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Efficacy and profitability of fungicide use to manage Curvularia leaf spot of maize

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A R T I C L E I N F O

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A B S T R A C T

Maize leaf spot disease, caused by *Curvularia lunata*, significantly reduces maize yield. Maize growers in China have been continuously challenged with controlling this disease. Maize is a tall and densely planted crop, and the motivation of farmers to perform disease control is affected by various aspects, such as the timing of disease control, types of fungicides and application methods, the effectiveness of the fungicides, and profitability. This study used maize inoculated and not inoculated with the pathogen *Curvularia lunata* under three conditions: fungicide treatment applied post-inoculation, fungicide treatment applied pre-inoculation, and fungicide application without inoculation (natural field conditions). We selected maize plants at growth stage V12 and performed a single fungicide application, then compared the characteristics and profitability of six types of fungicides on maize leaf spot disease. Under these conditions, all fungicide treatments resulted in a significantly lower number of leaf spots and lower disease index than the control group ($P < 0.05$), and the grain yield of all fungicide treatments was higher than the control. Under a net return of \$0/ha (D = \$0/ha), the probability of profit was within the range of 0.470–1.00. In terms of yield increase and probability of profit for achieving a net return of \$225/ha (D = \$225/ha) under all conditions, we found that 18.7% propiconazole·azoxystrobin was effective in protecting, preventing, and improving plant health. However, it was less effective in treating the disease, indicating that it can be applied during the early growth stages to prevent subsequent leaf diseases. The characteristics observed using 250 g/L pyraclostrobin and 125 g/L epoxiconazole was similar to that of 18.7% propiconazole·azoxystrobin. However, because of the higher cost, the probability of profit of 250 g/L pyraclostrobin was lower than 0.695 (D = \$225/ha), and the probability of profit of 125 g/L epoxiconazole was lower than 0.624 (D = \$225/ha) because of the smaller improvement in yield. Seventy-five percent trifloxystrobin·tebuconazole showed great effect on disease treatment and plant health improvement but was less effective for crop protection and disease prevention, suggesting that this fungicide is suitable for later application. Seventeen percent pyraclostrobin·epoxiconazole was only efficient at disease prevention, whereas 23% kresoxim-methyl·epoxiconazole was effective in treating the disease; regardless, their probabilities of profit were all lower than 0.629 (D = \$225/ha).

1. Introduction

Maize, *Zea mays* L., is the most widely grown crop in China, with an annual cultivated area of approximately 35 million hectares. The main growing regions include; North and Northeastern China, Southwestern and Northwestern China, all for spring maize, and Huanghuaihai for summer maize (Chen et al., 2015). The climate types, ecological conditions and farming systems and other environmental characteristics

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vary across different regions, resulting in different characteristics in foliar diseases. For example, northern corn leaf blight is present in Northeastern and North China spring maize, while Culvularia leaf spot disease, Southern corn leaf blight disease, and Southern corn rust are common in Huanghuaihai summer maize. There is high foliar disease pressure like gray leaf spot and northern corn leaf blight in Southwestern China (Wang et al., 2006; Zhao et al., 2015).

The Huanghuaihai region in China is one of the major cultivation areas of maize, encompassing more than 10 million hectares, with a farming system of wheat-maize rotation (Hao et al., 2017). This region has a complex climate that exhibits great variability. June–September is the growing season for maize. The accumulation of straw returns and diseased plant debris from previous years results in abundant amounts of pathogens. From 2015 to 2018, there was a severe outbreak of the southern corn rust across the southern areas of the Huanghuaihai region. For southern corn leaf blight, any highly susceptible Huanghuaihai cultivars had been rejected for the national cultivar registration; most varieties exhibit relatively good resistance to this disease. Most varieties are susceptible or highly susceptible to Curvularia leaf spot disease (Wang et al., 2006); therefore, it poses potential threats to maize production.

The pathogen of leaf spot disease is mainly transmitted by air. The pathogen causes sudden outbreaks and severe symptoms, which usually appear during mid- and late-growth stages, as well as during the grain-filling period. Combined with the fact that maize is a tall, densely planted crop, this has led to many difficulties for fungicide application and disease control (Wang and Li, 2009). Compared to growers in the U. S., maize growers in China generally have a smaller planting area; the average area is 0.7 ha for most maize growers in the Huanghuaihai region. Growers who have adopted large-scale planting ($\geq$6.7ha) are fewer than 1.5% (Jia et al., 2017). Most growers do not perform any control for foliar diseases and pests during the later growth stages; foliar disease management is far behind that in the U.S. (Esker et al., 2018). Due to the small planting scale, which is unfavorable for fungicide application by agricultural aircrafts and large agricultural machines, experts in China proposed a strategy to move the timing of disease control forward to manage diseases and pests during the later growth stages (Wang and Wang, 2019). However, whether maize growers take action to control foliar disease depends on the timing of fungicide application, the type and cost of fungicides as well as the effectiveness of disease control. In countries such as the U.S., in-depth studies on the selection of fungicides, timing of application, cost, and approaches to increase yield and profits during foliar disease management have been conducted (Ward et al., 1997a, 1997b; Munkvold et al., 2001; Bradley and Ames, 2010; Blandino et al., 2012; Mallowa et al., 2015; Tedford et al., 2017). Thus far, there are very few studies in China regarding this topic as only a few researchers have ventured into the detailed characteristics and functions of fungicides in maize foliar disease management.

