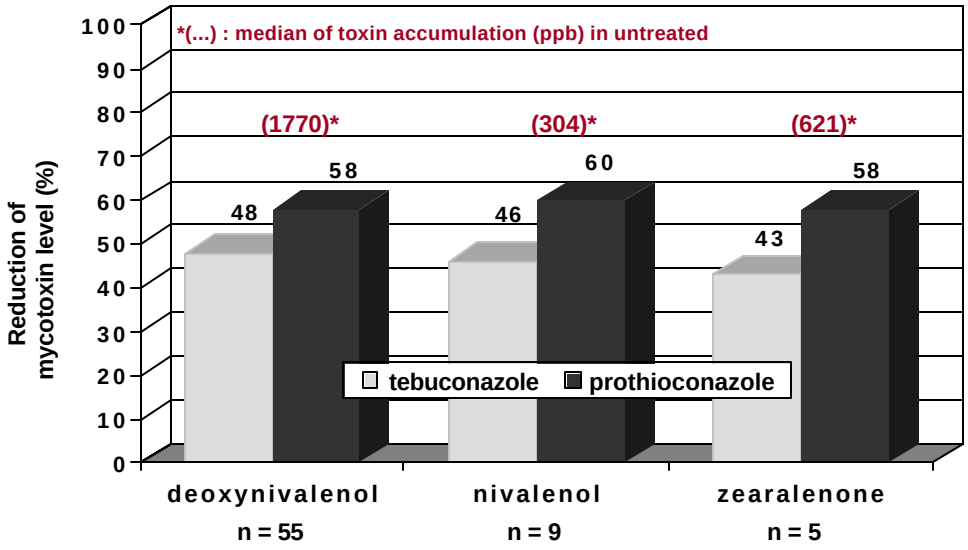


Figure 3. Effect of prothioconazole on reduction of mycotoxin level in wheat samples (n = number of trials), Europe 1998-2002.

THE USE OF *TRICHODERMA ASPERELLUM* AND THE YEAST *CRYPTOCOCCUS NODAENSIS* IN RUSSIA TO REDUCE FUSARIUM HEAD BLIGHT

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ABSTRACT

A unique biocontrol strategy that combines seed pretreatment with a biofungicide "Mycol" (*Trichoderma asperellum* strain GJS 03-35) with spraying wheat plants during flowering with the yeast *Cryptococcus nodaensis* OH 182.9 (NRRL Y-30216) to reliably reduce FHB development have been developed. Tests of the "Mycol" preparation and the yeast OH 182.9 (EOD) have been performed on the spring wheat "Ivolga" in greenhouse conditions (the Moscow region) and on the winter wheat "Kupava" in field trials in the North Caucasian region. An isolate of *F. graminearum* was used to insure adequate levels of disease development in greenhouse and field experiments. Fusarium head blight (FHB) severity and incidence, as well as mycotoxin accumulation in wheat grains, was studied for single or combination treatments with the biological preparations. Mycol (in concentrations 0,1; 0,5; 1,0; 2,0 kg / 1 tone of seeds) was used for wheat seed pretreatment. The yeast preparation EOD ($2,0E•10^7$ cfu/ml) was applied by spraying wheat plants during flowering. Chemical pesticides (Raxyl, TMTD) and a biological preparation Agat-25K were used as alternative control seed treatments. In greenhouse experiments, inoculations of heads with either biological preparation 4 h prior to inoculation with conidia of *F. graminearum* significantly reduced FHB severity. For treatments consisting of Mycol and EOD, 1000 grain weights were equivalent or higher than for control plants (both infected, and not infected). Wheat seeds obtained from the plants protected by these biological preparations germinated rapidly and possessed high germination rates compared to the FHB control. In field trials, Mycol treatments clearly reduced FHB symptoms, apparently providing an immunizing effect against FHB. Mycol reduced FHB severity and enhanced yield of the wheat varieties used. The effect of Mycol used at a minimum test-dose (0,1 kg / 1 tone) was not so pronounced. The greatest reduction of FHB development was observed at a dose of Mycol of 1,0 kg per 1 tone of seeds used in combination with EOD spraying. Experimental results support the contention that the offered technology has good prospects in controlling FHB. The work was executed within the framework of partner ISTC project !2336p.

ACKNOWLEDGEMENT

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EFFECTS OF TILLAGE PRACTICES ON DON CONTENT IN BARLEY

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ABSTRACT

Six tillage systems in barley (*Hordeum vulgare L.*) monoculture have been studied to compare chisel and no-till with conventional tillage. The experiment, conducted at the Research Farm of Agriculture and Agri-Food Canada at Normandin (Quebec), was initiated in 1990. The treatments were: T1: Conventional (fall molboard plowing and spring harrowing – 2 passes with a cultivator); T2: Chisel (fall) and spring harrowing (2 passes with a cultivator); T3: Chisel (fall) and spring harrowing (1 pass with a cultivator); T4: No tillage (fall) and spring harrowing (1 pass with a rotative harrow); T5: No tillage (fall) and spring harrowing (1 pass with a cultivator); T6: No-till (no tillage the previous fall and no harrowing in spring). Treatments were laid out in a complete randomized block design with four replications. Plot size was 10 m X 10 m. Barley seeding rate was 170 kg ha^{-1}. Because fusarium head blight (FHB) has become the most important cereal disease in Northern Quebec, DON content was measured in 2003 to determine the effect of soil tillage on FHB incidence in barley. According to treatments, mycotoxin content varied from 2.2 to 7.4 ppm. DON content was higher for no-till treatment (T6) than for other treatments (T1, T2, T3, T4, and T5). Because the fungus that causes FHB survives on residue left on soil, and according to the results of this trial, tillage practices that bury cereal residue could be used to reduce the amount of inoculum.

THE *FUSARIUM GRAMINEARUM* PKS12 GENE IS RESPONSIBLE FOR THE SYNTHESIS OF THE POLYKETIDE AUROFUSARIN

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ABSTRACT

The red pigmentation of *Fusarium graminearum* and related species is due to the deposition of aurofusarin in the cell walls. To identify the polyketide responsible for the biosynthesis of this pigment random mutagenesis of *F. pseudograminearum* using *Agrobacterium* mediated transformation was carried out. Several mutants were identified that had altered pigmentation and plasmid rescue was carried out to identify the insertional events. All mutants had integration of the T-DNA in a region upstream from a putative transcription factor with homology to the aflatoxin gene, *aflR*. This region of the *F. graminearum* genome contain genes typical of polyketide gene clusters and identifies *?pks12* as the gene responsible for the synthesis of aurofusarin. Comparative PCR analyses of the aurofusarin gene cluster in *F. graminearum*, *F. culmorum*, and *F. pseudograminearum* show conserved organisation. The expression of individual genes in the cluster were analysed by RT-PCR, *?pks12* is silenced in all mutants and most of the adjacent genes show reduced levels of transcripts. To confirm that *?pks12* encodes the precursor for aurofusarin, targeted mutagenesis was carried out. All disruptants showed an albino phenotype. Physiological studies of the *?pks12* mutants were carried out to access the function of the aurofusarin. The *?pks12* mutants have higher growth rate and a 10-fold increase in conidia production compared to the wild type indicating that the pigment negatively affects growth rate. Infection studies were carried out on barley roots in a sterile culture system and by inoculation of wheat heads. The aurofusarin deficient mutants were fully virulent and it is concluded that this compound is not important for pathogenicity. HPLC analyses of aurofusarin deficient mutants confirmed the absence of aurofusarin in the mutants. In addition, these analyses showed that there is an increase in the level of the mycotoxin, zearalenone.

ALTERING AGRONOMIC PRACTICES TO REDUCE THE EFFECT OF FUSARIUM HEAD BLIGHT ON DURUM

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ABSTRACT

Fusarium head blight (FHB) has become an important disease of cereals in moist regions of western Canada. This disease has played an important role in contributing to lower grain yields and substantial downgrading of durum wheat (*Triticum turgidum* L. var. *durum*). The objective of this study, conducted at three locations on the Canadian prairie, two in Saskatchewan and one in Manitoba, from 2001 to 2003, was to determine the effect of seeding density, nitrogen supply, fungicide treatment, and durum wheat cultivar on FHB development, grain quality, grade protection and economic return. A four-way factorial design was used with two seed densities (150 and 300 viable seeds m^{-2}), two nitrogen rates (75 and 100% of recommended rate), three cultivars (AC Avonlea, AC Morse and AC Navigator), and four fungicide treatments (no application, Tilt at flag leaf, Folicur at anthesis and Tilt at flag leaf followed by Folicur at anthesis). Increasing the seed density decreased FHB at 4 out of the 7 site year when FHB occurred, however increasing the seed density tended to increase leaf disease severity. The application of Folicur did not affect fusarium levels. The application of Tilt and /or Folicur decreased leaf disease at 6 out of 9 site years and affected yield at 5 out of 9 site years. There was no consistent effect from nitrogen or cultivar.

EFFICACY OF TRIAZOLE FUNGICIDES FOR FHB CONTROL WITH VARIOUS ADJUVANTS AND SPLIT TIMINGS OF APPLICATION

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OBJECTIVE

To improve the efficacy of triazole fungicides in control of FHB using adjuvants and appropriate timings of application.

INTRODUCTION

Currently, the triazole fungicide Folicur (tebuconazole) has special exemptions in some states within the United States (US) for use on wheat and barley to suppress Fusarium head blight (FHB). Another triazole, JAU 6476 (prothioconazole), an experimental product from Bayer CropScience, is being tested in the US for suppression of FHB. A standard adjuvant recommended for use with tebuconazole is Induce, a petroleum-based non-ionic surfactant. Various private companies in the US sell non-ionic surfactants similar to Induce, or have other adjuvants for sale that are silicone-based or are encapsulating products (Thomsan, L, 1998). With so many products on the market, more information is needed about their efficacy with the triazoles. Preliminary tests indicated few differences among adjuvants when combined with Folicur (Jordhal et al. 2001).

Timing of application also is known to affect efficacy of fungicide applications for FHB control. In North Dakota, three spring cereals vulnerable to FHB are hard red spring wheat, durum wheat, and spring barley. The question on whether multiple infections of these crops by *Fusarium graminearum* can be controlled with a single, appropriately timed application of a triazole fungicide needs to be answered. Preliminary results indicated this may be possible in hard red spring wheat cultivars, but not in barley (Jordahl et al. 2003). Neate et al. (2003) reported that split applications of fungicide to barley did not provide a significant advantage over a single application under low disease pressure. Further studies with durum wheat and barley were needed.

MATERIALS AND METHODS

Hard red spring wheat ('Grandin'), durum wheat ('Monroe') and spring barley ('Robust') were grown in the greenhouse, and then exposed to single or multiple inoculations of *Fusarium graminearum* and single or multiple fungicide applications at various heading stages. For the adjuvant studies, all fungicide applications were applied once, at early flowering (Feekes 10.51) in spring wheat and durum wheat, and at early full head emergence (Feekes 10.5) in barley (Table 1). Adjuvants were mixed with 4 fl oz/acre of Folicur (tebuconazole) or with 5.7 fl oz/acre of JAU 6476 (prothioconazole) (Table 1). Experimental adjuvants were provided by Agrilliance LLC.

For the timing of application studies, inoculations and or fungicide applications were applied at head half emerged (Feekes growth stage 10.3), early flowering or full heading (Feekes 10.51 in wheat, Feekes 10.5 in barley), and at kernel watery ripe stage (Feekes 10.54) (Table 2). Folicur at 4 fl oz/acre or at reduced/split rates was used in the timing studies. For both the adjuvant and the application timing studies, fungicide applications were made using a track sprayer mounted with XR8001 flat fan nozzles oriented forward and backward at 60° angle from vertical, delivering 18.3 gpa at 40 psi. Plants were inoculated with a mixture of three *F. graminearum* isolates, delivered at a rate of 10,000 spores/ml, 20 ml/pot, per spray event with a DeVilbiss atomizer, 4 hrs after the fungicide was applied. Immediately following inoculation, plants were misted for 48 hours using a closed mist system at or near 100% RH at 23^0C (+ or – 5^0 C).

FHB incidence, head severity and field severity (incidence x head severity) were determined at kernel soft dough stage. Field severity values were analyzed using ANOVA at the 95% and 90% confidence intervals.

RESULTS

Adjuvants: Across grain classes and the two triazoles tested, no significant differences were observed among adjuvants tested, when analyzed at the 95% confidence level, but some differences were observed when adjuvant treatments alone were compared at the 90% confidence level (Table 1). The adjuvants Placement and the experimental adjuvant #1 (supplied by Agrilliance LLC) were consistent in having high FHB field severity values, while Preference and the combination of experimental adjuvant #1 plus Preference were consistently low in FHB field severity values across all grain classes and triazole treatments. Placement is an encapsulating adjuvant, Preference is a crop-based non-ionic surfactant and the composition of the experimental adjuvants is proprietary at this time. In general, adjuvants tested did not perform better than Induce adjuvant, except in barley, where some adjuvant combinations were better than Induce, when analyzed at the 90% confidence level.

Timing Studies: For barley, multiple inoculations significantly increased FHB field severities over single applications, with three inoculations and no fungicide treatment resulting in a 68.3% field severity (Table 2). With three inoculation events, a multiple, 3-way split application of Folicur (total product applied was 4 fl oz/acre) reduced FHB field severity to 42%, as compared to a 52% field severity with a single full rate application applied at full head emergence. FHB field severity values were the lowest with a single inoculation and when a full rate of Folicur was applied at early head emergence or at kernel watery ripe stage.

In durum, FHB field severity values were as high as 81.9% with three inoculations. With three inoculation events, a single full rate application of Folicur at Feekes 10.51 reduced the disease level significantly, to 18.5%. Three split rate multiple applications in combination with the three inoculations resulted in similar disease levels (20% field severity) as the single full rate application (Table 2).

CONCLUSIONS

Adjuvants: Some registered and experimental adjuvants will provide a slight enhanced control of FHB over the standard non-ionic product, when combined with Folicur or JAU 6476. Other adjuvants are not as satisfactory as the standard non-ionic surfactants commonly used.

Timing studies: In durum, a single, appropriate timing of a full rate of Folicur fungicide may significantly reduce FHB field severity caused by multiple infection events. In barley, multiple infection events are difficult to control with a single, full rate application or with multiple reduced rate fungicide applications. Multiple applications of higher rates may be necessary for FHB suppression in barley under severe disease pressure, or products with greater efficacy than Folicur may be needed.

ACKNOWLEDGEMENT

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Table 1. Effect of adjuvants on efficacy of triazole fungicides for control of FHB for each grain class, averaged across two triazole fungicides (Folicur = tebuconazole, applied at 4 fl oz/acre, and JAU 6476 = prothioconazole, applied 5.7 fl oz/acre; both Bayer CropScience products).

Adjuvant Treatments[a]	Rate/acre	Average FHB field severity		
		Spring wheat[b]	Durum wheat[c]	Barley[d]
Untreated		22.9	47.5	48.7
Induce	0.125%v/v	2.3	6.3	12.4
Exp. # 1 + Preference	2 fl oz + 0.25%v/v	1.9	5.3	6.7
Placement+ Preference	2 fl oz + 0.25%v/v	2.6	4.8	9.3
Exp. # 2	1.0% v/v	2.7	6.4	14.0
Exp. # 3	0.5% v/v	2.1	8.0	8.5
Rivet	0.5% v/v	2.4	3.2	12.0
Preference	0.25% v/v	1.3	4.2	7.7
Placement	2 fl oz	3.2	10.2	11.6
Exp. # 1	2 fl oz	2.9	11.8	11.3
	LSD 0.05	4.6	16.6	10.6
	LSD 0.10 (trts only)	1.1	6.6	4.1

[a] Induce adjuvant provided by Bayer CropScience; all other adjuvants provided by Agrilliance LLC; all applied to either Folicur (4 fl oz/acre) or JAU 6476 (5.7 fl oz/acre)
[b] Average of four trials; two with Folicur and two with JAU 6476
[c] Average of four trials; two with Folicur and one with JAU 6476
[d] Average of four trials; two with Folicur and two with JAU 6476

Table 2. Effect of single and multiple inoculations and Folicur fungicide (4 fl oz/acre + 0.125% v/v Induce adjuvant) applications on FHB field severity in barley and durum wheat, 2003-2004 greenhouse tests.

Trt #	Folicur Rate/ac	Fungicide application timing Feekes grwth stg[a]	Inoculation timing Feekes grwth stg[a]	**Barley** FHB field severity[b] %	**Durum** FHB field severity[b] %
1	--	--	10.5 (10.51)	9.1	22.3
2	--	--	10.54	10	0.8
3	--	--	10.5 (10.51) 10.54	42.3	65.7
4	--	--	10.3 10.5 (10.51) 10.54	68.3	81.9
5	4 Fl oz	10.5 (10.51)	10.5 (10.51)	0.5	1.5
6	4 Fl oz	10.54	10.54	0.1	0.2
7	2 Fl oz 2 Fl oz	10.5 (10.51) 10.54	10.5 (10.51) 10.54	14.7	25.7
8	1 Fl oz 2 Fl oz 1 Fl oz	10.3 10.5 (10.51) 10.54	10.3 10.5 (10.51) 10.54	42	20
9	4 Fl oz	10.5 (10.51)	10.5 (10.51) 10.54	13.8	17.9
10	4 Fl oz	10.5 (10.51)	10.3 10.5 (10.51) 10.54	52	18.5
11	4 Fl oz	10.54	10.5 (10.51) 10.54	35.3	46.6
12	4 Fl oz	10.54	10.5 (10.51) no inoc.	8	41.5

[a] Feekes 10.3 = head half emerged; Feekes 10.5 = early full head emergence; Feekes 10.51 = early flowering in wheat; Feekes 10.54 = kernel watery ripe stage

[b] Field severity = Incidence x Head Severity

INTEGRATED STUDY OF AERIAL APPLICATION PARAMETERS TO IMPROVE CONTROL OF FHB WITH FUNGICIDES

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ABSTRACT

A study of aerial application methods for fungicidal control of Fusarium head blight (FHB) was done in 2004 as an interdisciplinary effort between Pathology and Ag. Engineering researchers from North Dakota, Minnesota, and the USDA/ARS Aerial Application research team from College Station, Texas. Three commercial hard red spring wheat fields were identified for study, one in east central and one in northeast North Dakota, one in northeast North Dakota, and one in northwest Minnesota. Fungicide treatments were applied with an Air Tractor AT-402B using CP-03 nozzles during the week of July 5th, when crop growth stage was at early flowering. Two spray parameters, droplet size (175 and 350 µm) and water volume (3, 5 and 10 gpa), were tested at each of the three locations, with tTreatments were arranged as a 2 3 x 3 x 3 (3^3)farrangedactorial in a randomized complete block design with three replicates. The two three factors tested were droplet size (175 and 350 µm), and spray volume (3, 5 and 10 gpa), and location (St. Thomas, Crookston, and Hunter).Data was analyzed as a 3 x 3 x 2 factorial, using SAS and the GLM procedure. The College Station, Texas, USDA/ARS Aerial Application Research Unit, using food grade dye and various methods of deposition measurement, studied spray deposition on the wheat heads. Results of the deposition studies are being submitted for publication in the *Crop Science* journal. In general, smaller spray rates with larger droplet sizes tended to result in greater deposition of active ingredient on the wheat heads. For determination of disease control, with the application methods, Folicur (tebuconazole) fungicide was applied at 4 fl oz/A with an addition of 0.125% v/v of Activator 90 non-ionic surfactant. Disease evaluations were made approximately three weeks following fungicide application, at soft dough stage of kernel development. Grain was harvested by the farmer cooperators using commercial harvest equipment, and yields were determined using weigh wagons. Sub-samples were saved for test weight and DON determinations. FHB incidences across locations and treatments ranged from 8.8 to 32.3%, field severities ranged from 0.4 to 2.3%, and yields ranged from 56.3 to 76.4 bu. /Aacre. FHB generally was significantly decreased and yield increased with fungicide treatments, but few significant differences among treatments were observed. Location differences were significant (P = 0.1) across all parameters measured except test weight. Test weights were significantly different by droplet size (58.6 lb/bu with 175µm compared to 58.9 lb/bu with 350µm). Location x gallon/acre interactions were significant for FHB incidence, FHB field severity and test weight. A 3-way interaction between location x gallon/acre x droplet size also was significant. Therefore, additional field testing will be necessary to determine disease differences among application methods across locations.

ACKNOWLEDGEMENT

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RESULTS OF THE UNIFORM FUNGICIDE TRIAL ON BARLEY, NORTH DAKOTA, 2004

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ABSTRACT

As part of the national uniform scab fungicide trial, six fungicide treatments were compared for control of Fusarium head blight (FHB) in 'Robust' spring barley at the Fargo, ND Agriculture Experiment Station. The barley was drilled into wheat stubble on April 28, 2004. Herbicide applications of Puma + Harmony GT + MCPA ester were made at the 5-leaf stage. Corn grain inoculated with *Fusarium graminearum* was spread evenly among plots. Following head emergence, a misting system provided added water to the plots when the nighttime humidity dropped below 90%. Fungicides were applied on July 1, at early full head emergence (Feekes 10.5). Applications were with a backpack-type sprayer equipped with two XR8001 flat fan nozzles oriented toward the grain head at a 30 degree angle from the horizontal. The fungicides were applied at 18.5 gpa with 40 psi. Disease notes were taken at soft dough stage of development and the crop was harvested on August 17th. The fungicide treatments included Folicur (tebuconazole) at 4 fl oz/A, Tilt (propiconazole) at 4 fl oz/A, a Bayer Co. experimental compound JAU 6476 (prothioconazole) at 5 fl oz/A, JAU 6476 at 2.85 fl oz/A + Folicur at 3.17 fl oz/A, a Valent Co. experimental compound V-10116 (metconazole) at 6 fl oz/A, and V-10116 at 4 fl oz/A. Results indicated that all treatments significantly reduced FHB field severity and DON (deoxynivalenol) and all treatments significantly increased yield over the untreated check. Fungicide treatments did not differ significantly from each other, but the experimental products generally provided slightly better disease control than the Folicur or Tilt. FHB field severity reductions ranged from 72.5 to 85%, DON reductions ranged from 48.9 to 69%, and yield increases ranged from 9.6 to 13.9%. Results of this trial will be published in *Fungicide and Nematicide Tests.*

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-9-053. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

WHEAT UNIFORM FUNGICIDE TRIALS, ND, 2004

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OBJECTIVE

To evaluate experimental fungicides for control of Fusarium head blight (scab) and leaf diseases in hard red spring and durum wheat in North Dakota.

INTRODUCTION

Uniform fungicide trials have been established across grain classes and environments as part of the U.S. Wheat and Barley Scab Initiative (McMullen and Milus 2002). The purpose of these trials is to evaluate efficacy of fungicides in reducing Fusarium head blight severity (FHB), Fusarium damaged kernels (FDK), and deoxynivalenol (DON) levels. North Dakota continues to participate in these trials and tests fungicides at several locations across grain classes and cultivars.

MATERIALS AND METHODS

A uniform set of six fungicide treatments were evaluated on hard red spring and durum wheat in ND in 2004 (Table 1). Fungicides tested included Folicur (tebuconazole), which had a Section 18 exemption for use on wheat in ND in 2004, JAU 6476 (prothioconazole), an experimental fungicide from Bayer CropScience, and V-10116, an experimental product from Valent. Artificial inoculum in the form of inoculated grain was dispersed in plots at Fargo and Langdon, wheat straw was distributed at Carrington, and natural inoculum was the source of infections at Minot. Natural rainfall was augmented by mist irrigation at Fargo and Langdon and by some overhead irrigation at Carrington.

All treatments were applied at early flowering (Feekes 10.51) with a CO_2 backpack type sprayer, equipped with XR8001 nozzles mounted at a 60^0 angle forward and backward toward the grain heads. Water volume was 18-20 gpa applied at 40 psi. Disease ratings were taken at soft dough kernel stage. Plots were harvested with small plot combines. DON levels were determined by the NDSU Veterinary Toxicology Lab. Plots were in a Randomized Complete Block design and data were statistically analyzed across locations using ANOVA.

The uniform trial was established at four locations: Fargo in the southeast; Langdon in the northeast; Carrington in the central part of the state; and at Minot in the north central region. Each site represents different environment, soil type, and cropping practices. Fungicides were evaluated over two wheat classes, 'Reeder' hard red spring wheat and 'Lebsock' durum wheat.

RESULTS AND DISCUSSION

FHB field severities varied across sites and wheat class. Field severity on untreated spring wheat averaged as high as 12.5% at Fargo on spring wheat, but was less than 1% on spring wheat at Langdon and on durum wheat at Minot. Because of the very low levels of FHB on spring wheat at Langdon and on durum wheat at Minot, Table 1 contains data only from hard red spring wheat at Fargo and Carrington and from durum wheat at Langdon. All fungicide treatments significantly reduced FHB field severity over the untreated check, and the combination treatment of JAU 6476 + Folicur had the lowest FHB field severity among fungicide treatments (Table 1). All treatments significantly reduced FHB DON ppm, with the two V -10116 resulting in the lowest DON levels. All treatments increased yield, from six to 12.7 bu, with the high rate of V-10116 resulting in the highest yield improvement.

Test weights were significantly improved by most treatments. All fungicide treatments significantly reduced the level of leaf disease from the untreated check but did not differ from each other.

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Table 1. Effect of fungicides on fungal leaf disease and FHB field severity, DON, FDK, yield and test wt., averaged across Carrington, Fargo and Langdon, ND locations and across spring wheat and durum wheat grain classes, 2004.

Treatment and rate/acre[1]		FHB FS[2] %	DON[3] ppm	FKD[4] %	Leaf disease[5] % severity	Yield Bu/A	Test wt Lbs/bu
Untreated check		10.6 a	9.5 a	10.1 a	35.2 a	55.5 c	55.2 c
Folicur 3.6 EC	4 fl oz	4.5 bc	5.8 bc	7.2 a	11.5 b	65.6 ab	57.2 ab
Tilt 3.6 EC	4 fl oz	5.5 b	6.3 b	6.7 a	14.0 b	61.6 bc	56.3 bc
JAU6476 480SC	5 fl oz	3.6 bcd	5.3 bcd	5.2 a	9.3 b	67.2 ab	57.6 ab
JAU6476 480SC + Folicur 3.6F	2.85 fl oz 3.17 fl oz	2.2 d	5.4 bcd	5.8 a	8.0 b	67.4 ab	57.7 a
V-10116 1.81 FL	6 fl oz	2.7 cd	4.7 cd	5.5 a	10.5 b	68.2 a	57.4 ab
V-10116 1.81 FL	4 fl oz	3.4 cd	4.6 d	5.7 a	11.3 b	65.9 ab	57.7 a

Numbers followed by different letters are significantly different at the 95% confidence level, using LSD analysis.
[1] All fungicide treatments had 0.125% Induce added; JAU6476 (prothiocoanzole) is an experimental fungicide from Bayer; V-10116 (metconazole) is an experimental fungicide from Valent;
[2] FHB FS = Fusarium head blight field severity; field severity = incidence x head severity;
[3] DON (deoxynivalenol = vomitoxin) levels were only available from Carrington and Fargo at time of this report;
[4] FDK = Fusarium damaged kernels; data from Fargo and Langdon sites available at time of this report; and
[5] Leaf spot diseases primarily tan spot and Septoria leaf spot complex.

PROTHIOCONAZOLE FUNGICIDES AGAINST FHB IN WHEAT, 2003/2004 RESULTS

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OBJECTIVES

According to many field and other tests the prothioconazole fungicides are superior against FHB in wheat. Our data originated mostly from dry years; and the difference between tebuconazole and prothioconazole fungicides were small. 2004 was much more humid, therefore the chance was there to detect differences between them.

INTRODUCTION

Most presentations of the Chemical Control Sessions of the National Fusarium Head Blight Forums dealing with AMS 21619 showed better performance that the tebuconazole fungicides did. However, the difference was not always convincing and the efficacy was often poor. We come to the conclusion that incomplete spraying technology explains much of the diverging results. According to Bayer the following covering was found on the heads: 30-39 of the head was covered on the front side, but only 1-2.1 % on the back side. As the translocation between palea and glume or grain is poor, and no translocation occurs from leaves to head, the fungicide concentration will be very uneven. Some landing spores will be inhibited to infect, but other not. Therefore significant infection can be observed on treated plots even they were sprayed with the most effective fungicide. When the fungicides cannot be placed uniformly on the whole surface of the head, the fungicides are not responsible for the moderate or low effect.

MATERIAL AND METHODS

The tests were made in 2003 and 2004. Three cultivars with differing resistance were used (Zugoly, Samson, Bence), three plot replicates (5 m²) for a cultivar were used for a fungicide treatment. Within each plot four isolates of *Fusarium* were used in three replicates as head of groups consisting of 15-20 heads (Mesterházy et al. 2003). Spraying terming: full flowering. Inoculation: one and three days after fungicide treatment in 2113 and 2004, respectively. Evaluation: FHB, FDK, yield loss and DON contamination. Fungicides: Kolfugo S 1.5 l/ha, 20 % carbendazime, Caramba 1.0 (2000) and 1.2 L/ha (2001), metconazole 60 g/L, Falcon 460EC 250 g spiroxamine, 167 g tebuconazole and 43 g triadimenole in one liter. In 2003 AMS 21619 and Prosaro (125 g prothioconazole and 125 g tebuconazole/L) was tested in two concentrations. The Input, (125 g prothioconazole and 500 g spiroxamine) was tested first in 2004. For 2004The composition of the other fungicides is presented at Mesterházy (2003).

RESULTS AND DISCUSSION

Table 1 shows the 2003 data. The prothioconazole fungicide (Prosaro) showed somewhat better results than Folicur Solo did across isolates and cultivars, but the difference according to different traits were not always significant. When we see the heaviest epidemics of the twelve, the situation looks somewhat different (Table 2). The Fusarium check for the two F. culmorum isolates was very high, more than 70 %. For isolate 12551 the difference between Falcon 460 EC and Prosaro 1 l/ha is significant, but the difference for Folicur Solo is not. Compared to the other isolates, here is a tendency for an increasing difference.

Table 3 presents the DON data for Zugoly according to isolates. The case is similar we have seen at FDK values in Table 2.

In 2004, the situation is different. Of the data I present only the FDK data. Table 4 presents the FDK data on the three cultivars on the isolate Fg 12377. This isolate produced the most severe symptoms. For this reason the differences between fungicides can be seen the best. Zugoly is the susceptible cultivar; here only the two prothioconazole fungicides performed well and this performance differed significantly from Folicur Solo or Falcon, the best fungicides until now. For the more resistant Samson also the tebuconazole fungicides gave identical results with Prosaro and Input, the rest had significantly higher indices. For the more resistant cultivar Bence all fungicides were good except Tango Star. It is remarkable that FDK values in Bence are about the value of Zugoly for Prosaro and Input. For the other isolates the values were zero or lower than one percent.

The mean values across isolates and cultivars are shown in Table 5. Prosaro 1.0 L/ha differed significantly from Solo at every trait, even the differences are smaller than presented in Table 4. Efficacies are for Prosaro 95-96 %for FHB and FDK and at 70 % for yield response.

The new prothioconazole fungicides are very promising also for the susceptible variety group where control was problematic in the last years by tebuconazole fungicides. Even they were the most effective fungicides the most sensitive cultivars could not be protected. The most sensitive cultivars can be protected successfully only with the prothioconazole fungicides, more resistant varieties can be protected successfully also with other compounds. Wilcoxson (1996) stated that a fungicide control is acceptable when infection severity, in our case FDK is lower than five percent. The Hungarian rules classify scabby kernels as dangerous part and the limit is maximally two percent. When efficacy is 50 %, all staples above five % infection severity cannot be used. When efficacy is 90 %, 20 % natural infection severity is the limit. When we consider DON limit value at 1 ppm, the situation will not be better much better, see Table 3. Of course, this is valid at full cover with fungicides. As present spraying technology cannot achieve this, the most important task is to improve spraying technology.

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Table 1. Fungicide control of FHB in wheat, general means across cultivars and isolates, 2003.

cultivars and isolates, 2003

Fungicide	Traits			
	FHB %	FDK %	Yield loss %	DON ppm
Prosaro 1.0	1.75	2.42	6.41	0.84
Prosaro 0.8	2.31	2.68	8.10	1.16
F. Solo 1.0 +Kolf. 1.5	2.49	5.13	12.15	1.04
Falcon 460EC 0.8	2.82	5.71	14.17	1.52
Folicur Solo 1.0	3.05	5.18	11.80	1.21
Caramba 1.2	3.29	11.31	13.21	3.14
Juwel 1.0	4.92	10.50	14.31	2.71
Tango Star 1.2	4.93	7.00	13.43	1.47
Kolfugo 1.5	15.81	20.77	22.78	5.67
Fusarium check	20.72	26.52	32.52	17.24
Mean	6.21	9.72	14.89	3.60
LSD 5 %	2.15	3.13	4.19	1.46

Table 2. Chemical control of FHB in wheat. FDK values on susceptible Zugoly according to isolates, 2003.

Fungicides and rates	Isolates				Gen.
	12377Fg	44Fg	12375Fc	12551Fc	Mean
Prosaro 0.8	0.00	0.04	0.58	2.51	0.78
Prosaro 1.0	0.11	0.07	1.13	4.20	1.38
Folicur Solo 1.0	0.27	0.29	4.42	4.13	2.28
Tango Star 1.2	0.80	0.47	2.51	6.89	2.67
F. Solo 1.0 +Kolf. 1.5	0.56	0.22	3.02	9.78	3.39
Falcon 460EC 0.8	0.13	0.07	4.13	11.22	3.89
Juwel 1.0	0.91	0.36	5.24	21.16	6.92
Caramba 1.2	1.13	1.20	21.13	18.65	10.53
Kolfugo S 1.5	2.73	1.07	26.98	54.73	21.38
Fus. check	4.98	13.73	74.11	79.00	42.96
Mean	1.16	1.75	14.33	21.23	8.01
LSD 5 %	12,12	12,12	12,12	12,12	5,42

Table 3. Chemical control of FHB. DON contamination (ppm) for cv Zugoly, 2003.

Fungicides	Isolates				Mean
	12377Fg	44Fg	12375Fc	12551Fc	
Prosaro 1.0	0.00	0.00	0.07	1.50	0.39
F. Solo 1.0 +Kolf. 1.5	0.03	0.00	1.30	1.47	0.70
Prosaro 0.8	0.20	0.00	0.20	1.37	0.44
Folicur Solo 1.0	0.20	0.00	0.83	1.10	0.53
Tango Star 1.2	0.27	0.00	1.07	2.63	0.99
Falcon 460EC 0.8	0.13	0.03	1.83	5.57	1.89
Juwel 1.0	0.53	0.37	2.60	9.80	3.33
Caramba 1.5	0.23	0.53	15.50	13.73	7.50
Kolfugo 1.5	0.93	0.13	12.37	19.80	8.31
Fusarium check	1.83	5.13	94.03	66.67	41.92
Mean	0.42	0.54	11.43	11.01	5.85
LSD 5 %	5.09	5.09	5.09	5.09	2.55

Table 4. Fungicides against FHB in wheat, FDK data for Zugoly, isolate Fg. 12377.

Treatments	Cultivars			Mean
	Zugoly	Samson	Bence	
Prosaro 1.0	**5.00**	**2.33**	**5.44**	4.26
Input 1.0	**2.33**	**0.56**	**3.89**	2.26
Falcon 0.8	28.89	**3.56**	**7.33**	13.26
Folicur Solo 1.0	32.78	**1.89**	**8.33**	14.33
Kolfugo S 1.5	52.22	47.78	**6.00**	35.33
Tango Star 1.0	66.67	43.33	29.44	46.48
Fusarium check	70.00	73.33	27.78	57.04
Mean	36.84	13,19	7,24	15,96
LSD 5 %	9.64	9.64	9.64	5.57

EFFICACY OF FUNGICIDES AND BIOCONTROL AGENTS ON FHB OF WHEAT IN ARKANSAS, 2004

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OBJECTIVE

To identify fungicides and biological control agents that are effective against Fusarium head blight (FHB).

INTRODUCTION

Identifying fungicides and biocontrol agents that reduce incidence and severity of FHB and levels of mycotoxins in the grain could have widespread benefits to growers and users of all market classes of wheat in the event of FHB epidemics. This test in Arkansas was part of the Uniform Fungicide and Biocontrol Trial that is coordinated by the Chemical and Biological Control Committee.

MATERIALS AND METHODS

The moderately susceptible soft red winter wheat cultivar 'Agripro Patton' was planted at the University Farm at Fayetteville on 9 October 2003. Seed was treated with Dividend fungicide (1 fl oz / cwt) for loose smut, Stagonosproa blotch, and seedling diseases and Gaucho insecticide (3 fl oz / cwt) for aphids and barley yellow dwarf. Individual plots were 7 rows by 13 ft. Plots were fertilized with a total of 100 lb nitrogen as ammonium nitrate (75 lb applied on 5 March and 25 lb applied on 5 April). Ryegrass and broadleaf weeds were controlled with recommended herbicides. Infested corn kernel inoculum of Fusarium graminearum was applied to the plots on 31 March at the rate of 6 kernels / sq ft. Fungicides were applied in a randomized complete block design with six replications on 26 April when 50% of the main stems had begun to flower. Treatments were applied at the rate of 20 gal per acre. The mist system operated on several days between 31 March and 26 April to promote sporulation on the inoculum and for eight 10-minute periods between midnight and 8:00 am on nine mornings (27 and 28 April, and 4, 6, 8, 12, 15, 19, and 21 May) to promote infection. On 20 May, plots were rated for the percentage of foliage with stripe rust that developed naturally late in the season. On 25 May, 50 heads per plot were sampled randomly and evaluated for FHB incidence and head severity, and plot severity was calculated. Plots were harvested with a plot combine on 14 June, and grain was passed once through a seed cleaner before test weight and percentage of scabby grain were measured.

