Integrated Use of Pyraclostrobin and Epoxiconazole for the Control of Fusarium Head Blight of Wheat in Anhui Province of China

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Abstract

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Fusarium asiaticum and *F. graminearum* are the primary causal agents of Fusarium head blight (FHB) of wheat in China. Carbendazim (a benzimidazole fungicide, MBC), has been extensively used for the control of FHB, resulting in severe MBC resistance in China. This article presents the baseline sensitivity of *F. asiaticum* and *F. graminearum* isolates from Anhui Province of China to fungicides pyraclostrobin (a quinone outside inhibitor) and epoxiconazole (a sterol demethylation inhibitor). In the presence of salicylhydroxamic acid, the 50% effective concentration (EC₅₀) values for pyraclostrobin in inhibiting mycelial growth of the 126 *F. asiaticum* isolates and 63 *F. graminearum* isolates were 0.012 to 0.135 µg/ml and 0.010 to 0.105 µg/ml, and the EC₅₀ values for pyraclostrobin in inhibiting conidium germination of the *F. asiaticum* and *F. graminearum* populations were 0.047 to 0.291 and 0.042 to 0.255 µg/ml, respectively. The EC₅₀ values for epoxiconazole in inhibiting mycelial growth of the *F. asiaticum* and

F. graminearum populations were 0.12 to 0.95 and 0.16 to 0.93 µg/ml, respectively. All of the baseline sensitivity curves were unimodal. This study also suggested that there was no cross-resistance between MBC and pyraclostrobin or epoxiconazole. In the protective and curative tests, pyraclostrobin and epoxiconazole applied at 200 and 300 µg/ml exhibited over 75% protective and curative control efficacy in all treatments. In field trials, both pyraclostrobin and epoxiconazole at 225 g a.i./ha provided over 80% efficacy in 2010 and 2011 at both sites where MBC resistance occurred, suggesting excellent activity against FHB. Interestingly, integrated use of pyraclostrobin + epoxiconazole applied at 150 + 150 g a.i./ha provided over 85% efficacy at both sites in 2010 and 2011. Pyraclostrobin and epoxiconazole should be good alternatives to MBC for the control of FHB, and integrated use of these two fungicides might achieve greater efficacy.

Fusarium head blight (FHB) is a devastating disease of cereals which can cause huge losses in epidemic years, becoming a major threat to the wheat and barley industry worldwide, and is responsible for extensive damage to wheat in humid and semihumid regions of the world (2). In China, FHB generally occurs in the middle and lower reaches of the Yangtze River, the Huaihe River Valley, and the eastern coastal region (6). However, an increase in the disease in the northern and western wheat-growing areas of China has occurred in the last decade (6). FHB not only results in 5 to 15% loss of grain yield in years of moderate epidemic and up to 40% in years of severe epidemic but also causes a decrease in grain quality because of the production of mycotoxins such as deoxynivalenol, nivalenol, and zearalenone (3,20,22). Although a number of *Fusarium* spp. can cause FHB, the primary etiological agents of this disease belong to the *Fusarium graminearum* species complex of B-tricothecene toxin producers, which contain at least 11 phylogenetic species, including *F. acaciae-mearnsii*, *F. asiaticum*, *F. austroamericanum*, *F. boothii*, *F. brasiliense*, *F. cortaderiae*, *F. gerlachii*, *F. graminearum*, *F. meridionale*, *F. mesoamericanum*, and *F. vorosii* (18,21,25,27). Different *Fusarium* spp. may be associated with FHB in different regions of the world because of different cropping systems and climatic conditions (17,26). In China, *F. asiaticum* and *F. graminearum* are the major causal agents of FHB, although other *Fusarium* spp. (such as *F. cul-*

morum, *F. avenaceum*, and *F. tricinctum*) were also isolated occasionally from infected wheat heads (27). In a previous study, in total, 299 isolates collected from various epidemic regions of China were analyzed and the results showed that 231 isolates (77.3%) belonged to *F. asiaticum* and the remaining 68 isolates were *F. graminearum* (31). These two species are also predominant in the populations of FHB pathogens in Japan (23).

To control FHB, fungicide sprays have been used during the last few decades because only a few cultivars were resistant to *F. graminearum* isolates. In the past 30 years, carbendazim (MBC) has been the major fungicide for FHB control in China, especially in Anhui Province. However, the effectiveness of MBC has been threatened by the emergence of resistant pathogen populations in the field (4,7,8).

