Fusarium Head Blight Inoculum: Species Prevalence and *Gibberella zeae* Spore Type

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ABSTRACT

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The objectives of this study were to examine the relative abundance of *Gibberella zeae* ascospores and conidia and other *Fusarium* species on wheat spikes in a field environment, to relate inoculum counts of *G. zeae* to airborne spore counts, and to evaluate an inoculum bioassay technique. The inoculum levels of *Fusarium* species and airborne spores of *G. zeae* were measured in North Dakota during the 1999, 2000, and 2001 growing seasons. Spores were collected from wheat spikes in a 24-h potted-plant bioassay in a fallowed field and in a spring wheat plot bioassay. Inoculum levels of *Fusarium* species were assessed by placing a solution recovered from bioassays on selective medium; meanwhile, ascospores and conidia of *G. zeae* were enumerated microscopically. A Burkard cyclonic sampler measured airborne spore levels in the fallowed field. Wheat spikes were inoculated with known concentrations of conidia or ascospores, and rinsate was put on selective medium at different intervals to compare recovery rates. Known concentrations of both spore types were also applied directly to selective medium to compare with recovery of spore types from inoculated spikes. *Fusarium graminearum* was the most prevalent *Fusarium* species on wheat spikes, although *F. moniliforme* and *F. poae* counts were highest on some days. Approximately twice as many ascospores were recovered in both the 24-h potted-plant field bioassay and the cyclonic sampler as were conidia. Significantly more colonies were recovered from wheat spikes after conidial inoculation than after ascospore inoculation at an identical concentration regardless of time of rinsate collection. Colony numbers did not differ significantly following application of ascospores and conidia to selective medium. Results confirm the predominance of *G. zeae* inoculum in North America but indicate conidia play an important role in the primary disease cycle.

Additional keywords: epidemiology, scab

Fusarium head blight (scab) of wheat and barley is caused primarily by the pathogen *Gibberella zeae* (Schwein.) Petch (anamorph: *Fusarium graminearum*) in North America (1,8,17). *Fusarium sporotrichioides*, *F. equiseti*, *F. moniliforme*, and other *Fusarium* species can cause head blight also but are less able to do so than *G. zeae* (16). Crop debris, particularly cereal stubble, gives rise to inoculum in the form of ascospores and conidia when colonized by *G. zeae* (3,17,19,21). Prevalence of airborne ascospores has been linked to such factors as rainfall and humidity (4,11,13). Given the appropriate environmental conditions for inoculum dispersal and disease development, an outbreak can

decimate a crop (1,8,17). Although ascospores and conidia are equally infective (15), at least one study found many fewer conidia present in airborne samples taken over an infected corn-kernel inoculated field, suggesting that ascospores exerted greater disease pressure on the host than conidia (4).

A bioassay has been used by researchers to assess the relative abundance of *Fusarium* species on wheat spikes in multiple growing regions (5). Although results have been published using the technique, the relative adherence of conidia and ascospores to the wheat spikes has not yet been examined; moreover, spore recovery relative to time after inoculation has not been studied. In another study (S. Markell and L. Franci, unpublished), ascospore levels collected from a Burkard volumetric sampler have not corresponded well to inoculum levels collected from the aforementioned bioassay. This disparity suggests that conidia may play a larger role in Fusarium head blight epidemics than previously thought.

The goals of this research were to assess the abundance of *Fusarium* species present on wheat spikes exposed to field conditions, evaluate the recovery efficiency of

the inoculum bioassay, and determine the relative contribution of conidia and ascospores as potential inoculum sources. In addition, a preliminary analysis was conducted for the relationship between environmental variables and inoculum levels.

MATERIALS AND METHODS

Field inoculum. Research to obtain relative levels of *Fusarium* species on wheat spikes was conducted at the North Dakota State University Experiment Station in Fargo. The hard red spring wheat cultivar Norm (susceptible to Fusarium head blight) was planted on wheat straw left from the previous growing season. The period in which spikes were in anthesis was extended by multiple planting dates in a randomized complete block design replicated three times. In 1999, Norm was planted on 29 April, and 19 and 24 May; in 2000, plantings were on 25 April, and 3 and 10 May; and in 2001, plantings were on 3, 14, and 25 May. Treatment 1 from the first planting date was covered with clear plastic to speed seedling growth (10), while treatments 2, 3, and 4 (one treatment from each planting date) were left uncovered. As wheat emerged, plastic was cut to allow for air and moisture exchange. Each of the 12 blocks measured 12.2 × 1.2 m and was surrounded by a 1.2-m fallow border.

