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Establishment of baseline sensitivity of *Rhizoctonia solani* to thifluzamide in maize and its field application

Xiangfeng Yao[1,2,*], Dianlong Shang[1,2,*], Zhihua Qiao[1,2], Haoyong Yu[1,2], Shiang Sun[1,2], Xiangdong Li[1], Jiwang Zhang[3] and Xingyin Jiang[1,2]

[1]College of Plant Protection, Shandong Agricultural University, Tai'an, Shandong 271018, P.R. China; [2]Key Laboratory of Pesticide Toxicology & Application Technique, Tai'an, Shandong 271018, P.R. China and and [3]State Key Laboratory of Crop Biology, College of Agronomy, Shandong Agricultural University, Tai'an, Shandong 271018, P.R. China

*These two authors contributed equally to the present study.

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Author for correspondence:
Xingyin Jiang, E-mail: xyjiang@sdau.edu.cn, 1210971102@qq.com

Abstract

In recent years, banded leaf sheath blight in maize (*Zea mays* L.) has become an important disease that seriously affects quality and yield. This paper aims to evaluate the sensitivity of *Rhizoctonia solani* Kuhn to thifluzamide on maize, to clarify the effect of seed coating using a thifluzamide suspension agent on safety and physiological indicators and to determine the effectiveness of control of banded leaf sheath blight in the field. In this study, the thifluzamide sensitivity of 102 strains of *R. solani* in maize from Shandong was determined using the mycelial growth rate method; the average half-maximal effective concentration value (EC_{50}) was 0.086 ± 0.004 μg/ml and displayed a unimodal frequency distribution, indicating that thifluzamide had strong inhibitory activity on the mycelial growth of *R. solani* in maize. In an indoor pot test, the root activities under 24 g a.i./100 kg seed were found to increase by 78.01%, compared with the control. Similarly, chlorophyll content increased most significantly at this dose, by 32.3%. Thifluzamide (FS) could significantly increase the per-plot yield. Among the examined dosages, 48 g a.i./100 kg seed had the most significant treatment effect, with the yield rate increasing by 15.7% and 14.1%, respectively, in 2017 and 2018 compared with the control. The field effectiveness against banded leaf sheath blight in maize was highest at the dosage of 48 g a.i./100 kg seed for a seed dressing with thifluzamide (FS). These results indicate that thifluzamide has enormous potential for controlling banded leaf sheath blight in maize.

Introduction

To promote the integrated control of air pollution in support of an ecological civilization, in recent years, straw burning has been completely prohibited, and straw mulching has been widely promoted throughout China. However, due to improper treatment, straw mulching has provided habitat for many soil-borne pathogens. As an important cereal crop in the global agricultural economy (Feng *et al.*, 2020), maize (*Zea mays* L.) is critical for increased grain yield, but the incidence of banded leaf sheath blight in maize has been increasing annually, resulting in a decline in the quality and yield of maize (Singh *et al.*, 2018). Currently, farmers have a weak sense of prevention and control of banded leaf sheath blight in maize, and there is little use of control agents. Therefore, the development of safe, effective agents for the prevention and treatment of this disease is urgently needed.

Globally, *Rhizoctonia* spp. (Irzykowska *et al.*, 2005) are destructive soil-borne pathogens of many crops, which can utilize organic residues in the soil during the saprophytic period to survive as mycelium (mycelium or sclerotia) (Baker and Martinson, 1970; Pascual *et al.*, 2000, 2001). Banded leaf sheath blight in maize is a soil-borne disease caused by fungi in the soil habitat (Hirrel, 1988) such as *Rhizoctonia cerealis* (Vander Hoeven), *Rhizoctonia solani* (Kuhn) and *Rhizoctonia zeae* (Voorhees). *R. solani* is a dominant pathogen on maize in Shandong Province, China (Zhao *et al.*, 2006). The sexual stage is *Thanatephorus cucumeris* (Frank) Donk, and strains include AG-1-IA, AG-1-IB, AG-1-IC, AG2-1, AG2-2IIIB, AG2-2IV, AG2-3, AG-3, AG-5, AG-6, AG-7, AG-8, AG-9, AG-11, AG-BI, AG-A and AG-K (Ogoshi, 1987; Sneh *et al.*, 1991; Jhm *et al.*, 1997). The isolated strain AG-1-IA readily causes banded leaf sheath blight in maize (Li *et al.*, 1998). The disease can occur from the seedling period to the late growth period and can be severe without crop rotation (Pascual *et al.*, 2000, 2001). The infection begins at the base of the leaf sheath, and peak damage occurs during the periods from tasselling (VT) to grain filling. Initially, the leaf sheaths have dark green hygrophanous spots that gradually develop into cloud-shaped/wavy or irregular lesions from the bottom upwards. The lesions are brown with the colour gradually becoming darker from the inside to the

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outside; the lesions continue to expand and result in the rotting of the leaf sheaths. In severe cases, the stems rot and become lodged/broken (Jackson *et al.*, 2007; Woli *et al.*, 2014), and the ears and grains become infested, causing insufficient grain filling, which seriously affects the quality and yield of the maize (Hooda *et al.*, 2017).

At present, the methods for preventing and controlling banded leaf sheath blight in maize primarily include agricultural control, biological control and chemical control; of these, agricultural control refers to the comprehensive measures of agricultural technology adopted to prevent and control crop diseases, such as regulation of sowing time, intercropping, crop rotation, rational fertilization, etc. However, agricultural control has limited effectiveness and is time- and labour-consuming (Qiu *et al.*, 2010). Biological control has become an important area of research in plant protection in recent years. Tagele *et al.* (2018) found that multi-trait *Burkholderia contaminans* KNU17BI1 has great potential to control banded leaf sheath blight in maize caused by *R. solani* AG-1-IA, but the control is not ideal due to the growth environment constraints. Hence, chemical control is still the most important prevention and control method in agricultural production. A previous study showed that the control efficacy of 25% triadimefon wettable powder for banded leaf sheath blight in maize could reach 44.2% when a 200-fold solution is applied for soil disinfection (Li, 2003), and the control efficacy of 20% Jinggang mycin (AF) in fertilizer can exceed 80.1% (Teng *et al.*, 2008). In addition, triazole fungicides such as tebuconazole have been used (Malik *et al.*, 2018). Traditional control methods involve foliar spraying during the maize tasselling stage, which is limited by the height of the maize plants and is time-consuming and laborious. Thifluzamide is a succinate dehydrogenase inhibitor (SDHI) fungicide, which interferes with succinate ubiquinone reductase in the mitochondrial electron transport chain of fungi and it can be used as a foliar spray or for soil treatment and can be rapidly absorbed by plants (Mu *et al.*, 2017). Thifluzamide is primarily used to prevent and control diseases caused by *Rhizoctonia* spp. of the phylum Basidiomycota (Hu *et al.*, 2014; Mu *et al.*, 2017).