Fungicide application is most effective at wheat anthesis, the time of primary infection. Applications of fungicides with a mode of action alternative to MBC would be necessary for management of MBC resistance in FHB. The sterol demethylation inhibitor (DMI) fungicides inhibit the sterol C-14 α -demethylation of 24-methylenedihydrolanosterol, a precursor of ergosterol in fungi (27). DMIs comprise a large number of commercially successful fungicides and were first used in agriculture in the 1970s. Many field studies showed that DMI fungicides such as tebuconazole and epoxiconazole are effective against *Fusarium* spp. (14,15). Pyraclostrobin, belonging to a relatively new group of fungicides, quinone outside inhibitors (QoIs), had been introduced for use on fruit and vegetables in China. DMIs and QoIs have a broad spectrum of activity against a large number of fungal pathogens on various crops. Both of the fungicide classes mentioned have modes of action different from that of MBC. No fieldwork has been reported evaluating the application of the mixture of pyraclostrobin and epoxiconazole for the control of FHB.

The objectives of this study were to (i) establish the baseline sensitivity of *F. asiaticum* and *F. graminearum* isolates to pyraclostrobin and epoxiconazole using the field isolates from Anhui

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Province of China, (ii) determine the cross-resistance patterns between MBC and pyraclostrobin or epoxiconazole, (iii) investigate the protective and curative activity of the two fungicides against FHB, and (iv) test the efficacy of pyraclostrobin and epoxiconazole in controlling this disease.

Materials and Methods

Fungicides and media. Pyraclostrobin (25% Cabrio, EC) and epoxiconazole (12.5% Opus, SC) were provided by BASF Co., China and were dissolved in sterile water to 1.0 mg/ml for the stock solution. Potato sucrose agar (PSA) was used in isolating and storing the tested isolates. Mung bean broth (MBB), 1 liter of H₂O amended with 50 g of mung bean, was used in producing conidia. Water agar (WA) medium was consisted of 20 g of agar per liter of water.

Isolation and identification of *F. asiaticum* and *F. graminearum*. Wheat samples were collected from commercial wheat fields in Anhui Province, where pyraclostrobin and epoxiconazole had likely never been used. The climate in the regions is humid and subtropical. The samples were collected during the harvest season of 2010 and 2011. Identification of *F. asiaticum* and *F. graminearum* species is described by Suga et al (23). In total, 126 and 63 single-spore isolates of *F. asiaticum* and *F. graminearum*, respectively, were purified and collected from the samples. Among these isolates, 25 *F. asiaticum* isolates were confirmed with resistance to MBC, and all the *F. graminearum* isolates were sensitive to MBC based on the previous study (28).

Determination of sensitivity of growth to pyraclostrobin and epoxiconazole. To evaluate the sensitivity of mycelial growth to pyraclostrobin and epoxiconazole, the 126 *F. asiaticum* isolates and 63 *F. graminearum* isolates were used to determine the concentration of the fungicide causing a 50% reduction in the growth rate compared with an unamended control (EC₅₀ values). Autoclaved PSA was amended with pyraclostrobin or epoxiconazole to obtain final concentrations of 0, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 µg/ml. Sensitivity tests for pyraclostrobin were conducted in the presence of salicylhydroxamic acid (SHAM), a specific inhibitor of alternative oxidase (29,30). The final concentration of SHAM (Sigma, China) was 100 µg/ml for each fungicide treatment. An inverted mycelial plug (5 mm in diameter), cut from the edge of a 3-day-old colony, was transferred to 9-cm petri dishes containing the amended media. Three replicates per concentration were used and all the tests were repeated twice. The mycelial growth was measured after 3 days of incubation at 25°C in a growth chamber (12-h photoperiod). Two perpendicular diameters of each fungal colony were measured and averaged (the diameter of the plug was subtracted). The EC₅₀ values were determined by probit analysis (5).

