ETIOLOGY, EPIDEMIOLOGY AND DISEASE FORECASTING

EFFECTS OF DON ON BARLEY LEAF TISSUES, SUMMARY OF RESULTS.

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ABSTRACT

DON, a trichothecene toxin produced by *Fusarium graminearum*, is postulated to have a role in pathogenesis in Fusarium head blight of barley and wheat. To understand possible roles of DON, we investigated effects of the toxin in healthy tissues. Portions of epidermis were removed from 1.1 cm barley leaf segments and the segments floated with exposed mesophyll cells in contact with DON solutions. Within 2-4 days, DON at 10-90 ppm had a bleaching effect on chloroplast pigments and damaged cell membranes, including the plasmalemma (Bushnell et al., 2002; 2005). Pending more complete publication of this work (Bushnell et al., 2007), the major findings and conclusions of this project are presented here.

1. The bleaching of leaf tissues was light dependent. Tissues turned white in light as they lost chlorophylls a and b, as well as carotenoids pigments. In the dark, tissues remained green but became flaccid and easily damaged when manipulated with forceps.

2. In both light and dark, DON damaged integrity of the plasmalemma as shown by electrolyte leakage and uptake of Evans blue. Damage was somewhat greater in dark than in light. We conclude that toxicity of DON is not light dependent and that photobleaching is a secondary consequence of damage to cell membranes and not a direct cause of cell degradation.

3. The first membrane to be damaged, as viewed by transmission electron microscopy, was the tonoplast (Bushnell et al., 2005). This allowed cytoplasm to disperse into the vacuole, an irreversible step toward cell death. This preceded dissolution of the plasmalemma, mitochondria, and chloroplasts, as well as the loss of chloroplast pigments.

4. Damage to membranes and chloroplasts apparently is related to the known ability of DON to inhibit cytoplasmic protein synthesis. Cycloheximide, an inhibitor of protein synthesis in cytoplasm of eukaryotes, caused photobleaching of barley leaf segments in our experiments much like the photobleaching caused by DON. Chloramphenicol, an inhibitor of protein synthesis in chloroplasts and prokaryotes, had little or no effect on leaf pigmentation.

5. Ca^{2+} added at 10 mM to test solutions greatly increased toxicity of DON. With Ca^{2+}, DON at 10 ppm bleached leaf segments; without Ca^{2+}, concentrations of 30 ppm or higher were required. The reasons for DON's effectiveness need investigation. In any case, differences in sensitivity to DON among genotypes of barley and wheat or plants at different stages of development, may relate to variations in Ca^{2+} availability within tissues.

6. Fumonisin B_1 (FB_1), a non-trichothecene toxin produced by *F. moniliforme*, caused photobleaching and membrane damage in jimsonweed leaves as reported by Abbas et al. (1992). The results were remarkably

similar to effects of DON in our experiments with barley leaves. FB_1 is known to inhibit ceramide synthesis, disrupting sphingolipid metabolism. Direct comparisons of effects of DON and FB_1 are warranted.

7. As summarized elsewhere (Bushnell et al., 2007), indirect lines of evidence indicate that degradation of cell membranes in DON-treated barley cells is a consequence of programmed cell death (PCD). DON is known to induce PCD (apoptosis) in animal cells. Dissolution of the tonoplast, as occurred in response to DON, is a primary event in plant PCD. Furthermore, Ca^{2+} is required and can enhance plant PCD, much as it enhanced DON toxicity. Both cyclohexamide and FB_1, which cause photobleaching in plants, have induced PCD in animal cells. Finally, anti-PCD genes introduced in plants have provided partial resistance to several necrotrophic pathogens. The role of PCD in response to DON needs additional physiological and molecular investigation, including the apparent link between PCD and inhibition of protein synthesis.

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DURATION OF POST-FLOWERING MOISTURE AFFECTS FHB AND DON IN WHEAT.

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ABSTRACT

Our understanding of how environmental and host genetic influences interact to determine DON concentrations in small-grain spikes is incomplete. High levels of DON have sometimes been observed in the absence of abundant disease symptoms. This multi-year experiment explores the influences of post-flowering moisture duration, infection timing, and cultivar resistance differences on FHB and DON in winter wheat. We conducted two one-year trials of a split-plot experiment in a misted nursery in Kinston, North Carolina. The main plots were four durations of post-flowering misting (0, 10, 20, or 30 days). Sub-plots were soft red winter wheat cultivars with different degrees and putative types of resistance to FHB. In 2005, one susceptible cultivar and six moderately resistant cultivars were planted; in 2006, an additional susceptible cultivar was added to the experiment. Within each irrigation regime, one plot of each cultivar was inoculated with 10^5 *Fusarium graminearum* macroconidia/ml using a backpack sprayer at flowering. In another plot of each cultivar under each irrigation regime, 40 heads received individual inoculation with a spray bottle at each of the following times: 0, 10, or 20 days post-flowering, or never. All treatment combinations were replicated three times. Spike samples were collected at normal harvest time and dissected into glume, rachis, and grain fractions. In addition, samples were randomly gathered from plots backpack-inoculated at flowering at approximately 10-day intervals from two weeks post-flowering through harvest time. Tombstone percentages were determined. DON was assayed in all tissue fractions by ELISA. In 2005, samples from replicated plots inoculated at flowering and misted for 30 days were also assayed for fungal DNA concentration using RT-PCR, and in 2006 this assay is being conducted on samples from all treatment combinations.