Maize seed coating technology has also been widely used in maize planting. Through seed coating, the active ingredients of fungicides/pesticides are slowly released, which can, to some extent, enhance plant resistance and promote plant growth (Pereira and Oliveira, 2005; Kunkur *et al.*, 2007) and thus have beneficial effects on maize (Avelar *et al.*, 2012). In China, thifluzamide has achieved good control as an agent against rice sheath blight. However, this effectiveness has not been documented for maize, and no study on the control of banded leaf sheath blight in maize by seed dressing with thifluzamide has been reported. Hence, the objectives of this study were to establish the baseline sensitivity of *R. solani* isolates to thifluzamide using the field isolates from Shandong Province of China, and determine the effects of seed treatment of thifluzamide on maize growth and control efficacy of banded leaf sheath blight in maize, to provide a basis for the scientific and reasonable application of thifluzamide to banded leaf sheath blight in maize.

Materials and methods

Fungal strains, maize variety and fungicide treatments

Fungal strains

In 2017–2018, diseased leaf sheaths, leaves and stalks of maize with evidence of banded leaf sheath blight were collected from six regions of Shandong, China: Tai'an (TA), Linyi (LY), Weifang (WF), Laiwu (LW), Rizhao (RZ) and Qingdao (QD) (detailed location information is shown in Table S1 of the Supplementary Material). Upon isolation and purification, 102 strains of *R. solani* in maize were obtained. The sampling fields were never exposed to any thifluzamide fungicide or other SDHIs. The identities of all isolates in the study were confirmed by morphology, phylogenetic analysis and pathogenicity testing. Isolates were held for long-term storage in cryogenic tubes with a 15% glycerol solution at −80°C. The maize variety used in this study was Zhengdan 958 (Henan Goldoctor Seed Co., Ltd., China). The fungicides included thifluzamide (a.i. 96%, TC, Shandong Kangqiao Bio-technology Co., Ltd.); tebuconazole (a.i. 94.7%, TC, Shandong Weifang Runfeng Chemical Co., Ltd.); thifluzamide (a.i. 24%, FS, made in the laboratory and containing 3% FS3000 (dispersing agent), 2% FS7PG (dispersing agent), 2% XG (xanthan gum), 56.5% deionized water, 2.5% magnesium aluminium silicate, 0.5% white carbon black, 0.5% LXC (wetting agent), 0.5% D625 (emulsifier), 0.5% EP60P (dispersing agent for oil phase) and 8% film former) and tebuconazole (a.i. 60 g/l, FS, Bayer CropScience, Co., Ltd., China).

Establishment of baseline sensitivity of Rhizoctonia solani to thifluzamide in maize

The mycelial growth rate method was used to determine the susceptibility of each of the 102 strains to thifluzamide, and a baseline sensitivity was established. The thifluzamide (0.5 g) was dissolved in acetone (10 ml) and was prepared as a 500-μg/ml stock solution with 0.1% Tween 80 and sterilized deionized water (990 ml). Using the stock solution for dilution, potato dextrose agar (PDA) plates amended with thifluzamide concentrations of 1, 0.5, 0.25, 0.125 and 0.0625 μg/ml were prepared; a PDA plate with the same volume of sterilized water was used as a control. A puncher (5 mm in diameter) was sterilized; mycelial plugs (5 × 5 mm) were cut from the periphery of 3-day-old colonies of each isolate, and the mycelial disc was transferred to a plate with the mycelia facing downwards. Four replicates were included for each treatment. The plates were incubated at 25°C for 4 days, and the colony diameter (minus the original diameter of the inoculation plug) was determined as the average of two perpendicular measurements. To calculate the mycelial growth inhibition rate, a toxic regression equation was established to obtain the half-maximal effective concentration (EC_{50}) value. The experiment was performed twice.

Safety test for maize

The safety test was designed with reference to 'Crop safety evaluation criteria for farm chemicals' and 'Indoor test methods for crop safety evaluation of seed treatment agents' (NY/T1965.3-2013, People's Republic of China Agricultural Industry Standard). The experimental setup was as follows: before seed sowing, fully developed maize seeds of uniform size were selected for disinfection and placed in sterilized river sand (60–70 mesh) within germination boxes (acrylonitrile butadiene styrene material, transparent, 360 mm × 29 mm × 12 mm) with the moisture content of the container controlled at 60–80%. For each treatment, 1 kg of seed was dressed uniformly and air-dried. The thifluzamide (24% FS) dosages were set at 192 g a.i./100 kg seed, 96 g a.i./100 kg seed, 48 g a.i./100 kg seed, 24 g a.i./100 kg seed, 12 g a.i./100 kg seed, 6 g a.i./100 kg seed and a control (CK). Thus, seven treatments were included with four replicates of 50 seeds per treatment. The germination boxes were maintained in a GXZ light incubator (Ningbo Jiangnan Instrument Factory, Zhejiang, China) at 28°C and kept under light for 14 h. The germination potential was calculated on day 4, and on the 7th day after establishment, the germination rate, seedling height, root length, root number and fresh plant weight were measured and the germination index (*Gi*) and vigour index (*Vi*) were calculated. The experiment was performed three times.

Germination potential: The percentage of normal germination seeds of maize in the number of tested seeds on the 4th day.

Germination rate: The percentage of normal germination seeds of maize in the number of tested seeds on the 7th day.

$$\text{Germination index } (Gi) = \sum \frac{Gt}{Dt} \tag{1}$$

$$\text{Vigour index } (Vi) = S\sum \frac{Gt}{Dt} = S \times Gi \tag{2}$$

Here, Gt is the number of germinated seedlings on the Tth day, Dt is the corresponding number of days needed for germination and S is the fresh weight per plant on the 7th day.