Meanwhile, international companies that manufacture agricultural chemicals have developed fungicides with multiple functions and characteristics, such as stronger absorption and a broader spectrum, providing both disease prevention and treatment, and long-lasting effectiveness. In addition, these chemicals exhibit multiple functions, such as improving plant health with focus on impacting plant growth, ensuring plants stay green longer, reducing senescence, and increasing yield. Therefore, this study selected six fungicides that were developed by international companies that are promoted and applied in China. We performed a single application of each fungicide on maize that was artificially inoculated with Curvularia leaf spot pathogen (*Curvularia lunata*) before and after fungicide application during maize growth stage V12. We compared samples with fungicide applications to samples under natural field conditions to analyze the characteristics of fungicides in protecting and preventing disease, as well as in improving plant health. Using three different environmental conditions, we performed a comparative analysis of fungicide characteristics in terms of disease control, effectiveness, and economic benefits, such as yield increases for potential use by maize growers.

2. Materials and methods

2.1. Field experiment location and design

The experiment location was the National Crop Variety Regional Trial Site in Changge City, Henan Province, China (34°10′22.51″N/113°52′6.73″E). The site had medium-level fertile soil, wheat was the preceding crop, and routine field management was applied in fertilization, irrigation, and weeding.

The main cultivated maize variety in the Huanghuaihai region, Zhengdan 958, was selected for this study. This variety is resistant to southern corn leaf blight, and is highly susceptible to Curvularia leaf spot disease and southern corn rust. Three experimental conditions were established in the field: (i) Fungicide treatment applied post-inoculation, for the investigation of the effectiveness in disease treatment; (ii) Fungicide treatment applied pre-inoculation, to investigate the effect in protection and disease prevention of the fungicide; and (iii) Fungicide application only, without inoculation (natural field conditions), to investigate the effect of the fungicide on plant health. Each condition consisted of six fungicide treatments and a water control, making a total of seven treatments (Table 1). The plot area was 42 m^2, with rows of 10-m in length, 0.6-m intervals between rows, with 7 rows in each plot. There were three replicate plots and a randomized complete block design was used. On June 4, 2018, seeds were sowed manually at a density of 67,500 plants/ha. Plants were harvested on October 2, 2018.

2.2. Fungicide applications and inoculation

The fungicides and their application amounts were as recommended in the user manual (Table 1), with 600 L of water applied to each hectare. At growth stage V12, fungicides were applied onto the leaves using an electric backpack sprayer. When applying different fungicides in the field, the sprayer was washed thrice with water.

A laboratory strain stock of *Curvularia lunata* was used in this study. After propagation on sorghum grains, the pathogen was washed off the grains with water and a spore suspension was made (2×10^5 spores/mL). This suspension was then used for manual spray inoculation as described previously (Wang et al., 2010).

Condition i: On July 12, 2018, experimental plots were inoculated at the V12 growth stage (12 visible leaf collars). At 7 d post inoculation (July 19), fungicide treatments were applied at the V14 growth stage (14 visible leaf collars) with the sprayer. Condition ii: Fungicide treatments were applied on July 19, 2018, followed by inoculation of the 16 visible leaf collars on July 27, 2018. Condition iii: On July 19, 2018, fungicides were applied to experimental plots.

2.3. Data collection

Disease data. Using the disease severity ratings of Wang et al. (2010) and Munkvold et al. (2001), as well as the standard assessment diagrams for evaluation of gray leaf spot lesion area on maize (Ward et al., 1997b), we graded the percentage of leaf spot lesions as grades 1–9. Each numerical value corresponded to a percentage interval of foliar surfaces exhibiting visible symptoms according to the following: 1 = 0–5%, 3 = 6–10%; 5 = 11–30%, 7 = 31–70%, 9 > 71%. On September 20, 2018 (R5 stage), we evaluated the severity of foliar disease from the ear leaf and above as a percent of the leaf area with symptoms by selecting three rows at the center of each plot and 10 sequential stalks in each row, and the disease index was calculated (Liu et al., 2016; Wang et al., 2005; Harveson et al., 2014). The disease index (I) was calculated using the formula $I = (1 \times n_1 + 3 \times n_3 + 5 \times n_5 + 7 \times n_7 + 9 \times n_9)/9 \times (n_1 + \ldots + n_9) \times 100$, where n_i = numbers of plants at the ith scale of infection severity.

In addition, three maize stalks were randomly selected from the

Table 1
Treatments, dosage and cost of pesticide, and fungicides used in this study.

Active ingredients and brand name	Manufacturer	Formulation	Dosage/ha	$/ha
18.7% propiconazole·azoxystrobin (Quilt Xcel)	Syngenta Nantong Crop Preservation Co., Ltd.	Emulsion	1050 mL	40.4
75% trifloxystrobin·tebuconazole (Nativo)	Bayer Crop Science China Co., Ltd.	Water dispersible granule	225 g	33.7
250 g/L pyraclostrobin (Cabrio)	BASF Plant Protection (Jiangsu) Co., Ltd.	Emulsifiable concentrate	450 mL	89.9
17% pyraclostrobin·epoxiconazole (Opera)	BASF Plant Protection (Jiangsu) Co., Ltd.	Emulsion	750 mL	78.7
125 g/L epoxiconazole (Opus)	BASF Plant Protection (Jiangsu) Co., Ltd.	Emulsion	450 mL	33.7
23% kresoxim-methyl·epoxiconazole (Allegro)	BASF Plant Protection (Jiangsu) Co., Ltd.	Emulsion	600 mL	51.7
Untreated control				

middle three rows of each plot as representatives. The ear leaf and the third leaf from the top were separated, placed on a board with a ruler in the background, and photographed with a Canon EOS60D camera. Image analysis was performed using ImageJ software (https://imagej.nih.gov/ij/download.html). On each leaf, two fixed areas were randomly selected along both sides of the veins, and the number of leaf spots were counted with the naked eye. Lastly, the number of leaf spots per square centimeter was calculated; the average values were used in statistical analysis.

