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Special Report

Dynamics of Carbendazim-Resistance Frequency of Pathogens Associated with the Epidemic of *Fusarium* Head BlightLishu Yi,^{1,2} Meixin Yang,^{1,3} Cees Waalwijk,³ Jin Xu,¹ Jingsheng Xu,¹ Orsolya Molnár,⁴ Wanquan Chen,^{1,2} Jie Feng,¹ and Hao Zhang^{1,2,†} ¹ State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China² National Agricultural Experimental Station for Plant Protection, Gangu Ministry of Agriculture and Rural Affairs, Tianshui, China³ Wageningen University and Research Center, Wageningen, the Netherlands⁴ ELKH Centre for Agricultural Research, Plant Protection Institute, Budapest, Hungary

Abstract

Carbendazim resistance was detected using 4,701 *Fusarium graminearum* species complex isolates collected from major wheat-producing regions in China from 2018 to 2020. A total of 348 carbendazim-resistant isolates were identified. The majority of carbendazim-resistant isolates were detected in Jiangsu and Anhui Provinces. In total, 227 and 88 isolates were obtained from each of the Jiangsu and Anhui Provinces, with a high resistance frequency of 41.12 and 20.56%, respectively. The predominant resistant isolates harboring point mutations were F167Y (79.31%), followed by E198Q (16.38%) and F200Y (4.31%). Compared with *F. graminearum*, *F. asiaticum* isolates were more likely to produce carbendazim resistance. In this study, we first detected carbendazim-resistant isolates

in Hebei, Shaanxi, Sichuan, and Hunan Provinces. In Jiangsu, Anhui, and Zhejiang, the frequency of carbendazim-resistant isolates maintained a high level, resulting in stable carbendazim-resistant populations. We also found the dynamic of carbendazim-resistance frequency in most provinces showed similar trends to the epidemic of *Fusarium* Head Blight (FHB). Our results facilitate the understanding of the current situation of carbendazim resistance of FHB pathogens and will be helpful for fungicides selection in different wheat-producing areas in China.

Keywords: carbendazim, *Fusarium graminearum* species complex, *Fusarium* head blight, resistance frequency

Isolates from the *Fusarium graminearum* species complex (FGSC) are the most frequent causal agents of *Fusarium* head blight (FHB), causing a devastating disease of wheat. Epidemics of FHB not only lead to massive economic losses but also result in mycotoxin contaminated grains which pose significant risks to human and domestic animals (Atanassov et al. 1994; Chen et al. 2007). Most wheat cultivars planted in China are susceptible to FHB; therefore, application of chemical fungicides has been the main method of controlling FHB.

Methyl benzimidazole carbamate (MBC) fungicides, especially carbendazim, perform an effective control on FHB when applied during wheat heading and flowering (Chen et al. 2007; Liu et al. 2010; Zhou and Wang 2001). MBCs inhibit microtubule assembly during nuclear division by binding to the β -tubulin protein and thereby prevent the mycelial growth and germ-tube elongation

(Leroux 1998; Leroux et al. 1998). Resistance of benzimidazole is associated with point mutations in the β -tubulin gene, which lead to reduction in binding affinity between MBC and target sites (Hollomon et al. 1998). Previous studies evidenced that point mutations at codons 6, 50, 167, 198, 200, or 240 of the β -tubulin gene are responsible for resistance to carbendazim in pathogens (Baraldi et al. 2003; Koenraadt et al. 1992; Ma et al. 2003; McKay et al. 1998).

Two homologous β -tubulin genes (*Tub1* and *Tub2*) have been identified in FGSC. In previous studies, resistant and sensitive isolates proved to have identical *Tub1* sequences showing *Tub1* is conserved, and resistance of FGSC isolates to MBC is due to non-synonymous mutations in *Tub2* (Chen et al. 2007, 2009). To date, at least five types of point mutations (F167Y, E198L, E198K, E198Q, and F200Y) of *Tub2* conferring MBC resistance have been detected in the field isolates (Chen et al. 2009, 2015; Liu et al. 2010; Qiu et al. 2011). Among the five point mutations, F167Y, E198Q, and F200Y were the main genotypes, which covered 99.5% resistant FGSC strains (Liu et al. 2014).

