Differences in *Fusarium* Species in *brown midrib* Sorghum and in Air Populations in Production Fields

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ABSTRACT

Several *Fusarium* spp. cause sorghum (*Sorghum bicolor*) grain mold, resulting in deterioration and mycotoxin production in the field and during storage. Fungal isolates from the air (2005 to 2006) and from leaves and grain from wild-type and *brown midrib* (*bmr*-6 and *bmr12* plants (2002 to 2003) were collected from two locations. Compared with the wild type, *bmr* plants have reduced lignin content, altered cell wall composition, and different levels of phenolic intermediates. Multilocus maximum-likelihood analysis identified two *Fusarium thapsinum* operational taxonomic units (OTU). One was identified at greater frequency in grain and leaves of *bmr* and wild-type plants but was infrequently detected in air.

Nine *F. graminearum* OTU were identified: one was detected at low levels in grain and leaves while the rest were only detected in air. Wright's *F* statistic (*F_{ST}*) indicated that *Fusarium* air populations differentiated between locations during crop anthesis but did not differ during vegetative growth, grain development, and maturity. *F_{ST}* also indicated that *Fusarium* populations from wild-type grain were differentiated from those in *bmr6* or *bmr12* grain at one location but, at the second location, populations from wild-type and *bmr6* grain were more similar. Thus, impairing monolignol biosynthesis substantially effected *Fusarium* populations but environment had a strong influence.

Grain mold disease, a major constraint to sorghum (*Sorghum bicolor* (L.) Moench) production throughout the world (Sharma et al. 2011), most likely occurs following infection of flowers and developing grain by airborne spores (asexual or sexual) (Bandyopadhyay et al. 1991; Denis and Girard 1980; Navi et al. 2005; Tarekegn et al. 2006). Sorghum grain mold disease results in reduced quality of grain harvested from the plant, and can cause rapid deterioration in storage (Audilakshmi et al. 2011; Castor and Frederiksen 1982; Navi 2006).

Numerous fungal species belonging to several genera (e.g., *Alternaria*, *Cochliobolus*, *Fusarium*, and *Leptosphaeria*) have been reported as causative agents of grain mold (Funnell-Harris et al. 2013b; Navi et al. 2005; Tarekegn et al. 2006). *Fusarium* spp. that are members of the *Fusarium fujikuroi* species complex are common in grain crops (Alves dos Reis et al. 2010; Boutigny et al. 2012; Funnell-Harris et al. 2010b; Han et al. 2014; Sreenivasa et al. 2008). Other *Fusarium* spp. such as *F. anthophilum* (A. Braun) Wollenw. 1916, *F. nelsonii* Marasas & Logrieco 1998, *F. sporotrichioides* Sherb. 1915, *F. graminearum* Schwabe 1839 (synonym *Gibberella zeae* (Schwein.) Petch 1936), and members of the *F. incarnatum*-*F. equiseti* (FIESC) and *F. chlamydosporum* species complexes, also

have been isolated from sorghum grain (Funnell-Harris et al. 2010b, 2013b; Lincy et al. 2011; Sreenivasa et al. 2008). In addition to reduced grain quality, many of these species can produce mycotoxins that contaminate grain, which is of considerable concern for both humans and livestock (Bhat et al. 1997; Bowman and Hagler 1991; Shotwell et al. 1980).

Because airborne infection is the most likely source for grain mold, studies have been conducted that examine fungal spores sampled from air over or within sorghum fields. Using a semiselective medium to trap spores, *Fusarium* spp. isolates were collected from within sorghum fields, and both cultural and molecular techniques were used for identification (Funnell-Harris and Pedersen 2011). In general, spores found in sorghum field air samples were consistent with genera and species also identified from infected grain (Bandyopadhyay et al. 1991; Funnell-Harris and Pedersen 2011). Previous research also indicated that populations of *Fusarium* spp. are associated with grain mold diseases, as well as stalk, root, and crown diseases of grasses, rather than a single *Fusarium* sp. or pathovar (Funnell-Harris et al. 2010b; Parikka et al. 2012; Petrovic et al. 2013). It has also been shown that multiple *Fusarium* genotypes can be present in locally harvested maize kernels or wheat grains (Del Ponte et al. 2015; Morales-Rodríguez et al. 2007; Waalwijk et al. 2004). *Fusarium* spp. also may be found systemically from other infected plant parts to infect grain, as has been demonstrated in maize with the *Fusarium* ear rot pathogen, *F. verticillioides* (Munkvold and Carlton 1997; Murillo-Williams and Munkvold 2008). Plant genetic background and single gene differences may play a role in determining *Fusarium* spp. populations within plants (Funnell and Pedersen 2006; Funnell-Harris et al. 2010b; Gatch and Munkvold 2002).

Plants with *brown midrib* (*bmr*) mutations, which result in reduced lignin compared with wild-type counterparts, were previously shown to have differing *Fusarium* genotypes recovered from sorghum grain

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compared with near-isogenic wild-type lines (Funnell and Pedersen 2006; Funnell-Harris et al. 2010b; Oliver et al. 2005; Palmer et al. 2008). Sorghum is a model crop for C_4 grasses (Pedersen et al. 2008) and, therefore, was the ideal species in which to incorporate two *bmr* mutant loci, *bmr6* and *bmr12*, into six elite grain sorghum genetic backgrounds (Pedersen et al. 2006). It was subsequently demonstrated that biomass from the resulting mutant lines had increased feed efficiency in livestock (Oliver et al. 2005) and increased conversion efficiency into ethanol (Dien et al. 2009). The wild-type *Bmr6* and *Bmr12* genes encode a cinnamyl alcohol dehydrogenase and a caffeic acid *O*-methyltransferase, which are enzymes in the monolignol biosynthesis pathway (Bout and Vermerris 2003; Saballos et al. 2009; Sattler et al. 2009, 2012). In general, reduced colonization of grain from *bmr6* and *bmr12* lines by *Fusarium* spp.

