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Screening Fungicides for Controlling Wheat Crown Rot Caused by *Fusarium pseudograminearum* across Hebei Province in China

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Abstract: Wheat Fusarium crown rot (FCR) is caused predominantly by *Fusarium pseudograminearum* across most wheat-producing countries; this fungal disease needs a specific combination of fungicides to control it. In this research, the efficacy of four fungicides against *F. pseudograminearum* is tested using in vitro assays. Our results showed that fludioxonil had an EC_{50} of 0.0447 mg/L, followed by difenoconazole (0.3845 mg/L) and tebuconazole (0.4919 mg/L). Azoxystrobin (2.6019 mg/L) was also effective. Commercially available fungicides with the first three ingredients as active ingredients were further tested for the control of FCR. Cruiser Plus and Celest presented higher efficacies in an environmentally controlled pot assay. Further testing in the field achieved a higher level of control by Cruiser Plus than Celest at the seedling (72.34% vs. 62.55%) and adult (56.76% vs. 47.78%) stages in a field plot experiment. When tested in naturally infected wheat fields in Linzhang, Hebei Province, applications of the two fungicides resulted in relative control efficacies of 45.17% and 38.57%, respectively, and grain yields were increased by dressing with Cruiser Plus (8.7%) and with Celest (5.3%). Furthermore, seed dressing combined with additional spraying in early spring resulted in significantly better control of FCR and higher grain yield than seed dressing treatment alone (10.4% and 7.4%, respectively). Similar results were obtained when tested in Xian County, Hebei Province, with a disease control efficacy of 40.36–59.91% and a yield increase of 4.2–7.5%. Integrated measures of dressing (Cruiser Plus and Celest) combined with spraying (Horizon) showed higher control efficacy to FCR.

Keywords: *Triticum aestivum*; Fusarium disease; soilborne pathogens; disease index; integrated pest management

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the major cereal crops grown worldwide, and *Fusarium* spp., including *F. pseudograminearum*, *F. graminearum*, and *F. culmorum*, are reported as the main causal agents for Fusarium crown rot (FCR) on wheat. FCR has been described in many arid and semi-arid wheat-growing continents and countries. After crown rot from *F. pseudograminearum* was initially reported in Australia [1], low levels of occurrence of this pathogen were reported in New Zealand [2]. Compared with other root pathogens on wheat, crown rot is mainly caused by *F. culmorum* and *F. pseudograminearum*, resulting in damage to the plant as well as lower grain yields in America [3,4]. This disease is also present in Europe [5,6], Africa [7], the Middle East [8,9], and China [10–12].

FCR causes disease throughout the entire growth stage of wheat, with brown necrosis at the stem collum region at the seedling stage. It causes the death of young seedlings before or soon after emergence in severe occurrences. Later, infections of this disease can cause brown lesions at the base of the stem, and they can progress to most parts of the stem later in the season. As a consequence, the overall growth of the wheat crop is affected, and

the plants produce white-blighted heads as well as abortive seeds, leading to significant yield losses, possibly all over the world. In Australia, yield losses of 8% to 36% in bread wheat and 24% to 52% in durum wheat were reported [13]. In Xinxiang, Henan Province, in 2019, the average diseased rate and white head rate reached 59% and 37%, respectively, with a yield loss of 70.6% when the wheat crown rot disease was serious [14]. Due to the application of agricultural control measures, including no-tillage and stubble retention practices, and the drier growing conditions experienced in wheat regions in recent years, this disease has been reported to be increasing in Jiangsu, Anhui, Henan, Shandong, and Hebei in China. *F. pseudograminearum* is the major pathogen causing FCR in the main wheat-growing regions in China. This pathogen is also associated with Fusarium head blight (FHB) in winter wheat [15,16]. Morphological characteristics of this pathogen show that it can produce teleomorph (*Gibberella coronicola*) when overwintering on diseased straw stubbles, and the subsequent spores can infect wheat florets to cause FHB [17]. FHB can also cause significant yield losses and reduced quality since this pathogen can specifically produce mycotoxins such as deoxynivalenol, zearalenone, and nivalenol in affected grains; these mycotoxins are harmful to human and livestock animals [18].