MBC fungicides, particularly carbendazim and thiophanate-methyl, have been used extensively to control FHB in China for over 40 years (Ye and Zhou 1985; Yuan and Zhou 2005). However, the efficacy of this type of fungicides is decreasing due to rapid development of fungicide resistance. MBC resistance has been reported in many fungal species, such as *Botrytis cinerea*, *Cladobotryum dendroides*, *Helminthosporium solani*, *Monilinia fructicola*, *Sclerotinia sclerotiorum*, *Tapesia yallundae*, and *Venturia inaequalis* (Bollen and Scholten 1971; Duan et al. 2018; Leroux et al. 1998; Liu et al. 2013, 2016, 2018; Ma et al. 2003; Schroeder and Provvidenti 1969; Zhu et al. 2016). In China, Ye and Zhou (1985) started to monitor MBC resistance of FHB pathogen. The first MBC-resistant field isolate of *F. graminearum* was detected

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in 1992 in the Zhejiang Province (Zhou et al. 1994), and the frequency of resistant isolates has been increasing in the middle and lower reaches of the Yangtze River (Liu et al. 2010). More recently, MBC-resistant isolates have gradually spread towards the Huanghuai River Basin in northern China (Dai 2013). The efficacy of MBCs for FHB control in Jiangsu, Zhejiang, and Shanghai decreased dramatically with the increase of resistant populations (Chen et al. 2007; Liu et al. 2010; Wang et al. 2002). The frequency monitoring of MBC-resistant isolates during 1985 to 2008 in Jiangsu Province revealed that the frequency of resistant isolates was closely related to the severity of FHB epidemics (Y. N. Zhang et al. 2009). The tendency to increase dosage and frequency of carbendazim applications with the severity of FHB epidemics led to high selective pressure of MBC, thereby contributing to an increase of the resistance frequency in subsequent years. Studies have shown that 10% of carbendazim-resistant *Fusarium* isolates can largely reduce control efficacy by 37.5% (Liu et al. 2014). Carbendazim-resistant strains had higher trichothecene production than carbendazim-sensitive strains (Y.-J. Zhang et al. 2009), which means that the resistance not only heavily influences the control efficacy of carbendazim but also raises the risk of mycotoxin contamination of wheat. Therefore, to control FHB efficiently, it is necessary to monitor the development of MBC-resistant *Fusarium* populations in China.

A conventional method for detecting the presence of MBC-resistant isolates is by inoculating pathogens isolated from field samples in fungicide-containing media. However, this procedure is

highly time-consuming, labor-intensive, and easily contaminated by bacteria. Several single-nucleotide-polymorphism-based molecular detection techniques have been developed and used in the detection of MBC-resistant isolates. The PIRA-PCR approach was used to detect the resistant isolates harboring F167Y or F200Y (Luo et al. 2009). Allele-specific PCR primers for the detection of resistant strains with F167Y, E198Q, or F200Y mutations were also designed Liu et al. (2010). A point mutation at codon 167 was detected by cycleave PCR (Hou et al. 2011). A simple on-site loop-mediated isothermal amplification method was developed for the specific detection of the F167Y and F200Y genotype (Duan et al. 2014, 2016). Zhang et al. (2016) developed a single-nucleotide-polymorphism-based genotyping assay for simultaneous detection of different carbendazim-resistant genotypes in the FGSC. Compared with the traditional mycelial growth inhibition assay, the above molecular detection techniques are more rapid, specific, and accurate.

There were several studies investigating the frequency of carbendazim resistance of FHB pathogens in China. Liu et al. (2010) and Liu et al. (2014) monitored the frequency of *F. graminearum* resistant isolates from 2008 to 2012 in the main wheat production areas in China. However, in the last 10 years, only regional surveys were reported, such as carbendazim resistance of *F. graminearum* from 2016 to 2018 in Henan Province (Liu et al. 2019). Along with the change of fungicides, the current status of MBC resistance of FHB pathogens on a national scale is largely unknown. Hence, the objectives of this study were (i) to use allele-specific PCR to detect

