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Reduced Virulence of Trichothecene-Nonproducing Mutants of *Gibberella zeae* in Wheat Field Tests

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We have analyzed the role of trichothecene toxins in the virulence of the fungus *Gibberella zeae* (anamorph, *Fusarium graminearum*) on wheat (*Triticum aestivum*). Trichothecene-nonproducing mutants of *G. zeae* were obtained by disrupting *Tri5*, the gene encoding trichodiene synthase, which catalyzes the first committed step in the trichothecene biosynthetic pathway. Trichothecene-nonproducing mutants appear to be normal in growth and development under laboratory conditions. One such mutant was selfed to generate a meiotic revertant that lost the disruption vector and recovered trichothecene production. In the present study, virulence was assessed in 1994 and 1995 by controlled field inoculation of *G. zeae* spore suspensions into flowering wheat heads. Trichothecene-nonproducing (*Tri5*$^-$) mutants were less virulent than the trichothecene-producing (*Tri5*$^+$) parental and revertant strains in their ability to cause head scab on field-grown wheat. Although trichothecene-nonproducing strains colonized wheat heads, the infected heads showed less disease by several parameters we tested, including head bleaching symptoms, seed weight, seed viability, and trichothecene contamination. This evidence indicates that trichothecenes are virulence factors in wheat head scab.

Some plant pathogenic fungi can attack a wide range of plant species. *Gibberella zeae* (Schwein.) Petch (anamorph, *Fusarium graminearum*) is noteworthy in the diversity of plants and plant tissues on which it can cause disease. *G. zeae* causes head scab and root rot of wheat, barley, oats, and rice, as well as maize ear and stalk rot, and root and stem rots of potatoes, legumes, and many other plant species. *G. zeae* is also a ubiquitous soil saprophyte, surviving well between crops on plant debris in the soil. Under certain environmental conditions, *G. zeae* is an especially virulent pathogen of wheat. If humid spring weather induces abundant fungal sporulation while wheat heads are at anthesis, then severe head scab epidemics can develop. Current farm programs that encourage less tillage for control of soil erosion create an environment rich in crop residues that is conducive to wheat head scab (Sutton 1982; Bai and Shaner 1994; Parry et al. 1995).

Wheat head scab (also called head blight) first appears as premature bleaching of individual spikelets of the head. Such bleached spikelets often contain shriveled, discolored seeds, or chalky seeds aptly called "tombstones" (Parry et al. 1995). Scab reduces both grain yield and quality; scabby seeds often fail to germinate or produce blighted seedlings. In addition, *G. zeae* contaminates grain with deoxynivalenol, also called vomitoxin, which is one of a family of trichothecene toxins that are harmful to human and animal health upon ingestion. Trichothecenes are potent protein synthesis inhibitors in a wide range of eukaryotic organisms, including animals, fungi, and higher plants (Wei and McLaughlin 1974). Experiments with chemically pure trichothecenes at low dosage levels in animals have reproduced many of the symptoms observed in moldy grain toxicoses, including nausea, vomiting, diarrhea, feed refusal, anemia immunosuppression, and hemorrhage of the skin, lungs, and gastrointestinal tract (Marasas et al. 1984). Historical and epidemiological data indicate an association between consumption of grain contaminated with trichothecenes and human disease outbreaks in the former Soviet Union in the 1940s, and more recently in India and Japan (Marasas et al. 1984).

Trichothecenes comprise a large family of sesquiterpene epoxides. Like many other microbial antibiotic biosynthetic genes, *Fusarium* trichothecene biosynthetic pathway genes are closely linked and constitute a gene cluster. Characterization of this gene cluster is still in progress, but results to date indicate that 10 genes involved in trichothecene biosynthesis are localized to a 25-kb region of chromosomal DNA in *F. sporotrichioides* (Hohn et al. 1995). This 25-kb region contains two genes encoding cytochrome P450 monooxygenases involved in trichothecene oxygenation, at least two genes encoding specific acylases, and a gene that encodes a transcription fac-

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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.

tor that regulates the expression of other pathway genes. The cluster also contains *Tri5*, the gene encoding trichodiene synthase, which catalyzes the first step in trichothecene biosynthesis. Transformation-mediated gene disruption of *Tri5* blocks the biosynthesis of trichodiene and all trichothecenes in *F. sporotrichioides, G. pulicaris,* and *G. zeae* (Hohn et al. 1995; Hohn and Desjardins 1992; Proctor et al. 1995).