The complexity of the disease caused by *Fusarium* spp. on wheat is due to the lack of genetic resistance/tolerance sources to relevant diseases, and such potential contamination of the wheat grains from mycotoxins also requires novel effective control strategies for the diseases mentioned above. Therefore, it is especially important to reduce the inoculum of the potential pathogens to control FCR specifically. There are different methods for controlling FCR. Resistant cultivars [19], management of crop residues and rotation [20,21], and fertilizers have been shown to be effective in controlling FCR disease to some extent [22–24]. The lack of high resistance germplasms to wheat FCR is a specific challenge for breeding resistant cultivars [25,26]. Even though there have been some reports about the development of some biocontrol agents against this pathogen, there have been no commercial agents available for application in field tests yet [27–31].

The application of chemical agents, such as seed dressing and spraying, is considered the most effective way to protect wheat against FCR [23]. However, it is desirable to use highly efficacious chemicals to achieve maximum control with lower application rates. Fields that are not controlled will result in severe disease and massive losses compared with controlled ones. Publications on choosing an effective fungicide targeting FCR are scarce. Therefore, this research examines the effective concentration for 50% growth inhibition (EC_{50}) of four technical-grade fungicides against *F. pseudograminearum* with in vitro assays so as to optimize the most promising fungicides when tested as a seed dressing or in combination with spraying to control FCR in the field.

2. Materials and Methods

2.1. Fungal Strain, Wheat Cultivar, Fungicides

A strain of *F. pseudograminearum* was isolated from Hebei Province, and it was preserved in the Technological Innovation Center for Biological Control of Crop Diseases and Insect Pests of Hebei Province, China. A wheat cultivar (Jimai22) that is susceptible to FCR and widely grown in Hebei Province was employed for pot assays in a greenhouse as well as in a field assay.

Four technical-grade fungicides were tested in our in vitro assays, including azoxystrobin (95% active ingredient a.i.), difenoconazole (97% a.i.), fludioxonil (98% a.i.), and tebuconazole (95% a.i.) (Weiyuan, Hebei, China). Stock solutions of fludioxonil were obtained by dissolving it in methyl alcohol. Azoxystrobin, difenoconazole, and tebuconazole were dissolved in acetone, respectively. PDA plates were amended with fludioxonil to give serially final concentrations of 0.03, 0.09, 0.20, 0.40, and 0.80 mg a.i./L, with azoxystrobin to give concentrations of 1.0, 2.0, 4.0, 8.0, and 16.0 mg a.i./L, with difenoconazole to give concentrations of 0.1, 0.4, 1.6, 6.4, and 25.6 mg a.i./L, and with tebuconazole to give concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 mg a.i./L. PDA plates amended with 0.1% (v/v) methyl alcohol or acetone served as control.

Celest (2.5% fludioxonil FS; Syngenta, Basel, Switzerland), Dividend (3% difenoconazole FS; Syngenta, Basel, Switzerland), Cruiser Plus (a fixed-dose combination of 22.6% thiamethoxam+ 2.2% fludioxonil+ 2.2% difenoconazole, Syngenta, Basel, Switzerland), and Raxil (6.0% tebuconazole FS; Bayer Crop Science, Leverkusen, Germany), with active ingredients as mentioned above, were used as dressing agents in pot assays. The efficacious ones were used for seed dressing in the field. Horizon (43% tebuconazole SC; Bayer CropScience, Leverkusen, Germany) was used for spraying in early spring.