Although wheat straw from a Fusarium head blight-susceptible cultivar served as a base inoculum reservoir in all years, artificial inoculum was added in 2000 to compensate for low disease intensity in the 1999 season. To prepare the inoculum, a hyphal tip of *G. zeae* field isolate Hagar94 was transferred to half-strength potato dextrose agar (PDA) in a 100 × 15 mm petri plate and allowed to grow 1 week. Stainless steel cake pans measuring 23 × 33 × 8 cm were filled nearly half full with corn kernels and 600 ml of distilled water. Kernels were allowed to soak overnight and then were autoclaved for 3 h. The colonized agar was blended in 500 ml of sterile distilled water and aseptically mixed with the corn kernels. Cake pans were kept covered in a growth chamber at 22°C. The mixture was stirred aseptically after 1 week and incubated two more weeks. Corn kernels were spread on plots and interior border rows at a rate of 10 g/m² at stem elongation, Zadoks 30 to 35 (22).

The plots were sampled daily at 1000 h between heading and early milk, Zadoks

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58 to 72. Five spikes from each replication were clipped and placed in a solution of 50 ml of sterile distilled water and 1 drop of Tween 20 surfactant. The solution was shaken vigorously for 2 min, decanted, and frozen at -80°C until later assessment.

Inoculum levels were similarly assayed on potted Norm wheat plants, which had been grown in the greenhouse and then isolated in a growth chamber for 1 week before heading. Once in anthesis, three pots of Norm were transferred to a fallow field environment with pots spaced approximately 25 m apart in a line. One pot of Norm was left in the growth chamber as a control. Pots were transferred between 0730 and 0800 h and remained in the fallow field for a 24-h period. The 3,500 m^2 fallow field contained wheat stubble from the previous year's crop and, in 2000 only, stubble plus corn kernel inoculum that was spread at 8.2 g/m^2 . Upon collection from the field, four spikes from each pot were clipped immediately and placed in an autoclaved solution of 40 ml of distilled water and 1 drop of Tween 20. The solution was shaken vigorously for 2 min, decanted, and frozen until later assessment. The study ran from days 177 to 202, 172 to 200, and 168 to 194 in 1999, 2000, and 2001, respectively.

To determine species abundance, a 0.5-ml aliquot of thawed solution was transferred under sterile conditions to Komada's medium, an antibiotic, *Fusarium*-selective medium (2). Cultures were grown at room temperature and under light conditions according to Tschanz et al. (18). After 1 week, colonies were categorized by color and type of growth. Sample colonies of each type were transferred to half-strength potato dextrose agar (PDA), carrot agar, and/or carnation leaf agar for identification of *Fusarium* species (2,9). The number of colonies identified in each specific category was multiplied by the percentage of colonies in each category to derive the population levels of each *Fusarium* species.

Environmental conditions were recorded every 30 min with an automatic weather station (Campbell Scientific model CR10X, Provo, UT) in a field adjacent to the aforementioned field plots. Sensors monitored surface wetness as electrical resistance (kilo-ohms) at the canopy; relative humidity (percent) and temperature (degrees Celsius) at 2 m; and rainfall (mm), solar radiation ($\text{KJ}/\text{m}^2\text{s}$), wind speed (m/s), and wind direction (degrees) at 3 m. Inoculum levels and environmental summaries for a 24-h and an 8-h period before assay were correlated using the Minitab statistical program (Minitab, Inc. Release 12, State College, PA).