In 2005, disease levels were very light. There was no significant relationship between post-flowering moisture duration and either disease or DON levels. No treatment combination was identified in which visual symptoms were low while DON levels were high. In plots backpack-inoculated at flowering and misted for 30 days, the quantity of fungal DNA in each tissue type was positively correlated with the DON concentration. This result is consistent with the hypothesis that the degree of fungal infection within each tissue type determined how much DON was found there at harvest, as opposed (for example) to significant amounts of DON being translocated or leached into grain from infections in glumes or rachis. Further, fungal DNA in each tissue fraction was positively correlated with that in each other tissue fraction, suggesting that the relative degree of resistance to infection in each cultivar was similar among tissue types.

In 2006, a more severe FHB epidemic developed. Both FHB incidence and severity increased significantly ($P < 0.05$) as the duration of post-flowering misting increased up to 20 days. There was no further increase in incidence or severity after 30 vs. 20 days of misting. The disease severity present on individual cultivars did not vary by mist duration in either year analyzed individually, nor in both years combined. The 2006 data suggest that the number of moist days following flowering can significantly increase FHB severity. Assays of DON and RT-PCR for the 2006 samples are currently underway.

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FIELD RELEASE OF *GIBBERELLA ZEAE* GENETICALLY MODIFIED TO LACK ASCOSPORES.

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INTRODUCTION AND OBJECTIVE

Gibberella zeae (asexual state *Fusarium graminearum*) causes wheat head blight (WHB) epidemics worldwide, reducing seed yield and contaminating seed with deoxynivalenol (DON) and other trichothecene toxins. Since 1990, WHB epidemics on wheat and barley have increased in frequency and severity in North America. Reemergence of this plant disease has been associated with the intensification of wheat-maize crop rotations and use of conservation tillage practices that leave large amounts of maize stalk pieces and other crop residues on the soil surface (Parry et al. 1995, McMullen et al. 1997). *G. zeae* is self-fertile and can produce macroconidia (asexual spores) and perithecia with ascospores (sexual spores) during WHB epidemics. The importance of *G. zeae* spores in WHB had been recognized by the late nineteenth century, but the relative contribution of ascospores and macroconidia to WHB epidemics is still debated. On the one hand, ascospores often are recovered at higher counts than macroconidia from traps that sample airborne spores in the field (Shaner 2003). On the other hand, some *Fusarium* species such as *F. avenaceum* and *F. culmorum*, which rarely or never produce ascospores, have produced severe WHB epidemics in Europe and Canada, indicating that macroconidia are sufficient to cause epidemics under some conditions (Parry et al. 1995, Waalwijk et al. 2003).

We previously generated *MAT*-deletion strains that lack ascospores and *MAT*-complemented strains that have regained ascospore production (Turgeon 1998, Desjardins et al. 2004). Both *MAT*-deletion and *MAT*-complemented strains were similar to the wild-type (WT) strain in production of macroconidia and DON in culture. Furthermore, in greenhouse tests, both *MAT*-deletion and *MAT*-complemented strains were virulent when their macroconidia were injected directly into wheat heads at anthesis, and the resulting infected wheat heads were contaminated with DON. The apparent absence of major pleiotropic effects of *MAT*-locus deletion on *G. zeae* radial growth, morphology, pigmentation, macroconidium production, DON production, and virulence on wheat in the greenhouse indicated that these mutants might be suitable for testing under field conditions. The objective of the present study was to investigate the importance of ascospores in WHB epidemics by conducting a series of controlled-release field tests in which *MAT*-deletion strains that lack ascospores were compared to WT and *MAT*-complemented strains that produce ascospores.

MATERIALS AND METHODS

Microbiology. *G. zeae* strain GZ3639 isolated from scabby wheat in Kansas was the WT and progenitor of all transformants (Table 1). All strains were generated and characterized as previously described (Desjardins et al. 2004). For wheat head injection, macroconidium inoculum (5×10^4 spores/ml) was prepared in mung bean liquid medium (Bai & Shaner 1996). Each head was injected with approx. 1000 spores, and controls with mung bean medium. A natural substrate inoculum for field tests was prepared from maize stalk pieces as described (Maldonado-Ramirez & Bergstrom 2000). Dried, mature maize stalks were cut into pieces approx. 15 cm long, sterilized by autoclaving three times, placed in 2.8 L Fernback flasks and inoculated with several blocks of fungal culture material cut from plates of V-8 juice agar medium.

Flasks were incubated for 4 weeks at $20 \pm 1°C$, under continuous illumination provided by an equal mixture of fluorescent white and black (General Electric BLB, 40 W) light bulbs. After 4 weeks, all fungal strains produced hyphae and macroconidia on the surfaces of the stalk pieces, but only WT and *MAT*-complemented strains also produced perithecia, which were checked microscopically to confirm the presence of mature ascospores. For fungal strain recovery, seeds were surface-disinfested and placed on a *Fusarium* selective medium (Nelson et al. 1983). *G. zeae* colonies from individual seeds were designated as isolates to distinguish them from the single-spored strains that were applied as treatments in the field tests. Because isolates were not purified by single-sporing, they may have contained more than one genotype. Isolates were subcultured to agar medium with hygromycin B (hyg) at 300 $\mu g\ ml^{-1}$ or geneticin (gen) at 1 $mg\ ml^{-1}$ and scored as resistant or sensitive by their radial growth. For selected isolates, DNA was purified and subjected to PCR amplification using standard methods and primers complementary to the *G. zeae Tri5* gene and to the *hygB* gene construct used for transformation (Desjardins et al. 2004).

