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Brief Report

Fungicidal spectrum and biological properties of a new fungicide, pyriofenone

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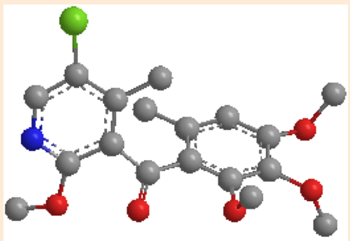
Supplementary material

Pyriofenone is a new fungicide developed by Ishihara Sangyo Kaisha, Ltd. To determine the fungicidal spectrum of pyriofenone, *in vivo* pot tests and *in vitro* mycelial growth-inhibition tests were conducted. Pyriofenone showed excellent activity against wheat and cucumber powdery mildew and moderate efficacy against rice blast in the pot tests. In the mycelial growth-inhibition tests, most fungi were not affected by pyriofenone except for *Botrytis cinerea*, *Helminthosporium sacchari*, *Pseudocercosporella herpotrichoides*, *Pyricularia oryzae*, *Rosellinia necatrix*, and *Verticillium dahliae*. The fungicidal properties of pyriofenone on powdery mildew in cucumber and wheat were evaluated precisely. Pyriofenone exhibited excellent preventive and residual activities. It had high rainfastness in the cucumber leaves against powdery mildew. Pyriofenone also showed inhibitory activity on lesion development upon application until 2 days after inoculation, and the lesion expansion and sporulation of the cucumber powdery mildew fungus were effectively controlled. Furthermore, pyriofenone showed translaminar and vapor activities.



Pyriofenone (IKF-309)

Keywords: pyriofenone, fungicidal spectrum, fungicidal properties.

Introduction

Fungi that cause powdery mildew affect many economically important crop species. They infect the leaves, stems, flowers, and fruit of nearly 10,000 species of angiosperms.[1)] During the cropping season of each crop, fungicides are sprayed many times to control powdery mildew. The widespread appearance of strains with increased tolerance to existing powdery mildew fungicides such as benzimidazoles, demethylation inhibitors (DMIs), or strobilurins in recent years has resulted in a significant drop in the efficacy of these active ingredients.[2)] Therefore, to avoid the development of fungicide-resistant strains, fungicides that have different modes of action are used in spray rotation programs.[3–5)] Consequently, there has been an urgent need for new mildewcides with novel modes of action to ensure the highly effective control of powdery mildew.[2)]

Pyriofenone, (5-chloro-2-methoxy-4-methyl-3-pyridyl) (4,5,6-trimethoxy-*o*-tolyl) methanone (code name: IKF-309; trade names: Property®, PROLIVO®, Kusabi®, Unicicut®), is a new fungicide developed by Ishihara Sangyo Kaisha, Ltd.[6–9)] It belongs to the aryl phenyl ketone fungicide group.[9)] Official tests conducted from 2008 by the Japan Plant Protection Association (JPPA) revealed that pyriofenone was efficient against powdery mildew diseases of wheat, cucumber, strawberry, and eggplant. It was introduced into the Japanese market in 2013 as a 26.8% w/w (300 g/L) suspension concentrate (SC) formulation.[10)]

In this paper, we describe the fungicidal spectrum of pyriofenone *in vivo* and *in vitro*, as well as the biological properties obtained from pot tests against powdery mildew in cucumber and wheat.

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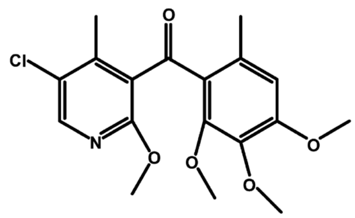

Fig. 1. Chemical structure of pyriofenone.

Materials and methods

1. Chemicals and formulations

The active ingredient or the SC formulation (300 g/L suspension concentrate for all crops except for wheat or 180 g/L suspension concentrate for wheat) of pyriofenone (Fig. 1) was used in this study.

2. Pot tests against various plant diseases

2.1. Plant diseases

Pot tests were used to evaluate the efficacy of pyriofenone in controlling various plant diseases listed in Supplemental Table S1; wheat powdery mildew, wheat glume blotch, wheat brown rust, cucumber powdery mildew, cucumber anthracnose, cucumber downy mildew, tomato late blight, kidney bean gray mold, and rice blast.