Greenhouse pot test

The greenhouse pot test included a total of six treatments: the 24% thifluzamide (FS) at dosages of 48 g a.i./100 kg seed, 24 g a.i./100 kg seed, 12 g a.i./100 kg seed, and 6 g a.i./100 kg seed; the control agent tebuconazole at a dosage of 12 g a.i./100 kg seed and the CK. The root activity and chlorophyll content of the maize were sampled at the three-leaf stage. The root activity was determined by the triphenyl tetrazolium chloride (TTC) reduction method of Bai *et al.* (1994). The TTC with 95% ethanol constant volume was used as the standard liquid, and the colorimetric measurement was conducted at 485 nm. The standard curve of the root activity was plotted. A 0.2 g sample of maize seedling root was then placed in a 25 ml test tube and 5 ml each of a 0.4% TTC solution and a 0.1 mol/l phosphate buffer (pH 7.0) were added; the tube was sealed and placed in the dark at 37°C for 1 h, and 2 ml of 1 mol/l H_2SO_4 was then added to terminate the reaction. The maize seedling roots were then placed in a test tube containing 10 ml of methanol, shaken in an incubator shaker (HZQ-F100) at 35°C and immersed for 6 h. Using methanol as a reference solution, the absorbance of the sample was measured at 485 nm. The TTC reduction intensity was calculated according to formula (3). The experiment was performed three times.

$$\text{TTC} = (\text{TTF} \times 1000)/(G \times T) \tag{3}$$

Here, TTC is the TTC reduction intensity (μg/g h), TTF is the TTC reduction amount (mg), G is the root weight (g) and T is the reaction time (h).

The chlorophyll concentration was determined using the extraction method of Ming *et al.* (Arnon, 1949; Ming *et al.*, 2007). Briefly, both sides of the maize leaf were rinsed and wiped dry, the leaf was cut up and 0.5 g of the leaf blades were placed into a test tube, and 15 ml of a 2 : 1 mixture of acetone and ethanol was added to each tube. The tubes were incubated at 25°C in the dark for 16 h; during this period, the mixture was shaken six times with a Vortex shaker (Cole-Parmer). The extract was then diluted five times, and the absorbance was measured at 649 and 665 nm to calculate the chlorophyll content using equations (4), (5) and (6). The experiment was performed three times.

$$C_a = 13.95 \times A_{665} - 6.88 \times A_{649} \tag{4}$$

$$C_b = 24.96 \times A_{649} - 7.32 \times A_{665} \tag{5}$$

$$\text{ChL} = (C \times V_T)/(\text{FW} \times 1000) \tag{6}$$

Here, C_a is the concentration of chlorophyll a (mg/l), C_b is the concentration of chlorophyll b (mg/l), ChL is chlorophyll content (mg/g), C is the pigment concentration (mg/l), V_T is the extraction volume (ml), FW is the fresh weight of sample (g) and A represents the absorbance at a certain wavelength.

Field fungicide trial

The test site was established at Tai'an City in Ningyang County in field plots where the incidence of sheath blight was high. The test plots had a total acreage of 1000 m^2. All other treatments, such as fertilizers, were used in accordance with standard farm practice. In the 2017 test, seed sowing occurred on 21 June, and harvest occurred on 24 September; in the 2018 test, seed sowing occurred on 19 June, and harvest occurred on 21 September. Seeding was done with a maize socket seeder (Zhengzhou Minle Agricultural Machinery Co., Ltd.) by first adjusting the sowing depth to 30 mm. Sowing was implemented using the single-seed dibble seeding method with two rows per film mulching, a plant spacing of 22 cm and a row spacing of 45 cm. The dosages of 24% thifluzamide (FS) included 48 g a.i./100 kg seed, 24 g a.i./100 kg seed and 12 g a.i./100 kg seed; the control fungicide tebuconazole was applied at a dosage of 12 g a.i./100 kg seed, and seed dressing treatments without thifluzamide served as a control. Thus, there were a total of five treatments in a randomized block design with three replicates per treatment, and each plot was 30 m^2. Due to the large sample size in the field trials, a diagonal five-sites random sampling was done to ensure that each sample taken was random. Maize seedlings were evaluated as follows. One week after planting, five sites were sampled in each plot, and 30 plants were surveyed at each site. On the 10th day after sowing, five sites were sampled in each plot, and 15 plants were excavated to quantify plant height, stem thickness, root length and the number of fibrous roots. The fresh plants were weighed, and the root-to-crown ratio was calculated. Before the maize was harvested, five sites were sampled in each plot, and 10 plants were brought back to the laboratory for quantification of ear length, ear thickness, number of rows of grain per ear, number of grains per ear and the 100-grain weight. The yield per plot and the yield increase rate were also calculated. The condition index of banded leaf sheath blight was evaluated at the small bell stage, large bell stage, tasselling and pollen-shedding stage, silking stage, milk-ripening stage and wax-ripening stage. In each plot, five sites were diagonally sampled, and 20 plants were surveyed at each site to determine the number of diseased plants and the disease grades. The disease rate, condition index and control effectiveness were calculated according to equations (7), (8) and (9), respectively. The disease grading was conducted according to the grading standards of the International Maize and Wheat Improvement

Table 1. Grading standard for maize sheath blight

Disease grade	Typical value	Grading standard
0	0	No disease incidence in the whole plant
1	1	Disease incidence at sheaths, at and above the 4th sheath below the ear position
2	3	Disease incidence at sheaths, at and above the 3rd sheath below the ear position
3	5	Disease incidence at sheaths, at and above the 2nd sheath below the ear position
4	7	Disease incidence at sheaths, at and above the 1st sheath below the ear position
5	9	Disease incidence at the ear position and at sheaths above the ear position

Center (CIMMYT) (Liu *et al.*, 2013) (Table 1).

$$D = (N_d/N) \times 100 \quad (7)$$

$$X = \sum (N_i \times i) \times 100/(N \times 9) \quad (8)$$

$$P = (X_1 - X_2)/X_1 \times 100 \quad (9)$$

Here, D is the diseased plant rate, N_d is the number of diseased plants, N is the total number of plants, X is the disease index, N_i is the number of diseased plants at various levels, i is the representative values at various levels, P is the control efficacy, X_1 is the disease index in the control group and X_2 is the disease index in the treatment group.