Field test sampling and deoxynivalenol analysis. Field tests were conducted under permit from USDA-APHIS. Wheat heads were tagged after heading, and only tagged heads were harvested in order to avoid selection bias. Heads were harvested at maturity, individually hand-threshed, pooled, and weighed to determine yield for each plot. For field test 1, 100 seeds per plot were saved for fungal strain recovery analysis and the remaining seeds were randomly assigned to one of 5 pools for DON analysis. For field tests 2, 3 and 4, heads from each plot were randomly assigned to 1 of 2 pools; half of each pool was ground for DON analysis, and half was sampled for fungal strain recovery analysis. Seed samples were analyzed for DON (the only trichothecene detected at significant levels) by liquid chromatography-mass spectrometry (LC-MS), with triplicate injections of each extract (Plattner & Maragos 2003).

Statistical analyses. A randomized complete block design was utilized in the 2002 and 2003 field tests to compare seed yield, DON contamination, head bleaching symptoms, and fungal strain recovery among treatments. Levene's homogeneity of variance tests at the 5% alpha levels were performed to determine if any data transformations of the dependent variables were necessary. One-way ANOVA tests were performed for each field test to detect differences among treatments in seed yield, DON contamination, head bleaching symptoms, and fungal strain recovery. F-test statistical results were considered significant at $p \leq 0.05$. Duncan's multiple range tests (alpha = 5%) were used as the multiple comparison procedure to find treatment mean differences in dependent variables if a significant F-test statistic was obtained from ANOVA.

RESULTS

Field Test 1 Plan. Field test 1 was a nonreplicated maize stalk treatment test conducted in 2001 at the Christ family farm near Peoria, Illinois, which also was the location for tests 2 and 3. In April, 3 plots of susceptible spring wheat cultivar Wheaton (3×3 m) were planted and separated from each other by 3 m of cultivated ground to minimize cross-contamination. For tests 1-4, the field test site was surrounded by a perimeter of 10 m of cultivated ground. From seedling emergence to seed maturity, each plot was mist-irrigated for 30 min, 4 times daily. In May, 2 to 3 weeks before first anthesis, each of the 3 plots was surrounded by wire-mesh fencing and 100 fungal-treated or control maize stalk pieces were placed on the ground in each plot. An additional 100 pieces were placed in each plot 2 weeks later. **Results.** In field test 1, WT strain, one *MAT*-deletion strain, and control were compared (Table 2). During this test, conditions were conducive for development of WHB, with daytime temperatures within the range of 15-30°C for 30 of the 45 critical days from 1 June to mid-July. The WT strain reduced yield by 27% and increased DON contamination by 180% compared to control. The *MAT*-deletion strain did not reduce yield or increase DON when compared to control. Seed infection with *G. zeae* also was higher in the plot infected with the WT strain than in the other 2 plots. *G. zeae* isolates were recovered from 63% of seeds from the plot treated with the *MAT*-deletion strain, but hyg-resistant isolates were recovered from only 1% of seeds in this plot and from no seeds in the other 2 plots. These

data indicate that DON contamination in the plot treated with the *MAT*-deletion strain was due to cross-contamination by the WT strain or by naturally occurring *G. zeae* strains.

Field Test 2 Plan. Field test 2 was a replicated maize stalk treatment test conducted in Illinois in 2002. In April, 12 plots of susceptible spring wheat cultivar Norm (3 × 3 m) were planted, separated from each other by 3 m of cultivated ground, and the entire test site was surrounded by a wire-mesh fence. The test was a randomized complete design with 6 treatments and 2 replicate plots per treatment. Each plot was mist-irrigated for 15 min, 4 times daily and 100 maize stalk pieces were applied to plots twice, as in field test 1. **Results**. In field test 2, 3 ascospore-producing strains, 2 *MAT*-deletion strains, and control were compared (Table 2). During this test, conditions were not conducive for WHB, with daytime temperatures above 30°C for 33 of 46 critical days from mid-June to the end of July. The 3 ascospore-producing strains reduced yield an average of 17%, which was significantly different from controls ($p \leq 0.05$), and caused DON contamination an average of 0.9 µg/g, which was not significantly different from controls. The 2 *MAT*-deletion strains reduced yield an average of 12% and caused DON contamination an average of 0.5 µg/g, neither of which was significantly different from controls. None of the strains increased seed infection with *G. zeae*, which averaged 44% for all treatments. *G. zeae* isolates were recovered from 34% of seeds from the 4 plots where *MAT*-deletion strains had been applied, but hyg-resistant isolates were recovered from only 4% of seeds in these plots and 0.5% of seeds from other plots.

