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Abstract

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Sorghum grain, valuable for feed, food, and bioenergy, can be colonized by several *Fusarium* spp.; therefore, it was of interest to identify possible sources of conidia. Analysis of air and soil samples provided evidence for the presence of propagules from *Fusarium* genotypes that may cause grain infections. Soil population estimates of members of the *Gibberella fujikuroi* species complex, that includes sorghum pathogens and other *Fusarium* spp., suggested that adequate inoculum for systemic infections was present. Conidia in air samples within two sorghum fields were collected by passive trapping for 2 years. Subsampled *Fusarium* isolates indicated that numbers of *G. fujikuroi* increased from anthesis through maturity, which coincides with grain development stages vulnerable to *Fusarium* spp. Genotyping using translation elongation factor 1-α gene sequences revealed that spore trap isolates included members of *G. fujikuroi* that are sorghum pathogens: *Fusarium thapsinum, F. verticillioides, F. proliferatum*, and *F. andiyazi.* Also detected were *F. graminearum*, *F. subglutinans*, and several *F. incarnatum-F. equiseti* species complex haplotypes that colonize sorghum asymptomatically. All commonly found grain colonizers were detected from air samples in this study.

Sorghum grain is valuable for feed, food, and bioenergy. A C_4 crop that is drought and heat tolerant, sorghum can be grown in tropical, subtropical, and warm-temperate climates under subsistence farming conditions (45). In the United States, grain is produced primarily for livestock feed but, in other cultures, it is a food staple (45). Because it is gluten-free and contains nutrients and protective phytochemicals unique among grasses, food-quality sorghum is produced in the United States as an alternative grain (3,56). Sorghum grain can also be used in ethanol production for biofuels (59), and the distiller's grain coproduct is useable for ruminant livestock feed (1).

An advantage to growing sorghum grain for food or feed is that production of mycotoxins as a result of contaminating fungi can be considerably less than that on maize kernels (4,7). This may be due to reduced total colonization by mycotoxigenic fungi (4) or lower toxin production by genotypes infecting sorghum (11,25,35). Thus, sorghum grain not only has many uses, it also has the potential to be advantageous for food and feed under less-than-optimal growing or storage conditions. Nonetheless, dangerous levels of mycotoxins have resulted in sorghum, especially in stored grain (23). Natural infection of sorghum grain by *Fusarium* spp. can result in production of mycotoxins such as fumonisin B_1, moniliformin, zearalenone, T-2, and deoxynivalenol (7,20,27,29). Previously, we reported 10 *Fusarium* spp. or genotypes within *Fusarium* species complexes in grain of elite sorghum lines grown in Nebraska fields (16). Eight of these species have been reported to produce mycotoxins: five members of the *Gibberella fujikuroi* species complex, two genotypes in the *Fusarium incarnatum-F. equiseti* species complex (FIESC; formerly known as *F. bullatum* Sherb. and *F. pallidoroseum* (Cooke) Sacc.), and *F. graminearum* Schwabe. *Fusarium* spp. most commonly recovered are within *G. fujikuroi* (Sawada) Wollenw., particularly *F. thapsinum* Klittich, J.F. Leslie, P.E. Nelson & Marasas (16). It is of great interest to define potential sources for *Fusarium* spp. colonizing sorghum grain.

Fusarium spp. are major components of the complex that causes sorghum grain mold, a disease common in humid tropical and subtropical regions (5,54). It results in reduced grain yields but equally serious are affects on physical appearance and biochemical content of the grain (54,58) and the presence of mycotoxins (29). Sorghum is particularly vulnerable to grain mold pathogens at anthesis (flowering) through grain hard-dough stage but exposed parts of the grain can be infected until post maturity, as long as weather conditions are conducive to grain diseases and conidia of pathogens are present (6,12,14,33,34,39,52). However, previous work with maize provided evidence that *F. verticillioides* (Sacc.) Nirenberg, a member of *G. fujikuroi*, at a low rate via systemic infection, could colonize kernels of plants grown from inoculated seed (37); it is conceivable that infected grain could also result from systemically infected plants arising from endophytically infected sorghum seed or from seedlings infected through roots by soilborne fungi. Therefore, we screened soils for *Fusarium* spp. from sorghum research plots and production fields in Nebraska and monitored conidia from within and above the canopies of sorghum fields, using passive spore trapping on two fungal media, throughout two growing seasons. In this way, the availability of potential inoculum in soil, that included organic matter, and in air was determined. Isolates were identified by morphological traits (soil and air) and molecular identification using the 5′ portion of the translation elongation factor 1-α (*TEF*) gene (air) (19). In this way it was possible to determine whether infections by *Fusarium* spp. identified from sorghum grain grown in Nebraska could be associated with *Fusarium* genotypes found in soil or air.

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Materials and Methods

Soil screens. Soil from sorghum research plots at the University of Nebraska, Lincoln, Field Laboratories (UNL) on the east side of Lincoln and near Ithaca, NE, was sampled pre-planting in 2005 and post harvest in 2005 and 2006. Soil also was sampled at Lincoln at harvest 2004 and one time during the winter in 2005. Fields

at Ithaca were irrigated using an overhead sprinkler system. Soil was collected from relatively dry fields. The upper 2 cm of soil, which was often dry and dusty, and debris were removed and discarded from the surface; then, 0.3 to 0.4 liters of soil was collected, using a shovel, to 10 cm below the original soil surface. Three samples were taken randomly across each field, then combined (a total of 1.0 to 1.2 liters) and mixed. The research fields were rotated with other crops in alternate years and conventionally tilled. Following harvest, plant materials were disked into the soil during late fall if weather permitted and in the spring, prior to planting; larger plant pieces were left on the surface. The 2005 Lincoln field was in soybean-sorghum rotations since at least 2000; thus, the preplanting sample obtained in 2005 was following soybean grown during summer 2004. The 2006 Lincoln field was in corn-soybean rotation since at least 2000, with soybean planted in 2005. The order of preceding crops for the 2005 Ithaca field was soybean (2004), sorghum (2003), soybean (2002), wheat (2001), and oat (2000); thus, the preplanting sample obtained in 2005 was following soybean grown during summer 2004. The order of preceding crops for the Ithaca field sampled in 2006 was soybean (2005), sorghum (2004), soybean (2003), wheat (2002), and oat (2001). In soybean fields at both locations, glyphosate-resistant soybean lines were planted since at least 2000 and glyphosate, a low-persistence herbicide (46), was applied at the rate of 2.25 liters ha^{-1}. For sorghum culture, nitrogen fertilizer was applied prior to planting at both locations at 157 kg ha^{-1}. At Lincoln, propachlor (2-chloro-*N*-[1-methylethyl]-*N*-phenylacetamide) at 3.36 kg ha^{-1} and atrazine (6-chloro-*n*-ethyl-*N*′-[1-methylethyl]-1,3,5-triazine-2,4,diamine) at 1.1 kg ha^{-1} were applied immediately after planting for weed control. At Ithaca, atrazine was applied at 2.2 kg/ha immediately after planting, followed by an application of alachlor (2-chloro-2′,6′-diethyl-*N*-(methoxymethyl)acetanilide) at 4.75 liters/ha and atrazine at 1.1 kg/ha, approximately 14 days post emergence. The insecticide chlorpyrifos (phosphorothioic acid, *O*,*O*-diethethyl *O*-[3,4,5, trichloro-2-pyridinyl] ester) at 177 g/ha was applied at Ithaca in 2004 for grasshopper control. Producers' fields also were sampled, two on the northwest side of Lincoln (designated NW Lincoln A and B) and one near Ruskin, NE, post harvest, during 2005 and 2006. The NW Lincoln fields were in soybean-sorghum rotation while the Ruskin field was in 2-year soybean-sorghum rotation. These fields were not irrigated. Standard commercial sorghum and soybean production practices for south-central and southeastern Nebraska were used (http://www.extension.unl.edu/web/sorghum/home and http://www.extension.unl.edu/web/soybean/home).

