Analysis of *Fusarium graminearum* Species Complex from Wheat–Maize Rotation Regions in Henan (China)

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Abstract

Fusarium head blight (FHB) and maize stalk rot (MSR), caused by members of the *Fusarium graminearum* species complex (FGSC), are among the most destructive and economically important diseases in the world. Species identity and the trichothecene chemotype of 312 members of the FGSC from diseased wheat spikes and maize stalks in Henan was determined using phylogenetic analyses and a polymerase chain reaction trichothecene chemotype assay. *F. graminearum* sensu stricto accounted for more than 93% of the FGSC isolates associated with FHB ($N = 168$) and MSR ($N = 130$). The remaining isolates were *F. asiaticum*. Significant differences were found in the frequencies of the two species within the hosts ($P < 0.01$). However, the frequencies of the same species in FHB and MSR were similar ($P >$

0.05) for wheat and maize isolates, indicating that the composition of the FGSC with respect to wheat and maize in these fields varied little. The 15-acetyl-deoxynivalenol (15-ADON) trichothecene chemotype represented 92.7 and 98.5% of isolates from wheat ($N = 167$) and maize ($N = 130$), respectively. However, the 3-acetyl-deoxynivalenol chemotype was found in 6.7% of wheat isolates, and the nivalenol chemotype in 1.5% of MSR isolates and in 0.6% of FHB isolates. Mycelial growth at different concentrations of carbendazim and difenoconazole did not differ between *F. graminearum* sensu stricto and *F. asiaticum*. These results suggest that the 15-ADON chemotype of *F. graminearum* sensu stricto is the predominant pathogen that causes wheat- and maize-related diseases in this region.

Fusarium graminearum species complex (FGSC) is one of several common pathogens that can cause a variety of root, stalk, and ear rot diseases in crops. It not only can lead to a reduction in crop yield but also can produce a variety of mycotoxins that can enter the food chain. The FGSC contains at least 16 distinct species. Some species have specific geographical distributions. *F. asiaticum* and *F. graminearum* sensu stricto are abundant in China (O'Donnell et al. 2000, 2004; Sarver et al. 2011; Yli-Mattila et al. 2009; Zhang et al. 2012). Studies on species of the FGSC causing *Fusarium* head blight (FHB) in China have shown that *F. asiaticum* is dominant in the Yangtze River Basin and in the southwest of China, whereas *F. graminearum* sensu stricto is predominant in northeast China and in the Huanghuaihai region of northern and central China (Zhang et al. 2012).

Related studies have found that members of the FGSC exhibit host preference on wheat, maize, barley, and rice in certain regions. In China, Korea, and Brazil, *F. asiaticum* was in the majority of FGSC on wheat or rice in southern regions (Gomes et al. 2015; Lee et al. 2009; Zhang et al. 2012), *F. meridionale* was the predominant species on ears and stalks of maize in Brazil (Kuhnem et al. 2016), and *F. graminearum* sensu stricto was the predominant species on maize in Korea (Lee et al. 2012) and on wheat and maize roots in South Africa (Boutigny et al. 2011). However, *F. graminearum* sensu stricto is dominant on wheat in northern China and Brazil (Del Ponte et al. 2015; Zhang et al. 2012). The pathogen diversity of the FGSC appears to be host, region, agroecosystem, and climate dependent; however, the real reasons are not clear.

Trichothecenes produced by the FGSC not only reduce the safety of food and feed but also are related to the virulence of each pathogen. The FGSC mainly produces type B trichothecene mycotoxins, including nivalenol (NIV), 3-acetyl-deoxynivalenol (3-ADON), and 15-acetyl-deoxynivalenol (15-ADON) (Goswami and Kistler 2005; Miller et al. 1991; Ward et al. 2002). In China, studies related to the FGSC causing FHB indicated that *F. graminearum* sensu

stricto mainly produces 15-ADON and that *F. asiaticum* mainly produces 3-ADON or NIV (Zhang et al. 2007; Zhang et al. 2012; Zhang et al. 2013).

Henan, in northern and central China, was chosen because it is the largest winter wheat–summer maize rotation area (5.3 million hm^2 of wheat and 3.3 million hm^2 of maize) in the Huanghuaihai region. The wheat–maize cropping area covers more than 3.0 million hm^2 . Crop rotation, conservation tillage, and crop residue management may have contributed to the increase of FHB and maize stalk rot (MSR) in this region.