2.2. Inoculum Preparation for F. pseudograminearum

Wheat grain medium was prepared as described by Wei [32] for fungal inoculum. When the grain was cooled to room temperature, 8–10 agar plugs of *F. pseudograminearum* (0.6 cm in diameter) were cut from a fully colonized PDA (potato dextrose agar) plate and inoculated into the wheat grain in a bag, sealed, put in darkness at 25 ± 1 °C for about 10 days, and hand shaken every two days to mix the wheat grains and the *F. pseudograminearum* mycelium completely.

2.3. In Vitro Evaluation of Inhibition Effect on F. pseudograminearum

F. pseudograminearum isolates were assessed with the four fungicides using the mycelial growth rate method [33]. Plugs from new cultured (3–4 days) *F. pseudograminearum* strains were placed in the middle of the PDA plates and amended with a series of concentrations for each fungicide. Mean radial mycelial growth was measured for each treatment by crisscross after 3–4 days of incubation at 27 °C in the dark. Each treatment had three repetitions.

The relative inhibition rate of the in vitro assays was calculated according to the formulas below,

Colony diameter (cm) = colony diameter measured—fungal plug diameter (0.6 cm)

The in vitro toxicity was calculated as the effective concentration of the 50% growth inhibition (EC_{50}) value relative to the control. Relative inhibition (%) = ((colony diameter of control—colony diameter of treatment)/colony diameter of control) × 100

The relevant concentration (mg/L) of the fungicide was converted into a base-10 logarithmic value (x); these converted data and the inhibition of mycelial growth were analyzed using Data Processing System (DPS 7.05) software for linear regression analyses to achieve the virulence regression equation ($y = a + bx$) as well as the credible interval and correlation coefficient (r) results.

2.4. Pot Assay under Greenhouse Conditions

Sieved soil was autoclaved and maintained at 121 °C (0.1 MPa) for 1.5 h and dried at room temperature for around 24 h. This step was repeated, and the dried soils were used for planting in the pot assays. Chemical agents, including Celest, Cruiser Plus, Dividend, and Raxil, were individually diluted with water to the recommended concentration and sprayed on wheat seeds, respectively. The wheat grains were stirred during spraying to make the fungicides coat the seeds uniformly. Eight seeds were planted in a 10 cm diameter plastic pot along with the *F. pseudograminearum* inoculum. The autoclaved wheat grains were used as pathogen-free control. The pots were placed in a glass house at 23 ± 2 °C with a 12 h photoperiod/day. All 80 plants were sampled 35 days after inoculation, washed free of soil, and scored for disease severity. Disease severity at the seedling stage was rated on a 0–4 scale based on the symptoms observed on the crown with minor modification [34], where 0 = no symptoms (healthy crown); 1 = light browning on the crown; 2 = extension of browning but <50% width of the first sheath; 3 = extension of browning but >50% width of the first sheath; and 4 = dark brown color of the crown and extension to the second sheath or with died tillers.

Disease index (DI) and relative control efficacy were calculated as below.

Disease index = 100 × ∑(number of diseased plants at each grade × grade value)/total number of plants tested × the value of the highest grade

Relative control efficacy (%) = (DI of control − DI of treatment)/DI of control × 100

2.5. Plot Assay for the FCR

The plots were located in Baoding City, Hebei Province. Soil fertility levels and tillage management were the same as those in the field production. Celest and Cruiser Plus were selected as the test agents in the field plot assay due to their relatively higher control efficacy in the pot assay mentioned above. The two chemicals were applied by seed dressing, with a dose of 2 mL/kg seed. The subsequent dressed seeds were sown on 16 October 2016. Diseased grain inoculum was applied in the furrow when sowing. Plots treated with autoclaved grain seeds (no fungal inoculum) served as control. Each plot was 30 m^2 in area. There were three repetitions, and the plots were randomized in blocks. Samples were collected on 20 November 2016 and 14 May 2017 for assessments of disease severity at both the seedling and the adult stages, respectively. Around 30 plants were tested for each treatment.