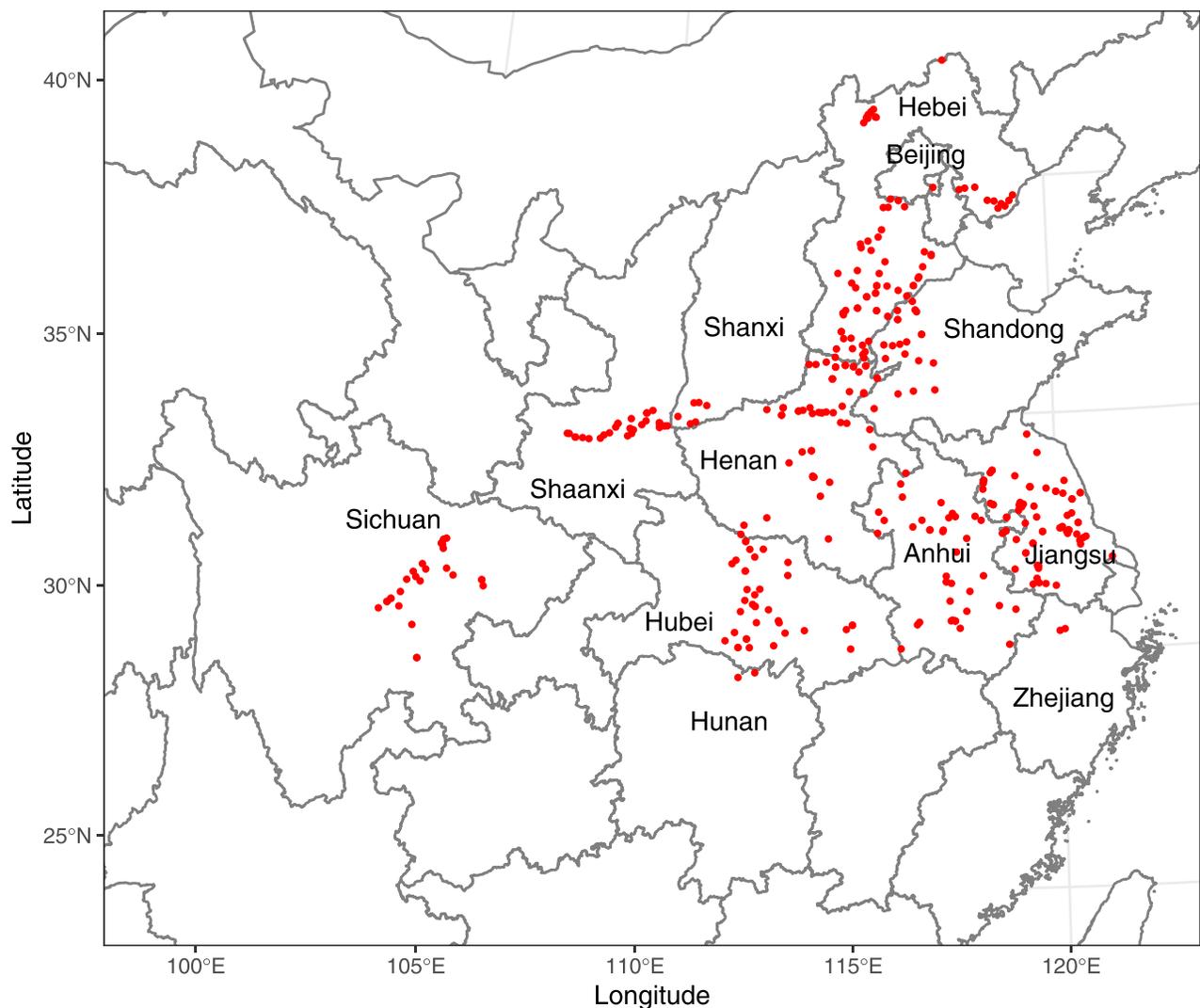


Fig. 1. Sampling sites map of *Fusarium* head blight collection in 11 provinces of China.

genotypes of carbendazim resistance among FGSC isolates collected from main wheat-growing areas in 2018, 2019, and 2020 in major wheat-growing regions in China; (ii) to clarify the resistance frequency of FGSC to carbendazim; (iii) to define the dynamics of carbendazim-resistance frequency in different provinces and years; and (iv) to determine the predominant resistant species and mutation types to carbendazim.

Materials and Methods

Fungal isolates

From 2018 to 2020, diseased wheat spikes with mature kernels were collected from 378 sampling sites in 11 provinces of China, including Hubei, Anhui, Jiangsu, Zhejiang, Hunan, Hebei, Henan, Shandong, Shanxi, Shaanxi, and Sichuan Provinces (Fig. 1). A total of 4,701 FGSC isolates were obtained by tissue isolation culture and single-spore separation (Zhang et al. 2008). Genomic DNA of FGSC isolates was extracted by using the SP Plant DNA Kit (Omega Biotek, Norcross, GA, U.S.A.) according to the manufacturer's instructions.

Species identification

The primers Fg16F and Fg16R (Nicholson et al. 1998) were used to identify the FGSC species *F. graminearum* (lineage 7) or *F. asiaticum* (lineage 6) when the expected 400- or 500-bp fragments were amplified, respectively. To validate the identification results of PCR amplification (Table 1), 30 strains were randomly selected to amplify a partial translation elongation factor (*TEF-1a*) gene sequence using primers EF1 and EF2 (Supplementary Table S1) (O'Donnell et al. 1998). The PCR products were sequenced by Shanghai Sangon Biological Engineering Co., Ltd. (Beijing, China) and compared in GenBank for species identification.

Detection of resistant isolates harboring F167Y, E198Q, and F200Y mutations

PCR primers Tub2-F, 167-RB, 198-RB, and 200-RB (Liu et al. 2010; Table 1) were used to amplify the partial sequence of *Tub2* gene. Three samples harboring the F167Y, E198Q, and F200Y mutations, respectively, were used as positive controls, while sterile distilled water was used as a negative control. As the resistant isolates with E198Q and F200Y mutations had similar amplified fragments, the resistant isolates carrying F167Y and F200Y mutations were identified with the same PCR reaction system, while the genotype of E198Q mutation was identified by a single PCR reaction system. The 398-, 494-, and 498-bp fragments were amplified from point mutation types of F167Y, E198Q, and F200Y in the *Tub2* gene, respectively, and could not be amplified in the sensitive strains. PCR was performed with the following parameters: preheating at 95°C for 3 min; followed by 35 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s; a final extension at 72°C for 5 min; and holding at 4°C. Finally, PCR products were separated by electrophoresis on 1.5% agarose gels.