TABLE 1. Mean monthly temperatures, precipitation, and irrigation at fields at Havelock in Lincoln, NE and at Mead, NE during sorghum growing months (May through October) during 2002, 2003, 2005, and 2006^a

Location	Year	Month	Mean temperature (°C)	Precipitation (mm)	Irrigation (mm) ^b
Lincoln	2002	May	15.6	121	0
		June	25.5	2	0
		July	27.3	14	0
		August	24.4	213	0
		September	20.1	39	0
Mead	2002	October	8.5	107	0
		May	15.4	65	0
		June	25.0	20	50
		July	26.6	31	50
		August	23.4	137	50
Lincoln	2003	September	19.5	36	0
		October	7.9	63	0
		May	15.6	69	0
		June	20.5	169	0
		July	26.0	26	0
Mead	2003	August	25.1	33	0
		September	17.0	97	0
		October	13.1	25	0
		May	15.1	97	0
		June	20.4	66	0
Lincoln	2005	July	25.3	22	25
		August	24.2	29	150
		September	16.2	53	0
		October	12.0	39	0
		May	17.0	84	0
Mead	2005	June	23.9	109	0
		July	25.9	135	0
		August	23.3	41	0
		September	21.4	4	0
		October	12.3	58	0
Lincoln	2006	May	16.5	70	38
		June	23.8	22	38
		July	25.5	55	114
		August	22.9	20	76
		September	20.9	12	0
Mead	2006	October	12.1	45	0
		May	18.0	43	0
		June	23.8	65	0
		July	26.6	65	0
		August	23.9	117	0
Lincoln	2006	September	16.1	109	0
		October	9.4	24	0
		May	17.7	13	13
		June	23.2	20	38
		July	25.5	39	38
Mead	2006	August	22.9	120	76
		September	16.1	114	0
		October	9.4	19	0

^a Based on data from the High Plains Regional Climate Center, University of Nebraska, Lincoln (<https://hprcc.unl.edu/awdn.php>).

^b Supplemental irrigation was applied at Mead using overhead irrigation, as needed. Sorghum was grown under dryland conditions at the Havelock field in Lincoln.

was observed when compared with grain from wild-type lines (Funnell and Pedersen 2006; Funnell-Harris et al. 2010b). Furthermore, *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg was significantly reduced in *bmr12* grain, and an FIESC genotype was not detected in *bmr12* grain; however, both *F. proliferatum* and the FIESC genotype were common in both wild-type and *bmr6* grain (Funnell-Harris et al. 2010b).

In previous studies, the 5' portion of the *translation elongation factor 1-α* (*TEF*) gene was used to confirm morphological identification (Geiser et al. 2004) of individual *Fusarium* isolates from air samples and from *bmr* and wild-type grain, and to conduct phylogenetic analysis of FIESC members from this grain (Funnell-Harris and Pedersen 2011; Funnell-Harris et al. 2010b). The purpose of the present study was to test the following two hypotheses: (i) air samples from two sorghum field locations in eastern Nebraska have significantly different *Fusarium* populations and (ii) sorghum *bmr* and wild-type genotypes (both grain and leaves) are colonized by different *Fusarium* spp. and operational taxonomic units (OTU). The two locations were approximately 37.1 km apart and differed in that one field was irrigated (Mead) and the other was dryland (Havelock). The fungal isolates collected from grain and leaves from six wild-type sorghum cultivars and *bmr6* and *bmr12* near-isogenic lines (Funnell and Pedersen 2006; Funnell-Harris et al. 2010b) and an expanded collection of isolates from air samples (Funnell-Harris and Pedersen 2011) were compared by sequence analysis at three phylogenetically informative loci: most of the internal transcribed spacer (ITS) region along with the 5'-variable region of the large subunit of nuclear ribosomal DNA (28S), and portions of the *histone-3* (*H3*) and *TEF* genes (Geiser et al. 2004; Glass and Donaldson 1995; O'Donnell and Cigelnik 1997). Phylogenetic analyses of OTU, defined herein as the taxon at a unique tip within the phylogenetic tree, were performed using maximum-likelihood analysis, whereas the level of population differentiation, based on sequence polymorphism, was determined using Wright's *F* statistic (*F_{ST}*) and *K* statistic (*K_{ST}*). DNA polymorphisms and phylogenies were analyzed for members of the *F. fujikuroi* species complex and for all other *Fusarium* spp., including those in the *F. graminearum* species complex (FGSC) and FIESC.

MATERIALS AND METHODS

Collection of *Fusarium* isolates. Near-isogenic sorghum lines *bmr6* and *bmr12* in the genetic backgrounds BTx623, BTx630, BTx631, RTx430, 'Redlan', and 'Wheatland' were developed and, along with the wild-type lines, maintained by United States Department of Agriculture–Agricultural Research Service, Lincoln, NE (Pedersen et al. 2006). All 18 lines were grown during 2002 and 2003 at Lincoln and Mead, NE (Funnell and Pedersen 2006; Funnell-Harris et al. 2010b) in four replicate plots per location and year. Eastern Nebraska has a humid continental climate with wet springs, warm to hot and humid summers and mild to cold and relatively dry autumns. Temperatures, precipitation and irrigation (Table 1), were recorded by the High Plains Regional Climate Center, University of Nebraska (UNL) (<https://hprcc.unl.edu/awdn.php>). Overhead sprinkler irrigation was applied to fields at Mead, NE, while Havelock fields in Lincoln lacked additional water applications. Observations have shown that when *Fusarium* isolates are collected from grain grown at both locations in the same year, more isolates are obtained from Mead than from Havelock, likely due to the application of overhead irrigation during anthesis and grain development (Table 1) (Funnell and Pedersen 2006; Funnell-Harris et al. 2010b, 2013b). A sample of approximately 20 mature grains (2002 to 2003) was collected from each of 10 randomly chosen plants (out of approximately 240 plants) per plot and the third leaf below the flag leaf (2002) was collected from each of five randomly chosen plants per plot, as previously described (Funnell and Pedersen 2006; Funnell-Harris et al. 2010b). To isolate *Fusarium* spp., grains from the 10 plants from one plot were mixed, a subsample of 15 to 20 grains was

surface disinfested, and 5 grains were aseptically placed onto each of two semiselective media: dichloran chloramphenicol peptone agar (DCPA) and pentachloronitrobenzene peptone agar (PCNB) (Funnell and Pedersen 2006; Funnell-Harris et al. 2010b). To isolate *Fusarium* spp. from leaf tissue, leaf disks (area = 1 cm²) were cut, surface disinfested, and aseptically placed onto DCPA medium at three leaf disks per each of five leaves from a plot and five disks per plate (Funnell and Pedersen 2006). All fungal colonies growing from each tissue onto the medium were transferred to half-strength potato dextrose agar (PDA; made with potato dextrose broth; Becton-Dickinson and Co., Sparks, MD). Further details of collection of *Fusarium* isolates from leaf tissues and grain are described by Funnell and Pedersen (2006) and Funnell-Harris et al. (2010b). For identification to species using morphological characteristics, presumptive *Fusarium* isolates were transferred to 1.5% water agar amended with 80 mM potassium chloride (KCl). *Fusarium* isolates were identified to species using colony, conidia, and conidiophore morphologies (Nelson et al. 1983), with identifications later confirmed by descriptions from Leslie and Summerell (2006), in addition to DNA sequences as described below. However, some *Fusarium* isolates with conidia, conidiophore, and colony characteristics did not fit well with a single species description; these were designated as *Fusarium* spp.

