Sensitivity of *Fusarium asiaticum* to a novel succinate dehydrogenase inhibitor fungicide pydiflumetofen

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ABSTRACT

Fusarium head blight (FHB) or scab mainly caused by *Fusarium asiaticum* is a devastating fungal disease in wheat. In China, carbendazim (MBC) and its mixture with other fungicides are the main fungicides for controlling the disease. But resistant populations have been developed seriously in the field. The fungicides for controlling the disease are limited. So screening novel fungicides for controlling FHB is urgent. The fungicide pydiflumetofen (development code number: SYN545974), 3-(Difluoromethyl)-N-methyl-N-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-1H-pyrazole-4-carboxamide, is a novel succinate dehydrogenase inhibitor (SDHI) that was developed by Syngenta. In the research, the baseline sensitivities for different isolates of *F. asiaticum* from various regions of China to pydiflumetofen were established. The 50% effective concentration (EC₅₀) value of pydiflumetofen on suppressing mycelial growth against 116 isolates of *F. asiaticum* was 0.019 to 0.2084 µg/ml and their average value was 0.0745 ± 0.0367 µg/ml. The EC₅₀ value of pydiflumetofen on suppressing conidium germination against 116 isolates of *F. asiaticum* was 0.0583 to 0.4237 µg/ml and their average value was 0.1813 ± 0.0861 µg/ml. The results indicated that the fungicide had strong inhibition effect on mycelial growth and conidium germination against *F. asiaticum* *in vitro*. The EC₅₀ values distribution of 116 isolates measured by mycelial growth assay or by conidium

germination assay was a unimodal curve and could be used for detecting any sensitivity changes of *F. asiaticum* populations to pydiflumetofen in the future. The results of cross-resistance analysis indicated that there was no positive or negative cross-resistance between pydiflumetofen and carbendazim / phenamacril. In field trials, though FHB incidence of control was very severe, pydiflumetofen at 125, 150 and 200 g ai ha⁻¹ all provided more than 80% control efficacy which was significantly higher than that of phenamacril at 375 g ai ha⁻¹ or MBC at 600 g ai ha⁻¹ in 2015 and 2016. In conclusion, pydiflumetofen had very high activity against *F. asiaticum* *in vitro* and excellent efficacy for controlling FHB could also manage carbendazim-resistant *F. asiaticum* populations in the field.

Key Words: Wheat scab; SDHI; Control disease; Resistance management

1. Introduction

Wheat is an very important grain crop in the world agriculture (Valipour, 2012, 2015, 2016a, 2016b). In wheat planting, Fusarium head blight (FHB) or scab is a common and devastating fungal disease in the world (Zhang et al., 2015; Abdullah et al., 2016; Bui et al., 2016). The disease not only causes large economic loss, but also results in damage in wheat quality because of contamination with mycotoxins (e.g. deoxynivalenol, nivalenol, zearalenone) in infested wheat grains (Audenaert et al., 2014; Ji et al., 2014; Jiang et al. 2016). The mycotoxins could inhibit protein synthesis, and cause emesis, feed refusal, and neural disturbance in human and animals (De Walle et al., 2010; Dong et al., 2016). In China, FHB mainly occurs in Jiangsu Province, Anhui Province, Zhejiang Province, Hubei Province, Hunan Province, Heilongjiang Province, Jiangxi Province and Shanghai (Fig.1). About 11 phylogenetic species of Fusarium spp. can cause FHB (Starkey et al., 2007; Yin et al., 2009). But the major Fusarium species in various regions may be different because of different climatic and planting systems (Hu et al., 2015). *F. asiaticum* and *F. graminearum* are the primary pathogens causing FHB in China (Yin et al., 2009; Zhang et al., 2014). Previous study showed that 77.3% isolates were *F. asiaticum* and 22.7% were *F. graminearum* in detected isolates from different places of China (Zhang et al., 2007).