Statistical analysis

Statistical analysis was performed using R software. Fisher's exact test was adopted to analyze the significant differences between carbendazim-resistant populations of *F. asiaticum* and *F. graminearum*.

Results

Overall *Fusarium* species

Sequencing the *TEF-1a* gene amplicon of 30 strains demonstrated that identification with primers EF1 and EF2 was consistent with amplification results of primers Fg16F and Fg16R, indicating that primers Fg16F and Fg16R could be used to identify the two *Fusarium* species. Sequencing information for 30 strains is shown in Supplementary Table S1. In total, 4,701 isolates were collected from all 378 sampling sites from 11 provinces (Fig. 1). Overall, *F. graminearum* was most abundant (59.33%, $n = 2,789$), followed by *F. asiaticum* (40.67%, $n = 1,912$; Table 2). In addition, *F. graminearum* was the main species in Shaanxi, Shanxi, Henan, Hebei, and Shandong in the Huanghuai Region, whereas *F. asiaticum* was the most frequent in the remaining provinces located in the Yangtze River Basin Region (Table 2).

Overall carbendazim resistance

A total of 348 isolates out of the 4,701 isolates were resistant to carbendazim, with an overall 7.40% of resistant isolates. Among 348 resistant isolates, the number of isolates-resistant with genotype of F167Y, E198Q, and F200Y were 276 (79.31%), 57 (16.38%), and 15 (4.31%), respectively (Table 2). Among the 3 years, the frequency of F167Y showed a decreasing trend from 84.5 to 76.5%, whereas E198Q increased from 11.3 to 19.1%. The F200Y type was at a similar level over the 3 years (Supplementary Fig. S1).

Carbendazim resistance related to species

Among the 2,789 *F. graminearum* isolates, 20 isolates were resistant to carbendazim, and of 1,912 *F. asiaticum* isolates, 328 were carbendazim resistant. On the national level, the frequency of carbendazim resistance in *F. asiaticum* (17.15%) was significantly higher ($P < 0.001$) than in *F. graminearum* (0.72%). Similar results were also observed at the provincial level: the carbendazim-resistance frequency of *F. asiaticum* was higher than *F. graminearum* in all provinces except Shanxi, where no carbendazim-resistant isolate was identified in either species. Fisher's exact test showed significantly higher frequency of carbendazim resistance of *F. asiaticum* in five provinces including Jiangsu and Anhui in the Yangtze River Basin Region and Hebei, Henan, and Shandong in the Huanghuai Region. In the remaining provinces, no significant differences were detected on the carbendazim-resistance frequency between the two species (Fig. 2). All three mutation types were present in both species: 79.6% F167Y, 16.2% E198Q, and 4.2% F200Y mutants were found in carbendazim-resistant *F. asiaticum*, while the frequency in carbendazim-resistant *F. graminearum* was 75.0, 2.0, and 0.5%, respectively. Fisher's exact test showed that there was no significant difference in the composition of mutation types between the carbendazim-resistant *F. asiaticum* and *F. graminearum* ($P = 0.64$).

Carbendazim resistance in different provinces

Carbendazim-resistant isolates were detected in almost all sampling provinces except Shanxi, where no carbendazim-resistant isolate was detected. The resistance frequency in different geographical regions was significantly different. The highest resistance frequency

Table 1. List of primers used in this study

Primer	Sequence (5'–3')	Description	Reference
Fg16F	CTCCGGATATGTTGCGTCAA	For specific detection of <i>Fusarium</i> species	Nicholson et al. (1998)
Fg16R	GGTAGGTATCCGACATGGCAA		
EF-1	ATGGGTAAGGAGGACAAGAC	For amplification of the partial <i>TEF-1a</i> sequence	O'Donnell et al. (1998)
EF-2	GGAAGTACCAGTGATCATGTT		
Tub2-F	TTGTAATTGCAAATTTCAACGTG	For specific detection of MBC-resistant isolates	Liu et al. (2010)
167-RB	GCGAGGGCATAACGGATT		
198-RB	CGTTATCGATACAGAAGGTCAG		
200-RB	GCCTCGTTATCGATACATT		

was observed in Jiangsu (41.12%), followed by Anhui (20.56%) and Zhejiang (8.57%). In other provinces, the resistance frequency was below 2% (Table 2). Among the carbendazim-resistant isolates in Hubei, Anhui, Jiangsu, Zhejiang, Hunan, Hebei, Henan, Shandong, Shanxi, Shaanxi, and Sichuan Provinces, the number of F167Y mutations was four, 67 (76.14%), 186 (81.94%), three, one, three, two, five, zero, four, and one, respectively, and the number of E198Q mutations was one, 19 (21.59%), 30 (13.22%), zero, one, two, three, one, zero, zero, and zero, respectively. Of the F200Y genotype, 11 of 13 isolates were detected in Jiangsu Province, and the other two were detected in the Anhui and Hubei Provinces, respectively.