RESULTS AND DISCUSSION

Relative to past years, little FHB developed in the plots even though sporulation was evident on the inoculum during flowering. None of the treatments had a significant effect on any of the FHB variables. All of the fungicides were highly effective against stripe rust, and TrigoCor 1448 significantly increased stripe rust severity. Tilt and V-10116 at 6 fl oz per acre increased yield significantly compared to nontreated check #2 (the check with the highest yield), but these yield increases appeared to be related to controlling stripe rust rather than FHB.

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Table 1. Effects of fungicides and biological control agents on Fusarium head blight, stripe rust, yield, and test weight of Agripro Patton soft red winter wheat at Fayetteville, AR, in 2004.

Treatment and amount of product per acre	Yield (bu/ac)	Test Wt. (lb/bu)	Plot Severity (%)	Infected Head Severity (%)	Incidence of Infected Heads	% Visual Scabby Seed	% Stripe Rust
Tilt 3.6EC 4.0 fl oz	100.3	54.7	7.8	24.0	0.3	2.0	0
V-10116 1.81FL 6 fl oz + 0.125% Induce	99.1	57.8	7.0	20.2	0.3	4.0	0
JAU6476 480SC 2.85 fl oz + Folicur 432SC 3.17 fl	98.7	55.3	6.1	22.7	0.3	3.0	0
1BC full strength broth 20 gal	95.3	56.5	6.5	18.7	0.4	3.0	32
Folicur 432SC 4 fl oz + 0.125% Induce	95.2	55.4	5.9	19.4	0.3	3.0	0
JAU6476 480SC 5 fl oz + 0.125% Induce	93.2	57.3	5.8	17.8	0.3	3.0	1
V-10116 1.81FL 4 fl oz + 0.125% Induce	91.9	57.0	6.0	18.1	0.3	2.0	0
TrigoCor 1448 full strength broth 20 gal	90.8	56.5	9.5	26.1	0.4	3.0	51
Nontreated check #2	90.7	55.9	5.3	14.8	0.3	3.0	34
C3R5 full strength broth 20 gal	89.6	55.4	4.4	14.7	0.3	3.0	40
AS 54.6 full strength broth 20 gal	88.6	54.5	6.5	18.3	0.4	3.0	32
Flutriafol 1.04EC 14 fl oz	87.7	56.7	7.7	21.7	0.4	4.0	0
Nontreated check #1	87.2	54.4	7.9	20.4	0.4	5.0	35
LSD (P=0.05)	8.0	NS	NS	NS	NS	NS	15

COMPETITIVE MICROSATELLITE PCR FOR MEASURING THE GROWTH OF *FUSARIUM GRAMINEARUM* AND *TRICHODERMA ATROVIRIDE* ON BT MAIZE RESIDUES

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ABSTRACT

In the last decade transgenic plants which contain genes encoding for insecticidal crystal proteins from *Bacillus thuringiensis* (Bt) have become increasingly popular. The biggest part was Bt maize, being cultivated on 9.1 billion hectares worldwide in 2003. This rapid increase in the global agricultural area cultivated with Bt maize could have an effect on microbially mediated processes and functions. Of particular interest is the survival of pathogenic organisms like mycotoxigenic *Fusarium* and their antagonists on maize residues. *Fusarium*-colonized maize residues serve as inoculum source for the infection of subsequent crops. We investigated whether the Bt-toxin in residues of genetically modified maize has an impact on the growth of the DON producing *Fusarium graminearum* strain GZ3639 and the potential antagonist *Trichoderma atroviride* strain P1. We developed two PCR assays to measure the growth of *F. graminearum* and *T. atroviride* in microcosms with residues of Bt maize. The PCR assays are based on a competitive PCR between microsatellite alleles of two strains of a haploid fungal species. Before DNA is extracted from a sample, a defined amount of mycelium of a second strain of the same species is added. The DNA of this second strain works as internal standard during DNA extraction and as competitor during the PCR amplification of a species-specific, polymorphic microsatellite. Using fluorescence-labeled primers, the amplification products can be separated and quantified with a sequencer. In contrast to other PCR assays, this method is not biased by a decreasing amount of plant DNA in the microcosm. The PCR assays have been used to measure the growth of *F. graminearum* and *T. atroviride* on -radiation-sterilized residues of two transgenic Bt maize varieties and their non transformed isogenic lines. Residues were collected at maturity on a field trial in 2002 and 2003. For one variety, we found a negative effect of the Bt transformation on the growth of *T. atroviride* for both years. On the same variety, *F. graminearum* grew less only on residues from 2003 but not from 2002. For the other variety, no significant effect was found in any year for both fungi. Generally the year had a greater impact on the growth of both fungi than variety or Bt transformation. Our result suggest, that Bt toxin has no direct effect on the growth of the two fungi, but isolines may carry further genetic differences in addition to the inserted Bt gene.

CHEMICAL CONTROL FOR FUSARIUM HEAD BLIGHT IN WINTER WHEAT AND MYCOTOXIN CONTAMINATION IN JAPAN

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ABSTRACT

The provisional standard of 1.1 ppm for deoxynivalenol (DON) in wheat was determined by Japanese government in 2002. Therefore, re-evaluation of registered fungicides and screening new candidates for control of mycotoxin contamination are considered mandatory. We tested totally 24 kinds of fungicides differing mode of action. Three experiments were conducted for two years. In paddy field, we sprayed fungicides at two days before flowering and 5 days after flowering. Inoculations were done at just flowering and 7 days after flowering. We used DON producer in 2002 and mixture of DON and nivalenol (NIV) producer were sprayed in 2003. An automatic sprinkler system was used to promote disease development. In addition, experiment in upland field was done in 2003. Corn grain inoculum of mixture of DON and NIV producer were used under natural rainfall condition. As a result in 2002, most of all fungicides controlled FHB disease severity, especially tebuconazole and captan and oxin-copper were highly effective. Azoxystrobin was not so effective but efficacy was about 40. As for DON in the same test, efficacy of DON was lower than that of disease severity. Tebuconazole, captan and oxin-copper decreased significantly DON level than control plot. On the contrary, azoxystrobin increased DON level significantly. In case of paddy field in 2003, most of all fungicides except trifulumizole were highly effective. The reason of failier in trifulumizole was unknown. As for mycotoxin in the same test, control of DON+NIV was difficult than disease severity. In the condition of 2003, two times application was not enough to decrease mycotoxin level. Thiophanate-methyl sol, cooper hydroxide, captan, and two-kinds of phosphorous acid, tebuconazole and metoconazole decreased significantly DON+NIV level than control plot. Trifulumizole was not effective both disease and toxin control. Azoxystrobin and mixture of azoxystrobin and propiconazole were effective for disease control but not for mycotoxins. We inoculated corn grain inoculum of DON+NIV mixture in 2003 of upland field to simulate natural infection. In this case, most of all fungicides were highly effective. To control disease severity, two times application was enough in this case. As for mycotoxin in the same test, control of DON+NIV was difficult than that of FHB. Efficacy of mycotoxin control was lower than that of paddy field, in which spore inoculation was done. It is possible that corn inoculum supply conidiospore continually during maturing period. Therefore, non visible infection might increase mycotoxin level. Thiophanate-methyl sol decreased significantly DON+NIV level than thiophanate-methyl powder. Tebuconazole and metoconazole were confirmed to decrease significantly DON+NIV level than control plot. On the other hand, azoxystrobin increased DON+NIV level, especially NIV level significantly. In this case mixture of propiconazole and azoxystrobin did not increase mycotoxin level but did not decrease. Interestingly, mode of action of kresoxim-methyl is similar to that of azoxystrobin, but effect on mycotoxin level seems to be different.

EFFECT OF STRAW MANAGEMENT AND TILLAGE SYSTEMS ON DON CONTENT IN CEREALS

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ABSTRACT

In Northern Quebec, Canada (Saguenay-Lac-Saint-Jean area), barley production is very important but fusarium head blight (FHB) has become a major problem in this region. In 2001, about 10 % of the barley acreage (1 800 ha) had a DON content superior to 2 mg kg^{-1}. In 2002, the problem was more severe with 40 % of the barley area infected with FHB. The economic losses associated with FHB in barley were estimated at 2 million Canadian dollars during those 2 years in the Saguenay-Lac-Saint-Jean region. In fall 2002 and spring 2003, several management systems (straw removal, use of disk harrow or cultivator) were applied in barley, oat and wheat fields to measure the effect of straw incorporation on DON content in grain harvested in 2003. Direct seeding, chiseling and moldboard plowing were also evaluated. No inoculation was performed on those trials. DON content in grain varied from 4.9 to 7.4 mg kg^{-1} in oat samples and from 5.9 to 8.9 mg kg^{-1} in wheat samples. For both oat and wheat, the treatments had no significant effect on DON content. DON content varied from 10.3 to 40.3 mg kg^{-1} in barley and was significantly higher in chisel and no-till treatments compared to moldboard. Straw management or spring harrowing had no effect on DON content in barley. The results from 2003 indicate that moldboard plowing can reduce DON content in barley. However, tillage system had no significant effect on mycotoxin content in wheat and oat. Straw management had also little effect on DON content in cereals.

USE OF GREEN MANURES TO INHIBIT *FUSARIUM GRAMINEARUM* ON WHEAT RESIDUES

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ABSTRACT

Fusarium head blight epidemics originate largely from inoculum associated with host residues, especially those of corn and small grain cereals. Research has shown that green manures (GMs) can have a significant effect on the intensity of a range of diseases on diverse range of crops, influencing pathogens directly through the breakdown of glucosinolates or by releasing fungitoxic compounds, or indirectly by influencing indigenous microbial populations. Preliminary studies have demonstrated that green manures may increase the population of streptomycetes and also that these microorganisms may affect the activity of *Fusarium graminearum*. In this way strategies that enhance the frequency or intensity of *Fusarium*-inhibitory streptomycetes in soil may contribute to a reduction in *F. graminearum* inoculum and thus disease development. The overall objective of this study was to quantify the effect of green manures on the frequency of soil-borne antagonists inhibitory to *F. graminearum* and to determine the intensity of *F. graminearum* inhibition. Three experiments were conducted in the greenhouse in 2004. *Fusarium*-infected wheat (*Triticum aestivum*) nodes, collected from naturally infected field sites, were incorporated into soils collected from wheat production fields in Minnesota. Three treatments, two green manures (common buckwheat [*Fagopyrum esculentum*] and sorghum-sudangrass [*Sorghum bicolor - S. vulgare* hybrid]), and a fallow (no green manure), were evaluated. The experiments were established in 10-inch pots each experiment in a randomized complete block design with 16 replicates. The *F. graminearum* populations on residue, and the frequency and intensity of *F. graminearum* inhibitors/ inhibitory activity in the soil were monitored over three months following the incorporation of the green manures grown for six weeks prior to incorporation. Total bacteria in soil were determined by soil dilution plating onto antibiotic amended oatmeal agar medium. Streptomycete densities were determined following dilution plating onto water agar overlaid with starch-casein agar; an adaptation of the method developed by Herr in 1959 (Phytopathology 49:270-273). *Fusarium*-inhibitory activity was determined by overlaying a soil dilution on water agar with a second water agar layer, incubating for three days at 28ºC and then overlaying with a suspension of *F. graminearum* macroconidia in molten potato dextrose water agar. These triple-layered plates were incubated for an additional three days prior to assessment. *Fusarium*-inhibition was determined by measuring the zones surrounding streptomycetes colonies where *F. graminearum* failed to grow. The total bacteria recovered from soil following the incorporation of buckwheat and sorghum-sudangrass tended to increase, with statistically significant differences detected for buckwheat in all experiments although only for sorghum-sudangrass in one of the three experiments. The density of streptomycetes recovered was not significantly influenced by the GMs, although a trend to increased numbers of streptomycetes was observed. The density of *F. graminearum*-inhibitory streptomycetes in soil increased significantly in both GM treatments in comparison to the fallow for at least one sampling time in each experiment. Both GMs were observed to increase the efficacy of *F. graminearum*-inhibitory streptomycetes, determined using the number and inhibition zone size in the triple-layer method, in comparison to the fallow, although the results were inconsistent. Green manures did not significantly impact the rate of residue decomposition or the population of *F. graminearum* in wheat residue. The low level of initial colonization of the wheat nodes by *F. graminearum*

may have reduced our power to detect differences among treatments. A field study has been established to further examine the impact of green manures on antagonists to *F. graminearum*. These results suggest that GMs might affect the soil population and activity of *Fusarium*-antagonists and thus may provide a complementary tool to reduce *Fusarium* inoculum.

STUDIES ON THE INTERACTION BETWEEN FUNGICIDES, SAPROPHYTIC MICROFLORA AND *MICRODOCHIUM NIVALE*,ON FUSARIUM HEAD BLIGHT IN WHEAT CAUSED BY *FUSARIUM CULMORUM*

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ABSTRACT

Microdochium nivale, a non-mycotoxigenic species involved in Fusarium head blight (FHB) complex and saprophytic microflora have been suggested to have an effect on the field performance of fungicides for control of FHB caused by *Fusarium* species. In a range of glasshouse experiments the effects of metconazole and azoxystrobin on the interactions between *Fusarium culmorum* and *M. nivale, Alternaria tenuissima* or *Cladosporium herbarum* and the development of FHB and deoxynivalenol (DON) production were studied. Fungicides metconazole and azoxystrobin were applied in all experiments at full ear emergence, wheat heads were inoculated with *F. culmorum* at mid-flowering, while *A. tenuissima*, *C. herbarum* or *M. nivale* were inoculated at ¾ ear emergence or 24 hours after *F. culmorum* inoculation (mid-flowering). When *A. tenuissima*, *C. herbarum* or *M. nivale* were introduced to wheat ears at ¾ ear emergence before inoculation with *F. culmorum* at mid-flowering resulted in an increase of FHB severity, *Tri*5 DNA and DON concentration in harvested grain, but in the case of *A. tenuissima* it was not significant. Inoculation with *C. herbarum* or *M. nivale* at ¾ ear emergence led to increased DON concentrations in grain compared to the control treatment by 34 and 151% respectively. Application of metconazole resulted in reduction of FHB severity, *Tri*5 DNA and DON concentration in grain in all of the trials. When azoxystrobin was applied after plants were inoculated with *M. nivale* at ¾ ear emergence, or before introduction of this fungus on ears after *F. culmorum* at mid-flowering, there was an increase in DON concentration in grain by 56% and 30% respectively. However this increase was not significantly different from the control treatment. This work indicates that poor performance of fungicides under field conditions and increased mycotoxin concentration in grain of wheat after application of particular fungicides, such as azoxystrobin, maybe due to the presence of non-target species such as *Alternaria* spp. or *Cladosporium* spp. or the non-toxin producing FHB species, *M. nivale*.

CROP ROTATION AND TILLAGE SYSTEM EFFECTS ON DON CONTENT IN WHEAT AND BARLEY PRODUCTION IN QUEBEC, CANADA

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ABSTRACT

Two field trials initiated in 1999 have been conducted for comparing the effect of crop sequences and two tillage systems on Fusarium head blight (FHB) in wheat and barley. At the Saint-Hyacinthe site, in the Montreal area, crops were maize (M), soybean (S) and wheat (W), and the two sequences were MSW and SMW. At the Normandin site, located 300 km north of Quebec city, crops were barley (B), canola (C), and field pea (P). The five 4-y crop rotations were BBBB, CPBB, PCBB, BCPB, and BPCB. The tillage treatments were conventional (moldboard plough) and reduced tillage (chisel at Normandin, and no-till at Saint-Hyacinthe) in fall. Experiments were exposed to natural infection. DON content in grain samples was analysed from 2001 to 2003. At Saint-Hyacinthe in 2001 and 2002 and Normandin in 2001, the treatments had no significant effect on DON content. However, in 2003 at Saint-Hyacinthe, DON content was significantly lower for the MSW x conventional tillage combination (2.7 ppm) than for the three other combinations (3.3 to 3.6 ppm). In 2002 at Normandin, DON content was significantly higher in reduced tillage (0.60 ppm) than in conventional tillage (0.36 ppm). In 2003, DON content was also significantly higher in reduced tillage but only for the three rotations in which barley was seeded the previous year (13.1, 14.1, and 16.4 ppm). There were no differences between the seven other combinations (4.8 to 6.5 ppm). These results suggest that in Quebec, in barley monoculture or when the previous year's crop is barley, ploughing crop residues may help to decrease the FHB incidence. They also indicate that rotation with canola and field pea during the 2 years preceding a barley crop may be effective in reducing DON content in either conventional or reduced tillage, and that only 1 year of soybean between maize and wheat was not effective under reduced tillage. However, none of the rotation x tillage combinations tested could, under a strong disease pressure, reduce the DON content under the maximum level of 1.0 ppm accepted by the swine industry. Therefore, growers should use as many control methods as they can to reduce the risk of infection by the pathogen, e.g. early seeding, resistant cultivars, rotation, and fungicides.

FUNGICIDE SPRAY DEPOSITION ON WHEAT HEADS FROM VARIOUS NOZZLE CONFIGURATIONS

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ABSTRACT

Fusarium head blight (FHB) epidemics in localized areas of the US have caused significant yield and quality losses of wheat and barley in recent years. Control of this disease has been difficult, because of the complex in the host/pathogen interaction. Fungicide application has become an accepted method for FHB control. Previous studies have shown paired nozzles pointed forward and backward provide better coverage and better FHB control than standard flat fan nozzles pointed downward. However, these studies did not address detailed parameters of spray deposition on varying wheat head structures and the relationship of this coverage to FHB control. The objective of this trial was to take initial steps in quantifying the parameters surrounding spray deposition on wheat heads and to identify methods whereby optimized fungicide application for efficacy can occur. Spring wheat (cvs Oxen and Ingot) were planted at the South Dakota State University Agronomy Farm and treated at anthesis (Feekes growth stage 10.51) in a single direction into the wind with a tank mixture of Folicur (tebuconazole) at a rate of 4 fl oz/a (292.30 ml/ha) and Induce adjuvant (0.125% v/v) supplemented with a fluorescent orange water soluble dye (3% v/v). The mixture was applied at 40 psi (275.79 kPa) at a rate of 18.6 gpa (173.97 l/ha). Nozzle configurations (treatments) included: 1) one flat fan nozzle pointing straight down (XR TeeJet 11002), 2) one flat fan nozzle angled 45° forward (XR TeeJet 10002), or 3) a twin-orifice flat fan nozzle (Twinjet TJ11002). Varieties and treatments were randomized in a 2 X 3 factorial design with four replications with varieties and nozzle types as factors. Plots were inoculated by spreading *Fusarium graminearum* (Fg4) inoculated corn (*Zea mays*) grain throughout the field and providing overhead mist irrigation on a 16 hr/8 hr on/off schedule (overnight mist) throughout anthesis. Wheat heads were evaluated for spray coverage and deposition pattern as well as for FHB incidence, head severity and total FHB damage and location of diseased spikelets relative to direction of sprayer travel. Further, plot yield, test weight, and *Fusarium* damaged kernels (FDK) was measured. Digital pictures of the incoming and outgoing side of the head were taken under UV light and spray coverage of the imaged was analyzed digitally. Light winds led to incomplete coverage of the head with the incoming spray side of the head showing greater spray deposition. All nozzle configurations provided reasonable spray coverage on the incoming side, while the side away from the application generally received little or no product, regardless of nozzle configuration. No nozzle tested in this trial overcame the problem of poor deposition on the back of the head, although differences were observed. Awns of the wheat plant collected a significant portion of the applied fungicide mix. Initial data on FHB infection appears to show that there may be an effect of applied fungicide penetrating to the rachis of the head and limiting FHB infection to single spikelets, thus reducing FHB spread within the head.

USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT 1: USE OF DIATOMACEOUS EARTH AS A CARRIER FOR FORMULATIONS OF THE ANTAGONIST *CRYPTOCOCCUS NODAENSIS* OH 182.9

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OBJECTIVES

To assess the feasibility of pilot-plant-scale production of a dried, formulated FHB biocontrol product composed of biomass of the FHB antagonist *C. nodaensis* OH 182.9 imbedded in a variety of diatomaceous earth matrices. Additionally, to assess the survival and efficacy of the dried product in laboratory and field tests, respectively.

INTRODUCTION

One of the most effective yeast strains discovered in our collaborative research (U.S. patented strain *Cryptococcus nodaensis* OH 182.9 (NRRL Y-30216), Schisler et al. 2003) was selected for testing against FHB in the 2001 Uniform Wheat Fungicide and Biocontrol Trial (UWFBT). Biomass of OH 182.9 was produced in our pilot plant facility, concentrated, frozen and used at 15 field trial sites across the United States. This biocontrol preparation significantly reduced FHB when results from all test sites were pooled (Milus et al., 2001) and compared favorably with the fungicide Folicur 3.6F (tebuconazole).

The development of a dried biocontrol product would have potential advantages of ease of handling, convenience in transportation, favorable economics and acceptance by consumers and commercial developers. In the development process, dehydration of antagonist biomass can adversely affect antagonist viability and efficacy (Wraight et al., 2001) as can cryoprotectants if they can be metabolized by the pathogen target (Schisler et al., 2004a). An air-dried OH 182.9 product would be more economically feasible for industrial production than a freeze-dried product. In work described elsewhere in these proceeding (Zhang et al., 2004), we discovered that cold temperature shock during production of biomass of OH 182.9 enhanced the survival of air-dried cells over time. Diatomaceous earth (DE) is an effective filter aid that can be used for embedding microbial biomass to produce a friable, moist product that can then be readily air-dried. The impact of a range of grades of DE on biocontrol agent survival and maintenance of efficacy in general and of OH 182.9 in particular has not been previously reported.

MATERIAL AND METHODS

Production of a dried diatomaceous earth product containing Cryptococcus nodaensis OH 182.9

A semidefined complete liquid medium (SDCL; Schisler, 2004a) with carbon:nitrogen ratio of 11 and total carbon loading of 14 g/L was used in all instances for production of biomass in liquid culture. For production of biomass in a B Braun D-100 fermentor, seed inoculum was produced in Fernbach flasks (1.5 L SDCL medium in 3.0 L capacity flask) at 25ºC and 250 rpm for 24 h. Starter inoculum was then added to the D-100 fermentor at approximately 5% (vol/vol) which resulted in an initial absorbance (A_{620}) of approximately 0.175. Reactor medium pH, temperature, dissolved O_2, antifoam, and agitation rate were monitored and/or maintained to insure near identical production runs. After 24h of incubation at 25ºC, the

reactor temperature was reduced to 15ºC to enhance OH 182.9 cell tolerance to drying stress (Zhang et al., 2004). After completion of biomass production at approximately 48h, cells in the broth were concentrated into a paste using a Sharples 12-V tubular bowl centrifuge. Cell paste was incorporated into various diatomaceous earth (DE) products (Table 1) at 150 ml paste to 1000 ml DE (vol/vol) using a blender. Friable DE products were then spread on trays, dried at approximately 95% RH and 25ºC for 24h to a final moisture content of approximately 5-7% (wet weight basis). DE products were then vacuum packed in foil pouches and stored for 21 weeks at 4ºC while being monitored periodically for cell survival (Fig 1).

Field testing of a dried Cryptococcus nodaensis OH 182.9 product

The soft red winter wheat cultivars Elkhart (susceptible) and Freedom (moderately resistant) were grown in both Peoria, IL and Wooster, OH. Cells of OH 182.9 dried in DE MN-51 and stored for 3-6 weeks were used in all field studies. Immediately prior to use, the DE product was rehydrated at 8:1 (water:product; wt/wt), agitated for 10 minutes, and decanted 5 minutes after agitation ceased (~1 x 10^7 CFU/ml). Additional field treatments were fresh biomass of OH 182.9 harvested from 48h Fernbach shake flasks (~5 x 10^7 CFU/ml), Folicur applied at the recommended rate, a water/tween 80 control and an untreated control. All treatments were applied at the beginning of wheat flowering at 80 gal/acre. Corn kernels colonized by *Gibberella zeae* were scattered through plots (~25 kernels/m^2) two weeks prior to wheat flowering and mist irrigation provided periodically for approximately one week after treatment application to promote FHB development. Heads were scored for disease incidence (presence or absence of disease symptoms) and severity using a 0-100% scale approximately three weeks after inoculation. Heads were then allowed to dry and threshed. Data for the deoxynivalenol content of grain is being tabulated (ongoing). Randomized complete block designs were used in both trials (n=6 in Peoria; n=4 in Wooster) and data analyzed using JMP software (ANOVA; SAS Inc., NC).

RESULTS AND DISCUSSION

Regardless of the DE used to make a dried OH 182.9 product, the product CFU's were virtually unchanged over the course of 21 weeks of storage at 4 C (Fig 1). Because the MN 51 product lost less CFU's immediately after drying than the others, this product was selected for pilot scale production and field efficacy testing.

Field performance of the dried DE product was variable across sites and wheat cultivars. Although disease level was relatively low in Peoria, Freedom wheat treated with rehydrated cells of OH 182.9 in DE (MN 51) had significantly lower disease severity compared to the untreated check (Table 2), tended lower in FHB disease incidence and had statistically identical severity compared to the Folicur treatment. No treatment effects were found on Elkhart in Peoria. In Wooster where the overall disease level was higher, the dried product did not reduce disease symptoms on either wheat cultivar while freshly produced cells of OH 182.9 significantly reduced severity on Freedom and Elkhart by as much as 56% and performed statistically better and worse than Folicur on Freedom and Elkhart, respectively (data not shown).

These results indicate that the development of a commercially feasible dried FHB biological control product containing OH 182.9 as an active ingredient is possible, especially in light of advancements made in developing commercial-scale production of stress tolerant cells of OH 182.9, UV protectants, and the discovery of new biological control strains that could be effectively combined with strain OH 182.9 (Schisler 2004b). Research devoted to optimizing biological control efficacy through the use of stickers, activators, and combinations of biocontrol agents with diverse modes of action, should position this biological control as an important tool to utilize in combination with reduced levels of fungicides, resistant varieties, improved spray coverage technologies and disease forecasting to minimize the impact of Fusarium head blight.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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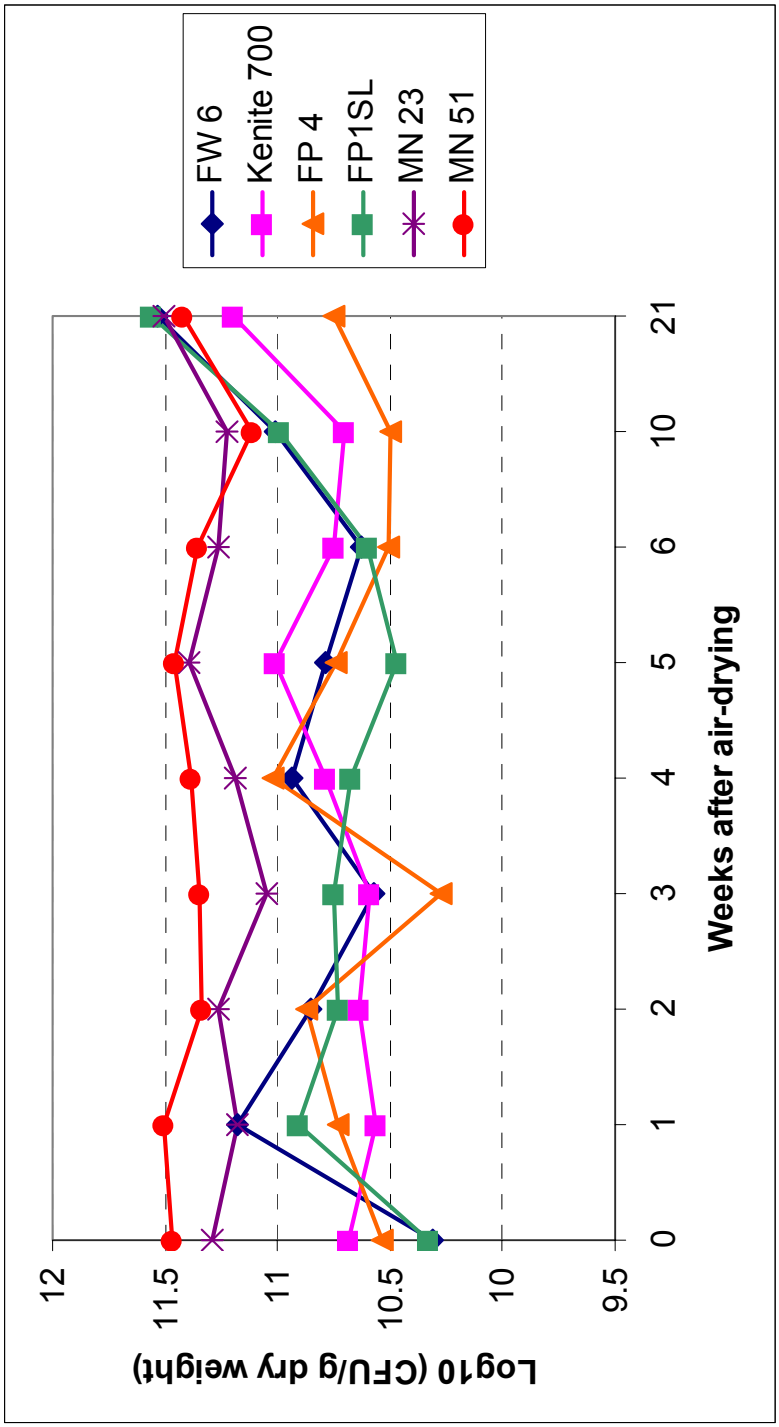

Figure 1. Survival of 100L fermentor-produced cells of *C. nodaensis* OH 182.9 after blending and air-drying in various grades of diatomaceous earth and storage at 4°C.

Table 1. Characteristics of various diatomaceous earth (DE) products used to formulate FHB antagonist *Cryptococcus nodaensis* OH 182.9.

DE Product[1]	pH	Permeability (Darcys)	Mean Particle Diameter (µm)
FW 6	9.0	0.48	18.0
Kenite 700	7.0	1.30	24.0
FP 4	8.8	0.30	15.0
FP1SL	6.5	0.07	12.5
MN 23	7.0	ND[2]	5.0
MN 51	7.5	ND	15.0

[1]Eagle-Picher Minerals, Inc.
[2]Not determined

Table 2. 2004 Peoria, IL field trial of an air-dried diatomaceous earth (MN 51) product containing FHB biocontrol agent *Cryptococcus nodaensis* OH 182.9 on winter wheat cultivar "Freedom"[1,2].

Treatment	DS (%)		DI (%)		100-kw (g)	
Untreated control	2.0	ab	9.2	a	3.76	a
Tween 80 control (0.036%)	1.4	bcd	7.8	a	3.85	a
Folicur 3.6F[3]	1.0	cd	6.1	a	3.67	a
Fresh OH182.9	1.3	bcd	7.8	a	3.73	a
DE dried OH182.9	1.1	cd	6.4	a	3.78	a

[1]Within a column, means without a letter in common are significantly different (P=0.05). Mean comparisons were performed on arc-sine transformed data. Back-transformed values are presented.
[2]DS = disease severity, DI = Disease incidence, 100-kw = 100-kernel weight
[3]Applied at recommended label rates.

BIOLOGICAL INACTIVATION OF FUMONISINS

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ABSTRACT

Fumonisins are a group of quite recently found mycotoxins mainly produced by *Fusarium moniliforme* and *Fusarium proliferatum*, which are very common contaminants of cereal grains, especially of maize. Among these the fumonisins B_1 and B_2 are produced most abundantly in nature and quantitatively may be of greatest toxicological concern (Sydenham *et al.*, 1992). Fumonisins have been shown to produce a wide range of pathological effects in animals, including the economically important disease symptoms of leucoencephalomalacia in horses and pulmonary oedema in swine. In addition, these compounds exhibit toxic effects to turkey poults and broiler chicks and cause nephrotoxicity, hepatotoxicity and hepatocellular carcinoma in rats. Although definite evidence of carcinogenicity in humans is lacking, oesophageal cancer occurs at greater frequency in world regions where corn is the dietary staple and levels of *Fusarium* and fumonisin contaminations are high.

The economic implications of animal feeds contaminated with high levels of fumonisins are significant (Shepard *et al.*, 1996), since contamination of corn and corn-based products with these mycotoxins is reported frequently and in many countries worldwide. This implies the need for appropriate decontamination strategies, and hereof biological detoxification - meaning the transformation of fumonisins via microorganisms or specific enzymes - seems to be promising. The microbial/enzymatic breakdown into compounds that are no longer toxic would provide a very gentle, effective and environmentally friendly way of deactivating fumonisins. Such a mechanism was already described for the yeast *Exophiala spinifera* as well as for an aerobic bacterium (Duvick *et al.*, 1998), in the course of complete metabolization of the toxin.

Based on these facts a project was initiated with the aim to find microorganisms with the capability to deactivate fumonisins through enzymatic transformation. The respective organism is intended to be used as part of a feed additive to ensure detoxification of fumonisins in the intestinal tract of animals during feed digestion. A screening for aerobic and anaerobic microorganisms with FB_1-transforming potential was realized by performing fumonisin-degradation experiments in liquid culture media as well as in buffered systems. Besides testing promising bacterial and yeast strains of culture collections, several different habitats, e.g. rumen fluid or intestinal segments of pigs, were investigated with regard to the presence of toxin-reducing, microbial activity. Different environmental samples were also under investigation, as for example soil. In one of these samples, fumonisin-reducing activity could be detected leading to trials to isolate the respective microorganisms.

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EFFECTS OF FUNGICIDES APPLIED AT ANTHESIS ON FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL IN WHEAT IN ANKARA AND SAKARYA PROVINCES

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ABSTRACT

Tebuconazole and Chlorothalonil reduced head blight incidence, visually scabby kernels and Deoxynivalenol concentration. The greatest reduction of FHB incidence with Tebuconazole(60 ml/100l). It was applied middle of the anthesis with spore suspension of *Fusarium graminearum* and *Fusarium culmorum*.

Fungicides treatment increased thousand kernel weight and yield. Deoxynivalenol content was determined from harvested seed samples during 2003. Samples were analyzed using high performance liquid chromatography (HPLC). Deoxynivalenol was detected range 0 to 4.28 µg/g.

Additional key words: wheat, deoxynivalenol, *Fusarium graminearum*, *Fusarium.culmorum*, Tebuconazole ,Chlorothalonil

POSSIBILITIES OF FHB *(FUSARIUM GRAMINEARUM* SCHWABE*)* CONTROL BY FUNGICIDES IN WHEAT

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ABSTRACT

The effect of wheat treatment with fungicides and their mixtures on FHB (Fusarium head blight) was assessed in a field experiment under conditions of artificial inoculation with *Fusarium graminearum*. The FHB infection level as the percentage of spike tissue necrotized by the pathogen was assessed in the field during the grain filling period. The number of scaby grains was determined in a laboratory using a "paper roll test" – germination test in fungicide medium containing active agent iprodione. Finely, mycotoxin DON content was evaluated immunologically by ELISA.

We assessed the efficacy of 24 different treatments with fungicides based on one as well as more agents and the TM-mixtures of these products. The infection level was compared with the inoculated and non-treated control.

The most effective reduction in DON content was found for triazoles tebuconazole, metconazole and mixture of tebuconazole+propiconazole at full rates. Mixtures of strobilurin product trifloxistrobin at the rate of 75 g/ha together with reduced rates of triazoles showed efficacy which was not statistically different in comparison with a full rate of tebuconazole in mixture with prochloraz.

The mixed fungicide Charisma (flusilazole+famoxadone) with declared efficacy against FHB did not show DON reduction alone but in mixture with half rate of tebuconazole (125 g/ha) DON content was reduced to 1/ 3 of the non-treated plot level. Also, the mixtures of Charisma with flusilazole, metconazole and prochloraz showed the increase in efficacy.

The use of reduced rates of stobilurin fungicide Amistar (azoxystrobin) – 0.3 and 0.6 l/ha in mixture with Artea fungicide (propiconazole+cyproconazole) reduced DON highly significantly, too. There were no differences between treatments based on this Artea fungicide (alone or in mixtures) in DON content but significantly more scaby grains were found after Artea alone as compared to both mixtures with Amistar.

Highly significant correlations were found between the following traits: FHB – DON and number of scaby grains - DON (positive levels), FHB - yield and DON - yield (negative levels). The significant correlation coefficient characterized the relationship yield – number of scaby grains (negative level).

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SIGNIFICANT ACCOMPLISHMENTS AND FUTURE ENDEAVORS IN CHEMICAL APPLICATION

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ABSTRACT

The seven variables that significantly influence fungicide efficacy are: application timing, active ingredient (AI) selection, "post application" weather, target plant type, application rate, deposition efficiency, and coverage uniformity. Currently US growers trying to control Fusarium head blight (FHB) on wheat and barley have only one fungicide available and it is available on EUP label ("Emergency Use Permit"). Folicur is Bayer product and its application timing and maximum rate are specified by the EUP. A second experimental chemistry numbered JAU6476 by Bayer is in the field testing stage. A label for a blended JAU 6476/ Folicur product is anticipated in one to two years. Growers can select the variety of wheat or barley they plant but they have no control of the weather. Recent North Dakota field studies have shown a direct and significant relationship between fungicide dose and efficacy but even two times the maximum rate does not guarantee 100% control of FHB, therefore rate reductions are not recommended. Thus the application technology researcher is left with two variables to optimize: deposition efficiency and coverage uniformity.