Disease severity at the adult stage was valued as a standard 0–4 stem browning scoring system as follows: 0 = no visible symptoms; 1 = visible lesions on the first internode; 2 = visible lesions on the first and second internodes; 3 = visible lesions on the first, second, and third internodes; and 4 = visible lesions on at least two internodes and the development of a white head or aborted tiller. Detailed disease index (DI) and relative efficacy values were calculated using the methods described in Section 2.4.

2.6. Field Experiments

Located at Linzhang and Xian County in Hebei Province, two continuous-wheat production fields were chosen for the field experiments because of their uniform and severe occurrence of wheat FCR. A standard base fertilizer (600 kg/ha) containing N, P, and K (18-20-7) was applied before sowing, and fertilizer (180 kg/ha; NK (25-5), Mindefu, Baoding, China) was applied at the seedling stage together with irrigation. The plot area was 0.2 ha for each treatment. Wheat seeds of the cultivar Jimai22 were dressed separately with Celest and Cruiser Plus (each with a dose of 2 mL/kg seed) in a rotary drum before sowing, and the dried seeds were sown in mid-October 2017. Undressed seeds were used as control to check the efficacy of the seed dressing chemicals on wheat growth parameters, including the plant density and growth of the seedlings (23–26 November 2017). A five-point sampling method was used to collect samples, about 30 plants for every 1 m^2. The incidence of disease was scored at the seedling stage (mid-March 2018) as described above. To half of the fungicides' dressing, we combined the treatment with Horizon (another fungicide) spraying (10 mL in 30 L water per 667 m^2) onto the base of the wheat stem on the same day after sampling. Relevant yield parameters (fertile tillers per m^2, panicle per spike, thousand grain weight) and disease severity were investigated before harvest (June 2018), and a standard nine-point sampling method was used to collect samples in the field so as to assess the occurrence of FCR in the field, with 10–15 plants for each point. Methods for calculating the disease index (DI) and relative efficacy are mentioned in Section 2.4, and grain yield was calculated as: Grain yield (kg/m^2) = fertile tillers per m^2 × grains per spike × thousand grain weight (TGW)/1000

2.7. Data Analysis

All experimentations for in vitro assays, pot trials, and field tests were performed with three repetitions. SPSS 21.0 software was used for statistical analyses. Duncans' new multiple-range test, at the $p < 0.05$ level of significance, was used to compare the differences among the control efficacies. Probit analysis was used to estimate the toxicological endpoints and to confect the curve.

3. Results

3.1. Fungicide Sensitivity Assay of F. pseudograminearum

The EC_{50} and regression equation of the tested fungicides against *F. pseudograminearum* are presented in Table 1. Chemical agent fludioxonil recorded the highest inhibition to *F. pseudograminearum*, with an EC_{50} value of 0.0447 mg/L, followed by difenoconazole and tebuconazole with 0.3845 mg/L and 0.4919 mg/L, respectively. Apart from these results, azoxystrobin was considered to have a relatively lower effect against *F. pseudograminearum*,

with an EC_{50} value of 2.6018 mg/L. There were significant differences in EC_{50} values for these four fungicides in terms of the inhibition effect on mycelial growth (Table 1).

Table 1. The EC_{50} values and the regression equation of the four tested fungicides to the mycelial growth of *F. pseudograminearum*.

Fungicide	Toxicity Regression Equation ($y = a + bx$)	The Correlation Coefficient (r)	EC_{50} (mg/L)	Credible Interval (95%)	Slope	*p*-Value
Azoxystrobin	$y = 3.8865 + 2.6816x$	0.9990	2.6018 ± 0.0021^{a}†	2.4625~2.7484	2.6816	0.0001
Difenoconazole	$y = 5.3641 + 0.8768x$	0.9972	0.3845 ± 0.0005^{c}	0.3128~0.4724	0.8768	0.0002
Fludioxonil	$y = 6.9780 + 1.4623x$	0.9317	0.0445 ± 0.0127^{d}	0.0201~0.0980	1.4623	0.0212
Tebuconazole	$y = 5.4074 + 1.3212x$	0.9822	0.4919 ± 0.0118^{b}	0.3395~0.7120	1.3212	0.0028

† Note: Different lowercase letters marked after the mean values identify significantly different means (Duncans' new multiple-range test ($p < 0.05$)). ($n = 3$, F = 53391.982, $df = 3$, $p = 0$).