Studies have been carried out on FHB to assess population structure, trichothecene chemotype, and geographical distributions of the FGSC. However, information is limited on the FGSC from maize fields in China. Consequently, in the present study, we aim to determine the differences in the FGSC composition, including the chemotypes of each species and their geographical distribution between FHB and MSR in wheat–maize cropping areas of Henan, and to assess whether *F. asiaticum* and *F. graminearum* sensu stricto differ in their pathogenicity and fungicide sensitivity toward wheat and maize.

Materials and Methods

Isolate collection. Wheat spikes and maize basal stalks that exhibited symptoms typical of FHB and MSR were harvested from wheat–maize rotation fields spanning 50 counties in Henan during 2011, 2012, and 2013 (Fig. 1).

Isolation, culture, and single-sporing followed the protocol described for MSR and FHB in Sun et al. (2014) and Gale et al. (2002), respectively. *Fusarium* isolates were initially identified morphologically (Leslie and Summerell 2006) and validated by species-specific polymerase chain reaction (PCR) assay (Rahjoo et al. 2008; Sun et al. 2014). DNA sequence-based identification of unidentified isolates was achieved using the translation elongation factor 1 α (*EF-1 α*) gene. DNA sequences were edited in SeqMan II 4.03 (Lasergene Sequence Analysis Software; DNASTAR), then searched for similarities using the BLAST similarity search programs in the National Center for Biotechnology Information and the *FUSARIUM-ID* v.1.0 database (<http://fusarium.cbio.psu.edu>) (Geiser et al. 2004). Isolates were purified using single sporing and stored for use. This procedure identified 312 isolates as members of the FGSC.

DNA extraction. Each isolate was grown on a potato dextrose agar (PDA) plate at 25°C for 1 week. The mycelium from each plate

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was harvested, frozen in liquid nitrogen, and then ground into fine powder. Genomic DNA from each isolate was extracted using a commercial fungal extraction kit (Shanghai Lifefeng Biotech) and stored at -20°C .

PCR amplification and sequencing. PCR amplification and sequencing of the *EF-1 α* gene and the 3-O-acetyltransferase (*Tri101*) gene were conducted using the primer pair EF1/EF2 (O'Donnell et al. 1998) and TRI1015B/TRI1013E (Proctor et al. 2009), respectively (Table 1). The sequencing method of O'Donnell et al. (2004) was followed and the PCR products were sequenced by Shanghai Sangon Biological Engineering Co., Ltd.

Phylogenetic analysis. DNA sequences were aligned and adjusted manually using DNASTar-SeqMan software. Reference sequences of the *EF-1 α* gene and *Tri101* gene representing all known NRRL strains of 16 clade species of the FGSC were downloaded from the GenBank database. Sequences were aligned in Clustal X 1.81 (www.clustal.org/clustal2/). Phylogenetic relationships were inferred in MEGA6.06 (Tamura et al. 2013) using the maximum-likelihood method with 1,000 bootstrap replicates. The best-fit model of molecular evolution was selected based on estimation of Bayesian Information Criterion scores.

Fig. 1. Map showing field samples of *Fusarium* head blight and maize stalk rot from the wheat-maize rotation region of Henan in 2011, 2012, and 2013.

Determination of trichothecene chemotypes. Trichothecene chemotypes were generated by multiplex PCR targeting *Tri3* and *Tri12* gene sequences under the conditions described by Starkey et al. (2007). PCR delineated FGSC and the other B-trichothecene producers as either the 3-ADON, 15-ADON, or NIV trichothecene chemotype. The *Tri3* gene primers were CON, 3NA, 3D15A, and 3D3A (Table 1). These primers produced amplified target DNA of approximately 840, 610, and 243 bp that contained the NIV, 15-ADON, and 3-ADON chemotype, respectively. The *Tri12* primers used were 12CON, 12NF, 12-15F, and 12-3F. These primers generated amplified target DNA of approximately 840, 670, and 410 bp that contained the NIV, 15-ADON, and 3-ADON chemotype, respectively (Table 1).