Deposition efficiency and coverage uniformity are affected by spray volume, drop size, and the methodology of droplet transport. North Dakota research documented that replacing one vertically mounted flat fan nozzle with two flat fan nozzles delivering 50% less solution oriented forward and backward and angle 60° downward from vertical improves efficacy. 2004 field studies extensively tested the effects of varying spray volumes and drop sizes for both aerial and ground application. Nearly all the treated plots were significantly better than the unsprayed checks, but due to low disease pressure resulting from below average summer temperature none of the application variables produced different effects. 2004 ground application deposition studies showed that the deposition on the grain heads increased as the nozzles are angled from zero to 60° from vertical for both standard hydraulic nozzle and air assist sprayer systems. Two hydraulic nozzles angle forward and backward were better than one or two nozzles angled forward. Preliminary 2004 results indicate that the local systemic activity of both Folicur and JAU 6476 was enough to overcome any differences in coverage uniformity between the several different application technologies tested. A normal season with greater disease pressure may produce different results.

The grower must be concerned about more than FHB control. Significant increases in yields and test weight are frequently caused by the fungicide's control of leaf diseases. Improvement in yield and quality factors as a result of fungicide efficacy improvement is difficult to delineate between FHB and foliar disease. Often control of FHB is quantified by the measurement of the toxin deoxynivalenol from the grain sample. Fungicide application that targets the grain head for only FHB control is often not feasible or practical from a grower's perspective. An economically sound spraying methodology must have both an AI and application equipment that target the whole plant disease complex.

FACTORS THAT CAN AFFECT FIELD EFFICACY OF BIOLOGICAL CONTROL AGAINST FUSARIUM HEAD BLIGHT

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ABSTRACT

Lysobacter enzymogenes C3 is a bacterial strain with biological control activity against Fusarium head blight (FHB) of wheat caused by *Fusarium graminearum*. While it is unique in having the potential to suppress FHB through direct antagonism and induction of host resistance, it is similar to other FHB biocontrol agents in that the achievement of consistent field control of FHB using C3 is a challenge. This poster summarizes greenhouse and field research conducted during the last three years to identify application factors and strategies that can influence the effectiveness of C3 in FHB biocontrol. C3 efficacy was found to be independent of cell concentration. Various dilutions of C3 broth cultures yielded similar levels of disease control in greenhouse experiments, while C3 efficacy in field experiments varied despite relatively uniform population levels of C3 being applied across trials. Timing of C3 application also was not an important factor as pre-anthesis applications of C3 in greenhouse and field experiments were as effective as C3 treatments made at the onset of anthesis. Among factors that were important to efficacy was uniformity of deposition. In the greenhouse, protection by C3 was localized to wheat spikelets to which the bacterium was applied. In the field, FHB control was achieved only in experiments in which C3 was applied in relatively high volumes that allow uniform coverage of wheat heads, whereas no efficacy was found in any experiment in which C3 was applied in low volumes that provide non-uniform treatment. Application strategies, including combining C3 with other biological control agents or with fungicides, will be examined as to their potentials for providing effective FHB control. In addition, results obtain using C3 will be discussed in relations to the use of biological agents in general as a tool for managing FHB.

RESULTS FROM THE 2004 STANDARDIZED EVALUATION OF BIOLOGICAL AGENTS FOR THE CONTROL OF FUSARIUM HEAD BLIGHT

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OBJECTIVE

To evaluate, using standardized methodology, a set of biological control agents for effectiveness in managing Fusarium head blight (FHB) in wheat and barley across a range of environmental conditions.

INTRODUCTION

Biological control agents with the potential for controlling FHB in the field have been identified (da Luz et al., 2003), the most extensively studied in the US being the yeast *Cryptococcus nodaensis* OH 182.9 (Khan et al., 2004), strains of *Bacillus* spp, including Trigocor 1448 (Stockwell et al., 2001) and 1BA (Draper et al., 2001), and *Lysobacter enzymogenes* strain C3 (Yuen and Jochum, 2002). These agents were effective when evaluated separately in field tests (Stockwell et al., 2001; Khan et al., 2004; Yuen and Jochum, 2002). To better gauge the potentials of biocontrol agents for commercial development, however, direct comparison of agents over a wide range of environmental conditions and crop genotypes is necessary. The Uniform Fungicide and Biological Control Trials (UFBT) supported by the USWBSI provides an avenue for wide-scale, standardized field testing (Milus et al., 2001), but for most biocontrol agents, systems for large scale propagation and formulation are unavailable, and thus it is difficult to evaluate biological agents in the same standardized trials as chemical fungicides. Informal efforts were undertaken in 2001 through 2003 to compare a set of biocontrol agents across several states, with procedures varying among the locations (Yuen et al., 2003). No one strain, however, was superior or consistently effective in these studies. But out of this collective effort came a mutual appreciation of the difficulties in working with microorganisms originating from different laboratories, along with the recognition that standardized methods are needed in order to compare results from one location to another. Given that biocontrol agents for FHB, in their current state, require special procedures as to propagation, handling and quality control, a USWBSI-funded program for uniform evaluation of biological agents on wheat and barley separate from the UFBT was initiated in 2004. The results of the 2004 efforts on wheat analyzed across locations are reported here.

MATERIALS AND METHODS

Five trials were conducted across four states on a range of classes (Table 1). A sixth trial in South Dakota on barley also was conducted as part of the uniform evaluation, but its results are reported in a separate paper. In each trial, four biological agents (Table 2) were tested. A culture of each organism was provided to the researcher in each location and inoculum for treatment was propagated by the researcher following instructions provided by the organism's supplier. The pre-application population of each agent in the inoculum was determined by the local researcher using dilution plating. In addition to the biological agents, there was a non-treated control and a treatment with the fungicide tebuconazole, as Folicur 432SC, 4.0 fl oz/

A, amended with 0.125% Induce. One application was made per treatment at early flowering (Feekes 10.51) in 20 gal/acre using a CO2-pressurized sprayer (approximately 40 psi) equipped with flat-fan nozzles oriented forward and backward. The size and number of replicate plots varied among trials. Some of the trials were inoculated with *Fusarium graminearum* and utilized mist irrigation systems to stimulate infection. In all trials, FHB incidence (% heads infected per plot), severity (%spikelets infected per diseased head), and index (plot severity) were determined from at least 40 heads per plot around 3 weeks after anthesis. The incidence of Fusarium-damaged, kernels (FDK) were determined after harvest. Samples from each plot were to USWBSI-designated laboratories for analysis of DON content. Results from all trials were analyzed together using ANOVA, with trials being treated as blocks. The trials conducted on two cultivars in Missouri were considered to be separate trials. Consult individual state trial reports for results at each location.

RESULTS AND DISCUSSION

FHB pressure varied considerable among the trials, with incidence ranging from 40 to 99% and severity ranging from 16 to 52% in the controls. None of the treatments with a biological agent or with Folicur 432SC had a significant effect on any disease parameter compared to the control across the trials (Table 3). The agents also were ineffective in all of the individual trials; Folicur 432SC provided a significant reduction in DON in the Missouri trial on 'Truman' but otherwise had no effect in individual trials (data not shown).

Biocontrol agent numbers in the inoculum cultures varied considerably among agents and among locations. In many instances, cell concentrations determined at the time of application were several orders of magnitude lower than expected. The low population numbers applied could have been a contributing factor to lack of efficacy in the biological treatments. This experience points to the need for better control over microorganism numbers when testing biological agents. The fact that Folicur also was ineffective across these trials is an indication that suppression of FHB under field conditions remains a difficult objective to achieve using biological or chemical treatments.

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Table 1. 2004 uniform biological control trial locations, wheat cultivars, and researchers.

State	Wheat market class and cultivar	PI and Institution
AR	Soft red winter wheat 'Agripro Patton'	E. Milus, University of Arkansas
MO	Soft red winter wheat 'Elkhart' and 'Truman'	L. Sweets, University of Missouri
NE	Hard red winter wheat '2137'	G. Yuen, University of Nebraska
SD	Hard red spring wheat 'Ingot'	B. Bleakley and M. Draper, South Dakota State University.

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Table 2. Biological control agents tested in 2004 uniform trials.

Organism	Supplier
Gram-positive bacterium AS 54.6	D. Schisler, NCUAR USDA-ARS, Peoria
Lysobacter enzymogenes C3R5	G. Yuen, University of Nebraska
Bacillus subtilis TrigoCor 1448	G. Bergstrom, Cornell University
Bacillus sp.1BC	B. Bleakley and M. Draper, South Dakota State University

Table 3. Results across five uniform biocontrol trials on wheat, 2004.

Treatment	% FHB incidence	% FHB severity	Index (%)	% FDK	DON (ppm)*
Non-treated control	60.9	29.6	18.7	5.2	3.7
Folicur 432SC	58.4	26.1	15.4	4.5	3.3
AS 54.6	63.6	29.3	19.6	5.8	3.7
C3R5	59.1	28.5	17.5	5.1	3.7
TrigoCor 1448	62.2	30.3	19.4	6.4	4.0
1BC	61.3	29.3	19.4	5.3	4.0

*Based on results from four trials.

USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT 2: COLD TEMPERATURE SHOCK DURING PRODUCTION OF *CRYPTOCOCCUS NODAENSIS* OH 182.9 ENHANCES CELL SURVIVAL AFTER AIR-DRYING

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OBJECTIVES

To investigate the effect of temperature during liquid cultivation of *C. nodaensis* OH 182.9 on cell survival after air-drying, and on biocontrol efficacy of air-dried products.

INTRODUCTION

Cryptococcus nodaensis OH 182.9 (NRRL Y-30216), isolated from the anthers of wheat, significantly reduced FHB under greenhouse and field conditions when applied as fresh cell preparations and frozen cell concentrate products (Khan et al., 2004; Milus et al., 2001; Schisler et al., 2002). Development of dried products of OH 182.9 would have potential advantages of ease of handling, convenience in transportation, favorable economics and consumer acceptance. Field data from the 2002 UWFBT indicated that the freeze-dried OH 182.9 product maintained viability but was not as effective in reducing FHB as was the frozen cell concentrate tested in 2001. It is possible that melezitose, a trisaccharide cryoprotectant that was used to enhance the tolerance of OH 182.9 to freeze-drying, stimulated pathogen activity. In order to avoid this problem, air-drying without the addition of cryoprotectants was selected as a preferred process for dehydrating biomass of OH 182.9 (Schisler et al., 2004). The specific objective of this project was to investigate the impact of heat and/or cold shock during liquid cultivation on the storage stability and biocontrol efficacy of OH 182.9 cells in an inert diatomaceous earth carrier.

MATERIALS AND METHODS

Effect of timing and duration of heat and/or cold temperatures (Experiment 1). The temperature extremes used for heat and cold treatments were 31ºC and 15ºC, respectively. Cultures of *C. nodaensis* OH 182.9 were initially grown in SDCL (Slininger et al., 1994) at 25ºC and 250 rpm for 20 or 26 h. Cultures of OH 182.9 are in late exponential growth after 20 h and early stationary growth after 26 h (data not shown). To test the effect of heat and cold temperature shock, OH 182.9 cells then were subjected to various heat and/or cold temperature extremes (Table 1). This experiment was conducted twice with 3 replications per treatment.

Effect of intensity and duration of cold temperatures (Experiment 2). Because results from the heat and cold shock experiments indicated the potential benefit of cold shock to long-term survival of dried OH 182.9 cells, experiments were conducted to optimize the timing and duration of cold shock during cell cultivation. The cold shock temperatures tested were 5, 10 and 15ºC applied at the late exponential stage of growth (20 h) for 28 h or 4 h. (Table 2).

Survival of cells after air-drying and storage. Harvested cultures were mixed with 10% diatomaceous earth (Hyflo, Celite Corporation) (w/v) and dewatered using vacuum filtration. The resulting *C. nodaensis* OH 182.9:diatomaceous earth formulations were milled in a food processor, and placed in shallow pans in an air-drying chamber at 60-70% RH for approximately 20 h or until the moisture content of the

formulations was less than 4% [(W_{wet}-W_{dry}) × 100% /W_{wet}]. The dried OH 182.9 formulations were vacuum packed in plastic bags and stored at 4ºC or room temperature (25ºC) for cell survival tests. Cell survival was assessed by suspending 50 mg of air-dried samples in 50 ml of weak (0.03%) phosphate buffer, mixing in a Stomacher 80 (Seward Inc., England) for 60 s, and dilution plating on 1/5 TSA. Since no colonies were recovered in some treatments when samples were stored at room temperature after 14 weeks, 500 mg samples were used instead of 50 mg for determining colony forming units (CFU). Data (CFU per gram of dry weight) was converted to logarithmic values and analyzed using JMP software (SAS Inc., NC)

Greenhouse bioassays of air-dried OH 182.9 products against FHB. Experiments were conducted in the greenhouse where temperatures ranged from 17-20ºC at night and 25-28ºC during the day. Two wheat (cultivar Norm) seedlings per plastic pot were grown in air-steam pasteurized potting mix in a growth chamber at 25ºC with a regime of 14 h light/ day for 7-8 weeks prior to use. Wheat heads were inoculated at anthesis by spraying cell suspensions of OH 182.9 products from Experiment 2 in weak PO_4 buffer with 0.036% Tween 80. One gram of an air-dried OH 182.9 product was added to 50 ml of PO_4 buffer and mixed in a Stomacher 80 (Seward Inc., England) for 60 s. This suspension (approx. 1-5×10^7 CFU/ml) was used to inoculate 4 plants representing a total of 12-16 heads. Heads were then challenged by spraying with 12 ml of a conidial suspension of *G. zeae* (1-2 ×10^4 conidia / ml) in weak PO_4 buffer with 0.036% Tween 80. Treated pots were arranged in a completely randomized design with four replications for each treatment. Each experiment was conducted 2 or 3 times. Wheat heads inoculated only with a suspension of *G. zeae* served as a disease control. Inoculated plants were incubated in a plastic humidity chamber for 3 days before being transferred to greenhouse benches. FHB severity was visually estimated using a 0 to 100% scale at 10-14 days after inoculation. Disease severity data were normalized using the arcsine transformation before analysis of variance (ANOVA).

RESULTS AND DISCUSSION

In general, air-dried cells from cultures incubated at 15ºC for 28 h after 20 h at 25ºC (T5, Table 1) maintained viability better than those from other treatments (Figure 1). Heat treatment during cell growth did not have a significant effect on cell survival. Exposure of cultures to cold temperatures during the late exponential growth stage of OH 182.9 (T5) significantly increased the storage stability of air-dried cells compared to cells exposed to cold during early stationary growth (Figure 1, T6).

A prolonged moderately cold shock after an initial period of normal incubation significantly enhanced storage stability with the highest $\log_{10}$CFU/g dry weight (7.6 for T5) at 18 weeks stored at room temperature after air-drying (Table 2, Figure 2). However, prolonged cold shock of cells at the lowest temperature (5ºC) tested had an adverse effect on cell survival, with the lowest $\log_{10}$CFU/g dry weight of 5.6 for T1 at 18 weeks, compared to a value of 6.2 for control cells (T7)(Figure 2). Similar results were observed from the air-dried OH 182.9 products stored at 4ºC (data not shown).

The biocontrol efficacy of air-dried products of OH 182.9 stored at 25ºC and 4ºC was assessed in the greenhouse. After air-drying, OH 182.9 products from T1, T3, T5, and T6 (Table 2, Table 3) significantly reduced disease severity of FHB compared to the untreated control (P = 0.05). After 6 weeks storage at 4ºC, air-dried OH 182.9 produced from T1, T2, T3, T5 and T7 retained biocontrol capacity. However, for OH 182.9 products stored at room temperature (25ºC), only air-dried products from T1, T3 and T5 significantly protected wheat plants from FHB. Fermentation conditions during OH 182.9 production and the storage conditions for dried cell products can be managed to enhance product stability and biological control performance.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Table 1. Descriptions of heat and cold temperature applications during liquid cultivation of OH 182.9 (Experiment 1).

Treatment	Application of heat and cold temperatures
T1	20 h 25ºC ? 2 h 31ºC ? 2 h 25ºC ? 24 h 15ºC
T2	26 h 25ºC ? 2 h 31ºC ? 2 h 25ºC ? 18 h 15ºC
T3	20 h 25ºC ? 2 h 31ºC ? 26 h 25ºC
T4	26 h 25ºC ? 2 h 31ºC ? 20 h 25ºC
T5	20 h 25ºC ? 28 h 15ºC
T6	26 h 25ºC ? 22 h 15ºC
T7	48 h 25ºC (standard control)

Table 2. Descriptions of cold temperature applications during the liquid cultivation of OH 182.9 (Experiment 2).

Treatment	Application of heat and cold temperatures
T1	20 h 25ºC ? 28 h 5ºC
T2	20 h 25ºC ? 4 h 5ºC ? 24 h 25ºC
T3	20 h 25ºC ? 28 h 10ºC
T4	20 h 25ºC ? 4 h 10ºC ? 24h 25ºC
T5	20 h 25ºC ? 28 h 15ºC
T6	20 h 25ºC ? 4 h 15ºC ? 24 h 25ºC
T7	48 h 25ºC (standard control)

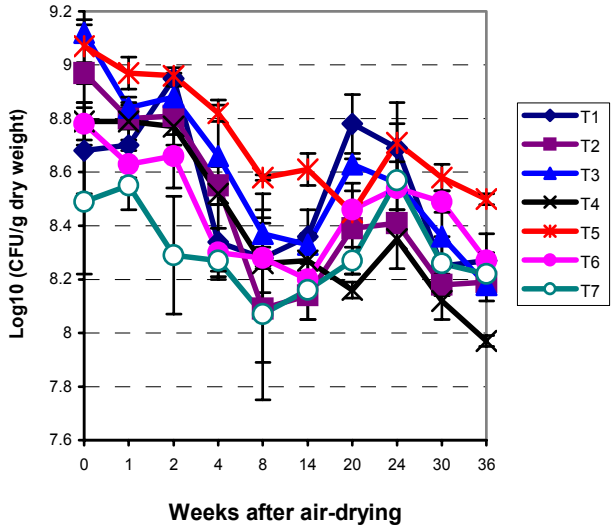

Figure 1. Effect of heat and/or cold temperatures on cell survival of OH 182.9 after air-drying (stored at 4ºC) (Experiment 1).

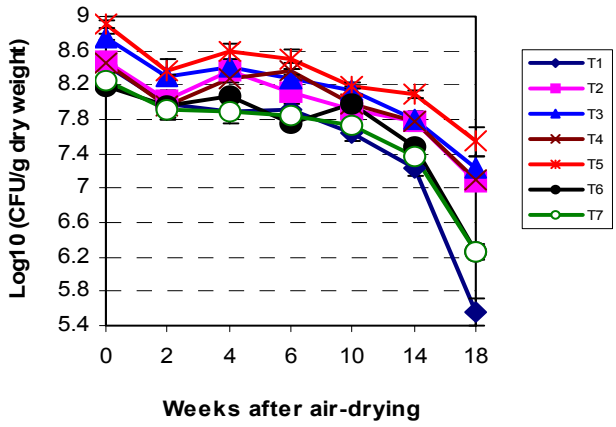

Figure 2. Impact of cold treatments during OH 182.9 biomass production on cell survival after air-drying (stored at 25ºC) (Experiment 2).

Table 3. Efficacy of cold shocked, air-dried OH 182.9 products from Experiment 2 in reducing FHB severity (%) in greenhouse tests[1].

		6 weeks after air-drying	
	After air-drying	25ºC	4ºC
Treatment	(% reduction vs CK)	(% reduction vs CK)	(% reduction vs CK)
T1	16 b (66)	35 b (53)	20 bc (51)
T2	28 ab (40)	55 ab (27)	22 bc (46)
T3	23 b (51)	42 b (44)	14 c (66)
T4	26 ab (45)	58 ab (23)	25 abc(39)
T5	23 b (51)	36 b (52)	10 c (76)
T6	23 b (51)	45 ab (40)	37 ab (10)
T7	31 ab (34)	49 ab (35)	13 c (68)
CK	47 a	75 a	41 a
$LSD_{0.05}$	24	33	17

[1]The cold temperature shock treatment regime utilized to produce cells in the air-dried products tested are described in Table 2.

FOOD SAFETY, TOXICOLOGY AND UTILIZATION OF MYCOTOXIN-CONTAMINATED GRAIN

Chairperson: Stephen Neate

RAPD ANALYSIS OF *FUSARIUM GRAMINEARUM* ISOLATES FROM ELECTRON BEAM IRRADIATED BARLEY

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ABSTRACT

Barley is affected by Fusarium head blight. The mycotoxins produced by *F. graminearum* affect the grain safety, malting and beer quality. Barley can be irradiated with electron beam radiation to reduce the fungal contamination, but there may be some *Fusarium* strains which can survive, grow and produce mycotoxins as irradiated barley is malted. Random amplified polymorphic DNA (RAPD) analysis was done to screen for mutations in *Fusarium* isolates from irradiated barley. FHB-infected and control barley samples, which were irradiated using electron beam radiation at doses of 0, 2, 4, 6, 8 and 10 KGy were obtained. *Fusarium* infection (FI) analysis was done on 100 barley seeds which were aseptically placed on half strength potato dextrose agar (HPDA) plates incubated at 25°C for 5 days. Single germinated spores of *Fusarium* isolates confirmed by FI analysis were transferred to carnation leaf agar and incubated at 25°C for 7 days. The fungal growth on the plates was identified to the species level microscopically based on morphological characteristics. The morphology, pigment and growth of these fungal colonies were confirmed by growing conidia of *Fusarium* isolates on HPDA plates which were incubated at 25°C for 7 days. Nine *F. graminearum* isolates were used for RAPD analysis to screen for mutations by comparing with a control *F. graminearum* isolate (FRC R-9821) and these isolates and reference strain (NRRL 6574) were grown on 25 g of autoclaved rice (40% moisture content) at 25°C for 14 days and analyzed for their ability to produce deoxynivalenol and related mycotoxins by high pressure liquid chromatography. The cluster analysis of the various *F. graminearum* isolates' RAPD profiles have confirmed that they can be divided into two groups. Group A consists of the reference strain (FRC R-9821) and four isolates obtained from control and irradiated (2KGy) barley. Group B consists of four isolates which were obtained from barley irradiated to 2, 4, 6 and 10 KGy and one control strains. Deoxynivalenol (DON) produced by both control and irradiated isolates ranged from none to 31.1 µg and 15-Acetyl deoxynivalenol (15-ADON) produced ranged from none to 105.34 µg. Some of the isolates obtained from irradiated barley retained their DON producing ability to some extent (ranged from 0.65-4.76 µg) but produced no ADON. The results from mycotoxin analysis indicate that irradiation may decrease mycotoxigenesis in *F. graminearum.* Further research has to be done to know whether irradiated barley can be used in malting industry, as irradiation is found to reduce the mycotoxigenecity of *F. graminearum* isolates obtained from irradiated barley.

BEAUVERICIN AND ENNIATINS IN FUSARIUM HEAD BLIGHT GRAINS

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ABSTRACT

Epidemics of Fusarium head blight (FHB) on cereal grains caused by several species of *Fusarium*, mainly reported as a complex, are becoming more frequent around the world. The disease reduces grain yield and quality, often causing grain to be unsuitable for human consumption, because some toxigenic *Fusarium* strains are capable to accumulate mycotoxins in cereal grains and derived foods and feeds. The pathogens mainly reported as causal FHB agents are strains of *F. graminearum* deoxynivalenol (DON) producer, but especially in cooler regions of Europe, other species of *Fusarium*, such as *F. culmorum*, (also a DON producer) and *F. avenaceum* (a moniliformin producer), can predominate. At this regard, recent investigations reported an increasing occurrence of strains of *F. avenaceum*, and its strictly related species *F. arthrosporioides*, as well as of *F. poae*, *F. sporotrichioides* and *F. tricinctum*, all producers of the esadepsipeptides beauvericin (BEA) and enniatins (ENs). The natural occurrence of high amounts of beauvericin (up to 3.5 mg/kg) and enniatins (up to 18.3 mg/kg of enniatin B) in FHB small grains, together with the relevant phytotoxic and zootoxic properties, suggest an examination of the potential role of these *Fusarium* metabolites in contributing to the severity of FHB and the toxicity of cereal grains.

Keywords: Fusarium Head Blight (FHB), Esadepsipeptides, Enniatins, Beauvericin, Mycotoxins, *Fusarium avenaceum*.

Fusarium Head Blight (FHB) of cereal grains - Fusarium head blight (FHB) of wheat and other small cereals is a severe disease world wide, causing reduction in crop yield often estimated up to 30 percent. In addition certain *Fusarium* strains are also capable of producing mycotoxins which can be formed already in infected plants standing in the field and then accumulate in stored grains under favourable fungal growth conditions. The occurrence of mycotoxins in cereal grains is of great concern, because their presence in foods and feeds is often associated with chronic or acute mycotoxicoses in livestock and, to a lesser extent, also in human. The *Fusarium* species predominantly reported around the world as causal FHB agent is *F. graminearum*, and the toxicological connected greatest problem is related essentially with the occurrence of deoxynivalenol or vomitoxin (DON). However, especially in cold northern European regions, other species of *Fusarium*, such as *F. culmorum* (also a DON producer), and *F. avenaceum* are important FHB agents (Bottalico, 1998).

Many strains of *F. avenaceum* from FHB small grains were found to produce several cyclic esadepsipeptides, including enniatins (ENs) and beauvericin (BEA) (Logrieco *et al*., 2002b; Morrison *et al*., 2002). In addition, some other *Fusarium* species from FHB grains, such as *F. poae*, *F. arthrosporioides* (*F. avenaceum*), *F. tricinctum* and *F. sporotrichioides* were proven to have the same relevance as esadepsipeptide producers (Nicholson *et al*., 2004). On the other hand, there are increasing evidences of the occurrence of high amounts of ENs and BEA in Finnish scab small grains (wheat, rye, oats and barley) (Logrieco *et al*., 2002b; Jestoi *et al*., 2004).

Enniatins were known for a long time as phytotoxins and associated with plant diseases characterized by wilt and necrosis (Gäumann *et al*., 1960), and some phytotoxic properties were recently reported also for BEA (Sagakuchi *et al*., 2000). Moreover, due to their ionophoric structure, BEA and ENs are able to exhibit many toxic effects on animal systems, starting from

the alteration of the ion transport across membranes, which may lead to the disruption of the cationic selectivity of cell wall, and ultimately to induce DNA fragmentation and cell death by apoptosis (Logrieco *et al*., 2002a; Macchia *et al*., 2002). These basic mechanisms, represent the behaviour leading to a large array of toxic ability, such as antimicrobial, insecticidal; and a strong cytotoxic activity on several cell lines of invertebrate, rodents, farm animals, and human (Ganassi *et al*., 2002; Calò *et al*., 2003, 2004; Fornelli *et al*., 2004). These findings on toxic potential of BEA and ENs in plant and animal systems, obviously stimulate us to a deeper examination of the significance of such esadepsipeptides and their producing *Fusarium* species in contributing to the FHB severity and grains toxicity.

Natural occurrence of Beauvericin and Enniatins - Beauvericin (BEA) is the main natural component of the beauvericin group, belonging to ENs family (Hamill *et al*., 1969). Besides BEA, five other compounds were purified from fumgal cultures, and designated as beauvericin A, C, D, E and F (Gupta *et al*., 1995; Fukuda *et al*., 2004), but they are not yet found as natural contaminants. The most important ENs, reported as natural contaminants, include: Enniatin A (ENA), Enniatin A1 (ENA1); Enniatin B (ENB); and, Enniatin B1 (ENB1) (Savard and Blackwell, 1994). Three new enniatins of the B series, designated as B_2, B_3 and B_4 were characterized from liquid culture of *F. acuminatum* and *F. compactum* by Visconti *et al*. (1992), but as far as we are aware they were not yet found as natural contaminants.

BEA was found in Finnish and Norwegian wheat and other small cereal grains mainly referred to the colonization of *F. avenaceum* (Logrieco *et al*., 2002b), but also to those of *F. poae* and *F. sporotrichiodes* (Yli-Mattila *et al*., 2004a; Jestoi *et al*., 2004; Uhlig and Ivanova, 2004). In particular, BEA was found in all samples of Finnish rye (up to 3.5 mg/kg) (Logrieco *et al*., 2002b); in Norwegian samples of wheat, barley and oats (up to 0.12 mg/kg) (Uhlig *et al*., 2004); in Finnish samples of wheat, barley and oats (up to 0.019 mg/kg), and at trace levels in Italian and Finnish samples of grain-based products (Jestoi *et al*., 2004). The natural occurrence of ENs in grains has been investigated less extensively than BEA. In fact, ENs have been found only in a few investigations in samples of small cereal grains and grain-based products from Finland and Norway (Logrieco *et al*., 2002b; Jestoi *et al*., 2004; Uhlig and Ivanova, 2004). Interestingly, ENs were reported at ppm levels in wheat (ENB, ENB1), rye (ENA1, ENB, ENB1), and barley (ENA1, ENB, ENB1), with concentrations of ENB as high as 18.3 and 9.76 mg/kg (ppm) in Finnish wheat and barley, respectively (Logrieco *et al*., 2002b; Uhlig and Ivanova, 2004; Jestoi *et al* , 2004). In particular, very high amounts of ENA (up to 6.9 mg/kg), ENB (up to 4.8 mg/kg); and ENB1 (up to 1.9 mg/kg) were found in Finnish rye samples by Logrieco *et al*. (2002b); whereas Uhlig and Ivanova (2004) found relevant amounts of ENs type B in Norwegian wheat, oats, and barley, but only trace levels of ENs type A.

Potential toxic role of BEA and ENs in FHB severity and toxicity of small cereals - The *Fusarium* species-complex causing FHB in northern Europe, depending on year crop season and cereal host, seems to be predominantly composed by *F. avenaceum*, *F. poae*, *F. arthrosporioides*, *F. sporotrichiodes* and *F. tricinctum* (Jestoi *et al*., 2004; Nicholson *et al*., 2004; Yli-Mattila *et al*., 2004b). As a consequence, the greatest mycotoxicological problem derived from FHB appears to be associated mainly with the occurrence of BEA and ENs and, at a lesser extent, with zearalenones and trichothecenes which are produced by *F. gaminearum*, *F. culmorum*, *F. poae* and *F. sporotrichiodes*. Both BEA and ENs exhibited phytotoxic and zootoxic activities in many bioassays, therefore, like for other mycotoxins formed in plant hosts, it could be suggested a possible role of BEA and ENs in plant pathogenesis and in grain toxicity.

Regarding the phytotoxic activities of ENs, it was reported that these metabolites are toxic in several plant systems, including: loss of turgor, leaf yellowing and marginal necrosis in tomato cuttings (Gäumann *et al*., 1960); inhibition of growth of wheat seedlings (Burmeister and Plattner, 1987); necrotic lesions on leaves (Hershenhorn *et al*., 1992) and on potato tu-

ber slices (Herrmann *et al*., 1996b); and inhibition of germination of a parasitic weed (Zonno and Vurro, 1999). Information on the toxicity of BEA against plant systems is limited to the induction of premature death of melon and tomato protoplasts (Sagakuchi *et al*., 2000; Paciolla *et al*., 2004).

In analogy with the role of DON in FHB severity, also for ENs was proposed a role during the plant infection process by *Fusarium* species synthesizing enniatins. To this regard, there are evidences that the virulence of modified strains of *F. avenaceum* was significantly reduced after disruption of the *esyn1* gene, which encodes for the multifunctional enzyme enniatin synthetase involved in enniatin biosynthesis (Herrmann *et al*., 1996a)

It appears that ENs exhibit more phytotoxic capability than BEA, which instead appears endowed with stronger zootoxic properties.

It was well established that the toxicity of BEA and ENs derives from their peculiar ionophoric property, and their capability to cause DNA fragmentation and cell death by apoptosis (Logrieco *et al*., 2002a). Consequently, the high cytotoxicity, especially for BEA, was confirmed in several cell line bioassays, including invertebrate, insects, rodents, livestock and human cell lines, and the ability of these metabolites to induce apoptosis is currently used as positive control in many physiopathological investigations. But, the data obtained on the biological activity of BEA and ENs in the few *in vivo* studies indicated a general low activity at the concentrations tested. In fact, besides a relatively low acute toxicity observed by intraperitoneal administration of ENs on mouse (McKee *et al*., 1997), no adverse effects were observed on growth and health parameters in several feeding trials on broiler and turkey (Leitgeb *et al*., 2000; Zollitsch *et al*., 2003).

The high contamination levels of the Finnish cereal grains, both for BEA (up to 3.5 mg/kg) (Logrieco *et al*., 2002b) and ENs (up to 18.3 and 9 mg/kg of ENA in wheat and barley, respectively) (Uhlig *et al*., 2004; Jestoi *et al*., 2004), besides the high toxigenic potential of FHB causing strains, suggest a deeper investigation on the chronic toxicity of these esadepsipeptides, particularly for ENB. Strains causing FHB were capable of producing in culture amounts of BEA (especially *F. poae*) and total ENs (especially *F. arthrosporioides*) up to 130 and 3000 mg/kg, respectively (Jestoi M., personal communication). In addition, the possible toxic interactions or synergistic effects of ENs and BEA with mycotoxins co-occurring in infected grains (especially MON, DON and NIV) (Golinski *et al*., 1997; Yli-Mattila *et al*., 2004a) should be deeper explored.

Finally, the phylogenetic and toxigenic relationships among the new entities within the monophyletic group *F. avenaceum*/*F. arthrosporioides*/*F. tricinctum*, which appear to play an important pathogenic and toxigenic role in FHB of small cereal grain of northern European countries (Yli-Matila *et al*., 2004a) should be better assessed. To this purpose it seems now possible the use of several molecular markers (Nicholson *et al*., 2004; Yli-Mattila *et al*., 2004b) to separate such strictly related molecular and morphological species even directly from the infected spikes, and then to evaluate the possibility to reconsider the taxonomic importance of *F. arthrosporioides* Sherb.

In conclusion, the widespread occurrence of *F. avenaceum* as the predominant agent of FHB of small cereal grains, together with its ability to produce high amounts of BEA and ENs which can occur at high levels in naturally infected grains, warrant more accurate investigations to establish the role of these *Fusarium* metabolites in the severity of FHB and the toxicity of scabby grains.

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HIGH-SPEED OPTICAL SORTING OF SOFT RED WINTER WHEAT FOR REMOVAL OF FUSARIUM-DAMAGED KERNELS

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ABSTRACT

Our previous work has examined the accuracy of a semi-automated wheat scab inspection system that is based on near-infrared (NIR) reflectance (1000 to 1700 nm) of individual kernels. Classification analysis has involved the application of various statistical classification techniques, including linear discriminant analysis (LDA), soft independent modeling of class analogy (SIMCA), partial least squares (PLS) regression, and non-parametric (*k*-nearest-neighbor) classification. Recent research has focused on the determination of the most suitable visible or near-infrared wavelengths that could be used in high-speed sorting for removal of FHB-infected soft red winter wheat kernels. Current technology in high-speed sorters limits the number of spectral wavelengths (regions) of the detectors to no more than two. Hence, the critical aspect of this study has been the search for the single wavelengths and best two-wavelength combinations that maximize class separation, using LDA. Four thousand eight hundred kernels from 100 commercial varieties, equally divided between normal and scab-damaged categories, were individually scanned in the extended visible (410-865 nm) and near-infrared (1031-1674 nm) regions. Single- and all combinations of two-wavelength LDA models were developed and characterized through cross-validation by the average correctness of classification percentages. Short visible (~420 nm) and moderate near-infrared (1450-1500 nm) wavelengths produced the highest single-term classification accuracies (at approximately 77% and 83%, respectively). The best two-term models occurred near the wavelengths of 500 and 550 nm for the visible region alone (94% accuracy), 1152 and 1248 nm for the near-infrared region alone (97%), and 750 and 1476 nm for the hybrid region (86%). These wavelengths are, therefore, considered of importance in the design of monochromatic and bichromatic high-speed sorters for scab-damage reduction. Ongoing research is presently examining the efficiency of high-speed sorting for Fusarium-damaged kernels, as measured by reduction in DON concentration. Approximately 40 5-kg commercial samples of soft red winter wheat have undergone as many as three successive sorts, using a commercial sorter outfitted with filters at 675 and 1470 nm. Results indicate a significant reduction in DON is achieved through sorting; however, this comes at the expense of false positives (good kernels diverted to reject stream) and the overall reduction in material available for processing.

INVESTIGATION OF FUSARIUM MYCOTOXINS IN UK WHEAT PRODUCTION

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OBJECTIVES

Determine the effects of agronomic factors on *Fusarium* mycotoxin levels in UK wheat grain over a five year period (2001 – 2005).

INTRODUCTION

Fusarium mycotoxins are produced on cereal grains by a wide range of *Fusarium* species. Many mycotoxins are produced in culture but the main ones found in cereals are the trichothecenes (which include deoxynivalenol (DON, also known as vomitoxin), nivalenol (NIV), HT2 and T2), zearalenone (ZEAR) and fumonisins. The *Fusarium* mycotoxins are produced predominantly in the field but levels can increase if stored under adverse conditions. On cereals the predominant mycotoxins worldwide are DON and zearalenone. Fumonisins are only usually found on maize.

The European Commission is currently considering maximum levels of *Fusarium* mycotoxins for unprocessed cereals and cereal foodstuffs intended for human consumption. Legislation for DON, zearalenone, HT2+T2 and fumonisins should be introduced within the next two to three years. Previous one year surveys of *Fusarium* mycotoxins have shown that levels are generally low within the UK (Turner et al., 1999; Prickett et al., 2000). However, it is not known how the levels of mycotoxins in wheat vary over different seasons. It is also not known how agronomic factors may affect mycotoxin levels in UK wheat.

MATERIALS AND METHODS

Three hundred grain samples are collected at each harvest. An equal number of samples were requested from each region: South, East, Central, West, North of England, Scotland and Northern Ireland.