Fungi from air samples were collected by passive spore trapping onto PCNB at the Havelock field in Lincoln, NE and at Mead, NE during 2005 and 2006, by exposing the medium in Petri dishes, as previously reported by Funnell-Harris and Pedersen (2011). Briefly, Petri dishes with PCNB were placed at three heights, two within and one above the canopy, throughout sorghum fields, four times during the growing season on rain- and irrigation-free days (Funnell-Harris and Pedersen 2011): during vegetative growth (designated as 1), at anthesis (2), during grain development (3), and at grain maturity (harvest) (4). The study also examined exposure of the medium to air for different predetermined times, from 5 min to 24 h. Numbers of fungi and *Fusarium* spp. colonies per square centimeter of agar medium per hour were previously analyzed and reported (Funnell-Harris and Pedersen 2011). Temperature, precipitation, and irrigation data from 2005 and 2006 also are shown in Table 1. Colonies randomly chosen from each exposed plate were transferred to PDA, then KCl, and identified to species as described above.

If multiple isolates of the same species were collected from the same plot (grain or leaf) or air-sampling plate, a representative isolate was chosen for further analysis. Representative grain, leaf, or air *Fusarium* isolates were single-conidium purified in the following way. Conidia from a KCl plate were gently dislodged using sterile purified water; then, a conidial suspension was streaked onto a peptone glucose agar plate (1% glucose, 0.5% peptone, 7 mM potassium phosphate monobasic, 2 mM magnesium sulfate heptahydrate, and 2.2% agar) amended with ampicillin (Sigma-Aldrich, St. Louis) at 100 µg ml⁻¹. The plate was incubated at room temperature for 24 to 28 h. Conidia were examined at ×25 power using a stereoscopic zoom microscope with a high-resolution objective (Nikon Instruments, Inc., Melville, NY) and an individual germinating conidium was transferred to the center of a PDA plate (60 by 15 mm) by cutting the agar around the germling using a flame-sterilized scalpel; in total, three germlings were transferred per isolate. Cultures were incubated at room temperature for 10 to 14 days, at which time the single-conidium-purified cultures were compared with the original isolate for morphological characteristics and a single isolate was chosen for each original culture. The single-conidium-purified isolates were stored (Funnell-Harris et al. 2013a) for further analyses. Amplification, sequencing, and comparison of sequences in *FUSARIUM*-ID was conducted on DNA from single-conidium-purified isolates as described below to confirm morphological identification and to update to the most current name (Geiser et al. 2013).

DNA isolation, PCR amplification, and sequencing. Sequencing of *TEF*, *H3*, and ITS was performed on DNA from 315 isolates: 186 obtained from air (74 from Mead and 112 from

Havelock in Lincoln), 93 from grain (62 from grain grown at Mead and 31 from Havelock), and 36 from leaf samples (8 from plants grown at Mead and 28 from Havelock). DNA was extracted from ground lyophilized mycelium from each isolate (Lee and Taylor 1990). For amplification of the 5' region of the *TEF* gene, primer ef1 (forward primer: 5'-ATGGGTAAGGA(A/G)GACAAGAC-3') and ef2 (reverse primer: 5'-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3') and reaction mixture and conditions as previously published were used (Geiser et al. 2004). The entire ITS region along with the 5' end of the 28S ribosomal RNA also was amplified using primers ITS5 (forward: 5'-GGAAGTAAAAGTCGTAACAAGG-3') and NL4 (reverse: GGTCCGTGTTTCAAGACGG), and the reaction mixture and conditions as previously published (O'Donnell 1996; O'Donnell and Cigelnik 1997; White et al. 1990). Amplification of *H3* was conducted using primers H3-1a (forward: 5'-ACTAAGCAGACCGCCCGCAGG-3') and H3-1b (reverse: 5'-GCGGGCGAGCTGGATGTCCTT-3') and the reaction mixture previously described (Glass and Donaldson 1995), with the following conditions: 94°C for 5 min; then 35 cycles of 92°C denaturation for 1 min, 56.5°C annealing for 1 min, and 72°C extension for 1 min; with a final extension at 72°C for 5 min. Amplification products were either gel purified or column purified using the E.Z.N.A. Ultra-Sep Gel Extraction kit (Omega Bio-tek, Norcross, GA) or Amicon Ultra-015 Centrifugal Filter Device (Millipore, Billerica, MA), respectively. Purified amplification products were sequenced using the same primers as for amplification. Sequences from opposite strands were assembled using Sequencher 4.10.1 (Gene Codes, Corp., Ann Arbor, MI) and consensus *TEF* sequences were compared with those in the *FUSARIUM*-ID database (<http://isolate.fusariumdb.org/guide.php>) (Geiser et al. 2004) and GenBank for consensus sequences for all three regions for identification.

Because of their prevalence in grains (Bandyopadhyay et al. 2007; Funnell-Harris et al. 2010b; Leslie et al. 2005), *Fusarium* isolates classified as members of the *F. fujikuroi* species complex were analyzed separately from all other *Fusarium* spp. *Fusarium* isolates were segregated into *F. fujikuroi* species complex according to their *TEF* sequence identification, based on pairwise sequence alignments with isolates curated in *FUSARIUM*-ID (Leslie and Summerell 2006; Leslie et al. 2005; Nelson et al. 1983; O'Donnell et al. 1998). The regions of *TEF* and *H3* genes and ITS targeted for sequencing, alignment, and phylogenetic analysis for *F. fujikuroi* species complex and for other *Fusarium* spp. in this study were based on published sequences for *F. verticillioides* (Sacc.) Nirenberg 1976 and *F. graminearum*, respectively. For ITS, sequences were aligned to positions 5 through 1,046 based on accession GQ168841 (Wicklow and Poling 2009) for the *F. fujikuroi* species complex and bases 97 through 1,113 of accession FJ240316 (O'Donnell et al. 2008) for other *Fusarium* spp. For *H3*, sequences were aligned to positions 2 through 475 based on accession number AY435467 (Saleh and Leslie 2004) for the *F. fujikuroi* species complex and positions 24 through 472 based on accession GQ915473 (Proctor et al. 2009) for other *Fusarium* spp. For *TEF*, sequences were aligned to positions 28 through 684 based on accession JX269007 (Funnell-Harris et al. 2013b) for the *F. fujikuroi* species complex isolates and positions 33 through 639 based on accession JF270252 for other *Fusarium* spp. (Funnell-Harris and Pedersen 2011). Representative sequences for each unique OTU were deposited into GenBank as accession numbers KX681483 to KX681488, KX681490 to KX681496, KX681498 to KX681534, KX681536 to KX681542, KX681544 to KX681575, KX681577 to KX681583, and KX681585 to KX681615. Accession numbers for sequences for each isolate as well as percent identities to sequences from representative isolates in *FUSARIUM*-ID (*TEF*) and BLAST searches in GenBank (ITS and *H3*) are detailed in Supplementary Table S1. Species or species complex identifications relied primarily on morphological characteristics and comparisons of *TEF* sequences and then, as needed, comparisons of ITS or *H3* sequences. Sequences from representative

isolates among those highly similar to sequences from isolates in this study are listed in the table.