Field Test 3 Plan. Field test 3 was a replicated, combined wheat head injection and maize stalk piece treatment test conducted in Illinois in 2003. In April, 12 plots of cultivar Norm (3 × 3 m) were planted as in field test 2. Two plots were used for injection of macroconidia into heads. The remaining 10 plots were used for maize stalk treatment, in a randomized complete design with 5 treatments and 2 replicate plots per treatment. Each plot was mist-irrigated for 30 min, 4 times daily. After stalk piece treatments were applied, all 12 plots individually were covered with lightshade cloth. Heads at mid-anthesis were injected with macroconidia in 2 replicate plots, each with 5 treatments and 60 heads per treatment. 100 maize stalk pieces were applied to plots twice, as in field test 1. **Results**. In field test 3, WT strain, 2 *MAT*-complemented strains, a *MAT*-deleted strain, and control were compared (Table 2). During this test, conditions were conducive for development of WHB, with daytime temperatures within the range of 15-30°C for 34 of the 45 critical days from June first to mid-July. Following head injection, all 4 strains caused an average head bleaching intensity of 92%, yield reduction of 54%, and DON contamination of 29 µg/g. All strains were significantly different from controls, but the *MAT*-deletion strain was not different from the ascospore-producing strains by any of the parameters tested (data not shown). Following maize stalk treatment, the 3 ascospore-producing strains reduced yield an average of 20% and caused DON contamination an average of 14.3 µg/g, both of which were significantly different from control. The *MAT*-deletion strain caused no yield loss and a DON contamination of 7.2 µg/g, which was not significantly different from control. None of the strains increased seed infection with *G. zeae,* which averaged 55% for all treatments. Despite the high level of seed infection with *G. zeae*, hyg-resistant, gen-sensitive isolates were recovered from only 3% of seeds from the 2 plots where the ascospore-nonproducing strain was applied. In contrast, hyg-resistant, gen-resistant isolates were recovered from an average of 34% of seeds, and accounted for 61% of the *G. zeae*, from the 4 plots where *MAT*-complemented strains were applied. Both classes of antibiotic-resistant isolates were each recovered from an average of 10% of seeds from plots where they were not applied.

Field Test 4 Plan. Field test 4 was a replicated, combined wheat head injection and maize stalk piece treatment test conducted in 2003 at the Purdue University Agronomy Farm near West Lafayette, Indiana. In September 2002, susceptible winter wheat cultivar Patterson was sown in rows spaced 18-cm apart. The entire test site was surrounded by wire-mesh fencing and 10 m of cultivated ground, and was not irrigated. For direct injection of macroconidia into heads, two blocks of 5 plots (each 1 m long and one row wide)

were delineated at opposite ends of the test site. Each plot was used for one treatment, with 50 heads per treatment. At mid-anthesis, heads were injected with macroconidia and groups of heads were covered with a plastic bag for 1 day. For maize stalk treatment, 10 plots (3×2.5 m) were delineated and separated from each other by 3 m of cultivated ground. The test was a randomized complete design with 5 treatments and 2 replicate plots per treatment. In April, 100 maize stalk pieces were placed on the ground in each plot. **Results**. In field test 4, WT strain, 2 *MAT*-complemented strains, a *MAT*-deleted strain, and control were compared (Table 2). In this test, disease levels were low for both head injection and maize stalk treatments, probably due to the lack of irrigation. Following head injection, the 4 strain treatments caused significant yield reductions, averaging 32%, but did not differ from controls in head bleaching or DON contamination (data not shown). In the maize stalk treatment, the 3 ascospore-producing strains tended to cause more yield reduction (average of 10%) and DON contamination (average of 5.2 $\mu g\ g^{-1}$) than the *MAT*-deletion strain (no yield reduction and 3.8 $\mu g\ g^{-1}$), but none of the treatments were significantly differently ($p \leq 0.05$) from controls. None of the strains increased head bleaching symptoms (data not shown) or seed infection with *G. zeae*, which was <10% for all plots. Hyg-resistant, gen-resistant isolates were recovered from an average of 2% of seeds from plots where *MAT*-complemented strains were applied. Hyg-resistant, gen-sensitive isolates were recovered from an average of 4% of seeds from plots where the *MAT*-deletion strain was applied.

DISCUSSION

The purpose of this study was to test the hypothesis that ascospores are the primary source of inoculum for WHB epidemics caused by *G. zeae*. To test this, we combined ecological and molecular approaches by conducting field tests under conditions that mimic natural WHB epidemics, and by using ascospore-nonproducing strains, generated by transformation-mediated *MAT*-locus deletion, and ascospore-producing strains, generated by *MAT*-locus complementation. In this complex ecological system, we were successful in obtaining WHB epidemics in field tests 1 and 3, but less successful in field tests 2 and 4. Overall, the 4 field tests of fungal-treated maize stalk pieces contained a combined total of 19 plots with ascospore-producing strains, 9 plots with ascospore-nonproducing strains, and 7 control plots. When compared to control plots, ascospore-producing strains caused a significant ($p \leq 0.05\%$) yield reduction in 58% (11 of 19) of plots where they were applied and a significant increase in DON contamination in 47% (9 of 19) of plots. In contrast, ascospore-nonproducing strains caused a significant yield reduction in only 22% (2 of 9) of plots where they were applied and no significant increase in DON contamination in any plots, as compared to control plots. In field tests 1 and 3, in which epidemics developed, the ascospore-nonproducing strains caused significantly less severe epidemics than did ascospore-producing strains. In tests 2 and 4, in which epidemics did not develop, differences between ascospore-producing and nonproducing strains may have been obscured by low levels of disease. This study demonstrates the feasibility of combining molecular and ecological approaches for analysis of a complex agroecosystem. This alternative approach has provided new evidence that ascospores can play a critical role in WHB epidemics, at least in Illinois. Ascospore-nonproducing strains of *G. zeae* could be useful tools to investigate the importance of ascospores in other agroecosystems, especially in regions of Europe where *G. zeae* appears to be displacing *F. culmorum* and *F. avenaceum* as the dominant WHB pathogen (Waalwijk et al. 2003). The importance of ascospores identifies the *G. zeae* sexual cycle as a potential target for control of a plant disease whose reemergence has serious consequences for farm economics and for food and feed safety worldwide.