To prevent loss of culturable microorganisms, the soils were immediately processed in the following way. For each soil sample, three to six 0.90- to 1.10-g subsamples were assembled and sample weights were recorded; numbers of each microorganism were normalized to 1.00 g. Each subsample was suspended in sterile purified water (Labconco, Kansas City, MO), a dilution series was prepared, and aliquots were plated onto two media. One-fifth-strength potato dextrose agar (PDA), prepared with potato dextrose broth (Becton, Dickinson and Co. [BD], Sparks, MO) and amended with 0.04% Triton X-100 (Calbiochem, EMD Biosciences, San Diego) and ampicillin at 50 mg/liter (Sigma-Aldrich, St. Louis), was used to estimate numbers of culturable fungal and fungal-like colonies. This medium allows for growth of many culturable fungi as well as some oomycetes (32) but, because growth is confined due to the detergent, the groups are difficult to distinguish on this medium. For estimation and isolation of *Fusarium* spp., a semiselective peptone agar, pentachloronitrobenzene (PCNB), was used, which was amended with the fungicide Terrachlor (75% PCNB; Uniroyal Co. Middlebury, CT) at 0.1%, and streptomycin at 1 g/liter (Sigma-Aldrich; 38). In a previous study, approximately 60% of colonies subcultured from PCNB plates applied with soil suspensions were *Fusarium* isolates (17).

Plates were incubated at room temperature (23°C) and fungal and oomycete colonies were enumerated 2 to 5 days following plating. From each soil, 10 to 15 single colonies were transferred from PCNB plates to one-half-strength PDA and allowed to grow 3 to 5 days at room temperature. Putative *Fusarium* spp. were assessed for conidia and conidiophore morphology by transferring an agar block from a PDA culture onto 1.5% agar containing 80 mM potassium chloride and allowed to grow 5 to 7 days at room temperature. Using colony characteristics on PDA and conidia and conidiophore structures on KCl agar, morphological identifications were determined using the taxonomic system of Nelson et al. (40). Species within the complex *G. fujikuroi*, as defined by Leslie et al. (27) and O'Donnell et al. (41), were grouped together for analyses; all other *Fusarium* spp. were also grouped together for analyses. Genera and species outside of *Fusarium* that were identified through the course of the study are mentioned in the results.

Statistical analyses of soil screens. All statistical analyses were performed with SAS v. 9.1. Total fungi and oomycete numbers (CFU per gram soil) growing on each medium were not normally distributed based on Shapiro-Wilk tests for normality and were subsequently log transformed prior to analysis of variance (61). These transformed data were analyzed using PROC MIXED. Replications were treated as random variables.

For analyses of morphological identifications, total numbers of fungi collected from each location and collection time were grouped by members of *G. fujikuroi* species complex, other *Fusarium* spp., and other fungi and percentages of total fungi in each group were calculated. Each group was separately analyzed with PROC MIXED. Where the effect of year was analyzed, location was treated as a random effect. Where the effect of location was analyzed, year was treated as a random effect.

Passive spore trapping. Passive spore trapping (26) in sorghum fields was conducted throughout two growing seasons, 2005 and 2006, at UNL's field laboratories at Lincoln and Ithaca. Fields at Ithaca were irrigated, using overhead sprinklers, while those at Lincoln were not. Four collections were conducted during each season at each location, determined by plant growth stage: during vegetative growth (prior to flowering), at anthesis (defined as approximately half the plants with half the anthers exerted), during grain development, and at harvest. By necessity, passive spore traps were set on days with little or no rainfall and with wind speeds less than 4 m/s or wind gusts less than 8 m/s. Days after planting when passive spore traps were set, plant growth stage, and weather conditions (temperature ranges, percent relative humidity, and precipitation or irrigation occurring up to 5 days prior to setting of traps) are summarized in Table 1. Passive spore traps were prepared by pouring 200 ml of medium, either one-fifth-strength PDA with amendments or PCNB, into 150-by-25-mm round, gridded petri dishes (Integrid, Falcon; BD Labware, Franklin Lakes, NJ). Four 1.8-m fiberglass ladders were set up diagonally across sorghum fields; then, a plate of each medium type was secured to ladder rungs at three heights: 0.3, 0.9, and 1.75 m. The two lower heights were within the canopy and the upper height was above the canopy. Plates were secured using general-purpose vinyl cleanroom tape, 2.5 cm wide (VWR, International LLC), by taping the edge of the bottom plate, then running the tape down one side of the plate, under the ladder rung, and to the opposite side and edge of the bottom plate. Plates were covered until the start of the experiment. Media in plates were exposed by uncovering for 5 min., 1 h, 8 h, and 24 h. One plate of each medium at each height was exposed for each length of time. Occasionally, plates exposed for 24 h and, rarely, 8 h were damaged due to animals, heavy dew, excessive dryness, or dust caused by nearby farming operations. Following exposure, plates were incubated in the laboratory at room temperature for 2 to 4 days, then counted, and colonies per centimeter squared per hour were determined. For each exposure time and each height, up to five colonies were transferred from PCNB plates onto one-half-strength PDA medium, then characterized as described above. Percentage of total fungi that were members of the *G. fujikuroi* species complex, and other *Fusarium* spp., as well as other fungi, were determined.