Analyses of DNA polymorphisms in *TEF*, *H3*, and ITS sequences. *TEF*, *H3*, and ITS consensus sequences obtained from isolates collected from air, grain, and leaves were aligned and trimmed using the MUSCLE aligner (Edgar 2004) implemented in MEGA 5 (Tamura et al. 2011). The individual alignments from each locus were imported into jMODELTEST (version 2.1.10) (Darriba et al. 2012) to select best-fit models of nucleotide substitution optimized for maximum-likelihood tree topology using Akaike Information Criteria. For ITS, TIM + I + G was selected as the best-fit model whereas TPM2uf + G and TIM2 + I were selected for *TEF* and *H3*, respectively. The alignments for the three loci were concatenated together using the catfasta2phymml script available at <https://github.com/nylander/catfasta2phymml>. Before phylogenetic analysis, redundant sequences likely derived from identical isolates were removed from the alignment using CD-HIT (Li and Godzik 2006), with a global sequence similarity cutoff of 99.5% to account for potential errors in sequencing or assembly. A representative sequence was chosen for inclusion into the phylogenetic analysis and the number of isolates with sequences highly similar (99.5%) to the representative sequence were enumerated. Maximum-likelihood trees were computed using GARLI (Zwicki 2006). Each locus was treated as a separate partition and allowed to evolve independently according to its optimal model of nucleotide substitution determined by jModelTest (ITS: TIM + I + G, *TEF*: TPM2uf + G, and *H3*: TIM2 + I). Evolution was simulated for 500,000 generations or until likelihood scores reached convergence, and nonparametric bootstrap analysis was performed to generate support for branching patterns ($n = 500$ pseudoreplicates). Bootstrap consensus trees were compiled using the sumtrees function of the Dendropy package (Sukumaran and Holder 2010). In total, three different phylogenetic trees were computed. For the *F. fujikuroi* species complex, a single phylogenetic

tree including all three loci was produced. However, *H3* did not amplify well for certain *Fusarium* spp. other than those in the *F. fujikuroi* species complex (for example, *F. sporotrichioides*, *F. graminearum*, and some members of the FIESC); therefore, two phylogenetic trees were constructed for this fungal group. The first tree included all three loci while the second tree included only ITS and *TEF*, so that taxa missing sequences for *H3* could be included in the phylogenetic comparisons.

F_{ST} and K_{ST} scores, along with P values for K_{ST} scores, were computed using the program DNASP (version 5) (Librado and Rozas 2009) using the Gene Flow and Population Differentiation Analysis. Sites with alignment gaps were considered as a fifth state and a permutation test was run on all possible pairwise treatment comparisons to generate statistical support for predicted population differentiation. For interpretation of level of differentiation as indicated by F_{ST} scores, the following scale was used: $F_{ST} \leq 0.15$ as low differentiation, $0.25 \geq F_{ST} > 0.15$ as moderate differentiation, and $F_{ST} > 0.25$ as strong differentiation (Karubian et al. 2015; Lowe and Allendorf 2010; Ottewill et al. 2016). For K_{ST} scores, significance was indicated at $P \leq 0.05$.

RESULTS

Maximum-likelihood analyses of *F. fujikuroi* species complex sequences from sorghum field isolates. OTU is defined herein as the taxon at a unique tip within the phylogenetic tree (Daly et al. 2001; McCaig et al. 1999). Multiple OTU were identified for each species: *F. proliferatum*, *F. thapsinum* Klittich, J. F. Leslie, P. E. Nelson & Marasas (synonym *G. thapsina* Klittich, J. F. Leslie, P. E. Nelson & Marasas), *F. subglutinans* (Wollenw. & Reinking) P. E. Nelson, Toussoun & Marasas 1983, and *F. verticillioides* (Fig. 1). *F. proliferatum*, *F. thapsinum*, *F. subglutinans*, and *F. verticillioides* each had the presence of sequences assigned on multiple tips within

Fig. 1. Maximum-likelihood analysis of sequences from isolates assigned to the *Fusarium fujikuroi* species complex from air samples over sorghum fields plus sorghum grain and leaf samples. Maximum-likelihood phylogenies were constructed using concatenated alignments of portions of the translation elongation factor 1- α (*TEF*) and histone-3 (*H3*) genes and the internal transcribed spacer (ITS) region, including the 5'-variable region of the large subunit of nuclear ribosomal DNA (ITS), with the program Garli. Bootstrap values ≥ 50 are presented. For isolate designations, the letter in the first position indicates location ("M" indicates "Mead" and "H" indicates "Havelock"), the next two digits indicate the year, and the letter in the fourth position indicates source ("S" indicates "seed" and "A" indicates "air"); all other digits and letters are specific to each isolate. In headings, for "Air" isolates, the numbers represent time points for collection during the growing season: 1 = during vegetative growth, 2 = at anthesis, 3 = during grain development, and 4 = at grain maturity (harvest). For "Seed" and "Leaf" isolates, "WT" indicates "wild-type" lines and *bmr6* and *bmr12* indicate near-isogenic lines having the brown midrib (*bmr*)-6 or 12 mutation in lignin biosynthesis.