Only few wheat cultivars are resistant to the disease FHB (Blandino et al., 2012). To control FHB, fungicides were widely used during the last few decades in China. The benzimidazole fungicides and their mixture with other fungicides are the main fungicides for controlling the disease. The benzimidazole fungicide carbendazim (MBC) inhibited cell division of plant pathogens and had been widely applied for almost 40 years in China (Qiu et al., 2012). However, MBC-resistant *F. asiaticum* and *F. graminearum* populations have increased in the field because of large amount use of MBC and climate changes in recent years (Chen and Zhou, 2009; Zhang et al., 2009). MBC had few control efficacy for protecting wheat in some areas of China, especially in the places where large MBC-resistant populations appeared. FHB has threatened the yield and quality of wheat. The fungicide phenamacril (development code number: JS399-19) is another fungicide widely used for controlling FHB in China, especially in Jiangsu and Anhui Province. It was a Fusarium-specific fungicide, especially to *F. graminearum* and *F. moniliforme*. Phenamacril had an excellent efficiency for controlling FHB in the field and had been applied for a few years (Li et al., 2008). But phenamacril-resistant mutants could be obtained easily in the laboratory and most resistant mutants had almost the same fitness levels as their parents and had moderate resistance or high resistance levels, which indicated that phenamacril may have a high resistance risk for controlling the disease in the field (Chen et al., 2008). So screening novel fungicides for controlling FHB is very urgent.

The fungicide pydiflumetofen developed by Syngenta in recent years (development code number: SYN545974), 3-(Difluoromethyl)-N-methyl-N-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-1H-pyrazole-4-carboxamide, is the first member of a new chemical group among the succinate dehydrogenase inhibitor fungicides (SDHI), the N-methoxy-(phenyl-ethyl)-pyrazole-carboxamides (Olaya et al., 2016). Its molecular formula is $C_{16}H_{16}Cl_3F_2N_3O_2$ and its structural formula was shown in Fig.2. SDHI fungicides could interfere the respiration of plant pathogens by inhibiting the activity of complex II enzymes, which are effective in controlling many plant diseases (Avenot and Michailides, 2010; Horsefield et al., 2006).

Up to now, the fungicide pydiflumetofen was registered only in Argentina for controlling end-of-cycle diseases (the diseases caused by *Septoria* spp., *Cercospora sojina* or *Cercospora kikuchii*) of soybean. In *Alternaria solani* isolates, the *in vitro* sensitivity revealed incomplete cross resistance patterns between pydiflumetofen and boscalid, penthiopyrad, benzovindiflupyr and fluopyram (Olaya et al., 2016). But little is known about the activity and cross resistance patterns of pydiflumetofen in *F. asiaticum*. So the objectives of the research were to: (i) understand sensitivity distribution of *F. asiaticum* populations to the novel fungicide pydiflumetofen by establishing baseline sensitivities of different *F. asiaticum* isolates collected from various regions of China to pydiflumetofen by mycelial growth assay and conidium germination assay respectively. (ii) determine whether there are positive or negative cross-resistance between pydiflumetofen and other widely used fungicides (MBC and phenamacril). (iii) test the efficacy of pydiflumetofen for controlling FHB in the field.

2. Materials and methods

2.1. Collection of *Fusarium asiaticum* isolates

Infected wheatears with typical symptoms of FHB were collected from fields in Jiangsu Province, Anhui Province, Hubei Province and Shanghai in 2015. All *F. asiaticum* isolates were separated from those infected wheatears and stored at 4 °C. Among the 116 isolates, 75 were collected from Jiangsu Province, 15 from Anhui Province, 14 from Hubei Province and 12 from Shanghai (Table 1). Pydiflumetofen had never been used to control *Fusarium* head blight of wheat before collecting diseased wheatears.

2.2. Fungicides and culture media

MBC (available ingredient 98%), phenamacril (95%) and pydiflumetofen (99.0%) were offered by Jiangsu Lanfeng Bio Chemical Limited Co. Ltd., Jiangsu Pesticide Research Institute Co. Ltd., and Syngenta (China) Investment Co. Ltd. respectively. MBC was dissolved into 0.1 mol/L hydrochloric acid (HCl) to get 10 mg/ml for stock solution while phenamacril or pydiflumetofen was dissolved into methanol to get 10

mg/ml. These stock solutions were used for testing the sensitivities of *F.asiaticum* to the fungicides *in vitro*.