Determination of sensitivity of conidium germination to pyraclostrobin. Pyraclostrobin was added to WA after sterilization to produce the concentrations of 0, 0.01, 0.02, 0.04, 0.1, 0.2, and 0.8 µg/ml. Sensitivity tests were also conducted in the presence of SHAM at a concentration of 100 µg/ml. Conidium suspensions containing 10⁵ conidia/ml derived from shake culture at 150 rpm and 25°C for 5 to 7 days in MBB were poured onto the surface of WA plates. Conidia were allowed to germinate at 25°C for 8 h. Germination was quantified at three sites by counting 100 conidia per site. A conidium was scored germinated if the germ tube had reached at least half the length of the conidium. Three plates for each concentration were used, and the experiment was performed twice. For each isolate, a linear regression of the percent inhibition relative to the control of spore germination versus the log₁₀ transformation for each of the seven concentrations of pyraclostrobin was obtained. EC₅₀, which was the fungicide concentration that resulted in 50% spore germination inhibition, was calculated with the regression equation for each isolate as described previously (29,30).

Cross-resistance patterns between MBC and pyraclostrobin or epoxiconazole. Sensitivity to MBC of the isolates used in this study was tested through a discriminatory dose test (7,28). Twelve

F. asiaticum isolates with differential sensitivity to MBC were arbitrarily chosen for the cross-resistance analysis. The 12 isolates which were sensitive or resistant to MBC were compared regarding their sensitivity to pyraclostrobin and epoxiconazole (EC₅₀) to determine cross-resistance between MBC and pyraclostrobin or epoxiconazole, as described previously (30). This experiment was performed twice.

Evaluation of protective and curative activity of pyraclostrobin and epoxiconazole against FHB. The *F. asiaticum* isolate GZ01, which was resistant to MBC, with stable pathogenicity on wheat (*unpublished data*), was used for inoculation on wheat plants in the protective and curative activity tests. To prepare inoculums of conidium suspension, the actively growing mycelial plugs from PSA plates were transferred to MBB for shake culture (150 rpm) at 25°C for 7 days, and the conidium suspensions were adjusted to 10⁶ conidia/ml by centrifugation. The experiment was performed in a wheat experimental field which was known to be naturally infested with *F. graminearum* complex, in Anhui Academy of Agricultural Sciences. The wheat field was divided into 24 plots and each plot was 0.5 by 0.5 m² and included at least 40 wheat plants. Each of the plots was separated by a 30-cm interval with untreated wheat plants.

To evaluate the protective and curative activity of pyraclostrobin and epoxiconazole, wheat plants (Huamai number 1, a susceptible wheat cultivar) were inoculated by injecting 10 µl of conidium suspension into a flowering grain in the middle of the spike. A minimum of 40 spikes per treatment were inoculated. The six treatments were: treatments 1 and 2, pyraclostrobin EC (25% Cabrio) commercial formulation applied at 200 and 300 µg/ml; treatments 3 and 4, epoxiconazole SC (12.5% Opus) commercial formulation applied at 200 and 300 µg/ml; treatment 5, 50% MBC WG commercial formulation applied at 750 µg/ml; and treatment 6, no treatment control (CK). Treatments were replicated four times and the randomized completed block design was laid out for the arrangement of the 24 plots. The experiment was repeated twice. No other fungicides were applied to the experimental plots. Other crop needs, such as fertilizers, used standard farm products. The wheat plants were at anthesis growth stage when sprayed with fungicides. Treatments were applied 24, 48h and 72 h before (protective treatments) and after (curative treatments) inoculation. Visual disease assessment was made 14 days after the inoculation. The severity of scabbed wheat heads was assessed using a scale as described by previous studies (13,24), with eight evaluation classes (0, 2, 5, 10, 25, 50, 75, and 90% area infected) and by applying the following formula: number of heads in each class × each evaluation class/total number of heads. Efficacy percentage was determined by applying Abbott's formula: (% infection control – % treated infection)/% infection control × 100 (13). The experiment was performed twice.