G. zeae spore type recovery. To compare ascospores and conidia recovered on the 24-h potted-plant bioassay, 10 ml of the solution from each pot was centrifuged for 15 min at 5,000 rpm. Nine milliliters of supernatant was decanted, and the three

remaining 1-ml samples were combined for each day (the control excluded) to create a daily sample. The daily sample was centrifuged again for 15 min at 5,000 rpm and the top 2 ml of solution decanted. If the colony forming units (CFU) for that day were found to be in the top 20% of all assayed days, the remaining solution was observed microscopically to count *G. zeae* conidia and ascospores. Conidia and ascospores were tabulated from a 15- μl aliquot placed on a microscope slide and scanned in parallel fields. Scanning stopped when either fields equivalent to 12 μl had been viewed, or more than 12 spores had been observed. Sixteen days were examined.

Airborne spores were also sampled in 2001 with a Burkard cyclonic spore sampler (Rickmansworth, UK) at the same field site as the 24-h bioassays. The volume of air sampled was approximately 1 m^3/h in 19- to 74-h intervals. Sample collection followed the protocol of Larson et al. (7), which resulted in a final volume of 1 ml. Three 15- μl drops of the sample were placed on a microscope slide, and conidia and ascospores were tabulated. Samples were collected from day 171 to day 212 in 2001.

Evaluation of bioassay: *G. zeae* spore type recovery. Adherence of *G. zeae* ascospores and conidia from wheat spikes was analyzed by direct inoculation of wheat spikes at mid-anthesis (Zadoks 65) with a known amount of each spore type. Inocula of ascospores and conidia were obtained from *G. zeae* field isolate Hagar94. Conidia and ascospores were produced on carnation leaf agar. Conidia were harvested from a 7- to 10-day-old culture and frozen until inoculation. Ascospores were harvested from the petri dish lid of a 7- to 10-day-old culture. Inoculum levels of 250 ascospores per spike, 250 conidia per spike, or water were applied to the wheat spikes with a paint spray gun (Badger Basic model 250, Franklin Park, IL) in an encasement designed to eliminate external air disturbance and ensure constant application levels. Clipped spikes were then placed in a moist incubator with their peduncles in water. Conidia-inoculated, ascospore-inoculated, and water-inoculated (control) spikes were sampled separately in groups of four at intervals of 0.5, 3, 7, and 24 h after inoculation using the field sampling protocol described above. Three repetitions of this experiment were performed.

A 0.5-ml aliquot of solution was transferred under sterile conditions to Komada's selective medium and incubated at 22°C . Colonies were counted, and comparisons were made among time intervals and spore type. A *t* test and linear regression were used to analyze results. To compare recovery of conidia and ascospores, three plates of Komada's medium were directly inoculated with 25, 50, 100, or 250 conidia or ascospores and allowed to grow as de-

scribed above. Three replications were performed.

RESULTS

Fusarium head blight occurred in the experimental fields in 1998, 1999, 2000, and 2001. Spring wheat planted in 1998 had a final disease severity of 21%. In 1999, disease severity in Grandin and Norm fields adjacent to the research plots was 6 and 1.3%, respectively. Grandin field severity in 2000 was approximately 7% and was less than 1% in 2001.

Field samples. Of the seven *Fusarium* species observed on the 24-h potted-plant bioassay in 1999, *F. graminearum* was the most prevalent species throughout the study (Fig. 1). *F. equiseti* and *F. sporotrichioides* were observed during the entire period, and *F. sporotrichioides* became most frequent late in the study, about the same time daily levels of *F. graminearum* declined (results not shown). *F. moniliforme*, *F. semitectum*, *F. culmorum*, and *F. sambucinum* were found sporadically in low numbers (Fig. 1). No *Fusarium* species were recovered from control spikes except for *F. moniliforme* on one occasion.

Treatments 1 and 4 were not sampled in 1999 due to unfavorable wheat growth. Three different *Fusarium* species were commonly recovered from treatments 2 and 3 in the Norm field plots (Fig. 1). *F. graminearum* was the dominant species in both plots, followed closely by *F. sporotrichioides*. *F. equiseti* was consistently recovered but in less frequency than either of the other two species.

F. graminearum and *F. moniliforme* were the most common of seven species recovered on the 24-h potted-plant bioassay throughout 2000 (Fig. 1). *F. graminearum* was less common than both *F. sporotrichioides* and *F. equiseti* in the beginning of the study, but became predominant as the period progressed. Recovery of both *F. equiseti* and *F. moniliforme* remained moderate to high until the latter part of the study when observations of both species became less frequent. *F. sporotrichioides*, *F. semitectum*, *F. sambucinum*, and *F. poae* were observed at low levels throughout the study. *F. moniliforme* was recovered from control plants twice during the study.