In China, the epidemic severity of FHB was significantly different from 2018 to 2020. A nationwide epidemic of FHB happened in 2018, with the diseased acreage of 7.1 million hectares, while the disease was mild in 2019 and 2020, with the acreage of 2.3 and 2.0 million hectares, respectively. To investigate the dynamic of the resistance frequency from 2018 to 2020, we excluded the sampling size lower than 50 isolates in 1 year to avoid the bias. After that, there are seven provinces which have data from more than 2 years. In Shaanxi, carbendazim-resistant isolates were first detected in 2020,

so we cannot conclude about the trend of development of resistant populations in this province. In four of the other six provinces (Anhui, Hebei, Henan, and Sichuan), a clear decrease of resistance frequency was observed, which was associated with the acreage of FHB in these provinces (Fig. 3). In Jiangsu, the carbendazim-resistance frequency was at a high level over the 3 years. It decreased from 40.83% in 2018 to 30.00% in 2019, which showed a similar trend of the acreage of FHB, but the highest frequency (47.04%) was observed in 2020, when the FHB was mild. In Hubei Province, the resistance frequency went up from 0.34% in 2018 to 2.46% in 2019 (Fig. 3, Table 2).

Discussion

In this study, we conducted a large-scale survey of carbendazim-resistance among 4,701 FGSC isolates collected at 378 sampling sites from 11 provinces covering almost all wheat-growing areas in China from 2018 to 2020. We found 348 isolates (7.40%) were resistant to carbendazim. A total of five mutation types of carbendazim-resistant isolates were reported in literature, and F167Y

Table 2. Isolated *Fusarium graminearum* species complex, carbendazim-resistant isolates, and resistance frequency distribution in 11 provinces during 2018 to 2020

Provinces	Years	Isolates			CarR ^a isolates						Resistance frequency (%)
		Total	FG ^b	FA ^c	Total (CarR)	Mutation types			FG	FA	
						167	198	200			
Sichuan	2018	217	117	100	1	1	0	0	0	1	0.46
	2019	34	15	19	0	0	0	0	0	0	0
	2020	226	84	142	0	0	0	0	0	0	0
	Total	477	216	261	1	1	0	0	0	1	0.21
Hubei	2018	296	35	261	1	1	0	0	0	1	0.34
	2019	244	30	214	6	3	1	2	0	6	2.46
	2020	4	0	4	0	0	0	0	0	0	0
	Total	544	65	479	7	4	1	2	0	7	1.29
Hunan	2019	192	4	188	2	1	1	0	0	2	1.04
	Total	192	4	188	2	1	1	0	0	2	1.04
Anhui	2018	65	27	38	20	17	3	0	0	20	30.77
	2019	160	31	129	30	22	8	0	6	24	18.75
	2020	203	24	179	38	28	8	2	3	35	18.72
	Total	428	82	346	88	67	19	2	9	79	20.56
Jiangsu	2018	169	29	140	69	60	5	4	0	69	40.83
	2019	130	10	120	39	35	2	2	0	39	30.00
	2020	253	26	227	119	91	23	5	4	115	47.04
	Total	552	65	487	227	186	30	11	4	223	41.12
Zhejiang	2020	35	1	34	3	3	0	0	0	3	8.57
	Total	35	1	34	3	3	0	0	0	3	8.57
Shaanxi	2018	236	236	0	0	0	0	0	0	0	0
	2019	53	41	12	0	0	0	0	0	0	0
	2020	53	46	7	4	4	0	0	3	1	7.55
	Total	342	323	19	4	4	0	0	3	1	1.17
Shanxi	2019	44	32	12	0	0	0	0	0	0	0
	2020	28	19	9	0	0	0	0	0	0	0
	Total	72	51	21	0	0	0	0	0	0	0
Henan	2018	159	126	33	4	1	3	0	0	4	2.51
	2019	238	230	8	1	1	0	0	1	0	0.42
	2020	335	327	8	0	0	0	0	0	0	0
	Total	732	683	49	5	2	3	0	1	4	0.68
Hebei	2018	295	292	3	2	2	0	0	0	2	0.68
	2019	24	22	2	2	0	2	0	2	0	8.33
	2020	705	692	13	1	1	0	0	1	0	0.14
	Total	1,024	1,006	18	5	3	2	0	3	2	0.49
Shandong	2018	27	27	0	0	0	0	0	0	0	0
	2020	276	266	10	6	5	1	0	0	6	2.17
	Total	303	293	10	6	5	1	0	0	6	1.98
Total		4,701	2,789	1,912	348	276	57	15	20	328	7.4

^a CarR = carbendazim resistant.