Pathogenicity assays. In all, 10 isolates of *F. graminearum* sensu stricto and *F. asiaticum* were analyzed for their pathogenicity on corn and wheat using the Petri plate assay of Broders et al. (2007) and Hao et al. (2013). Isolates were grown on PDA at 25°C for 4 days. A colony (5 mm in diameter) from each isolate was transferred to the center of a Petri dish (8 mm in diameter) which contained 15 ml of 1.7% water agar. The isolates were grown for 4 days. In total, 10 seeds of either corn hybrid 'Xundan26' or 20 seeds of wheat 'Zhengmai366' were surface disinfested in 3% NaClO and then plates were filled to within 1 cm of the rim. The plates were incubated at 25°C in darkness for 7 days. Percent seed germination and rot was determined according to the following scale: 1 = seed germinated, healthy seedlings were produced with no clear symptoms of infection; 2 = seed germination observed, rarely with signs of infection, 1 to 19% with lesions on seedling root; 3 = seed germinated, partially covered with mycelium, 20 to 75% with lesions on seedling root; 4 = seed germinated, completely covered with mycelium, more than 75% with lesions on seedling root; and 5 = seed did not germinate, completely covered with mycelium. We used three block replicates for each treatment (10 isolates and one control). There were three replicate plates for each isolate in each experiment. The entire procedure was repeated three times.

Fungicide sensitivity. The same 10 isolates used in the pathogenicity assay were used to assess sensitivity to carbendazim and difenoconazole (Shenyang Academy of Chemistry and Industry). Carbendazim and difenoconazole were dissolved in HCl and acetone, respectively. This stock solution was added to PDA cooled to approximately 55°C . Technical-grade carbendazim was evaluated at 0.1, 0.15, and 0.25 $\mu\text{g/ml}$, and unamended PDA was used as the control. Difenoconazole (technical-grade product) was evaluated at 0.5, 2.5, and 10 $\mu\text{g/ml}$, and unamended PDA was again used as the control. A colonized plug (5 mm in diameter) taken from the edge of each 6-day-old colony was transferred to the center of a plate containing each concentration of the fungicide. The colony diameter was measured in two places after 6 days of incubation at 25°C . For each isolate at each concentration, the percent growth inhibition was calculated by dividing the average colony diameter minus 5 mm for the

Table 1. Polymerase chain reaction primers used in the experiments

Primer name	Primer sequence (5'-3') ^x	Amplified target DNA (bp) ^y	Target gene	Citation ^z
EF1	ATGGGTAAGGARGACAAGAC	600	<i>EF-1α</i>	O'Donnell et al. 1998
EF2	GGARGTACCAGTSATCAGTT	...	...	...
TRI1015B	CCATGGGTCGCRGGCCARGTSAA	1,336	<i>TRI101</i>	Proctor et al. 2009
TRI1013E	AATCSCCRTCIGGYTTYTTNGGCAT	...	...	...
3CON	TGGCAAAGACTGGTTTAC	840 (NIV)	<i>Tri3</i>	Starkey et al. 2007
3NA	GTGCACAGAATATACGAGC	610 (15-ADON)	...	...
3D15A	ACTGACCCAAGCTGCCATC	243 (3-ADON)	...	...
3D3A	CGCATTGGCTAACACATG	...	...	...
12CON	CATGAGCATGGTGATGTC	840 (NIV)	<i>Tri12</i>	Starkey et al. 2007
12NF	TCTCCTCGTTGTATCTGG	670 (15-ADON)	...	...
12-15F	TACAGCGGTCGCAACTTC	410 (3-ADON)	...	...
12-3F	CTTTGGCAAGCCCGTGCA	...	...	...

^x R = A + G and Y = C + T.

^y Nivalenol (NIV), 3-acetyl-deoxynivalenol (3-ADON), and 15-acetyl-deoxynivalenol (15-ADON) chemotypes.

^z Reference from literature cited.

agar plug by the average colony diameter on nonfungicide amended media. The results were multiplied by 100. Three replicates were performed for each of the selected isolates. Tests were repeated twice. The results were analyzed using PROC GLM of SAS (v 9.2; SAS Institute, Inc.). These included analyzing the variation in sensitivity of isolates and species to the two different fungicides and the effect of each fungicide concentration on the efficacy of the two fungicides.