2.2. Application, inoculation, and evaluation

The active ingredient of pyriofenone was dissolved in acetone with 1% (v/v) Triton™ X-100 and suspended in water containing 0.2% (v/v) of the surfactant SHINDINE®. Test seedlings of wheat (cv. Norin 61), cucumber (cv. Sagami hanjiro), tomato (cv. Yellow pear), kidney bean (cv. Taisho kintoki), and rice (cv. Nipponbare) were sprayed with the test solutions until runoff using a handgun sprayer. After drying, the test plants were inoculated either by spraying aqueous spore suspensions (wheat glume blotch, cucumber powdery mildew, cucumber downy mildew, cucumber anthracnose, rice blast, and tomato late blight) or by dusting the spores on the leaves (wheat powdery mildew and brown rust). Mycelial discs or filter papers containing spore suspensions were placed on the detached leaves (kidney bean gray mold). The inoculated plants were incubated for 3–10 days at 20°C. Fungicidal activity was determined by visually observing the lesion area of the leaves.

3. Effect on mycelial growth of pathogenic fungi in vitro

3.1. Pathogens and cultures

Forty-two plant pathogenic fungi, which are listed in Supplemental Table S2, were used. All fungi collected in our laboratory were maintained on potato sucrose agar (PSA) at 20°C in the dark.

3.2. Effect on mycelial growth

Mycelial discs (4 mm in diameter) of test fungi, except for those of *Exobasidium reticulatum*, grown on PSA were cut from the margins of the colony and placed on PSA containing different concentrations of pyriofenone (0, 0.1, 1, 10, and 100 μg/mL). For testing of *E. reticulatum*, colonies on PSA were scraped and rubbed on PSA containing different concentrations of pyriofenone. After incubation at 20°C for 2–14 days, radial mycelial growth was measured. For *E. reticulatum*, mycelial growth was observed visually. The recorded mycelial growth was compared with that of the controls. The activity was expressed as the EC_{90} and EC_{50} (the concentrations inhibiting growth by 90% and 50%, respectively).

4. Pot tests against cucumber powdery mildew

4.1. Preventive activity

The SC formulation of pyriofenone was suspended in water containing 0.2% (v/v) of a surfactant SHINDINE®. Cucumber seedlings (cv. Sagami hanjiro, two-leaf stage) were sprayed on the adaxial surface of the first leaf with the test solutions using a handgun sprayer (20 mL/0.2 m^2; this water volume is equivalent to 1,000 L/ha). The treated seedlings were kept until the solutions were air dried. After the application and drying of the spray solution, the treated seedlings were inoculated by spraying with a conidial suspension of *Podosphaera xanthii* (5.0×10^5 conidia/mL) to the adaxial leaf surface and were incubated at 20°C for 10 days under fluorescent light. The fungicidal activity was evaluated by visually observing the lesion area and was expressed in terms of disease severity (0–100%). The control value was calculated from the following equation:

$$\text{Control value} = (1 - T/C) \times 100,$$

where T is the disease severity of the treated plot, and C is the disease severity of the untreated plot.

4.2. Residual activity

Cucumber seedlings were sprayed on the adaxial surface of the first leaf with the test solutions using the same method as described in section 4.1. After application and drying of the spray solution, the treated seedlings were kept in a glass house for 7 and 14 days before inoculation. Inoculation and evaluation were conducted using the same method described in section 4.1.

4.3. Rainfastness

4.3.1. Light rain condition test

Cucumber seedlings were sprayed on the adaxial surface of the

Table 1. Preventive activity and residual activity of pyriofenone on cucumber powdery mildew.

Application dose (μg/mL)	Preventive activity % conorol±S.D.[a]	Residual activity % conorol±S.D.[a]	
		7 days[b]	14 days[b]
100	NT[c]	NT	100
50	NT	100	100
25	NT	100	76±20
12.5	100	90±13	34±5
6.3	98±3	25±17	2±3
3.1	59±26	5±10	NT
1.6	44±27	NT	NT
0.8	31±22	NT	NT

[a] $n=8$. [b] Inoculation was carried out 7 or 14 days after treatment. [c] NT: Not Tested.

Table 2. Rainfastness of pyriofenone on cucumber powdery mildew.