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Table 1. *Gibberella zeae* strains used in this study.

Strain no.	Strain description	Genotype	Ascospore phenotype
GZ3639	Wild type	*MAT1-1/MAT1-2*	producer
GZ3639MT#39	Mock transformant	*MAT1-1/MAT1-2*	producer
GZ3639 MT#44	Mock transformant	*MAT1-1/MAT1-2*	producer
ΔMAT#14	Gene-deletion mutant	*mat1-1/mat1-2/hygB*^{R}	nonproducer
ΔMAT#40	Gene-deletion mutant	*mat1-1/mat1-2/hygB*^{R}	nonproducer
ΔMAT#73	Gene-deletion mutant	*mat1-1/mat1-2/hygB*^{R}	nonproducer
ΔMAT#78	Gene-deletion mutant	*mat1-1/mat1-2/hygB*^{R}	nonproducer
MAT-comp#9	Gene-complemented mutant of ΔMAT#78	*MAT1-1/MAT1-2/hygB*^{R}*/gen*^{R}	producer
MAT-comp#88	Gene-complemented mutant of ΔMAT#78	*MAT1-1/MAT1-2/hygB*^{R}*/gen*^{R}	producer

Table 2. Disease assessment and strain recovery after treatment with *G. zeae* on maize stalk pieces.

	Treatment		Disease assessment*		Strain recovery**		
Field Test	ascospore phenotype	strain	seed yield	DON	*G. zeae* (%)	HygR GenS	HygR GenR
1	producer	GZ3639	637	35.5	89	0	NA
	nonproducer	ΔMAT#14	855	9.7	63	2	NA
		control	869	12.6	53	0	NA
2	producer	GZ3639	698 c	1.7 c	59	0 a	NA
	producer	MT#39	828 abc	0.7 b	42	5 ab	NA
	producer	MT#44	723 bc	0.4 a	52	0 a	NA
	nonproducer	ΔMAT#40	752 bc	0.6 ab	38	10 b	NA
	nonproducer	ΔMAT#73	858 ab	0.4 a	30	11 b	NA
		control	908 a	0.6 ab	45	0 a	NA
3	producer	GZ3639	728 b	10.8 bc	54	10 a	15 a
	producer	MAT-comp#9	732 b	16.9 d	57	30 b	60 b
	producer	MAT-comp#88	692 b	15.1 cd	56	18 a	61 b
	nonproducer	ΔMAT#78	922 a	7.2 ab	56	6 a	25 a
		control	892 a	6.6 a	51	16 a	24 a
4	producer	GZ3639	790	4.7	10	3 a	20
	producer	MAT-comp#9	945	5.8	8	15 a	15
	producer	MAT-comp#88	835	5.0	10	0 a	25
	nonproducer	ΔMAT#78	960	3.8	8	48 b	14
		control	955	2.5	7	10 a	20

* Disease assessment methods: seed yield per head in mgs; DON = deoxynivalenol (μg/g seed dry weight) by LC-MS. Data are unreplicated (1 plot per treatment) in test 1, and are means of 2 replicate plots per treatment in tests 2, 3 and 4. For tests 2-4, means with the same letter are not different at $p \leq 0.05$; means without a letter indicate that none of the treatments were different. The total number of heads analyzed per treatment was 300 for test 1; approx. 600 for tests 2 and 3; 200 for test 4.

** *G. zeae* (%) = percentage seeds infected with *G. zeae*. HygRGenS = percentage *G. zeae* isolates hyg-resistant and gen-sensitive. HygRGenR = percentage *G. zeae* isolates hyg- and gen-resistant. Number of seeds tested per treatment was 63-100 for test 1, test 3, and control and ascospore producers in test 2; 500-800 for ascospore nonproducers in test 2; 300-450 for test 4. Number of isolates tested for hyg resistance per treatment was 50-82 for test 1; 20-40 for control and ascospore producers in test 2; 220-256 for ascospore nonproducers in test 2. Number of isolates tested per treatment for resistance to hyg and gen was 20-30. NA = not applicable because gen-resistant strains were not released in field tests 1 and 2.