Results

Species diversity. According to morphological and molecular identification, 494 *Fusarium* isolates were collected by sampling wheat–maize rotation fields in Henan during the 2011, 2012, and 2013 growing seasons. In all, 180 isolates of the FGSC from FHB

samples were collected and identified. Among the 314 isolates from MSR samples, the FGSC was most abundant (42.0%, $N = 132$) of isolates, followed by *F. proliferatum* (29.9%, $N = 94$) and *F. verticillioides* (28.0%, $N = 88$). Among the 312 isolates of the FGSC collected in this study, 180 isolates from wheat and 132 isolates from maize were nested within the FGSC.

Phylogenetic analysis of *EF-1 α* and *Tri101* gene sequences revealed that *F. graminearum* sensu stricto and *F. asiaticum* were recovered in our study of FHB and MSR in wheat–maize rotation fields in Henan. Among 312 isolates, 14 isolates (4.5%) belonged to *F. asiaticum*. The remaining 298 (95.5%) isolates belonged to *F. graminearum* sensu stricto (Fig. 2; Table 2). Among the isolates from wheat ($N = 180$), the majority were *F. graminearum* sensu

Fig. 2. Phylogeny of *Fusarium graminearum* species complex based on the translation elongation factor 1 α (*EF-1 α*) gene and the 3-O-acetyltransferase (*Tri101*) gene region. Bootstrap values (percentage, based on 1,000 replications) ≥ 60 are shown on branches. Some representative strains of *F. graminearum* species complex isolates in this study were selected in the phylogenetic tree with “W” and “M” codes obtained from wheat and maize, respectively. The remaining isolates were from the National Center for Biotechnology Information. *F. pseudograminearum* and one *F. culmorum* were used as outgroups.

stricto (93.3%, $N = 168$) but 6.7% were *F. asiaticum* ($N = 12$). Among 132 isolates from MSR, only 2 isolates (1.5%), from Luoyang City and Zhumadian City, were *F. asiaticum*. The remaining isolates (98.5%, $N = 130$) were *F. graminearum* sensu stricto (Table 2). Significant differences were found in the frequencies of *F. asiaticum* and *F. graminearum* sensu stricto by host ($P < 0.01$). However, the frequency of these species causing FHB and MSR were similar ($P > 0.05$) to those shown for wheat and maize isolates. This shows that the FGSC composition is similar for wheat and maize in the wheat–maize rotation fields in Henan. *F. graminearum* sensu stricto was the predominant FHB and MSR pathogen in Henan.

Trichothecene chemotypes. The trichothecene chemotype of 312 isolates was determined using two multiple PCR targeting *Tri3* and *Tri12*. The 15-ADON chemotype was predominant in isolates from wheat (92.7%) and maize (98.5%) in Henan and accounted for 297 (95.1%) of isolates (Table 3). The NIV chemotype comprised 0.6% ($N = 1$) of isolates from wheat and 1.5% ($N = 2$) of isolates from maize. In total, 12 isolates (6.7%) from wheat were the 3-ADON chemotype. The frequency of the 15-ADON or NIV chemotype among isolates from wheat and maize was not significantly different ($P > 0.05$). The 15-ADON chemotype was observed throughout Henan and was the most common chemotype in the wheat–maize fields.

Results indicated that, among the 168 isolates of *F. graminearum* sensu stricto from wheat, 167 isolates possessed the 15-ADON and 1 the NIV chemotype. All 12 isolates of *F. asiaticum* were the 3-ADON chemotype. In addition, 130 isolates of *F. graminearum* sensu stricto from maize were 15-ADON chemotype and 2 isolates of *F. asiaticum* were the NIV chemotype (Table 4).

Pathogenicity. All 10 representative isolates of the FGSC exhibited a high degree of pathogenicity to both maize and wheat when grown on Petri plates under the described conditions. Pathogenicity was scored 4, seed had germinated and were completely covered with mycelium, and more than 75% contained lesions on the seedling roots. *F. graminearum* sensu stricto and *F. asiaticum* also did not differ significantly in degree of pathogenicity on both corn and wheat when using the Petri plate assay (all data not shown).