We have begun to investigate the role of trichothecenes in a number of plant diseases caused by *Fusarium* species (Desjardins et al. 1989; Desjardins et al. 1992; Proctor et al. 1995). To determine if trichothecenes play a role in wheat head scab, we generated trichothecene-nonproducing mutants by disrupting the *Tri5* gene in *G. zeae*. These *Tri5*$^-$ mutants were reduced in virulence in preliminary tests of wheat seedling blight and head scab in the growth chamber (Proctor et al. 1995). A limitation of these laboratory virulence tests is that a growth chamber is a highly artificial environment. Wheat grown under these conditions is less healthy and vigorous than wheat grown in the field. We therefore tested our mutants under more complex and realistic agricultural conditions, on wheat growing at two different field sites. In the present report, we show that trichothecene-nonproducing mutants of *G. zeae* caused significantly less wheat head scab than the trichothecene-producing parental and revertant strains under agricultural field conditions.

RESULTS

A trichothecene-nonproducing mutant displays reduced virulence on wheat.

We first tested virulence of the trichothecene-nonproducing mutant GZT40 in Illinois in 1994 on hard red spring wheat cultivars Butte 86 and Wheaton, by inoculating flowering heads with approximately 1,000 spores of the wild-type strain GZ3639 (*Tri5*$^+$), the mutant strain GZT40 (*Tri5*$^-$), or with water. In field test 1, 1,800 wheat heads were individually inoculated. By 7 days after inoculation, heads began to show symptoms of premature spikelet bleaching. Between 7 and 18 days after inoculation, each head was assessed once or twice for disease symptoms by counting the number of heads with scab symptoms (disease incidence) and the number of bleached spikelets per head (disease severity). For both cultivars, disease severity was greater on heads inoculated with the wild-type strain than with the *Tri5*$^-$ mutant, but disease incidence was similar for the two treatments (Table 1). There was a low incidence of premature spikelet bleaching on control heads inoculated with water only, or on uninoculated heads. This relatively low incidence of natural infection was probably due to the occurrence of hot and dry weather during anthesis when heads were at their peak of susceptibility. In both cultivars Butte 86 and Wheaton, the mutant was less virulent than the wild-type strain when yield components were compared: number of seeds per head, individual seed weight, and total yield per head (Table 1). For cultivar Butte 86, total yield per head was reduced by 56% upon wild-type inoculation and reduced by 34% upon mutant inoculation compared to water-inoculated control heads. For cultivar Wheaton, total yield per head was reduced by 43% upon wild-type inoculation, and by 4% (not significant) upon mutant inoculation.

We conducted field test 2 in Indiana in 1995 to test with additional wheat cultivars the reduced virulence of the trichothecene-nonproducing mutant demonstrated in the 1994 Illinois field test. Soft red winter wheat cultivars Caldwell, Clark, and Patterson were inoculated and analyzed as described above. In field test 2, 3,600 wheat heads were inoculated. Spikelet bleaching symptoms were assessed at 23 days after inoculation. The disease incidence and severity data are presented in Table 1. Results from field test 2 with winter wheat generally confirmed those obtained the previous year with spring wheat. The trichothecene-nonproducing mutant was significantly less virulent than the wild-type strain in both disease incidence and disease severity on all three winter wheat cultivars tested, and

Table 1. Disease development and yield components of wheat following inoculation with *Gibberella zeae*[a]

Cultivar	Treatment	Disease incidence	Disease severity	Seeds per head (no.)	Total yield per head (mg)	Individual seed weight (mg)
Field test 1						
Butte 86	GZ3639	84 a	65 a	16 a	347 a	18 a
	GZT40	73 b	41 b	24 b	516 b	23 b
	Water	2 c	1 c	26 c	782 c	30 c
Wheaton	GZ3639	99 a	52 a	38 a	946 a	25 a
	GZT40	97 a	27 b	43 b	1,381 b	32 b
	Water	4 b	1 c	44 b	1,435 b	32 b
Field test 2						
Caldwell	GZ3639	100 a	48 a	15 a	340 a	22 a
	GZT40	75 b	23 b	14 a	430 a	30 b
	Water	50 c	13 c	13 a	387 a	30 b
Clark	GZ3639	100 a	83 a	15 a	367 a	24 a
	GZT40	59 b	33 b	25 b	900 b	37 b
	Water	17 c	26 b	25 b	870 b	35 b
Patterson	GZ3639	99 a	75 a	12 a	255 a	19 a
	GZT40	66 b	34 b	16 b	485 b	29 b
	Water	30 c	19 c	18 b	522 b	29 b

[a] Means within a column for each cultivar followed by a letter in common are not significantly different at $P < 0.05$ according to Fisher's protected LSD test. Disease incidence is the percentage of heads per plot that show scab symptoms; disease severity is the percentage of spikelets per head that show symptoms. Disease was assessed 18 and 23 days after inoculation for field tests 1 and 2, respectively. Each treatment group contained 100 inoculated heads and was replicated three times (field test 1) or four times (field test 2) in a randomized complete block design for each cultivar. All wheat heads harvested from each plot were assessed individually for each disease and yield parameter. Data are means derived from approximately 400 heads per treatment in field test 2, and from 250 to 300 heads per treatment in field test 1, except that individual head weight data for cultivar Butte 86 include only 175 heads because seeds from one plot were accidentally pooled before the individual heads were weighed.