Yield data. On October 2, 2018, the middle three rows were harvested (harvest area: 18 m^2) and threshed. Grain weight and grain moisture content were measured; yield per hectare was calculated using a water content of 14%.

2.4. Data analysis

The number of leaf spots, disease index, and grain yield trait were subjected to normality tests (the normal distribution and homogeneity of variances for the disease severity data were verified using PROC mixed and PROC UNIVARIATE in SAS software version 9.2, SAS Institute, Cary, NC, USA) and exhibited a normal distribution of the response variable. Thus, we used the GLM program in SAS 9.2 to perform the analysis of variance (ANOVA), and least significant differences ($P \leq 0.05$) for comparing treatment means were also calculated according to the GLM procedure of SAS. Fungicide applications were fixed effects, and block conditions were considered random factors. Exploratory analyses indicated large variations between inoculated and non-inoculated trials; therefore, each individual condition was analyzed separately.

The economic benefits for different fungicide treatments were calculated according to Munkvold et al. (2001). In this study, the currency exchange rate used was USD$1.0 = RMB6.675. In the Huanghuaihai region in China, maize growers mainly perform natural grain drying under the sun. Therefore, in our study, we did not include the cost for grain drying needed due to the difference in grain moisture content between fungicide application and control. This was different from the methods described by Munkvold et al. (2001). According to the cost of fungicide per hectare (Table 1), the cost of fungicide per application and yield increase resulted from fungicide application under different conditions, and the net return was calculated for each fungicide application. The prices of selected fungicides were the reference prices from retailers. Currently, the methods for fungicide application in Huanghuaihai are mainly by electric backpack sprayers, with a labor cost of $33.7/ha. In recent years, the price of maize in Huanghuaihai has fluctuated around $0.27/kg. We used $0.24/kg, $0.27/kg, and $0.30/kg to separately estimate the net return from yield increase.

The probability of a positive net return was determined using Bayesian statistics. As described by Munkvold et al., 2001, we employed two levels: one level with the break-even point D = $0/ha, which was the net profit of yield increase equal to the cost of fungicide application (fungicide cost + cost of application), and another level with a net return of $225 (in the Huanghuaihai region, this is equivalent to a net return of $6.675 for every 666.7 m^2, i.e., the daily salary per worker), which was acceptable to the maize growers. Grain prices of $0.24/kg, $0.27/kg, and $0.30/kg were used to separately calculate the probability of D = $0/ha and D = $225/ha.

3. Results

3.1. Fungicide applications for disease control

The present study employed three conditions: fungicide treatments applied post-inoculation, fungicide treatments applied prior to inoculation, and fungicide application only under non-inoculated conditions (natural field conditions). Under different environments, we compared the characteristics and effectiveness of different fungicides in controlling Curvularia leaf spot disease. ANOVA revealed that under the same conditions, the number of leaf spots on the ear leaf and the third leaf from the top varied with treatment ($P < 0.01$). No significant differences among blocks were observed. With fungicide treatment applied post-inoculation, the number of leaf spots on the ear leaf and the third leaf from the top in the control group was 2.81 and 2.38, respectively; treatments with six fungicides generated significantly different results compared to the control ($P < 0.05$, Table 2). In the fungicide treatment groups, a 37.3–51.8% decrease in the number of leaf spots on the ear leaf was observed compared to the control, whereas on the third leaf from the top, a 40.8–53.6% decrease in the number of leaf spots was detected. This indicated that the selected fungicides were effective in treating the disease, of which 250 g/L pyraclostrobin was the least effective. When fungicide treatment was applied prior to inoculation, the number of leaf spots on the ear leaf and the third leaf from the top per square centimeter in the control group was 3.25 and 2.60, respectively. The six fungicide treatments showed significant differences from the control group ($P < 0.05$, Table 2). However, no significant differences among the six fungicide treatments were observed. Compared to the control, the number of leaf spots on the ear leaf and the third leaf from the top decreased to 41.7–53.2% and 37.3–51.4%, respectively, indicating that all fungicide treatments had some effect on controlling Curvularia leaf spot disease. Under natural field conditions, the number of leaf spots per square centimeter on the ear leaf and the third leaf from the top were 1.52 and 1.17, respectively. Similarly, the six fungicide treatments were significantly different from the control ($P < 0.05$, Table 2). However, there were no significant differences among most of the fungicides. Compared to the control, the number of leaf spots on the ear leaf and the third leaf from the top decreased to 42.2–54.7% and 43.2–51.1%, respectively. Among the six fungicides, 18.7% propiconazole·azoxystrobin was the most effective.

