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Original Article

Control efficacy of cyflufenamid in the field and its fungicidal properties*

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The control efficacy of a novel fungicide, cyflufenamid, (*Z*)-*N*-[α-(cyclopropylmethoxyimino)-2,3-difluoro-6-(trifluoromethyl) benzyl]-2-phenylacetamide was studied. In field trials, a low dosage (25 ppm) of cyflufenamid (10%WG) showed excellent efficacy in almost all plants against powdery mildew caused by various pathogens in agricultural production. Cyflufenamid also had high efficacy against brown rot in stone fruits caused by *Monilinia fructicola*. The fungicidal properties of cyflufenamid were investigated to elucidate the high performance of the compound in the field. Pot tests against cucumber powdery mildew indicated that cyflufenamid has excellent preventive, curative, long residual, translaminar and vapor phase activities at low concentrations.

Keywords: cyflufenamid, control efficacies, fungicidal properties, powdery mildew, brown rot.

Introduction

Cyflufenamid, (*Z*)-*N*-[α-(cyclopropylmethoxyimino)-2,3-difluoro-6-(trifluoromethyl)benzyl]-2-phenylacetamide (Code Name: NF-149, Pancho®),[1] is a novel fungicide currently in development by Nippon Soda Co., Ltd. (Nisso) (Fig. 1). Cyflufenamid shows excellent control activity against powdery mildew in various plants and brown rot in stone fruits.[2–6] Cyflufenamid belongs to a new fungicide class, amidoximes. Its biological mode of action against pathogens is unique and differs from those of commercial fungicides such as benzimidazole, demethylation inhibitor (DMI) and strobilurin.[4]

The application of cyflufenamid in the field has been registered in Asia and Europe.[6] The first registration of cyflufenamid was received in Japan in 2002. A mixture formulation of cyflufenamid with triflumizole called Pancho TF®, a water-dispersible granule (WG) formulation [cyflufenamid 3.4%+ triflumizole 15% (w/w)], was also developed to avoid the early appearance of resistant strains to the fungicide according to Nisso's resistance risk management strategies. The mixture formulation was registered for strawberry, cucumber, watermelon, eggplant and green pepper plants in Japan in 2003. Mixture formulations of cyflufenamid with DMI fungicides were proposed for registration in Korea in 2003. In Europe, cyflufenamid was submitted to the EU Annex I inclusion in 2003 and is now under evaluation. Its EW (emulsion in water) formulation (50 g/liter) was proposed for registration for cereals in the UK, Germany, France, Belgium and Romania.

This paper describes the control efficacy of cyflufenamid in the field against powdery mildew in various plants and brown rot in stone fruits, and also describes the fungicidal properties

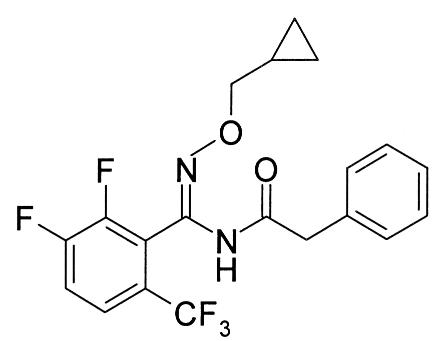

Fig. 1. Chemical structure of cyflufenamid.

* Studies on a novel fungicide, cyflufenamid (Part 2)
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of cyflufenamid, including its preventive, curative, residual, translaminar, translocative and vapor phase activities in pot tests against cucumber powdery mildew.

Materials and Methods

1. Chemicals and formulations

Cyflufenamid was synthesized and formulated as a 10% WG. Kresoxim-methyl [41.5% suspension concentrate (SC) and 47% WG], triadimefon [25% wettable powder (WP)], tebuconazole (40% SC), mepanipyrim (40% SC), tetraconazole [11.6% micro emulsion (ME)], bitertanol (25% WP), iprodione (50% WP and 40% SC) and triadimefon (5% WP) were purchased from commercial sources, and used as reference fungicides in the field and pot tests.

2. Field tests

2.1. Wheat powdery mildew

Seeds of winter wheat (*Triticum aestivum* cv. Chihoku) were sowed in a field at Bandai Agricultural Research Station, Nisso, Fukushima, Japan, on Oct. 2, 1996. Three replicates of $5\,m^2$ ($1\,m \times 5\,m$) per each plot were used. After the first symptoms of wheat powdery mildew by *Brumeria graminis* f. sp. *tritici* were observed on May 10, 1997, foliar spraying was performed on May 13 with a knapsack-type power sprayer with a water volume of 100 liters per 10 a. At that time, the growth stage of wheat was BBCH[7] (Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundessortenamt und Chemische Industrie) 37 (flag leaf just visible).