in all yield components tested for cultivars Clark and Patterson. For cultivar Clark, total yield per head was reduced by 58% upon wild-type inoculation, but was 3% (not significant) greater than the control upon mutant inoculation. For cultivar Patterson, total yield per head was reduced by 51% on wild-type inoculation, and was reduced by 8% (not significant) upon mutant inoculation. The number of seeds per head and total yield for cultivar Caldwell did not differ significantly among the three treatments. Frequent rain during anthesis was the probable cause of a high incidence of natural infection in water-inoculated control heads, especially of cultivar Caldwell (Table 1) and in uninoculated heads in the field plots (data not shown).

Trichothecene-producing revertants regain virulence on wheat.

Because trichothecene-nonproducing mutants were obtained by specific gene disruption, they are unlikely to have additional defects that fortuitously reduce virulence. However, to further test whether reduced virulence of *Tri5*$^-$ mutants is due specifically to loss of *Tri5* function, we generated *Tri5*$^+$ revertants of *G. zeae* by selfing GZT33, a *Tri5*$^-$ transformant that had undergone additive gene disruption (Proctor et al. 1995). To facilitate strain tracking during the field test, the revertant was retransformed to generate strains GZT106 and GZT108 which are hygromycin resistant and produce wild-type levels of trichothecenes. Spring wheat cultivar Wheaton was inoculated as described above with water, with spores of a trichothecene-producing strain (GZ3639, GZT106, or GZT108), or with spores of a trichothecene-nonproducing strain (GZT33 or GZT40). In field test 3, 1,800 wheat heads were individually inoculated. Spikelet bleaching symptoms were assessed at 12 and 19 days after inoculation. Due to cool, rainy weather during anthesis, there was a moderate incidence of natural infection in all the field plots. The results from field test 3 were similar to those obtained in the two earlier, more limited, field tests and are presented in Figures 1 and 2. Heads inoculated with either of the trichothecene-nonproducing mutants showed less scab than heads inoculated with the trichothecene-producing parent and revertant strains, whether assessed by spikelet bleaching, individual seed weight (Fig. 1), or by percent "tombstone" kernels (Fig. 2). The average individual seed weight was reduced by 32, 27, and 39% upon inoculation with *Tri5*$^+$ wild-type and revertant strains, but by only 3 and 12% (not significant) upon inoculation with *Tri5*$^-$ strains. Data on number of seeds per head and total yield per head could not be accurately determined for field test 3 because of selective grazing by rabbits of approximately 10% of the inoculated wheat heads in some plots.

Additional characterization of wheat seeds harvested from field tests 1, 2, and 3 demonstrated that poor seed quality was correlated with poor seed yield. Trichothecene toxin levels were higher in seeds from heads inoculated with *Tri5*$^+$ strains than in seeds from heads inoculated with *Tri5*$^-$ strains or with water (Table 2). The ability of the seeds to germinate and produce a viable wheat seedling was also reduced more following inoculation with *Tri5*$^+$ strains than with *Tri5*$^-$ strains (Table 2). The percentage of seeds infected with *Fusarium* was determined by plating surface disinfested seeds on a medium that was selective for *Fusarium* species and showed no consistent relationship with wheat yield and quality. Many apparently healthy seeds contained *Fusarium*, although the species and the amount of fungal biomass per seed could not be determined by this method. Percent *Fusarium* infection tended to be lower after inoculation with *Tri5*$^-$ strains than with *Tri5*$^+$ strains, but the difference was generally not statistically significant (Table 2).

Trichothecene-nonproducing mutants survive in wheat heads.

Although both trichothecene-nonproducing mutants GZT33 and GZT40 are less virulent than wild-type strains, PCR analysis of isolates from the harvested wheat seeds indicated that both GZT33 and GZT40 retained the ability to survive in wheat. In field tests 1 and 2, strain GZT40 was recovered from inoculated heads in all plots where it was introduced, and in field test 3, strains GZT33 and GZT40 were recovered from inoculated heads in all plots where they were introduced. The trichothecene-producing mutants GZT106 and GZT108 also retained the ability to survive in wheat. Wild-type strains