SYSTEMIC COLONIZATION AND PRODUCTION OF DEOXYNIVALENOL THROUGHOUT WHEAT PLANTS FOLLOWING INOCULATION OF CROWN TISSUE WITH *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Fusarium Head Blight (FHB), incited by *Fusarium graminearum (Fg)*, has re-emerged as major disease, causing devastating losses in U.S. wheat producing states for over a decade. FHB poses an additional threat to industry as grain is contaminated with mycotoxins, particularly deoxynivalenol (DON). Crown rot (CR), which can also be caused by *Fg,* is an important and chronic problem in Australia and other similarly arid countries. Although DON has been reported as an aggressiveness factor in FHB, its role in the development of CR is unknown. Experiments, conducted in Australia, with a transgenic isolate of *Fg* (hygromycin resistant), examined the ability of *Fg* to systemically colonize wheat after crown inoculation using the wheat cv. Kennedy (CR susceptible). Following inoculation, *Fg* was recovered from 100% of crowns and 55% of heads. DON was detected at 275ppm in crowns and 7ppm in asymptomatic heads. An experiment, conducted in the U.S., examined three isolates of *Fg*; an highly aggressive (FHB) (B86A11) and competent DON producing isolate; a transgenic isolate (GZ40) lacking the trichodiene synthase (Tri5) gene; and the wild-type isolate (GZ3639) used to produce GZ40. Following crown inoculation of the wheat cv. Wheaton (FHB susceptible) the isolates were recovered from 50-95% of crowns and 5-21% of kernels. The recovery of *Fg* was lowest for GZ40. DON was detected at levels of 91ppm and 23ppm in crowns and 3ppm and 2ppm in kernels, for B86A11 and GZ3639, respectively. DON was detected in a few GZ40 inoculated plants, but below 1ppm, and probably arose from cross contamination in the growth chamber. These experiments demonstrate that crown infection can lead to systemic fungal colonization of wheat and DON production in all tissues, including kernels. The results suggest partial role for DON contributing to the extent of systemic colonization from the crown.

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EXPANDED HOST RANGE OF *FUSARIUM GRAMINEARUM* TO POTATO AND SUGARBEET.

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ABSTRACT

Fusarium graminearum is a well known pathogen of cereals, particularly wheat, barley and corn, where it can cause yield losses, and quality losses due to the production of numerous mycotoxins. Recently, *Fusarium graminearum* was isolated from stored potato tubers and sugarbeet roots showing field dry rot symptoms in North Dakota and Minnesota. The objective of this study was to determine the host range and pathogenicity of *F. graminearum* isolates collected from diseased potatoes and sugarbeet, compared to known *F. graminearum* isolates collected from wheat. Thirty-five isolates (20 from potato, five from sugarbeet and ten from wheat) were tested for the ability to cause disease in potato, sugarbeet and wheat. Potatoes cv. Russet Burbank and sugarbeets cv. Phoenix were inoculated by removing a plug from the tuber/root and replacing it with a mycelial plug of the *F. graminearum* and incubated at 14°C for 4 weeks. Wheat plants cv. Grandin were inoculated at anthesis by spraying the spikelets with a conidial suspension (4 x 10^4)/ml), incubated for 48 hours and maintained in the greenhouse for three weeks before measuring infection. Disease severity for potato and sugarbeet was estimated by obtaining a ratio of infected tuber/root area to total tuber/root area. Disease severity for wheat was determined using a visual scale for Fusarium Head Blight (FHB). *F. graminearum* isolates were pathogenic to all three crops, regardless of the original host they were isolated from. Typical FHB symptoms were observed in wheat, and both potato and sugarbeet tubers/roots showed typical dry rot symptoms. *F.* graminearum was not pathogenic to sugarbeet seedlings. These findings have major epidemiological implications for crop rotations and other disease management strategies for *F. graminearum.*

DIGITAL IMAGE ANALYSES, RELATIVE CHLOROPHYLL CONTENT, AND MICROSCOPIC EVALUATION OF LEAVES OF FRONTANA AND ALSEN INOCULATED WITH FOUR ISOLATES OF *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Seedlings of spring wheat cultivars Frontana and Alsen were grown in Cone-tainers in the greenhouse to the two-leaf stage. At 14 days post-planting, seedlings were inoculated in a split-plot design, with cultivars as main-plots and fungal isolates as subplots. Four different isolates of *F. graminearum*, obtained from the University of Minnesota Small Grains Pathology Project, St. Paul, were separately inoculated onto the adaxial surface of the middle of each cultivar's primary leaves. Each cultivar-isolate or control combination had ten replicates. Water inoculated primary leaves of seedlings for each cultivar provided control comparisons. Following inoculation, plants were maintained at nearly one hundred percent relative humidity at 23°C for 72 h in an incubation chamber. Lighting was provided under a 12 h light:dark period while plants were incubating. Following incubation, plants were removed to lab benches beneath artificial lighting at temperatures from 21 to 23°C for another 24 h. At 96 h post-inoculation primary leaves were excised at their base near the ligule and placed on a photographic stage adaxial side upwards. Leaves with lesions were photographed using a high-resolution digital camera. Images were analyzed using the Assess digital image analysis software obtained from the American Phytopathological Society Press. Threshold levels of lesion area were established by setting the hue, saturation, and intensity indices of the program to discriminate lesions of inoculated leaves relative to non-inoculated control leaves to provide differentiation of chloroses and necroses versus healthy appearing leaf area. Leaves were then measured for their relative chlorophyll content at the point of inoculation using an Opti-Sciences CCM-200 chlorophyll content meter in two of the six experiments we conducted. Averaged over the inoculation treatments for the six experiments, mean percent lesion area of inoculated leaves of Frontana was 0.23 % and was significantly lower ($P<0.001$) than for Alsen, which was 6.31 %. Significant differences were observed among inoculation treatments ($P<0.001$) of *F. graminearum* and there was a significant cultivar by isolate interaction ($P<0.001$) for percent lesion area assessments. Over the two experiments where relative chlorophyll content was measured on inoculated primary leaves, mean measurements of Frontana showed significantly ($P<0.001$) more chlorophyll content (greener leaves measure with higher numbers) and measured 6.63 whereas Alsen's primary leaves measured 3.27. UV-microscopy and brightfield microscopic observations of inoculated primary leaves of the two cultivars will be discussed.