Pydiflumetofen (200 g/L pydiflumetofen, SC) was provided by Syngenta (China) Investment Co., Ltd. MBC (50 % DuoJunling, WP) and phenamacril (25% qingxijunzhi, SC) were purchased in Nanjing, Jiangsu Province. Pydiflumetofen (200 g/L pydiflumetofen, SC), carbendazim (50 % DuoJunling, WP) and phenamacril (25% qingxijunzhi, SC) were used for testing efficiency for controlling FHB in the field.

Potato dextrose agar (PDA) medium was made according to previous study and used for regular growth of *F.asiaticum* (Kuang et al., 2011). Carboxymethylcellulose (CMC) medium was prepared as previous description and used for inducing conidia production (Zheng et al., 2012). Water agar (WA) medium was made from 20 g agar per liter of distilled water and used for conidium germination.

2.3. Baseline sensitivity of mycelial growth of *F.asiaticum* populations to pydiflumetofen

The baseline sensitivity of mycelial growth of *F.asiaticum* populations to pydiflumetofen was set up by analyzing the distribution of 116 isolates EC₅₀ values. To understand the inhibition effect of pydiflumetofen on mycelial growth of *F.asiaticum* populations, 116 *F.asiaticum* isolates (table 1) were used to determine the 50% effective concentration (EC₅₀ values). Briefly, the stock solution of pydiflumetofen (10 mg/ml) was diluted to a series of concentrations of 4000, 1000, 250, 62.5, 15.625 µg/ml with methanol. 100 ml melted PDA culture medium was added with 0.1 ml above diluted solutions of pydiflumetofen to get a series of concentrations of 4, 1, 0.25, 0.0625, and 0.015625 µg/ml. Then the melted PDA culture medium was poured into petri dishes with 9 cm in diameter. The control was PDA plates containing the same volume of methanol. The isolates grew on PDA medium at 25 °C for three days and mycelial plugs in the edge of colony were prepared by hole puncher whose diameter was 5 mm. With mycelium upward, the mycelial plugs were shifted to the centre of PDA medium plates containing the fungicide pydiflumetofen with different concentrations. After cultured in an incubator at 25 °C for three days in darkness, the diameters of every mycelial colony on PDA

plates were measured twice perpendicularly and then averaged. Inhibition ratios of the fungicide to mycelial growth of *F.asiaticum* isolates in different concentrations were calculated by the formula: (the averaged diameter of control — the averaged diameter in some fungicide concentration) / (the averaged diameter of control — 5 mm of mycelial plug). EC₅₀ values were determined by the fitted regression line of the log-transformed percentage inhibition plotted against the log-transformed fungicide concentration (Brandt et al., 1988). There are three replicates in the experiment and the experiment was repeated twice.

2.4. Baseline sensitivity of conidium germination of *F.asiaticum* populations to pydiflumetofen

The baseline sensitivity of conidium germination of *F.asiaticum* populations to pydiflumetofen was set up by analyzing the distribution of 116 isolates EC₅₀ values. To understand the inhibition effect of pydiflumetofen on conidium germination of *F.asiaticum* populations, 116 isolates (table 1) were selected for detecting their sensitivities to pydiflumetofen. Briefly, the stock solution of pydiflumetofen (10 mg/ml) was diluted to a series of concentrations of 4000, 1000, 250, 62.5, 15.625 µg/ml with methanol. 100 ml melted WA culture medium was added with 0.1 ml above diluted solutions of pydiflumetofen to get a series of concentrations of 4, 1, 0.25, 0.0625, and 0.015625 µg/ml. Then the melted WA culture medium was poured into petri dishes with 9 cm in diameter. The control was WA plates containing the same volume of methanol. The isolates grew on PDA medium at 25 °C for three days. Five mycelial plugs in the edge of colony were prepared and shifted into 15 ml CMC medium in a 50 ml triangular flask. After cultured at 25 °C for 4 days in a shaker (175 rpm/min, 12 h illumination every day), conidia were filtered with two pieces of lens wiping paper and collected by centrifuging at 5000 rpm for 5 min. The conidia were suspended with sterile water and its concentration was adjusted to 1×10^5 /ml. 100 µl suspension of conidia was smeared on the surface of WA plates containing the fungicide pydiflumetofen with different concentrations. After cultured in an incubator at 25 °C for 8 h in darkness, germination was counted at three sites by the microscope (Primo Star, Zeiss, Germany) and 100 conidia were counted in each site. The standard