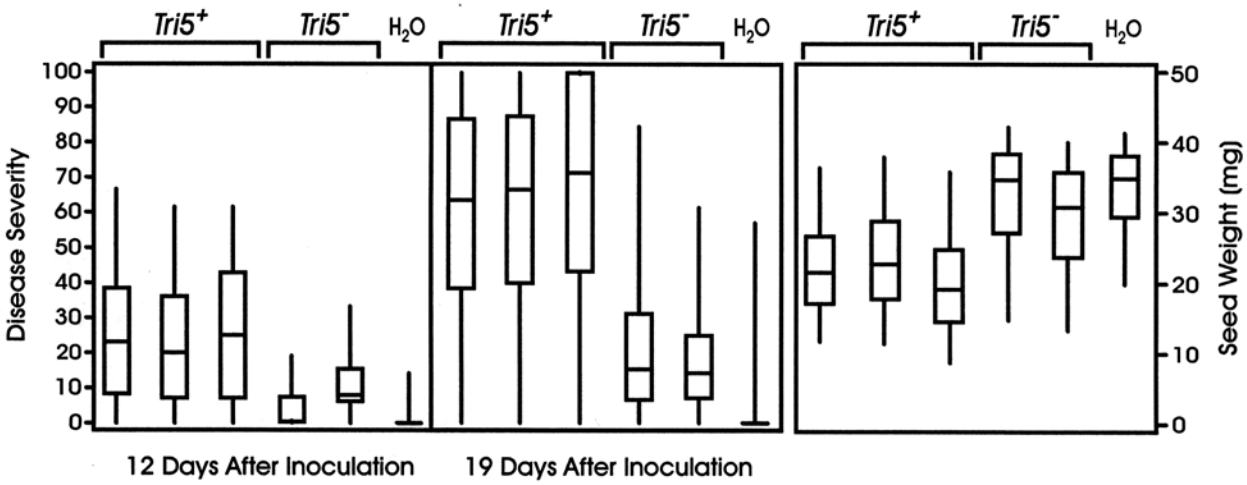

Fig. 1. Box plot statistics of disease severity and individual seed weight data from field test 3. The plots show the medians, 50%, and 95% ranges for cultivar Wheaton inoculated with *Gibberella zeae*. Treatments, from left to right in each plot, are: *Tri5*$^+$ strains GZ3639, GZT106, and GZT108, *Tri5*$^-$ strains GZT40 and GZT33, or water. The left axis indicates disease severity as percent blighted spikelets per head. The right axis indicates individual seed weight in milligrams. The number of wheat heads analyzed per box varied from 189 to 291.

of *G. zeae* were often present in seeds from heads inoculated with mutant strains, probably as a result of natural infection during the field test (data not shown).

DISCUSSION

Specific disruption of a gene can determine its importance in a complex biological system, particularly if loss of that gene causes a dramatic change. In this way, disruption of genes for biosynthesis of host-selective toxins by *Cochliobolus* species has substantiated the role of these toxins in causing disease on particular genotypes of maize (Panaccione et al. 1992; Yang et al. 1994). We have extended this approach to analysis of trichothecenes, a class of fungal toxins with potent and broad spectrum antibiotic activity, and their role in diseases caused by *Fusarium* species with a diverse range of plant hosts (Proctor et al. 1995; Desjardins et al. 1989; Desjardins et al. 1992). We have isolated trichothecene-nonproducing mutants of *G. zeae* by disrupting *Tri5*, which encodes trichodiene synthase, the first committed enzyme in the trichothecene biosynthetic pathway, and we have shown that these mutants are less virulent than wild-type strains in causing wheat head scab. For example, in three field tests, production of trichothecenes accounted for 40 to 100% of the loss of seed yield due to *G. zeae*. We have also demonstrated that virulence of a *Tri5*$^-$ disruptant could be restored to wild-type levels when a functional *Tri5* gene was recovered by meiotic recombination. The loss of trichothecene production appears to convert *G. zeae* into a relatively benign pathogen that survives in wheat heads and colonizes developing seeds, but no longer produces severe disease symptoms.

Previous virulence tests of genetically modified plant pathogenic fungi have been conducted in controlled experimental environments (Proctor et al. 1995; Panaccione et al. 1992; Yang et al. 1994). However, results of such laboratory and greenhouse studies could be misleading if plant health or microbial population dynamics differ significantly from those in the field. Because preliminary studies indicated a low risk associated with the release of our genetically modified strains of *G. zeae* into the environment, USDA-APHIS granted permission for the field tests described in this report. As we undertook the field tests, our most serious concern was that natural infection by indigenous strains of *G. zeae* might overwhelm our experiments. In fact, weather conditions during the time of inoculation and incubation were favorable for some natural scab infection in all of our field tests, and there was detectable contamination by wild-type strains in many of the plots that were inoculated with mutant strains. Contrary to our expectations, this natural infection did not obscure differences that resulted from inoculation of the wild-type strain compared to the trichothecene-nonproducing strains. In fact, differences between wheat plants inoculated with *Tri5*$^+$ strains and *Tri5*$^-$ strains were more dramatic in the field tests than in our previous controlled environment studies (Proctor et al.