STRATEGIES TO REDUCE *FUSARIUM* AND MYCOTOXIN CONTAMINATION IN NORWEGIAN CEREALS.

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ABSTRACT

Increasing levels of *Fusarium* toxins have been recorded in Norwegian cereals during the last few years. In 2004, some grain lots with unusually high levels of deoxynivalenol (DON) were recorded, in particular in oats (e.g. 25-30 mg/kg). Alarming levels of T-2 and HT-2 were recorded in oats in 2005. In Norway, only a limited number of check samples of grains used for food and fodder are analyzed for mycotoxin content. To reduce the risk of contaminated cereals entering the food and feed processing chain, a four-year project (2006-2009) was started at *Bioforsk-Norwegian Insititute for Agricultural and Environmental Research.* We are aiming to establish a three-step screening system in order to identify grain lots with high levels of *Fusarium* toxins: **1**- Identify 'high-risk' fields/lots, based on information on cultivation practice and climatic conditions, through the use of a FHB-prediction model. **2**-Analyze the 'high-risk' lots with a rapid test method selected due to its capacity to screen for *Fusarium* toxins in a large number of grain samples at low costs. **3**- Forward selected samples (based on analyzes in step 2) to chemical mycotoxin analyzes.

Fusarium avenaceum , *F. culmorum*, *F. poae* and *F. tricinctum* have been the most frequently recorded *Fusarium* species on cereals in Norway for many years. However, more recently also *F. graminearum* has occurred more frequently, and *F. langsethia* has been detected especially in oats. Investigations will be carried out to clarify if there has been a change in the composition of Fusarium species.

EFFECT OF CORN RESIDUE LEVEL, FUNGICIDE APPLICATION, AND CULTIVAR RESISTANCE LEVEL ON DISEASE INCIDENCE AND SEVERITY OF FUSARIUM HEAD BLIGHT AND DON CONCENTRATION.

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ABSTRACT

Previous cooperative experiments conducted in 2003 and 2004 showed that the presence of corn residue did increase the risk of Fusarium head blight (FHB) in many years, and that 14% or 80% ground cover often resulted in similar levels of disease intensity. The result also indicated that eliminating local sources of inoculum alone may not be enough to provide satisfactory reduction in disease and DON (deoxynivalenol) when disease pressure is extremely high. Experiments were conducted in IN, ND, OH, PA, and SD during the 2005 and 2006 growing seasons to evaluate the effect of corn residue level, fungicide application at flowering, cultivar resistance, and their interactions on FHB. The experiment was a split-split-plot design with three replications at each location. Treatments included two levels of corn residue (approximately 0 and 80% ground cover) as the main plot factor, fungicide treatment [Folicur (Tebucanazole) was applied at 50% anthesis (Zadoks 65)] as the sub-plot factor, and three winter wheat cultivars (‘Hopewell’, ‘Patterson’ and ‘Truman’) as the sub-sub plot factor in IN, OH and PA. The protocol varied at the ND and SD locations. At the SD location, two spring wheat cultivars (‘Alsen’ and ‘Norm’) were used. At the ND location, *Gibberella zeae*-colonized corn kernels were used to establish the main plots, and three spring wheat cultivars (‘Alsen’, ‘Argent’, and ‘Granite’) were used as the sub-sub plots; however, the fungicide sub-plot remained consistent. Disease incidence, severity and DON concentration varied between years and locations, and current analysis considers each location and year separately. In 2005, disease incidence, severity and DON concentration varied from 0 to 78% (mean 18%), 0 to 33% (mean 5%), and 0 to 4.1 ppm (mean 0.7 ppm), respectively. Winter wheat locations had very low disease (0-7% mean disease incidence), while spring wheat locations had moderate (35-51% mean disease incidence) disease intensity. Among locations with measurable disease intensity or DON, the effect of cultivar resistance was significant in the majority of cases. Two-way or three-way interactions were observed in several cases indicating that combinations of two or more management practice resulted in better control. This indicates the potential importance of integrating multiple management tactics.

INFLUENCE OF WEATHER ON THE ABUNDANCE OF *GIBBERELLA ZEAE* PROPAGULES WITHIN WHEAT CANOPIES: A LAG REGRESSION ANALYSIS.