Fungicide sensitivity. Analysis of variance showed that there was no difference between *F. graminearum* sensu stricto and *F. asiaticum* species in inhibiting mycelial growth at different concentrations of carbendazim or difenoconazole (Table 5). No significant difference was observed among the three *F. asiaticum* isolates ($P > 0.05$) but there was a significant difference ($P < 0.01$) among the seven *F. graminearum* sensu stricto isolates. Highly significant differences ($P < 0.01$) were observed between fungicides and between their different

concentrations. The effect of fungicide–concentration was also highly significant (Table 5).

There was a significant difference in the inhibition of mycelial growth at different carbendazim and difenoconazole concentrations (Table 6). An increase in concentration led to a significant increase in inhibition efficacy. Difenoconazole inhibited mycelial growth to 91.2% of the control at a concentration of 10 µg/ml. However, mycelial growth of 35 to 83.6% of the control was observed on media supplemented with carbendazim at a concentration of either 0.1, 0.15, or 0.25 µg/ml (Table 6). Additionally, the two fungicides provided greater inhibition in WLY11-2-1, MSP11-2-1, and MS105-6 isolates than MS29-1, WZX3, WPY11-3-1, and MS68-1 within the seven isolates of *F. graminearum* sensu stricto (Table 7).

Discussion

This study has described pathogen diversity of the FGSC in wheat–maize rotation fields in Henan for the first time and increased the body of information on pathogen detection and diversity of the FGSC. Phylogenetic analysis using the *EF-1α* gene and *Tri101* gene region sequences revealed two species within the FGSC, *F. graminearum* sensu stricto and *F. asiaticum*, from a winter wheat–summer maize cropping area in Huanghuaihai region. This is the first report of *F. graminearum* sensu stricto and *F. asiaticum* causing MSR in the world. Isolates used in this study were mainly collected from a winter

Table 4. Chemotypes of *Fusarium graminearum* sensu stricto (*F. graminearum*) and *F. asiaticum* from wheat and maize in wheat–maize rotation region of Henan^y

Chemotypes ^z	Wheat		Maize	
	<i>F. graminearum</i>	<i>F. asiaticum</i>	<i>F. graminearum</i>	<i>F. asiaticum</i>
3-ADON	0	12	0	0
15-ADON	167	0	130	0
NIV	1	0	0	2
<i>N</i>	168	12	130	2

^y Results are expressed as number of isolates relative to the total number of isolates (N).

^z Trichothecene chemotypes: 3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON), and nivalenol (NIV).

Table 5. Analysis of variance for *Fusarium asiaticum* and *F. graminearum* sensu stricto on fungicide sensitivity

Source	df	MS	<i>F</i>	<i>P</i> value
Rep	2	38.9	0.8	0.459
Between species	1	42.4	0.9	0.357
Isolates (species)	8	239.9	4.8	<0.0001
Within <i>F. asiaticum</i>	2	35.7	0.7	0.489
Within <i>F. graminearum</i> sensu stricto	6	308.0	6.2	<0.0001
Fungicide	1	2,883.4	58.0	<0.0001
Concentration	2	44,348.2	892.1	<0.0001
Fungicide × concentration	2	1,966.3	39.6	<0.0001
Error	163	49.7	...	...

Table 6. Growth of *Fusarium asiaticum* and *F. graminearum* sensu stricto evaluated on media amended with different concentrations of difenoconazole and carbendazim^z

Fungicide	Concentration (µg/ml)	Inhibition of mycelial growth to the control (%)
Difenoconazole	0.5	31.4c
	2.5	68.0b
	10.0	91.2a
Carbendazim	0.1	35.0c
	0.15	48.3b
	0.25	83.6a

^z Means in a column followed by the same letter do not differ significantly ($P = 0.05$).

Table 2. Diversity of *Fusarium graminearum* species complex within hosts in wheat–maize rotation region of Henan^z

Host	Isolates (%)		<i>N</i>
	<i>F. graminearum</i> sensu stricto	<i>F. asiaticum</i>	
Wheat	93.3	6.7	180
Maize	98.5	1.5	132

^z Results are expressed as percentage of isolates relative to the total number of isolates (N).

Table 3. Chemotype of *Fusarium graminearum* species complex isolates collected from wheat and maize in wheat–maize rotation region of Henan^y

Host	Isolates (%) per chemotype ^z			<i>N</i>
	15-ADON	3-ADON	NIV	
Wheat	92.7	6.7	0.6	180
Maize	98.5	0.0	1.5	132

^y Results are expressed as percentage of isolates relative to the total number of isolates (N).