^b FG = *F. graminearum*.

^c FA = *F. asiaticum*.

was the most common genotype, followed by E198Q (Chen et al. 2009; Liu et al. 2010). In agreement with these observations, among the 348 carbendazim-resistant isolates in this study, the majority of isolates (276, 79.31%) harbored the F167Y mutation, while 57 isolates (16.38%) carry the E198Q mutation, and 15 isolates (4.31%) contained the F200Y mutation. Isolates with the F167Y and E198Q mutations accounted for 95.69% of the resistant populations, which was consistent with the finding that F167Y and E198Q are the predominant mutation types demonstrated Liu et al. (2014).

F. asiaticum and *F. graminearum* were reported as the predominant species in China (Zhang et al. 2012). Both species were included in this study, 2,789 of 4,701 isolates (59.33%) were identified as *F. graminearum*, and the remaining 40.67% of isolates were *F. asiaticum*. There are several previous reports about the carbendazim resistance of FHB pathogens in China (Chen et al. 2009; Liu et al. 2010; Wang et al. 2002; Yin et al. 2009), but the two species were not distinguished in most of these studies. Dai (2013) identified 167 *F. graminearum* isolates and 168 *F. asiaticum* isolates from seven provinces in China: 19.6% of the *F. asiaticum* isolates were

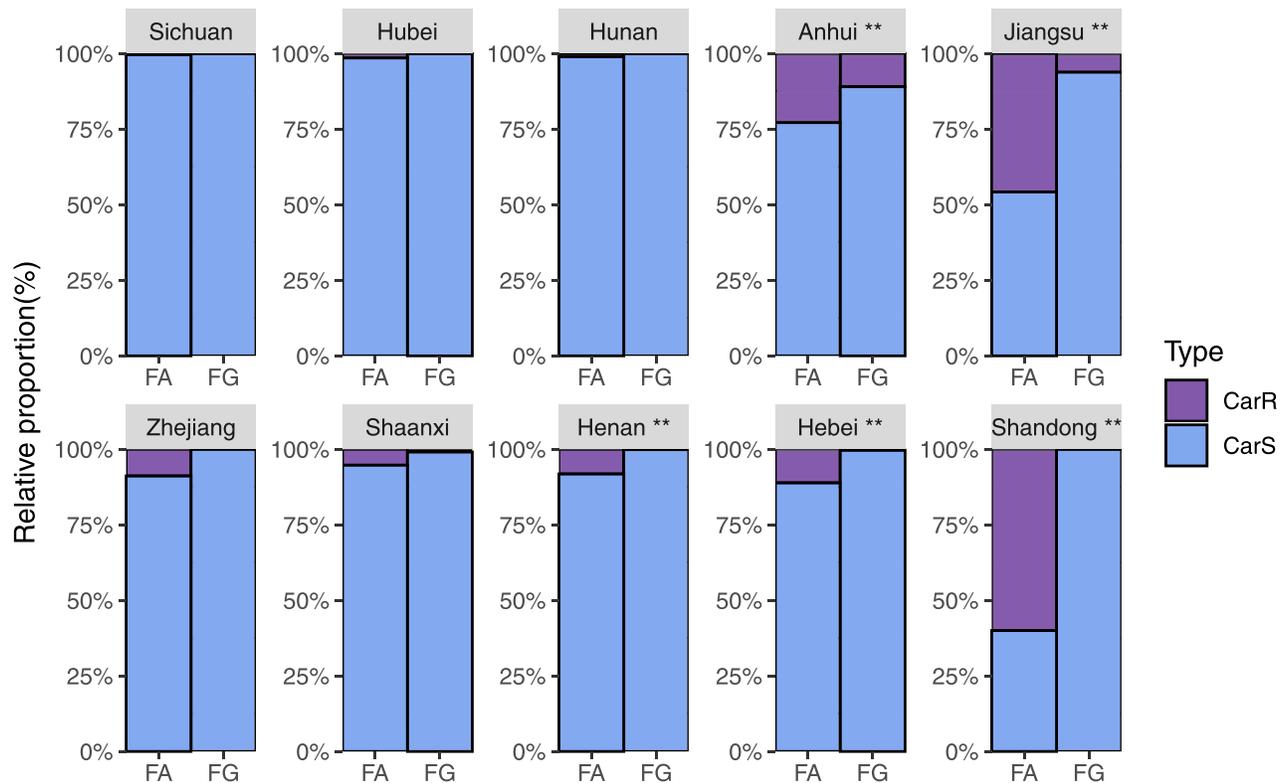


Fig. 2. Relative proportion of carbendazim-resistant isolates and sensitive isolates in *Fusarium asiaticum* and *F. graminearum*, respectively, in 10 provinces. CarR = carbendazim-resistant isolates; CarS = carbendazim sensitive isolates; FA = *F. asiaticum*; FG = *F. graminearum*; and ** = a significant difference was detected on the carbendazim-resistance frequency between *F. asiaticum* and *F. graminearum*. They were significantly related at $P = 0.05$.