of a conidium considered to be germinated was according to previous study (Chen et al., 2012b). There are three replicates in the experiment and the experiment was repeated twice. Inhibition ratios of the fungicide to conidium germination of *F. asiaticum* isolates in different concentrations were calculated by the formula: (the amount of germinated conidia of control — the amount of germinated conidia in some fungicide concentration) / the amount of germinated conidia of control. EC₅₀ value of pydiflumetofen to each strain was determined according to previous study (Chen et al., 2012b).

2.5. Cross-resistance pattern between pydiflumetofen and MBC / phenamacril

In order to understand the cross-resistance patterns between pydiflumetofen and the widely used fungicide MBC / phenamacril, nine *F. asiaticum* isolates were used for detecting their sensitivities to the fungicides (table 2). The strains (S1, S2, S3, R1, R4 and R6) were collected from Taizhou of Jiangsu Province in 2014 and the strains (Y-A, Y-C and J1) were phenamacril-resistant mutants induced in the lab. The sensitivities of selected strains to MBC were detected by a certain distinguish concentration, which was minimum inhibitory concentration (MIC value). When the MIC value was >1.4 µg/ml, the isolate was classified as resistant strain (Yuan and Zhou, 2005; Chen and Zhou, 2009). Their sensitivities to pydiflumetofen or phenamacril were determined by testing EC₅₀ values of mycelia growth on PDA culture medium plates containing different concentrations of fungicides (table 2). There are three replicates in the experiment and the experiment was repeated twice. Whether there are positive or negative cross-resistance between pydiflumetofen and MBC / phenamacril was determined by analyzing the relationships of sensitivities of selected strains to the fungicides.

2.6. Control efficacy of pydiflumetofen against *Fusarium* head blight in the field

A very susceptible wheat cultivar “Huaimai 33” to *F. asiaticum* was planted in the fields of Huaian, Jiangsu Province, where *Fusarium* resistance population to MBC has been reported (Chen et al., 2009; Zhang et al., 2013), in the wheat growing seasons of 2015 and 2016. The cultivar of “Huaimai 33” was planted according to normal agronomic practice. The experimental field was separated into 18 piece of

“box” by red string and the area of each piece was 30m² (5×6 m). There was 50 cm no treatment wheat as the guarding row among every piece. Five treatments were designed: (1-3) pydiflumetofen at 125, 150 and 200 g ai ha⁻¹, respectively. (4) MBC at 600 g ai ha⁻¹. (5) phenamacri at 375 g ai ha⁻¹. The control was spraying the same volume water as treatments. Each treatment had three replicates which were distributed randomly. Those fungicides above were sprayed using the same electric sprayer as previous study when wheats began flowering (Chen et al., 2012b; Li et al., 2008). The volume of spraying water dissolving fungicides was 750 L ha⁻¹. All experimental sites were naturally infected. The field trial was repeated twice in two experimental fields of Huaian. Generally, the disease symptom of FHB will appear at least 14 days after fungicides application. In each plot, 100 wheat heads were collected randomly. The disease severity was evaluated with eight assessment classes (0%, 2%, 5%, 10%, 25%, 50%, 75%, and 90% area infected) (Parry et al., 1984). The disease index was calculated as follows: ($\sum$ number of heads in each class $\times$ each evaluation class)/total number of heads. The control efficacy of fungicides against FHB in the field was determined by the following formula: (disease index in the water control - disease index in the treatment)/disease index in the water control $\times$ 100. The data of disease index and control efficacy were analyzed with variance analysis by the Fisher’s least significant difference (LSD) test at $P=0.05$ to determine whether the fungicides had a significant effect on controlling FHB.

2.7. Statistical analysis

In order to understand statistical variances between repeated experiments, all the data in the research were processed with the software of SPSS 14.0 (SPSS Inc., Chicago, IL). Fisher’s LSD test ($P = 0.05$) was used to determine whether there were significantly different among the data of EC₅₀ values, disease index and control efficacy.