An even number of samples were requested from each of the following categories:

1. Organic production
2. Conventional production with no head spray
3. Conventional production with straight strobilurin head spray
4. Conventional production with strobilurin /triazole mixture head spray
5. Conventional production with straight triazole head spray

Samples were analysed by RHM Technology, High Wycombe, UK by GC-MS analysis for ten trichothecenes and by the Central Science Laboratory, York, UK by HPLC analysis for zearalenone. The trichothecenes analysed were deoxynivalenol (DON), nivalenol (NIV), 3-acetylDON, 15-acetylDON, fusarenone X, T2 toxin, HT2 toxin, diacetoxyscirpenol (DAS), neosolaniol and T2 triol.

RESULTS AND DISCUSSION

Incidence of *Fusarium* mycotoxins in UK wheat in the first three years of the project, 2001-2003, was generally low with only four mycotoxins detected in more than 5% of samples tested. The five most dominant mycotoxins found are detailed in Tables 1, 2 and 3. Data for HT2 and T2 were combined as T2 is rapidly metabolised into HT2. HT2 was the major component of this combined data. It should be noted these results are from selected samples and not a strati-

fied survey so the average values obtained may not accurately represent the true UK averages.

The incidence and concentration of *Fusarium* mycotoxins in UK wheat were similar in each year tested. The most noticeable difference in the three years was the higher incidence of HT2+T2 in 2003 (Table 1, 2 and 3). The vast majority of samples were well below the current EU proposed maximum limit for DON in unprocessed wheat (1250 ppb) The concentration of DON found in UK wheat from 2001 to 2003 was generally low (average was 140 ppb) compared to levels found in other European countries and elsewhere in the world (Anon, 2001).

Preliminary statistical analysis of the combined data from 2001 to 2003 has shown a number of agronomic factors can affect DON levels in wheat grain. Other factors may be determined to have an effect once data from all five years have been analysed. The results to date indicate:

·Region where wheat is grown was a major factor and this can change with year. The South and East had higher levels of DON than the rest of the country.

·Maize as the previous crop increased the risk of higher DON levels.

·Minimum cultivation increased the risk of higher DON levels if following a cereal, in particular maize.

·The Fusarium head blight resistance in winter wheat varieties reduced DON levels in harvested grain.

·There was no measurable effect of fungicides used at current rates on DON levels and there was no difference between wheat samples from conventional and organic farms.

Visual assessments, using *Fusarium* damaged grain counts, were poor and inconsistent indicators of trichothecene levels in UK wheat.

Analysis of all 1500 samples over five years will provide a clear picture of *Fusarium* mycotoxin levels in UK wheat over a range of different seasons and will allow powerful statistical analysis of all agronomic factors. Results will aid the cereal industry to prepare for EU legislation on the maximum permissible levels of *Fusarium* mycotoxins in cereal grains and products. Results will also be used to advise growers of "Good Agricultural Practice" to minimise *Fusarium* mycotoxin levels in UK wheat production.

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Table 1. Mycotoxin content of UK wheat from the 2001 harvest (283 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90th%	95th%	Max
DON	80	80	32	133	223	5175
NIV	80	34	23	71	97	428
HT2+T2	30	<20	<20	22	32	214
ZEAR	4.9	<5	<5	6	9	188

Table 2. Mycotoxin content of UK wheat from the 2002 harvest (343 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90th%	95th%	Max
DON	78	116	30	211	470	3065
NIV	55	21	11	46	68	430
HT2+T2	16	<20	<20	<20	22	75
ZEAR	17	10.6	<5	19	38	707

Table 3. Mycotoxin content of UK wheat from the 2003 harvest (328 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90th%	95th%	Max
DON	89	218	38	346	594	10626
NIV	82	34	22	77	106	237
HT2+T2	69	22	18	44	55	199
ZEAR	13	7	<5	14	28	209

Means are based on an imputation of 1.67 (0.83 for zearalenone) for all samples below the limit of quantification (10 ppb; 5 ppb for zearalenone).

DOES BIOCHEMICAL COMPOSITION OF DURUM WHEAT KERNELS INFLUENCE THE TRICHOTHECENES B CONTAMINATION LEVELS?

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ABSTRACT

Commercial durum wheat varieties do not significantly differ for their level of resistance to Fusarium Head Blight (FBH). However, this does not exclude different sensitivities to trichothecenes B (TCTB) accumulation. In addition to FBH resistance, several mechanisms may influence the TCTB content of kernel such as degradation/conjugation of TCTB or occurrence of compounds inhibiting the toxinogenesis.

The aim of the present work was to investigate the eventual occurrence of different sensitivities to TCTB contamination among a collection of durum wheat lines derived from crosses involving *dicoccoïdes*, *dicoccum* or *polonicum* accessions with high yielding durum varieties and to identify the factors involved in this variability. Fifteen cultivars were inoculated at anthesis by a Nivalenol (NIV) producing *Fusarium* strain. Ergosterol and TCTB amounts were quantified on the harvested kernels. The TCTB contamination rates (measured as TCTB/ergosterol ratios) showed great variations depending on the considered genotype. Some lines appeared therefore as able to limit TCTB biosynthesis.

Two *Fusarium culmorum* strains, a Deoxynivalenol (DON) producing strain and a NIV one, were inoculated *in vitro* on kernels, bran and semolina from the Nefer durum wheat variety. If semolina resulted to be an excellent substrate for TCTB biosynthesis, bran induced a strong decrease in both DON and NIV yields compared to whole kernels. Moreover, in 30 days liquid cultures of both DON and NIV producing strains supplemented with 0.05g.l^{-1} of bran, the TCTB amounts were 10 fold lower than in the control flasks, meanwhile the fungal growth was similar in the different conditions. These results allowed us to conclude for the occurrence of some biochemical compounds inhibiting the TCTB biosynthesis in brans of Nefer durum wheat.

In accordance with the literature data and the biochemical composition of bran fractions, these inhibitors could be phenolic compounds, and more precisely some acid phenols. Thus, ten benzoic and cinnamic acids including ferulic and *p*-coumaric acids which are predominant in wheat bran, were added to liquid cultures of a DON producing strain at concentrations which do not affect fungal growth. Whatever the considered phenolic acid, higher TCTB amounts were obtained in the supplemented media. Therefore, in our experimental conditions, phenolic acids were shown to activate TCTB production. Moreover, this efficiency of activation appeared to strongly depend upon the antioxydant potential of the phenolic acid, the most antioxydant leading to the highest TCTB amount.

Our studies demonstrated that the choice of durum wheat genotype may influence the TCTB accumulation level of the yielded kernels. The different sensitivities to TCTB contamination may be ascribed to the biochemical composition of kernels. Brans were shown to contain TCTB biosynthesis inhibitors. Phenolic acids appeared

as not involved in this inhibition. Further studies are carried out in order to purify and identify the compounds of durum wheat bran inhibiting toxinogenesis.

|Abbreviations used: TCTB: trichothecene B; DON: deoxynivalenol; NIV: nivalenol

SIMPLE AND RAPID IMMUNOQUANTIFICATION OF FUSARIUM IN BARLEY AND ITS RELATIONSHIP WITH DON AND FHB SCORES

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ABSTRACT

A simple and accurate method of quantifying the presence of *Fusarium* graminearum limits our ability to understand disease epidemiology, develop control measures, or improve resistance via breeding or biotechnology. Breeding based upon visual scores of head blight incidence have greater heritability than selecting low deoxynivalenol (DON), but visual scoring is not easily standardized. Head blight scores (mean r = 0.21, std. dev. = 0.24) and DON (mean r = 0.46, std. dev. = 0.22) were poorly correlated among locations in the 2003 NABSEN field trials. Both visual scoring and DON analysis are time consuming, making neither amenable to multiple analysis for inter- or intra-experiment quality control assessments. Thus a better method of *Fusarium* quantification is needed to measure head blight that can be used as quality assurance methods for end users.

An ELISA test for *Fusarium* in small grains and corn was developed. Experimental error associated with visual scoring, DON analysis, and ELISA evaluation of *Fusarium* presence was evaluated in three replicated field experiments grown in Osnabrock, Langdon, and Casselton, ND. Each were visually scored for FHB, analyzed for DON by GC-EC, and analyzed for *F. graminearum* by species-specific indirect ELISA. Coefficients of variation and a correlation matrix were calculated for each response variable. We obtained 89 samples from grain elevators in North Dakota and correlated FHB, DON, and ELISA data with one another. In another study, we developed a method to sequentially extract DON and *Fusarium* antigens to quantify both within individual seeds, thus eliminating variability to sampling.

Mean coefficients of variability for the ELISA values were lower than FHB or DON in the field studies. Mean correlation coefficients among the field experiments were greater for ELISA vs. DON than for ELISA vs. FHB or DON vs. FHB. Similarly, correlations coefficients between ELISA and DON were greater than ELISA vs. FHB or DON vs. FHB for the grain elevator samples. Analysis of individual seeds for DON and ELISA did not improve goodness of fit among samples. Thus, we grew 3 isolates of *Fusarium graminearum* in 4 different media with 10 replications. Mycelial growth was less on Shenk Hildebrandt medium than other media, but other media did not differ from one another. *Fusarium* antigen per g of mycelium was not different among media or isolates. DON was highly variable within each medium, ranging from 0 to 26 ppm. Subsequent to these studies we have analyzed as many as 1000 barley samples (in duplicate) in one day using ELISA with an R^2 = 0.97 for the repeated measures. Consistency, speed, and ease of analyses make ELISA a superior method for quantifying *F. graminearum*.

DETOXIFICATION OF ZEARALENONE BY GENETICALLY MODIFIED ORGANISMS

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ABSTRACT

Zearalenone (ZEN) is a nonsteroidal estrogenic mycotoxin produced by numerous Fusarium species in pre- or post-harvest cereal grains, and causes severe reproductive problems on livestock. Previously, we isolated a ZEN-detoxifying gene, zhd101, from a fungus Clonostachys rosea (Takahashi-Ando et al., 2002). To reduce the mycotoxin-contamination level in food and feed, this gene is expected to be useful in establishing a reliable detoxification system with genetically modified organisms.

We constructed a codon-optimized zhd101 gene for expression in yeast (Takahashi-Ando et al., in press), which was recently reported to be applicable as a live vehicle for transgenic detoxification of toxic substances (Blanquet et al., 2003). When the transgenic yeast cells were added to the medium containing 2 µg/ml of ZEN, this mycotoxin was completely eliminated within 48h of incubation at 28°C. We also generated several transgenic rice and maize using an egfp::zhd101 fusion gene. Preliminary experiments with transgenic rice demonstrated in vivo and in vitro detoxification of ZEN by calluses (Takahashi-Ando et al., 2004) and leaf extracts, respectively. Transgenic maize also showed ZEN detoxification activity. The ZEN-detoxification activity of these transgenic cereal plants will be evaluated by an artificial inoculation assay using mycotoxigenic Fusarium graminearum.

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MYCOTOXINS AND CYTOTOXICITY OF FINNISH *FUSARIUM*-STRAINS GROWN ON RICE CULTURES

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OBJECTIVES

To screen the mycotoxins produced by Finnish *Fusarium*-strains in pure cultures grown on rice at three different environmental conditions, and to test the cultures for cytotoxicity in a bioassay using porcine kidney –cells.

INTRODUCTION

Several species of *Fusarium* are capable of producing a range of mycotoxins. Generally, the production of mycotoxins is a very complex and diverse process, the details of which have not been totally understood, although several affecting factors have been recognized (Hesseltine, 1976).

The type and amount of mycotoxin produced is determined mainly by the fungus, substrate and environmental conditions (Lacey, 1986). However, the different strains of the same species of fungi may differ remarkably in their toxin production capability due to the genetic differences. This fact may partly, in addition to the environmental conditions applied, explain the differences in the reported cases of *Fusarium*-species to produce specific mycotoxins.

Attention must be paid to the true identification of the species as well as to the metabolites produced. In fact, the current view is that the metabolite profile of a strain is regarded as an important parameter in the systemic taxonomics of a fungus supporting the genetic and morphological studies, thereby preventing possible misidentifications. The modern chemical techniques, e.g. mass spectrometry, enable the reliable determination of fungal metabolites providing more detailed data on the capabilities of the species to produce specified mycotoxins. In addition to the chemical analyses, *in vitro* –studies with cell cultures may provide complementary data on the biological properties of mycotoxins.

An identification and understanding of the factors that affect the mycotoxin production of fungi is crucial to the success of preventive actions to minimize the exposure of humans and animals to these toxic compounds.

MATERIALS AND METHODS

Fifteen different *Fusarium*-strains (Table 1.) were isolated from Finnish raw cereal samples harvested in 2001-2002 and identified by morphology and species-specific primers (Jestoi et al., 2004a). Polished rice (100 g) was autoclaved with 25 ml or 150 ml of deionized water (corresponding to a_w-values 0.973 and 0.997, respectively) and inoculated with 10 ml of spore suspension obtained from pure cultures on potato dextrose agar (PDA) –plates. The rice cultures were incubated (a_w 0.997 at 15°C and 25°C; a_w 0.973 at 25°C) for four weeks. After incubation, the cultures were air-dried and ground with a laboratory-mill.

The air-dried cultures were analysed for trichothecenes (deoxynivalenol – DON, fusarenon X – FX, 3-acetyldeoxynivalenol – 3-AcDON, diacetoxyscirpenol – DAS, nivalenol – NIV, HT-2 –toxin and T-2 –toxin), fusaproliferin (FUS), beauvericin (BEA), enniatins (ENN A, ENN A1, ENN B, ENN B1), moniliformin (MON) and zearalenone (ZEN) (Jestoi et al., 2004a,b; Eskola et al., 2001) using gas chromatography–mass

spectrometry (GC-MS), liquid chromatography–tandem mass spectrometry (LC–MS/MS) or high-performance liquid chromatography (HPLC) combined with fluorescence detection.

Porcine kidney (PK15) –cells were exposed to rice culture extracts and their cytotoxicity was assessed using the alamarBlue™ -assay (Nakayama, et al., 1997). In the assay, the metabolic activity of the exposed cells results in a chemical reduction of the colour reagent (resazurin à resorufin) and the absorbance of the affected cells is inversely proportional to their viability. Thus the absorbance can be used as an index of cell viability (O'Brien et al., 2000). PK15 -cells were exposed for 24 hours to filtered rice culture extracts (84 % acetonitrile in water), corresponding to 1 g of culture, and cytotoxicity was calculated relativ to the solvent control.

RESULTS AND DISCUSSION

All tested Finnish *Fusarium*-strains produced mycotoxins on rice media at the investigated culture conditions (Table 1.). BEA was produced by both strains of *F. poae* and *F. sporotrichioides*. Interestingly, *F. avenaceum/F. arthrosporioides*-, *F. tricinctum*- and *F. langsethiae* -strains did not produce BEA. Especially type-B ENNs were produced by one strain of *F. poae* and all strains of *F. avenaceum*/*F. arthrosporioides* and *F. tricinctum*. MON was produced by all *F. avenaceum/F. arthrosporioides*- and *F. tricinctum*-strains.

DON and 3AcDON were produced by *F. culmorum* and *F. graminearum*. FX was produced by only one *F. poae* –strain (p53). High concentrations of DAS were produced by *F. langsethiae*, but also in one culture of *F. poae* (p53) and in two cultures of *F. sporotrichioides* remarkable amounts of DAS could be detected. NIV was produced by all *F. poae*-, *F. sporotrichioides*- and *F. langsethiae* -strains. *F. culmorum* and *F. graminearum* produced only small amounts of NIV, suggesting that Finnish strains belong to chemotype IA, producing particularly DON and 3AcDON. HT-2 and T-2 were produced mainly by *F. sporotrichioides* and *F. langsethiae*. Significant production of ZEN was observed in the cultures of *F. culmorum* and *F. graminearum*. Smaller amounts of ZEN were also detected in one *F. tricinctum*- and *F. langsethiae* -cultures. FUS was not produced by any of the Finnish *Fusarium*-strains tested.

The capabilities of the Finnish *Fusarium*-strains to produce mycotoxins were generally in accordance with the available literature (e.g. Bottalico, 1997). Traces of specified mycotoxins were, however, detected in several cultures (Table 1.). These findings may be due to the contamination of the rice matrix used, rather than the toxins being produced by these strains, as the levels detected were clearly lower than the levels measured in cultures of recognised producers. Nevertheless, it is possible that very small amounts of mycotoxins can be produced also by other species than those earlier reported.

Environmental conditions had a tremendous effect on the production of some mycotoxins on the Finnish strains examined, as for some mycotoxins only minor changes in the production rates could be observed (Table 1.). For instance, the mycotoxin production of *F. poae* p57 was mainly favoured by high temperature and high water activity (a_w). *F. culmorum* p241, instead, produced higher amounts of mycotoxins at lower a_w or temperature (Figure 1.). Based on the data collected, it can be concluded that environmental (stress) factors may affect the nature and the amount of mycotoxins produced. This conclusion is in line with other published studies (e.g. Fanelli et al., 2003). To better understand the influence of different temperature/water activity-combinations on Finnish *Fusarium*-strains more studies are needed.

The metabolite profiles of *F. poae* p57 and *F. culmorum* p241 at three different environmental conditions, y-axis: mycotoxin produced (log µg/kg), x-axis: determined mycotoxins.

The cytotoxicity of Finnish *Fusarium*-strains grown on rice cultures was significantly ($p<0.05$) correlated (Spearman Rank Correlation) to the total mycotoxin concentrations determined (Figure 2.). In terms of single compounds, only DAS, HT-2 (main producers *F. sporotrichioides* and *F. langsethiae*) and NIV (main producer *F. poae*) correlated with

the cytotoxicity observed, this being in line with the findings of earlier studies (Visconti et al., 1992; Morrison et al., 2002a). Although *F. culmorum* was reported to be more toxic than *F. graminerum in vitro* (Morrison et al., 2002b), we could not confirm that observation. However, more data on toxic metabolites produced by *Fusarium* spp. is needed before the cytotoxicity of the complex mycotoxin mixtures can be resolved (Abbas et al., 1984).

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Table 1. The capabilities of Finnish *Fusarium*- strains to produce mycotoxins on rice cultures under three different environmental conditions (aw 0.997, 25 ºC; aw 0.973, 25 ºC; aw 0.997, 15 ºC).

Strain	BEA	ENN A	ENN A1	ENN B	ENN B1	DON	FX	3AcDON	DAS	NIV	HT-2	T-2	ZEN	MON
***F.poae* p57** range (µg/kg)	+++ 90-3930	+++ 1470-11200	+++ 8200-77700	+++ 34100-235000	+++ 41400-254900	±± n.d.-<100	± n.d.-<100	n.d.	± n.d.-<100	+++ 340-2150	n.d.	± n.d.-<100	± n.d.-<100	n.d.
***F.poae* p53** range (µg/kg)	+++ 2540-131400	n.d.	n.d.	± n.d.-<100	n.d.	n.d.	++ n.d.-11700	n.d.	++ n.d.-10800	+++ 2370-35000	±± n.d.-<100	±± n.d.-<100	n.d.	n.d.
***F.culmorum* p241** range (µg/kg)	n.d.	n.d.	n.d.	n.d.	n.d.	+++ 200-118700	± n.d.-<100	+++ <100-3500	n.d.	+ n.d.-320	n.d.	+ n.d.-150	+++ 320-2618000	n.d.
***F.culmorum* p251** range (µg/kg)	± n.d.-<50	n.d.	n.d.	± n.d.-<100	n.d.	+++ 550-36500	n.d.	+++ 260-12800	± n.d.-<100	± n.d.-<100	n.d.	n.d.	+++ 262000-1726000	n.d.
***F.sporotrichioides* p139** range (µg/kg)	+++ (150-4310)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+ n.d.-240	+ n.d.-6570	+ n.d.-7900	+++ 5790-24650	+++ 124000-182000	± n.d.-<100	n.d.
***F.sporotrichioides* p46** range (µg/kg)	+++ (100-11500)	± n.d.-<50	n.d.	n.d.	n.d.	n.d.	n.d.	+ n.d.-110	++ n.d.-7590	++ n.d.-1950	+++ 4660-30000	+++ 5130-219000	± n.d.-<100	n.d.
***F.graminearum* p6** range (µg/kg)	±± n.d.-150	n.d.	n.d.	n.d.	n.d.	+++ 240-26300	n.d.	+++ <100-57300	n.d.	++ n.d.-270	n.d.	± n.d.-<100	+++ 38200-174000	n.d.
***F.graminearum* p11** range (µg/kg)	± n.d.-50	n.d.	n.d.	+ n.d.-500	± n.d.-<100	+++ 220-57700	n.d.	+++ <100-12100	± n.d.-<100	+ n.d.-250	n.d.	±± n.d.-250	+++ 343000-1944000	n.d.
***F.arthrosporioides* p84** range (µg/kg)	n.d.	++ n.d.-310	+++ <50-3990	+++ 2890-226400	+++ 1340-46700	±± n.d.-<100	n.d.	n.d.	n.d.	n.d.	n.d.	± n.d.-<100	+ n.d.-340	+++ 39200-59650
***F.arthrosporioides* p75** range (µg/kg)	n.d.	+++ 2280-25100	+++ 13500-144400	+++ 183700-1900000	+++ 115400-1155000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+ n.d.-270	+++ 120-22800
***F.avenaceum* p191** range (µg/kg)	n.d.	+ n.d.-700	+ n.d.-4830	+++ 270-213400	++ n.d.-50400	± n.d.-<100	n.d.	n.d.	n.d.	n.d.	n.d.	± n.d.-<100	n.d.	+++ 250-45550
***F.avenaceum* p228** range (µg/kg)	n.d.	++ n.d.-280	++ n.d.-4970	+++ 110-130800	++ 9600-13800	n.d.	n.d.	n.d.	± n.d.-<100	n.d.	± n.d.-<100	± n.d.-<100	+ n.d.-100	+++ 3800-91650
***F.tricinctum* p105** range (µg/kg)	n.d.	+++ 60-11100	+++ 410-70600	+++ 600-724900	+++ 800-339500	+ n.d.-230	n.d.	n.d.	± n.d.-<100	n.d.	± n.d.-<100	+ n.d.-260	n.d.	++ n.d.-14700
***F.tricinctum* p113** range (µg/kg)	n.d.	+++ <50-11900	+++ 70-52200	+++ 170-227200	+++ 240-164400	± n.d.-<100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	++ n.d.-34000	+++ 310-2140
***F.langsethiae* 113** range (µg/kg)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+ n.d.-120	++ n.d.-60700	+++ 2830-4440	+++ 3770-10630	+++ <100-49000	++ n.d.-1990	n.d.

+++ = mycotoxin produced in all three environmental conditions; ++ = mycotoxin produced in two environmental conditions,
+ = abundant mycotoxin production in one environmental condition; = traces of mycotoxin detected, n.d. = mycotoxin not detected in any of the environmental conditions tested.

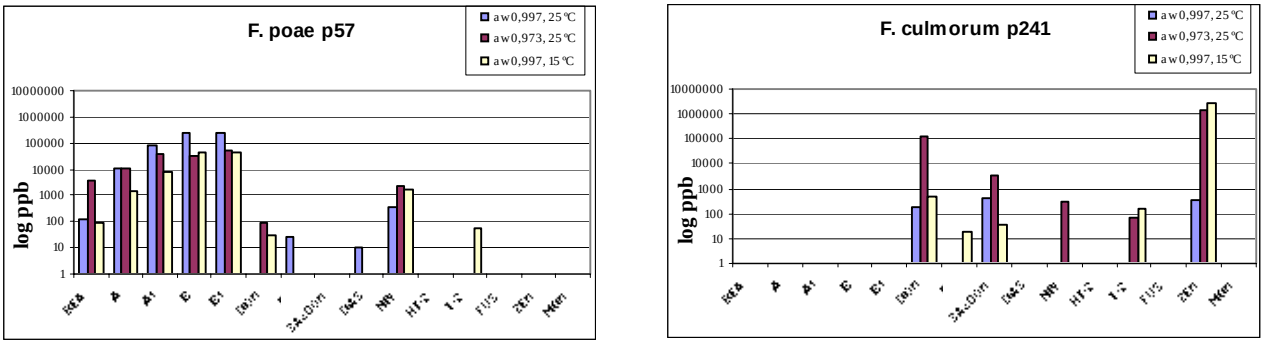

Figure 1. The metabolite profiles of *F. poae* p57 and *F. culmorum* p241 at three different environmental conditions, y-axis: mycotoxin produced (log µg/kg), x-axis: determined mycotoxins.

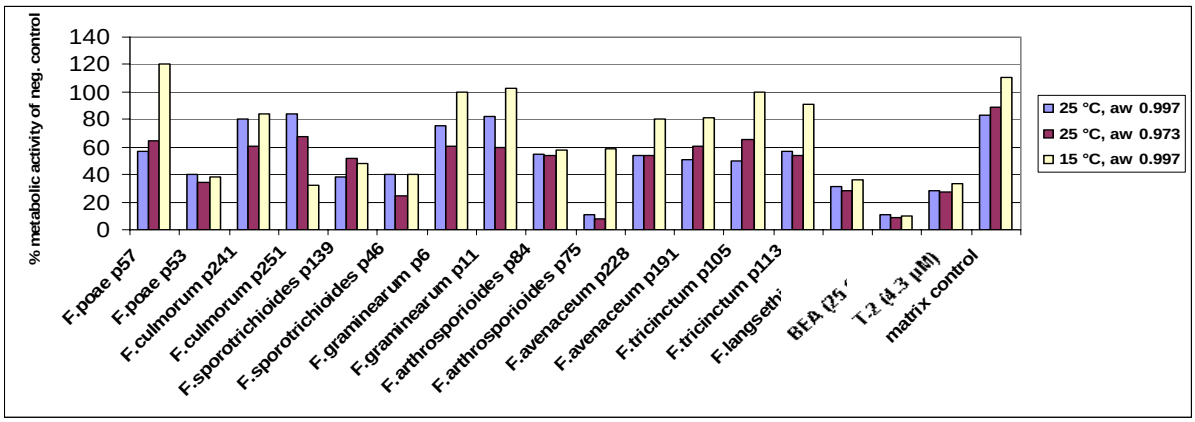

Figure 2. The cytotoxicity of Finnish *Fusarium* –strains (1 µg culture/2 × 10^4 cells) grown on rice medium at different environmental conditions. x-axis: the strains studied and BEA and T-2 standards (positive controls); y-axis the percentage of the metabolic activity of the exposed porcine kidney -cells compared with the extraction solvent (84 % acetonitrile in water).

DETECTION AND QUANTIFICATION OF *FUSARIUM* SPP. IN CEREAL SAMPLES

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ABSTRACT

To screen cereal samples for the presence of mycotoxin producing *Fusarium* spp. and to study the interaction between these fungi and their host plants, species-specific and highly sensitive detection methods are needed. In this study, species-specific PCR-primers designed for the detection of *F. poae, F. sporotrichioides* and the new species *F. langsethiae* are described. In cereal samples infections lower than 1% could be detected by this PCR assay. Four additional primers were developed to detect subgroups of *F. poae* representing the four different ITS genotypes present in *F. poae*. The primer pair developed for the detection of *F. langsethiae* could be used to distinguish between *F. langsethiae* and the morphologically similar *F. poae*. The described PCR assays are highly sensitive and enable the detection of *Fusarium* genomic DNA in concentrations as low as 5-50 fg. High levels of *F. poae* and/or *F. langsethiae* were detected in random field cereal samples but only low levels of *F. sporotrichioides* were found. To allow a quantitative detection of the species of *Fusarium* producing the mycotoxins most frequently detected in samples of small grain cereals in Norway, several TaqMan real-time PCR assays were developed. One assay allowed the detection and quantification of *F. avenaceum*. Another TaqMan assay was used for the quantification of the total amounts of *F. langsethiae* and *F. sporotrichioides*. In Norway these *Fusarium* species are the two most important producers of the highly toxic type A trichothecenes, T-2 and HT-2 toxins. To determine the total amount of trichothecene-producing *Fusarium* (both type A and type B trichothecenes) a TaqMan assay based on the *Tri5* gene of the trichothecene synthesis pathway was developed. The possible correlation between the amount of *Fusarium* DNA detected by these assays and corresponding mycotoxin content found in cereal samples will be discussed.

EVALUATION OF OZONE AND HYDROGEN PEROXIDE TREATMENTS FOR PREVENTING THE POST-HARVEST *FUSARIUM* INFECTION IN MALTING BARLEY

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ABSTRACT

Utilization of *Fusarium* infected barley for malting may lead to mycotoxin production and decreased malt quality. Methods for treatment of *Fusarium* infected barley may prevent these safety and quality defects and allow use of otherwise good quality barley. Gaseous ozone and hydrogen peroxide were evaluated for effectiveness in reducing *Fusarium* infection (FI) while maintaining germinative energy (GE) in two barley samples (sound and infected). Gaseous ozone treatments (GOT) included concentrations of 11 and 26 mg/g for 0, 15, 30, and 60 minutes. Hydrogen peroxide (HP) treatments included 0, 5, 10, and 15% concentrations with exposure times of 0, 5, 10, 15, 20, and 30 minutes. For GOT, in naturally *Fusarium*-infected barley, a statistically significant ($P<0.05$) decrease of 24-36% in FI occurred within 15 minutes of exposure at either concentration. GE was significantly ($P<0.05$) affected (11-20%) by 30 minutes at both concentrations in naturally *Fusarium* infected barley but not in sound barley. For HP, FI was significantly decreased (50-98%) within 5 minutes of exposure. With the exception of two treatments (10% and 15% HP agitated for 20 minutes) GE was not statistically significantly different from the control in naturally *Fusarium* infected barley. In sound barley, HP had no significant ($P>0.05$) effect on GE. The results suggest that GOT and HP may have potential for treatment of *Fusarium* infected malting barley.

CAFFEINE AS INTERNAL STANDARD FOR HIGH PRESSURE LIQUID CHROMATOGRAPHY ANALYSIS OF DEOXYNIVALENOL IN WHEAT SAMPLES

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ABSTRACT

A method was developed to utilize caffeine as an internal standard for analytical detection of deoxynivalenol (DON) in wheat samples using high pressure liquid chromatography (HPLC) with ultraviolet detection. Ground wheat samples (25 g) were extracted with 100 mL aqueous acetonitrile (84:16) and spiked with 30 µg caffeine (CAF) dissolved in extraction solvent. Samples were blended for 3 minutes at high speed then a 5 mL aliquot passed through an alumina-charcoal column for clean-up. The resulting extract was evaporated in a 50°C water bath under nitrogen. The residue was dissolved in aqueous methanol (20%) and microfiltered into autosampler vials. Analytes were separated with linear aqueous methanol solvent gradient (85:15 ramping to 80:20 over 20 min, flow rate 0.7 mL/min) with UV detection at 220 nm. Retention times for DON and CAF were approximately 9.0 min and 16.0 minutes, respectively. Fungal culture material (124 ppm) was added to commercial wheat flour (no detectable DON) to achieve mean DON concentrations of 0.26, 0.51, 1.99, and 5.96 ppm. Three replicates of each concentration were analyzed in one day and repeated four different days. An internal standard curve was developed plotting sample concentration against area response ratios (DON/CAF). The correlation coefficient (r) was 0.985 across days and there was no significant difference between or among days of analysis. Recovery analysis of spiked samples at 1.00 ppm was 100% in this system. This method will allow analysis of samples using an easily accessible, stable, and inexpensive compound that is not likely to be found in most food samples of interest.

POSSIBLE WAYS TO UTILIZE MYCOTOXIN CONTAMINATED GRAIN

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ABSTRACT

There is a strong tendency that for securing food and feed safety requirements, more and more countries will set up limit values for toxin contamination. Industry applied until now toxin limits. When toxin limits will be officially introduced, millions of tons of grain can be qualified as dangerous waste material. In this case the farmers will bear all risks and they should pay for everything even they are not fully responsible for the damage. The social consequences are now clear in the Red River Valley and elsewhere (McMullen 2003). For this reason economic utilization(s) of the contaminated grain must be found. Without this a well working system cannot be developed.

The food and feed industry is excluded, therefore other industrial uses remain. Now three possibilities have greater chances:

a. Direct energy production. Wheat grains have energy content comparable to middle quality coal (Ruckenbauer and Reichart 1994). When a coal price would be paid for the contaminated grain and some additional sum would be paid it would lessen the financial damage and could help farmers to survive. For this economists should count out, whether movable plants should be built and positioned into the given area for a while or existing electric plants can accept it. It should be decided whether heat or electricity is more economic. The use of straw may increase the profitability when not used otherwise.

b. Usage for gasoline production. The European Union plans that it will use the agricultural and forest waste materials for fuel production as renewable energy sources. With the present oil prices above 50 USD this is economic now. The EU will replace 30-40 % of the diesel fuel by this new resource within ten years. For this regional plants will be built with a capacity of 480 t fuel/day capacity. The first Sundiesel experimental plant is working now. It seems that oil need grows exponentially, but not the resources. The Middle East provides a number of risk factors for the world economy. The gasoline production of this art can lessen the tension.

c. Alcohol production for industrial use or as fuel. The problem is the large amount of waste material that is also toxin contaminated. Drying and burning could be a solution, the question: profitability.

d. Other industrial uses. From there is no special information, but their need is not in range of million tons, but may help.

We are convinced that the economic utilization of the toxin contaminated grain can be a good business for the farmers who cannot sell their contaminated grains now. For this reason national and international efforts are needed to find solutions that help producers and utilize products that are not marketable now. This would also stabilize commodity prices.

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THE RELATIONSHIPS BETWEEN FUSARIUM HEAD BLIGHT VISUAL SYMPTOMS, *FUSARIUM* BIOMASS AND DEOXYNIVALENOL LEVELS IN BARLEY

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ABSTRACT

For more than a decade Fusarium Head Blight (FHB) has resulted in large portions of the upper mid-west six-rowed barley crop being rejected for malting due to levels of the toxin deoxynivalenol (DON) in the grain exceeding 0.5 ppm. Currently, screening for disease resistance involves a combination of visual scoring of disease symptoms and DON analysis. The Barley Pathology and 6-rowed Barley breeding program at NDSU undertake more than 5,500 DON analyses each year and to reduce costs and time also do more than 45,000 visual scores on barley heads, yet it is not clear that visual scores are a good predictor of DON. Recently a *F. graminearum* species-specific indirect ELISA technique has been developed that can quantify fungal biomass of *Fusarium* sp.. Our objectives were twofold, 1) to investigate the relationship between FHB visual score, DON and the Fusarium ELISA, and 2) to determine the impact on total spike DON by kernels with differing visual scores. Visual scoring was done at hard dough stage of development on seven cultivars grown in different environments in North Dakota in 2003. Individual kernels with characteristic dark brown lesions occupying > 25% of the kernel were scored and determined as a percentage of the total number of kernels on the head. Heads were dissected and kernels separated into those displaying FHB symptoms on >25% of the kernel, 1-25% of the kernels, or no symptoms. Single kernels were used for ELISA and DON analysis. For ELISA, antigens were extracted from whole seed, diluted in coating buffer and coated onto microtiter plates followed by indirect ELISA using monoclonal antibodies. DON in the single kernel barley samples was determined by gas chromatography with electron capture detection. When regressions were done between FHB and DON, R^2 values differed widely between cultivars and environments, but when pooled, gave a R^2=0.39. The R^2 values for ELISA and DON also differed widely between cultivars and environments, but when pooled, gave a R^2=0.73. When separated into different symptom classes, the R^2 values for both ELISA and DON or FHB and DON were greatest for the kernels showing >25% symptoms and very low for kernels showing 1-25% or zero symptoms. Kernels showing >25% symptoms had the highest average DON levels with individual kernels up to 700 ppm. However, 70/205 of these kernels tested zero for DON. Of kernels showing 1-25% lesion coverage, 140/205 tested zero for DON and the remainder between 1-65 ppm with one kernel 488 ppm. Of asymptomatic kernels, 173/215 tested zero for DON and the remainder tested 1-4 ppm. It is clear that the kernels showing zero or 1-25% lesion coverage are making a small contribution to the DON level and that some visual symptoms are not associated with DON accumulation.

ADVERSE HEALTH EFFECTS OF TRICHOTHECENE MYCOTOXINS

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ABSTRACT

During head blight of wheat and barley, deoxynivalenol (DON or "vomitoxin") and other trichothecene mycotoxins are elaborated that can potentially cause adverse health effects in individuals who consume the infected grain. Although DON is regulated in the U.S. at 1 ppm in finished food, the European Economic Union and Codex Alimenterius have proposed much lower limits for consumption based on rodent studies. A further concern is that although agricultural workers are exposed to airborne DON during harvest, threshing and milling of infected wheat and barley, virtually nothing is known about the adverse effects of inhaling this toxin. *In vitro* studies suggest that the keys steps for DON toxicity are induction of stress signaling and cytokine expression in white blood cells which ultimately can mediate acute and chronic illness. We have used multiparameter flow cytometric analysis to measure the sensitivity of human white blood cells to induction of cytokines by DON. In assessing blood from 8 individuals, some were found to be much more sensitive to DON's effects than others. The minimal threshold for induction of IL-1 beta, IL-6 and IL-8 was 100 ng/ml. Activation of the mitogen-activated protein kinase p38 was found to precede and be a requisite for inducing IL-1 beta, IL-6 and IL-8 with the minimum threshold for activation being 25 ng/ml. These are critical observations because they suggest: (1)p38 activation is a biomarker for DON toxicity and for which humans are 4-fold more sensitive than mice, (2) higher concentrations are needed for cytokine induction and (3) some people may be resistant to DON whereas others are sensitive perhaps due to genetic and non-genetic factors (eg.prior/ ongoing infections, diet, medication). To accurately measure the hazardous potential of trichothecene to humans, it is essential to relate these in vitro studies to threshold dose, duration of exposure, exposure route, and magnitude of toxic effects in the mouse model. The resultant data can be used by the regulatory agencies and the wheat and barley industries for improved accurate, safety assessments relative to consumption of grain products and inhalation of grain dust.