^z Trichothecene chemotypes: 3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON), and nivalenol (NIV).

wheat–summer maize cropping area under a double cropping system. Previous studies focused on Chinese wheat scab but little research has been conducted on maize FGSC in the wheat–maize cropping system, especially as it relates to a comparative study of FGSC between wheat and maize. Previous studies associated with wheat scab have shown that *F. graminearum* sensu stricto is the predominant species in the north of China, whereas *F. asiaticum* is predominant in the south. The cooler temperatures (annual average temperature 15°C or lower) in the north of China appear to favor *F. graminearum* sensu stricto and the warmer south (annual average temperature above 15°C) appears to favor *F. asiaticum*. The majority of *F. asiaticum* isolates were collected from these warmer regions in which FHB epidemics occurred often (Gale et al. 2002; Zhang et al. 2007; Zhang et al. 2012; Zhang et al. 2013). All isolates from the Zhejiang wheat fields in eastern China were *F. asiaticum* (Gale et al. 2002). Analysis of wheat scab FGSC from 15 provinces in China found that *F. graminearum* sensu stricto and *F. asiaticum* co-exist in most provinces of the Yangtze River Basin and the Huaihe River Basin (Zhang et al. 2012). However, *F. asiaticum* was dominant in the Yangtze River Basin and in southwestern China. *F. graminearum* sensu stricto was dominant in the northeast and Huanghuaihai regions. An obvious shift in species domination from *F. asiaticum* to *F. graminearum* sensu stricto from south to north existed; 35 *F. graminearum* sensu stricto and 3 *F. asiaticum* isolates were identified from 20 sampling points in the Henan wheat area (Zhang et al. 2012). These results are consistent with this study, which demonstrates the presence of *F. graminearum* sensu stricto and *F. asiaticum* in wheat scab in Henan. *F. graminearum* sensu stricto was the dominant pathogen. The distribution of *F. asiaticum* did not have a defined geographical distribution, which is likely due to the limited number of sampling points.

Ndoye et al. (2012) studied maize ear rot in China and showed that the NIV chemotype of *F. asiaticum* was dominant in the warmer south and 15-ADON chemotype of *F. graminearum* sensu stricto was dominant in the colder north. However, the toxin chemotype of *F. asiaticum* from wheat in the same region was different. We have found that, for both wheat and maize, the dominant species was 15-ADON chemotype of *F. graminearum* sensu stricto. The FGSC from maize ear rot in South Korea contained *F. meridionale*, *F. boothii*, *F. asiaticum*, and *F. graminearum* sensu stricto (Lee et al. 2012); *F. graminearum* sensu stricto was the dominant species (75%) but in Korean rice, *F. asiaticum* was the dominant species (78.5%), followed by *F. graminearum* sensu stricto (Lee et al. 2009). There are significant differences between hosts; the authors believed that *F. asiaticum* had a preference and adapted to rice (Lee et al. 2012). Boutigny et al. (2011), studying species-specific differences in host preference, found that, in South Africa, *F. graminearum* sensu stricto, *F. boothii*, and *F. meridionale* were the three dominant

species in the FGSC from wheat, maize, and barley. *F. graminearum* sensu stricto was the dominant species in wheat and barley. *F. boothii* was the dominant species in maize ears. *F. graminearum* sensu stricto was the dominant species in maize roots, followed by *F. boothii* and *F. meridionale*, showing that different species in the same area of South Africa had certain host preferences and adaptability. In Brazil, *F. graminearum* sensu stricto is dominant in wheat (Astolfi et al. 2012; Del Ponte et al. 2015), *F. asiaticum* is dominant in rice (Gomes et al. 2015), and *F. meridionale* is the predominant species on maize ears and stalks (Kuhnem et al. 2016).