the phylogenetic tree. One OTU within each species occurred more frequently among all the air and plant (grain and leaf) samples than the others. For example, the *F. proliferatum* OTU represented by the grain isolate M03S_A2_91 was common in air samples from both locations, nearly throughout the growing seasons, and was commonly isolated from leaves from Havelock and grain from both locations. Most other *F. proliferatum* OTU were detected at a much lower frequency and in one or only a few environments (Fig. 1). The exception was one of two *Fusarium* sp. OTU, represented by isolate H03S_C1_2, which grouped in the same clade as most *F. proliferatum* OTU and was detected in grain and air samples from both Mead and Havelock. This isolate, and isolates H06A_4K_1b and M02S_C1_5, had characteristics that placed them into the *F. fujikuroi* species complex but could not be definitively identified to species. There were only two *F. thapsinum* OTU identified using the three concatenated loci (Fig. 1). The OTU represented by isolate H03S_A1_2 was frequently recovered from plant tissues at Mead in grain and at Havelock in grain and leaves collected at harvest in 2002 and 2003. However, it was detected only at low levels from air samples during the 2005 and 2006 growing seasons. The other *F. thapsinum* OTU, represented by isolate M02S_A1_92, was detected in grain only at low levels and only detected in air samples once at Havelock. There also were only two *F. subglutinans* OTU identified (Fig. 1). The predominant OTU, represented by isolate M02S_A1_92, was isolated at most air sampling time points at both locations; however, this OTU was isolated from plant tissues (grain and leaves) only at Mead. Although there were three *F. verticillioides* OTU identified, this species was detected at relatively low levels in the environments screened during this study (Fig. 1). The most common OTU, represented by isolate M03S_C1_5a, was detected in air samples at harvest at both locations and in plant tissues (grain and leaves) at Mead.

In summary, four species and three *Fusarium* sp. OTU from within the *F. fujikuroi* species complex were found in association with sorghum and air samples over sorghum fields. Only *F. proliferatum*, *F. thapsinum*, and one of the *Fusarium* sp. OTU (represented by isolate H03S_C1_2) were present in plants and air at both locations. For *F. proliferatum* and *F. thapsinum*, only a single OTU each (M03S_A2_91 and H03S_A1_2, respectively) was present in air and plant samples at both Mead and Havelock.

Maximum-likelihood analyses of sequences from other *Fusarium* spp. and species complexes in plant and air samples from sorghum fields. Among the numerous OTU identified after concatenation of ITS, *TEF*, and *H3* sequences, the most frequent were members of the FGSC and the FIESC (Fig. 2A). This study detected nine *F. graminearum* OTU but the most commonly detected in most samples, and the only one detected in plant tissues, was the OTU represented by isolate M02S_C2_3. For FIESC, 10 OTU were detected, representing eight different genotypes (O'Donnell et al. 2009), with 3 OTU for genotype FIESC 25-a,b,c, the only FIESC genotype with multiple OTU (Fig. 2A). Most of the FIESC genotypes were rarely detected in sorghum plant tissues or only detected in air samples. FIESC 18-a,b was detected in air samples at both locations during 2005 and 2006 and detected in grain grown at Mead during 2002 and 2003. The most commonly detected OTU of FIESC 25-a,b,c, represented by the leaf isolate H02L_C3_11a, was only found at Havelock but was detected in air, grain, and leaf samples (Fig. 2A). Although at low levels, FIESC 24-a was present in air and plant samples (grain at Mead and both grain and leaf tissue at Havelock) at both locations.

Because there were difficulties amplifying the *H3* gene in a few genotypes of FIESC and in *F. graminearum* and *F. sporotrichioides*, a reanalysis conducted by concatenating ITS and *TEF* sequences was done (Fig. 2B). When sequences with more than 99.5% sequence identity were collapsed, fewer representative OTU resulted than when all three sequences were used in analysis. Among the four FGSC OTU, isolate M02S_C2_3 still represented the most common OTU in air samples at both locations (Fig. 2B). This isolate was placed in the same clade as two *F. armeniacum* (G. A. Forbes,

Windels & L. W. Burgess) L. W. Burgess & Summerell OTU and a *Fusarium* sp. The *Fusarium* sp. OTU, represented by isolate H05A_1D_4, was present at low levels in air samples and plants (leaf tissue at Mead and both grain and leaf tissue at Havelock) at both locations (Fig. 2B). This analysis resulted in OTU for nine FIESC genotypes, with one FIESC 4-b air isolate in the same clade as FIESC 1-a, 4-b isolates (Fig. 2B). There were only two OTU for FIESC 25-a, b,c in this analysis, including the one represented by the leaf isolate H02L_C3_11a, which was predominately found in plants (grain and leaves) sampled at Havelock but was also detected in grain from Mead.

F_{ST} and K_{ST} analyses of *Fusarium* sequences from *bmr6*, *bmr12*, and wild-type plants. F_{ST} analysis, which indicates the degree of genetic differentiation between populations, was performed on all *Fusarium* isolates cultured from wild-type, *bmr6*, and *bmr12* grain (Havelock, NE and Mead, NE) and leaves (Havelock only) (Table 2) by comparing sequences from *TEF*, ITS, and *H3* sequences. F_{ST} , a value between 0 (identical) and 1 (entirely differentiated), indicated that populations in wild-type and *bmr6* grain grown at Havelock were highly similar. However, the F_{ST} values from Mead were much higher, indicating that the populations of *Fusarium* isolates from wild-type, *bmr6*, and *bmr12* grain grown at Mead were more different from one another than those from wild-type and *bmr6* grain grown at Havelock (Table 2). These results indicated that sorghum genotypes and location (environment) effected *Fusarium* OTU in the grain. Although adequate numbers of leaf isolates were collected from wild-type and *bmr6* plants only from the Havelock field, there were indications that these populations also were strongly differentiated (Table 2); environmental influences could not be indicated for leaf populations.

K_{ST} was also conducted to provide further insights into the F_{ST} results. Because K_{ST} values were generated on two groups, the *F. fujikuroi* species complex and all other *Fusarium* spp. (Figs. 1 and 2, respectively), they indicated what fungal groups may have contributed to observed differentiation between the populations (Table 3). Comparing *TEF*, *H3*, and ITS sequences (the same sequences used for F_{ST} analysis) between populations in wild-type and *bmr* grain grown at Mead provided evidence that *Fusarium* spp. OTU not within the *F. fujikuroi* species complex (other *Fusarium* spp.) may have contributed to observed differences in these populations indicated by F_{ST} analysis (Tables 2 and 3; Fig. 2A). However, K_{ST} values do not support significant differences in grain and leaf OTU populations at $P < 0.05$ (Tables 2 and 3; Figs. 1 and 2).

F_{ST} and K_{ST} analyses of *Fusarium* sequences from air samples at sorghum fields. *Fusarium* populations from air samples at Havelock and Mead over two growing seasons were compared using concatenated sequences from *TEF*, *H3*, and ITS from all *Fusarium* isolates. F_{ST} values for all pairwise comparisons between each time point at Havelock were very close to zero or had low differentiation, which indicated that the populations were highly similar (Table 4). For *Fusarium* populations in air samples, comparisons between time points 2 (anthesis) and 4 (grain maturity) indicated similar levels of low differentiation at both locations, while other comparisons between time points at each location indicated that those populations were essentially the same (close to zero) (Table 4). The populations at the same time point taken at different locations also were compared (Table 4). In this case, populations taken at time point 2 (during anthesis) were strongly differentiated whereas F_{ST} at time point 1 (vegetative growth) and the two later time points (3 and 4 = grain development and maturity, respectively) were nearly 0, suggesting a high degree of similarity.