Data analysis

All data were analysed using the SAS statistical software package (version 9.2; SAS). The EC_{50} values were calculated from the sensitivity tests described above using the fitted regression line of the probit of the percent inhibition plotted against the $\log_{10}$-transformed fungicide concentration (Finney, 1971; Chen *et al.*, 2008). After analysis, Fieller's theorem was used to determine the standard errors (S.E.) and confidence intervals for the EC_{50} values (Finney, 1971). Data from the other experiments were analysed using analysis of variance (ANOVA), taking account of the design and treatment structure of the experiments. To detect differences between treatments, the means of the control efficacy were arcsine-transformed and then compared using Fisher's least significant difference test (LSD, $P < 0.05$). The POLYANOVA model allowed an assessment of factor by partitioning variance into linear and non-linear (quadratic) contrasts.

Results

Establishment of baseline sensitivity of Rhizoctonia solani to thifluzamide in maize

The sensitivity of 102 strains of *R. solani* in maize to thifluzamide was determined using the mycelial growth rate method. *R. solani*

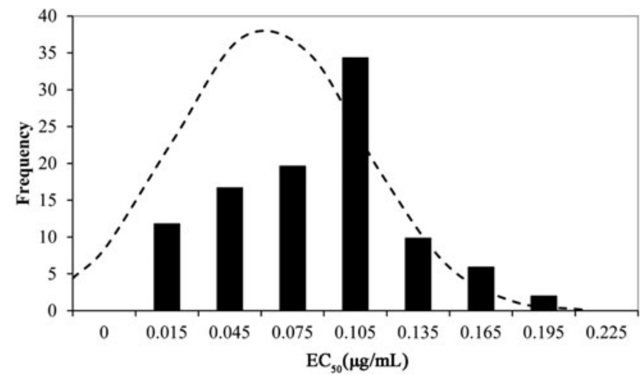

Fig. 1. Frequency distributions of 50% effective concentration (EC_{50}) of 102 *R. solani* in corn isolates treated with thifluzamide based on mycelial growth. EC_{50} values were calculated by performing a regression of the percentage relative growth against the $\log_{10}$ fungicide concentration.

was highly sensitive to thifluzamide, with an EC_{50} range of 0.0103–0.1942 and an EC_{50} average of 0.086 ± 0.004 μg/ml. The skewness = 0.298, kurt = −0.298 and P = 0.0884 (>0.05), which is consistent with a continuous skewed normal distribution, and the sensitivity frequency distribution had a continuous unimodal curve (Fig. 1) that can be used as the baseline sensitivity of *R. solani* in maize to thifluzamide in the Shandong region.

Safety of thifluzamide in maize

Thifluzamide (24% FS) was generally safe for maize, but excessive use (192 g a.i./100 kg seed) had an adverse effect on indicators, including plant height, root length, fresh mass, germination rate, shoot ratio, germination index and vigour index. When the dosage was 6–96 g a.i./100 kg seed, the maize was safe and promotes the emergence of maize seedlings to a certain extent, and the dosage of 12 g a.i./100 kg seed increased plant height, root length, root number, germination rate, shoot ratio and germination index. The dosage of 6 g a.i./100 kg seed had the most favourable effect on the fresh mass and vigour index (Table 2).

Effects of thifluzamide on root activity and chlorophyll content

Seed dressing with thifluzamide at 6–48 g a.i./100 kg seed improved the root activity and increased the chlorophyll content of maize seedlings; the dosages of 12 and 24 g a.i./100 kg seed had the most significant beneficial effect on root activity and outperformed the tebuconazole treatment; the dosages of 12 g a.i./100 kg seed increased the chlorophyll content better than other dosages (Figs 2 and 3).

Effect of thifluzamide on field emergence of maize

Three dosages of 24% thifluzamide (FS) increased the emergence rate and seedling growth of maize to varying degrees. Among them, in 2017 and 2018, the 24 g a.i./100 kg seed dosage had the most favourable effect on the seedling emergence rate, plant height, main root length, fibrous root number and plant fresh weight. In 2017, the seedling emergence rate was 13.12% higher than the control, and the plant height, main root length, fibrous root number and plant fresh weight were increased by 4.16 cm, 2.94 cm, 0.87 and 0.64 g, respectively. The dosage of 12 g a.i./100 kg seed had a greater promotional effect on stem thickness, which was 0.75 mm higher than that of the control (Table 3). Three doses of

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Table 2. Safety of thifluzamide in maize[a]

Dosage[b] (g a.i./100 kg seed)	Plant height (cm)	Root length (cm)	Root number (piece)	Fresh mass (g)	Germination rate (%)	Shoot ratio (%)	Germination index (%)	Vigour index (%)
192	8.0 ± 1.1[c]	7.2 ± 0.46	5.0 ± 0.21	1.2 ± 0.05	66.7 ± 1.3	1.5 ± 0.04	9.7 ± 0.08	11.2 ± 0.14
96	14.8 ± 0.76	11.7 ± 0.01	5.1 ± 0.05	1.7 ± 0.17	90.0 ± 1.7	1.7 ± 0.06	13.6 ± 0.09	22.7 ± 0.09
48	13.5 ± 0.45	13.0 ± 0.93	5.2 ± 0.71	1.7 ± 0.20	93.3 ± 1.4	2.0 ± 0.15	14.3 ± 0.07	24.1 ± 0.20
24	14.5 ± 0.01	13.5 ± 0.65	5.3 ± 0.32	1.7 ± 0.17	95.0 ± 2.1	1.9 ± 0.04	14.9 ± 0.02	25.4 ± 0.08
12	18.0 ± 0.02	13.8 ± 0.25	5.3 ± 0.23	1.8 ± 0.07	98.3 ± 1.0	2.1 ± 0.05	15.6 ± 0.01	27.4 ± 0.07
6	15.5 ± 0.26	13.4 ± 0.64	5.2 ± 0.15	1.8 ± 0.04	98.3 ± 1.3	1.9 ± 0.1	15.5 ± 0.01	28.4 ± 0.07
CK	13.1 ± 0.16	9.1 ± 0.24	4.4 ± 0.28	1.5 ± 0.07	76.7 ± 1.4	1.7 ± 0.12	12.3 ± 0.02	17.9 ± 0.21
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a]The experiments performed in the laboratory in 2017.
[b]'Dosage' means the effective concentration.
[c]Values are means ± s.e. analysed by Fisher's LSD test at $P < 0.05$.