3. Results

3.1. Sensitivity of mycelial growth of *F.asiaticum* to pydiflumetofen

The inhibition effect of pydiflumetofen on mycelial growth of the isolate H1 at a series of concentrations of 0, 0.015625, 0.0625, 0.25, 1 and 4 µg/ml was showed in Fig.3, which suggest that the fungicide pydiflumetofen have a strong inhibition effect on its mycelial growth.

There was no variation to pydiflumetofen sensitivity in the 116 isolates of *F.asiaticum* by mycelial growth assay. No resistant population were found. EC₅₀ values of 116 isolates to pydiflumetofen were from 0.019 to 0.2084 µg/ml and their average value was 0.0745 ± 0.0367 µg/ml. The EC₅₀ values distribution of 116 isolates was a unimodal curve (Fig.4) and the range-of-variation factor was 10.97 (the maximum EC₅₀ value / the minimum EC₅₀ value).

3.2. Sensitivity of conidium germination of *F. asiaticum* populations to pydiflumetofen

There was no variation to pydiflumetofen sensitivity in the 116 isolates of *F.asiaticum* by conidium germination assay. No resistant population were found. EC₅₀ values of conidium germination of 116 isolates to pydiflumetofen were from 0.0583 to 0.4237 µg/ml and their average value was 0.1813 ± 0.0861 µg/ml. The EC₅₀ values distribution of 116 isolates was a unimodal curve (Fig.5) and the range-of-variation factor was 7.27.

3.3. Cross-resistance between pydiflumetofen and MBC / phenamacril

According to statistical analysis of EC₅₀ values, there were significant differences in sensitivity to pydiflumetofen or phenamacril between tested strains (Table 3). But there was no correlation in sensitivities between pydiflumetofen and MBC / phenamacril.

The strains that were sensitive to MBC / phenamacril were sensitive to pydiflumetofen and the strains that were resistant to MBC / phenamacril were still sensitive to pydiflumetofen. The results suggested there was no positive or negative cross-resistance between pydiflumetofen and MBC / phenamacril.

3.4. Efficacy of pydiflumetofen in controlling *Fusarium* head blight

The fungicide pydiflumetofen had excellent activity against FHB. The disease severity of FHB in all experimental fields was related to the weather prior to and

during the period of wheat flowering, especially the moisture (Chen et al., 2012b). In the field trails in 2015 and 2016, there were severe visual disease and pydiflumetofen at 125, 150 and 200 g ai ha⁻¹ all provided more than 80% control efficacy that was much better than that of MBC at 600 g ai ha⁻¹ (Table 4). But there were no significantly differences among the control efficacies at three dosages of pydiflumetofen ($P > 0.05$). In addition, the control efficacy of 25% phenamacril (SC) in 2015 and 2016 was significantly lower than that of pydiflumetofen ($P < 0.05$).

4. Discussion

Pydiflumetofen is a very special and novel SDHI fungicide. It belongs to a new chemical group of N-methoxy-(phenyl-ethyl)-pyrazole-carboxamides (Olaya et al., 2016). The fungicide had strong inhibition effect on mycelial growth and conidia germination of *F.asiaticum*. The average EC₅₀ value of baseline sensitivity for mycelial growth of 116 *F.asiaticum* isolates was 0.0745 ± 0.0367 µg/ml and lower than that of Fusarium-specific fungicide phenamacril (Chen et al., 2008; Li et al., 2008), which indicated that pydiflumetofen had more inhibition activity to mycelial growth of *F.asiaticum* than phenamacril *in vitro*. The average EC₅₀ value of baseline sensitivity for conidia germination was also very low, which indicated that pydiflumetofen could strongly inhibit the respiration of conidium when it germinated. From the results of conidia germination assay, we could speculated that pydiflumetofen may have a strong inhibition on ascospore germination and block primary infection of *F.asiaticum* in wheat.

The range-of-variation factors of baseline sensitivity curves were all short. No resistant mutants were found. The baseline sensitivity of EC₅₀ values distribution established by two different methods could be very useful for detecting any sensitivity changes in mycelial growth or conidium germination of *F. asiaticum* populations to pydiflumetofen in the future.