REAL-TIME PCR FOR QUANTIFICATION OF TOXIGENIC *FUSARIUM* SPECIES IN BARLEY AND MALT

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ABSTRACT

Fusarium species are potential mycotoxin producers in cereals. In near future EU is going to set the maximum limit values for some *Fusarium* toxins in unprocessed cereals and cereal products. Mycotoxin analyses are expensive and time-consuming. Hence, a rapid and reliable quantification method for toxigenic *Fusarium* species is needed for evaluation of the mycotoxin risk in cereal-based industry. We have applied real-time PCR technique for the quantification of trichothecene-producing *Fusarium* species present in barley and malt samples (the TMTRI assay, S. Klemsdal unpubl. sequences). PCR results were compared to the amount of trichothecenes in the samples. Furthermore, highly toxigenic *Fusarium graminearum* was quantified in cereals by real-time PCR (the TMFg12 assay, T. Yli-Mattila unpubl. sequences). DNA was extracted from ground kernels (0.1 g) using FastDNA Spin Kit for Soil and analysed in a LightCycler® system using fluorigenic TaqMan probes. Both naturally and artificially contaminated grains were analysed. The TMTRI assay and the TMFg12 assay enabled the quantification of trichothecene-producing *Fusarium* species and *F. graminearum* present in barley and malt samples, respectively. Both TaqMan assays were regarded as sensitive and reproducible. Linearity of the assays was at least 3-4 log units when determined using pure *Fusarium* DNA. The amount of *Fusarium* DNA analysed with the TMTRI-trichothecene assay correlated with the DON content in Finnish barley samples. The TMFg12 assay for *F. graminearum* gave a good estimation about the DON content in North American barley and malt samples. The amounts of DON and *F. graminearum* in Finnish barley were found to be naturally low.

ROLE OF GLUTATHIONE IN TRICHOTHECENE TOXICITY IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

In a yeast cDNA library screen intended to identify genes mediating increased DON resistance when overexpressed, we found the yeast *CYS4* gene. *CYS4* encodes cystathionine beta-synthase, which synthesizes cystathionine, the immediate precursor of L-cysteine. Cysteine is required for the two-step synthesis of glutathione (GSH) mediated by the *GSH1* and *GSH2* gene products. Since many toxins are known to be detoxified by GSH conjugation, we speculated about a possible role of GSH in DON detoxification by yeast. Yeast has a specific GSH uptake transporter, we therefore tested whether exogenous GSH protects yeast against DON toxicity. Exogenous GSH indeed increased resistance against DON and 3-Acetyl-DON (3-ADON), but not against the closely related trichothecenes nivalenol (NIV), trichothecin (TTC) and 15-Acetyl-DON (15-ADON). Using LC-MS/MS, clear evidence for formation of a previously unknown DON-glutathione conjugate was obtained. Efforts to prepare larger amounts of this conjugate in order to characterize its chemical structure (by NMR) and biological properties (using a wheat germ *in vitro* translation assay) are currently under way. In order to find out which enzyme is responsible for conjugate formation we have performed a systematic gene knock-out approach (gene disruption of all putative yeast glutathione-S-transferases (GSTs)). We identified one gene, where inactivation leads to increased sensitivity against DON. Deleting this candidate gene also increased sensitivity against 3-ADON and CDNB, the latter is a widely used test substrate for GSTs. We have generated overexpression constructs (with and without a *c-MYC-6xHis*-tag for affinity pruification). Isolation and biochemical characterisation of the protein encoded by this candidate gene is currently under way. In an attempt to identify *Arabidopsis* GSTs capable of DON-GSH conjugate formation, several DON-induced *Arabidopsis* GST genes where tested. The candidate *Arabidopsis* cDNAs where cloned into a yeast expression vector and tested for their ability to increase DON resistance in yeast. Unfortunately this approach was so far not successful.

ACKNOWLEDGEMENT

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A NOVEL *FUSARIUM* METABOLITE WITH BIOLOGICAL ACTIVITY

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ABSTRACT

A novel *Fusarium* metabolite with a hydrocarbon-like basic structure was purified from the methanolic extract from the rice culture of a *F. avenaceum* strain, which had been isolated from Norwegian grain. The metabolite was discovered by bio-assay guided fractionation of culture extracts and was cytotoxic against the porcine kidney epithelial cell line PK-15. Other *Fusarium* spp. that were found to produce the compound in rice culture include *F. tricinctum*, *F. langsethiae* and *F. poae*. The results from the structure elucidation using NMR- and mass spectroscopy will be presented.

WORLDWIDE REGULATIONS FOR *FUSARIUM* MYCOTOXINS

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ABSTRACT

Regulations for mycotoxins have been established in food and animal feed in many countries since the late 1960s to protect the consumer from the harmful affects that mycotoxins may cause. Various factors influence the decision-making process of setting limits for mycotoxins. These include scientific factors such as data about effects on man and animals, the level of human exposure, as well as the availability of methods of sampling and analysis. Economical factors such as commercial interests and sufficiency of food supply have their impact as well. Over the last 2 decades various international inquiries on worldwide limits and regulations for mycotoxins were published. The latest completed inquiry resulted in the publication "Worldwide regulations for mycotoxins in food and feed in 2003 (FAO Food and Nutrition Paper 81, 2004). On a worldwide basis, approximately 100 countries had mycotoxin regulations or guideline limits for food and/or feed in 2003, an increase of approx. 30 % as compared to 1995. The total population in these countries represents approx. 90 % of the world's inhabitants. *Fusarium* mycotoxins for which (proposed) limits and regulations existed in 2003 include deoxynivalenol, diacetoxyscirpenol, the fumonisins B_1, B_2 and B_3, HT-2 toxin, T-2 toxin and zearalenone. Deoxynivalenol, zearalenone and fumonisins were among the most regulated. At least 41 countries have set regulatory or guideline limits for deoxynivalenol in food or feed. Whereas in 1995 this trichothecene was only sporadically regulated, it has become a toxin of high concern in monitoring programmes and amongst regulatory authorities since the late 1990's when mg/kg concentrations were reported to occur in cereals and cereal products, particularly in Europe. Tolerance levels for deoxynivalenol in wheat (flour) range from 300-2000 µg/kg, with a peak at 750 µg/kg. The latter is dominated by the countries of the EU, where this (unofficial) guideline tolerance level is applied since several years for deoxynivalenol in flour used as raw material. Zearalenone is now regulated in food and feed in 28 countries as compared to 6 in 1995. Limits for this toxin in maize and other cereals currently vary from 50 to 1000 µg/kg with more of the tolerance levels set at the higher end of this range than at the lower end. Whereas in 1995 fumonisins were only subject of regulations in one country, this number has now increased to 6, with limits for maize ranging from 1000-3000 µg/kg. Although proportionally a very significant increase, the number of fumonisins-regulating countries is too small to draw meaningful conclusions about generally agreed limits. Comparing the situations in 1995 and 2003, regulations for *Fusarium* mycotoxins have increased, and they have become more diverse and detailed with newer requirements regarding official procedures for sampling and analytical methodology. These developments reflect the general concerns that governments have regarding the potential effects of *Fusarium* mycotoxins on the health of humans and animals.

EFFECT OF HARVESTING TIME ON INCIDENCE OF *FUSARIUM* SPECIES AND ACCUMULATION OF DEOXYNIVALENOL IN KERNELS OF SILAGE CORN IN ONTARIO

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ABSTRACT

The effect of three harvesting times (75, 50, and 25% milkline) on the incidence of kernelborne *Fusarium* spp. was examined using four silage corn hybrids (Maizex Leafy 4, NK Enerfeast 1, Pioneer 37M81, and TMF94) in Ottawa, Ontario in 2001 and 2002. Eleven *Fusarium* spp. were isolated over the two years. *Fusarium subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas was the dominant species recovered from 28.8% of the kernels. Other frequently isolated species were *F. oxysporum* Schlecht. (2.6%), *F. graminearum* Schwabe (2.5%), *F. proliferatum* (Matsushima) Nirenb. (0.3%), and *F. sporotrichioides* Sherb. (0.2%). The remaining six species, *F. avenaceum* (Fr.) Sacc., *F. crookwellense* Burgess, Nelson, & Toussoun, *F. culmorum* (W.G. Sm.) Sacc., *F. equiseti* (Corda) Sacc., *F. poae* (Peck) Wollenw., and *F. solani* (Mart.) Sacc., were recovered from < 0.1% of the kernels. The incidences of *F. subglutinans* increased from 20.9 to 26.7, and to 38.7%; *F. graminearum* from 1.7 to 2.9, and to 3.1%; and, total *Fusarium* spp. from 28.7 to 32.2, and to 42.3%, when harvested at 75, 50, and 25% milkline, respectively. The incidences of the other species and deoxynivalenol (DON) levels in kernels were not affected by harvesting time. Of the four silage corn hybrids, TMF94 had significantly greater incidence of *Fusarium* spp. and DON concentration than the others, indicating a genotypic variation in resistance to kernelborne *Fusarium* spp.

REAL-TIME PCR DETECTION AND QUANTIFICATION OF *FUSARIUM POAE* AS COMPARED TO MYCOTOXIN PRODUCTION IN GRAINS IN FINLAND

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OBJECTIVES

To develop a fluorogenic (TaqMan) real-time polymerase chain reaction (PCR) -assay for species-specific detection of *F. poae* in barley, wheat and oats based on species-specific differences in intergenic spacer region (IGS) sequences.

INTRODUCTION

Species-specific primers based on IGS sequences for end-point PCR have been developed (Konstantinova and Yli-Mattila, 2004), and in this study these primers were tested together with DyNAmo SYBR Green kit (Finnzymes) and rox dye. In addition, TaqMan primers and probe were designed for the same DNA region and the correlation between *F. poae* DNA and mycotoxins in grain samples was studied.

The mycotoxins produced by *F. poae* include e.g. diacetoxyscirpenol (DAS), monoacetoxycirpenols (MAS), scirpentriol (SCR), nivalenol (NIV) and fusarenon-X (FX) (Pettersson 1991, Liu et al., 1998, Torp and Langseth 1999, Thrane et al., 2004). The reports of *F. poae* isolates to produce type-A trichothecenes (HT-2 –toxin, T-2 –toxin and neosolaniol) are contradictory (Torp and Langseth, 1999, Abramson et al., 1994, Sugiura et al., 1993). These variable findings may, however, be due to the misidentification of *F. langsethiae* isolates, which are morphologically similar to *F. poae* (Yli-Mattila et al., 2004a). According to our results (Jestoi et al., 2004) Finnish *F. poae* isolates produce beauvericin (BEA) and enniatins (ENNs) in addition to DAS, NIV and FX.

MATERIALS AND METHODS

Grain samples - The specificity of the TaqMan primers and probe were studied using DNA from eight *Fusarium* species (Yli-Mattila et al., unpublished results). Finnish grain samples (15 barley, 16 one oats and 23 wheat) harvested in 2002 and in 2003 were used for the analysis (Yli-Mattila et al., 2004b, unpublished results). Some samples were from field plots, which were artificially inoculated with the spores of different *Fusarium* species (Jestoi et al., unpublished results, Yli-Mattila et al., unpublished results).

Molecular analyses - DNA was extracted from grain samples according to the modified method of Taylor et al. (2001). The final volume of DNA extract from 10 g of grains was ca. 50 µl and the total DN concentration was usually 1-10 ng/µl. Before PC the DNA concentration was diluted to 1 ng/µl or th results obtained were divided by the total DNA concentration.

For isolation of genomic DNA, fungi were grown for 4 to 6 days at 24°C on potato dextrose agar (PDA) plates. DNA was extracted with chloroform/octanol method as described in Yli-Mattila et al. (2004a,b). In barley, DNA was also extracted from ground kernels (c20-c25 and c27-c28) using FastDNA Spin Kit for Soil (Q-BIOgene, Sarlin et al., 2004, unpublished results).

DyNAmo SYBR Green kit (Finnzymes) with rox dye was used for SYBR Green quantitative PCR in GeneAmp 5700 cycler (PE Biosystems). Amplification for quantitative PCR was performed in 96-well Optical Reaction Plates (ABgene) sealed with Optical Adhesive Covers (ABgene) in a GeneAmp5700 sequence detector. The PCR program for SYBR Green PCR consisted of 2 min at 50°C, 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 60°s at 60°C (Yli-Mattila et al., unpublished results). TaqMan amplification reactions were performed in the GeneAmp5700 sequence detector in 25 µl containing 12.5 µl Absolute qPCR Rox master mix, 100 ν M TMpoaef and TMpoaer primers, 100µM TMpoae probe and DNA sample containing 1-6 ng DNA with the standard PCR protocol. The probe was labelled at the 5´ends with TET (tetrachloro-6-carboxy-fluorescein) and at the 3´end with 3'Eclipse Dark Quencher (Yli-Mattila et al., unpublished results).

To determine the sensitivity and range of the assay, 1 ng of genomic DNA from *F. poae* strain 02-65 was serially diluted by a factor 10 and used as a template. The specificity of the primers was tested against genomic DNA of eight *Fusarium* species.

Mycological and toxin analyses - Fresh and dried grain samples (200 seeds per sample) from the years 2002-2003 were used for the analysis of *Fusarium* spp. The isolation and morphological identification of the fungi took place as described in Yli-Mattila et al. (2004b). With the samples of the year 2003 *Fusarium*-specific Peptone PCNB plates were used (Nash and Snyder Medium modified by Nelson et al 1983). Mycotoxins (deoxynivalenol, FX, DAS, 3-acetyldeoxynivalenol, NIV, HT-2, T-2, BEA, ENNs, moniliformin) were analysed as described in Jestoi et al. (2004).

Statistical analyses - R^2 (= square of regression coefficients), regression slope and P(a) (= significance of regression slope) were calculated by the program SigmaPlot 2001 version 7.1 (SPSS Inc). The original DNA and mycotoxin concentrations were transformed to logarithmic values.

RESULTS AND DISCUSSION

TaqMan reactions - The PoaeIGS/CNL12 primer pair displayed a linear range of at least two orders of magnitude from $10^{-3.5}$ to $10^{-1.5}$ µg/µ l with pure DN from *F. poae* isolates. In grain samples containing *F. poae* DNA the *F. poae* specific peak and occasionally also an unspecific peak was found in the melting curve, which made it difficult to estimate the exact amount of *F. poae* specific DNA. On gel, only one major specific PCR product (ca. 306 bp) was obtained by the primer pair in the DNA samples from *F. poae* and grain samples was found, but the unspecific products made it difficult to estimate the amount of *F. poae*-specific PCR product.

Primers and probe designed for *F. poae* successfully amplified DNA extracted from pure cultures and from grain samples. The primers and probe displayed a linear range of at least four orders of magnitude with pure DNA from *F. poae* isolates. The primers and probe amplified DNA extracted only from *F. poae* strains.

Correlation between molecular, morphological and mycotoxin results - NIV and ENNs contamination levels were generally higher in 2002 than in 2003. A significant correlation was found between *F. poae* DNA and NIV ($p<0.05$) and ENNs ($p<0.01$) levels in barley (Figure 1).

In wheat and oats the correlation was not as clear, which may be due to the lower levels of NIV and ENNs found. But also in wheat higher *F. poae* DNA levels were found in samples with high NIV levels, especially in 2002. No clear correlation was found between the levels of other mycotoxins and *F. poae* DNA (Yli-Mattila et al., 2004, unpublished results) in grain samples, although BEA levels were also somewhat higher in the few samples with high *F. poae* DNA levels in 2002.

This study is one of first reports of fluorogenic PCR detection assays for *F. poae* in grains (Waalwijk et al., 2004). Artificial infection increased *F. poae* contamination level only in barley in 2003. In 2002 there

was a strong natural infection in all artificially infected barley samples. In 2003 the highest *F. poae* contamination levels were found in oats.

SYBR Green real-time quantitative PCR was suitable for estimating the amount of *F. poae* DNA in pure cultures and it can also be used for estimating the amount of *F. poae* DNA from the melting curve obtained from grain samples. However, more manual calculations are required for the estimation and the results were not as reproducible as in Taqman real-time quantitative PCR. Also the correlation with toxin levels was not good. Due to variable amounts of unspecific PCR products in SYBR green realtime quantitative PCR, it was difficult to get comparable and reproducible results from the grain samples between separate PCR runs.

According to the results of the present study Taqman primers and probes designed are really specific for *F. poae*. With other *Fusarium* species at least one thousand times more DNA is required to get the threshold value with a cycle number below 35. Thus, the primers and probe can be used for estimating the DNA levels of *F. poae*.

For most of the samples, there was a clear correlation between *F. poae* DNA and NIV and ENNs contamination levels. For some samples, however, the determined mycotoxin contamination levels were high, even though the DNA levels were low. This may be due to a large number of small colonies or low number of bigger colonies on grains. The environmental conditions in the fields (Jestoi et al. 2004) may also have an influence on the type and amount of mycotoxin produced.

In 2002 one oats and seven barley samples having high levels of NIV also had high levels of *F. poae* DNA. In wheat both *F. poae* DNA and NIV levels were generally lower. In 2003 both NIV and *F. poae* specific DNA levels were lower in barley than in 2002.

It is possible to use the *F. poae* DNA concentration levels to eliminate the grain samples containing high amounts of NIV and ENNs in barley and oats. In the combined barley samples of 2002-2003 the average NIV level in the seven samples containing the highest levels (>2 x 10^{-3} ng/ng total DNA) of *F. poae* DNA was 4100 µg/kg, while in the rest of 29 samples the average level was 77µg/kg. In the combined oats samples of 2002-2003 the average NIV level in the five samples containing the highest levels (>10^{-4} ng/ng total DNA) of *F. poae* DNA was ca. 190 µg/kg, while in the rest of 13 samples the average level was 85 µg/kg.

Also in Sweden *F. poae* seems to be the most important NIV producer (Pettersson 1991), while strains of other *Fusarium* species, such as *F. culmorum, F. graminearum* (Bottalico et al. 2002), *F. sporotrichioides* and *F. langsethiae* (Jestoi et al., 2004) may also be able to produce NIV. The ENNs levels in the seven barley samples with the highest *F. poae* DNA (>2 x 10^{-3}ng/ng total DNA) was 10400 µg/kg, while in the rest of the 19 samples the average level was 320 µg/kg. A correlation was also found between *F. avenaceum/F. arthrosporioides* DNA and ENNs levels in barley (Paavanen-Huhtala et al., 2004, unpublished results). These results are in accordance with the results of Jestoi et al., (2004), according to which some *F. poae* strains are effective in producing NIV and ENNs.

ACKNOWLEDGEMENTS

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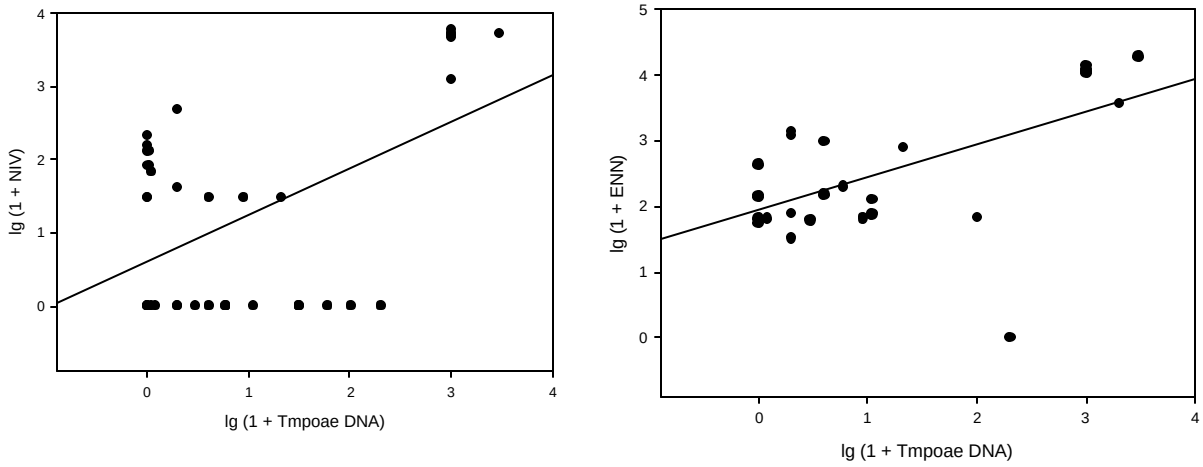

Figure 1. Correlation between *F. poae* DNA (ng/ng total DNA) and mycotoxins (µg/kg) nivalenol (left, R^2 = 0.21, P(a) = 0.043) and enniatins (right, R^2 = 0.35, P(a) = 0.0015) in Finnish barley samples harvested in 2002-2003.

PHENOTYPIC EXPRESSION BY DIFFERENT STRAINS OF *FUSARIUM GRAMINEARUM* AS AFFECTED BY SUBSTRATE AND TEMPERATURE

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ABSTRACT

We investigated the phenotypic expression of biomass, pigments and mycotoxins by *Fusarium graminearum* as affected by strain type, substrate, and temperature. Twelve isolates known to have phenotypic and genetic diversity were used for this experiment. Each isolate of *F. graminearum* was inoculated in triplicate into 25 g of 40% moisture content autoclaved white rice, hard red spring wheat, corn, and barley in half pint canning jars. Approximately 10^4 spores in 1 ml were added to each jar. The cultures were incubated at 15°C, 25°C and 35°C and 80% humidity for two weeks with 12 hr light cycle in an environmental chamber. The fifth substrate was wheat that was grown at 22-25°C with supplemental lighting in a greenhouse. The heads of Grandin wheat, a moderately susceptible hard red spring variety, were sprayed with spore suspension of *Fusarium graminearum* isolates at a concentration of 10,000 macroconidia per ml until dripping at anthesis stage. The samples were analyzed for deoxynivalenol (DON),15-acetyldeoxynivalenol (15Ac-DON), 3-acetyldeoxynivalenol (3Ac-DON), nivalenol (NIV) and zearalenone (ZEN). Ergosterol was measured as an estimate of fungal biomass. We found that all the Fusarium strains used in this experiment belonged to 15Ac-DON chemotype, and ZEN normally was produced along with B trichothecene. The mycotoxin production and the ratio among DON, 15-AcDON and ZEN varied with *Fusarium graminearum* strain type, substrate and temperature. Even though interactions among media, temperature and substrate existed, the following trends were found. Rice was the best medium for mycotoxin production, followed by corn and wheat, while barley and greenhouse wheat were the worst media for mycotoxin production. The 15°C and 25°C promoted mycotoxin production much more than 35°C did. And also visible mycelium growth and pigment production were observed in the four grain cultures incubated at 15°C and 25°C, while at 35°C, sparse white mycelium was observed in some samples of rice culture only. Ergosterol contents in samples were in agreement with the observations mentioned above. Additional detailed results and further discussion will be presented.

PATHOGENESIS, EPIDEMIOLOGY AND DISEASE FORECASTING

Chairperson: Thomas Miedaner

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.

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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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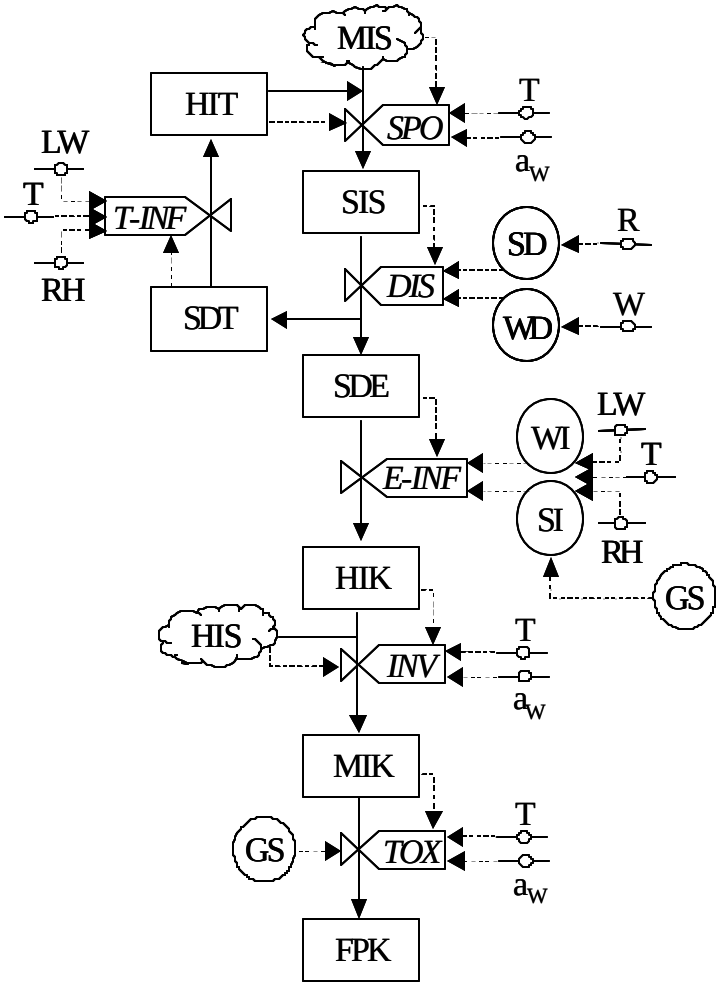

Figure 1. – Relational diagram of a dynamic model simulating the life cycle of *F. verticillioides* on maize.

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 =

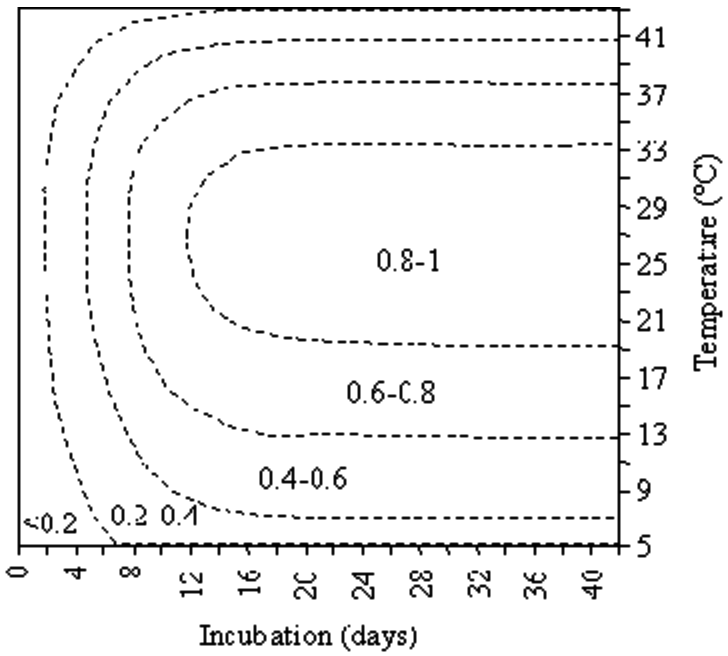

Figure 2. – Sporulation rate of *F. verticillioides* on a semolina based medium under different temperature regimes.

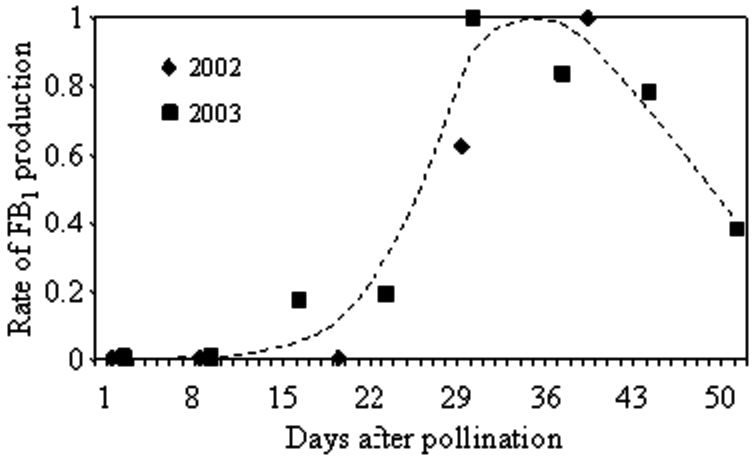

Figure 3. – Rate of fumonisin production on artificial media prepared with maize ears collected on different days after pollination.FC

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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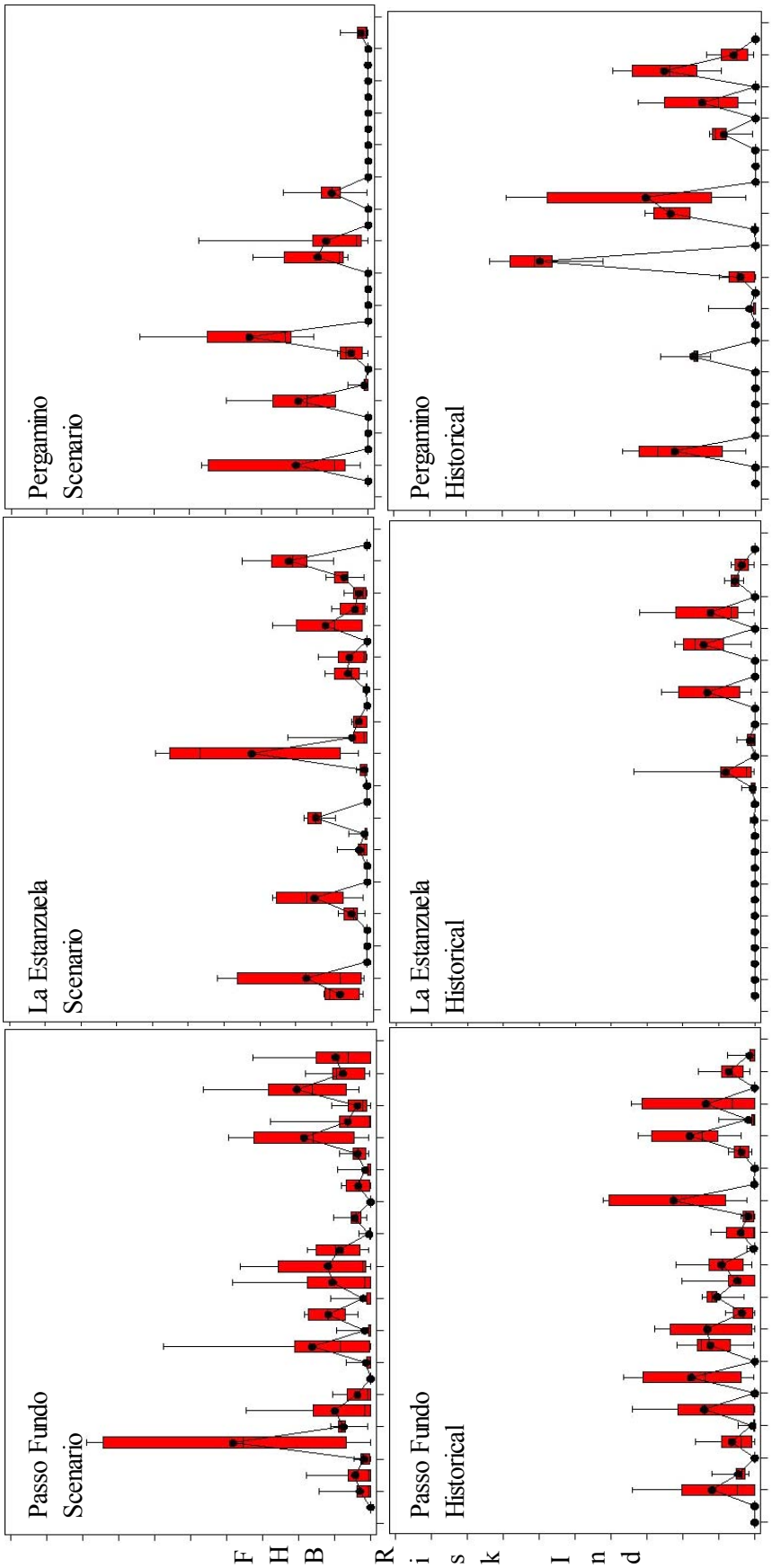

Figure 1. Simulated Fusarium Head Blight risk index under a scenario and historical weather data (1970-2000) from different locations. The shades strips from bottom to top correspond to non epidemic, light, moderate and severe, respectively.

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.

ACKNOWLEDGEMENTS

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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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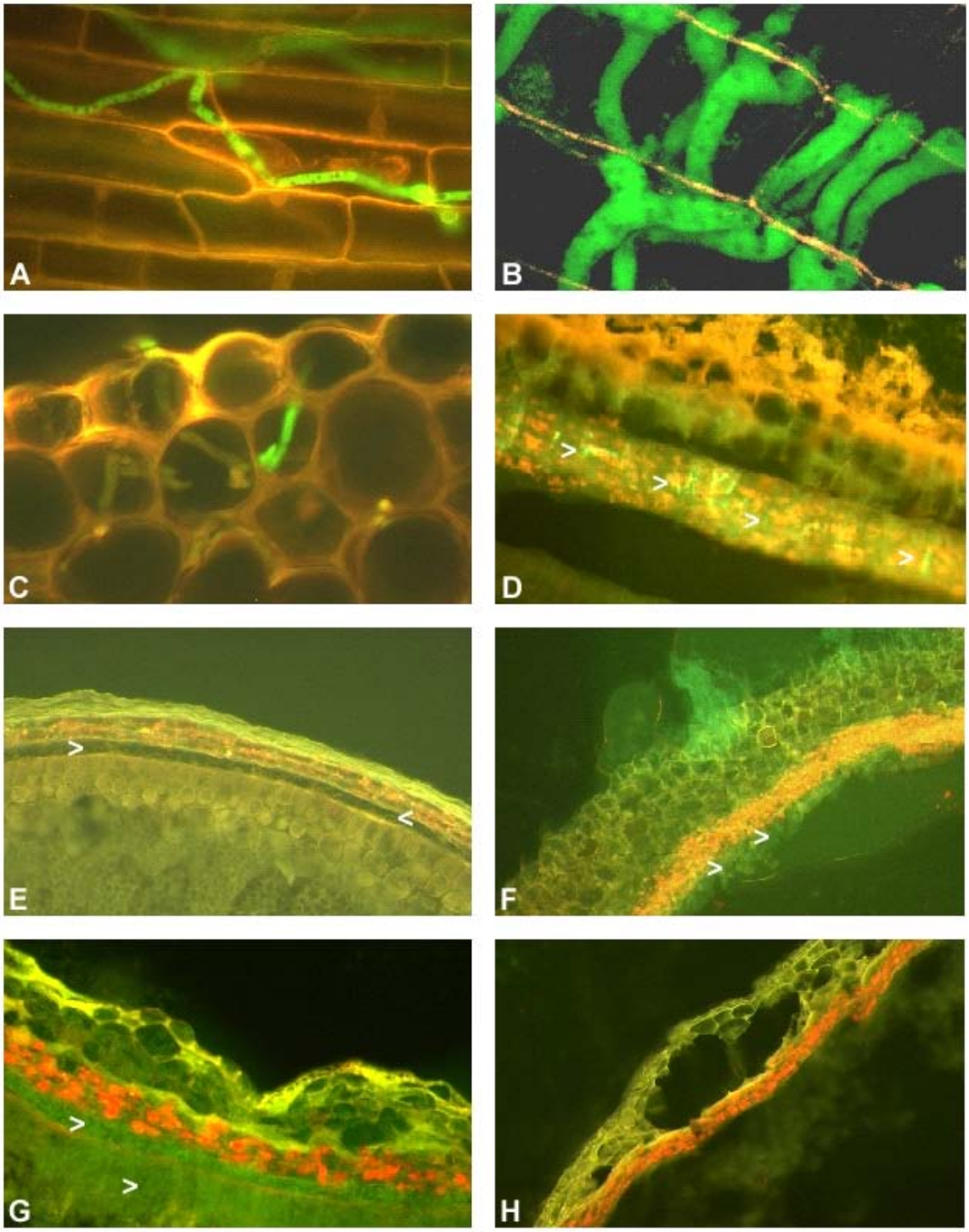

Figure 1. Microscopic pictures of barley caryopses inoculated with GFP-marked strains of *F. graminearum*. A: Fungal hypha of ΔTri5 strain in epicarp cells. B: Hyphae of WT strain passing trough cell walls of epicarp cells (A, B: cv. Golden Promise, 48 hai). C: Hyphae of ΔTri5 strain in hypodermis (cv. 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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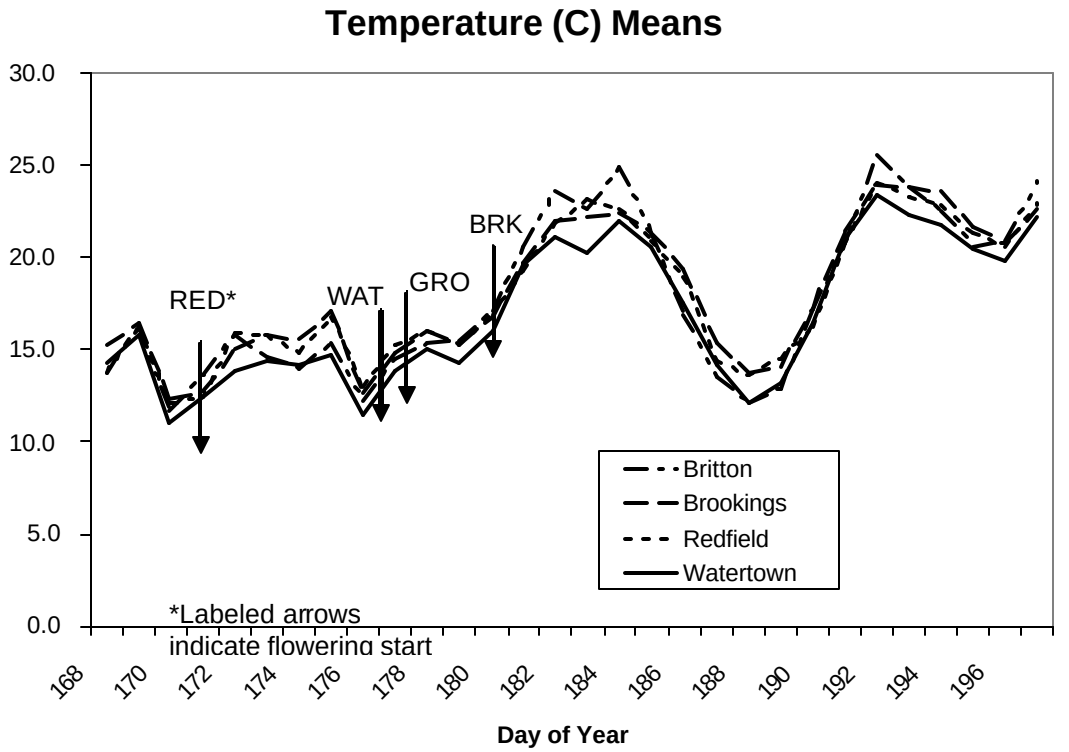
Table 1. Field Locations and Disease Data

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%



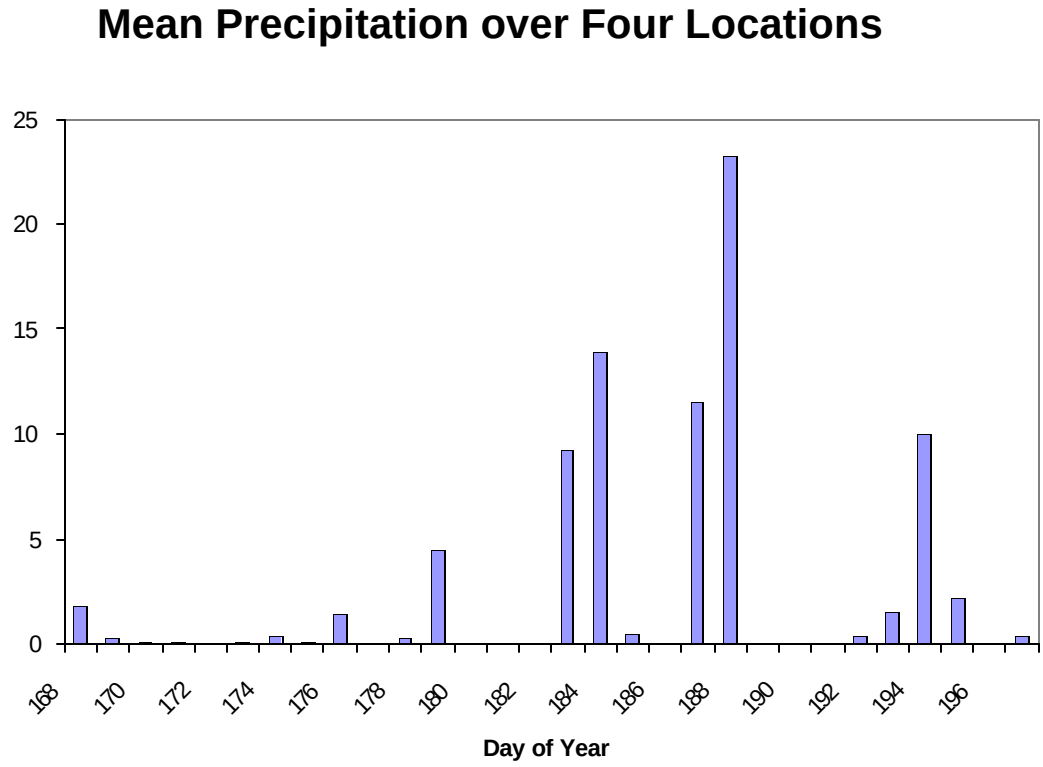


Figure 1. Temperature and Precipitation for FHB Monitoring Locations, 2004.