K_{ST} analysis supported population differentiation between time point 1 and the three later time points sampled at Havelock among the *F. fujikuroi* species complex OTU but not all other *Fusarium* spp., which was not apparent when using F_{ST} assessment alone (Tables 3 and 4). K_{ST} analysis also indicated that differences between populations at the two locations were due to differences among *F. fujikuroi* species complex isolates and all other *Fusarium* spp.

DISCUSSION

The first hypothesis tested was that air samples from two sorghum field locations in Nebraska have significantly different *Fusarium* populations. Although this appears to be a straightforward hypothesis, it was not supported throughout the growing season. Results from F_{ST} and K_{ST} showed that, during anthesis of the sorghum crop growing season (time point 2), populations of aerial propagules of *Fusarium* spp. were strongly differentiated (Tables 3 and 4) but, early in the growing season (vegetative growth) and at later time points during the season (grain development and harvest), there was less differentiation between the populations at the two locations. The time periods from flowering through physiological maturity are when grain infections are likely to occur (Menkir et al. 1996; Navi et al. 2005). Thus, it is expected that grain pathogens would be prevalent in air samples during these time periods (Bandyopadhyay et al. 1991); however, in the current study, the greatest differences in infections between the two locations would occur if conditions were conducive to infection at anthesis (Navi et al. 2005). Less is known about leaf infections of sorghum and other grasses (Larran et al. 2007; Murillo-Williams and Munkvold 2008; Wagacha et al. 2012). Presumably, *Fusarium* spp. could infect leaves by way of aerial spores, spores dispersed from infected debris by rain or irrigation splash, or through systemic infections of panicles or roots (Bateman et al. 2007; Funnell-Harris and Pedersen 2011; Funnell-Harris et al. 2010a; Murillo-Williams and Munkvold 2008).

The second hypothesis tested was that sorghum *bmr* and wild-type genotypes are colonized by different *Fusarium* spp. and OTU. A previous study indicated that grain of near-isogenic *bmr* lines had unique differences in *Fusarium* spp. compared with the wild type for both members of the *F. fujikuroi* species complex and all other *Fusarium* spp. (Funnell-Harris et al. 2010b). F_{ST} analysis of population differentiation when considering *TEF*, *H3*, and ITS sequence comparisons among all isolates collected supported this previous observation and showed population differentiation at the

TABLE 2. Genetic similarity between populations, as indicated by Wright's F statistic (F_{ST}), of *Fusarium* grain and leaf isolates from three sorghum genotypes grown at two locations (Havelock field in Lincoln, NE and Mead, NE), and between grain and leaf isolates of the same plant genotype grown at Havelock, that could be attributed to differences in the concatenated sequences of portions of the *translation elongation factor* and *histone-3* genes and the internal transcribed spacer region^a

Location, comparison of	Plant genotype ^b		
	WT	<i>bmr6</i>	<i>bmr12</i>
Grain isolates			
Havelock			
WT grain	0	-0.1186	utd
<i>bmr6</i> grain	...	0	utd
Mead			
WT grain	0	0.3536*	0.2331*
<i>bmr6</i> grain	...	0	0.2976*
Leaf isolates (Havelock, only)			
WT leaf	0	0.4049*	utd
<i>bmr6</i> leaf	...	0	utd
Same plant genotype (Havelock)			
Grain versus leaf	0.1476	0.1336	utd

^a Sequences were analyzed using DNASP, version 5.10.1 (<http://www.ub.edu/dnasp/>). F_{ST} indicates variation between two populations: "0" is identical while "1" is completely differentiated. Negative values are treated as "0". A population compared with itself is represented by "0".

^b Genotypes: wild type (WT), *brown midrib-6* (*bmr6*), and *bmr12*. For purposes of this discussion, an asterisk (*) indicates moderate to strong differentiation ($F_{ST} \geq 0.15$). All other comparisons are considered to have low differentiation. Unable to determine (utd) indicates that not enough isolates from *bmr12* grain and leaf isolates were collected for sequence analysis. Additionally, not enough isolates from leaves from plants grown at Mead were collected for sequence analysis.

OTU level (Table 2). The populations were subdivided into the *F. fujikuroi* species complex and all other *Fusarium* spp. for K_{ST} analysis, which suggested that this differentiation was due to differences in all other *Fusarium* spp. rather than to differences in

TABLE 3. K statistics (K_{ST}) and P values for *Fusarium* population comparisons of isolates collected at Mead, NE and at the Havelock field in Lincoln, NE^a

Isolates, location, comparison ^c	FFSC-TEF-H3-ITS ^b		OF-TEF-H3-ITS		OF-TEF-ITS	
	K_{ST}	P	K_{ST}	P	K_{ST}	P
Plant isolates						
Mead						
WT & <i>bmr6</i> grain	-0.0123	0.82	0.0880	0.07	-0.0978	0.89
WT & <i>bmr12</i> grain	0.0068	0.27	0.0774	0.08	-0.0286	0.73
<i>bmr6</i> & <i>bmr12</i> grain	0.0113	0.21	-0.0950	0.70	-0.1400	0.90
Havelock						
WT & <i>bmr6</i> grain	-0.0212	0.58	-0.0399	0.82	-0.0389	0.88
WT & <i>bmr12</i> grain	-0.1744	0.81	na	na	na	na
<i>bmr6</i> & <i>bmr12</i> grain	-0.1891	0.86	na	na	na	na
WT & <i>bmr6</i> leaf	-0.2287	0.97	-0.1290	1.00	-0.0978	0.90
WT & <i>bmr12</i> leaf	-0.1748	0.91	-0.0324	0.74	-0.0286	0.73
<i>bmr6</i> & <i>bmr12</i> leaf	na	na	-0.0611	0.67	-0.1400	0.89
WT grain & leaf	-0.0038	0.39	-0.0180	0.54	0.0316	0.2
<i>bmr6</i> grain & leaf	-0.2234	0.98	-0.0832	0.97	-0.1370	0.98
<i>bmr12</i> grain & leaf	-0.2630	0.87	na	na	na	na
Air isolates						
Mead						
TP 1 & 2	0.0068	0.33	-0.0207	0.76	-0.0190	0.93
TP 1 & 3	-0.0221	0.68	0.0223	0.13	0.0173	0.13
TP 1 & 4	0.0047	0.32	-0.0059	0.53	-0.0104	0.64
TP 2 & 3	0.0481	0.13	0.0032	0.32	0.0053	0.30
TP 2 & 4	0.0605	0.16	0.0014	0.44	-0.0083	0.56
TP 3 & 4	-0.0282	0.82	0.0032	0.38	0.0006	0.43
Havelock						
TP 1 & 2	0.0399	0.05	0.0018	0.28	-0.0043	0.49
TP 1 & 3	0.0125	0.08	0.0035	0.30	-0.0018	0.40
TP 1 & 4	0.0059	0.15	0.0559	0.02	0.0464	0.02
TP 2 & 3	-0.0040	0.76	-0.0059	0.70	-0.0090	0.82
TP 2 & 4	0.0451	0.18	0.0090	0.16	0.0247	0.03
TP 3 & 4	0.0135	0.67	0.0216	0.05	0.0321	0.03
Mead & Havelock						
TP 1	-0.0181	0.62	0.0972	<0.01	0.0925	<0.01
TP 2	0.2077	0.05	0.0204	0.07	0.0393	0.02
TP 3	-0.0406	0.84	-0.0070	0.58	0.0037	0.29
TP 4	-0.0250	0.86	-0.0106	0.76	-0.0085	0.59