Field trial for pyraclostrobin and epoxiconazole in controlling FHB. Winter wheat 'Yannong 19', a cultivar susceptible to FHB, was sown in fields located in Bengbu city and Chuzhou city, Anhui Province, where MBC resistance has been reported, during wheat growth seasons of 2010 and 2011. The two fields were known to be naturally infested with *F. graminearum* isolates. Each of the fields was divided into 28 plots and each plot had an area of 30 m² (5 by 6 m). Each of the plots was separated by a 50-cm interval with untreated wheat plants. The seven treatments were: treatments 1 and 2, pyraclostrobin EC (25% Cabrio) commercial formulation applied at 150 and 225 g a.i. ha⁻¹; treatments 3 and 4, epoxiconazole SC (12.5% Opus) commercial formulation applied at 150 and 225 g a.i. ha⁻¹; treatment 5, 50% MBC WG commercial formulation applied at 562.5 g a.i. ha⁻¹; treatment 6, a blend of pyraclostrobin EC (25% Cabrio) + epoxiconazole SC (12.5% Opus) commercial formulations applied at 150 + 150 g a.i. ha⁻¹; and treatment 7, no treatment control (CK). The volume of water delivered was 750 liters/ha. The cultivar was planted following the same agronomic practice. Fungicides were applied upon initiation of flowering at Feekes growth stage (GS) 10.5.1, anthesis. The sprayer was a Jacto Heavy-Duty HD400 (Agrolex, Singapore) with

a nozzle diameter of 1.0 mm and operating pressure of 4 to 5 kg/cm². Treatment plots were laid out as a randomized complete block design with four replicates. No other fungicides were applied to the experimental plots. All the other crop needs, such as herbicides and fertilizers, used standard farm products. Visual disease assessment was made at least 14 days after fungicide application based on the percentage of ears exhibiting disease symptoms. Efficacies of products for FHB in the field was evaluated based on a 100-head sample collected from each plot. The percentage of diseased heads and the infected area of the head were determined for each plot. The disease severity and fungicide efficacy were reported as previously described (13,24).

Data analysis. Data from repeated experiments were combined for analysis because variances between experiments were homogeneous. All data were processed with the SIGMASTAT Statistical Software Package (version 11; SPSS Science). EC₅₀ values were calculated from the sensitivity tests described above from the fitted regression line of the probit of the percent inhibition plotted against the log-transformed fungicide concentration (6).

Results

Sensitivity of growth to pyraclostrobin and epoxiconazole. The EC₅₀ values for pyraclostrobin in inhibiting mycelial growth of the 126 *F. asiaticum* isolates and 63 *F. graminearum* isolates on PSA plates were 0.012 to 0.135 and 0.010 to 0.105 µg/ml, respectively, with average EC₅₀ values of 0.064 ± 0.028 and 0.052 ± 0.017 µg/ml, respectively (Fig. 1A and B). Both of the two baseline sensitivity curves were unimodal (Fig. 1A and B), representing range-of-variation factors of 11.25 and 10.50 for *F. asiaticum* and *F. graminearum*, respectively.

The EC₅₀ values for epoxiconazole in inhibiting mycelial growth of the *F. asiaticum* and *F. graminearum* populations on PSA plates were 0.12 to 0.95 and 0.16 to 0.93 µg/ml, respectively, with the average EC₅₀ values of 0.46 ± 0.18 and 0.42 ± 0.13 µg/ml, respectively (Fig. 2A and B). Both of the two baseline sensitivity curves were also unimodal (Fig. 2A and B), representing range-of-variation factors of 7.92 and 5.81 for *F. asiaticum* and *F. graminearum*, respectively.

Fig. 1. Frequency distribution of sensitivity of mycelial growth (baseline sensitivity) of A, *Fusarium asiaticum* and B, *F. graminearum* to pyraclostrobin (in the presence of salicylhydroxamic acid).

In the presence of SHAM, pyraclostrobin exhibited stronger inhibitory activity against mycelial growth of *F. asiaticum* isolates and *F. graminearum* than that of epoxiconazole. The sensitivity data can be used as baselines for monitoring any future changes in sensitivity to pyraclostrobin and epoxiconazole by *F. asiaticum* and *F. graminearum* populations. Such baseline data are essential for assessing resistance risk, managing field-resistance, and monitoring resistance levels in the pathogen populations.

Sensitivity of conidium germination to pyraclostrobin. The EC₅₀ values for pyraclostrobin in inhibiting conidium germination on WA plates of the *F. asiaticum* and *F. graminearum* populations were 0.047 to 0.291 and 0.042 to 0.255 µg/ml, respectively, with the average EC₅₀ values of 0.102 ± 0.041 and 0.117 ± 0.055 µg/ml, respectively (Fig. 3A and B). Both of the two baseline sensitivity curves were unimodal (Fig. 3A and B), representing range-of-variation factors of 6.19 and 6.07 for *F. asiaticum* and *F. graminearum*, respectively. These data indicated that conidium germination of *F. asiaticum* and *F. graminearum* was very sensitive to pyraclostrobin. This baseline data would also be essential for monitoring any further insensitivity or resistance to pyraclostrobin in *F. asiaticum* and *F. graminearum* isolates.