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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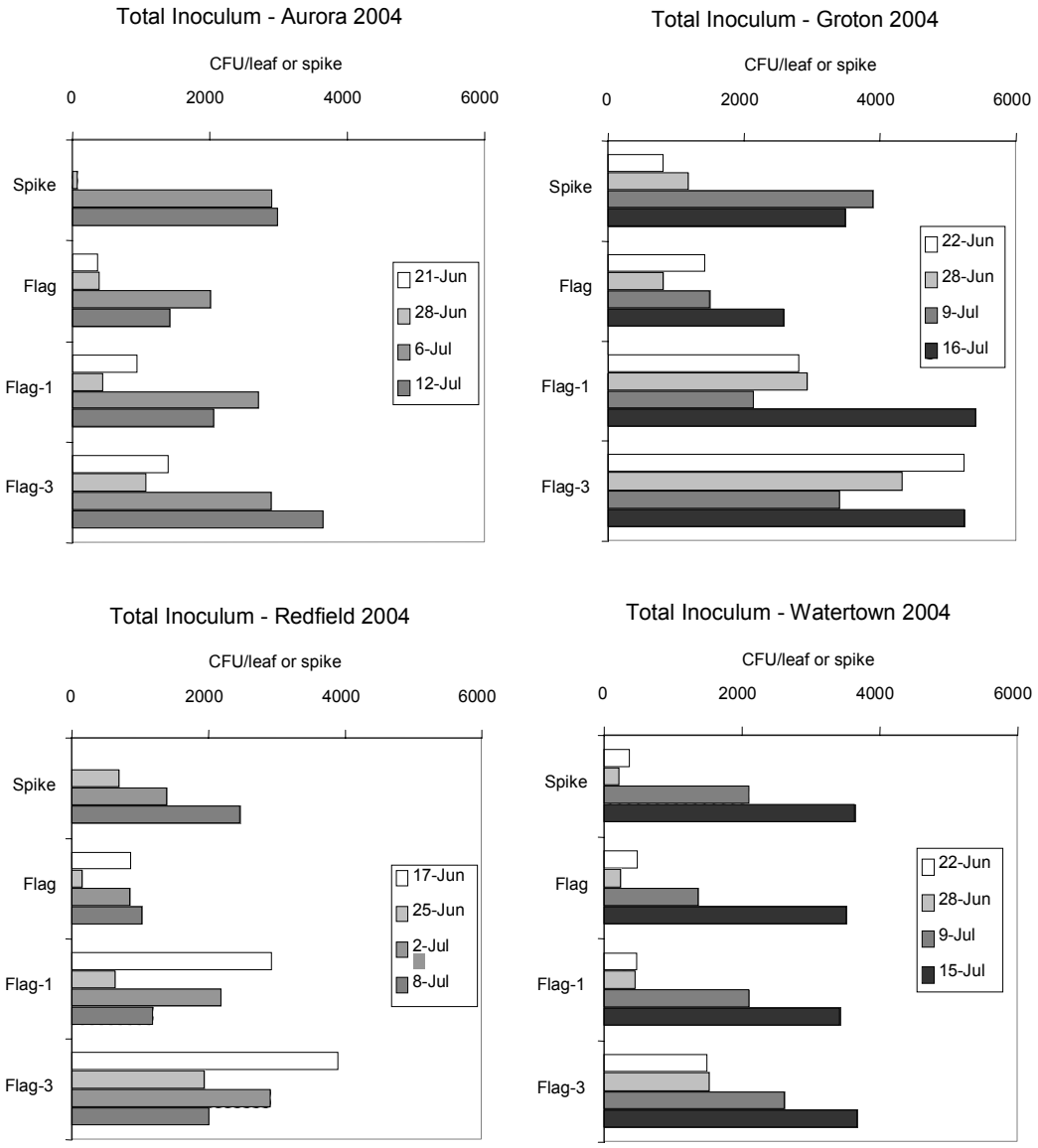

Figure 1. Inoculum estimates for four locations in 2004.
Each graph represents four sampling times, and four plant components (spike, flag leaf, flag leaf-1, and flag leaf-3 (usually the lowermost healthy leaf). Values represent all *Fusarium graminearum* type colonies on Komada's medium.

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).

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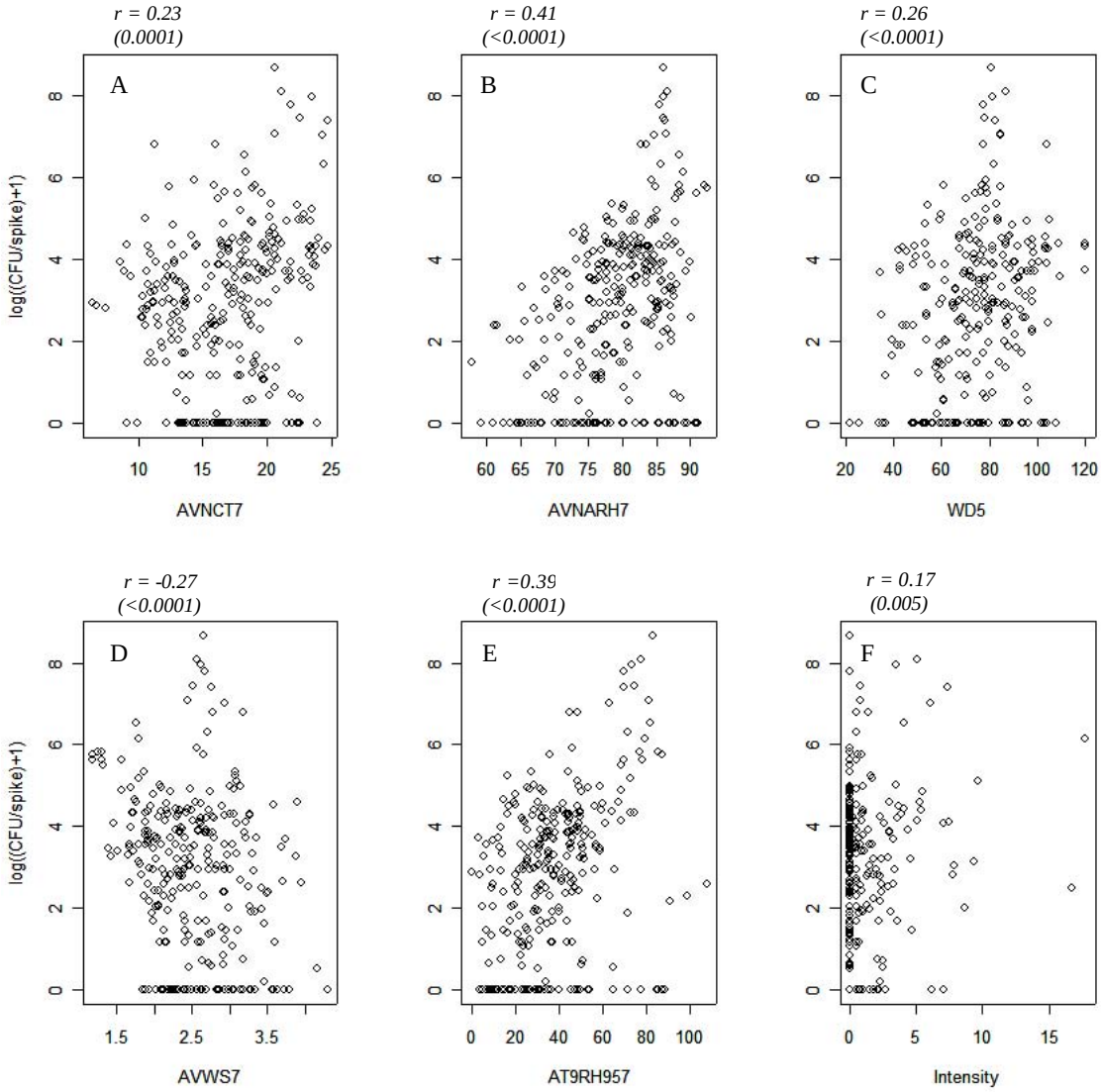

Figure 1. Scatter plots depicting the relationship between log-transformed CFU/spike and AVNCT = average nighttime wheat canopy temperature (^{o}C) (A); AVNARH = average nighttime air relative humidity (%) (B); WD = surface wetness duration (h) (C); average wind speed (m/s) (D); number of hours with 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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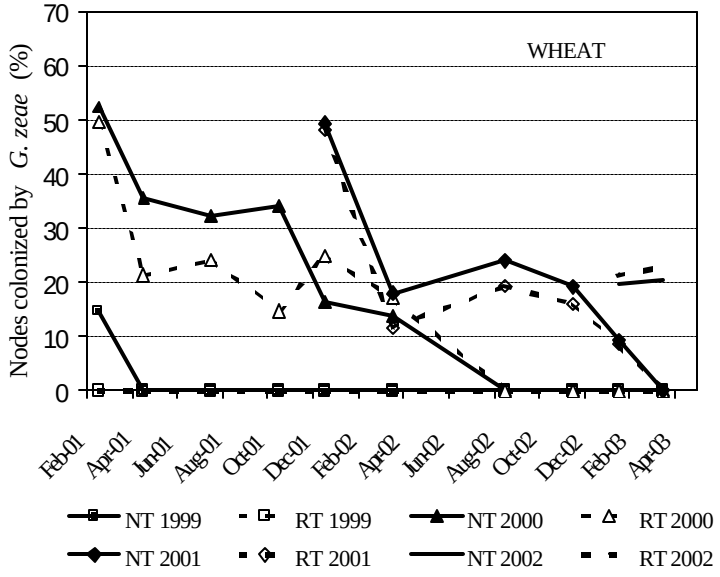

Figure 1. Percentage of wheat nodes from which *G. zeae* was recovered in different residue age and tillage systems from February 2001 to March 2003 (RT: reduced tillage; NT: no till). Values are mean percentages of all residue pieces sampled.

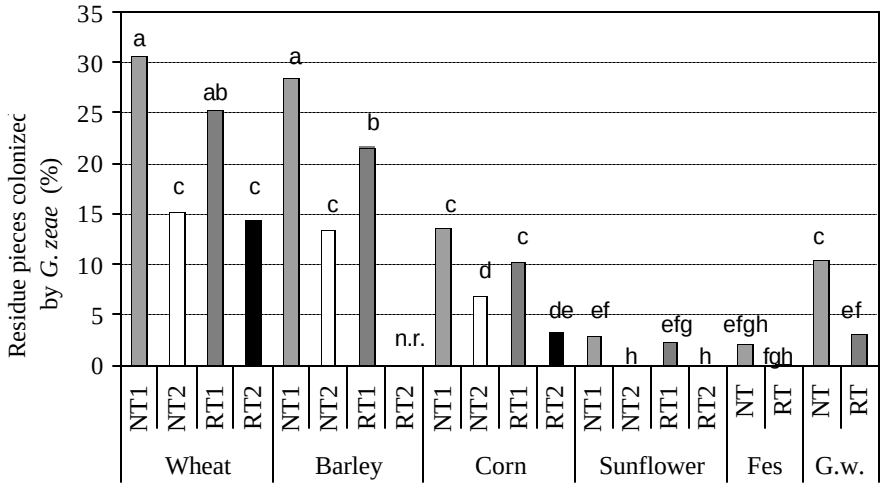

Figure 2. Mean *Gibberella zeae* colonization of different residue types, residue age, and tillage systems from February 2001 to March 2003.Values are mean percentages of all residue pieces sampled. Values with different letters are significantly different at *P=0.0001* based on likelihood ratio statistics. 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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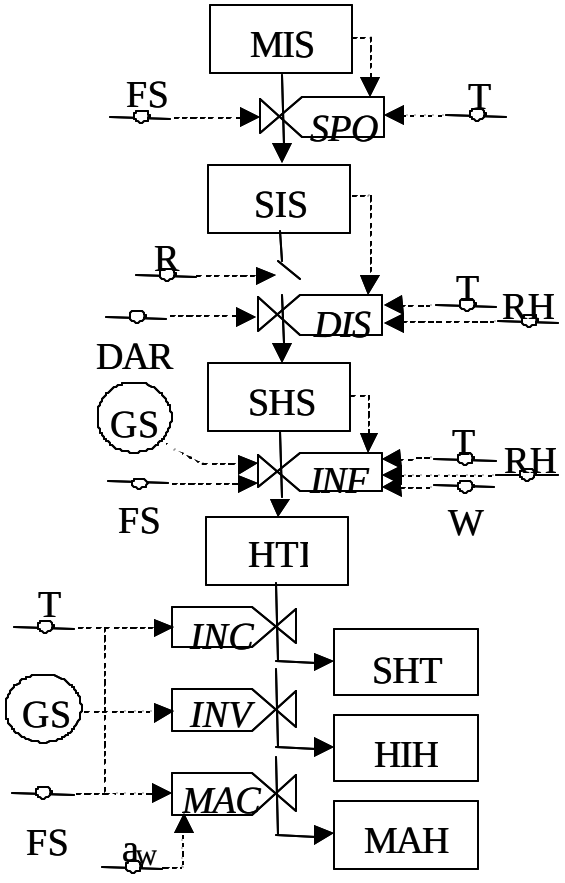

Figure 1. Relational diagram of the model.

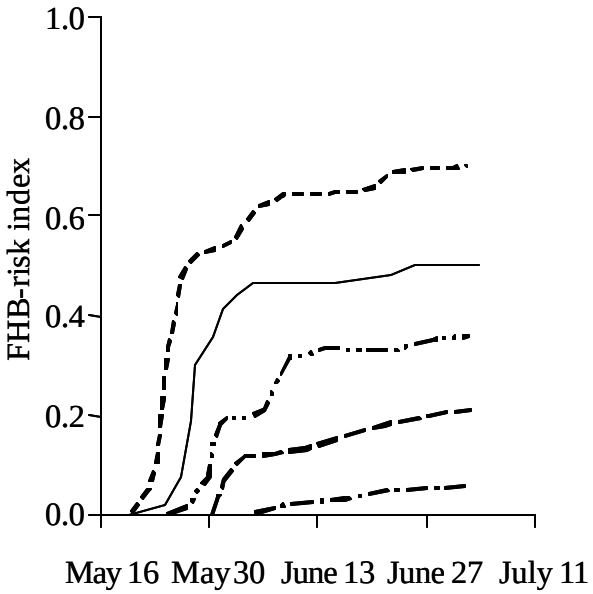

Figure 2. Dynamics of the FHB-risk index under different epidemiological conditions (Po valley, Italy, 2002).

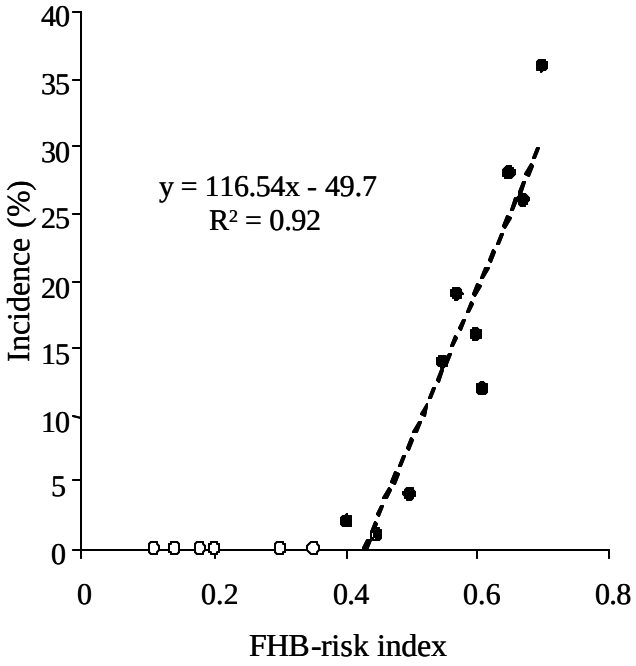

Figure 3. Relationship between the FHB-risk index for *F. graminearum* and the % of wheat kernels affected by the fungus (white points not used to calculate the regressione line).

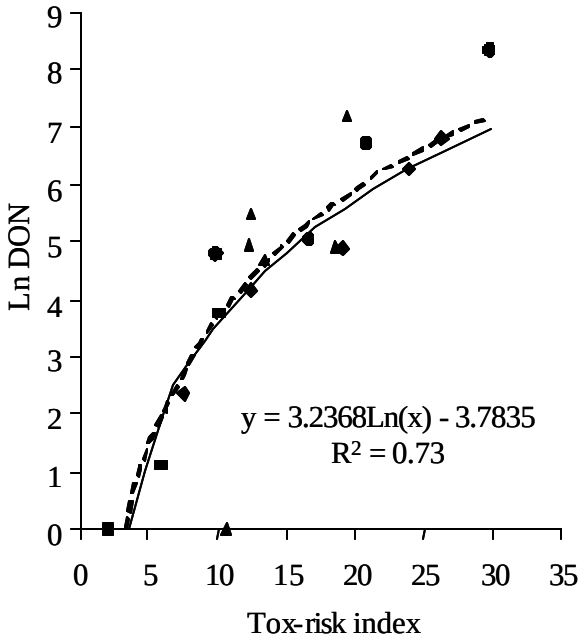

Figure 4. Relationship between the Tox-risk index for *F. graminearum* and *F. culmorum* and the content of DON in wheat kernels (points are actual data from different experiments; --- is the regression fitting these data; — is the estimate made by the model).

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

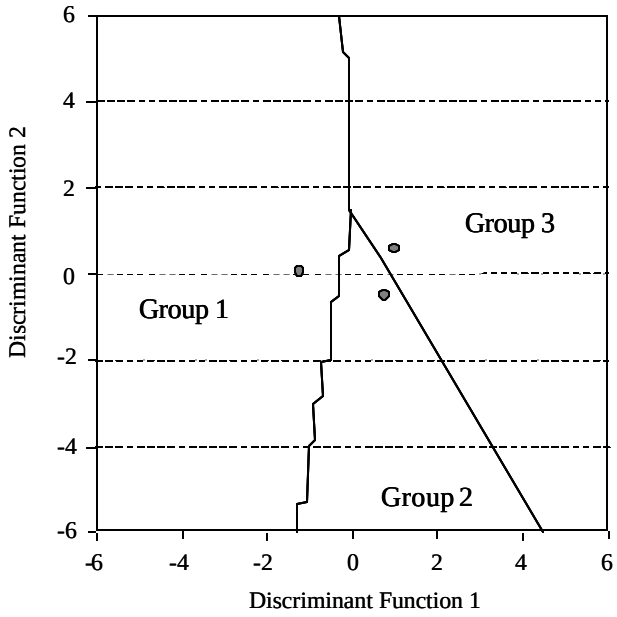

Figure 1. Territorial map resulted from the stepwise discriminate analysis: Group 1, no DON in kernels; Group 2, low DON; Group 3, high DON. Points on the map represent centroids.

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.

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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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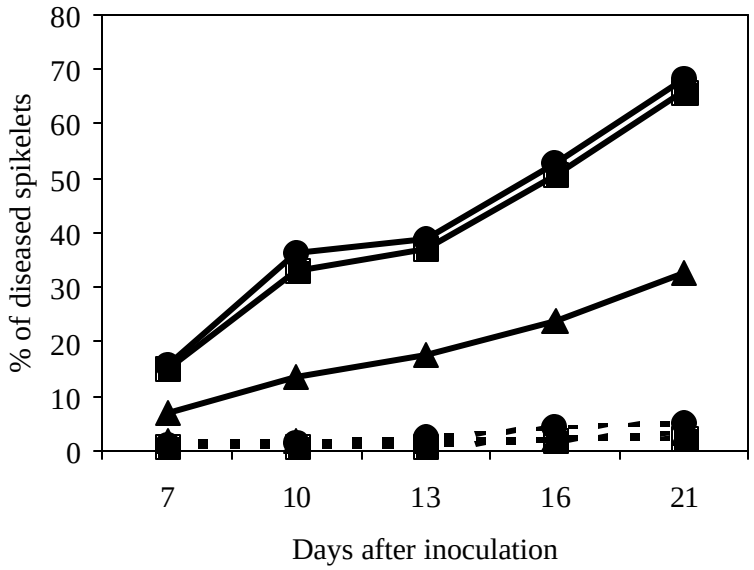

Figure 1. Wheat FHB severity depending on time post inoculation of the *F. graminearum* wild type strains (continuous lines) FG06 (?), FG25 (?), and FG2311 (¦) in comparison to respective *tri5* deletion mutants (dashed lines). Each fungus was tested at least on 20 spikes.

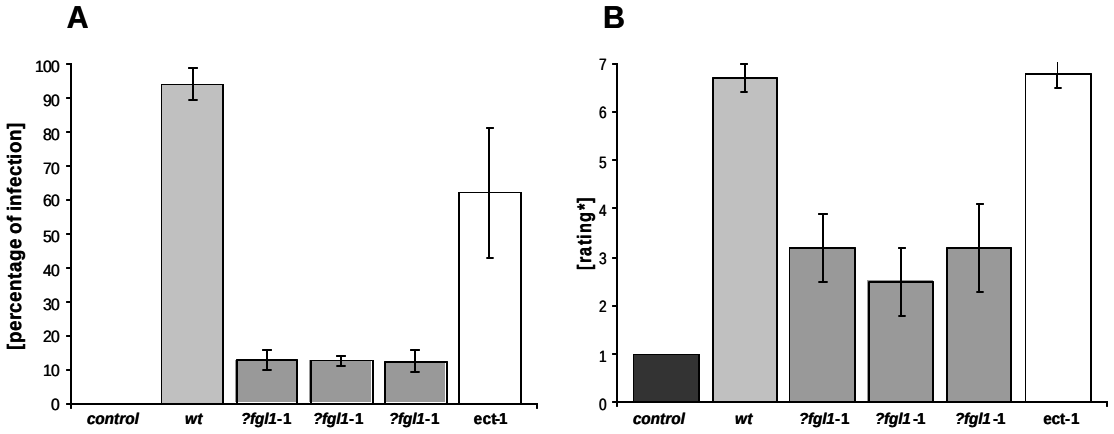

Figure 2. Infection of cereal flowers with *F. graminearum* wild type and ?fgl1strains.

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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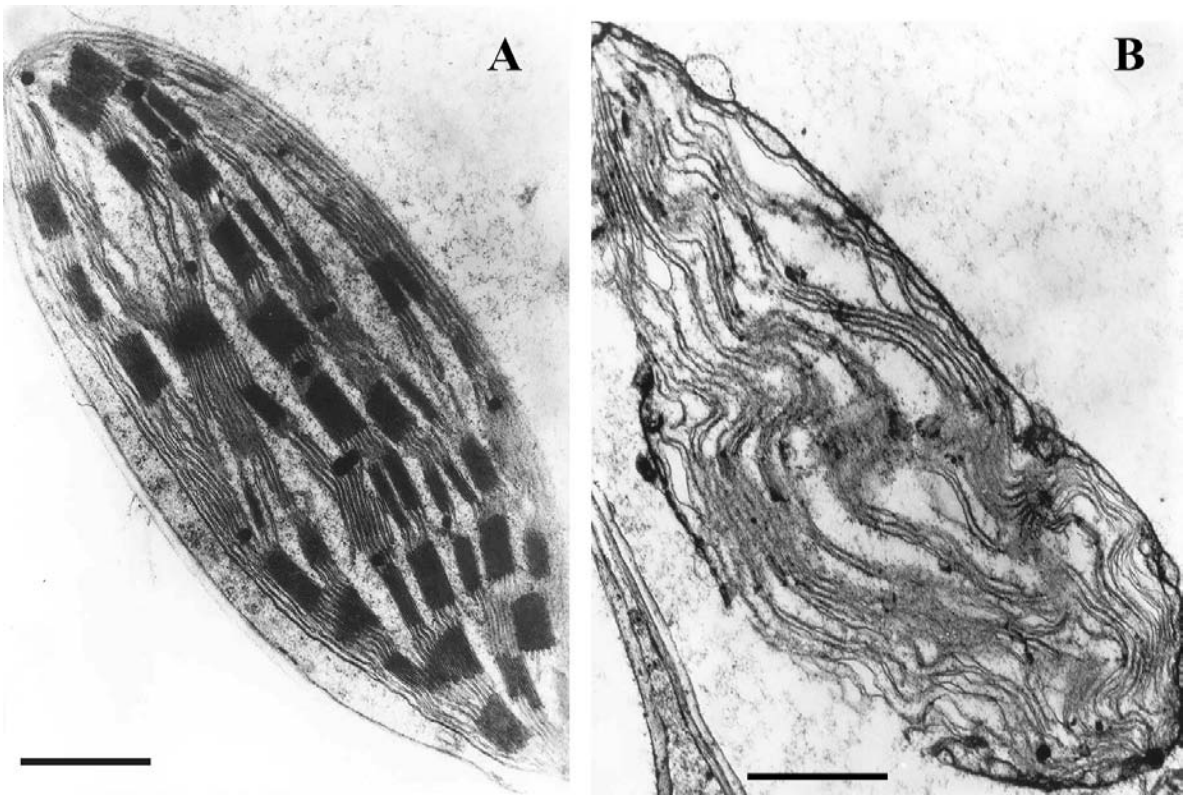

Figure 1. Well-developed inner membrane organization of chloroplasts in leaf mesophyll cells in control plants (A). In the susceptible cv. Pavla (B) treated by fusariotoxins, thylakoids are not organized in grana their arrangement is disturbed. Bars represent 1 µm.

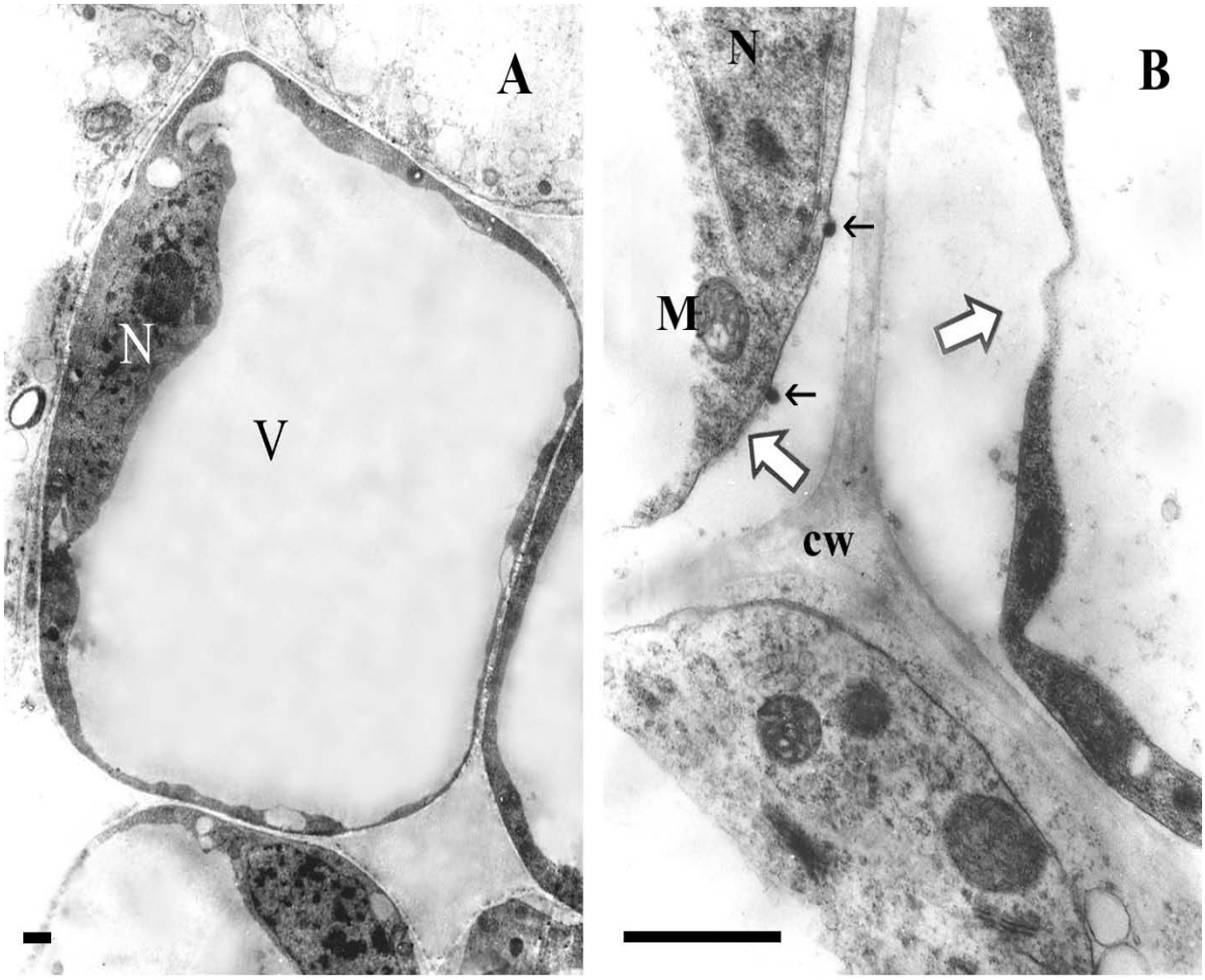

Figure 2. The ultrastructure of control **(A)** and fusariotoxins-treated **(B)** root cells of the susceptible cv. Pavla. Arrows in B indicate protoplast retreat due to plasmolysis, small arrows the osmiophilic globules associated with plasma membrane. M – mitochondria, cw – cell wall, N – nucleus,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

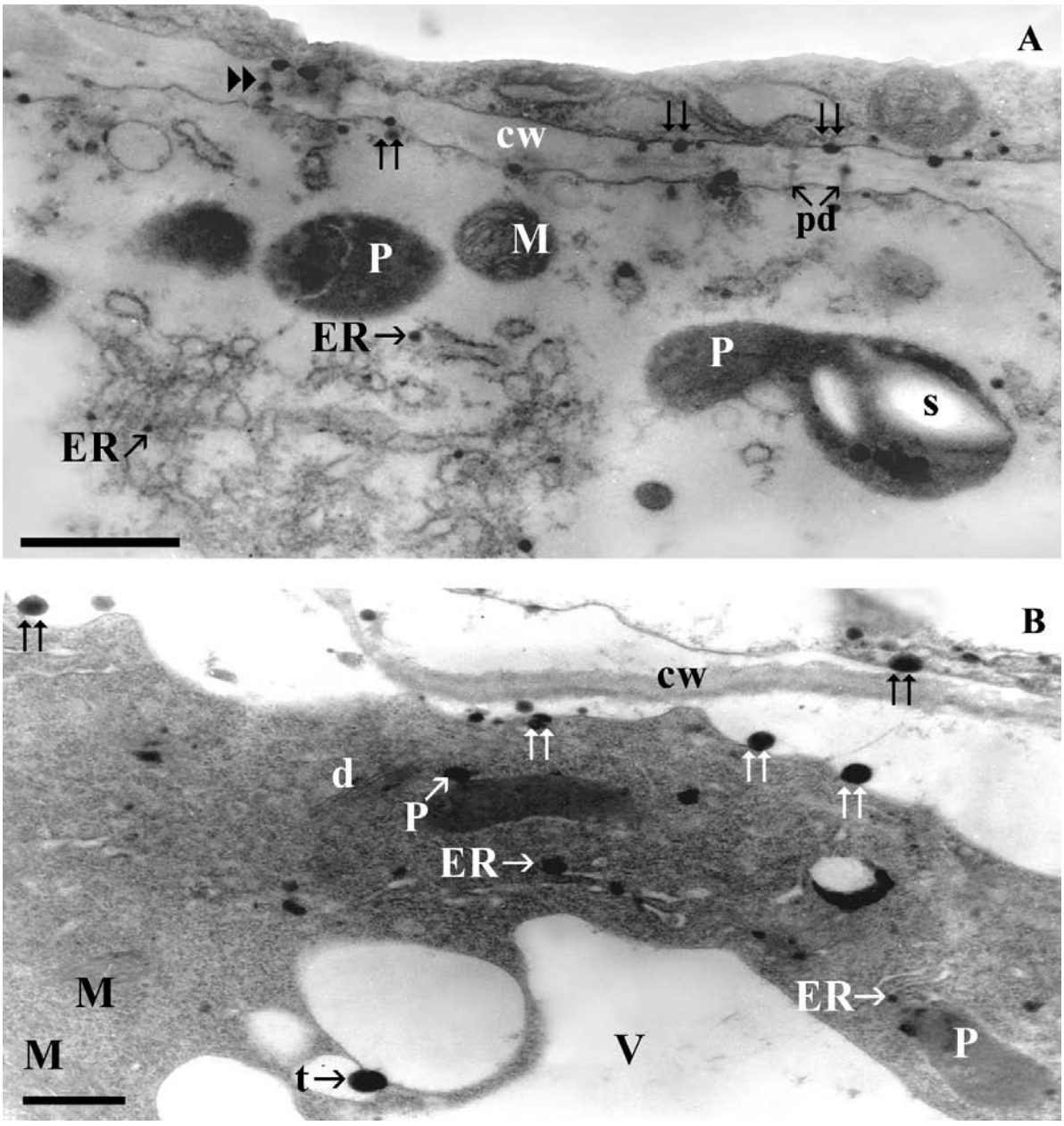

Figure 3. Ultrastructure of cells of young root cortex of the susceptible cv. Pavla treated by fusariotoxins. **A**. Osmiophilic granules were associated with plasmodesmata-pd (A, double arrowheads), plasma membrane (double arrows), endoplasmatic reticulum (ER) (**A, B**), plastids (P), cw – cell wall 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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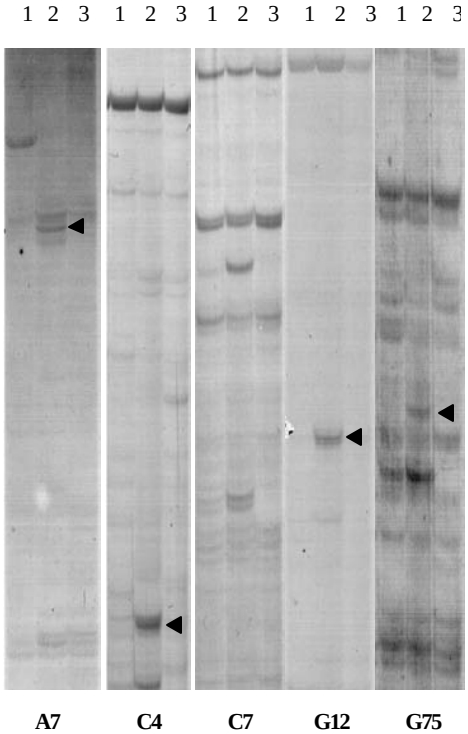

Figure 1. Differential display of mRNAs of wheat cultivars 'Sumai 3' and 'Wheaton' in 32 hai. Lane1, 2, 3 are the FHB-inoculated 'Wheaton', the FHB-inoculated 'Sumai 3', and the water-inoculated 'Sumai 3', respectively. Arrows point to the cDNAs cloned.