Seven different *Fusarium* species were also recovered from the field plots in 2000 (Fig. 1). Low levels of all species were observed in treatments 3 and 4, and data were not analyzed further. Treatments 1 and 2 reached anthesis simultaneously, so collections of sample dates overlapped (Fig. 2). The highest counts of *G. zeae* inoculum were recovered from both plots on days 179 and 183, contradicting what was recovered during the 24-h bioassay (Fig. 2). *F. sporotrichioides*, *F. equiseti*, and *F. moniliforme* were all observed frequently throughout the study in moderately low levels (Fig. 1). Low levels of *F. sam-*

bucinum, *F. chlamydosporum*, and *F. avenaceum* were also observed.

F. graminearum was the most frequently isolated *Fusarium* species for the 24-h potted-plant bioassays in 2001. *F. sporotrichioides*, *F. moniliforme*, and *F. equiseti*

were found in moderate amounts throughout the study (Fig. 1). The highest levels of *F. graminearum* recovered throughout the 3-year study were found in 2001 (Fig. 2).

Time of maturation differed among treatments 2, 3, and 4 in 2001, which re-

sulted in a 24-day continual sampling window in the wheat plots (Fig. 2). The only high inoculum levels recovered from treatment 2 were of *F. graminearum* in the first few days it was sampled; all species levels were low after that. High levels of *F.*

*All *F. poae* in the 2001 24 h bioassay was recovered on only two days in the study

Fig. 1. Percent abundance of *Fusarium* species by year on wheat spikes in a 24-h potted-plant bioassay and the combined field plot bioassays.

graminearum appeared on the last few days sampled in treatment 4. Very high levels of *F. poae* occurring during 2 days of collection (day 201 and day 203) accounted for 60% of all *Fusarium* species recovered during the entire study (Fig. 1).

Inoculum recovery and environment.

Environmental conditions were not correlated with daily *G. zeae* inoculum levels (potted plants) in any of the years of the study. Multiple regression analysis found no significant relationship between *G. zeae*

inoculum levels and 24-h environmental summaries in any of the years. Regression between environmental conditions during the last 8 h of each 24-h potted-plant bioassay and inoculum levels revealed no significant association.

Fig. 2. Daily levels of *Fusarium graminearum* colony forming units (CFU) recovered from wheat spikes in a 24-h potted-plant bioassay and field plot bioassays and millimeters of rainfall in 1999, 2000, and 2001 field studies.

Rainfall frequency was similar in all years, with at least trace amounts of rainfall recorded on 31, 38, and 37% of the days in 1999, 2000, and 2001, respectively. However, rainfall amounts were highest in 2000. Five days with 6 mm or more of rain were observed in 2000, while only 3 and 2 days in 1999 and 2001, respectively, recorded rain periods higher than 6 mm (Fig. 2).

G. zeae spore type recovery. A daily fluctuation in the spore type ratio was found when the 24-h potted-plant rinsates were enumerated microscopically (Table 1). Both spore types were observed on all days with five exceptions: day 190 in 1999 when neither spore type was found, and days 174 to 177 in 2001, when only ascospores were observed. When both spore types were recovered, the composition of inoculum per sample varied from 40 to 90% ascospores, with an average of approximately two ascospores to every one conidium.

Of the 108 spores collected by the Burkard volumetric spore sampler that were identified microscopically in 2001, 62 were ascospores (Table 2). The time series was characterized by several high spore counts, consisting of both ascospores and conidia, over relatively short time periods; for instance, in three separate 26-hour or less sampling periods, 12, 13, and 14 spores were recovered.

Significantly more CFU, at all postinoculation time intervals, were recovered from Norm wheat spikes when inoculated with conidia than when inoculated with ascospores (Table 3). CFU recovered from direct application of ascospores and conidia to Komada's medium were not significantly different based on a two sample *t* test comparing each concentration separately (Table 4).