ANOVA of disease index revealed a significant difference using fungicide treatment that was applied post-inoculation ($P < 0.05$). There was a highly significant difference among fungicide treatments applied prior to inoculation and under natural field conditions ($P < 0.01$). For fungicide treatments applied post-inoculation, disease severity in the control group was mainly 7 and 9, and a disease index of 80.6. The disease indexes of the six fungicides were around 60, which was significantly different from that of the control ($P < 0.05$, Table 3). However, the differences among the six fungicides were not significant. Compared to the control group, the effectiveness in disease prevention was 19.2–26.8%, and disease treatment was less effective. When fungicide treatments were applied prior to inoculation, the disease severity in the control group was mostly 7 and 9, and the disease index was 87.3. For the six fungicide treatments, the disease index was between 53.1 and 67.9, which was significantly different from the control

Table 2
Effects of fungicide treatments on leaf spots (N/cm2) for ear-leaf and third leaf from the top under different conditions.

Treatments	Ear-leaf			Third leaf		
	i	ii	iii	i	ii	iii
18.7% propiconazole·azoxystrobin	1.53 cb	1.52b	0.69c	1.3b	1.34b	0.54b
75% trifloxystrobin·tebuconazole	1.35c	1.87b	0.78 cb	1.1b	1.26b	0.62b
250 g/L pyraclostrobin	1.76b	1.88b	0.75 cb	1.41b	1.63b	0.58b
17% pyraclostrobin·epoxiconazole	1.54 cb	1.59b	0.77 cb	1.32b	1.46b	0.67b
125 g/L epoxiconazole	1.39 cb	1.89b	0.81 cb	1.07b	1.5b	0.57b
23% kresoxim-methyl·epoxiconazole	1.44 cb	1.79b	0.88b	1.03b	1.38b	0.66b
Untreated control	2.81a	3.25a	1.52a	2.38a	2.6a	1.17a

Means followed by the same letter within a column are not significantly different according to the least significant difference (LSD) test at $P = 0.05$.

Table 3
Effects of fungicide treatments on disease index and grain yield under different conditions.

Treatments	Disease index			Grain yield (kg/ha)		
	i	ii	iii	i	ii	iii
18.7% propiconazole·azoxystrobin	65.1b	57.7 b	13.3c	6874.5a	7576.8a	8319.3a
75% trifloxystrobin·tebuconazole	63.6b	67.9b	18.5 cb	7353.9a	6385.4bc	8421.9a
250 g/L pyraclostrobin	62.7b	65.7b	17.6 cb	6783.2ba	7294.6a	8083.7ba
17% pyraclostrobin·epoxiconazole	61.1b	53.1b	16.0 cb	6492.6bc	7258.6a	7340.0ba
125 g/L epoxiconazole	59.0b	62.0b	21.9b	6760.1ba	6982.3 ab	7746.8ba
23% kresoxim-methyl·epoxiconazole	59.9b	53.1b	23.5b	6996.5ba	6830.9 ab	7020.6ba
Untreated control	80.6a	87.3a	33.0a	5951.0c	5891.7c	6712.1b

Means followed by the same letter within a column are not significantly different according to the least significant difference (LSD) test at $P = 0.05$.

($P < 0.05$, Table 3). Similarly, there was no significant differences among the six fungicide treatments. Compared to the control, the effectiveness in disease prevention was 22.2–39.2%. Among the fungicide treatments, 17% kresoxim-methyl·epoxiconazole and 23% kresoxim-methyl·epoxiconazole were more effective in preventing leaf spot disease. Under natural field conditions, the disease severity of the control group was mostly 3, with a few reaching 5, and the disease index was 33. Again, the six fungicide treatments were significantly different from the control ($P < 0.05$, Table 3). In contrast, no significant differences were observed among most fungicide treatments. The effectiveness in disease control was 28.8–59.7%, of which 18.7% propiconazole·azoxystrobin and 17% pyraclostrobin·epoxiconazole showed a disease prevention effectiveness of >50%.

3.2. Effects of fungicide applications on yield

ANOVA of yield trait under the three conditions exhibited no significant difference among treatments under natural field conditions ($P > 0.05$). There was a highly significant difference among treatments under two inoculation conditions ($P < 0.01$). Under the three environmental conditions, the grain yield of all fungicide treatments was higher than the control, suggesting that the fungicides selected in this study were effective in increasing maize yield. With fungicide treatment applied post-inoculation, except for the 17% pyraclostrobin·epoxiconazole treatment that had no significant difference from the control, the remaining five fungicide treatments generated significantly higher yields than the control; approximately a 9.1–23.6% increase in maize yield. Among these, 75% trifloxystrobin·tebuconazole treatment resulted in the highest yield increase (23.6%, Table 3). When fungicide treatments were applied prior to inoculation, except for 75% trifloxystrobin·tebuconazole that showed no significant difference from the control, the remaining five fungicide treatments had significantly higher yields than the control, with an 8.4–28.6% increase in yield. Treatment with 18.7% propiconazole·azoxystrobin resulted in the highest yield increase. Under natural field conditions, the yield increase with fungicide treatments was 4.6–25.5%. With the exception of the 18.7% propiconazole·azoxystrobin and 75% trifloxystrobin·tebuconazole treatments that had significantly higher yield increases than the control, the other four treatments showed no significant differences relative to the control (Table 3). The 18.7% pyraclostrobin·azoxystrobin treatment was effective in increasing yield under different environmental conditions, followed by 250 g/L pyraclostrobin and 125 g/L epoxiconazole treatments. The 75% trifloxystrobin tebuconazole treatment was effective in increasing yield under fungicide treatments that were applied post-inoculation and under natural field conditions.