Disease severity in the field was evaluated on May 20, 27 and Jun. 9 using a disease index from 0 (no apparent symptoms) to 4 (severe disease). The disease degree (DD) of each plot was calculated by the following equation.

$$\mathrm{DD}=(S/4\times N)\times 100$$

S represents the sum of indices in the plot and N represents the number of plants in the plot. The results indicated the average infection degree in the three plots.

2.2. Strawberry powdery mildew

Strawberry sets (*Fragaria ananassa* cv. Nyohou) were bedded out in a greenhouse at Haibara Agricultural Research Center, Nisso, Shizuoka, Japan, on Nov. 2, 1997. The trial was laid out in randomized blocks with 3 replicates of 8 plants per plot. After the first symptoms of strawberry powdery mildew by *Sphaerotheca aphanis* var. *aphanis* were observed on May 5, 1998, foliar spraying was performed on May 8 (1 application), May 8 and 18 (2 applications), or May 8, 15 and 22 (3 applications), with a knapsack-type power sprayer with a water volume of 150 liters per 10 a. Disease severity in the field was evaluated on May 18, Jun. 1 and 16 with a disease rating from 0 (no apparent symptoms) to 4 (severe disease). The disease degree of each plot was calculated using the same formula as in Section 2.1. The results indicated the average infection degree in the three plots.

2.3. Cucumber powdery mildew

Cucumber seedlings (*Cucumis sativus* cv. Hokushin) were bedded out in the greenhouse at Haibara Agricultural Research Center, Nisso, Shizuoka, Japan, on Sep. 2, 2000. The trial was laid out in randomized blocks with 3 replicates of 10 plants per plot. Diseased cucumber pots inoculated with a strobilurin-resistant strain of *Sphaerotheca cucurbitae* as an inoculum were placed in the test field. After the first symptoms of cucumber powdery mildew were observed on Sep. 25, foliar spraying was performed on Sep. 26 (1 application) or Sep. 26 and Oct. 3 (2 applications), with a knapsack-type power sprayer with a water volume of 200 liters per 10 a. Disease severity in the field was evaluated on Oct. 3, 10, 17 and 25 with a disease rating from 0 (no apparent symptoms) to 4 (severe disease). The disease degree of each plot was calculated using the same formula as in Section 2.1. The results indicated the average infection degree in the three plots.

2.4. Peach brown rot

Eleven-year-old peach (*Prunus persica* var. *vulgaris* cv. Akatsuki) trees planted in a field at Bandai Agricultural Research Station, Nisso, Fukushima, Japan were used in the trial. The trial was laid out in randomized blocks with 3 replicates of 1 tree per plot. Foliar spraying was performed on Jul. 18, 29 and Aug. 9, 1997 with a knapsack-type power sprayer with a water volume of 400 liters per 10 a. The first symptoms of peach brown rot by *Monilinia fructicola* were observed on Aug. 15. After harvesting on Aug. 20, the fruit from each plot was stored in plastic containers at room temperature. Disease severity was evaluated on Aug. 22, 25 and 28 by counting the number of diseased peaches. The percentage of diseased fruit in each plot was calculated. The results indicated the average % diseased fruit in the three plots.

2.5. Cherry brown rot

Twelve-year-old cherry (*Prunus avium* cv. Koukanishiki) trees planted in a field at Bandai Agricultural Research Station, Nisso, Fukushima, Japan were used in the trial. The trial was laid out in randomized blocks with 2 replicates of 1 tree per plot. Foliar spraying was performed on Jun. 10 and 18, 1997 with a knapsack-type power sprayer with a water volume of 400 liters per 10 a. The first symptoms of cherry brown rot by *M. fructicola* were observed on Jun. 15. Disease severity was evaluated on Jun. 18 and 24 by counting the number of diseased cherries on the tree. The percentage of diseased fruit in each plot was calculated. The results indicated the average % diseased fruit in the two plots.