DISCUSSION

Our results agree with surveys of wheat and barley in the northern prairie regions

of the United States and Canada that identified *F. graminearum* as the dominant species causing Fusarium head blight (1,4,6,14,20). Temporal fluctuation in levels of all *Fusarium* species were observed in each of the 3 years, suggesting that some less pathogenic species could influence disease intensity outcome, either alone or through an interaction with *F. graminearum*.

Inoculum levels coincided among collected samples from treatments that overlapped (Fig. 2). In 2000, when all collection days of treatments 1 and 2 overlapped, a strong relationship was observed between both *F. graminearum* (Fig. 2) and *F. sporotrichioides* (results not shown). Inoculum levels were also in agreement during three overlapping days in 1999. The consistent

Table 2. *Gibberella zeae* ascospores and conidia observed microscopically in samples collected from a Burkard volumetric spore sampler in 2001

Sample period		Time (h)	Ascospores	Conidia
Start (day)	End (day)			
171	172	27	2	0
172	173	22	0	0
173	174	29	0	0
174	175	24	0	0
175	176	24	2	3
176	177	24	12	2
177	178	28	3	0
178	179	23	0	0
179	180	24	2	1
180	181	20	5	0
181	183	51	0	0
183	184	26	0	0
184	186	46	0	0
186	187	26	0	2
187	188	19	0	0
188	189	24	0	0
189	190	27	0	0
190	191	24	0	0
191	192	24	0	0
192	193	24	0	0
193	194	25	0	0
194	195	20	Sample lost	
195	197	52		
197	198	24	0	0
198	199	24	10	3
199	200	25	1	4
200	201	24	1	1
201	204	72	9	3
204	205	22	1	0
205	206	23	1	0
206	207	27	4	3
207	208	20	1	0
208	211	74	0	0
211	212	26	5	7
Total		993	62	30

Table 1. *Gibberella zeae* ascospores and conidia observed microscopically in samples of washed wheat spikes of the 24-h bioassay from a field environment

	Day ^y	Ascospores	Conidia	Ascospores/ Conidia	μl sampled	CFU/head ^z
1999	181	9	6	1.5	13.5	180
	182	9	10	0.9	13.5	134
	184	13	7	1.9	16.5	53.2
	185	5	8	0.6	6	119
	186	9	8	1.1	15	149
	187	4	1	4	19.5	70
	189	10	4	2.5	9	125
	190	0	0	0	12	74
2000	188	4	1	4	12	75.6
	189	17	5	3.4	12	67.9
2001	169	18	2	9	11	81.9
	172	7	2	3.5	12	328.6
	174	4	0	NA	13	314.0
	175	3	0	NA	13	129.6
	176	4	0	NA	13	126.0
	177	5	0	NA	12	172.6

^y Plants were collected at 0800 on day of year listed.

^z Inoculum recovered on Komada's medium from potted plants.

recovery of similar inoculum levels in overlapping assays supports the consistency of the technique.

Inoculum levels from the 24-h potted-plant bioassay were comparable to and supportive of data from other field experiments (4). Inoculum levels fluctuated considerably (Fig. 2) throughout the study. In 1999 and 2000, several high spikes in inoculum levels were recorded while wheat plants were in the most susceptible stages of growth (approximate anthesis days were 179 to 183 and 191 to 189, respectively), while no such high inoculum levels were observed while plants were highly susceptible in 2001 (approximate anthesis days were 181 to 186). Although inoculum availability is only one component necessary for disease, our field severities during the 3 years of the study tended to mirror the presence of high inoculum levels during susceptible growth stages.

Generally, more CFU were recovered following rainfall events, but this was not a consistent phenomenon. For example, the 4 days in 1999 with the highest inoculum levels recovered from the potted-plant bioassay (days 181, 182, 186, and 189) had experienced rainfall within the last 24 h, but rainfall had not occurred on days 184, 185, and 187, when relatively high levels of inoculum were observed (Fig. 2). Further study of the relationship between inoculum counts and environmental factors is necessary prior to use in disease forecasting.

In 2001, low inoculum levels while wheat was in susceptible growth stages was likely a consequence of a 168-mm rainfall on day 172, immediately before the study began (not shown in Fig. 2). Both the corn kernel inoculum and natural wheat stubble were submerged or washed away in the week or more that the field was flooded.