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ABSTRACT

The development of Fusarium head blight (FHB) and the accumulation of deoxynivalenol (DON) are largely dependent on the availability and abundance of inoculum of *Gibberella zeae* under the right set of environmental conditions. An understanding of the dynamic associations among inoculum potential, toxin production, weather, and disease intensity would be invaluable to our ongoing efforts to predict the risk of FHB and DON. In an effort to characterize the association between weather variables and inoculum within wheat canopies, spikes were sampled and assayed for propagules of *G. zeae* from plots established in OH, PA, ND, SD and IN from 1998 through 2005. Spikes were collected daily from each field from Feekes growth stage 10 through 11.2, placed in sterile distilled water, and washed to dislodge propagules. Samples of spike wash suspensions were transferred to Petri plates containing Komada selective medium, and *G. zeae* was identified based on morphology of colonies and spores. Inoculum abundance was quantified as the number of colony-forming units per spike (CFU/spike). Ambient weather data were collected and summarized for different periods prior to sampling of spikes. A total of 35 individual weather variables were generated. Polynomial distributed lag regression analysis was used to identify weather variables and the period of time that best predicted spore abundance. Linear mixed models were then used to simultaneously determine the effects of location, year within location, and weather variables on abundance. Inoculum density (based on log-transformed CFU/spike) within wheat canopy was statistically related to weather conditions both on the day of sampling and several days prior to sampling. The response to weather conditions was distributed over nine days, and the functional relationship (linear, quadratic etc) between weather and spore abundance varied with the predictor variable. The most significant predictors of log CFU/spike were average relative humidity, wetness duration, average daily air temperature, and rainfall intensity. Study location also had a major effect on inoculum abundance. Current research focuses on accounting for serial correlation of responses within location-years, and on identifying location-specific determinants of spore density.

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ENVIRONMENTAL FACTORS INFLUENCING FUSARIUM HEAD BLIGHT OF BARLEY IN THE NORTHERN GREAT PLAINS.

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ABSTRACT

We are investigating the relationship between environmental factors, crop stage, and barley genotype with Fusarium head blight (FHB) and DON accumulation in the grain. This project is associated with the established spring and winter wheat FHB-modeling efforts and aims to produce the information required to either validate one of the current wheat models for barley, or generate unique models.

Varieties of regionally adapted barley of both 2- and 6-row types were planted at multiple locations in the Northern Great Plains during the 2005 and 2006 growing seasons. At least three varieties were common to each location. Plots were un-irrigated, a minimum of 1.5m x 4.6m in size, and replicated four times in a RCBD. Additional varieties were planted based upon availability and local producer preference. Crop stage was monitored regularly throughout the season and the date at which each plot was at Feekes 10.5 stage was noted. No additional inoculum was introduced into the plots. The incidence and severity of FHB was recorded on a minimum of 25 heads per plot at the soft-dough stage (approximately 21 days after heading). Environmental variables consisting of temperature, relative humidity, and precipitation were recorded with an on-site, or nearby, weather station.

Over the past two seasons, we have successfully collected data for 24 of the 26 locations planted. These represent a range of disease intensities with some varieties at locations in 2005 having almost 100% incidence of infection. Locations in western Minnesota all had relatively low disease (< 3%) in 2005, whereas those in the Dakotas had a much broader range (<1 to 25%). For 2006, a wide-spread drought in the region resulted in negligible disease at most locations. DON data is pending for the 2006 trials, however the concentrations in grain ranged from 0 to 3ppm for 2005.

Correlation analysis was conducted on the limited data set available and the environmental variables that were most associated with disease severity (field index) were pre-heading temperature and measurements of air moisture content (relative humidity, etc). In comparison, only the air moisture content after heading was significantly associated with final DON concentration. Additional results will be presented as data becomes available.

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TIMING OF INFECTION: THE EFFECTS ON FUSARIUM HEAD BLIGHT SEVERITY AND TOXIN ACCUMULATION IN WHEAT KERNELS.

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ABSTRACT

There have been recent reports of wheat grain which appears to be free of Fusarium Head Blight symptoms, but contains significant levels (≥ 2ppm) of the mycotoxin deoxynivalenol (DON). We are investigating the role of infection timing relative to host growth stage in the development of asymptomatic wheat kernels with significant DON. Three hard red winter wheat varieties, Hopewell (moderately susceptible), Valor (moderately susceptible) and Truman (moderately resistant), were used in this field study. The cultivars were subjected to four treatments: (1) ambient conditions (no supplemental moisture), (2) misting during anthesis only, (3) misting during both anthesis and grain-fill stages and (4) misting during grain-fill only. Movable greenhouses and mist chambers were employed to prevent rain or to add moisture as needed. All plots were spray inoculated with four DON-producing *Fusarium graminearum* isolates at both anthesis and late milk stages of growth. Plants were misted overnight for four consecutive nights post-inoculation. Disease incidence and severity were measured in the field prior to harvest. Following harvest, yield and the percentage of *Fusarium*-damaged kernels were also assessed. High Pressure Liquid Chromatography was used to analyze DON content of the samples and statistical analysis was performed using proc MIXED of SAS (version 9.1, SAS Institute, Cary, NC). The disease incidence in treatments 1 and 4 were significantly lower ($P \leq 0.05$) than treatments 2 and 3. Increased moisture during anthesis also resulted in significantly higher ($P \leq 0.05$) disease severity regardless of variety. Although treatments 1 and 4 did not differ in terms of incidence and severity, kernels from treatment 4 contained significantly higher ($P \leq 0.05$) DON than treatment 1. Within treatment 4, Truman (1.5 ppm) contained significantly less ($P \leq 0.05$) DON than the more susceptible cultivars Hopewell and Valor which contained an average of 3.0 ppm and 3.7 ppm DON, respectively. This study suggests that late infections, facilitated by moisture during grain-filling stages of kernel development, may result in low disease intensity yet kernels containing significant levels of DON.

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