^a Operational taxonomic units (OTU) were determined by sequence analysis of portions of the *translation elongation factor 1-α* (*TEF*) and *histone-3* (*H3*) genes and the internal transcribed spacer (ITS) region that includes the 5'-variable region of the 28S ribosomal RNA. Samples were taken from *brown midrib* (*bmr*)-6, *bmr12*, and wild-type (WT) grain (Havelock and Mead) and leaves (Havelock) and air over sorghum fields (Havelock and Mead). Time points (TP) for air samples were 1 = during vegetative growth, 2 = at anthesis, 3 = during grain development, and 4 = at grain maturity (harvest). *Fusarium* isolates were divided into two groups for analysis: those in the *Fusarium fujikuroi* species complex (FFSC) and all other *Fusarium* (OF) species. Comparisons are based on results of Wright's F statistic and K_{ST} analyses for detecting genetic differentiation between two populations. When $P \leq 0.05$, the two populations are considered significantly differentiated (values in bold). Not applicable (na) indicates that not enough isolates from *bmr12* grain and leaf isolates were collected at Havelock for sequence analysis. Additionally, not enough isolates from leaves from plants grown at Mead were collected for sequence analysis.

^b *F. fujikuroi* species complex (FFSP) OTU were analyzed using the concatenated sequences of *TEF*, *H3*, and ITS. OF haplotypes were analyzed using the concatenated sequences of *TEF*, *H3*, and ITS, and *TEF* and ITS, to account for DNA from a few other *Fusarium* spp. that did not consistently amplify using the *H3* primers.

^c Comparisons (separated by an ampersand) are between populations in plant samples (grain or leaf) from two plant genotypes grown at the same location, between two air sample TP at the same location, or air samples from two locations conducted at the same TP.

members of the *F. fujikuroi* species complex (Table 3). However, both analyses showed that such differences were apparent at Mead, NE but not at the Havelock field in Lincoln, indicating that environment could determine whether differences in *Fusarium* populations between *bmr* and wild-type lines were observed (Tables 2, 3, and 4). The *bmr6* and *bmr12* lines in this study had mutations that impair lignin biosynthesis (Bout and Vermerris 2003; Palmer et al. 2008; Saballos et al. 2009; Sattler et al. 2009, 2012). Previous studies had shown that each mutation affects lignin content, composition, and levels of other phenolic intermediates but that genetic background also had an effect on these traits (Oliver et al. 2005; Palmer et al. 2008). Another study provided evidence that sorghum genotype affected the microorganisms associated with the rhizosphere (Funnell-Harris et al. 2010a). In the current study, analyses across six genetic backgrounds suggested that both *bmr6* and *bmr12* loci had a moderate to strong effect on grain- and leaf-infecting *Fusarium* OTU at the Mead location but much less effect at Havelock.

F_{ST} and K_{ST} are indicators of the proportion of variability between populations that could be attributed to sequence differences (Hudson et al. 1992a,b). K_{ST} provides a statistical measurement that operates maximally when populations within a sample number approximately 50. Although, in the current study, population numbers were relatively low, differentiation in air populations between the two locations was indicated at earlier time points. K_{ST} analyses also supported differentiation of populations between the two locations for time point 2 (Tables 3 and 4). Combining F_{ST} and K_{ST} analyses suggested that differences between the air populations at the two locations may have been due to other *Fusarium* spp. at time point 1 and both the *F. fujikuroi* species complex and other *Fusarium* spp. at time point 2. For example, at time points 1 and 2, there were several *F. graminearum* isolates represented by OTU

M02S_C2_3 at Havelock and fewer at Mead (Fig. 2) whereas, at time point 2, *F. subglutinans* OTU M02S_A1_92 was more apparent at Mead than at Havelock (Fig. 1). Therefore, combining F_{ST} and K_{ST} analyses was informative on population differentiation within *Fusarium* groups in different environments.

OTU is commonly used in microbial ecology to describe groups of individuals that share sequence similarity above a certain threshold, usually at 97% nucleotide sequence similarity or higher (Daly et al. 2001; Fierer et al. 2007; McCaig et al. 1999). This approach was used in the current study to group fungal isolates with at least 99.5% sequence similarity in order to determine how the abundance of each group of fungi changed over time (air samples) or how fungi were present in leaves and grain collected from different sorghum genotypes. For the phylogenetic analysis presented, a representative sequence from each OTU (generally the most abundant sequence within the OTU) was used to construct the tree. Although multiple OTU were assigned to the same fungal species complexes in several cases, it is beyond the scope of the work to determine whether these OTU represent new variants, strains, or haplotypes within these species complexes.

Among the members of the *F. fujikuroi* species complex, there were multiple OTU detected for each species, or *Fusarium* isolates not yet identified to species that grouped in the same clade as OTU with specific epithets, for air, grain, and leaf isolates. However, there was just one dominant OTU found in plant tissues for each species (Fig. 1). The *F. subglutinans* OTU represented by isolate M02S_A1_92, was detected in air samples taken at both locations (2005 and 2006) but only in plant tissues (grain and leaves) grown at Mead (2002 and 2003). These data suggested that environmental conditions at irrigated Mead fields were more conducive for infection by this OTU than conditions at the dryland Havelock field. One *F. thapsinum* OTU, represented by isolate H03S_A1_2, was most prevalent in grain and leaf samples; however, both OTU were found in grain and leaf samples but detected in air samples only at low levels. In another study conducted at Mead and Havelock during 2005, *F. thapsinum* was detected in grain from both locations, indicating that infection of sorghum grain by this species was common despite low detection levels in air (Funnell-Harris et al. 2013b); however, further analyses will need to be conducted to determine whether the same OTU were present in both air and plant tissues during that year.