3.3. Probabilities of profit of fungicide applications

The main purpose of applying fungicides is to increase yield and revenue through disease management. The probability for profit with a net return D = \$0/ha from fungicide application was within the range of 0.470–1.00 (Table 4). To achieve a net return of \$225/ha (D = \$225/ha), the probability varied within a range of 0.001–0.842 for fungicides applied post-inoculation. Treatment with 75% trifloxystrobin·tebuconazole had the highest probability that varied within the range of 0.676–0.842. Under the condition of fungicide treatments applied prior to inoculation, the probability varied within the range of 0.047–0.908. Among these, 18.7% propiconazole·azoxystrobin treatment had the highest probability of profit (0.818–0.908), whereas the 75% trifloxystrobin·tebuconazole treatment had the lowest probability. Under natural field conditions, the probability varied within the range of 0.086–0.927, of which the 18.7% propiconazole treatment showed the highest probability of profit (0.826–0.927), followed by the 75% trifloxystrobin·tebuconazole treatment (0.752–0.837). In summary, 18.7% propiconazole·azoxystrobin and 75% trifloxystrobin·tebuconazole treatments revealed better profit potential in two conditions; however, none of the fungicides had a probability higher than 0.500 under all three conditions.

4. Discussion

To prevent and control maize leaf spot disease, the most economical and effective approach is to breed new disease-resistant varieties (Wang and Li, 2009; Esker et al., 2018). However, in the Huanghuaihai region, the two varieties that are most widely cultivated are Zhengdan 958 and Pioneer 335, and most of the newly approved varieties from recent years are susceptible or highly susceptible to Curvularia leaf spot disease

Table 4
Probability of profit for fungicide applications by artificial spraying evaluated under different conditions.

Conditions	Fungicides	Net return = $0/ha "break-even point"			Net return = $225/ha		
		$0.24/kg	$0.27/kg	0.30/kg	$0.24/kg	$0.27/kg	0.30/kg
i	18.7% propiconazole·azoxystrobin	0.999	1.000	1.000	0.005	0.030	0.179
	75% trifloxystrobin·tebuconazole	0.980	0.982	0.983	0.676	0.780	0.842
	250 g/L pyraclostrobin	0.986	0.992	0.995	0.001	0.004	0.012
	17% pyraclostrobin·epoxiconazole	0.709	0.819	0.879	0.001	0.002	0.004
	125 g/L epoxiconazole	0.926	0.934	0.940	0.119	0.203	0.302
	23% kresoxim-methyl·epoxiconazole	0.999	0.999	0.999	0.028	0.163	0.542
ii	18.7% propiconazole·azoxystrobin	0.984	0.985	0.986	0.818	0.875	0.908
	75% trifloxystrobin·tebuconazole	0.721	0.748	0.768	0.047	0.075	0.111
	250 g/L pyraclostrobin	0.945	0.952	0.958	0.457	0.595	0.695
	17% pyraclostrobin·epoxiconazole	0.870	0.881	0.890	0.478	0.564	0.629
	125 g/L epoxiconazole	0.961	0.965	0.967	0.364	0.508	0.624
	23% kresoxim-methyl·epoxiconazole	0.876	0.889	0.898	0.229	0.325	0.418
iii	18.7% propiconazole·azoxystrobin	0.991	0.991	0.992	0.826	0.892	0.927
	75% trifloxystrobin·tebuconazole	0.952	0.955	0.957	0.752	0.803	0.837
	250 g/L pyraclostrobin	0.950	0.957	0.962	0.425	0.574	0.685
	17% pyraclostrobin·epoxiconazole	0.613	0.648	0.675	0.104	0.148	0.196
	125 g/L epoxiconazole	0.862	0.870	0.877	0.387	0.469	0.537
	23% kresoxim-methyl·epoxiconazole	0.470	0.495	0.515	0.086	0.115	0.144

(Wang et al., 2006). Similarly, hybrids in the maize regional trials of the Huanghuaihai and Henan Province identified by our laboratory were mostly susceptible or highly susceptible to Curvularia leaf spot disease. Cultivating disease-susceptible varieties in areas with a history of severe leaf spot outbreaks could result in a risk of production.

Chinese maize growers generally hesitate in adapting measures to control maize foliar disease, such as optimizing the timing of disease control, the use of appropriate fungicides, methods for fungicide application, and the performance of yield increase and economic benefits after fungicide treatments. This study was based on the fact that maize growers in Huanghuaihai have a relatively small planting area and are familiar with the characteristics of artificial sprays. We performed a single fungicide application at growth stage V12, and then compared the effectiveness of six fungicides for disease control and economic benefits. The timing of crop disease control, as well as the number of fungicide applications, have significant impacts on disease management. For example, to control Fusarium head blight in wheat, fungicides applied at the early flowering stage are the most effective (Cowger et al., 2016). The application of fungicides, propiconazole or mancozeb, at growth stages VT to R1 significantly reduced gray leaf spot severity and increased yield (Munkvold et al., 2001). A previous study involving maize seed production in Iowa suggested that the optimal time for one application is at stage VT (tasseling), whereas the optimal time for two applications is at stages V7–V8 and VT. In that study, the greatest net returns resulted from treatments in which fungicide sprays were initiated soon after disease detection and continued for at least three consecutive application dates (Wegulo et al., 1997). Ward et al. (1997b) found that the use of fungicides to control gray leaf spot in maize was most effective in controlling disease and preventing yield loss when sprays were initiated at disease severity levels of 2–3% and continued until physiological maturity. The best timing for foliar northern corn leaf blight control were observed with applications from the mid-stem elongation to the milk stage. Furthermore, treatments at the mid-stem elongation and flowering stages significantly increased grain yield when a mixture of azoxystrobin and propiconazole were used (Blandino et al., 2012), which is the same as the application of 18.7% propiconazole·azoxystrobin in the present study. However, artificial spraying of fungicides during silking and pollination stages is unrealistic in China. This study showed that, at growth stage V12, certain fungicides could effectively control Curvularia leaf spot disease and significantly increase grain yield.