Molecular identification. Representative isolates (n = 144) from subsampled colonies from each PCNB plate were single-co-

nidium purified. For each isolate, DNA was extracted from ground lyophilized mycelium (24) and the 5′ region of the *TEF* gene was polymerase chain reaction (PCR) amplified using primers EF-1 and EF-2 (19). Amplification products were sequenced and sequences from opposite strands were assembled using Sequencher 4.10.1 (Gene Codes Corp., Ann Arbor, MI). Assembled sequences were compared with those in the publically available FUSARIUM-ID database (http://isolate.fusariumdb.org) (19). Sequences were submitted to GenBank, accession numbers JF270168 to JF270311. Sequences of some FIESC isolates and one *F. oxysporum* species complex had equivalent similarity scores to multiple haplotypes in the database; in these cases, all highly similar haplotypes were listed (e.g., 1-a,c,4-b). Genotypes of *F. thapsinum, F. subglutinans* (Wollenw. & Reinking) P.E. Nelson, Toussoun & Marasas, and *F. proliferatum* were defined in the following way for the current study. *TEF* sequences from air isolates similar to *F. thapsinum* were highly similar to *Fusarium* sp. isolate number 1851 (100% similarity) and a *G. fujikuroi* isolate identified as *F. thapsinum* (99% similarity) when compared with sequences in the FUSARIUM-ID database. When the sequences from grain isolates from a previous study were reassessed in the updated FUSARIUM-ID database, isolates previously identified as *F. thapsinum* yielded the same result (*Fusarium* sp. no. 1851 [100% similarity] and *G. fujikuroi* anamorph *F. thapsinum* [99% similarity]). For the purposes of this study, this group of *Fusarium* isolates with identical *TEF* genotypes will be designated as *F. thapsinum*. When *TEF* sequences from air isolates from the present study and grain isolates that were identified as *F. subglutinans* in a previous study (16,17) were submitted to the updated FUSARIUM-ID database, they were identified as *F. subglutinans* or *Fusarium* sp. isolate number 1862, which were highly similar genotypes (near 100% similarity). Therefore, these genotypes will be designated as *F. subglutinans* for the present study. Sequences from the *TEF* region from some air isolates were identified as being from *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg but a few isolates also had high similarity with the *TEF* sequence of *Fusarium* sp. isolate number 1858 (99 to 100% similarity); isolates with these *TEF* genotypes will be referred to as *F. proliferatum* in the present study.

Statistical analyses of passive spore traps. For analysis of total number of fungal numbers growing on each medium, counts from different exposure times were standardized by calculating colonies per centimeter squared per hour. Nonparametric analyses were performed using the NPAR1WAY procedure. The SAVAGE option, recommended for exponential distributions or data with large shifts in extreme values (21,50), was used to assign rank scores to individual observations. No significant differences were observed for each medium by year ($P \geq 0.40$) or location ($P \geq 0.38$). However, it was known that Ithaca was irrigated while Lincoln was not and that environments should be expected to be dissimilar. Therefore, results were analyzed by environment (location and year), and significant differences became apparent. For morphological identifications, total number of fungi collected from each location, collection time, and sampling height were grouped to include members of the *G. fujikuroi* species complex, other *Fusarium* spp., or other fungi and percentages of total fungi in each group were calculated. Each group was analyzed using the NPAR1WAY procedure as described above.

Results

Soil sampling. During 2005, soil samples were taken prior to planting and at harvest from fields at Lincoln and Ithaca, NE, that had been planted with soybean during the 2004 growing season. When comparing numbers of total culturable fungi and oomycetes from soil collected at the two 2005 seasons, there were significant effects of location ($P = 0.05$), season ($P < 0.01$), and their interactions ($P = 0.01$). At Lincoln, mean CFU per gram of soil declined significantly ($P < 0.01$) from 1.05×10^5 in the spring to 1.1×10^4 at harvest. At Ithaca, there were no significant differences ($P = 0.24$) and the means at both seasons were 6.5×10^4 CFU g^{-1} of soil. Soil also was sampled from Lincoln, at harvest 2004 and in winter and spring 2005, to determine whether total fungal and oomycete numbers can change during winter weather typically found in Nebraska. When comparing 2004 harvest and 2005 winter samples, a 2.5 fold decrease, from 3.39×10^5 to 1.32×10^5 CFU g^{-1} of soil, was observed ($P = 0.01$), but there was no further significant change in mean numbers in the 2005 spring sample ($P = 0.52$). Comparisons also were made between mean numbers of total culturable fungi and oomycetes at harvest at Lincoln and Ithaca during 2005 and 2006; location and year effects were apparent ($P \leq 0.01$) but not their interactions ($P = 0.27$). The mean numbers of culturable fungi and oomycetes at Lincoln in 2005 was 1.12×10^4 and in 2006 6.61×10^4 CFU g^{-1} of soil whereas, at Ithaca, the 2005 and 2006 post-harvest counts were 5.01×10^4 and

Table 1. Temperature range and percent relative humidity (RH) during and rainfall or irrigation 5 days prior to the 24-h time period in which passive spore traps were set

Passive spore trap	DAP[x]	Temp. (°C)[y]	RH (%)	Precipitation and irrigation (cm)[z]	Days prior to sampling
Lincoln 2005					
Vegetative	56	26 ± 9	68.1	0.0	>5
Anthesis	71	21 ± 11	62.7	11.3	2
Grain development	112	24 ± 10	71.6	0.2	1
Maturity	145	17 ± 13	52.2	1.1	5
Ithaca 2005					
Vegetative	58	31 ± 9	65.4	2.4	2
Anthesis	71	27 ± 7	69.4	0.1	0
Grain development	112	28 ± 9	66.7	0.3	0
Maturity	145	18 ± 17	49.7	0.0	>5
Lincoln 2006					
Vegetative	40	29 ± 8	55.3	2.3	4
Anthesis	60	22 ± 8	69.8	0.6	1
Grain development	95	21 ± 12	64.1	0.0	>5
Maturity	141	11 ± 5	45.3	0.0	>5
Ithaca 2006					
Vegetative	62	32 ± 12	56.6	0.0	> 5
Anthesis	72	27 ± 11	70.0	3.8	1 (I)
Grain development	99	20 ± 11	68.7	1.1	2
				3.8	4 (I)
Maturity	162	10 ± 10	67.1	0.4	1

[x] Days after planting. Fields were planted at Lincoln on 18 May 2005 and 15 June 2006 and at Ithaca on 23 May 2005 and 18 May 2006.
[y] Approximate temperature ranges are indicated by mean and standard deviations of high and low temperatures.
[z] Centimeters of precipitation or irrigation (I) 5 days or less prior to passive spore trapping are shown. Combination of 0 cm for "Precipitation and irrigation" and >5 for "Days prior to sampling" indicates that no precipitation or irrigation events had occurred for at least 5 days prior to setting passive spore traps.

1.32×10^5 CFU g^{-1} of soil, respectively. When comparing the mean numbers of culturable fungi and oomycetes at Lincoln and Ithaca with two other fields in Lincoln and a field at a distance in Ruskin, NE, again, there were location and year effects ($P \leq 0.01$) but no effects of their interactions ($P = 0.20$). The mean fungal and oomycete CFU per gram of soil ranged from 1.23×10^4 at Lincoln in 2005 to 1.70×10^5 at NW Lincoln field A in 2006.