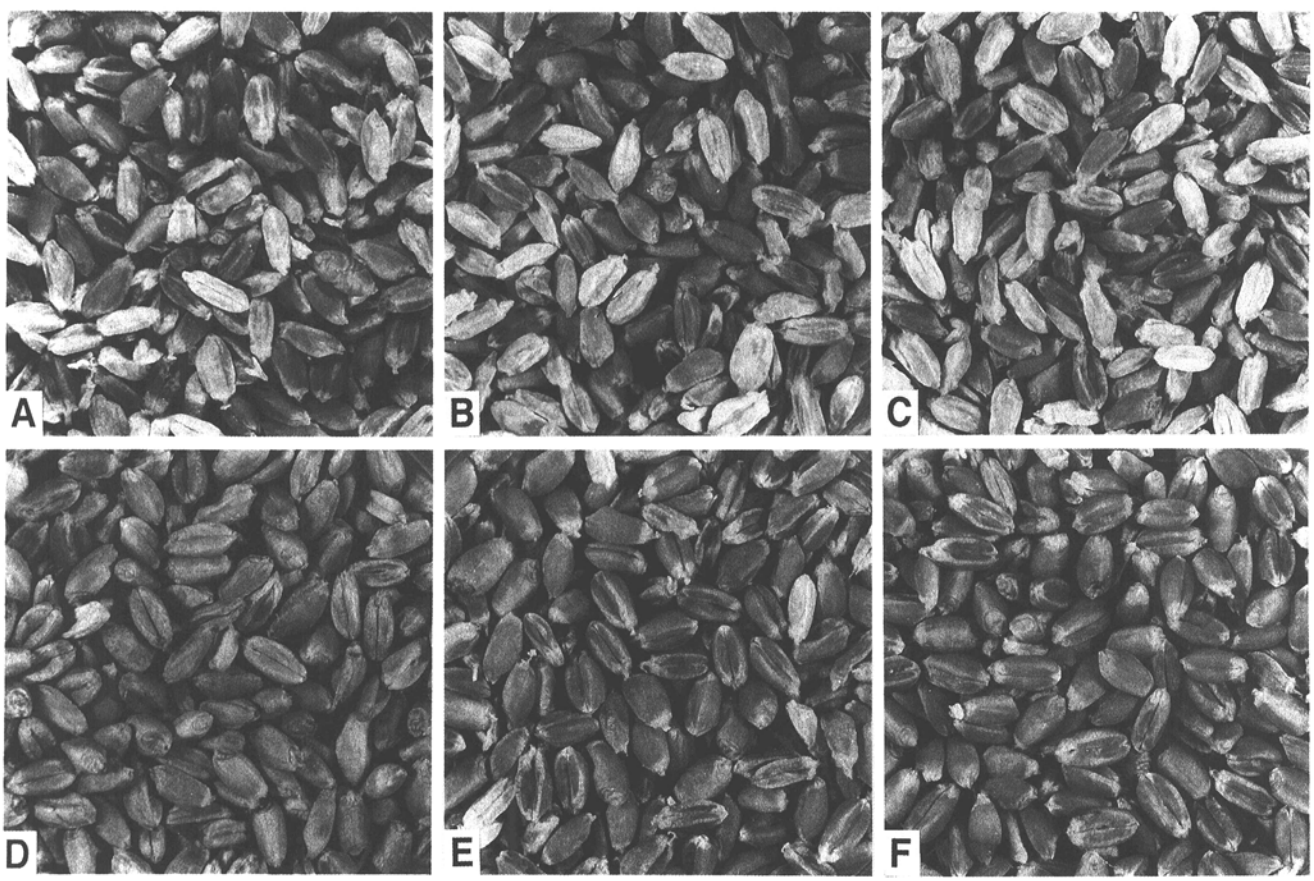

Fig. 2. Comparison of frequency of "tombstone" kernels among seed harvested from field test 3 heads of cultivar Wheaton inoculated with *Gibberella zeae Tri5*$^+$ strains **(A)** GZ3639, **(B)** GZT106, **(C)** GZT108, with *Tri5*$^-$strains **(D)** GZT40, **(E)** GZT33, or with water **(F)**.

1995). The spread of wild-type strains into plots inoculated with *Tri5*⁻ strains may have been too late for damage to develop before the wheat matured. Prior infection by the trichothecene-nonproducing strains may also have conferred some protection against the effect of the wild-type strains.