3.2. Pot Efficacy of Seed Dressing at the Seedling Stage

Seeds of wheat cultivar Jimai22 were treated with four fungicides with high efficacy in the in vitro assay, and water was used as the control. The results were assessed at the seedling stage, and Cruiser Plus and Celest presented two of the best results, with relative control efficacies of 58.40% and 57.21%, respectively. When used at the recommended dose rates of 2.0 mg/kg seed, no significant differences were observed between these two fungicides. Dividend was less efficacious (52.07%) and Raxil least efficacious (49.25%) compared to Cruiser Plus and Celest. The latter two fungicides were significantly less efficacious than Cruiser Plus and Celest ($p = 0.004$) (Table 2).

Table 2. Relative control efficacies of four fungicides to wheat FCR in a pot assay.

Fungicides	RCE (%)
Raxil	49.25 ± 3.29^{b}†
Dividend	52.07 ± 1.65^{ab}
Cruiser Plus	58.40 ± 1.95^{a}
Celest	57.21 ± 2.20^{a}
Control	-

† Note: Different lowercase letters marked after the mean values identify significantly different means (Duncans' new multiple-range test ($p < 0.05$)). This experiment was independently replicated, with similar results ($n = 3$, F = 10.036, $df = 3$).

3.3. Field Plot Assay Using Artificial Inoculation

At the seedling stage, dressing with Cruiser Plus and Celest presented a lower disease index (DI) at 6.39 and 8.65, respectively (Table 3), and these results were significantly lower than that of the control (23.11) ($p = 0$). However, at the adult stage, even with the higher DI values assessed (15.71 and 18.97, respectively), the disease index values for adult wheat dressed with the two fungicides were also significantly lower than that of the control ($p = 0$). As for the relative control efficacy at the seedling stage, Cruiser Plus and Celest scored 72.34% and 62.55%, respectively. At the adult stage, Cruiser Plus (56.76%) again showed much greater efficacy than Celest (47.78%), and these results are consistent with the findings obtained at the seeding stage (Table 3).

Table 3. Relative control efficacy of wheat FCR by two chemical agents, Cruiser Plus and Celest.

Treatments	Seedling Stage		Adult Stage	
	DI	RCE (%)	DI	RCE (%)
Cruiser Plus	6.39 ± 1.38^{c}	72.34	15.71 ± 2.00^{b}†	56.76
Celest	8.65 ± 0.84^{b}	62.55	18.97 ± 0.77^{b}	47.78
Control	23.11 ± 0.34^{a}	-	36.33 ± 2.55^{a}	-
F	272.071		99.816	
df	2		2	

† Note: Different lowercase letters marked following the mean values identify significantly different means (Duncans' new multiple-range test ($p < 0.05$, $n = 3$)).

3.4. Control Efficacy of Cruiser Plus and Celest in the Field

In order to test the relative control efficacy of Cruiser Plus and Celest under natural infection in the field, two sites with an annual occurrence of FCR disease, with *F. pseudograminearum* as the dominant pathogen, according to our investigation, were selected. Dead seedlings were observed in the control, with nearly no dead seedlings but only dead tillers in the dressed field (Figure 1). After the investigation of the growth status of wheat plants at the seedling stage, Cruiser Plus promoted wheat growth, with a greater number of seedlings per square meter (7.8% higher than the control), also at rooting and tillering (8.1% and 20.5% higher, respectively). These results were significantly greater than that of the control. By contrast, Celest had no obvious positive effect on seedling density, tillering, or root growth (Table 4).

(**A**) (**B**) (**C**) (**D**)

Figure 1. Dead seedlings (**left**) and dead tillers (**B**, **right**) in the field. (**A**), the healthy seedling; (**B**), the dead tiller; (**C**,**D**), the infected sheath.