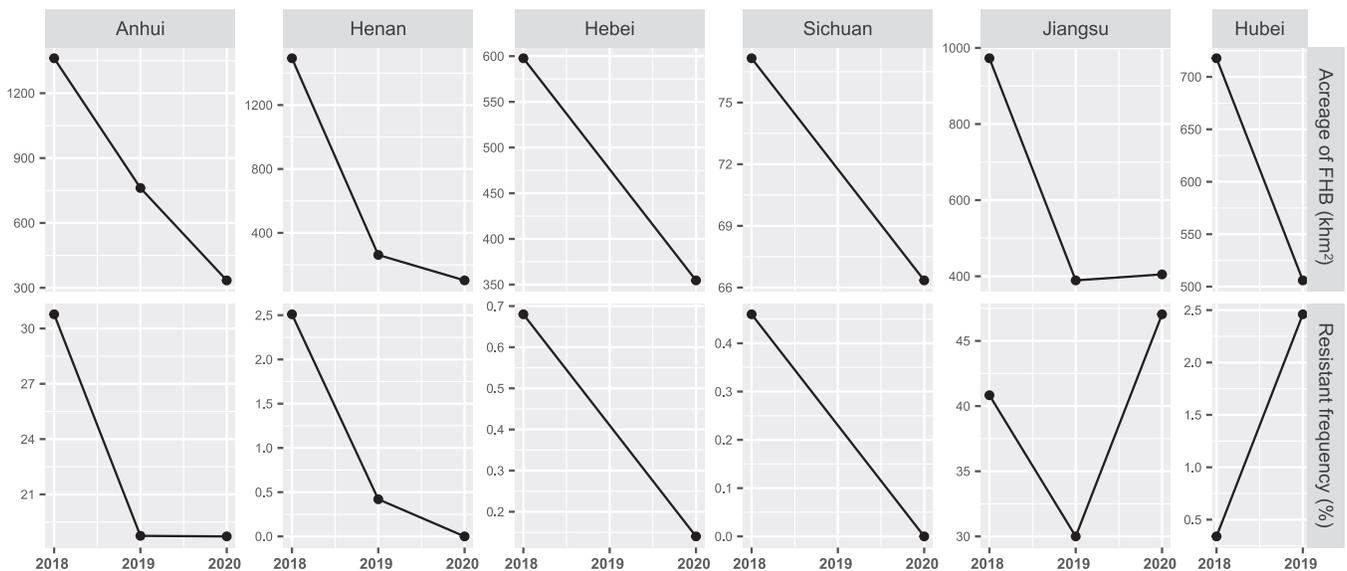


Fig. 3. The dynamics of the acreage of *Fusarium* head blight and carbendazim-resistant frequency in six provinces, which had a number of isolates more than 50 in 1 year among 2018 to 2020.

resistant to carbendazim, while no resistant isolates were detected in *F. graminearum*. This study is the first report of carbendazim resistance in *F. graminearum* in five provinces. However, the frequency of carbendazim resistance was unbalanced between the two species. Significantly higher ($P < 0.001$) carbendazim-resistance frequency of *F. asiaticum* (17.15%) was observed at the national scale compared to *F. graminearum* (0.72%). A previous study suggested that in the lower reaches of the Yangtze River, where *F. asiaticum* is the dominant species with a long history and high dosage of carbendazim usage, only *F. asiaticum* resistance to carbendazim was observed. The authors thought the larger population size made *F. asiaticum* more likely to develop resistant genotypes against carbendazim (Zhang et al. 2016). However, in this study, the carbendazim-resistance frequency of *F. asiaticum* was higher than *F. graminearum* in all provinces except Shanxi, where no carbendazim-resistant isolate was found, including provinces where *F. graminearum* was the dominant population (e.g., Henan, Hebei and Shandong Provinces). Also in five provinces, the resistance frequency of *F. asiaticum* was significantly higher than *F. graminearum* at $P < 0.05$ (Fig. 2). The results indicated that carbendazim resistance is not only related to the population size, but frequencies also vary between the two species. In this study, we found that *F. asiaticum* isolates are more likely to develop resistance to carbendazim than *F. graminearum*, but there is no preference of the resistant mutation types between the two species.