The conclusion that trichothecenes are virulence factors in wheat head scab caused by *G. zeae* is consistent with our earlier findings that trichothecenes are virulence factors in parsnip root rot caused by *F. sporotrichioides* and *G. pulicaris* (Desjardins et al. 1989; Desjardins et al. 1992). Perhaps the next question is why are trichothecene-nonproducing strains less virulent? Studies of trichothcene toxicity in animal and fungal cells and cell-free systems indicate that toxic effects seem to be the result of inhibition of protein synthesis and, specifically, of peptidyl transferase (Wei and McLaughlin 1974). Trichothecenes are growth inhibitors of wheat and a wide variety of other plants (Miller and Arnison 1986; Wang and Miller 1988). The trichothecene target site in plants is likely to be protein synthesis, but this hypothesis remains to be proven in a plant in vitro translation system. Any hypothesis that trichothecenes are virulence factors because they inhibit plant protein synthesis and, thereby, alter the host response to infection has to accommodate the fact that *Tri5*⁻ trichothecene-nonproducing mutants of *G. pulicaris* retain wild-type virulence on potato tubers (Desjardins et al. 1992). Recent studies suggest several mechanisms that may account for the apparent lack of response of potato tuber tissue to trichothecene production, including degradation of trichothecenes, inhibition of their biosynthesis, or an altered trichothecene target site (Miller and Arnison 1986; Wang and Miller 1988; Desjardins et al. 1988; Iglesias and Ballesta 1994).

Historically, trichothecenes have been notorious for causing acute and chronic disease symptoms in humans and animals that eat scabby wheat and other contaminated grains (Marasas et al. 1984). This study demonstrates that trichothecenes also play an important role in the ability of a fungal species to cause disease symptoms on its plant host as well.

Table 2. Quality of wheat seed following inoculation with *Gibberella zeae*[w]

Cultivar	Treatment	Percent viability[x]	Percent infection with *Fusarium*[x]	Trichothecenes µg/g dry wt[y]
Field test 1				
Butte 86	GZ3639	25 a	73 a	6.2 a
	GZT40	50 b	67 a	0.8 b
	Water	84 c	29 b	0 c
Wheaton	GZ3639	33 a	73 a	3.1 a
	GZT40	77 b	61 b	0.5 b
	Water	87 c	27 c	0.4 b
Field test 3				
Wheaton	GZ3639	46 a	67 a	8.1 a
	GZT106	52 a	67 a	9.2 a
	GZT108	42 a	77 a	8.5 a
	GZT33	81 b	51 a	1.6 b
	GZT40	87 b	44 a	1.3 b
	Water	91 b	67 a	1.1 b
Field test 2				
Caldwell	GZ3639	84 a	60 a	11.9 a
	GZT40	90 b	41 b	8.0 b
	Water	91 b	34 b	8.6 ab
Clark	GZ3639	68 a	72 a	12.0 a
	GZT40	93 b	47 b	6.9 b
	Water	92 b	28 b	5.7 b
Patterson	GZ3639	62 a	88 a	14.5 a
	GZT40	87 a	62 ab	6.1 c
	Water	84 a	42 b	10.6 b

[w] Means within a column for each cultivar followed by a letter in common are not significantly different at $P < 0.05$ according to Fisher's protected LSD test. Heads from each treatment plot were randomly assigned to one, two, or three bulked seed pools per plot. Random samples were taken from these bulked seed pools for quality analysis.

[x] Seeds were surface disinfested, planted in trays of vermiculite, and incubated at 22°C with a cycle of 16 h light/8 h dark. Green seedlings were counted 7 to 9 days after planting. Percent infection with *Fusarium* was determined by culturing seeds on a selective medium (Nelson et al. 1983). Viability and infection data are expressed as means for three (field tests 1 and 3) or four (field test 2) replicate plots per treatment: 900 seeds per treatment of cultivar Wheaton in field test 1, 350 to 450 seeds of cultivar Wheaton in field test 3, 450 seeds of cultivar Butte 86, and 100 seeds of cultivars Caldwell, Clark, and Patterson.

[y] Trichothecenes were quantitated as described in Table 3. For field tests 1 and 3, data are means of three replicate plots per treatment, with one 10-g sample analyzed per each bulked seed pool of each plot. For field test 2, data are means of four replicate plots per treatment, with one 4- to 12-g sample analyzed per plot.

MATERIALS AND METHODS

Fungal strains and inoculum preparation.

The *G. zeae* strains used in this study are described in Table 3 and were maintained and cultured as described (Proctor et al. 1995). A virulent strain isolated from scabby wheat in Kansas (Bowden and Leslie 1992) and designated GZ3639 was the trichothecene-producing parent of the mutant strains in this study and served as the wild-type strain for all field tests. Vegetative compatibility was used to confirm the lineage of strains GZT33, GZT40, GZT106, and GZT108 from strain GZ3639 (Table 3). Nitrate-nonutilizing mutants were generated from each strain and tested for complementation by standard methods (Bowden and Leslie 1992). All mutants were vegetatively compatible with strain GZ3639 from which they were derived.