No significant differences were recorded for fertile tillers treated with seed dressing alone by the above two fungicides, dressing + spraying, and the control. However, the grains numbers and TGW of the dressing + spraying treatment were significantly higher than those of the seed dressing treatment only (Table 4). For the final yield, both the treatments of dressing (Cruiser Plus) and dressing (Cruiser Plus) + spraying (Horizon) showed yield increases of 8.7% and 10.3%, respectively, which were significantly higher than those of dressing with Celest (5.3%) and dressing (Celest) + spraying (Horizon) (7.4%) (Table 4).

The disease index decreased significantly at the seedling stage as well as at the adult stage; fungicide dressing performed better than the blank control (Figure 2, Table 5). The relative control efficacies of the two fungicides of Cruiser Plus and Celest were 45.17% and 38.57%, respectively, at the seedling stage and 35.76% and 31.04%, respectively, at the adult stage. When Horizon fungicide was applied as a spray in spring, the relative control efficacy was enhanced by 6.71% and 12.18%, respectively, compared with dressing only (Table 5). As a whole, our findings indicate that Cruiser Plus shows a better effect on germination, tillering, and relative control efficacy than Celest at both the seedling stage and the adult stage. The combined treatment of seed dressing + spraying was superior to seed dressing alone and increased grain yield significantly. Similar results were obtained when the same treatments were performed in Xian County, Hebei Province (Tables S1 and S2).

(**A**) (**B**) (**C**)

Figure 2. White spikes in the field in Linzhang. (**A**), No dressing (control); (**B**), dressing by Cruiser Plus; (**C**), dressing by Celest.

Table 4. Influence of seed dressing on wheat growth and yield parameters (Linzhang 2017).

Treatments	Wheat Growth at Seedling Stage			Parameters at Adult Stage				
	Density Plant/m^2	Tiller/Plant	Secondary Roots	Fertile Tillers/m^2	Grain/Spike	TGW (g)	Yield/kg/m^2	Increased Yield (%)
Cruiser Plus	1565 ± 48.80 ^{a}†	3.7 ± 0.13 ^{a}	13.5 ± 0.13 ^{a}	616 ± 22.17 ^{a}	33.6 ± 0.38 ^{ab}	44.5 ± 0.35 ^{ab}	0.783 ± 0.01 ^{ab}	8.7
Celest	1418 ± 26.86 ^{b}	3.4 ± 0.16 ^{b}	12.7 ± 0.23 ^{ab}	619 ± 32.70 ^{a}	33.2 ± 0.35 ^{b}	43.4 ± 0.52 ^{c}	0.758 ± 0.02 ^{b}	5.3
Cruiser Plus + Horizon	-	-	-	622 ± 38.69 ^{a}	33.7 ± 0.33 ^{a}	44.6 ± 0.56 ^{a}	0.795 ± 0.03 ^{a}	10.4
Celest + Horizon	-	-	-	627 ± 40.07 ^{a}	33.2 ± 0.27 ^{b}	43.7 ± 0.89 ^{bc}	0.773 ± 0.02 ^{ab}	7.4
Control	1452 ± 71.39 ^{b}	3.4 ± 0.14 ^{b}	11.2 ± 0.29 ^{b}	624 ± 38.93 ^{a}	32.1 ± 0.19 ^{c}	42.3 ± 0.58 ^{d}	0.720 ± 0.03 ^{c}	
F	10.870	8.258	134.390	0.071	21.355	12.239	8.394	
df	2	2	2	4	4	4	4	
p-value	0.002	0.006	0	0.990	0	0	0	

† Note: Different lowercase letters marked following the mean values identify significantly different means (Duncans' new multiple-range test ($p < 0.05$)).

Table 5. Relative control efficacy of the two agents in the field and their combined treatments (Linzhang 2017).