Leaf spot disease causes withering and necrosis on the leaves of maize. It significantly affects photosynthesis and leads to an imbalance in the relationship of source-sink; thus, affecting yield. This study considered previous research that measured maize photosynthesis using the ear leaf as the functional leaf (Sun et al., 2013; Li et al., 2017), as well as previous studies that discovered the ear leaf contains the highest amount of chlorophyll (Zuo et al., 1987). Based on these studies, we selected and used the ear leaf and the third leaf from the top, quantified leaf spots, and studied the effectiveness of disease control. Under the condition of fungicide applied post-inoculation, the number of leaf spots on both the ear leaf and the third leaf from the top was lower than that under fungicide application prior to inoculation and under natural field conditions. The same trend was observed for disease index. There was a significant positive correlation between the number of leaf spots and disease index (data unpublished). There was an eight-day difference between the timing of inoculations under these two conditions; generally, an earlier inoculation causes the disease to be more severe. However, our results suggest the opposite. In general, the variety Zhengdan 958 has about 20 leaves, 5–6 leaves near the top of the ear; the ear leaf was usually the 15th or 16th leaf. This may be due to the fact that when inoculation was performed earlier, there were approximately 13 leaves on the plant under the condition of fungicide applied post-inoculation. And on September 20, 2018 (R5 stage), we found that the leaves below the ear had mostly withered. However, when inoculation was conducted under fungicide application prior to inoculation, there were approximately 15 leaves, and the terminal leaf that had contact with the pathogen during spraying inoculation was the three-ear leaf on the adult plant; therefore, there was a higher number of leaf spots and a larger disease index than during the earlier inoculation period. In addition, we also observed an inconsistency between the decrease in leaf spots and the effectiveness of disease prevention in some of the fungicide treatments. This may be due to differences in sampling or errors derived from manually counting the number of leaf spots and conducting a visual rating of disease severity. In future studies, an image analysis system should be employed to automatically identify the functional leaf area and perform quantifications when studying the traits of disease severity (Barbedo, 2014; Mahlein, 2016). This supplementation would decrease human error and increase the accuracy of the experiments.

In this study, because there mostly was no significant difference in the number of leaf spots and disease index among treatments under the same conditions, we mainly used yield increase from fungicide treatments under different conditions to estimate the characteristics and functions of fungicides. Comparative analysis indicated that in all six fungicides used, 18.7% propiconazole·azoxystrobin had at least a 15% increase in grain yield compared to the control under all three conditions (Table 3). This was effective in terms of yield increase and profitability, with net returns all above $150/ha. For fungicides applied prior to inoculation, as well as under natural field conditions, the net returns were above \$300/ha. By comparing the three conditions, we found that 18.7% propiconazole·azoxystrobin was effective in plant protection, disease prevention, and improvement of plant health, although it was less effective in disease treatment. This indicates that 18.7% propiconazole·azoxystrobin can be used for early fungicide applications to prevent subsequent foliar diseases. This is consistent with results of Blandino et al. (2012) on northern corn leaf blight. This fungicide was also effective in controlling northern corn leaf blight and southern corn leaf blight under natural conditions (Wang et al., 2015). Munkvold et al. (2001) also found that fungicide propiconazole significantly reduced gray leaf spot severity and increased yield. Azoxystrobin + propiconazole application on maize can control northern corn leaf blight and maximize grain yield (Blandino et al., 2012), indicating that 18.7% propiconazole·azoxystrobin is a broad-spectrum fungicide for controlling maize leaf spot diseases. Treatment with 250 g/L pyraclostrobin was also effective in increasing yield under all three conditions (>14%). This fungicide had similar characteristics and functions as 18.7% propiconazole·azoxystrobin but has a higher fungicide cost (\$90/ha); therefore, there is a lower net return. The fungicide application of 125 g/L epoxiconazole had some effectiveness under the three conditions; however, the yield increase was relatively low and it had a low net return. Its characteristics and functions were similar to 18.7% propiconazole·azoxystrobin and 250 g/L pyraclostrobin. Treatment with 75% trifloxystrobin·tebuconazole exhibited a 20% grain yield (Table 3) under both post-inoculation and natural field conditions, and the net returns of all two conditions were above \$300/ha, but the yield increase was lower if the fungicide was applied pre-inoculation. This indicated that the 75% trifloxystrobin·tebuconazole treatment was effective for disease treatment and the improvement of plant health, but less effective in disease prevention, suggesting that this fungicide is more suitable for later stages. Moreover, in China, this fungicide was found to be effective in treating northern leaf corn blight (Zhuo and Bai, 2015) and rice blast (Yu et al., 2012) during the promotion of crop applications, indicating that it is a broad-spectrum treatment. In addition, 17% pyraclostrobin·epoxiconazole exhibited functions in protection, but due to its high cost (\$78.7/ha), the net return was average. Twenty-three percent kresoxim-methyl·epoxiconazole was only effective in treating the disease; the profit was average. In summary, to control maize leaf spot disease, we recommend an early artificial or mechanical application of 18.7% propiconazole·azoxystrobin. In addition, we recommend 75% trifloxystrobin·tebuconazole for later applications using mechanical spraying methods.