Cross-resistance patterns. There was significant difference ($P = 0.05$) in sensitivity to pyraclostrobin or epoxiconazole between MBC-sensitive and -resistant isolates based on the EC₅₀ values (Table 1). However, there was no correlation between this difference in sensitivity to MBC and pyraclostrobin or epoxiconazole. This suggests that there is no cross-resistance between MBC and pyraclostrobin or epoxiconazole.

Protective and curative activity of pyraclostrobin and epoxiconazole against FHB. When the fungicides were applied 1, 3, and 5 days before inoculations, the average control efficacy of pyraclostrobin at 200 or 300 µg/ml was more than 80% in all cases, indicating excellent protective activity against FHB. Moreover, epoxiconazole applied at 200 or 300 µg/ml provided 78.2 to 89.8% protective control efficacy (Table 2). Unfortunately, MBC applied at 750 µg/ml provided significantly less protective control efficacy than pyraclostrobin or epoxiconazole in corresponding treatments (Table 2).

Also, pyraclostrobin applied 1 and 3 days after inoculations at 200 or 300 µg/ml exhibited over 80% curative control efficacy

Fig. 2. Frequency distribution of sensitivity of mycelial growth (baseline sensitivity) of A, *Fusarium asiaticum* and B, *F. graminearum* to epoxiconazole.

against FHB but less than 80% curative control efficacy when applied 5 days after inoculations (Table 2). However, in all cases, epoxiconazole applied at 200 or 300 µg/ml provided excellent curative control efficacy of over 80% (Table 2). Worse still, MBC applied at 750 µg/ml exhibited less than 70% curative control efficacy in each treatment (Table 2).

Control efficacy of pyraclostrobin and epoxiconazole in wheat fields. The incidence of FHB at both sites was correlated with moisture period during the flowering stage. In the two sites, both pyraclostrobin and epoxiconazole at 225 g a.i./ha provided over 80% control efficacy in 2010 and 2011, suggesting excellent activity against FHB (Table 3). Also, control efficacy of pyraclostrobin and epoxiconazole at 150 g a.i./ha at both sites was greater than 70%, which was significantly higher than that of MBC applied at 562.5 g a.i./ha (Table 3). Interestingly, integrated use of pyraclostrobin + epoxiconazole applied at 150 + 150 g a.i./ha provided over 85% control efficacy at both sites in 2010 and 2011,

which was significantly better than those of other treatments in the field trials (Table 3).

Discussion

Application of fungicide for managing FHB has been relied upon over the last few decades, because few cultivars with effective genetic resistance are available. The benzimidazole fungicides, particularly MBC, have been routinely used for more than 30 years during each period of wheat heading and flowering, in areas with warm and moist weather, to control FHB in China (32). A distinct advantage of MBC was its systemic activity which not only protected plants from infection but also provided disease control when applied after the early stages of infection. However, based on MBC-resistance monitoring, the frequency of resistant isolates in some regions of China increased gradually, and the efficacy of MBC against FHB decreased dramatically after 1998 (6). In a sense, the effectiveness of MBC was reduced due to the selection of a resistant pathogen population in the field (28). Because of this selection for resistance, China now is facing a challenge to find alternative fungicides for managing FHB on wheat. Therefore, introduction of an alternative fungicide with a different mode of action for MBC resistance and consistent control of FHB is urgent. In theory, when fungicides are introduced with specific targets to control, each of the targets requires a baseline sensitivity to be established so that product use strategies can be monitored and future resistance can be detected (19). The mode of actions of pyraclostrobin (a QoI) and epoxiconazole (a DMI), which were not widely used for the control of FHB in China, have been proven to be different from that of MBC (a benzimidazole fungicide). This study also suggested that there was no cross-resistance between MBC and pyraclostrobin or epoxiconazole. This could be explained by the difference in mode of action between MBC, pyraclostrobin, or epoxiconazole (Table 1). Previous studies showed that the spore germination of fungal pathogens was always more sensitive to QoI fungicides than that of mycelial growth, and protective use of these fungicides exhibited greater control efficacy for fungal diseases in plants (12). In this study, pyraclostrobin showed strong antifungal activity of inhibiting both mycelial growth and conidium germination in the presence of SHAM (Table 1). Some plant antioxidants, such as flavone presented in the host, quenched the active oxygen species from reaching the level sufficient for inducing an alternative oxidase pathway during QoI action, playing a role similar to SHAM (12). In fact, our study has shown that SHAM greatly improved the activity of pyraclostrobin against mycelial growth and conidial germination of *F. graminearum* isolates (data not shown). Nevertheless, the presence of SHAM would favorably exhibit the activity of pyraclostrobin in the in vitro test.