Approximately twice as many ascospores as conidia were observed in the 24-

h potted-plant bioassay as well as the Burkard volumetric spore sample. Although this ratio may have been affected by several factors, it is interesting that conidia were often present on days with a high measured inoculum load (Table 1). Presumably, the probability of a successful infection event is greatest under conditions when inoculum levels are high, so these periods are important to blighting potential. Observation of both conidia and ascospores during these critical periods indicates that both spore types play a role as inoculum sources of *Fusarium* head blight.

Attempts to validate experimentally the 2:1 spore type ratio observed under field conditions produced results that are open to several interpretations. Ascospore inoculation of wheat spikes resulted in fewer recovered CFU than conidial inoculation at the same concentration (Table 3), which could be due to differential adherence, spore clumping, or germination. Spore counts after direct application on Komada's medium did not differ significantly, suggesting that germination and clumping properties of both spore types were similar in aggregate (Table 4). Most likely, ascospores adhered more efficiently to wheat spikes than did conidia. Pinkerton et al. (12) showed that ascospores of *Anisogramma anomala*, the pathogen causing Eastern filbert blight on European hazelnut (*Corylus avellana*), adhered irreversibly to certain vegetative tissues on hazelnut after only 5 min. Moreover, *G. zeae* ascospores readily adhere to the wall of the cyclonic sampler, which was redressed by the collection protocol (7). Therefore, *G. zeae* adherence over even the shortest time period tested (0.5 h) seems plausible. However, if more ascospores than conidia adhered to spikes, then the ascospore/conidia ratio in the washed wheat spike solution should have been closer to unity than the

ratio for the cyclonic sampler solution. In contrast to this expectation, both samples recovered about two ascospores to one conidium (Tables 1 and 2).

One problem experienced on some days, particularly those when rain had occurred, was that it was often difficult to get a potted-plant bioassay sample clean, yet concentrated enough, to view either spore type. In some samples, so much dirt had been splashed on the wheat spikes that, although a high level of colonies were recovered on assayed spikes, few if any spores could be identified microscopically and therefore went uncounted. This was unfortunate, considering rainfall has been linked to high inoculum and disease levels (4,8,13,17). Similar problems were noted when microscopically viewing samples of conidia and ascospores collected from the Burkard cyclonic spore sampler, although not nearly as severe. Despite being closer to the ground, the small cross-sectional surface area (28 mm²) of the air intake orifice may have limited the amount of unwanted material that entered the sample, unlike the wheat spike bioassay, which collected more unwanted material on its high surface area. However, we presume an equal chance of seeing conidia as ascospores for each collection method so the observed spore ratio should be unaffected.

Observation of both conidia and ascospores on wheat spikes and the implications for overall inoculum level and infection by each spore type are useful to understanding the dynamics of both the pathogen and the disease. Clearly, any attempt to manage head blight by focusing on inoculum will have to take both spore types into consideration. Further research concerning the source of conidia and their fate on wheat spikes will decipher more thoroughly the disease cycle of *Fusarium* head blight.

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Table 3. Average recovery of *Gibberella zeae* ascospores and conidia from Norm wheat heads inoculated with equal numbers of spores at four different postinoculation times

	CFU/head				Regression	
	0.5 h	3 h	7 h	24 h	Slope	Linear fit
Conidia/head	153.33 a ^z	120 a	120 a	33.33 a	-0.139	Yes
Ascospores/head	26.67 b	40 b	46.67 b	13.33 b	-0.714	No
Conidia/ascospore	5.75	3	2.57	2.5		

^z Numbers within a column followed by the same letter are not significantly different according to a *t* test with $\alpha = 0.05$.

Table 4. Average number of colony forming units (CFU) recovered on Komada's medium when known quantities of *Gibberella zeae* ascospores and conidia were applied

Application rate spores/plate	Conidia (μ_1) CFU/plate	Ascospore (μ_2) CFU/plate	<i>P</i> value ^z ($\mu_1 = \mu_2$)
25	10	5	0.21
50	15	9	0.22
100	35	21	0.08
250	72	53	0.25

^z $P > 0.05$ indicates no evidence of significant difference between spore types based on three replications.

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