Application dose (μg/mL)	% conorol±S.D.[a]				
	10 mm/hr		40 mm/hr		
	1 hr[b]	2 hr[b]	1 hr[b]	3 hr[b]	6 hr[b]
100	100	100	100	95±0	72±5
50	100	97±2	NT[c]	NT	NT
25	83±14	61±27	NT	NT	NT
12.5	44±26	18±14	NT	NT	NT
6.3	5±4	9±11	NT	NT	NT

[a] n=8 (10 mm/hr), n=4 (40 mm/hr). [b] Rainfall duration. [c] NT: Not Tested.

first leaf with the test solutions using the same method as described in section 4.1. One hour after application and drying of the spray solution, the seedlings were treated with artificial rain (10 mm/hr) for 1 or 2 hr using an artificial rain generator (Daiki Rika Kogyo, DIK-6000). Twenty-four hours after spraying, the seedlings were inoculated with the pathogen. Inoculation and evaluation were conducted using the same method as described in section 4.1.

4.3.2. Heavy rain condition test

Cucumber seedlings were sprayed with the test solutions using the same method as described in section 4.3.1. The seedlings were treated with artificial rain (40 mm/hr) 1 hr after application for 1 hr, 3 hr, and 6 hr continuously using an artificial rain generator. The seedlings were inoculated with the pathogen 24 hr after the artificial rain. Inoculation and evaluation were conducted using the same method as described in section 4.1.

4.4. Curative activity

The cucumber seedlings were inoculated by spraying a conidial suspension of *Podosphaera xanthii* onto the adaxial leaf surface and were incubated at 20°C under fluorescent light for 24 hr or 2 days before application. The application was conducted using the same method described in section 4.1. After application and drying of the spray solution, the treated seedlings were incubated for 8 or 9 additional days under the same conditions. The evaluation was conducted using the same method as described in section 4.1.

4.5. Effect on lesion expansion

The cucumber seedlings were inoculated with droplets of 10 μL of spore suspension of *Podosphaera xanthii* (5.0×10^5 conidia/mL) onto the adaxial leaf surface (six droplets/leaf). Seedlings were incubated at 20°C under fluorescent light for 6 days. The test solution was sprayed onto the adaxial and abaxial surfaces of the first leaves of inoculated seedlings with a handgun sprayer until runoff 6 days after inoculation. After application, the treated seedlings were incubated for 7 days under the same conditions. The diameter of each lesion was measured 6 days after inoculation and 7 days after application as the initial and final values, respectively. The values of lesion expansion were calculated by subtracting the initial value from the final value. The control value was calculated using the same equation as in section 4.1.

4.6. Effect on sporulation

Inoculation and application were conducted using the same method as described in section 4.5. The conidia were counted 6 days after inoculation as the initial value and 7 days after application as the final value. Three leaves were washed with water (20 mL), and the conidia were counted using a hemocytometer. The diameter of each lesion was measured at the same time.

4.7. Translaminar activity

The fungicides were sprayed on the adaxial or abaxial surfaces of the first leaves of the cucumber seedlings. Treated seedlings were incubated for 5 days in a greenhouse. The opposite side of the fungicide-treated surface was inoculated with a conidial suspension of *Podosphaera xanthii* (5.0×10^5 conidia/mL). Seedlings were incubated at 20°C under fluorescent light for 10 to 11 days. The evaluation was conducted using the same method as described in section 4.1.

5. Pot tests against wheat powdery mildew

5.1. Vapor activity

The SC formulation of pyriofenone was suspended in water containing 0.2% (v/v) of a surfactant SHINDINE®. Wheat seedlings (cv. Norin 61) were sprayed with the test solutions using a laboratory-scale track sprayer (90 g a.i./ha; water volume is equivalent to 200 L/ha). Fungicide-treated seedlings and untreated seedlings were placed in a plastic case, as shown in Supplemental Fig. S1, and were dusted with conidia of *Blumeria graminis*. The plastic case was covered with paper and incubated at 20°C for 7 days. The fungicidal activity was determined by visually observing the lesion areas of the leaves.

Results

1. Pot tests against various plant diseases

Pyriofenone's preventive activity against various plant diseases was investigated through pot tests. Pyriofenone showed excellent efficacy against wheat powdery mildew and cucumber powdery mildew. Efficacy against rice blast was detected at a high dose. There was no efficacy against other plant diseases in the pot tests (Supplemental Table S1). These results indicate that pyriofenone has excellent preventive activity against powdery mildew.

Table 3. Curative activity of pyriofenone on cucumber powdery mildew.

Application dose (μg/mL)	% conorol±S.D.[a]	
	1 DAI[b]	2 DAI[b]
25	NT[c]	97±5
12.5	100	96±5
6.3	97±7	77±16
3.1	80±21	43±26
1.6	37±26	17±10

[a] n=8. [b] Application after 1 day or 2 days after inoculation. [c] NT: Not Tested.

Table 4. Effect of pyriofenone on cucumber powdery mildew lesion expansion.