High carbendazim-resistance frequency in the middle and lower reaches of the Yangtze River in China, including Jiangsu, Zhejiang, and Anhui Provinces, has been reported in the past few years. The long history of continued use of carbendazim and other benzimidazole fungicides in these regions was thought to be the main reason (Chen et al. 2009; Liu et al. 2010, 2014; Wang et al. 2002; Yin et al. 2009). High resistance frequency decreased the control efficiency of carbendazim, so the government did not recommend using benzimidazole fungicides for FHB control in the guideline in these regions, and fungicides without cross-resistance like tebuconazole and phenamacril were used instead in the past 10 years. Now in these areas, the usage of MBC has been greatly reduced. However, in this study, we found the carbendazim-resistance frequency in this area still maintains a high level from 2018 to 2020; the resistance frequency in Jiangsu (41.12%), Anhui (20.56%), and Zhejiang (8.57%) were even higher than the results from previous studies, with 33.3% in Jiangsu in 2012 (Liu et al. 2014), 8.2% in Anhui from 2010 to 2012 (Chen et al. 2015), and 4.4% in Zhejiang in 2010 (Liu et al. 2014). This may be due to the high fitness of the carbendazim-resistant population. Fungicide-resistant pathogens usually have a fitness cost, but Y.-J. Zhang et al. (2009) detected higher amounts of DNA and trichothecene production in wheat heads infected by carbendazim-resistant *F. graminearum* isolates, and the FHB incidence was identical between resistant and sensitive populations. Wang et al. (2021) evaluated the effects of various temperatures on biological fitness of carbendazim-resistant and -sensitive isolates; conidia of F167Y displayed strong adaptability to low temperatures; and higher aurofusarin and trichothecene production was detected in resistant isolates. Therefore, within a short period, reducing the dosage and usage of MBCs at such a high level of resistance frequency shows a minor effect on reducing the carbendazim-resistant populations.

Except the middle and lower reaches of the Yangtze River Region, FHB is generally mild in other wheat-producing areas, especially in Northern China, so farmers applied MBC less frequently. From 2008 to 2012, Liu et al. (2010) and Liu et al. (2014) monitored the carbendazim resistance in Hebei, Shandong, Shanxi, and Sichuan Provinces and found no carbendazim-resistant isolates in these regions. In 2010, low carbendazim-resistance frequency was detected in Shandong (2.00%), Hubei (1.39%), and Henan (0.43%) Provinces (Shao et al. 2011). Liu et al. 2019 screened 1,132 isolates in Henan Province from 2016 to 2018; the carbendazim-resistance frequency varied from 0.43 to 4.30%. In this study, we also detected low carbendazim-resistance frequency in 2018 (2.51%) and 2019 (0.42%), while in 2020, no carbendazim-resistant strains were found

in Henan Province. Also, in this study, we firstly detected carbendazim-resistant isolates in Hebei, Shaanxi, Sichuan, and Hunan Provinces. This was especially true in Hebei, where resistant isolates were identified in all 3 years. Overall, carbendazim-resistant isolates were detected in 10 of 11 provinces in this investigation, indicating the carbendazim-resistant population not only occurred in the traditional FHB epidemic regions like Jiangsu and Anhui but have also developed throughout the wheat-growing areas in China.

When severe plant disease outbreaks occur, growers are likely to use fungicides more frequently, which may lead to high selection pressure for the resistant pathogens. In China, a nationwide epidemic of FHB happened in 2018, while the disease was mild in 2019 and 2020. In this study, we found the carbendazim-resistance frequency associated with the epidemic of FHB in most provinces (Fig. 3). This suggested rotation of fungicides with different modes of action is important for avoiding a rapid increase of the resistant populations in epidemic years of FHB. It is worth noting that the frequency of carbendazim resistance in Jiangsu Province persisted at a high level in the 3 years, and the highest resistance frequency was 47.04% in 2020 when the disease was quite mild. This may be due to the carbendazim-resistant isolates in Jiangsu Province having been developed into a stable population, and the selection pressure has little effect on them. Also, we found the dynamics of the frequency of resistant mutation types was correlated with the epidemic of the disease. The highest frequency of F167Y was observed in the epidemic year 2018 and decreased in the following 2 years, while E198Q showed an opposite trend. It is reported that *F. graminearum* harboring the F167Y and F200Y mutations were moderately resistant to MBC fungicides and that the E198Q type mutant showed low resistance (Chen et al. 2009; Liu et al. 2014). So, application of high-dosage carbendazim in the epidemic year would be a strong selection pressure for higher resistant strains. We speculate the moderately resistant F200Y mutants have some fitness defects that caused its low frequency in all the 3 years.

In summary, to prevent the further development of resistant populations of carbendazim, it is recommended to apply fungicides with different mechanisms such as tebuconazole and phenamacril instead of carbendazim in regions showing high levels of MBC resistance, particularly in Jiangsu and Anhui Provinces. In regions where the frequency of resistance is low or has not yet been detected, MBC could be applied in mixes or in rotation with different mechanisms of fungicides.

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