When considering fungal colonies grown on *Fusarium* semiselective medium (PCNB) for soil collected at the two seasons during 2005, again, there were significant decreases in the number of CFU per gram of soil at both locations. Location effects were not significant ($P = 0.61$) but season effects ($P < 0.01$) and location–season interactions ($P = 0.05$) were. Mean CFU per gram of soil decreased 10-fold (from 7.94×10^4 to 7.24×10^3) at Lincoln and 4-fold (from 4.17×10^4 to 1.07×10^4) at Ithaca. When comparing mean CFU per gram of soil in samples from Lincoln and Ithaca during 2005 and 2006, selected on PCNB, year effects were observed ($P < 0.01$) but not location effects ($P = 0.13$); during 2006, mean CFU per gram of soil was 3.5-fold higher than in 2005 (3.24×10^4 versus 8.91×10^3 CFU g^{-1}). When comparing mean CFU per gram of soil from samples from these fields, selected on PCNB, with those from two other fields in Lincoln and one near Ruskin, there were significant location and year effects ($P < 0.01$) but their interactions were not significant ($P = 0.11$). The UNL Lincoln field had significantly fewer CFU per gram of soil of colonies selected on PCNB in both years than the NW Lincoln field A (7.24×10^3 versus 1.82×10^4 CFU g^{-1} for 2005 [$P = 0.02$] and 2.34×10^4 versus 9.77×10^4 for 2006 [$P < 0.01$]) but had similar mean fungal numbers in both years to those from soil samples from NW Lincoln field B ($P \geq 0.27$). No other comparisons of mean numbers of fungal colonies per gram of soil sampled from different fields and selected on PCNB plates were significantly different in both years.

Fungal colonies were subsampled from PCNB plates and, using morphological characteristics, were grouped as members of the *G. fujikuroi* species complex, other *Fusarium* spp., or other fungi. Comparing percentages of isolates collected from Lincoln or Ithaca fields at harvest in 2005 and 2006, there were no significant differences attributed to year ($P \geq 0.47$). Therefore, results from each year were combined. Percentages of *G. fujikuroi* at the two locations (9.5% for Lincoln and 13.0% for Ithaca; standard deviation (SD) = 6.9) and of other *Fusarium* spp. (67.5% for Lincoln and 79.0% for Ithaca; SD = 18.1) were not significantly different at the two locations ($P \geq 0.70$). When comparing percentages of *G. fujikuroi* and other *Fusarium* spp. from soils at Lincoln and Ithaca with those obtained from two fields in NW Lincoln and from the Ruskin field, there also were no significant differences ($P \geq 0.53$).

Fusarium spp. recovered at Lincoln sorghum fields in 2005, as determined using Nelson et al. (40), were *F. graminearum, F. merismoides* Corda*, F. thapsinum*, and *Fusarium* spp. *Fusarium* spp. identified from 2005 Ithaca fields were *F. aquaeductuum* (Rabenh. & Radlk.) Lagerh. & Rabenh., *F. merismoides, F. solani* (Mart.) Sacc., *F. subglutinans, F. thapsinum*, and *Fusarium* spp. In 2006, *F. chlamydosporum* Wollenw. & Reinking, *F. equiseti* (Corda) Sacc., *F. lateritium* Nees, *F. semitectum* Berk. & Ravenel (also known as *F. pallidoroseum*), *F. solani*, and *Fusarium* spp. were recovered at Lincoln and *F. anthophilum* (A. Braun) Wollenw., *F. equiseti, F. proliferatum*, *F. solani*, *F. verticillioides*, and *Fusarium* spp. were found at Ithaca. Also in 2006, an *Alternaria* sp. was recovered at Lincoln and *Microdochium nivale* (Fr.) Samuels & I.C. Hallett was recovered at Ithaca from PCNB plates.

At Lincoln sorghum production fields in 2005, *F. merismoides, F. solani, F. thapsinum*, and *Fusarium* spp. were obtained whereas, at Ruskin production fields, *F. graminearum, F. solani, F. thapsinum*, and *Fusarium* spp. were found. In 2006, *F. avenaceum* (Fr.) Sacc., *F. chlamydosporum, F. equiseti, F. lateritium, F. semitectum, F. solani, F. subglutinans, F. thapsinum, F. verticillioides*, and *Fusarium* spp. were recovered from Lincoln production fields and *F. semitectum, F. solani*, and *F. subglutinans* were found at Ruskin.

Passive spore trapping. Savage scores were generated and compared for fungal numbers (colonies per centimeter squared per hour) passively collected and grown on two media (one-fifth-strength PDA and PCNB) at four environments (Lincoln and

Table 2. Means and rank mean scores (RMS) of colonies per centimeter squared per hour as calculated from fungal colony numbers on passive spore traps on two media (potato dextrose agar [PDA] and pentachloronitrobenzene [PCNB]) exposed for different times at four environments[w]

	PDA			PCNB		
Exposure time	***N*[x]**	**Mean[y]**	**RMS[z]**	***N*[x]**	**Mean[y]**	**RMS[z]**
Lincoln 2005						
5 min	12	12.78 ± 9.99	0.7631 a	12	7.51 ± 7.32	0.5392 a
1 h	12	6.68 ± 3.79	0.0685 a	12	4.15 ± 2.97	–0.0486 a
8 h	12	3.95 ± 2.63	–0.4242 b	12	2.97 ± 2.16	–0.2886 a
24 h	6	0.79 ± 0.66	–0.8147 c	3	0.42 ± 0.25	–0.8081 a
Lincoln 2006						
5 min	12	18.88 ± 14.45	0.9307 a	12	7.44 ± 8.59	0.3778 ab
1 h	12	12.23 ± 8.40	0.3833 a	12	6.81 ± 5.16	0.5561 a
8 h	11	2.60 ± 0.47	–0.5070 b	11	1.75 ± 0.67	–0.3851 b
24 h	12	0.83 ± 0.06	–0.8493 c	9	0.59 ± 0.31	–0.7744 c
Ithaca 2005						
5 min	12	12.11 ± 12.78	0.8486 a	12	5.90 ± 5.86	0.7106 a
1 h	12	5.36 ± 2.60	–0.0290 b	12	2.60 ± 1.72	–0.0692 a
8 h	8	3.33 ± 1.05	–0.4531 b	8	2.14 ± 1.52	–0.2421 a
24 h	7	1.65 ± 0.25	–0.8872 c	7	0.68 ± 0.28	–0.8114 b
Ithaca 2006						
5 min	12	17.37 ± 12.23	0.9714 a	12	7.46 ± 7.67	0.6201 ab
1 h	12	8.75 ± 2.60	0.3029 a	12	5.37 ± 3.31	0.2334 a
8 h	12	3.31 ± 0.71	–0.5480 b	11	2.89 ± 0.94	–0.2241 b
24 h	10	1.05 ± 0.25	–0.8717 c	10	0.88 ± 0.28	–0.7778 c

[w] Counts on plates were normalized for different exposure times by calculating colonies per centimeter squared per hour. For convenience to the reader, means and standard deviations are shown. The two media were one-fifth-strength PDA and a peptone-based agar containing the fungicide PCNB. PDA allows growth of culturable fungi while PCNB is semiselective for *Fusarium* spp. The four environments were Lincoln, NE in 2005 and 2006 and Ithaca, NE in 2005 and 2006. Fields at Ithaca were irrigated.

[x] Number of observations. Some 24-h plates and a few 8-h plates were damaged due to animals, heavy dew, excessive dryness, or dust caused by nearby farming operations.

[y] For convenience to the reader, means (colonies per centimeter squared per hour) with standard deviations are shown.