Fungal inoculum was prepared in two different ways. For spring wheat tests in Illinois, inoculum was produced by washing spores (macroconidia) from cultures grown on V8

Table 3. *Gibberella zeae* strains used in this study

Strain no.	Strain description	Genotype	Trichothecenes µg/ml[a]	Source or reference
GZ3639	Wild type	*Tri5*⁺; *hygB*^{S}	157	Procter et al. 1995
GZT33	Additive gene disruptant of GZ3639	*Tri5*⁻; *hygB*^{R}	0	Procter et al. 1995
GZT40	Gene replacement of GZ3639	*Tri5*⁻;*hygB*^{R}	0	Procter et al. 1995
GZT106	Retagged revertant of GZT33	*Tri5*⁺;*hygB*^{R}	85	...[b]
GZT108	Retagged revertant of GZT33	*Tri5*⁺; *hygB*^{R}	100	...[b]

[a] Trichothecenes were quantitated as described (Proctor et al. 1995) and are expressed as micrograms of triacetylated deoxynivalenol per gram of cornmeal culture medium. Data are the average of two replicate cultures after 1 week of incubation.

[b] The revertant strain was retagged by transformation with vector pUCH2-8 by previously described methods (Proctor et al. 1995, and R. H. P., T. M. H., and S. P. M., unpublished results) and two transformants were selected for field testing.

juice agar for 7 to 10 days. For winter wheat tests in Indiana, inoculum was produced in a mung bean liquor medium. To prepare this medium, 40 g of mung beans was steamed in 1 liter of water for 20 min, filtered, and autoclaved. Mung bean medium, 100 ml in a 200-ml Erlenmyer flask, was inoculated with a 3-mm^2 disk from an agar culture, and the flasks were shaken at 24°C for 4 days. Following this period of incubation, the liquid cultures were stored in a refrigerator until use. Before inoculation, spore suspensions were filtered to remove hyphal fragments. For all field tests, spore suspensions were adjusted to 5×10^4 spores per ml.

Field tests.

Three field tests were conducted and are numbered in chronological order. For the 1994 test in Illinois (field test 1), hard red spring wheat cultivars Wheaton and Butte 86 were hand sown at the Walter Christ farm 24 km west of Peoria in adjacent 20- × 20-m blocks on 25 March 1994. In May, some plants were uprooted to form 3- × 3-m plots spaced 2 m apart. There were nine plots of each cultivar. For the 1995 test at the same site in Illinois (field test 3), cultivar Wheaton was hand sown in a 13- × 22-m block on 19 April, 1995. In June, some plants were uprooted to form 18 1.5- × 1.5-m plots spaced 1 to 1.5 m apart. For the 1995 test in Indiana (field test 2), soft red winter wheat cultivars Caldwell, Clark, and Patterson were sown with a conventional wheat drill in rows spaced 18-cm apart at the Purdue University Agronomy Farm in early October 1994. In the spring, shortly before heads emerged, plots 1 m long and three rows wide were delineated by mowing alleys approximately 1.5-m wide.

The same method of inoculation was used in all three field tests. When each cultivar reached the mid-anthesis stage of growth, 100 culms in each plot were tagged with colored tape just below the head. These tagged heads were inoculated by injecting a drop of spore suspension into one floret of a central spikelet. This method of inoculation delivered approximately 20 µl of inoculum containing 1,000 spores. Control plants were inoculated with sterile water. In 1994, cultivar Butte 86 was inoculated with spores on 7 and 8 June, and cultivar Wheaton on 9 and 10 June. Butte 86 and Wheaton control plants were inoculated with water on 13 and 14 June. In 1995, cultivar Clark was inoculated on 23 May; cultivar Patterson on 24 May, cultivar Caldwell on 25 May, and cultivar Wheaton on 22, 23, 24, and 25 June.

Postinoculation treatments differed in the three field tests. For field test 1, each inoculated head was covered with a sleeve of dialysis tubing, which was left on for the duration of the field experiment. For field test 2, each plot was covered with a plastic bag, which was left in place for 3 days. For field test 3, the heads were left uncovered. When the wheat was ripe, the tagged heads were harvested, individually threshed, and stored at –20°C.

Identification of fungal strains.