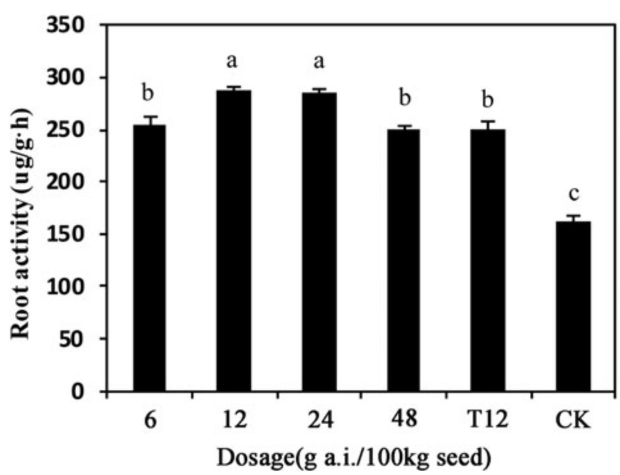

Fig. 2. Effect of seed dressing with thifluzamide on the root activity of maize seedlings. T_{12} represents a dosage of 12 g a.i./100 kg seed of the control fungicide tebuconazole. Values are means ± s.e.. Values with the same letter are without significant difference according to Fisher's LSD test at $P = 0.05$.

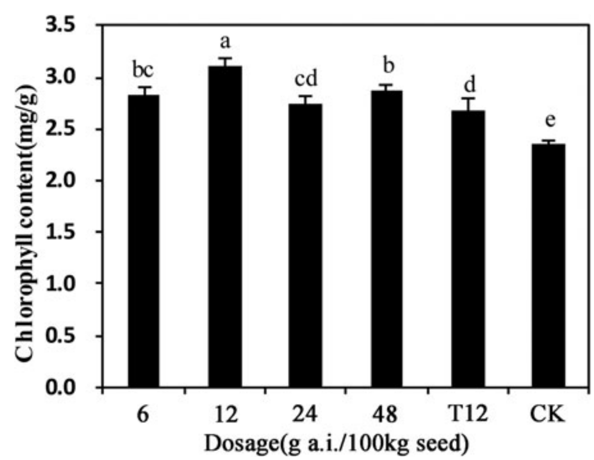

Fig. 3. Effect of seed dressing with thifluzamide on the chlorophyll content of maize seedlings. T_{12} represents a dosage of 12 g a.i./100 kg seed of the control fungicide tebuconazole. Values are means ± s.e.. Values with the same letter are without significant difference according to Fisher's LSD test at $P = 0.05$.

thifluzamide (FS) significantly increased the maize root-to-crown ratio, which was higher than under the tebuconazole treatment. The 2018 study validated the 2017 conclusions. Three dosages of 24% thifluzamide (FS) increased the emergence rate and seedling growth of maize to varying degrees (Table 4).

Effects of thifluzamide on maize yield

Three doses of thifluzamide increased the ear length, ear thickness, number of rows per ear and number of grains per ear in the field test of this study. The laboratory seed investigation showed that thifluzamide (FS) significantly increased the 100-grain weight of maize and the yield per plot. The 48 g a.i./100 kg seed treatment increased the 100-grain weight by 12.47% (2017) and 13.44% (2018) compared with the control, leading to a yield increase of 15.72% (2017) and 14.11% (2018) (Tables 5 and 6).

Effects of thifluzamide on the prevention of banded leaf sheath blight in maize in the field

In the field test of this study, we found that there were fewer incidences of banded leaf sheath blight in maize from the seedling stage to the large bell stage, during which the control effectiveness was high. The tasselling and pollen-shedding stage was the period of disease spread, with high temperature and humidity conditions that were conducive to the spread of sheath blight, and the maturity stage was the period when the disease surged abruptly. The two-year field trial showed that three doses of thifluzamide (FS) had effective control over banded leaf sheath blight in maize throughout the entire growth period and significantly reduced the incidence of banded leaf sheath blight in maize during the high-incidence period. Among these, the dosage of 48 g a.i./100 kg seed had the optimal field control effectiveness, with control rates during the small bell stage, large bell stage, tasselling and

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Table 3. Effect of thifluzamide on field emergence of maize (2017)[a]

Fungicide	Dosage[b] (g a.i./100 kg seed)	Emergence rate (%)	Plant height (cm)	Stem thickness (mm)	Main root length (cm)	Fibrous root number (piece)	Fresh weight (g)	Shoot ratio
Thifluzamide (FS) 24%	48	92.7 ± 1.2[c]	17.0 ± 0.05	2.9 ± 0.11	14.5 ± 0.15	3.5 ± 0.21	2.1 ± 0.15	0.57 ± 0.02
	24	95.6 ± 0.84	18.6 ± 0.08	3.1 ± 0.12	16.2 ± 0.07	3.9 ± 0.09	2.5 ± 0.20	0.61 ± 0.02
	12	93.8 ± 1.9	18.1 ± 0.05	3.2 ± 0.11	15.6 ± 0.16	3.8 ± 0.04	2.3 ± 0.23	0.55 ± 0.02
Tebuconazole (FS) 60 g/l	12	95.1 ± 1.7	17.0 ± 0.13	3.0 ± 0.03	15.4 ± 0.08	3.6 ± 0.15	2.3 ± 0.12	0.54 ± 0.02
CK	–	82.4 ± 1.9	14.5 ± 0.12	2.4 ± 0.09	13.3 ± 0.35	3.0 ± 0.13	1.9 ± 0.13	0.39 ± 0.02
P value	–	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.0293
Linear[d]	–	0.223	<0.001	0.003	0.004	<0.001	0.059	0.728
Quadratic[d]	–	0.097	<0.001	0.960	0.010	0.002	0.034	0.026

[a]The experiments performed in the field in 2017.
[b]'Dosage' means the effective concentration.
[c]Values are means ± s.e. analysed by Fisher's LSD test at $P < 0.05$.
[d]Linear effect of thifluzamide (FS) 24% dosage; quadratic effect of thifluzamide (FS) 24% dosage.