[z] Savage scores, generated for each exposure time of each medium at each environment, were compared using NPAR1WAY procedure (50). Pairwise comparisons were conducted between exposure times, within an environment, for colonies per centimeter squared per hour calculated from colonies on each medium. Letters next to RMS that are different indicate that χ^2s are significantly different at $P \leq 0.05$.

Ithaca, 2005 and 2006). Comparisons were made for plate height in stand (0.3, 0.9, and 1.75 m), exposure time (5 min., 1 h, 8 h, and 24 h), and plant growth stage (vegetative, anthesis, grain development, and maturity). There were no significant differences in means with regard to plate heights in each environment for colonies per centimeter squared per hour growing on PDA or PCNB ($P \geq 0.50$). Thus, the three heights were treated as replicates for each exposure time.

Comparisons between Savage scores generated from numbers of fungi (colonies per centimeter squared per hour) collected on PDA for each exposure time showed that, for all environments, they were significantly different ($P < 0.01$; Table 2). The greatest numbers of colonies per centimeter squared per hour were obtained for the 5-min exposure time. Pairwise comparisons between the different exposure times showed that the 5-min and 1-h exposure times were not significantly different for colonies per centimeter squared per hour ($P \geq 0.07$), except for the field at Ithaca, NE in 2005 ($P = 0.03$). PDA medium exposed for 24 h had significantly fewer colonies per centimeter squared per hour than all other exposure times ($P \leq 0.04$; Table 2).

Comparisons of Savage scores generated from numbers of fungi (colonies per centimeter squared per hour) growing on PCNB plates following each exposure time resulted in significant differences at Lincoln fields in 2006 and Ithaca in both years; exposure times at Lincoln in 2005 yielded colonies per centimeter squared per hour that were not significantly different ($P = 0.07$; Table 2). For all four environments, pairwise comparisons between the 5-min exposure time and the 1- or 8-h exposure times showed the scores resulted with χ^2 probabilities that were not significantly different ($P \geq 0.06$).

For each environment, Savage scores were generated and compared for colonies per hour for sampling at each plant growth stage and for each medium. For colonies per centimeter squared per hour growing on PDA, plant growth stage was significant from both locations in 2005 ($P < 0.01$) but not in 2006 ($P \geq 0.26$). Nonetheless, there was a trend toward increased colonies per centimeter squared per hour being collected at anthesis compared with colonies collected at the vegetative stage (Table 3). When considering colonies per centimeter squared per hour, as calculated from colonies growing on PCNB following collection at each growth stage, this trend of increasing number through the growing season was again apparent, especially at Lincoln fields in both years. At Ithaca fields, an increase was noticed at maturity in 2005 and at grain development in 2006 (Table 3).

Morphological characterization. Fungi were characterized by colony, conidia, and conidiophore morphologies and identified as belonging to one of three groups: *Fusarium* spp. in the *G. fujikuroi* species complex, other *Fusarium* spp., or other fungi. Savage scores were generated and compared for percentage of total fungi in each group, by environment, for height and exposure time of plate from which colonies were obtained, and plant stage at which colonies were collected. Considering each environment, each time point, and each fungal group, there were no significant differences between heights of plates within the plant stand ($P \geq 0.13$). In only one instance did pairwise comparisons indicate a significant difference ($P = 0.04$) when comparing percentages of other fungi at the 0.3- and 1.75-m heights collected at Ithaca in 2006. Because there were no significant differences in all other pairwise comparisons for the three fungal groups ($P \geq 0.07$), plates at different heights were considered replications in other analyses.

Comparisons of Savage scores for percentage of total fungi collected from PCNB plates for each exposure time resulted in significant differences for members of the *G. fujikuroi* species complex at Lincoln 2006 ($P = 0.01$; Table 4). The shorter exposure times of 5 min or 1 h resulted in percentages of *G. fujikuroi* isolates that were not significantly lower than one or more of the longer exposure times. With regard to percentages of total fungi that were other *Fusarium* spp., differences were significant for the Ithaca 2006 environment ($P = 0.01$). In this case, the 5-min exposure time had significantly lower percentages than all other exposure times (Table 4). The percentages of total fungi that were other *Fusarium* spp. collected during 1-h exposure times were similar to longer exposure times at all four environments. The Savage scores for

Table 3. Means and rank mean scores (RMS) for colonies per centimeter squared per hour calculated from fungal colonies collected as a result of passive spore trapping on two media (potato dextrose agar [PDA] and pentachloronitrobenzene [PCNB]) at four plant stages during the growing season at four environments[w]

	PDA			PCNB		
Plant stage	***N*[x]**	**Mean[y]**	**RMS[z]**	***N*[x]**	**Mean[y]**	**RMS[z]**
Lincoln 2005						
Vegetative	12	0.75 ± 0.69	–0.8057 a	11	0.37 ± 0.17	–0.8275 a
Anthesis	12	6.20 ± 1.72	–0.1081 b	10	4.04 ± 1.19	–0.0973 b
Grain development	9	9.63 ± 10.14	0.3942 bc	9	7.32 ± 6.68	0.4653 bc
Maturity	9	11.70 ± 5.74	0.6568 c	9	7.03 ± 4.21	0.5917 c
Lincoln 2006						
Vegetative	12	3.64 ± 2.23	–0.3833 a	11	1.13 ± 0.98	–0.5991 a
Anthesis	12	13.51 ± 15.75	0.4016 a	10	5.39 ± 4.73	0.2007 b
Grain development	11	8.35 ± 10.27	–0.0505 a	12	6.51 ± 5.70	0.4261 b
Maturity	12	9.63 ± 10.41	0.0352 a	11	4.70 ± 9.06	–0.0506 ab
Ithaca 2005						
Vegetative	10	2.72 ± 1.34	–0.6206 a	10	2.09 ± 1.95	–0.3030 ab
Anthesis	12	6.21 ± 4.10	0.1572 b	11	2.94 ± 1.82	0.0432 a
Grain development	11	4.08 ± 2.31	–0.3126 ab	12	1.26 ± 0.85	–0.5974 b
Maturity	6	17.61 ± 16.21	1.4740 c	6	8.83 ± 7.06	1.4647 c
Ithaca 2006						
Vegetative	12	4.24 ± 1.93	–0.3506 a	9	1.69 ± 1.06	–0.5601 a
Anthesis	12	11.1 ± 11.82	0.3093 a	12	4.85 ± 3.99	0.1057 ab
Grain development	12	9.08 ± 8.82	0.1276 a	12	7.78 ± 7.22	0.7124 b
Maturity	10	6.60 ± 8.60	–0.1448 a	12	2.31 ± 1.78	–0.3981 a

[w] Counts on plates were normalized for different exposure times by calculating colonies per centimeter squared per hour. For convenience to the reader, means and standard deviations are shown. The two media were one-fifth-strength PDA and a peptone-based agar containing the fungicide PCNB. PDA allows growth of culturable fungi while PCNB is semiselective for *Fusarium* spp. The four environments were Lincoln, NE in 2005 and 2006 and Ithaca, NE in 2005 and 2006. Fields at Ithaca were irrigated.