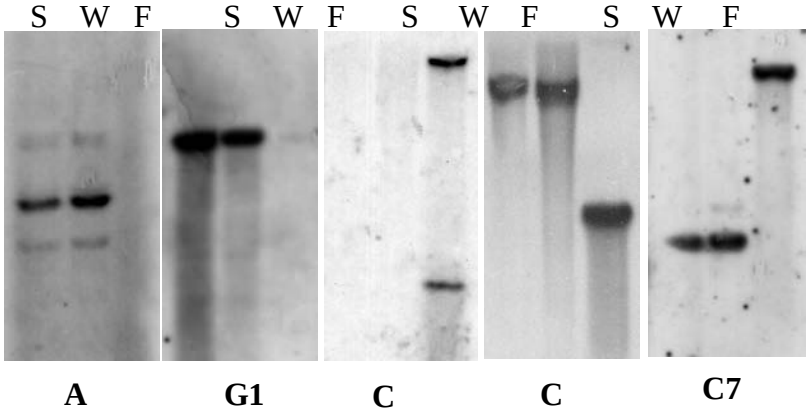

Figure 2. Southern analysis of the five differentially expressed cDNAs. S: 'Sumai 3'; W: 'Wheaton'; F: *F. graminearum. Genomic DNA was* digested with *Eco*RI.

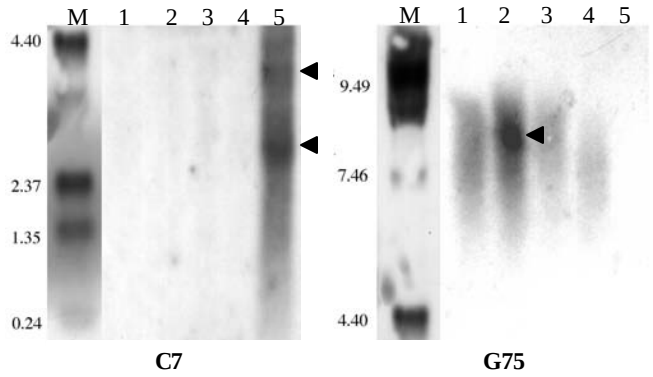

Figure 3. Northern analyses of mRNA with cDNAs C7 and G75 as the probes. Lane 1: the water-inoculated 'Suami3'; Lane 2: the FHB-inoculated 'Sumai 3'; Lane 3: the FHB-inoculated 'Wheaton'; Lane 4: the water-inoculated Wheaton; Lane 5: *F. graminearum*; M: RNA ladders (kb).

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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Agriculture, under Agreement No. 59-0790-9-045. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

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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.

TAXONOMY, POPULATION GENETICS AND GENOMICS OF *FUSARIUM* SPP.

Chairperson: John Leslie

FUSARIUM GENOMICS ACTIVITIES IN THE AUSTRIAN GENOME PROGRAM GEN-AU

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OBJECTIVES

Our working hypothesis is that *Fusarium graminearum* is able to overcome host resistance by producing small molecules suppressing defense gene expression. We intend to utilize the tools of genomics to identify fungal virulence factors and to use the knowledge gained to search for plant resistance mechanism which are able to at least partly antagonize the fungal virulence factors. The improved understanding of the interaction between plant and pathogen should be utilized for development of new chemical control strategies, development of new screening and selection tools for breeders, and in biotechnological approaches aiming to improve *Fusarium* resistance.

INTRODUCTION

In 2002 the Austrian Federal Ministry for Education, Science and Culture has established the national genome program GEN-AU (http://www.gen-au.at/). In the pilot project FUSARIUM (coordinated by G. Adam) an interdisciplinary team of researchers from BOKU and IFA Tulln, the TU Vienna, the Austrian Research Center Seibersdorf (ARCS) and from the wheat breeding company Saatzucht Donau (SZD) found together to approach the Fusarium head blight problem.

MATERIAL AND METHODS

As a first step towards the goal to get new insights into fungal virulence mechanisms by using the tools of genomics, we have supported the development of the *Fusarium graminearum* genome database by the subcontractor MIPS (http://mips.gsf.de/genre/proj/fusarium/). Several team members are involved in efforts to improve the available tools for functional genomics of *Fusarium* (e.g. repeated gene disruption using the Cre/lox system). Changes in the metabolite spectrum of *Fusarium* gene disruptions were characterized using HPLC-MS/MS techniques. Model organisms like yeast and *Arabidopsis thaliana* were used to characterize and identify toxin resistance mechanisms and to clone detoxification genes.

RESULTS AND DISCUSSION

As a first successful example we have identified a gene from the model plant *A. thaliana* encoding a UDP-glucosyltransferase, which is able to detoxify the known *Fusarium* virulence factor deoxynivalenol (Poppenberger *et al*., 2003). DON-glucoside is also formed in wheat (*Dall'Asta et al., 2004*), and this detoxification reaction seems to be a very important

resistance mechanism against *Fusarium* also in wheat (see contribution of M. Lemmens).

In collaboration with Prof. F. Trail (Michigan State University) we are currently investigating the effect of disruption of individual polyketide synthase (PKS) genes on virulence of *F. graminearum* and on its metabolite spectrum. Preliminary results suggest that inactivation of a PKS gene necessary for zearalenone (ZON) production leads to a moderate reduction in aggressiveness of the mutant on wheat heads. We have investigated the effect of ZON on the model plant *Arabidopsis thaliana*. Results of Affymetrix microarray experiments and characterization of signal transduction mutants indicate that ZON represses genes encoding proteins involved in cell wall modification/reinforcement, most likely by interfering with the ethylene signaling pathway. We have identified an *Arabiodpsis* gene encoding a UDP glucosyltransferase inactivating zearalenone (Poppenberger *et al*., in preparation).

We have furthermore identified a *Fusarium* ZON detoxification gene which seems to play a role in self protection (Mitterbauer *et al*., in preparation). Inactivation of the predicted sulfotransferase gene leads to loss of production of ZON and ZON-sulfate.

One result supporting the suppressor hypothesis is the recent identification of a new virulence gene. Deletion of this gene leads to highly pleiotropic changes in the metabolite spectrum of *F. graminearum* and a nearly complete loss in the ability to cause disease. We are currently working on the development of a bioassay allowing high throughput screening for inhibitors of this potential target for *Fusarium* control.

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DNA CHIP FOR MONITORING EXPRESSION OF *FUSARIUM SPP*. SECONDARY METABOLITES IN CEREALS

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ABSTRACT

We have developed a DNA chip to facilitate expression studies of genes involved in the synthesis of secondary metabolites in *Fusarium graminearum* and related species. The genome sequence from *F. graminearum* was used to design a PCR probe that corresponds to 9 genes from the trichothecene cluster, 8 ABC transporters, 15 polyketid synthases, 21 peptidsynthetases, and 6 genes from the aurofusarin cluster. As an internal standard for fungal growth, probes corresponding to the constitutive expressed genes, b-tubulin and glyceraldehydes-3-phosphate, gpd, were generated. The DNA chips were spotted using a Genetix Q-pix Robot (contact spotting) and scanned using a ArrayWoRx scanner (Applied Prescision Inc.). The chip design included one replication of the target genes and several copies of the internal standards.

Secondary metabolites are expressed relatively late in the fungal life cycle and under stress. Some of these substances have a detrimental effect on the quality of RNA and the amounts that can be purified. Contaminating compounds or degradation of RNA prepared from older cultures has resulted in a lowered efficiency of the incorporation of Cy3/Cy5. Several different methods are tested to overcome this problem. Analyses of aurofusarin deficient mutants indicate that this pigment may in part be responsible for the problem. Preliminary results from comparison of 3 different *F. culmorum* isolates showed a significant difference in the regulation of two ABC transporters and *Tri*101 (trichothecene 3-O-acetyl transferase). To determine how gene expression correlates to the presence of secondary metabolites a time series experiment using *F. culmorum* is performed. The trichothecenes are analysed by HPLC and the expression profile of the *tri* genes determined by DNA chip analyses. To increase the sensitivity of the assay allowing the use of smaller amounts of RNA from infected plant material a Gensisphere 3DNA array 900™ kit is tested.

FUNCTIONAL ANALYSIS OF TRICHOTHECENE BIOSYNTHETIC GENES VIA HETEROLOGOUS EXPRESSION IN A TRICHOTHECENE-NONPRODUCING *FUSARIUM* SPECIES

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ABSTRACT

The biosynthesis of trichothecene mycotoxins by *Fusarium sporotrichioides* and *F. graminearum* involves a complex biochemical pathway that begins with the cyclization of farnesyl pyrophosphate to the sesquiterpene hydrocarbon trichodiene and continues with multiple oxygenation, cyclization and esterification reactions. While almost all of the steps in the pathway have been identified, there are still some questions regarding gene function, particularly of several of the P450 enzymes involved in the oxygenation steps. In previous studies using *F. sporotrichioides*, disruption of the P450 monooxygenase-encoding gene *Tri4* blocked trichothecene production and led to the accumulation of trichodiene. Therefore, trichodiene is the likely substrate of the TRI4 protein. To further elucidate the function of the TRI4 protein, we heterologously expressed the *F. graminearum Tri4* (*FgTri4*) in *F. verticillioides*, which does not produce trichothecenes. Transgenic *F. verticillioides* carrying *FgTri4* under the control of a fumonisin biosynthetic gene (*FUM8*) promoter converted exogenous trichodiene to isotrichodermin. Conversion of trichodiene to isotrichodermin requires seven steps. Previous studies indicate that two of these reactions are non-enzymatic, and feeding studies done here indicate that wild-type *F. verticillioides* can convert isotrichodermol to isotrichodermin. Thus, the remaining four oxygenation reactions required for the conversion of trichodiene to isotrichotriol must be catalyzed by the TRI4 protein, suggesting that it is a multifunctional monooxygenase. Using a similar strategy, we analyzed the expression of FgTRI1 and FsTRI1. We have shown the usefulness of using a transgenic expression system to determine function of unknown genes which should be helpful in analyzing the many genes that are being identified in genomic projects.

MONITORING OF FHB USING PCR FOR QUALITATIVE AND QUANTITATIVE DETECTION OF *FUSARIUM* SPP

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ABSTRACT

Since the 1990ies Fusarium head blight (FHB) and the resulting mycotoxin (mainly DON) contamination of the crops are a great problem in winter wheat production in Bavaria. Main objective is to elaborate a forecasting system for FHB and DON production based on the correlation of actual epidemiological data gained in the field and the corresponding meteorological parameters captured by weather stations in spatial proximity. As a first step in our project field trials using *Fusarium graminearum* infected maize stubbles as inoculum were conducted in 2004. Two different cultivars of winter wheat were planted at two different locations and the development of FHB was monitored throughout the growing period. Samples were taken two times a week. For qualitative and quantitative determination of *Fusarium* in these samples PCR-based methods were elaborated. For the quantitative approach realtime PCR using SYBR green and Taqman ® probes are chosen. First results with the newly designed primers and probes are shown. As a prerequisite for the risk assessment of DON production in a next step a test for expression of the tri 5 gene, coding for the key enzyme in the production of thrichothecene mycotoxins, is to be established.

CROSS FERTILITY OF *GIBBERELLA ZEAE*

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ABSTRACT

O'Donnell *et al*. (2000) divided *Gibberella zeae* into seven phylogenetic lineages and this was extended to eight lineages by Ward et al. (2002). Recently, the lineages were extended to nine and given species rank by O'Donnell et al. (2004). Bowden and Leslie (1999) had previously shown cross fertility between some strains later placed in different species in the *Gibberella zeae* clade by O'Donnell et al. The objective of this study was to estimate the potential for genetic exchange between these lineages or species by quantifying cross fertility in the laboratory. Crosses were conducted on carrot agar as described by Bowden and Leslie (1999). Three strains of *G. zeae* lineage 7 with an insertion in the *MAT1-2* locus that renders them heterothallic were used as females (Lee et al., 2003). Standardized suspensions of macroconidia from strains of each of the nine lineages were used as males to fertilize the females. At least two male strains were used for each lineage except lineage 1. On day 10 after fertilization, carrot agar plates were inverted in a 40 cm spore settling tower made of 10 cm PVC pipe. Fertility was measured by counting ascospores deposited overnight on water agar plates at the bottom of the spore settling tower. Homothallic cultures and unfertilized heterothallic strains served as controls. Cross fertility was highly variable and differed for the three female strains. All males from all lineages produced viable progeny with at least one lineage 7 female strain. Individual pairings of lineage 7 females with males of lineages 1, 4, 5, 6, and 9 showed fertility levels comparable to lineage 7 x 7 crosses. Pairings with representatives of lineages 2, 3, and 8 are in progress. No evidence for consistent fertility barriers between the lineages (or species) of O'Donnell et al. (2004) and lineage 7 has been found.

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GENETIC MAPPING IN *GIBBERELLA ZEAE*

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ABSTRACT

Fusarium graminearum is the asexual conidial stage of a haploid fungus that can self-fertilize to produce the sexual ascospore stage, *Gibberella zeae*. Although predominantly an inbreeder, *G. zeae* has been shown to outcross in laboratory studies (Bowden and Leslie, 1999). Crosses can be fertile both within and between the lineages of *G. zeae* described by O'Donnell et al. (2000). A difficulty in genetic studies of fungi with mixed mating systems is distinguishing selfings from outcrosses. One solution is to use pairs of complementary auxotrophic markers, such as nitrate nonutilizing mutants, to detect recombinants among random ascospore progeny (Bowden and Leslie, 1999). In this fungus, all asci in individual perithecia result from one fertilization event and contain either selfed or outcrossed ascospore progeny, but not both. When the ascospores from an individual perithecium are collected, single segregating markers can be used to detect outcrosses. Another solution is to create obligate outcrossing strains by deleting portions of the mating type (*MAT*) locus (Lee et al., 2003). Segregation occurs in the F_1 generation in haploid fungi so recombinant population development can be rapid.

These genetic techniques make genetic mapping possible in this fungus. To date, we are aware of three genetic maps of *G. zeae* (Bowden et al, 2002; Jurgenson et al., 2002; and Gale and Kistler, http://www.broad.mit.edu/annotation/fungi/fusarium/maps.html). Markers utilized to construct the maps include Amplified Fragment Length Polymorphisms (AFLP), Restriction Fragment Length Polymorphisms (RFLP), Cleaved Amplified Polymorphic Sequences (CAPS), Derived Cleaved Amplified Polymorphic Sequences (dCAPS), and Simple Sequence Repeats (SSR). The genomic sequence of *G. zeae* (http://www.broad.mit.edu/annotation/fungi/fusarium/index.html) has been a valuable tool for development of some of these markers. A variety of software has been used for haploid linkage analysis including Map Maker, MapManager QTX, and JoinMap.

The genetic maps of *G. zeae* are useful for many purposes. For example, the linkage map of Jurgenson et al. (2002) was used to select an unbiased set of unlinked AFLP markers for population diversity studies of *G. zeae* (Zeller et al., 2003, 2004). Genetic maps can be used to assist or validate the genomic sequence assembly. Comparative mapping is useful for understanding differences in genome organization between species, lineages, or strains. Perhaps most importantly, genetic maps are valuable tools to dissect the genetic basis of important traits of the pathogen such as toxin production, fertility, or aggressiveness.

The map of Jurgenson et al. was based on a cross between a Japanese barley strain (R-5470, lineage 6) and a Kansas wheat strain (Z-3639, lineage 7). This cross was chosen because it was polymorphic for trichothecene toxin type (nivalenol vs. deoxynivalenol), toxin amount, colony color, female fertility, and AFLP banding patterns. The map contains 1048 polymorphic markers, primarily AFLPs, which map to 468 unique loci on nine linkage groups. The total map length is approximately 1300 cM with an average interval of 2.8 map units between loci. The map was used to position the deoxynivalenol/nivalenol switch in the trichothecene gene cluster. This was corroborated when the *Tri13* gene in the trichothecene cluster was proved to be the switch (Lee et al. 2002). We also located a major gene for toxin accumulation (*TOX1*) which was tightly linked to female fertility (*PERI1*) and colony color (*PIG1*)

In collaboration with colleagues at the University of Hohenheim, we conducted a QTL analysis of the aggressiveness of the progeny of the Jurgenson et al. mapping cross (Cumagun et al., 2004). We identified two major QTLs for pathogenicity or aggressiveness. One locus (*PATH1*) maps on linkage group IV near *TOX1*, which controls toxin amount. Progeny producing little or no detectable trichothecene toxin had very low pathogenicity, an effect that had previously been reported (Proctor et al., 1995). The other QTL maps on linkage group I and is centered on the trichothecene gene cluster that contains the deoxynivalenol vs. nivalenol switch. Progeny producing deoxynivalenol were, on average, twice as aggressive as those producing nivalenol. Both the high aggressiveness and high pathogenicity alleles were from the Kansas parent. It was interesting that no transgressive segregation for aggressiveness was detected in this cross between lineages 6 and 7.

The release of the genomic sequence of *G. zeae* strain PH-1 by the Broad Institute provided an opportunity to align the sequence assembly with our linkage map. We used 7 sequenced structural genes and 130 sequenced AFLP markers from all nine linkage groups of the genetic map (Lee et al., 2004). One hundred and fifteen markers were associated with nine supercontigs of the genomic sequence. The alignments of linkage groups with supercontigs allowed the assembly of four putative chromosomes that anchored 99% of the genomic sequence. Co-linearity of the physical and genetics maps was very high, though there was some evidence of inversions on two chromosomes. The map by Gale and Kistler also showed a high degree of co-linearity and anchored 99.8% of the genomic sequence. The supercontigs joined by the two maps are in agreement. These results validate both the genomic sequence assembly and the linkage maps.

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ANALYSIS OF INTRA- AND INTER-SPECIES GENETIC EVOLUTION OF EUROPEAN WHEAT PATHOGENIC *FUSARIUM* FUNGI

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ABSTRACT

Fusarium head blight (FHB) is an important disease of wheat, barley and maize world-wide. *Fusarium* fungi exhibit an extraordinary degree of biodiversity with respect to morphological, physiological and ecological characteristics. This study used AFLP analysis, to examine the inter- and intra-species genetic diversity of 80 *Fusarium* wheat-pathogenic isolates representing five species (*F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *M. nivale*) and originating from Ireland, the UK, Hungary and Italy. Nine other *F. graminearum* isolates representing the different geographic lineages and isolates of seven other *Fusarium* species were included in this study. Isolates were identified morphologically and by species-specific PCR analysis. At the intra-species level, UPGMA cluster analysis of AFLP data revealed that the *F. avenaceum*, *F. culmorum* and *F. graminearum* isolates showed the highest level of genetic similarity. The most genetically diverse species were *F. poae* and *M. nivale*. Principal coordinate analysis of AFLP data generally confirmed the same cluster profile as did the dendrograms. The present study also found a relationship between genetic diversity and country of origin of the isolates within certain species; no relationship was found between genetic diversity and growth or pathogenicity of the isolates.

PHYSICAL MAPPING OF THE *FUSARIUM GRAMINEARUM* GENOME

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ABSTRACT

Fusarium head blight, caused by *Fusarium graminearum*, is a major disease in wheat and barley. To enable genomics studies of *F. graminearum*, we developed a bacterial artificial chromosome (BAC)-based physical map and integrated it with the genome sequence and genetic map. We developed two complementary genomic libraries with average insert sizes of 107 and 95 kb, which were estimated to exhibit 23-fold genome coverage. We fingerprinted 4,224 BAC clones and developed a physical map consisting of 112 contigs. Using lower stringency parameters for contig assembly, 112 contigs were assembled into 26 contig groups covering 36.4 Mb. The physical map was confirmed by comparing our map to the genome sequence posted on the *F. graminearum* database website (http://www.broad.mit.edu/cgi-bin/annotation/fusarium). Our results show high consistency of the physical map with the genome sequence. Among the 112 contigs used for BAC assembly, 3 contigs did not match with any of the genomic sequences registered in database. To further validate the physical map and to integrate the physical map and the genetic map, we selected 30 genetic markers evenly covering the whole genome and conducted PCR-based screening of the BAC clones. The physical map was consistent with the genetic map throughout the entire genome. Our current physical map, integrated with the genome sequence and genetic map, will enable advanced studies such as gene cloning, comparative mapping, and elucidation of *F. graminearum* genome organization.

POLYMERASE CHAIN REACTION BASED ASSAYS FOR THE DETECTION AND IDENTIFICATION OF *FUSARIUM* SPECIES IN MYCELIAL CULTURES AND GRAINS

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OBJECTIVES

1. To use species-specific PCR assays to identify nine *Fusarium* species collected from cereals in Canada.

2. To screen cereal samples for Fusaria using PCR and whole seed plating techniques.

INTRODUCTION

Fusarium head blight (FHB) is one of the major fungal diseases of cereals in Canada and worldwide. *Fusarium graminearum* Schwabe is the predominant FHB pathogen in Manitoba and southeastern Saskatchewan (Clear and Patrick, 2000). The other principal FHB pathogens in Canada are *F. avenaceum* (Corda ex Fr.) Sacc. and *F. culmorum* (W.G. Smith) Sacc. Several other common *Fusarium* species also infect cereal grains in Canada, including *F. acuminatum* Ell. & Ev., *F. equiseti* (Corda) Sacc., *F. poae* (Peck) Wollenw., and *F. sporotrichioides* Sherb. (Clear et al., 2000a, Clear et al., 2000b, Clear and Patrick 1993).

The traditional diagnostic method for the detection and identification of *Fusarium* species in pure culture or in infected grain is usually based on the observation of the micro and macro morphological features of cultures developing on a nonselective agar medium. Besides requiring a growth period of several days, many of the diagnostic characters can be altered by the culture conditions. In addition, when culturing fungi from seed, only viable fungi that are able to compete well enough to be visually detectable are recorded. Polymerase chain reaction (PCR) is a sensitive and rapid method that can be used for detection and screening of *Fusarium* species in infected grain samples. In this study, species-specific PCR assay was used for the detection and identification of nine *Fusarium* species in pure mycelial cultures and grain samples.

MATERIALS AND METHODS

Grain samples – The wheat samples were composites of producer deliveries from 39 crop districts across western Canada. The other grain samples were from individual producers.

Mycological analysis – 200 seeds from each grain sample were surface disinfected and placed onto potato dextrose agar (PDA) for 5 days and analyzed according to Clear and Patrick (2000).

Fungal cultures – For each culture in this study, initial fungal isolations were made by transferring mycelia growing from infected cereal seed (or for three isolates of *F. pseudograminearum*, from straw) to a petri plate containing PDA. After 5 to 10 days of growth at room temperature and under UV light, a spore suspension was prepared and spread onto a fresh PDA plate and incubated for 18 h at room temperature. A single germinating conidium was then removed from the PDA plate, transferred to an SNA plate (Nirenberg 1981), and after 7 days growth stored in a plastic bag at 4°C for up to one year.

DNA extraction – For DNA extraction, mycelia from the SNA plates were transferred to PDA plates and incubated in the dark at room temperature for 7 to 10 days. The mycelia were then harvested and freeze-dried prior to DNA extraction. DNA was also extracted from fresh mycelial cultures without freeze drying. For cereal samples, a 20g sample was ground in a coffee grinder and then a subsample of 0.2g was

taken for DNA extraction. Sodium dodecyl sulphate (SDS) based buffer was used for DNA extraction.

Polymerase chain reaction – PCR for single species detection was performed in 96-well plates containing 25 µL of a reaction mixture consisting of 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH8.3), 0.2 mM of each of the four dNTPs, 0.25 µM of each oligonucleotide primer (Table 1), 1.5 µL DNA solution and 0.15 units of *Taq* DNA polymerase (Applied Biosystems, Foster, CA). AccuPrime™ *Taq* DNA polymerase (Invitrogen, Burlington, CA) was used for multiplex PCR according to the manufacturer's recommendations. DNA amplification was performed in an MJ Research PTC-200 Thermal Cycler using an initial 3.0 min denaturation at 95°C; and then 38 cycles of 30 s at 9°C, 20 s at 62°C, and 45 s at 72°C, followed by a final extension of 5 min at 72°C. Annealing temperatures of 56°C and 57°C were used for *F. acuminatum* and *F. pseudograminearum* PCR reactions, respectively.

RESULTS AND DISCUSSION

PCR detection of Fusarium species – Twelve *F. acuminatum*, nine *F. avenaceum*, seven *F. crookwellense*, 12 *F. culmorum*, 11 *F. equiseti*, 77 *F. graminearum* (72 isolates from Canada and 5 isolates from Australia), 10 *F. poae*, 23 *F. pseudograminearum* (five of them from Australia) and 10 *F. sporotrichioides* isolates were correctly identified using species-specific PCR (data not shown). The primers for *F. acuminatum*, *F. crookwellense*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. poae*, *F. pseudograminearum* and *F. sporotrichioides* are specific as there was no cross reaction when each specific primer was used to amplify DNA from the other *Fusarium* species. One of the primer sets for *F. avenaceum* (FaF/FaR) also amplified DNA fragments from *F. acuminatum*. The other primer set (J1AF/R) was specific and did not amplify DNA from the other eight *Fusarium* species.

A multiplex PCR reaction was developed for the simultaneous detection of the three most important mycotoxin producing *Fusarium* species (*F. culmorum*, *F. graminearum* and *F. sporotrichioides*).

Detection of *Fusarium* species in cereal grains – Most grain samples were infected with several *Fusarium* species. A comparison between the results for PCR and whole seed plating for six *Fusarium* species is shown in Table 2. *Fusarium avenaceum* was the most often detected species by both PCR (using FaF/FaR primer) and whole seed agar plating (Table 2). The *Fusarium avenaceum* specific primer (J1AF/R) was less sensitive than FaF/FaR primer (amplifies both *F. avenaceum* and *F. acuminatum*) in amplification of infected grain samples. Four of the six species were detected in more samples by PCR than by whole seed plating, with only *F. poae* and *F. sporotrichioides* being detected slightly more often by whole seed plating than by PCR (Table 2). Three species, *F. crookwellense*, *F. acuminatum* and *F. pseudograminearum*, were not included in Table 2. For *F. crookwellense*, only one sample of barley from New Brunswick revealed a positive reaction with PCR and whole seed agar plating. *Fusarium acuminatum* was detected in several grain samples when assayed with whole seed agar plating. However, only those samples that had a relatively high frequency of seed infection by *Fusarium acuminatum* (~35%) were positive with the PCR assay (data not shown). *Fusarium pseudograminearum* was not detected in any samples by either PCR (85 samples) or whole seed agar plating (82 samples), reflecting the rare occurrence of this species infecting cereal seed in western Canada.

Out of 474 comparisons (using *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. poae*, and *F. sporotrichioides*) between PCR and whole seed agar plating results, a discrepancy occurred 83 times (Table 2). Fifty-four times a *Fusarium* species-specific PCR amplification was obtained even though the target species was not observed during whole seed agar plating, whereas in 29 comparisons the reverse was true (Table 2). In all samples where a discrepancy was noted the level of the target species in the plating results was very low. Most importantly, *F. graminearum* was detected more often by PCR (50 of 85 samples) than by whole seed agar plating (36 of 82 samples), and the PCR-based method was able to accurately distinguish between *F. graminearum* and *F. pseudograminearum*. Failure of whole seed

agar plating to detect species that PCR detected may be due to the removal of fungal material during surface disinfestations, failure to detect the Fusaria due to competition on the plate, or lack of viability of the target species. Differences between PCR and whole seed agar plating results may also be due to variation in the two sampling techniques and to low levels of the target species on the grain. In this trial, whole seed agar plating was done on 200 individual kernels; whereas 0.2g subsample from 20g ground seed was used for DNA extraction and PCR analyses.

In summary, species-specific PCR assay was successfully used for the identification of nine *Fusarium* species. The PCR assay was also used for the detection of *Fusarium* species in several types of cereal grain. The PCR assay used in this study can be used for routine detection and identification of *Fusarium* species in mycelial cultures and grain samples in Canada.

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Table 1. List of primer sequences, expected DNA fragment length and sources of primers.

Primer name	Target	Sequence (5' – 3')	Size (bp)	Source*
FAC F	F. acuminatum	GGGATATCGGGCCTCA	600	A
FAC R		GGGATATCGGCAAGATCG		
FaF	F. avenaceum	CAAGCATTGTCGCCACTCTC	920	B
FaR		GTTTGGCTCTACCGGGACTG		
J1AF	F. avenaceum	GCTAATTCTTAACTTACTAGGGGCC	220	C
J1AR		CTGTAATAGGTTATTTACATGGGCG		
CroA F	F. crookwellense	CTCAGTGTCCACCGCGTTGCGTAG	842	D
CroA R		CTCAGTGTCCCAATCAAATAGTCC		
FC01F	F. culmorum	ATGGTGAACTCGTCGTGGC	570	E
FC01R		CCCTTCTTACGCCAATCTCG		
FEF1	F. equiseti	CATACCTATACGTTGCCTCG	400	F
FER1		TTACCAGTAACGAGGTGTATG		
FG11F	F. graminearum	CTCCGGATATGTTGCGTCAA	450	E
FG11R		GGTAGGTATCCGACATGGCAA		
FP82F	F. poae	CAAGCAAACAGGCTCTTCACC	220	G
FP82R		TGTTCCACCTCAGTGACAGGTT		
AF330109CF	F. sporotrichioides	AAAAGCCCAAATTGCTGATG	332	H
AF330109CR		TGGCATGTTCATTGTCACCT		
FP1-1	F. pseudograminearum	CGGGGTAGTTTCACATTTCYG	523	I
FP1-2		GAGAATGTGATGASGACAATA		

*Sources of primers – A = Williams et al., 2002; B = Doohan et al., 1998; C = Turner et al., 1998; D = Yoder et al., 1998; E = Nicholson et al., 1998; F = Mishra et al., 2003; G = Parry and Nicholson 1996; H = Genbank AF330109 (primer designed by authors); I = Aoki and O'Donnel, 1999.

Table 2. Summary of Fusarium detection in grain samples using PCR and whole seed agar plating.

Fusarium species	Crop	PCR # +ve	PCR #-ve	PCR TTL	Whole seed # +ve	Whole seed #-ve	Whole seed TTL	PCR + & WS -	PCR - WS +	Similarity %
F. avenaceum[a]	CWRS	37	1	38	32	6	38	4	0	34/38 (89.5)
	CWAD	26	0	26	23	3	26	3	0	23/26 (88.5)
	Barley	8	2	10	9	1	10	0	1	9/10 (90.0)
	Oat	3	1	4	3	1	4	0	0	4/4 (100.0)
	Corn[b]	0	6	6	0	3	-	-	-	-
	Rye	1	0	1	1	0	1	0	0	1/1 (100.0)
F. culmorum	CWRS	5	33	38	4	34	38	5	4	29/38 (76.3)
	CWAD	11	15	26	10	16	26	3	2	21/26 (80.8)
	Barley	0	10	10	1	9	10	0	1	9/10 (90.0)
	Oat	1	3	4	1	3	4	1	0	3/4 (75.0)
	Corn	0	6	6	0	3	-	-	-	-
	Rye	0	1	1	0	1	1	0	0	1/1 (100.0)
F. equiseti	CWRS	20	18	38	13	25	38	12	5	21/38 (55.3)
	CWAD	21	5	26	19	7	26	4	2	20/26 (76.9)
	Barley	8	2	10	7	3	10	3	1	6/10 (60.0)
	Oat	1	3	4	1	3	4	0	0	4/4 (100.0)
	Corn	0	6	6	1	2	-	-	-	-
	Rye	1	0	1	1	0	1	0	0	1/1 (100.0)
F. graminearum	CWRS	21	17	38	14	24	38	8	1	29/38 (76.3)
	CWAD	13	13	26	10	16	26	4	1	21/26 (80.7)
	Barley	9	1	10	9	1	10	0	0	10/10 (100.0)
	Oat	3	1	4	2	2	4	1	0	3/4 (75.0)
	Corn	3	3	6	0	3	-	-	-	-
	Rye	1	0	1	1	0	1	0	0	1/1 (100.0)
F. poae	CWRS	14	24	38	18	20	38	0	4	34/38 (89.5)
	CWAD	9	17	26	9	17	26	1	1	24/26 (92.3)
	Barley	6	4	10	9	1	10	0	3	7/10 (70.0)
	Oat	2	2	4	3	1	4	0	1	3/4 (75.0)
	Corn	0	6	6	0	3	-	-	-	-
	Rye	0	1	1	0	1	1	0	0	1/1 (100.0)
F. sporotrichioides	CWRS	20	18	38	21	17	38	3	0	35/38 (92.1)
	CWAD	15	11	26	19	7	26	2	2	22/26 (84.6)
	Barley	9	1	10	9	1	10	0	0	10/10 (100.0)
	Oat	3	1	4	3	1	4	0	0	4/4 (100.0)
	Corn	0	6	6	0	3	-	-	-	-
	Rye	1	0	1	1	0	1	0	0	1/1 (100.0)

[a] FaF/FaR primer set was used for the PCR assay. [b]Three corn samples were received ground, and thus whole seed agar plating was carried out for only three of the six corn samples. WS = whole seed agar plating method. Two hundred seeds were used for whole seed agar plating assay, whereas 0.2g from 20g ground sample was used for DNA extraction and PCR assay.

IMPLICATION OF DISRUPTION OF THE FUM12 GENE ON THE FUMONISINS PRODUCTION BY *FUSARIUM VERTICILLIOIDES*

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ABSTRACT

Fumonisins are polyketide-derived mycotoxins produced by *Fusarium* species of the *Gibberella fujikuroi* complex. Wild type strains of the fungus produce predominantly four B-series fumonisins, designated FB1, FB2, FB3 and FB4. Recently, a cluster of 15 putative fumonisin biosynthetic genes (*FUM*) was described in *F. verticillioides*. We have now conducted a functional analysis of the *FUM12* gene that is predicted to encode a cytochrome P-450 monooxygenase. Therefore, we generated *FUM12* disrupted mutants (*FUM12*) of the wild-type *F.verticillioides* strain MUCL 43478 (M-3125). HPLC analyses revealed that the FB1 and FB3 production of *FUM12*- mutants was reduced by over 98% and was at least doubled for FB2 compared to the progenitor strain. These results indicate that the *FUM12* protein catalyses the hydroxylation of the C-10 of the fumonisin backbone. As the phenotype of the mutants is identical to that of previously described mutants with defective alleles at the meiotically defined *fum2* locus, it appears that *FUM12* and *fum2* locus are the same gene which is now renamed *FUM2*.

IDENTIFICATION OF GENES EXPRESSED BY *FUSARIUM GRAMINEARUM* DURING INFECTION OF WHEAT

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ABSTRACT

Our initial studies have shown that the *Fusarium graminearum* species complex is comprised of strains belonging to at least nine biogeographically structured cryptic species that may differ significantly in aggressiveness on wheat and mycotoxin production. To study this host-pathogen interaction at a genomic level and identify fungal genes expressed during initial infection (48 hours after inoculation), cDNA libraries were created by suppression subtractive hybridization. One such library was constructed using RNA isolated from wheat heads inoculated with a highly aggressive strain (tester) or with water (driver). The ESTs sequenced from this library could be assembled into 182 contigs and 630 singletons. Of these, 349 ESTs were determined to be of fungal origin according to their matches to the *F. graminearum* genome sequence. These sequences were compared with ESTs from libraries created using the *F. graminearum* grown under various culture conditions and the whole genome sequences of *Magnaporthe grisea* and *Neurospora crassa*. Putative functions of genes corresponding to the fungal ESTs obtained from this library were predicted based on comparisons with sequences from publicly available databases. Interestingly, nearly 56% of the fungal ESTs were mitochondria related. Also, a significant number of ESTs with no known homologs in currently available databases were observed. These are believed to be new open reading frames specific to *F. graminearum*. Additionally, candidate genes potentially involved in pathogenicity were identified e.g. those corresponding to genes coding for an ABC transporter, amino acid permease, polyketide synthase, histidine kinase and the regulatory gene alcR. Some of these have been selected for targeted deletion mutagenesis using gene replacement. Analysis of these sequences and the methods used for successful gene replacement in *F. graminearum* will be presented.

DEVELOPMENT OF AN AFFYMETRIX GENECHIP MICROARRAY USING THE GEN-AU / MIPS *FUSARIUM GRAMINEARUM* GENOME DATABASE

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ABSTRACT

Shortly after public release of the genome sequence of the plant pathogenic fungus *Fusarium graminearum* by the Broad Institute, automated draft gene calls were processed at MIPS and also at the Broad Institute. For both predicted gene sets, a variety of bioinformatics methods were applied at MIPS using the PEDANT system. Manual inspection of the calls using different gene prediction programs and EST sequences were also conducted to examine underpredicted genes and verify predicted coding regions. In order to reduce mis-designed probe sets, manual inspection of genes, at least the most interesting targets, is desirable before the design of an Affymetrix GeneChip microarray design.

With the help of the *Fusarium* community we manually processed ~860 entries; 408 of the calls were altered or added as completely new calls. To integrate all different calls as well as the results of the applied bioinformatics methods, the *F. graminearum* Genome Database was created (http://mips.gsf.de/genre/proj/fusarium/). However, only 6.1 % of the putative 14,000 *Fusarium* genes were manually processed.