In this study, the baseline sensitivities of *F. asiaticum* and *F. graminearum* isolates to pyraclostrobin and epoxiconazole were also

Fig. 3. Frequency distribution of sensitivity of conidium germination (baseline sensitivity) of **A**, *Fusarium asiaticum* and **B**, *F. graminearum* to pyraclostrobin (in the presence of salicylhydroxamic acid).

Table 1. Cross-resistance between carbendazim and pyraclostrobin or epoxiconazole in *Fusarium asiaticum*

Isolates	Sensitivity to carbendazim ^z	EC ₅₀ for ^y		
		Pyraclostrobin (µg/ml)		Epoxiconazole (µg/ml)
		Inhibiting growth	Inhibiting germination	Inhibiting growth
TTZ01	S	0.052 f	0.122 a	0.323 k
TTZ02	S	0.054 f	0.077 cde	0.581 f
TTZ03	S	0.058 ef	0.072 ef	0.622 e
LJ09	S	0.082 bc	0.052 g	0.763 b
LJ13	S	0.074 cd	0.091 bcd	0.535 g
LK07	S	0.088 ab	0.064 efg	0.852 a
FY01	R	0.068 d	0.092 bc	0.427 h
FY03	R	0.092 a	0.062 efg	0.393 j
FY06	R	0.072 d	0.106 ab	0.715 c
GZ02	R	0.066 de	0.057 fg	0.577 f
GZ06	R	0.055 f	0.100 b	0.412 i
GZ08	R	0.067 d	0.074 def	0.646 d

^y EC₅₀ = 50% effective concentration. Mean values with the same letters within the same column were not significantly different with a least significant difference test at *P* = 0.05.

^z S and R indicate sensitive and resistant, respectively, to carbendazim.

established. The results showed that the selected isolates were very sensitive to these two fungicides and the low values and the relatively short range of sensitivity variation indicated the absence of resistant subpopulations among the isolates used in this study. Therefore, the sensitivity data can be used as baseline sensitivity for monitoring any future changes in sensitivity to pyraclostrobin and epoxiconazole in *F. asiaticum* and *F. graminearum* populations. Such baseline data are essential for assessing resistance risk, managing field resistance, and monitoring resistance levels in the pathogen populations.

In the protective and curative tests, pyraclostrobin applied at 200 and 300 µg/ml exhibited higher protective control efficacy than that of epoxiconazole applied at 200 and 300 µg/ml (Table 2). This could be explained by the specific activity of pyraclostrobin against conidium germination by inhibiting delivery of electrons for energy production (10,12). However, in the curative tests, epoxiconazole applied at 200 and 300 µg/ml exhibited over 80% curative control efficacy, indicating stable activity against FHB (Table 2). Therefore, integrated use of pyraclostrobin + epoxiconazole might provide ideal control of FHB. This was further confirmed by field trials in this study. The results of field trials showed that integrated use of pyraclostrobin + epoxiconazole applied at 150 + 150 g a.i./ha provided over 85% control efficacy at both sites in 2010 and 2011, an increase compared with other treatments in the field trials (Table 3). In addition, single use of pyraclostrobin or epoxiconazole applied at

150 and 225 g a.i./ha at both sites also provided over 70 and 80% control efficacy, respectively, against FHB (Table 3). MBC applied at 562.5 g a.i./ha in all cases only provided less than 67% control efficacy (Table 3).