There are many methods available for analyzing economic benefits that result from crop disease management through fungicide applications. In this study, net profit was calculated as the difference between increased income by comparing fungicide treatment and control, and the fungicide + application cost (Wegulo et al., 1997; Munkvold et al., 2001; Cowger et al., 2016). This study employed the calculation method of Munkvold et al. (2001). We used Bayesion statistical methods to calculate the probability of profit when D = \$0/ha and D = \$225/ha. In terms of fungicide application cost, we considered the relatively small planting scale and fungicide application methods used by farmers in the Huanghuaihai region and performed calculations for artificial spraying. The cost of application was \$33.7/ha. The cost of fungicides was \$33.7–89.9/ha. The sum of these two values was much higher than costs of fungicide applications in the U.S., which were approximately \$39/ha (Munkvold et al., 2001). Similarly, Esker et al. (2018) found that maize growers in the US Midwest considered \$37.06/ha as an acceptable cost of fungicide application. In comparison, the cost of fungicide application in China is much higher. In this study, we set the price of maize as \$240/t, \$270/t, and \$300/t (=\$0.24/kg, \$0.27/kg, and \$0.30/kg). However, in the U.S., the price is approximately \$100/t; therefore, the price of maize in China is also higher than in the U.S. The net return was set to D = \$225/ha to calculate the probability of each fungicide; this value was also much higher than values in the U.S., which are estimated at D = \$25/ha (Munkvold et al., 2001). The data mentioned above suggest that, overall, the cost of maize planting in China is much higher than in the U.S. However, this study found that an early application of 18.7% propiconazole·azoxystrobin or a later application of 75% trifloxystrobin·tebuconazole had a relatively high probability of profit (approximately 0.800).

This study investigated the control of Curvularia leaf spot in maize under different environmental conditions; however, in recent years, southern corn rust has also occurred and was relatively severe in some areas of the Huanghuaihai region. In 2015, severe southern corn rust occurred in a large area of the Huanghuaihai region, but southern corn rust was minor in our experimental site in 2018. Thus, it was difficult to investigate the effect of the selected fungicides on southern corn rust, which requires further investigation. In addition, there were severe pest outbreaks during the maize grain-filling period in Henan Province. To minimize the cost of fungicide application in the field, maize disease and pest control could reference the "one spray, three preventions" approach used in wheat farming in the Huanghuaihai region. This approach suggests spraying before flowering to prevent disease, pests, and dry-hot wind. A single combined agrochemical application should be performed at growth stage V12 to prevent maize diseases and pests. At the same time, it could prevent pollen abortion and low fruit setting caused by continuous high temperatures and drought around the silking and pollinating periods. This requires further investigation. Finally, the economic benefits calculated in this study were based on estimates of small-scale artificial spraying fungicides in the field. Tedford et al. (2017) showed that plot size can influence yield benefits from fungicides on corn. Studies on whether mechanical spraying or unmanned aerial vehicles leads to increased yield are required. Moreover, further investigations involving mechanical fungicide applications in a larger field area are needed in order to obtain more accurate data.

Declaration of competing interest

The authors declare that they have no competing interests.

CRediT authorship contribution statement

Jun-Jie Hao: Conceptualization, Investigation, Resources, Writing - original draft, Writing - review & editing. **Wei-Ling Zhu:** Supervision, Conceptualization. **Yong-Qiang Li:** Investigation, Data curation, Writing - original draft. **Jia-Zhong Liu:** Investigation. **Shu-Na Xie:** Investigation. **Jing Sun:** Project administration. **Zhong-Dong Dong:** Data curation, Formal analysis, Software, Writing - review & editing.

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References

Barbedo, J.G.A., 2014. An automatic method to detect and measure leaf disease symptoms using digital image processing. Plant Dis. 98, 1709–1716.
Blandino, M., Galeazzi, M., Savoia, W., Reyneri, A., 2012. Timing of azoxystrobin + propiconazole application on maize to control northern corn leaf blight and maximize grain yield. Field Crop. Res. 139, 20–29.
Bradley, C.A., Ames, K.A., 2010. Effect of foliar fungicides on corn with simulated hail damage. Plant Dis. 94, 83–86.
Chen, H., Wang, Q.Z., Zhou, H., 2015. Empirical analysis of corn spatial distribution variation in China. Econ. Geogr. 35 (8), 165–171.
Cowger, C., Weisz, R., Arellano, C., Murphy, P., 2016. Profitability of integrated management of Fusarium head blight in North Carolina winter wheat. Phytopathology 106, 814–823.
Esker, P.D., Shah, D.A., Bradley, C.A., Conley, S.P., Paul, P.A., Robertson, A.E., 2018. Perceptions of Midwestern crop advisors and growers on foliar fungicide adoption and use in maize. Phytopathology 108 (9), 1078–1088.
Hao, J.J., Xie, S.N., Sun, J., Yang, G.Q., Liu, J.Z., Xu, F., Ru, Y.Y., Song, Y.L., 2017. Analysis of *Fusarium graminearum* species complex from wheat–maize rotation regions in Henan (China). Plant Dis. 101, 720–725.