Table 4. Effect of thifluzamide on field emergence of maize (2018)[a]

Fungicide	Dosage[b] (g a.i./100 kg seed)	Emergence rate (%)	Plant height (cm)	Stem thickness (mm)	Main root length (cm)	Fibrous root number (piece)	Fresh weight (g)	Shoot ratio
Thifluzamide (FS) 24%	48	99.3 ± 0.33[c]	16.7 ± 0.21	2.9 ± 0.04	14.2 ± 0.00	3.4 ± 0.17	2.3 ± 0.08	0.58 ± 0.03
	24	97.3 ± 0.67	19.1 ± 0.24	3.2 ± 0.03	16.1 ± 0.04	4.4 ± 0.25	2.7 ± 0.08	0.62 ± 0.02
	12	92.7 ± 0.88	18.1 ± 0.34	3.1 ± 0.03	14.8 ± 0.14	3.8 ± 0.20	2.4 ± 0.09	0.55 ± 0.01
Tebuconazole (FS) 60 g/l	12	98.7 ± 0.33	17.4 ± 0.54	3.0 ± 0.01	15.6 ± 0.14	3.8 ± 0.25	2.7 ± 0.09	0.60 ± 0.01
CK	–	90.0 ± 0.58	14.8 ± 0.13	2.5 ± 0.00	13.9 ± 0.02	3.2 ± 0.10	2.2 ± 0.07	0.42 ± 0.02
P value	–	<0.01	<0.01	<0.01	<0.01	<0.01	0.0123	<0.01
Linear[d]	–	0.001	0.004	0.001	<0.001	0.068	0.020	0.729
Quadratic[d]	–	0.027	0.005	0.019	<0.001	0.011	<0.001	0.068

[a]The experiments performed in the field in 2018.
[b]'Dosage' means the effective concentration.
[c]Values are means ± s.e. analysed by Fisher's LSD test at $P < 0.05$.
[d]Linear effect of thifluzamide (FS) 24% dosage; quadratic effect of thifluzamide (FS) 24% dosage.

pollen-shedding stage, silking stage, milk-ripening stage and wax-ripening stage of 100, 66.7, 52.8, 67.8, 68.5 and 62.7% (2017), respectively, and 75, 63.2, 50.9, 53.6, 61.4 and 55.9% (2018). The disease rate in the plots treated with the seed dressing of thifluzamide was significantly higher during the period from the late wax-ripening stage to maize harvest than during other stages (Table 7).

Discussion

As a fungicide, thifluzamide inhibits the synthesis of succinate dehydrogenase (Sierotzki and Scalliet, 2013), thereby preventing pathogens from transporting electrons within their mitochondria (Sun *et al*., 2015), and inhibiting their growth (He *et al*., 2017). Studies have shown that thifluzamide has high inhibitory activity against *R. solani* and can be used as a more effective substitute for boscalid and Jinggang mycin to control sheath blight (Chen *et al*., 2012). Hence, we established the baseline sensitivity of *R. solani* in maize to thifluzamide, and found that the fungus was highly sensitive to this compound. Of the 55 fungicides listed by the Fungicide Resistance Action Committee (FRAC), the SDHI class has the fastest rate of growth among the new compounds that have been produced and marketed (Sierotzki and Scalliet, 2013). As an SDHI fungicide, thifluzamide has high biological activity, but it only has a single site of action, so there is a high risk of drug resistance (Ajayi-Oyetunde *et al*., 2016). A previous study found that the risk of resistance to thifluzamide is moderate in *R. solani*, which can develop resistance to quinone outside inhibitor fungicides, and the FRAC states that the use of this fungicide should be in accordance with the manufacturer's recommended

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Table 5. Effects of thifluzamide on maize yield (2017)[a]

Fungicide	Dosage[b] (g a.i./100 kg seed)	Ear length (cm)	Ear width (cm)	Row (numbers/ ear)	Ear grain number (grains/ear)	Hundred-grain weight (g)	Plot yield (kg)	Yield increase (%)
Thifluzamide (FS) 24%	48	22.9 ± 0.30[c]	17.1 ± 0.06	15.5 ± 0.38	516.6 ± 0.68	32.7 ± 0.25	25.8 ± 0.64	15.72
	24	21.2 ± 0.52	16.7 ± 0.08	15.3 ± 0.05	506.8 ± 3.9	31.5 ± 0.01	24.3 ± 0.29	9.18
	12	20.2 ± 0.22	16.4 ± 0.31	15.1 ± 0.31	508.5 ± 0.64	31.2 ± 0.46	24.0 ± 0.14	8.00
Tebuconazole (FS) 60 g/l	12	20.2 ± 0.21	17.1 ± 0.21	15.0 ± 0.34	507.9 ± 1.7	31.3 ± 0.10	23.4 ± 0.27	5.18
CK	–	17.8 ± 0.20	15.8 ± 0.20	14.7 ± 0.38	471.7 ± 2.9	29.1 ± 0.07	22.3 ± 0.63	–
P value	–	<0.01	<0.01	0.7812	<0.01	<0.01	<0.01	<0.01
Linear[d]	–	0.002	0.002	0.059	<0.001	0.001	0.047	<0.001
Quadratic[d]	–	0.817	0.324	0.495	0.002	0.318	0.295	0.034

[a]The experiments performed in the field in 2017.
[b]'Dosage' means the effective concentration.
[c]Values are means ± S.E. analysed by Fisher's LSD test at $P < 0.05$.
[d]Linear effect of thifluzamide (FS) 24% dosage; quadratic effect of thifluzamide (FS) 24% dosage.