Treatments	Seedling Stage		Adult Stage		Increased Control Efficacies (%)	
	DI	RCE (%)	DI	RCE (%)	Dressing + Spraying vs. Dressing	Cruiser Plus vs. Celest
Cruiser Plus	6.98 ± 0.67 ^{b}	45.17	17.69 ± 0.96 ^{b}†	35.76		15.21
Celest	7.82 ± 0.44 ^{b}	38.57	18.99 ± 1.66 ^{b}	31.04		
Cruiser Plus + Horizon	-	-	17.03 ± 1.39 ^{b}	38.16	6.71	9.59
Celest + Horizon	-	-	17.95 ± 0.56 ^{b}	34.82	12.18	
Control	12.73 ^{a}	-	27.54 ± 3.27 ^{a}	-		
F	47.900		17.133			
df	2		4			
p-value	0		0			

† Note: Different lowercase letters marked following the mean values identify significantly different means (Duncans' new multiple-range test, $p < 0.05$, $n = 3$).

4. Discussion

FCR is considered one of the most problematic fungal diseases for wheat production in China. Chemical control through seed dressing has proven to be an effective, fast-acting, and highly economical approach to protecting wheat against the disease. We compared four fungicides in designed in vitro tests. The results show that these fungicides (fludioxonil, difenoconazole, tebuconazole, and azoxystrobin) can effectively inhibit the mycelial growth of *F. pseudograminearum*.

From our pot assays, all the above four fungicides, with relatively low EC_{50} values, were shown to be effective as seed dressings at commercially recommended rates in reducing disease. Two fungicides (Celest and Cruiser Plus) with much better results were further tested in the field. With fludioxonil as the active ingredient, Celest can inhibit the phosphorylation of glucose, which inhibits the growth of the fungal mycelium, leading to an increase in the seed emergence rate [35]. Difenoconazole has been reported to be an effective fungicide as a seed dressing to increase seedling emergence significantly against *Fusarium* spp. and, at the same time, reduce the number of rotted roots and increase healthy grains per spike and yield [36]. The fungicide Cruiser Plus is a compound agent with equal quantities of the active ingredients of fludioxonil and difenoconazole (2.2% each). When applied to small plots and large field experiments, Cruiser Plus exhibited better control than Celest (2.5% fludioxonil).

According to Hysing and Wiik [35], the fungicide Celest Extra Formula M (CEFM, difenoconazole + fludioxonil) or Celest Formula M (CFM, fludioxonil) had no significant effect on most agronomic characters, including yield. However, in our study, Celest, which has the same ingredients as CFM, had a significant effect on plant density, tillering, and the production of secondary roots. Dressing with Cruiser Plus showed a positive effect on TGW and, thus, on yield. In Xian County, the yields were higher, with 22.45% and 19.53% increases, respectively. Therefore, it is necessary to exploit a fixed-dose combination of fungicides with different modes of action and/or to employ mixed fungicides to control a specific disease so as to reduce the development of relevant fungicide resistance. As seed dressings have a limited period of protection, the combined use of spraying and seed dressing against severe disease outbreaks should improve the control efficacy.

Tebuconazole can affect ergosterol biosynthesis, and it is considered one of the most effective fungicides for controlling diseases caused by *Fusarium* spp. [37,38]. This chemical can also prevent the formation of mycotoxins produced by *F. culmorum* and *F. graminearum* [36]. Akgül et al. [22] also showed a 93.9% reduction in disease severity after spraying with tebuconazole. We tried an additional spray in early spring using Horizon (tebuconazole as the active ingredient), and a significant effect was achieved, comparing the control of Cruiser Plus (2.2% difenoconazole + 2.2% fludioxonil) with Celest (2.5% fludioxonil) in the field assay; there was a 15.21% increase using the dressing treatment and a 9.59% increase using the dressing + spraying treatment.