Harveson, R.M., Nielsen, K.A., Eskridge, K.M., 2014. Utilizing a preplant soil test for predicting and estimating root rot severity in sugar beet in the Central High Plains of the United States. Plant Dis. 98, 1248–1252.

Jia, J.Q., Li, X.D., Wang, S.H., 2017. Effect of the grain support policy adjustment to the different scale framer's planting decisions—based on survey data of Shandong, Hebei and Henan Province. Reform Econ. Syst. 1, 89–95.

Li, G.H., Liu, P.P., Zhao, Bi, Dong, S.T., Liu, P., Zhang, J.W., Tian, C.X., He, Z.J., 2017. Effects of water conditions and controlled release urea on yield and leaf senescence physiological characteristics in summer maize. Chin. J. Appl. Ecol. 28 (2), 571–580.

Liu, L., Tan, J., Zhang, Y.D., Li, H.Y., Bi, Y.Q., Yu, L.J., Jeffers, D.P., Kang, M.S., Fan, X. M., 2016. QTL mapping for gray leaf spot resistance in a tropical maize population. Plant Dis. 100, 304–312.

Mahlein, A.K., 2016. Plant disease detection by imaging sensors-parallels and specific demands for precision agriculture and plant phenotyping. Plant Dis. 100 (2), 241–251.

Mallowa, S.O., Esker, P.D., Paul, P.A., Bradley, C.A., Chapara, V.R., Conley, S.P., Robertson, A.E., 2015. Effect of maize hybrid and foliar fungicides on yield under low foliar disease severity conditions. Phytopathology 105, 1080–1089.

Munkvold, G.P., Martinson, C.A., Shriver, J.M., Dixon, P.M., 2001. Probabilities for profitable fungicide use against gray leaf spot in hybrid maize. Phytopathology 91, 477–484.

Sun, X.F., Ding, Z.S., Hou, H.P., Ge, J.Z., Tang, L.Y., Zhao, M., 2013. Post-anthesis photosynthetic assimilation and the changes of carbon and nitrogenin different varieties of spring maize. Acta Agron. Sin. 39 (7), 1284–1292.

Tedford, E.C., Kriss, A.B., Geater, C., Saini, M., Battles, B., Smelser, R.B., Fithian, W.A., 2017. Plot size can influence yield benefits from fungicides on corn. Crop Protect. 91, 66–73.

Wang, X.M., Jin, Q.M., Shi, J., Wang, Z.Y., Li, X., 2006. The status of maize diseases and the possible effect of variety resistance on disease occurrence in the future. Acta Phytopathol. Sin. 36 (1), 1–11.

Wang, X.M., Shi, J., Jin, Q.M., Li, X., Sun, S.X., 2010. Field Manual of Corn Diseases and Pest. China Agricultural Science and Technology Press, China, pp. 264–265.

Wang, X.M., Li, H.J., 2009. Resistance breeding-best strategy of control leaf spot diseases on maize. Crops 2, 1–4.

Wang, X.M., Gong, S.Y., Liu, J.Y., Zhang, Q.F., Yuan, L.Z., Xie, Y.L., Li, W.X., Duan, C.X., Li, J.L., 2015. Techniques for controlling leaf diseases using fungicides on maize. Crops 3, 150–154.

Wang, Z., Wang, X., 2019. Current status and management strategies for corn pest and diseases in China. Plant Prot. 45 (1), 1–11.

Wang, Z.L., Li, L.H., He, Z.H., Duan, X.Y., Zhou, Y.L., Chen, X.M., Lillemo, M., Singh, R. P., Wang, H., Xia, X.C., 2005. Seedling and adult plant resistance to powdery mildew in Chinese bread wheat cultivars and lines. Plant Dis. 89, 457–463.

Ward, J.M.J., Laing, M.D., Nowell, D.C., 1997a. Chemical control of maize grey leaf spot. Crop Protect. 16 (3), 265–271.

Ward, J.M.J., Laing, M.D., Rijkenberg, F.H.J., 1997b. Frequency and timing of fungicide applications for the control of gray leaf spot in maize. Plant Dis. 81, 41–48.

Wegulo, S.N., Martinson, C.A., Rivera-C, J.M., Nutter Jr., F.W., 1997. Model for economic analysis of fungicide usage in hybrid corn seed production. Plant Dis. 81, 415–422.

Yu, S.H., Wang, H.F., Zhu, X.Z., 2012. A preliminary report of trifloxystrobin-tebuconazole on controlling rice blast. Biol. Disa. Sci. 35 (3), 276–278.

Zhao, L.P., Wang, X.M., Duan, C.X., Long, S.S., Li, X., Li, H.L., He, Y.Q., Jin, Q.M., Wu, X. F., Song, F.J., 2015. Occurrence status and future spreading areas of maize gray leaf spot in China. Sci. Agric. Sin. 48 (18), 3612–3626.

Zhuo, D.Y., Bai, X.F., 2015. Control effect of 75% trifloxystrobin·tebuconazole WG against northern corn leaf blight in maize. Modern Agric. Sci. Tech. 17, 148–150.

Zuo, B.Y., Li, S.Y., Kuang, T.Y., Duan, X.C., 1987. The changes of ultrastructure and chloropyll content of chloroplast of leaves in different ranks in maize. Acta Agron. Sin. 13 (3), 213–218.