During the manual gene modeling and correction procedure it appeared that the MIPS draft gene call set performed significantly better than the Broad set. Therefore we produced a combined gene call set for the Affymetrix GeneChip design with the order of preference "manually processed new calls" > "MIPS draft set" > "Broad set". To reduce the number of ~26,000 gene calls, the ORF sequences were truncated to 500 bases towards the 3' end and all redundant call names were added to the preferred ones as an alias. This approach resulted in a set of 16,926 calls. The set of full length ORF sequences and an additional 611 EST- and rRNA-sequences were submitted to Affymetrix for initial computation of probe sets. After 3 rounds of chip design proposals, the sets were approved for mask design. First chip experiments for validation are on the way.

FUNCTIONAL GENOMICS OF *FUSARIUM GRAMINEARUM*

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ABSTRACT

We are conducting gene profiling experiments using *Fusarium graminearum* ~4.5K uniEST cDNA microarrays and targeted gene disruptions to identify and functionally test candidate genes suspected to be involved in *F. graminearum* (*Fg*) pathogenicity. Fourteen *F. graminearum* cDNA libraries have been constructed from fungal cultures grown under a variety of conditions to generate a collection of ~10,000 expressed sequence tags (ESTs) which group into ~5500 contigs/singletons. A 4.5K unigene *F. graminearum* cDNA microarray has been printed in-house and array hybridization experiments are underway to explore metabolite biosynthesis and genes induced upon plant contact. Initial array hybridizations have compared expression profiles of *Fg* grown in liquid culture under conditions of trichothecene production over a 12-day period versus *Fg* in log phase. Metabolite analysis (HPLC/NMR) of ethyl acetate extracts of these liquid cultures has indicated increasing production of 15A-DON over the growth period as well as the presence of a second metabolite, butenolide.

Functional analysis of candidate genes through gene disruption or modification is the focus of a collaborative project between the USDA and Agriculture & Agri-Food Canada. For example, an analysis of *Fg* ESTs revealed a hotspot of gene expression from a putative novel biosynthetic gene cluster. Eight consecutive predicted genes are represented by a total of 51 *Fg* ESTs isolated from trichothecene-producing culture conditions. In addition, five of the genes are represented by six ESTs originating from *Fg*-infected wheat or barley libraries, suggesting these genes are expressed *in planta.* Northern analysis conducted on seven of the eight genes showed coordinated induction of gene expression, beginning at 4 days post induction and peaking at approximately 10 days post induction in liquid culture. Expression analysis of the eighth gene is in progress. Two of these genes have been initially targeted for gene disruption.

TYPE-A TRICHOTHECENE PRODUCTION BY *FUSARIUM* IN A PHYLOGENETIC CONTEXT

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ABSTRACT

Most Fusaria causing wheat and barley head scab produce the type-B trichothecene deoxynivalenol and other 8-keto trichothecenes such as 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol. However, some fungi found on head-blighted wheat are known to produce the more toxic type-A trichothecenes such as diacetoxyscirpenol. Our objective is to fully elucidate species and species boundaries within the trichothecene-producing group of *Fusarium* and to determine the trichothecene mycotoxin profile of all species within the group. We analyzed a set of 57 suspected type-A producers from the Penn State Fusarium Research Center collection and the NRRL collection at Peoria using molecular phylogenetics and HPLC-MS for trichothecenes. For HPLC–MS analysis, cultures were grown on autoclaved rice grains and subjected to analysis for ten trichothecenes: T-2 tetraol, nivalenol, deoxynivalenol, fusarenon-X, neosolaniol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, diacetoxyscirpenol, HT-2, and T-2 toxin. Peak identities were determined using mass spectral analysis and comparison to standards. DNA sequences were generated from seven nuclear genes, EF1-a translation elongation factor, phosphate permease, 28S rDNA, *Tri101, Tri4, Tri5* and Bð-tubulin and subjected to phylogenetic analysis using maximum parsimony as the optimality criterion. Trichothecene-producers resolved to four well-supported clades. Most type-B trichothecene producers associated with head scab fell into a single clade, termed the *Gibberella zeae* complex, which is derived from within the others. Our initial observations indicate that there are species within each of the three type-A producer clades that produce type-B trichothecenes exclusively or in addition to type-A compounds. Species capable of producing both type-A and type-B are found in two of three type-A clades. Previous reports have noted this phenomenon only for *F. kyushuense* and recently for *F. poae*. We will report our ongoing work to define species and mycotoxigenic potential within the trichothecene-producing Fusaria.

THE ALIGNMENT BETWEEN PHYSICAL AND GENETIC MAPS OF *GIBBERELLA ZEAE*

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ABSTRACT

Jurgenson et al. (2002) previously published a genetic map of *Gibberella zeae* (*Fusarium graminearum*) based on a cross between Kansas strain Z-3639 (lineage 7) and Japanese strain R-5470 (lineage 6). The genetic map was based on 1048 AFLP markers and consisted of nine linkage groups. We aligned the genetic map with the first assembly of the genomic sequence of strain PH-1 (lineage 7) that was released by The Broad Institute (Cambridge, MA). We used 7 sequenced structural genes and 130 sequenced AFLP markers from all nine linkage groups (LG) of the genetic map. One hundred and fifteen markers were associated with nine supercontigs (SC) of the genomic sequence. LG1, LG7, LG8 and LG9 aligned with SC2 and SC5; LG2 aligned with SC3, SC8 and SC9; LG 3 aligned with SC4 and SC6; and LG4, LG5 and LG6 aligned with SC1 and SC7. Approximately 99% of the sequence was anchored to the genetic map, indicating the high quality of the sequence assembly and the relative completeness and validity of the genetic map. The alignments grouped the linkage groups and supercontigs into four sets, suggesting that there are four chromosomes in this fungus.

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MOLECULAR AND PHENOTYPIC DIVERSITY WITHIN AND AMONG POPULATIONS OF *FUSARIUM GRAMINEARUM*

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ABSTRACT

We aimed for the analysis of genetic variation in populations of *Fusarium graminearum* collected on a world-wide, regional, and local basis and within a crossing progeny by molecular markers (RAPD, AFLP) and phenotypic traits. The traits were: Aggressiveness on a susceptible host, deoxynivalenol [DON] and nivalenol [NIV] production, and host colonization. The amount of genetic variation is an indicator for the evolutionary potential of a pathogen and provides an important clue for risk analysis of host resistance. Analysis of the world-wide collection showed that practically all isolates were mycotoxin producers, mostly of the DON (22/26), but some of the NIV (4/26) chemotype. NIV producer were from Germany, Italy, and Brazil and generally lower aggressive. AFLP profiles of 38 isolates of the world collection showed a high level of molecular diversity (mean GS 0.21). No grouping with respect to geographical origin or host species occurred. On a regional basis (southwestern Germany) also a significant ($P<0.01$) genetic variation for aggressiveness was found, isolates displayed a similar genotypic range than the world collection. Similarly, molecular diversity was high even on the local scale of two small (about 10.000 m²) wheat fields amounting to 46 to 64% of the maximal genotypic diversity (G_{max}). In total, 70 isolates yielded 53 multi-locus haplotypes within the largest German population. Allele frequencies ranged from 0.01 and 0.97 in this local population compared to 0.04 to 0.92 in the world collection. A hierarchical analysis of diversity for RAPD bands and aggressiveness showed that 84 and 54% of total variance, respectively, was already found within sampling sites of about 1 m². Within single-field populations from Hungary and Canada a lower, but still considerable genotypic variation (27-28% of G_{max}) was detected. Crossing of two medium aggressive isolates from Europe (Germany, Hungary) resulted in a progeny of 155 isolates displaying a significant ($P<0.01$) and large segregation variance for aggressiveness, host colonization, and DON production. Moreover, significant transgression directed towards higher aggressiveness, colonization and DON production was found consistently across three environments. This illustrates that different alleles for these traits were present in both parents that were obviously combined to a higher performance. The large molecular and phenotypic diversity of *F. graminearum* even on small spatial scales might be most likely caused by a large population size of the pathogen and regular recombination with some extent of outcrossing. This enlarges allele diversity, but favourable genotypes can still be maintained by asexual propagation. The frequent alternation between saprophytic and parasitic life cycle might additionally support a high degree of polymorphism within populations. In conclusion, the results reflect a high evolutionary potential of *F. graminearum* that might allow a non-specific adaptation to host resistance, especially when highly effective resistance sources are used on large acreages.

VARIABILITY AND STRUCTURE OF THE IGS REGION IN THE *GIBBERELLA FUJIKUROI* SPECIES COMPLEX

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ABSTRACT

The intergenic spacer of the rDNA units is located between 28S and 18S genes of the nuclear rDNA in filamentous fungi with a length of about 2,5 kb in species of *Fusarium*. This region shows a high variability which is widely used to analyse variability at intraspecific level or among closely related species. Variability is due to nucleotide substitutions and to differences in length, which are attributed to duplications or deletions during crossing-over events. However, IGS region also contains a number of relevant conserved motifs such as those motifs controlling rDNA expression. We report the results of the comparative analysis of the primary structure of the IGS region to unravel the presence of repetitions and/or functional motifs in the *Gibberella fujikuroi* species complex and closely related species. This complex is especially relevant because it includes cosmopolitan pathogens of important agricultural plant species and they are important source of mycotoxins. Several short (14 nt) and long (120 nt) repetitions have been identified which showed variability in the number and position, in the case of the short repeats, causing the occurrence of characteristic specific patterns and differences in length which may be accompanying the speciation in this complex. These patterns could be explained by recombination events reducing or increasing the number of repetitions and its relative position.

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MATING TYPES DETERMINATION IN *FUSARIUM PHYLLOPHILUM* AND *FUSARIUM RAMIGENUM*

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ABSTRACT

F. phyllophilum and *F. ramigenum* are two species newly described within the *Gibberella fujikuroi* complex (Nirenberg and O'Donnell, 1998). In previous studies, it was demonstrated that they were fumonisin producers (Fotso *et al.*, 2002; Van Hove *et al.*, 2002). As only fragmentary data concerning their sexual stage exist, we decided to focus our study i) on their mating identification, ii) on the search of their sexual stage, and iii) on their phylogenetic relationships within the complex.

Therefore, the primers Gfmat1a and Gfmat1b, previously developed by Steenkamp *et al.* (2000) for identification of the mating type in the *Gibberella fujikuroi* complex, were used to characterize the isolates. Interestingly, two *F. ramigenum* strains (MUCL 43904 and 7612) presented two different mating sequences (*MAT-1* and *MAT-2*). Furthermore, two *F. phyllophilum* strains (MUCL 239 and 43905) were identified as *MAT-1* strains, while two *F. phyllophilum*-like strains (MUCL 27661 and 44479) were identified as *MAT-2* strains. Phylogenetic analyses clearly separated *F. ramigenum* and *F. phyllophilum* from the other sequenced species, particularly those for which the sexual stage has already been described (mating population A to I). The analyses also demonstrated the close relationships of the two species with *F. verticillioides*.

Diallele crosses were tested on six different media, and under different light conditions. Besides the development of perithecia in compatible *F. verticillioides* crosses used as control, development of mature perithecia was observed in *F. ramigenum* crosses, what is the first report of the sexual form for this species. On basis of integration of the various results (morphological, molecular and crosses), we propose to create a new mating type population J and its teleomorphic stage *Gibberella ramigena*. Additionnal data are expected for *F. phyllophilum*.

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VARIATION AMONG ISOLATES OF *FUSARIUM GRAMINEARUM* ASSOCIATED WITH FUSARIUM HEAD SCAB IN INDIA

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ABSTRACT

Head scab of wheat caused by *Fusarium spp*. is characterized by bleaching of the wheat spike, shriveled kernels and accumulation of mycotoxins which may cause various ailments in humans and animals. Understanding the variability of the fungal population associated with head scab could improve disease control strategies. The main aim was to investigate the genotypic and pathogenic variability within *F. graminearum* in relation to geographic origin. In the present study, *F. graminearum* were isolated from diseased spikes sampled from naturally infected wheat from Punjab, Tamil Nadu and high Himalayas of Himachal Pradesh during 2000-2002. Inter and intra species specific aggressiveness assessed could also be noticed on the basis of head scab rating on different varieties of wheat. *F. graminearum* isolates of Dalang Maidan and Wellington were significantly more aggressive than Ludhiana and Gurdaspur isolates of *F. graminearum*. Fusarium head scab ratings were more on varieties Sonalika, HD 29 and PBW 222, irrespective of the isolate used. Randomly amplified polymorphic DNA (RAPD) was used to study genetic variation in natural pathogen populations of F. graminearum (15 isolates). A screening of sixty one 10-mer oligonucleotide RAPD primers (OPAA 1-20, OPAC 1-20, OPAD 1-20, OPV 14) revealed 19 RAPD primers to yield informative (polymorph), strong and reproducible DNA amplicons (bands) by PCR. The number and size of the amplified fragments varied with different primers. The amplification products were in the range of 300 bp to 1.2 Kb. Maximum number of bands (11) were scored with primer OPAD 12 followed by 10 bands with OPAA 12. The genetically most similar isolates belonged to Punjab viz., G 31 and L 23 (92.65 %) while the Lahaul valley isolate (D 3) and the Wellington isolate (W 3) were found genetically most dissimilar (47.79 %). Cluster analysis of band sharing coefficients separated isolates of *F. graminearum* into four clusters. This study has shown that there is a considerable pathogenic and genotypic variability among *F. graminearum* isolates obtained from infected wheat earheads from different geographic regions of India.

VARIABILITY OF *FUSARIUM CULMORUM* – AGENTS OF HEAD BLIGHT

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OBJECTIVES

Variability of Fusarium culmorum to pathogenic and toxic will study.

Strains of *F. culmorum* will characterize on 5 rye cultivars.

INTRODUCTION

Scab or Fusarium head blight (FHB) is a serious disease in many cereal crops Russia. This disease is the most dangerous to the winter rye. FHB can significantly reduce rye grain yield and quality (Novozhilov K.V., Levitin M.M., 1990; Monastirskij O.A. 2000; G. Gang et al., 1998; Ivaschenko V.G. et al, 2004).

The mycotoxins produced by *Fusarium* spp. in infected grains are harmful to animals and human health (Bilai V.I., 1955; Bamburg et all, 1968; Mirocha et all, 1976; Ivaschenko V.G. et al, 2004; Levitin M.M., 1994; S. L. Walker et al., 2001).

The selection of winter rye cultivars immune to FHB considers the very perspective. But it is impossible without study pathogenic and toxic qualities of frequent fungus.

Fusarium culmorum (W.G.Sm.) Sacc. is one of frequent pathogens of scab in Moscow region.

MATERIALS AND METHODS

Genotypes *F. culmorum* with various morphological - cultural types ("C") of colonies were received on potato agar plates at 24°C during 2 weeks.

With the purpose of pathogenic and toxic qualities sterile seeds were inoculated with conidial suspensions and culture filtrates of the 4 genotypes *F. culmorum*. Inoculated and control (treated by adding 5 ml sterile water) seeds were allowed to continue germination for 3 days, on filter paper with low moisture level (25 seeds/Petri dish). In fours reiteration samples were evaluated for each experiment. For each genotype, records of following parameters are taken:- the germination's percent of seeds; - length of root of seeds.

Accounting information of control taken for 100 %.

RESULTS AND DISCUSSION

Genotypes *F. culmorum* with various MCT significantly differed pathogenic and toxic quality. The analysis of variance has showed significant influence of factors (cultivars and strains) on percent of seed germination of winter rye (fig. 1, 2). Each bar represents the mean of experiments with ten replications for cultivar-strain combinations averaged across 4 strains *Fusarium culmorum* or 5 winter rye cultivars with the purpose of revealing their influence on percent of seed germination. Cultivars or strains with a letter in common above the bar do not differ significantly according to Tukey's multiple test (a= 0.05)*.

The strain MCT IV significantly differed ($P<0.05$) from strains "C" I, "C" III and "C" V according to Fisher's criterion. Strain MCT IV showed the greatest pathogenic and toxic quality. It was reduced percent of seed germination of winter rye on 50 % in comparison with control. Differences between strains *F. culmorum w*ith MKT I, III and V were inessential.

They were reduced percent of seed germination of seeds on 61 % on average.

Distinctions in percent of seed germination on cultivars were significant (P<0.05). The least susceptible to cultural suspension and culture filtrates were cultures of rye Purga and Falenskaja 4 – percent of seed germination was 67 % in comparison with control. Genotype of rye Vjatka 2 was the most susceptible to studied strains. Percent of seed germination was 50 % in average.

Influence of cultivar-strain interactions was also statistically significant (P<0.05), separate distinctions between values of average percent of seed germination have been insignificant and marked with identical letters above the bars (fig. 3, 4). Each bar represents the mean of experiments with ten replications for cultivar-strain combinations averaged across 4 strains *Fusarium culmorum* or 5 winter rye cultivars with the purpose of revealing their influence on percent of seed germination. Cultivars or strains with a letter in common above the bar do not differ significantly according to Tukey's multiple test (a = 0.05)*.

The analysis of variance has showed significant influence of factors (cultivars and strains) on length of root of seeds of winter rye. The strains MCT IV and MCT I significantly differed (P<0.05) from strains "C" III and "C" V according to Fisher's criterion (fig. 5, 6). These strains were characterized high pathogenic qualities. Strain MCT V was characterized less pathogenic qualities to length of root. Strain MCT IV was characterized high toxic qualities to length of root, and strain MCT I was characterized less toxic qualities.

Distinctions in length of root of seeds of winter rye were significant (P<0.05). The least susceptible to cultural suspension were culture of rye Falenskaja 4 – length of root was 66 % in comparison with control. The least susceptible to culture filtrates were culture of rye Falenskaja 4, Purga and Bezenchukskaja 88. The most susceptible to culture filtrates were culture of rye Snezhana.

So *F. culmorum* showed high variability pathogenic and toxic qualities. It is necessary to note at the selection of resistance cultivars.

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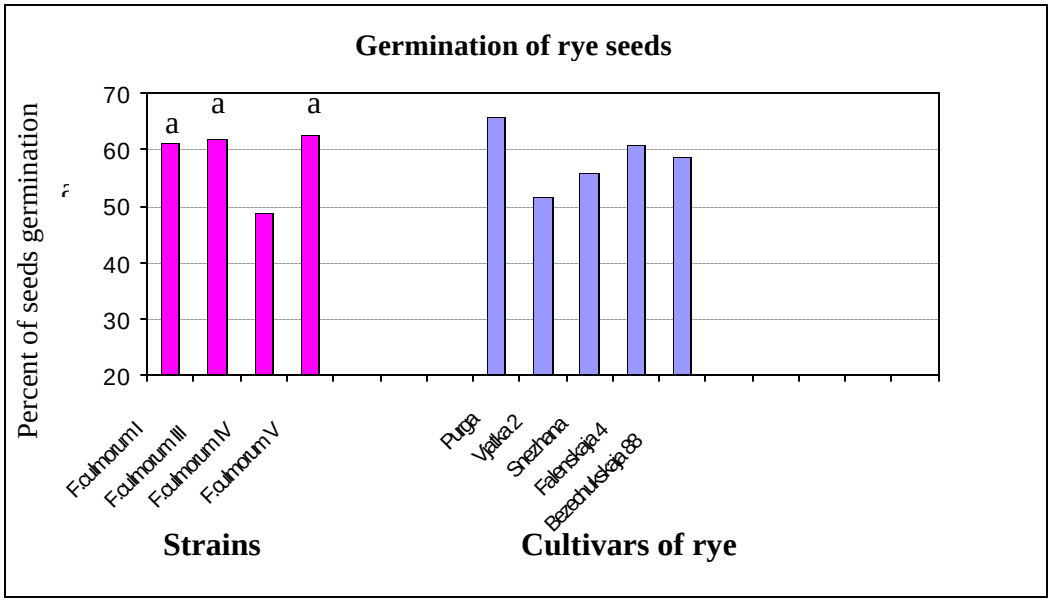

Figure 1. Mean percent of seed germination of five winter rye cultivars inoculated with conidial suspension of four strains *Fusarium culmorum* with various morphological - cultural types (? C?) of colonies (I, III, IV and V*).*

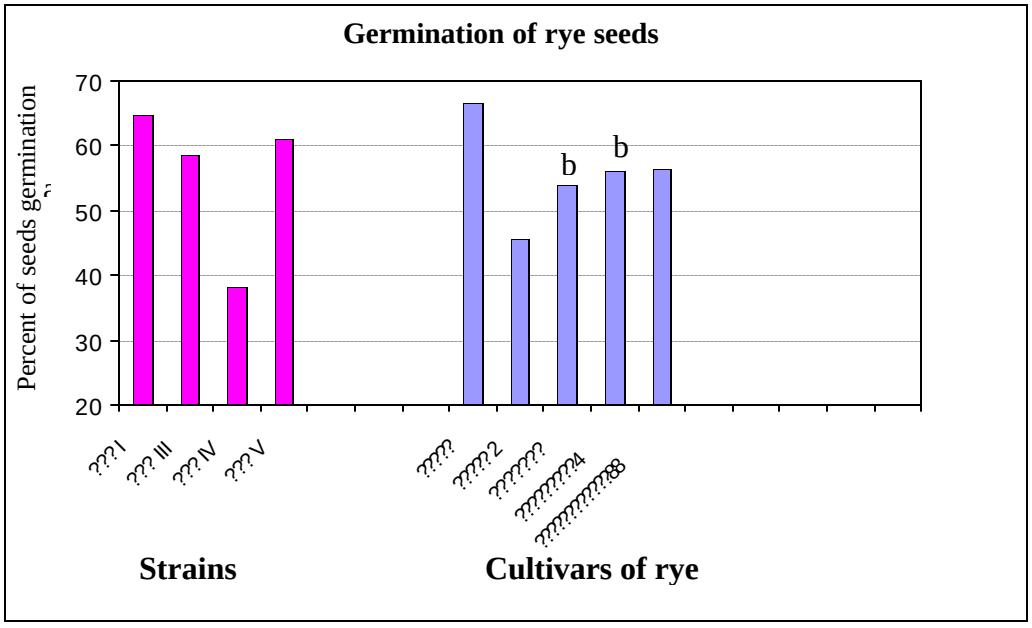

Figure 2. Mean percent of seed germination of five winter rye cultivars inoculated with culture filtrates of four strains *Fusarium culmorum* with various ? C? of colonies (I, III, IV and V*).*

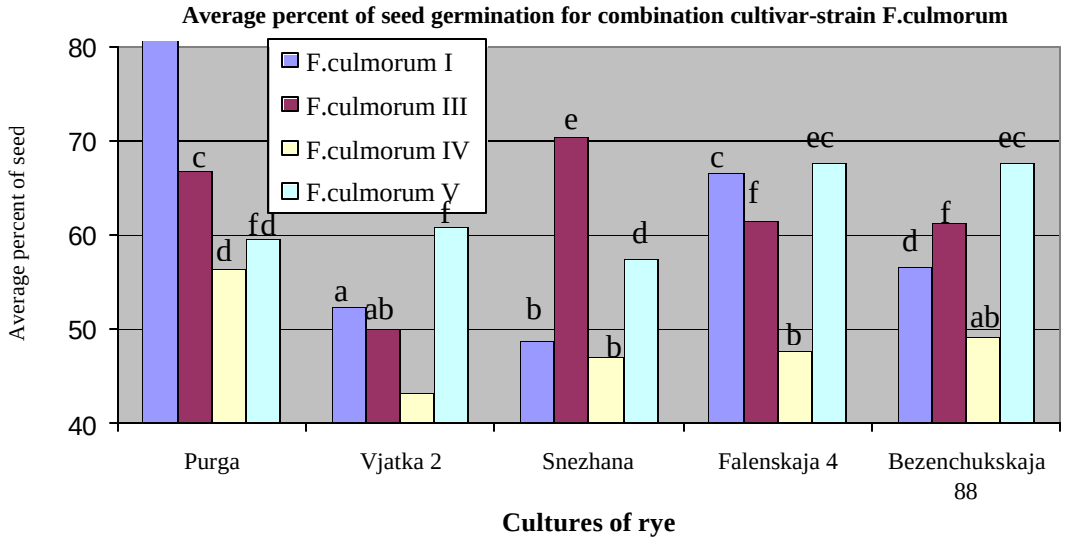

Figure3. Mean percent of seed germination of five winter rye cultivars inoculated with conidial suspension of four strains *Fusarium culmorum* with various ? C? of colonies (I, III, IV and V*).*

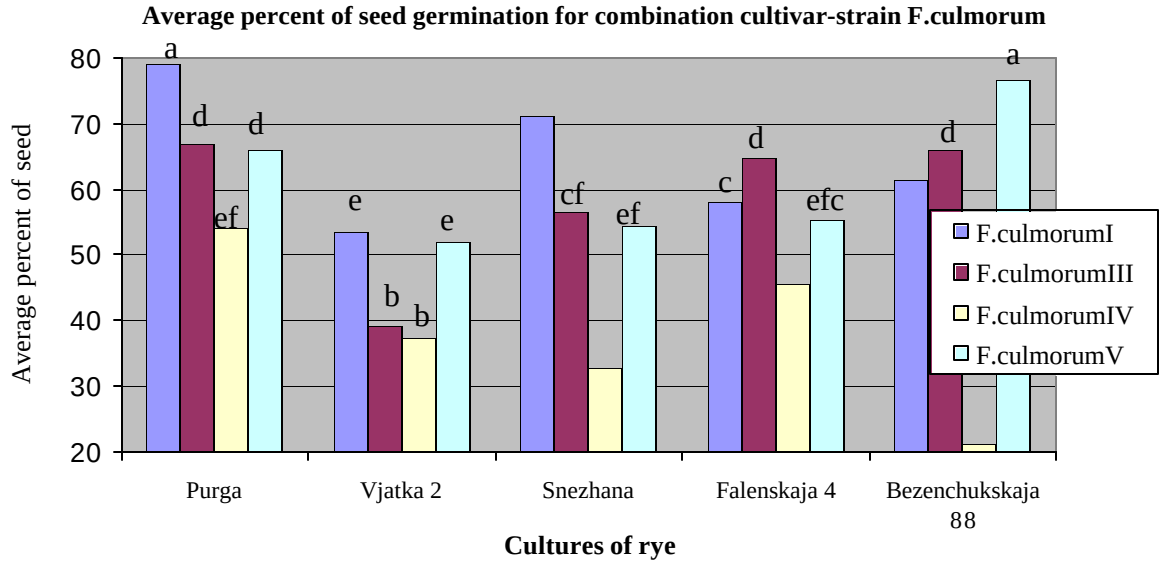

Figure 4. Mean percent of seed germination of five winter rye cultivars inoculated with culture filtrates of four strains *Fusarium culmorum* with various ? C? of colonies (I, III, IV and V*).*

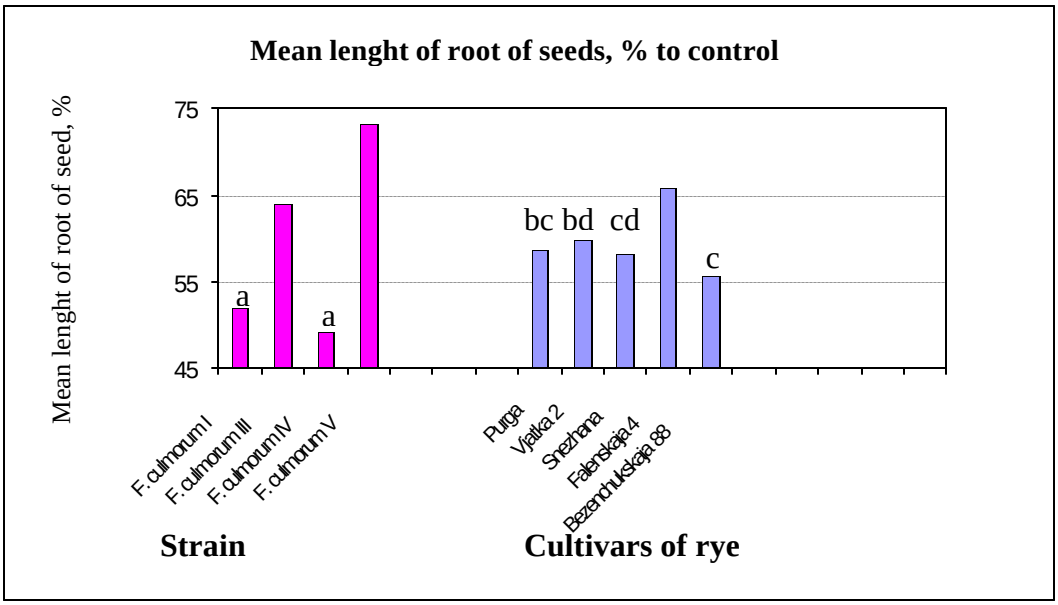

Figure 5. Mean length of root of seeds of five winter rye cultivars inoculated with conidial suspension of four strains *Fusarium culmorum* with various morphological - cultural types (? C?) of colonies (I, III, IV and V*).*

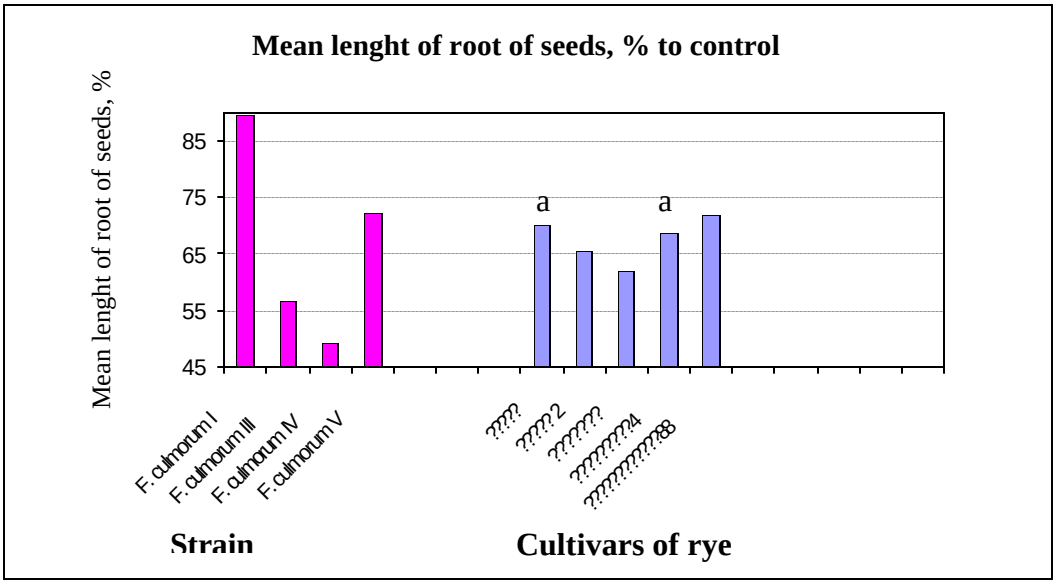

Figure 6. Mean length of root of seeds of five winter rye cultivars inoculated with culture filtrates of four strains *Fusarium culmorum* with various morphological - cultural types (? C?) of colonies (I, III, IV and V*).*

MYCOTOXIN PRODUCTION AND LINEAGE DISTRIBUTION IN CENTRAL EUROPEAN ISOLATES OF THE *FUSARIUM GRAMINEARUM* CLADE

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ABSTRACT

Fusarium head blight caused mainly by *Fusarium graminearum* and *F. culmorum* is the most important disease of wheat in Central Europe. Contamination of wheat by the mycotoxins produced by these and other species is the most serious effect of FHB, since these mycotoxins are harmful to both humans and animals. We examined the mycotoxin producing abilities, aggressiveness and molecular variability of *Fusarium graminearum* isolates using different techniques. Altogether 26 Hungarian, three Austrian isolates and representatives of eight species identified in the *F. graminearum* clade were involved in this study. Mycotoxin producing abilities of the isolates were tested by GC-MS and HPLC. The mycotoxins tested included type B trichothecenes (deoxynivalenol, 3- and 15-acetyl-deoxynivalenol, nivalenol, 4-acetyl-nivalenol (fusarenone X)) and zearalenone. All but one of the isolates produced zearalenone. The Central-European isolates were found to belong to chemotype I (producing deoxynivalenol). Most isolates produced both 15- and 3-acetyl-DON, but the majority of them produced more 15-acetyl-deoxynivalenol than 3-acetyl-deoxynivalenol suggesting that they belong to chemotype 1b. Huge differences were observed among DON producing abilities of the isolates (54-16.000 mg kg^{-1}). All *F. graminearum* isolates were found to be highly pathogenic in *in vitro* aggressiveness tests. During previous studies, most Central-European isolates were found to belong to lineage 7 characteristic to the Northern hemisphere, with the exception of one Hungarian isolate based on RAPD and IGS-RFLP data, and two other Hungarian isolates based on mitochondrial DNA RFLP analysis. We carried out sequence analysis of a putative reductase gene to ascertain the taxonomic position of these isolates. Sequence data confirmed that these isolates do not belong to the *F. graminearum* species, but represent isolates of the species *F. boothii* and a so far undescribed species closely related to *F. asiaticum*. The presence of mating type gene homologs have also been studied in the Central European isolates. All isolates carried both mating type idiomorphs in accordance with previous findings. Further work is in progress to compare the pathogenicity of the isolates belonging to different lineages in field tests.

USING GENOMICS TO UNDERSTAND THE LIFE CYCLE OF *GIBBERELLA ZEAE*

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ABSTRACT

In the last two years, the genome of *Gibberella zeae* has been sequenced and genome-based microarray chips have become available. These tremendous resources will provide an opportunity to study the life cycle of this important plant pathogen at a level not previously within reach. Over the last several years, we have characterized the development of perithecia *in vitro*, and more recently, we have elucidated the process of colonization of wheat tissue which leads to perithecium production. The latter studies have revealed the formation of vascular occlusions which prevent mycelia from colonizing the stem tissue. We have also been investigating the mechanism of forcible spore discharge in ascospore-producing fungi. For over 100 years, the working hypothesis has been that turgor pressure drives ascospore ejection. For the first time, we have shown 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 controlling mechanism of ascospore discharge. These findings shed light on the environmental factors that influence spore discharge in the field.

How can we use genomics to extend these studies? We have begun to elucidate the gene expression shifts that accompany sexual development *in vitro* using a limited EST-based microarray. 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. We will be able to extend this survey to all of the genes in the genome with the new genome chip, and pinpoint those important for each developmental stage. We can identify the genes regulated by the DNA binding protein controlling discharge of spores, and thereby uncover the process whereby environmental factors are translated into spore release.

We have already used the genomic sequence to understand the production of mycotoxins. 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. We used the recently released genomic sequence 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.

These studies provide new targets for control of this devastating pathogen. Elucidation of the developmental processes underlying the disease cycle and the response of the pathogen to the environment is an important step towards developing integrated control strategies. This research provides information about infection pathways and serves as a basis for these and future investigations into the genetics of host-pathogen interactions.

GECCO: A BIOINFORMATICS TOOL FOR COMPARATIVE ANALYSIS OF FUNGAL GENOMES

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ABSTRACT

Decisive steps in the interactions between hosts and pathogens occur at the onset of infection. During these stages the fungal biomass is still very limited and the majority of mRNAs will originate from the host. To get a better understanding of the genes involved in the infection process we generated cDNA libraries of infected plants. In order to avoid sequencing large numbers of ESTs, before obtaining fungal genes, we have developed a procedure that specifically enriches for fungal sequences during the early (and later stages) of infection. This allowed us to generate large datasets containing pathogen genes involved in the early stages of pathogenesis in various pathosystems. Together with the huge amounts of data available through several genome-sequencing efforts (Whitehead, Sanger, TIGR, JGI/DOE, Genescope) there is a great need for bioinformatics tools to mine and compare these large datasets. A bioinformatics platform has been developed that performs automated analysis of sequence datasets and allows for fast and robust comparison of different databases. Among 1724 *F. graminearum* in *planta* unigenes, we identified three ABC transporters that were not present in the annotated genome of *Magnaporthe grisea*, two of which were also absent in the *Neurospora crassa* genome. Among 4452 *in planta* unigenes from *Mycosphaerella graminicola*, we identified 41 unigenes involved in signal transduction, four of which were not previously identified in *N. crassa*.

AN EVOLUTIONARY FRAMEWORK FOR TACKLING FUSARIUM HEAD BLIGHT; SPECIES RECOGNITION, TOXIN EVOLUTION, AND BIOGEOGRAPHY OF THE *FUSARIUM GRAMINEARUM* SPECIES COMPLEX

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ABSTRACT

Effective disease control programs that minimize the threat of FHB to the producers, processors, and consumers of wheat and barley require a detailed understanding of pathogen diversity. Although the primary etiological agent of FHB, *Fusarium graminearum*, has been regarded as a single, panmictic species worldwide, we previously used genealogical concordance phylogenetic species recognition (GCPSR) to demonstrate that this morphospecies actually consists of at least nine phylogenetically distinct and biogeographically structured species (the *Fg* complex).

Here we report the identification of several additional species within the *Fg* complex, including a novel species isolated from wheat within the U.S. In addition to the extraordinary species diversity that we have discovered, we have also demonstrated that the virulence-associated trichothecene mycotoxin genes are under a novel form of balancing selection, which may have important consequences for the fitness and aggressiveness of FHB pathogens on particular hosts or in particular environments. It appears that only a fraction of FHB species/chemotype diversity is currently represented within North America. Therefore, the introduction of novel FHB pathogens or chemotypes via global trade in agricultural products has the potential to exacerbate the FHB problem in the U.S. Using a unique multi-locus DNA sequence database (11 nuclear genes, 13.6 kb of DNA sequence) we have developed a high-throughput single tube assay for the simultaneous identification of all known B-trichothecene FHB species and chemotypes in order to improve disease surveillance efforts and to facilitate a greater understanding of the ecology, epidemiology, and population dynamics of these FHB pathogens.