Benzimidazole fungicides resistance in *F. asiaticum* resulted from amino acid mutations in the β_2 -tubulin gene (Chen et al., 2009; Qiu et al., 2012). A single-point

mutation at codon 167 or 200 (F167Y; F200Y) yielded moderate resistance to MBC while a single point mutation at codon 198 (E198K) yielded high resistance. In the field, more than 90 % of the MBC-resistant isolates had the point mutation at codon 167 while less than 10 % had the point mutation at codon 198 and 200 (Hou et al., 2013). The mutations in myosin-5 conferred resistance to phenamacril in *F. asiaticum* (Zheng et al., 2015; Zhang et al., 2015). In the lab, point mutations A135T, V151M, P204S, I434M, A577T, R580G/H or I581F led to low resistance. Point mutations S418R, I424R and A577G were responsible for moderate resistance and point mutations K216R/E, S217P/L, or E420K/G/D conferred high resistance (Li et al., 2016).

The resistance mechanism and cross resistance patterns across SDHI fungicides are complex because the fungicides target succinate dehydrogenase complex II contains four subunits SdhA, SdhB, SdhC, SdhD and the single-point mutation in SdhB, SdhC or SdhD could all confer resistance of fungal plant pathogen species to the fungicides (Stammler et al., 2014). Until now, there are no reports about resistance mechanism of *F. asiaticum* to SDHI fungicides because this class fungicides except pydiflumetofen had poor fungicidal potency on *F. asiaticum* and were not used for controlling FHB. In other fungal plant pathogens (e.g. *Ustilago maydis*, *Aspergillus oryzae*, *Pyrenophora teres*, *Botrytis cinerea*), many point mutation types had been reported (Keon et al., 1991; Shima et al., 2009; Stammler et al., 2007, 2014; Yin et al., 2011; Veloukas et al., 2011; Angelini et al., 2010). For example, in *Pyrenophora teres*, a single point mutation SdhB-H277Y, SdhC-N75S, -G79R, -H134R, -S135R, SdhD-D124N/E, -H134R, or -D145G could result in resistance (Stammler et al., 2014). Usually, there are cross resistance among the same class fungicides (Chen et al., 2012a). But not all of SDHI fungicides appeared cross resistance because of differences in mutation types. For example, *Alternaria solani* isolates resistant to boscalid remained sensitive to fluopyram, and most of moderately resistant and resistant isolates were sensitive to penthiopyrad (Gudmestad et al., 2013).

In China, MBC-resistant *F. asiaticum* populations in the field has formed and the control efficacy of MBC was worse and worse, especially in Jiangsu and Anhui

Province (Chen et al., 2015; Zhang et al., 2013; Zhang et al., 2009). In this study, MBC at 600 g ai ha⁻¹ only provided 44.7 % and 40.3 % control efficacy in 2015 and 2016 respectively (table 4). Resistant mutants in the field could not be inhibited by increasing the amount of fungicide. Phenamacril may had a high resistance risk. Though we monitored phenamacril resistance for three years and did not find *Fusarium* resistant populations in the field, resistant populations may appear in near future and appropriate managements against resistance should be considered. Pydiflumetofen, carbendazim and phenamacril have different mode of actions and there was no cross-resistance between them. So pydiflumetofen could manage carbendazim or phenamacril-resistant *F.asiaticum* populations in the field.

The resistance risk of SDHI fungicides was identified to be medium level to high level by FRAC (Fungicide Resistance Action Committee) because of their single targets. Though pydiflumetofen had an excellent efficiency for control FHB, it should not be used singly. In order to delay the emergence of resistant population in the field, it may be applied with other fungicides of different mode of actions in the future. Nowadays, only few cultivars of wheat are resistant to *F.asiaticum* isolates and the effective fungicides for controlling FHB are limited, so pydiflumetofen will be very popular with farmers.

Pydiflumetofen provided a strong fungicidal potency to a wide range of plant pathogen species (Olaya et al., 2016), which indicated that its binding domain in the complex II enzymes may be very conserved. So, in the field, the probability of the emergence of resistant strains in a few years may be very low.

In conclusion, the research first reported the baseline sensitivities of *F. asiaticum* populations to pydiflumetofen and the cross-resistance patterns between pydiflumetofen and phenamacril / MBC. Pydiflumetofen is an excellent fungicide for controlling FHB. But its resistance risk and dynamics in wheat are still unknown, which should be further studied.