Several OTU were identified among the other *Fusarium* spp. but most were only detected from air samples. When using the concatenated *TEF*, *H3*, and ITS sequences, the most commonly detected OTU from air samples was *F. graminearum*, represented by isolate M02S_C2_3, which constituted 54% of the colonies sampled for sequencing (Fig. 2A). The closely related species also found in North America, *F. pseudograminearum* O'Donnell & T. Aoki 1999 (Aoki and O'Donnell 1999; Smiley et al. 2016), was not isolated in this study. One of three OTU of FIESC genotype 25-a,b,c (represented by isolate H02L_C3_11a) was only detected in air, grain, and leaf samples from the Havelock field in Lincoln, NE. Another FIESC OTU, genotype 18-a,b represented by isolate M02S_A1_4, was detected in air samples from both Mead and Havelock but only detected in grain grown at Mead. These last two cases provided evidence that environmental conditions may affect sporulation or sorghum infection by different members of FIESC (Bottalico and Perrone 2002; Dorn et al. 2011).

Inconsistent amplification of the *H3* gene fragment from isolates of a few species occurred. Although most (93.0%) of the *Fusarium* isolates analyzed in the present study had an amplified *H3* fragment, DNA from a few FIESC, *F. graminearum*, and *F. sporotrichioides* genotypes did not consistently yield amplification products. Therefore, ITS and *TEF* sequences were concatenated and the analysis was repeated. Results for FIESC genotypes were similar between the two analyses. However, three *F. graminearum* OTU, two *F. armeniacum* OTU, and one *Fusarium* sp. OTU were all grouped in the same clade. Little is known of *F. armeniacum* (Burgess and Summerell 2000;

TABLE 4. Genetic similarity between populations, as indicated by Wright's F-statistic (F_{ST}), of *Fusarium* air isolates collected at sorghum fields in Lincoln, NE (Havelock field) and Mead, NE, during vegetative growth (time point 1), anthesis (time point 2), grain development (time point 3), and grain maturity (4) over 2 years^a

Location, comparison	F_{ST}
Population, different times ^b	
Havelock	
1 versus 2	0.0709
1 versus 3	0.0242
1 versus 4	0.0156
2 versus 3	-0.0078
2 versus 4	0.1077
3 versus 4	0.0281
Mead	
1 versus 2	-0.0587
1 versus 3	0.0509
1 versus 4	-0.1210
2 versus 3	0.0248
2 versus 4	0.1017
3 versus 4	-0.0720
Two locations, same time ^c	
1	-0.0395
2	0.3264*
3	-0.07945
4	-0.1113

^a F_{ST} values were determined based on concatenated alignments of portions of the translation elongation factor and histone-3 genes plus the internal transcribed spacer region. Sequences were analyzed using DNASP, version 5.10.1 (<http://www.ub.edu/dnasp/>). F_{ST} indicates variation between two populations: "0" = identical whereas "1" = completely differentiated. Negative values are treated as "0". For purposes of this discussion, an asterisk (*) represents moderate to strong differentiation ($F_{ST} > 0.15$); all other comparisons have low differentiation.

^b Comparisons of populations at different sampling time points.

^c Comparisons between air populations collected at two locations at the same time point.

Burgess et al. 1993). It originally was considered a subspecies of *F. acuminatum* but was reassigned to its own species due to its greater toxigenicity (Wing et al. 1993) and electrophoretic karyotype differences (Nagy and Hornok 1994). *F. armeniacum* has been isolated from grain crops and grasses (Burgess et al. 1993; Funnell-Harris et al. 2010b; Kommedahl et al. 1979; Nagy and Hornok 1994) and, unlike *F. graminearum*, was shown to produce T-2 trichothecenes (Wing et al. 1993). Despite differences in mycotoxin production, the current study provides evidence of the phylogenetic relationships between these two species.

In summary, screening of air, grain, and leaf isolates from multiple environments indicated that, within a species group, one or two OTU were dominant across most environments tested. Exceptions included *F. subglutinans* and FIESC 18-a,b OTU that were present in air samples at both locations but only present in grain (*F. subglutinans* and FIESC 18-a,b) and leaves (*F. subglutinans*) taken at Mead; and the dominant FIESC 25-a,b,c OTU, which was present only in air and plant samples (grain and leaves) taken from Havelock. On the other hand, the *F. thapsinum* OTU that was dominant in grain samples from both locations, representing 62.1% of *F. fujikuroi* species complex grain isolates and 44.1% of total grain isolates, was detected only at low levels from air samples. A likely explanation is the dominance of maize production in this region of Nebraska (Healey et al. 2011), which could result in the presence of conidia primarily from maize pathogens such as *F. proliferatum* and *F. graminearum* (Jurjevic et al. 2005; Schmale and Bergstrom 2004). This could explain the greater number of OTU from these maize pathogens in air samples identified for each of these species in the present study (Figs. 1 and 2). Although additional investigations are required to determine the aerial patterns and pathogenicity of different *F. thapsinum* OTU, these results lend further support to the previously proposed idea that *F. thapsinum* may be particularly aggressive toward and perhaps specialized on sorghum (Leslie et al. 2004). The present study also provided evidence indicating that air populations at two locations in Nebraska were differentiated earlier in the growing season (e.g., during anthesis) but were much more similar later (during grain development), when infection by pathogens would likely occur (Funnell-Harris and Pedersen 2008; Navi et al. 2005). This study presented evidence that fungal populations in grain and leaf samples from *bmr6*, *bmr12*, and wild-type lines across six genetic backgrounds may be different, which suggested that changes to lignin biosynthesis, and the consequential changes to lignin content, its composition, or levels of other phenolic compounds, have a substantial effect on infection by *Fusarium* spp. It had been previously demonstrated that *Fusarium* pathogens could be inhibited during in vitro growth assays by phenolic compounds that were elevated in some *bmr* lines, which led to the hypothesis that free phenolics accumulating in *bmr* plants acted as a resistance mechanism (Funnell-Harris et al. 2014). However, it was recently demonstrated that free phenolic extracts from *bmr6* plants did not inhibit in vitro growth of *F. thapsinum*; thus, it was proposed that cell-wall-bound phenolics or inducible defense compounds, as a result of increased salicylic acid levels in *bmr* plants, may reduce in planta growth of some fungal pathogens (Funnell-Harris et al. 2010b, 2017).

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