Table 6. Effects of thifluzamide on maize yield (2018)[a]

Fungicide	Dosage[b] (g a.i./100 kg seed)	Ear length (cm)	Ear width (cm)	Row (numbers/ ear)	Ear grain number (grains/ear)	Hundred-grain weight (g)	Plot yield (kg)	Yield increase (%)
Thifluzamide (FS) 24%	48	22.7 ± 0.11[c]	17.9 ± 0.23	14.6 ± 0.34	516.5 ± 0.95	33.3 ± 0.23	26.3 ± 0.08	14.11
	24	20.4 ± 0.60	16.6 ± 0.16	14.5 ± 0.25	508.2 ± 1.1	32.4 ± 0.16	24.8 ± 0.29	7.50
	12	19.9 ± 0.24	16.3 ± 0.19	14.7 ± 0.37	504.3 ± 1.2	30.5 ± 0.09	24.3 ± 0.63	5.20
Tebuconazole (FS) 60 g/l	12	21.2 ± 0.23	17.4 ± 0.14	14.5 ± 0.07	509.7 ± 1.2	32.1 ± 0.13	25.1 ± 0.37	8.93
CK	–	17.7 ± 1.00	15.9 ± 0.10	14.7 ± 0.56	482.4 ± 1.3	29.3 ± 0.25	23.1 ± 0.16	–
P value	–	<0.01	<0.01	0.9827	<0.01	<0.01	<0.01	<0.01
Linear[d]	–	<0.001	<0.001	0.297	<0.001	<0.001	<0.001	<0.001
Quadratic[d]	–	0.224	0.013	0.892	<0.001	0.001	0.024	0.027

[a]The experiments performed in the field in 2018.
[b]'Dosage' means the effective concentration.
[c]Values are means ± S.E. analysed by Fisher's LSD test at $P < 0.05$.
[d]Linear effect of thifluzamide (FS) 24% dosage; quadratic effect of thifluzamide (FS) 24% dosage.

effective dose with particular attention to adhering to safety intervals (Li *et al*., 2011). In this study, we did not spray, and we reduced the number of fungicide applications, and the optimal dosage was determined in the indoor safety test and the greenhouse pot experiment using the seed dressing method. When the thifluzamide dosage (24% FS) was 6–96 g a.i./100 kg seed, seed coating with this fungicide was safe for maize. The field study found that the seed coating treatment at the dosage of 48 g a.i./100 kg seed had the highest field control effectiveness on banded leaf sheath blight in maize and could provide a theoretical basis for control using thifluzamide.

Many studies have shown that SDHI fungicides provide good health protection for plants and can promote crop growth and enhance the ability of crops to tolerate adverse environments. A previous study by De Lapeyre de Bellaire and Dubois (1997) showed that benodanil (a fungicide) can prevent and control diseases caused by *Rhizoctonia* in a variety of crops and can increase yield, and field trials have found that carboxin can stimulate wheat growth and increase yield (Gupta and Gajbhiye, 2004). When thifluzamide is applied at 240 g/l, rice leaves become broader, thicker and greener, and rice stalks exhibit enhanced toughness, which promotes robust growth. Worthing and Walker (1991) found that compound products such as Emesto and EverGol (the main active ingredient of both is penflufen) can improve crop viability, improve resistance in plants and increase crop quality. A greenhouse pot test in this study preliminarily determined the effects of seed coating using a thifluzamide suspension agent on the root activity and chlorophyll content of maize, and showed that the fungicide had a significant promotional growth effect and has further research value.

Thifluzamide has a strong adsorption capacity in the soil, but its adsorption intensity is weak with 19.5–54.0% digestion in 90 days (Gupta and Gajbhiye, 2004). In the field test of this study, the disease rate of banded leaf sheath blight in maize in each plot treated with thifluzamide (FS) was found to significantly increase after the late milk-ripening stage, but the effectiveness

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Table 7. Effects of thifluzamide on the prevention of maize sheath blight in the field in 2017 and 2018[a]

			2017			2018		
Growth period	Fungicide	Dosage[b] (g a.i./100 kg seed)	Disease rate (%)	Condition index (%)	Control effect (%)	Disease rate (%)	Condition index (%)	Control effect (%)
Small bell stage	Thifluzamide (FS) 24%	48	0 ± 0.00[c]	0 ± 0.00	100 ± 0.00	3.3 ± 0.43	0.4 ± 0.14	75 ± 0.32
		24	0 ± 0.00	0 ± 0.00	100 ± 0.00	5.0 ± 0.11	0.6 ± 0.01	62.5 ± 0.23
		12	1.7 ± 0.43	0.19 ± 0.14	66.9 ± 0.33	8.3 ± 0.18	0.9 ± 0.06	37.4 ± 0.56
	Tebuconazole (FS) 60 g/l	12	1.7 ± 0.43	0.19 ± 0.14	66.9 ± 0.44	6.7 ± 0.61	0.7 ± 0.20	50 ± 0.34
	CK	–	5 ± 0.55	0.56 ± 0.18	–	13.3 ± 0.27	1.5 ± 0.08	–
P value	–	–	0.364	0.365	<0.01	<0.01	<0.01	<0.01
Linear[d]	–	–	0.175	0.099	<0.001	<0.001	<0.001	<0.001
Quadratic[d]	–	–	0.211	0.142	<0.001	<0.001	<0.001	<0.001
Large bell stage	Thifluzamide (FS) 24%	48	5 ± 0.55	0.56 ± 0.07	66.7 ± 0.45	11.7 ± 0.40	1.3 ± 0.12	63.2 ± 0.26
		24	6.7 ± 0.18	0.74 ± 0.59	55.6 ± 0.20	11.7 ± 0.15	1.7 ± 0.12	52.7 ± 0.80
		12	6.7 ± 0.67	0.74 ± 0.63	55.6 ± 0.60	16.7 ± 0.34	1.9 ± 0.10	47.4 ± 0.42
	Tebuconazole (FS) 60 g/l	12	8.33 ± 0.33	0.93 ± 0.14	44.6 ± 0.36	26.7 ± 0.11	3 ± 0.03	15.8 ± 0.62
	CK	–	15 ± 0.45	1.7 ± 0.73	–	28.3 ± 0.21	3.5 ± 0.06	–
P value	–	–	0.449	0.245	<0.01	0.029	0.030	<0.01
Linear[d]	–	–	0.111	0.711	<0.001	0.007	0.013	<0.001
Quadratic[d]	–	–	0.660	0.913	0.027	0.014	0.966	0.700
Tasseling and pollen-shedding stage	Thifluzamide (FS) 24%	48	18.3 ± 0.24	3.2 ± 0.16	52.8 ± 0.33	26.7 ± 0.20	5.2 ± 0.93	50.9 ± 0.58
		24	20 ± 0.63	4.1 ± 0.22	38.9 ± 0.28	35 ± 0.45	6.5 ± 0.21	38.6 ± 0.54
		12	25 ± 0.32	5 ± 0.28	25 ± 0.53	33.3 ± 0.30	6.7 ± 0.21	36.9 ± 0.52
	Tebuconazole (FS) 60 g/l	12	21.7 ± 0.63	4.6 ± 0.21	30.6 ± 0.64	36.7 ± 0.19	7.8 ± 0.69	26.4 ± 0.25
	CK	–	30 ± 0.18	6.7 ± 0.25	–	45 ± 0.29	10.6 ± 1.62	–
P value	–	–	0.444	0.428	<0.01	<0.01	<0.01	<0.01
Linear[d]	–	–	0.109	0.487	<0.001	0.001	0.029	<0.001
Quadratic[d]	–	–	0.845	0.500	0.002	0.007	0.250	<0.001
Silking stage	Thifluzamide (FS) 24%	48	26.7 ± 0.20	5.2 ± 0.41	67.8 ± 0.42	31.7 ± 0.35	8.3 ± 0.33	53.6 ± 0.53
		24	41.7 ± 0.27	9.1 ± 0.18	43.7 ± 0.79	41.7 ± 0.33	10.6 ± 0.60	41.2 ± 0.64
		12	36.7 ± 0.26	9.3 ± 1.28	42.5 ± 0.62	38.3 ± 0.27	10.9 ± 1.19	39.2 ± 0.94
	Tebuconazole (FS) 60 g/l	12	41.7 ± 0.27	12 ± 1.29	25.3 ± 1.31	46.7 ± 0.27	14.8 ± 2.37	17.5 ± 0.57
	CK	–	55 ± 0.17	17 ± 1.92	–	51.7 ± 0.11	18 ± 0.76	–
P value	–	–	0.012	0.021	<0.01	0.045	0.011	<0.01
Linear[d]	–	–	<0.001	<0.001	<0.001	0.007	<0.001	<0.001
Quadratic[d]	–	–	0.001	<0.001	<0.001	0.025	0.004	<0.001
Milk-ripeness stage	Thifluzamide (FS) 24%	48	31.7 ± 0.65	5.4 ± 2.59	68.5 ± 0.23	48.3 ± 0.20	9.1 ± 0.09	61.4 ± 0.43
		24	41.7 ± 0.67	9.4 ± 2.25	44.6 ± 0.25	60. ± 0.20	13.7 ± 1.06	41.7 ± 0.35
		12	40 ± 0.34	9.6 ± 1.27	43.5 ± 0.60	53.3 ± 0.32	16.3 ± 0.38	30.7 ± 0.18
	Tebuconazole (FS) 60 g/l	12	48.3 ± 0.25	12 ± 1.52	29.4 ± 0.30	56.7 ± 0.10	17.8 ± 0.72	24.4 ± 0.12
	CK	–	60 ± 0.17	17 ± 0.79	–	61.7 ± 0.24	23.5 ± 2.34	–
P value	–	–	0.020	0.038	<0.01	0.042	0.015	<0.01