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483

484

Table1. Places of 116 *Fusarium asiaticum* isolates collected from diseased wheat ear in 2015

Province	Farm code	Isolates tested
Jiangsu	Huaian	23
	Yancheng	21
	Nantong	15
	Yangzhou	16
Anhui	Fengyang	15
Hubei	Jinzhou	14
Shanghai	Songjiang	12

Table 2. Concentrations of fungicides used to determine cross-resistance

Fungicides	Type of isolates ^a	Fungicide concentrations (µg/ml)
Pydiflumetofen	S	0, 0.015625, 0.0625, 0.25, 1, 4
Phenamacril	S	0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8
Phenamacril	R	0, 12.5, 25, 50, 100, 200, 400

^a S and R represent sensitive and resistant, respectively

499 Table 3. Cross-resistance between pydiflumetofen and MBC / phenamacril^a

Strains	MIC ^b to MBC (µg/ml)	EC ₅₀ (µg/ml) ^c	
		Pydiflumetofen	Phenamacril
S1	1.4 (S)	0.0762 (S) de	0.1238 (S) d
S2	1.4 (S)	0.0653 (S) fg	0.1245 (S) d
S3	1.4 (S)	0.1262 (S) a	0.0977 (S) d
R1	>100 (R)	0.1059 (S) b	0.1085 (S) d
R4	>100 (R)	0.0836 (S) cd	0.1140 (S) d
R6	>100 (R)	0.0897 (S) c	0.1562 (S) d
Y-A	1.4 (S)	0.0570 (S) g	162.3 (R) b
Y-C	1.4 (S)	0.0710 (S) ef	171.6 (R) a
J1	1.4 (S)	0.1326 (S) a	136.8 (R) c

500 ^a The letters S and R in bracket represent sensitive and resistant.501 ^bMIC indicates minimum inhibitory concentration.502 ^c There were no significant differences between the mean values with the same letters in the same column ($P >$
503 0.05).507 Table 4. Efficacy of pydiflumetofen in controlling FHB in Huaian, Jangsu Province, China in
508 2015 and 2016

Treatment ^b	Dosage (g ai ha ⁻¹)	2015 ^a		2016 ^a	
		Disease index	Control efficacy (%)	Disease index	Control efficacy (%)
200g/L Pydiflumetofen (SC)	125	5.29 d	82.0 a	6.05 d	81.1 a
	150	5.25 d	82.1 a	6.09 d	80.9 a
	200	5.13 d	82.5 a	5.92 d	81.5 a
50% carbendazim (WP)	600	16.25 b	44.7 c	19.08 b	40.3 c
25% phenamacril (SC)	375	7.84 c	73.3 b	9.1 c	71.5 b
Water	—	29.36 a	—	31.94 a	—

509 ^a 100 wheat heads with the symptom of FHB were collected randomly in each plot after the fungicides were
510 applied for at least 14 days . The disease severity was evaluated with eight assessment classes (Parry et al., 1984).511 The control efficacy of fungicides against FHB in the field was evaluated according to the disease severity of 100
512 wheat heads and calculated described previously (Li et al., 2008, Parry et al., 1984). Values in each column
513 followed by the same capital letters were not significantly different ($P > 0.05$).514 ^b SC and WP represent suspension concentrates, and wettable powder respectively.

515

Figure captions

Fig.1 Distribution of Fusarium head blight (FHB) of wheat in China

Fig.2 The structural formula of pydiflumetofen

Fig.3 The inhibition effect of pydiflumetofen on the isolate H1 of *Fusarium asiaticum* in different concentration of pydiflumetofen

Fig.4 Sensitivity distribution of 116 isolates of *Fusarium asiaticum* to pydiflumetofen as determined by mycelial growth assays

Fig.5 Sensitivity distribution of 116 isolates of *Fusarium asiaticum* to pydiflumetofen as determined by conidium germination assays

CK

0.015625 $\mu\text{g/ml}$

0.0625 $\mu\text{g/ml}$

0.25 $\mu\text{g/ml}$

1 $\mu\text{g/ml}$

4 $\mu\text{g/ml}$