[x] Number of observations.

[y] For convenience to the reader, means (colonies per centimeter squared per hour) with standard deviations are shown.

[z] Savage scores, generated for each exposure time of each medium at each environment, were compared using NPAR1WAY procedure (50). Pairwise comparisons were conducted between exposure times, within an environment, for colonies per centimeter squared per hour calculated from colonies on each medium. Letters next to rank mean scores (RMS) that are different indicate that χ^2s are significantly different at $P \leq 0.05$.

other fungi were significant when considering percentages of total fungi collected during different plate exposure times for Lincoln 2005 and Ithaca 2006 ($P \leq 0.04$). For the four environments, the greatest percentages of other fungi were obtained from plates exposed for 5 min. At Lincoln in 2005, the percentage of other fungi obtained from PCNB medium exposed for 5 min (78.5 ± 24.9) was significantly greater than this percentage on plates exposed for 1 h (29.7 ± 22.3; $P < 0.01$); and, at Ithaca in 2006, the percentage of other fungi collected from plates exposed for 5 min (45.4 ± 31.1) was significantly greater than those collected from the 1-h (11.7 ± 19.9) or 24-h (10.0 ± 31.6) exposure times ($P \leq 0.03$).

When considering collection of fungi at each plant developmental stage, pairwise comparisons of Savage scores indicated that percentage of total fungi that were members of the *G. fujikuroi* species complex collected at the vegetative stage were significantly less than those for fungal isolates collected during grain development or maturity at Lincoln in 2006 ($P \leq 0.04$). However, there was a trend for percentages of *G. fujikuroi* to be greater at grain development or maturity than during vegetative growth or at anthesis (Fig. 1). Savage scores for percentages of other *Fusarium* spp. were significant for Lincoln 2006 ($P = 0.02$) but there were no apparent trends across environments in mean percentages as suggested for *G. fujikuroi* isolates (Fig. 1). Mean percentages of other fungi collected from PCNB spore trap plates (semiselective for *Fusarium*) spp. varied widely from zero (anthesis, Lincoln 2006) to 65.6% ± 31.0 (maturity, Lincoln 2005). Savage scores of these percentages were significant for Lincoln and Ithaca in 2006 ($P \leq 0.02$); however, there were no clear trends across environments. Approximately half of the other fungi recovered from the PCNB passive spore traps were in the genus *Alternaria*.

Molecular identification. Sequence analysis of a portion of the *TEF* gene from representative *Fusarium* isolates subsampled from PCNB passive spore traps were genotyped by comparison with sequences of type isolates in the FUSARIUM-ID database (Table 5). From three environments, many of the isolates were *F. graminearum*: 49.1% at Lincoln and 25.5% at Ithaca in 2005 and 23.7% in Lincoln in 2006. Thirteen different FIESC haplotypes were detected, and genotypes in the *F. oxysporum* and *F. solani* species complexes also were detected. Four known pathogens of sorghum (*F. thapsinum, F. verticillioides, F. proliferatum*, and *Fusarium andiyazi* Marasas, Rheeder, Lampr., K.A. Zeller & J.F. Leslie) were recovered by subsampling PCNB medium, and several asymptomatic colonizers of sorghum (*F. subglutinans, F. graminearum*, and members of FIESC) also were detected.

Discussion

There is an abundance of literature on airborne conidia of *Fusarium* spp. responsible for head blight of wheat, ear rot of maize, and pine pitch canker of Monterey pine. These studies had indicated that a warm and humid environment is important for release of spores (18,44,51,60) but it also had been evident that inoculum could be detected at a range of temperatures and moisture conditions (13,18,48,51). Under controlled environmental conditions, Tonapi et al. (57) determined the ideal temperature (28°C) and humidity (98%) for sporulation of an isolate of *Fusarium moniliforme* J. Sheld. (a complex that includes *F. verticillioides, F. thapsinum, F. subglutinans*, and *F. proliferatum*; 27). In the present study, temperatures during anthesis (Lincoln 2005 and Ithaca 2005 and 2006) or grain development (Lincoln 2005 and Ithaca 2005) certainly met this ideal (Table 1) but it was unclear whether conditions, including those following rain or irrigation events, affected the number of airborne *Fusarium* conidia (Table 3; Figure 1). Percentage of fungal colonies that were *Fusarium* spp., subsampled from spore traps, tended to be greater later in the growing season: at flowering for Lincoln in 2006, during grain development for Lincoln in 2005 and Ithaca in 2006, or at grain maturity for Ithaca in 2005. Bandyopadhyay et al. (6) monitored fungal spores over a sorghum plot from anthesis until after grain maturity using an active method: a 24-h Hirst trap, which pumps in air and directs it toward a sticky microscope slide. In their study, increases in airborne *Fusarium* spp. later in grain development also were noticed. In the present study, passive spore traps were set and colonies were

Table 4. Means and rank mean scores (RMS) for percentage of total fungi that were members of the *Gibberella fujikuroi* species complex or other *Fusarium* spp., collected during passive spore trapping on pentachloronitrobenzene (PCNB) plates for different exposure times, during the growing season at sorghum field locations in Nebraska[x]

		G. fujikuroi (%)		Other *Fusarium* spp. (%)	
Exposure time	***N*[y]**	**Mean**	**RMS[z]**	**Mean**	**RMS[z]**
Lincoln 2005					
5 min	11	10.5 ± 16.8	0.0550 a	10.7 ± 20.7	–0.6285 b
1 h	12	14.2 ± 18.2	0.3079 a	56.1 ± 26.8	0.3608 a
8 h	12	4.2 ± 9.7	–0.3012 a	45.0 ± 34.6	0.2423 a
24 h	3	6.7 ± 11.5	–0.2285 a	33.3 ± 38.2	–0.1078 ab
Lincoln 2006					
5 min	12	11.0 ± 12.0	–0.4742 c	60.3 ± 40.5	0.1975 a
1 h	12	28.6 ± 23.6	0.2440 ab	61.0 ± 31.6	0.0580 a
8 h	9	38.3 ± 30.2	0.7382 a	40.8 ± 33.8	–0.3751 a
24 h	10	12.7 ± 13.9	–0.3881 bc	62.3 ± 26.9	0.0309 a
Ithaca 2005					
5 min	12	18.3 ± 25.1	0.0393 a	15.8 ± 21.0	–0.4085 b
1 h	12	14.0 ± 16.7	–0.2226 a	36.3 ± 29.5	0.2529 ab
8 h	8	13.1 ± 18.7	–0.1929 a	37.1 ± 29.7	0.3988 a
24 h	8	29.8 ± 22.0	0.4677 a	24.8 ± 21.3	–0.1654 b
Ithaca 2006					
5 min	12	30.8 ± 18.3	–0.1898 a	23.9 ± 23.2	–0.6067 c
1 h	12	33.6 ± 21.1	0.0347 a	54.8 ± 20.6	0.1122 ab
8 h	12	32.8 ± 28.5	0.2358 a	44.2 ± 26.6	–0.1458 b
24 h	10	23.1 ± 25.5	–0.0969 a	66.9 ± 33.7	0.7684 a

[x] Fungi were grouped as members of *Gibberella fujikuroi* species complex, other *Fusarium* spp., or other fungi. For convenience to the reader, means and standard deviations are shown. PCNB is a peptone-based agar containing the fungicide PCNB and is semiselective for *Fusarium* spp. The four environments were Lincoln, NE in 2005 and 2006 and Ithaca, NE in 2005 and 2006. Fields at Ithaca were irrigated. Percentages of *G. fujikuroi* at Lincoln in 2006 and of other *Fusarium* spp. at Ithaca in 2006 were significant ($P = 0.01$). Percentages of other fungi also were significant at Lincoln in 2005 and Ithaca in 2006 ($P \leq 0.04$) (*data not shown*). All other comparisons were not significant ($P \geq 0.06$).