(*Continued*)

Table 7. (*Continued.*)

Growth period	Fungicide	Dosage[b] (g a.i./100 kg seed)	2017			2018		
			Disease rate (%)	Condition index (%)	Control effect (%)	Disease rate (%)	Condition index (%)	Control effect (%)
Linear[d]	–	–	<0.001	0.031	<0.001	0.035	0.042	<0.001
Quadratic[d]	–	–	<0.001	0.714	<0.001	0.627	0.114	<0.001
Wax-ripeness stage	Thifluzamide (FS) 24%	48	38.3 ± 1.67	9.8 ± 1.59	62.7 ± 0.36	51.7 ± 0.17	13.9 ± 1.83	55.9 ± 0.20
		24	53.3 ± 2.53	13.3 ± 2.19	49.3 ± 0.37	66 ± 0.28	21.5 ± 2.88	31.8 ± 0.18
		12	41.7 ± 1.95	13.5 ± 0.61	48.6 ± 0.14	53.3 ± 0.18	21.9 ± 0.65	30.6 ± 0.42
	Tebuconazole (FS) 60 g/l	12	50 ± 1.66	17.8 ± 1.04	32.4 ± 0.28	61.7 ± 0.21	24.6 ± 2.15	21.8 ± 0.42
	CK	–	60 ± 0.01	26.3 ± 0.48	–	66.7 ± 0.34	31.5 ± 1.08	–
P value	–	–	<0.01	<0.01	<0.01	0.047	0.042	<0.01
Linear[d]	–	–	<0.001	0.018	<0.001	0.021	0.030	<0.001
Quadratic[d]	–	–	<0.001	0.369	<0.001	<0.001	0.367	0.001

[a]The experiments performed in the field in 2017 and 2018.
[b]'Dosage' means the effective concentration.
[c]Values are means ± s.e. analysed by Fisher's LSD test at $P < 0.05$.
[d]Linear effect of thifluzamide (FS) 24% dosage; quadratic effect of thifluzamide (FS) 24% dosage.

was still higher than that of the blank control and the control fungicide. It can basically guarantee that no other pesticides will be applied to control banded leaf sheath blight in maize during the entire growth period. Jiang *et al.* (2000) stated that the control of banded leaf sheath blight in maize should be based on agricultural methods, and seed treatment with chemical agents should be the primary approach. A study by Xue *et al.* (2008) showed that the control effectiveness of banded leaf sheath blight in maize was significantly different when the fungicide application occurred during different growth stages and that the jointing stage was the best period for application. Using the traditional fungicide Jinggang mycin as an example, although two consecutive applications by spraying the leaf sheath in the early tasselling stage is effective, the application method is time-consuming, laborious and causes severe air pollution at high dosages that are unsafe for natural enemies, humans and livestock and has caused the chemical to be banned in many countries. In addition, spraying is ineffective for controlling soil-borne diseases and has a short duration of effectiveness. Furthermore, multiple applications are required, and the awareness of disease control is minimal among farmers. Therefore, it is necessary to develop efficient, safe, time-saving fungicides. In this study, the effectiveness of a thifluzamide suspension (FS) on banded leaf sheath blight in maize in the field was significantly greater than the seed dressing with the control fungicide, tebuconazole. Compared with traditional fungicidal agents and fungicide application methods, thifluzamide (FS) has the advantages of an increased utilization rate, precise application and reduced application frequency, all of which save seeds and fungicide, as well as reduced production costs and broad prospects for development.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0021859620000295.

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Conflict of interest. None.

Ethical standards. Not applicable.

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