Trial No.	Number of evaluated lesion	Average value of expansion of lesion mm±S.D.[a]		Conotrol value
		pyriofenone treated[b]	untreated	
1	54	0.33±0.43	4.28±0.56	92.3
2	90	0.14±0.29	4.26±0.58	96.7
3	90	0.16±0.26	4.14±0.52	96.1
4	72	0.18±0.31	3.93±0.65	95.4
Ave.		0.20±0.32	4.15±0.58	95.2

[a] Trial 1; n=54, Trial 2; n=90, Trial 3; n=90, Trial 4; n=72. [b] Practical use dose rate; 100 μg/mL

2. Effect on mycelial growth and the fungicidal spectrum

The fungicidal activity of pyriofenone against various fungi was investigated using a mycelial growth-inhibition test. Pyriofenone did not exhibit strong inhibition of any fungal growth tested. The EC_{90} values of pyriofenone against 42 fungi were greater than 100 μg/mL (Supplemental Table S2). However, the EC_{50} values against *Botrytis cinerea*, *Helminthosporium sacchari*, *Pseudocercosporella herpotrichoides*, *Pyricularia oryzae*, *Rosellinia necatrix*, and *Verticillium dahliae* were less than 100 μg/mL (Supplemental Table S2).

3. Pot tests against cucumber powdery mildew

3.1. Preventive and residual activity

At 6.3 μg/mL, pyriofenone showed excellent preventive activity against cucumber powdery mildew (Table 1). To evaluate the residual activity of pyriofenone, inoculation was carried out after maintenance for 7 or 14 days in a glass house after treatment. Pyriofenone showed excellent residual activity at 12.5 μg/mL (7 days after treatment) and at 25 μg/mL (14 days after treatment) (Table 1).

3.2. Rainfastness

Under light rain conditions (10 mm/hr), pyriofenone exhibited excellent activity at 25 μg/mL with 1 hr of rain and at 50 μg/mL with 2 hr of rain (Table 2). The effects of heavy (40 mm/hr) and continuous (until 6 hr) rain were investigated next. Pyriofenone showed a high level of rainfastness at 100 μg/mL under these severe conditions. It exhibited good rainfastness even when long continuous rainfall (6 hr) was applied immediately after drying (Table 2).

3.3. Curative activity

In the curative activity test, pyriofenone showed excellent activity at 6.3 μg/mL (applied 24 hr after inoculation) and at 12.5 μg/mL (applied 2 days after inoculation) (Table 3). However, it did not inhibit lesion development after 3 days of inoculation (data not shown).

3.4. Effect on lesion expansion and sporulation

Pyriofenone treatment strongly suppressed lesion expansion. The average control value was 95.2% at 100 μg/mL (Table 4, Supplemental Fig. S2). Pyriofenone also effectively suppressed sporulation at the same dosage (Table 5). These results suggest that pyriofenone can control secondary infection and prevent spread of the disease.

3.5. Translaminar activity

Pyriofenone showed good translaminar activity from the abaxial to the adaxial surface of the leaf and from the adaxial to the abaxial surface of the leaf (Table 6). This activity is thought to be effective against powdery mildew fungi that have already developed in infected leaves.

4. Pot tests against wheat powdery mildew

4.1. Vapor activity

Pyriofenone showed excellent vapor activity with an inhibition rate of 96.3% at a distance of 0–6.5 cm from the treated pot, 81.5% at 6.5–13 cm, and 59.0% at 13–19.5 cm (Supplemental Fig. S1, Supplemental Table S3). This activity may result in good efficacy under conditions where a small volume of spray does not adequately cover the stems and leaves.

Discussion

Powdery mildew pathogens continue to infect a large number of important crops.[1,2] The widespread emergence of strains with increased tolerance to existing powdery mildew fungicides in recent years has resulted in a significant decrease in the efficacy of these active ingredients. Consequently, there has been an urgent need for new mildewcides with a novel mode of action.[2–5] Pyriofenone (code name: IKF-309; trade names: Property®, PROLIVO®, Kusabi®, Unicicut®) is a novel fungicide discovered and developed by Ishihara Sangyo Kaisha, Ltd.[6–9] It is a new compound based on the benzophenone moiety.[9] Official tests conducted since 2008 revealed that pyriofenone was effective against powdery mildew disease in wheat, cucumber, strawberry, and eggplant. It was introduced into the Japanese market in 2013 as SC formulation.[10]

In the present study, pot tests in a glass house and *in vitro* mycelial growth-inhibition tests were conducted to determine the fungicidal spectrum of pyriofenone. Pyriofenone showed excellent activity against powdery mildew in wheat and cucumber in a pot test. Although pyriofenone showed moderate effi-

Table 5. Effect of pyriofenone on cucumber powdery mildew sporulation.