[y] Number of observations. Some 24-h plates and a few 8-h plates were damaged due to animals, heavy dew, excessive dryness, or dust caused by nearby farming operations.

[z] Pairwise comparisons were conducted between exposure times, within an environment, for percentage of total fungi for each fungal group. Letters next to RMS that are different indicate that χ^2 probabilities were significantly different at $P \leq 0.05$.

subsampled to assess morphological characteristics; then, a subset of these isolates were further genotyped using a recently available molecular technique. The relatively inexpensive use of media in passive spore trapping allowed for setting multiple traps at different locations. To assess the possibility of differential availabilities of spores at different heights within the canopy, the traps were set near the ground, at mid-plant height, and above the canopy. Senescing lower leaves (6) as well as debris left from the previous season's crop (in this case, soybean) could act as substrate for fungal colonization and conidiation (10,43). That *Fusarium* spp. can survive in crop residues on soil surfaces, even over harsh winters, has been well established (31,43). In a study conducted in India, spore concentrations of *Fusarium* spp. and other fungal genera were shown to vary with distance above the ground in a sorghum

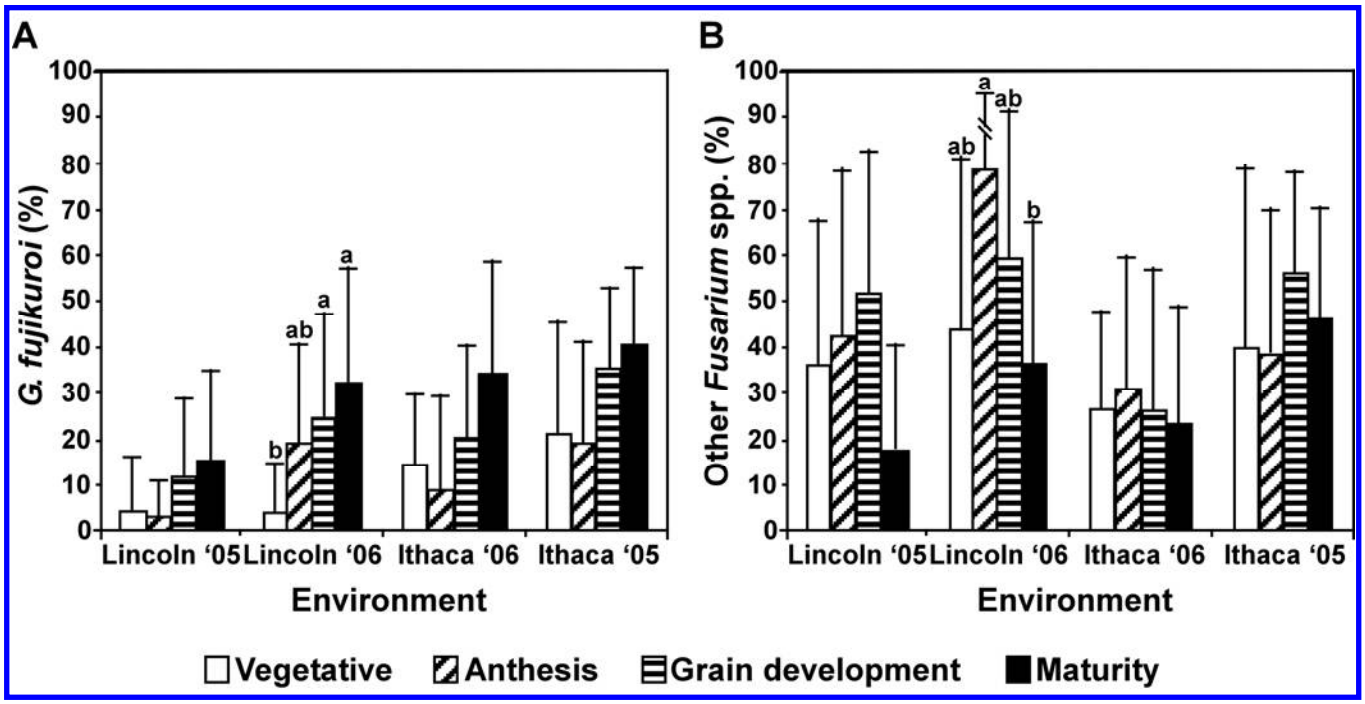

Fig. 1. Percentages of fungal isolates obtained from passive spore trapping throughout the sorghum growing season at four environments, using a medium (pentachloronitrobenzene) semiselective for *Fusarium* spp., that were **A,** members of the *Gibberella fujikuroi* species complex as previously defined (27,41) or **B,** other *Fusarium* spp. Air samples were collected at four plant developmental stages: vegetative growth, anthesis, grain development, and maturity. Then, colonies were subsampled, genotyped by morphological characteristics, and grouped as members of the *G. fujikuroi* species complex, other *Fusarium* spp., or other fungi. Percentages of total fungi for each fungal group were compared using χ^2 analysis of Savage scores. Means and standard deviations were calculated to construct histograms. Pairwise comparisons of Savage scores of percentages of *G. fujikuroi* and of other *Fusarium* spp. at developmental stages during passive spore trapping at Lincoln 2006, were significant ($P \leq 0.05$) as indicated by different letters. Standard deviation of percentage of other *Fusarium* spp. at Lincoln 2006 during anthesis was 21.4.