To our knowledge, the present study is the first report on the baseline sensitivity of Chinese *F. asiaticum* and *F. graminearum* populations to pyraclostrobin and epoxiconazole and the excellent protective and curative efficacy of these two fungicides in controlling FHB. Taken together, pyraclostrobin and epoxiconazole should be good alternatives to MBC for the control of FHB. However, this does not mean that pyraclostrobin and epoxiconazole could be extensively used for disease control because these two QoIs and DMIs are also at-risk fungicides, and resistance to QoIs and DMIs has already reported in previous studies (1,9,11,16,29). Therefore, these two fungicides should be carefully used, such as alternative applications or tank-mixing with other effective fungicides with different modes of action.

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Table 2. Protective and curative activity of pyraclostrobin and epoxiconazole against Fusarium head blight of wheat

Treatment ^z	Dosage (µg ml ⁻¹)	Average disease severity (%) ^y					
		Protective activity			Curative activity		
		1 day	3 days	5 days	1 days	3 days	5 days
25% pyraclostrobin EC	200	3.82 c	4.53 cd	5.93 c	3.57 d	5.16 c	7.52 c
	300	2.56 d	3.46 d	4.69 d	2.62 e	3.81 d	7.01 d
12.5% epoxiconazole SC	200	4.45 c	5.75 c	6.31 c	4.65 c	3.97 d	5.88 e
	300	2.91 d	4.27 cd	6.52 c	2.78 e	2.75 e	5.31 e
50% carbendazim WG	750	9.86 b	10.52 b	11.68 b	11.73 b	10.63 b	10.17 b
CK	...	28.63 a	32.64 a	29.92 a	31.13 a	27.86 a	30.35 a

^y Visual disease assessment was made 14 days after inoculation. The severity of scabbed wheat heads was assessed using a scale as described by previous studies (13,24), with eight evaluation classes (0, 2, 5, 10, 25, 50, 75, and 90% area infected) by applying the following formula: number of heads in each class × each evaluation class/total number of heads. Efficacy percentage was determined by applying Abbott's formula: (% infection control – % treated infection)/% infection control × 100. Mean values with the same letters within the same column were not significantly different ($P > 0.05$, Fisher's least significant difference).

^z EC, SC, WG, and CK represent emulsifiable concentrates, suspension concentrates, water-dispersible granules, and aqueous solutions, respectively.

Table 3. Efficacy of pyraclostrobin and epoxiconazole in controlling Fusarium head blight of wheat^y

Site, treatment ^z	Dosage (g a.i./ha)	Average disease severity (%)		Control efficacy (%)	
		2010	2011	2010	2011
Chuzhou City					
25% pyraclostrobin EC	150	2.68	0.97	75.5 c	71.1 c
	225	1.79	0.66	83.7 b	80.4 b
12.5% epoxiconazole SC	150	2.77	0.94	74.7 c	72.0 c
	225	1.92	0.62	82.5 b	81.5 b
25% pyraclostrobin EC + 12.5% epoxiconazole SC	150 + 150	1.25	0.42	88.6 a	87.5 a
50% carbendazim WG	562.5	3.69	1.32	66.3 d	60.7 d
CK	...	10.96	3.36	...	...
Bengbu City					
25% pyraclostrobin EC	150	3.96	1.31	75.1 c	71.3 c
	225	2.74	0.86	82.8 b	81.1 b
12.5% epoxiconazole SC	150	3.88	1.15	75.8 c	74.8 c
	225	2.89	0.90	81.9 b	80.2 b
25% pyraclostrobin EC + 12.5% epoxiconazole SC	150 + 150	1.96	0.63	87.7 a	86.2 a
50% carbendazim WG	562.5	6.05	1.72	62.0 d	62.3 d
CK	...	15.93	4.56	...	...

^y Visual disease assessment was made at least 14 days after inoculation. The severity of scabbed wheat heads was assessed using a scale as described by previous studies (13,24), with eight evaluation classes (0, 2, 5, 10, 25, 50, 75, and 90% area infected) by applying the following formula: number of heads in each class × each evaluation class/total number of heads. Efficacy percentage was determined by applying Abbott's formula: (% infection control – % treated infection)/% infection control × 100. Mean values with the same letters within the same column were not significantly different ($P > 0.05$, Fisher's least significant difference).

^z EC, SC, WG, and CK represent emulsifiable concentrates, suspension concentrates, water-dispersible granules, and aqueous solutions, respectively.

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