	Initial value[a]	Final value[b]	
		pyriofenone treated[c]	untreated
Total number of conidia on 3 leaves (×10^4 cells)	9.2±2.4[d]	7±1.5	166.1±33.4
Total area of lesions on 3 leaves (mm^2)	187.4±11.7	216±17.6	858.6±67.9
Number of conidia per unit area (cells/mm^2)	495.7±135.0	323.1±60.5	1952.6±435.7

[a] Counting and measuring 6 days after inoculation. [b] Counting and measuring 7 days after application. [c] Practical use dose rate; 100 μg/mL was applied. [d] n=3.

Table 6. Translaminar activity of pyriofenone on cucumber powdery mildew.

Pyriofenone concentration (μg/mL)	% control±S.D.[a]	
	abaxial to adaxial	adaxial to abaxial
400	94±9	77±20
200	84±15	53±35
100	61±29	32±30
50	39±24	18±20

[a] $n=8$.

cacy against rice blast in the pot test, it had no activity against wheat glume blotch, brown rust, cucumber anthracnose, downy mildew, tomato late blight, and kidney bean gray mold (Supplemental Table S1). These results indicate that pyriofenone is specifically effective against powdery mildew on several important crops. On the other hand, in mycelial growth-inhibition tests, pyriofenone showed moderate antifungal activity against *B. cinerea*, *H. sacchari*, *P. herpotrichoides*, *P. oryzae*, *R. necatrix*, and *V. dahliae*, although most other fungi were not affected by pyriofenone (Supplemental Table S2). These findings are interesting because pyriofenone may have the ability to enhance the activities of other fungicides when they are mixed with pyriofenone.[10] The side effects of pyriofenone should be evaluated in detail in the future.

The fungicidal properties of pyriofenone in cucumber powdery mildew were evaluated in various pot tests. Pyriofenone exhibited excellent preventive and residual activity against cucumber powdery mildew (Table 1). It exhibited good rainfastness, even when long continuous rainfall was applied immediately after drying (Table 2). Pyriofenone showed inhibitory activity on lesion formation with application up to 2 days after inoculation (Table 3). Furthermore, pyriofenone controlled lesion expansion and sporulation of cucumber powdery mildew (Supplemental Fig. S2, Tables 4 and 5). These results suggest that pyriofenone can control secondary infection and prevent disease spread. These properties seem to be important for pyriofenone's good performance in the field.

Pyriofenone also showed good translaminar activity (Table 6). This activity is thought to be effective against powdery mildew fungi that have already developed in infected leaves, leading to good curative efficacy.[2] Pyriofenone showed excellent activity against powdery mildew in wheat and cucumber by good vapor action, which resulted in good performance even under conditions where a small volume of spray did not cover stems and leaves (Supplemental Table S3 and data not shown). Both good translaminar activity and vapor action also may lead to a high control effect, even in the case of uneven spraying.[10] In this study, pyriofenone showed good biological properties against powdery mildew in various pot tests. Based on these results, pyriofenone is expected to be useful in controlling powdery mildew in the field.

Powdery mildew attacks many economically important crop species.[1] To control powdery mildew, fungicides are sprayed many times during the cropping season of each crop. Generally, the causal pathogens of powdery mildew have high resistance risks to fungicides because they produce many conidia with a very short period of alternation of generations.[11,12] Therefore, to control powdery mildew and to avoid the development of fungicide-resistant strains, the use of fungicides with different modes of action in spray rotation programs is necessary.[2–5] In a preliminary study, the sensitivity of field isolates of cucumber powdery mildew fungus collected from various locations in Japan against pyriofenone and other commercial fungicides was monitored, and pyriofenone did not show cross resistance to triflumizole, azoxystrobin, and cyflufenamid,[10,13] which suggests that pyriofenone has a novel mode of action. Pyriofenone is thought to be a powerful tool for rotation spray programs. The monitoring of pyriofenone sensitivity should be evaluated more precisely in the future.

Electronic supplementary materials

The online version of this article contains supplementary materials (Supplemental Fig. S1, Fig. S2, Supplemental Table S1, Table S2 and Table S3), which are available at https:// www. jstage.jst.go.jp/browse/jpestics/.

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