Table 5. Numbers of each *Fusarium* genotype obtained by subsampling passive spore traps at Lincoln and Ithaca, NE in 2005 and 2006, as indicated by sequence analysis of the translation elongation factor 1-α (*TEF*) gene[w]

	Lincoln		Ithaca	
***Fusarium* genotype[x]**	**2005**	**2006**	**2005**	**2006**
*F. andiyazi**	…	1 (99)	…	…
F. graminearum	27 (99–100)	12 (100)	9 (99–100)	…
*F. proliferatum**[y]	1 (100)	15 (99–100)	2 (100)	2 (99–100)
F. subglutinans[y]	2 (100)	4 (100)	…	2 (100)
*F. thapsinum**	…	4 (99)	3 (99)	1 (99)
*F. verticillioides**	2 (100)	…	…	…
FIESC 1-a, c	2 (95)	…	3 (94–95)	…
FIESC 1-a, c; 4-b	1 (94)	…	4 (94)	…
FIESC 1-c	1 (100)	…	7 (100)	…
FIESC 18-a, b	6 (100)	2 (100)	5 (100)	…
FIESC 24-a	1 (100)	…	1 (100)	…
FIESC 25-a, b, c	3 (99–100)	…	…	…
FIESC 25-a, b, c; 26-a, b	2 (98)	…	…	…
FIESC (others)	2 (99–100)	1 (100)	3 (94–99)	…
F. oxysporum SC 216, 161, 51	1 (100)	…	…	…
F. solani SC 3+4jj	…	1 (100)	…	…
Fusarium sp. (no. 1304)[z]	4 (99–100)	…	1 (100)	…
G. fujikuroi SC (no. 1857)[z]	…	7 (100)	…	1 (100)

[w] The 5′ region of the *TEF* gene was polymerase chain reaction amplified, sequenced, and compared with sequences in the FUSARIUM-ID database to obtain molecular identifications. Percent similarity to genotype in FUSARIUM-ID database is indicated in parentheses; … indicates that the genotype was not detected from subset of *Fusarium* spp. from given environment.

[x] FIESC = *Fusarium incarnatum-F. equiseti* species complex and SC = species complex. Genotypes of FIESC that were detected only once were grouped in "others." Haplotypes of species complexes are indicated. Asterisk indicates that species is a known sorghum pathogen.

[y] Both *F. proliferatum* and *F. subglutinans* include two *TEF* genotypes each.

[z] Number indicates isolate in FUSARIUM-ID database.

field (55). However, in the present study, there no significant differences noticed in plates at different heights with regard to *Fusarium* spp.

Molecular genotyping, comparing sequences of a portion of the *TEF* gene to those in a database (FUSARIUM-ID) (19), allowed comparison of *Fusarium* genotypes subsampled from passive spore traps with those that had been identified from grain (16). In a previous study, *F. thapsinum* was the predominant colonizer of grain across 3 years (2002 to 2004) and accounted for nearly 50% of *Fusarium* isolates recovered from grain using PCNB selection (16). In passive air samples from the present study, *F. thapsinum* was detected at somewhat low levels at Lincoln in 2006 and at Ithaca during both years (Table 4). During another study of *Fusarium* spp. from grain sampled from plants grown at UNL fields in 2005, morphological characteristics indicated that the predominant *Fusarium* spp. recovered from grain at both locations was likely *F. thapsinum* (D. L. Funnell-Harris, J. F. Pedersen, and L. K. Prom, *unpublished*). In Nebraska, sorghum production fields are primarily found near the southern border (2), while the major crops in production near UNL research fields are corn and soybean (www.Nebraskacorn.org; http://quickstats.nass.usda.gov/). If *F. thapsinum* conidia were below the level of detection during the process of passive spore trapping and subsampling of PCNB medium, or intermittent screening did not occur on days in which *F. thapsinum* conidia were at higher levels (13), they could have been missed. Leslie and associates (28) proposed that, because *F. thapsinum* was so commonly found on sorghum and less so on other possible hosts (22), it may be specific to and aggressive on sorghum. Therefore, this apparent selection process may not require a huge abundance of inoculum. This is consistent with what we have previously observed with grain colonization by *Fusarium* spp.: *F. thapsinum* was frequently recovered across locations, years, and sorghum genotypes we have screened while other *Fusarium* spp. that commonly colonize sorghum grain could be specific to particular sorghum genotypes (16).

Fusarium isolates subsampled from passive spore traps had *TEF* sequences highly similar to members of the *G. fujikuroi* species complex, *F. verticillioides, F. proliferatum, F. subglutinans*, and *F. andiyazi*, members of the *F. oxysporum* and *F. solani* species complexes, *F. graminearum*, several FIESC haplotypes, and a few isolates designated as "*Fusarium* sp." in the FUSARIUM-ID database. *F. verticillioides, F. proliferatum, F. subglutinans*, and *F. graminearum* have been shown to be associated with ear rot diseases of maize (30); therefore, it is not surprising to recover these from passive spore traps in Lincoln and Ithaca fields. We previously reported isolating from sorghum grain *Fusarium* spp. with *TEF* genotypes of *F. verticillioides, F. proliferatum, F. subglutinans, F. graminearum*, and FIESC haplotypes, in addition to *F. thapsinum* (16). A reevaluation of *TEF* sequences from FIESC isolates from this previous study (accession numbers EF152428, GQ339783 through GQ339793 and GU116571 through GU116586) (16), using the updated FUSARIUM-ID database (42), revealed that all of the genotypes previously recovered from sorghum grain were also found on passive spore traps: 1-a, c; 1-a, c, 4-b; 24-a; 18-a, b; and 25-a, b, c (Table 5).

Members of the *G. fujikuroi* species complex, *F. thapsinum* and *F. proliferatum*, as well as other *Fusarium* spp., such as *F. graminearum* and members of the FIESC, are capable of infecting sorghum roots. Under environmental conditions conducive to such infections, they can result in Fusarium stalk rot disease but also can be apparently asymptomatic (9,15,27,47). In maize, kernels infected with a green fluorescent protein (GFP)-marked strain of *F. verticillioides* (closely related to *F. thapsinum*) yielded plants, at a low rate, that lacked stalk disease symptoms but produced kernels with *F. verticillioides* carrying GFP (37). Thus, it is conceivable that the presence of root-infecting fungi in seeded fields might also result in systemically infected sorghum plants that yield colonized grain. By subsampling PCNB medium spread with soil suspensions and using morphological characters for identification, we can estimate that the mean populations of *G. fujikuroi* and other *Fusarium* spp. were 2.56×10^3 and 2.13×10^4 CFU/g of soil at Lincoln and 1.06×10^4 and 5.49×10^4 CFU/g of soil at Ithaca, respectively. It is possible that these levels of inocula may be adequate to colonize seed and seedling roots of sorghum (15). Colonization of maize seedling roots by *Fusarium* spp. up to 10 days after planting was associated with 4 to 17 CFU/g of soil (53) while populations of *Fusarium virguliforme* O'Donnell & T. Akoi (formerly known as *F. solani* (Mart.) Sacc. f. sp. *glycines*), part of a disease complex with soybean cyst nematode, ranged from approximately 1.4 to 2.8×10^3 CFU/g of soil in fields with soybean sudden death syndrome (49).

In summary, we conducted air and soil analyses of sorghum fields in order to determine whether *Fusarium* spp. infecting grain might come from one of these two sources, either by direct infection of grain during development or by systemic infection via the germinating seed or roots. Members of *G. fujikuroi* increased after anthesis through grain maturity, which is consistent with previous reports of grain diseases of sorghum (6,8,39). All *Fusarium* genotypes commonly identified from grain in a previous study were detected in air samples from at least one environment. Screens of soil samples from two research fields and production fields at two other locations suggested that estimated numbers of *G. fujikuroi* CFU per gram of soil could provide inoculum for systemic infections (36,37).

Acknowledgments

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