In field tests 1 and 3, seeds harvested from inoculated heads were surface disinfested with 0.5% sodium hypochlorite and cultured on a *Fusarium* selective medium (Nelson et al. 1983). In field test 2, spikelets harvested from inoculated heads were cultured on the selective medium. *Fusarium* colonies were transferred to potato dextrose agar to facilitate identification (Nelson et al. 1983). Putative *G. zeae* strains were subcultured to individual plates of V8 juice agar medium. Mycelium harvested from these plates was lyophilized, resuspended in DNA extraction buffer (Hohn and Desjardins 1992), and extracted with phenol/chloroform. DNA was further purified from the resulting aqueous phase using GeneClean (BIO 101, Vista, CA). PCR reactions employed the isolated DNA as template and primer pairs capable of specifically identifying wild type and transformed fungal strains. The PCR product band pattern from each field isolate was compared to those from wild type and mutant strains to determine the genotype of each field isolate.

Test protocol.

Studies described in this report were conducted under terms outlined in permits 94-006-01 and 95-003-01 granted by the United States Department of Agriculture, Animal and Plant Health Inspection Service.

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LITERATURE CITED

Bai, G., and Shaner, G. 1994. Scab of wheat: Prospects for control. Plant Dis. 78:760-766.

Bowden, R. L., and Leslie, J. F. 1992. Nitrate-nonutilizing mutants of *Gibberella zeae* (*Fusarium graminearum*) and their use in determining vegetative compatibility. Exp. Mycol. 16:308-315.

Desjardins, A. E., Spencer, G. F., Plattner, R. D., and Beremand, M. N. 1989. Furanocoumarin phytoalexins, trichothecene toxins and infection of *Pastinaca sativa* by *Fusarium sporotrichioides*. Phytopathology 79:170-175.

Desjardins, A. E., Hohn, T. M., and McCormick, S. P. 1992. Effect of gene disruption of trichodiene synthase on the virulence of *Gibberella pulicaris*. Mol. Plant-Microbe Interact. 5:214-222.

Desjardins, A. E., Plattner, R. D., and Spencer, G. F. 1988. Inhibition of trichothecene toxin biosynthesis by naturally occurring shikimate aromatics. Phytochemistry 27:767-771.

Hohn, T. M., Desjardins, A. E., McCormick, S. P., and Proctor, R. H. 1995. Biosynthesis of trichothecenes, genetic and molecular aspects. Pages 239-248 in: Molecular Approaches to Food Safety. M. Eklund, J. L. Richard, and K. Mise, eds. Alaken Inc., Ft. Collins, CO.

Hohn, T. M., and Desjardins, A. E. 1992. Isolation and gene disruption of the *Tox5* gene encoding trichodiene synthase in *Gibberella pulicaris*. Mol. Plant-Microbe Interact. 5:249-256.

Iglesias, M., and Ballesta, P. G. 1994. Mechanism of resistance to the antibiotic trichothecin in the producing fungi. Eur. J. Biochem. 223:447-453.

Marasas, W. F. O., Nelson, P. E., and Toussoun, T. A. 1984. Toxigenic *Fusarium* Species: Identity and Mycotoxicology. The Pennsylvania State University Press, University Park, PA. 328 pages.

Miller, J. D., and Arnison, P. G. 1986. Degradation of deoxynivalenol by suspension cultures of the *Fusarium* head blight resistant wheat cultivar Frontana. Can. J. Plant Pathol. 8:147-150.

Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* Species: An Illustrated Manual for Identification. The Pennsylvania State University Press, University Park, PA. 193 pages.

Panaccione, D. G., Scott-Craig, J. S., Pocard, J.-A., and Walton, J. D. 1992. A cyclic peptide synthetase gene required for pathogenicity of the fungus *Cochliobolus carbonum* on maize. Proc. Natl. Acad. Sci. USA 89:6590-6594.

Parry, D. W., Jenkinson, P., and McLeod, L. 1995. *Fusarium* ear blight (scab) in small grain cereals—a review. Plant Pathol. 44:207-238.

Proctor, R. H., Hohn, T. M., and McCormick, S. P. 1995. Reduced virulence of *Gibberella zeae* caused by disruption of a trichothecene toxin biosynthetic gene. Mol. Plant-Microbe Interact. 8:593-601.

Sutton, J. C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Plant Pathol. 4:195-209.

Wang, Y. Z., and Miller, J. D. 1988. Effects of *Fusarium graminearum* metabolites on wheat tissue in relation to *Fusarium* head blight resistance. J. Phytopathol. 122:118-125.

Wei, C.-M., and McLaughlin, C. S. 1974. Structure-function relationship in the 12,13-epoxytrichothecenes, novel inhibitors of protein synthesis. Biochem. Biophys. Res. Commun. 57:838-844.

Yang, G., Turgeon, B. G., and Yoder, O. C. 1994. Toxin-deficient mutants from a toxin-sensitive transformant of *Cochliobolus heterostrophus*. Genetics 137:751-757.