Higher control efficacies were detected when we compared the seed dressing and seed dressing + spraying treatments using Cruiser Plus and Celest in the field assay. Results from these treatments showed an increase of 6.71% and 12.18% in Linzhang and 21.24% and 11.99% in Xian County, respectively.

The seed dressing treatment using Cruiser Plus and the combined treatment showed higher final yields than treatments with Celest only. The increase in the florets per spike and the TGW may have played a key role in the final yield. Such results also indicated that a dressing treatment using a single fungicide such as Cruiser Plus, combined with spraying, may also improve the disease tolerance or the overall compensation ability of wheat plants. Consequently, our research revealed that Cruiser Plus, with the additional active ingredients of difenoconazole and thiamethoxam, can be used in areas with more serious diseases and larger populations of insects, while Celest may be used in areas with relatively mild pest infestations.

It can also be possible to integrate the use of fungicides and biological agents to increase control efficacy. Though there are no commercial biological control agents currently

available to control FCR, a range of studies have proved the effects of such a combination approach. For instance, *Bacillus* strains and *Trichoderma* together may also play major roles in stimulating host defenses and significantly protecting the plant against FCR in glasshouse assays [23]. Additive protection against FCR has been achieved with combined applications of biocontrol agents and chemical fungicides [23]. Nanochitin whisker (NC) was reported to have positively increased control efficacy when mixed with tebuconazole against FCR and to be beneficial for seedling growth, leading to an overall reduced quantity of chemical fungicide applied [29].

Regardless of the mode of action of the fungicides, there is a need to develop sustained-released formulations to improve the persistence of the chemical so that the wheat crop can be protected for a longer period. In addition, efficacious agents with different modes of action should be further screened to delay the development of fungicide resistance.

Chemical control plays an important role in integrated pest management (IPM) [39], especially in dealing with emergent pests and seedborne/soilborne pathogens. Pesticide-treated wheat seed is commonly used in America [40], the United Kingdom [41], and China [42]. Though there are many advantages in using fungicides, there are negative effects on humans, the environment, and the biota, especially for plants and soil [43]. A few priorities should be established to reduce the use of fungicides in controlling FCR, and providing detailed information such as active ingredients, potential targets, and risk exposures for different types of pesticides is useful before seed treatments [44]. This may also help farmers make decisions about seed selection so as to avoid the use of secondary dressings by providing advice and knowledge about IPM management for a specific crop, such as wheat. When the disease risk of a key potential soilborne pathogen is low, such prior knowledge is significant for farmers because the use of treated seeds can be avoided [45]. Additionally, combined key crop management strategies, such as cultivating moderately resistant varieties, sowing-date adaptation to reduce the contacting of the pathogens, reducing pathogen populations by deep plowing, and rolling after sowing to improve seed germination, seedling emergence, and, thereby, the quality of the crop, should be established to help control the disease [45]. Ultimately, efforts should be made by farmers, researchers, and regulators to protect the environment from the adverse effects of pesticides for sustainable crop protection strategies in the long term.

5. Conclusions

Fludioxonil, as well as difenoconazole and tebuconazole, shows a high mycelium inhibition of *F. pseudograminearum*. Cruiser Plus and Celest are efficacious agents to control wheat FCR in the field; they promote an increased yield production of 5.3%~8.7% when used solely as dressing agents. Higher yield production is achieved when combined with the spraying of Horizon fungicide in early spring. More agents with different modes of action should be screened to delay the development of fungicide resistance, and integrated measures should be considered for sustainable crop protection in the longer term.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12101643/s1, Table S1: Influence of seed dressing on wheat growth and yield parameters (Xian County 2017), Table S2: Relative control efficacy of the two agents in the field and their combined treatments (Xian County 2017).

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Abbreviations

FCR	Fusarium crown rot
FHB	Fusarium head blight
EC_{50}	effective concentration for 50% growth inhibition
WP	wettable powder
SC	suspension concentrate
PDA	potato dextrose agar
DI	disease index
RCE	relative control efficacy
TGW	thousand grain weight
IPM	integrated pest management

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