Among the 132 isolates obtained from MSR samples, this study has identified only two NIV-type *F. asiaticum* isolates from Zhumadian and Luoyang. In addition, 12 3-ADON-type *F. asiaticum* isolates were collected from Zhengzhou, Hebi, and Zhumadian. One NIV-type isolate of *F. graminearum* sensu stricto was found in wheat in Hebi, while the other *F. graminearum* sensu stricto isolates produced 15-ADON chemotypes. Studies have shown that *F. graminearum* sensu stricto populations from Asia mainly produced 15-ADON, *F. asiaticum* populations from Asia mainly produced 3-ADON or NIV chemotypes, and NIV-type *F. graminearum* sensu stricto populations have not been found (Gale et al. 2002; Lee et al. 2009, 2012; Shen et al. 2012; Zhang et al. 2007; Zhang et al. 2012; Zhang et al. 2013). Gale et al. (2011) reported NIV-type *F. graminearum* sensu stricto populations where 79% of the isolates were prevalent on wheat in Louisiana. Our study is the first to demonstrate the existence of the NIV chemotype of *F. graminearum* sensu stricto in China.

From maize and wheat, although we obtained 2 and 12 *F. asiaticum* isolates and 130 and 168 *F. graminearum* sensu stricto isolates, respectively, no significant difference in frequency between isolates of *F. asiaticum* and *F. graminearum* sensu stricto between wheat and maize was found. A significant difference was observed in the frequency between the *F. asiaticum* and *F. graminearum* sensu stricto isolates within the same host. However, from species and chemotype studies, we found a difference in host between 3-ADON-type *F. asiaticum* strains. This may be the result of a limited number of sampling points and can be addressed by expanding the geographical range of the sampling points and the sample size. This study shows that the 15-ADON-type *F. graminearum* sensu stricto is the main pathogen that causes wheat- and maize-related diseases in Henan. Additional research on the effect of plant residue and tilling method on these related diseases will be of economic significance, because the FGSC can cause root, stalk, and ear rot diseases in wheat–maize rotation cropping fields.

This study identified isolates from wheat and maize and assayed their pathogenicity on both maize and wheat using a Petri plate assay; this method was also used to analyze differences in pathogenicity between different isolates of *Fusarium* on maize and soybeans (Broders et al. 2007; Hao et al. 2013). The results of this study have shown that the representative isolates all were scored 4; no difference between species was observed between chemotypes and between the isolates studied. Lamprecht et al. (2011) found a difference in pathogenicity between *F. boothii*, *F. graminearum* sensu stricto, and *F. meridionale* in causing maize root rot. In addition, these representative isolates did not show significant differences in inhibition of mycelial growth at the different concentrations of carbendazim and difenoconazole between species or between chemotypes, which was similar to the results of Tateishi et al. (2010), who analyzed metconazole sensitivity of *F. asiaticum* and *F. graminearum* sensu stricto from wheat in Japan. However, Zhang et al. (2013), who studied wheat scab FGSC from Jiangsu Province, China, found that the majority of NIV and 15-ADON-type isolates were sensitive to carbendazim, whereas many 3-ADON-type isolates were resistant to carbendazim. In addition, Zhang et al. (2012) found that 3-ADON-type isolates of *F. asiaticum* had a greater pathogenicity, growth rate, sporulation capacity, and resistance to benzimidazole than the NIV type of *F. asiaticum*. Isolates selected in the present study were sensitive to both fungicides. This may be a result of the small sample size of representative isolates. We found no isolates that were resistant to carbendazim and difenoconazole.

Table 7. Effect of *Fusarium asiaticum* and *F. graminearum* sensu stricto on fungicide sensitivity

Species, isolate	Host	Chemotype ^y	Inhibition of growth (%) ^z
<i>F. asiaticum</i>			
WZZ11-2-1	Wheat	3-ADON	59.9a
WZX1	Wheat	3-ADON	61.8a
WHX2-5	Wheat	3-ADON	59.1a
<i>F. graminearum</i> sensu stricto			
MS105-6	Maize	15-ADON	62.9a
MS29-1	Maize	15-ADON	58.2b
MSP11-2-1	Wheat	15-ADON	63.1a
WLY11-2-1	Wheat	15-ADON	64.1a
WZX3	Wheat	15-ADON	56.4bc
WPY11-3-1	Wheat	15-ADON	53.6bc
MS68-1	Maize	15-ADON	56.1bc

^y Trichothecene chemotypes: 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON).

^z Inhibition of mycelial growth to the control. Means followed by the same letter do not differ significantly ($P = 0.05$).

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