3. Pot tests

3.1. Preventive activity

Twenty-day-old cucumber seedlings (cv. Sagamihanjiro, 1.2 leaf stage) were grown in pots under standard conditions in the greenhouse. The test plants were sprayed with the test chemical solutions (at a range of concentrations) containing 0.01% Tween 20 on the adaxial surface of first leaves. After the solutions were air-dried, the treated plants were inoculated with spore dust of *Spaerotheca cucubitae*, and incubated at 20°C for 10 days under 12 hr light/12 hr dark. The evaluation of fungicidal activity was determined by observing the area of visible lesions and was expressed as the percentage of diseased leaf area (0 to 100%). The control value (CV) was calculated from the following equation.

$$CV=(1-T/C)\times 100$$

T represents the percentage of diseased leaf area in the treated seedlings and *C* represents the percentage of diseased leaf area in the non-treated seedlings. The results indicated the average CV in three replications.

3.2. Curative activity

The adaxial surface of the first leaves of cucumbers (cv. Sagamihanjiro, 1.2 leaf stage) was inoculated with spore dust of *S. cucubitae*, and incubated at 20°C under 12 hr light/12 hr dark. After 4 days of inoculation, the test plants were sprayed with chemical solutions prepared by the same method as in Section 3.1 on the adaxial surface of inoculated leaves. The test plants were incubated again at 20°C for 6 days under 12 hr light/12 hr dark. Fungicidal activity was evaluated using the same formula as in Section 3.1.

3.3. Residual activity

The adaxial surface of the first leaves of cucumbers (cv. Sagamihanjiro, 1.2 leaf stage) was sprayed with the test chemical solutions prepared by the same method as in Section 3.1. After the solutions were air-dried, the treated plants were kept in a greenhouse. After 10 days, the treated plants were inoculated with spore dust of *S. cucubitae*, and incubated at 20°C for 10 days under 12 hr light/12 hr dark. Fungicidal activity was evaluated using the same formula as in Section 3.1.

3.4. Translaminar activity

The adaxial surface of the first leaves of cucumbers (cv. Sagamihanjiro, 1.2 leaf stage) was sprayed with the test chemical solutions prepared by the same method as in Section 3.1. After the solutions were air-dried, the treated plants were kept in a greenhouse. After 10 days, the abaxial surface of the treated leaf was inoculated with spore dust of *S. cucubitae*, and incubated at 20°C for 10 days under 12 hr light/12 hr dark. Fungicidal activity was evaluated using the same formula as in Section 3.1.

3.5. Translocative activity

Cucumber seedlings (cv. Sagamihanjiro, 1.2 leaf stage) were removed from the pot and their roots were washed in water to remove soil and sand particles. The roots were then dipped into chemical solutions prepared by the same method as in Section 3.1 and incubated at 20°C for 5 days under 12 hr light/12 hr dark. The adaxial surface of the first leaves of treated seedlings was then inoculated with spore dust of *S. cucubitae*, and incubated at 20°C for 10 days under 12 hr light/12 hr dark. Fungicidal activity was evaluated using the same formula as in Section 3.1.

3.6. Vapor phase activity

Twenty-day-old cucumber seedlings (cv. Sagamihanjiro, 1.2 leaf stage) were used in this study. Chemical solutions (10 μl) prepared by the same method as in Section 3.1 were dropped onto small squares (5 mm×5 mm) of aluminum foil. After the solutions were air-dried, the squares of aluminum foil were placed onto the first leaves of seedlings. The seedlings were then inoculated with spore dust of *S. cucubitae*, and incubated at 20°C for 10 days under 12 hr light/12 hr dark. To evaluate the vapor action, the diameter (ϕ, mm) of the inhibition zone of lesion formation on the first leaf of each seedling was measured.

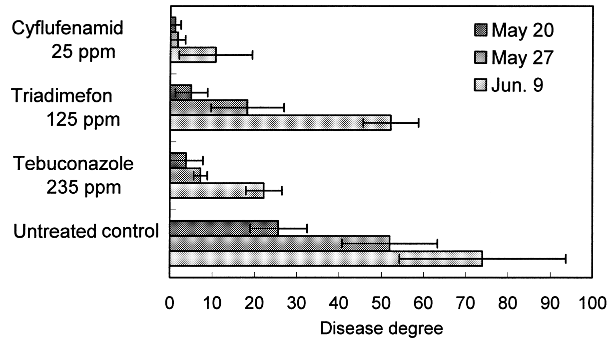

Fig. 2. Efficacy of cyflufenamid against wheat powdery mildew. Crop: Winter wheat (cv. Chihoku), 1×5 m/plot, 3 replications. Application: May 13, 1997 (100 liters/10 a). Assessment: May 20, 27 and Jun. 9, 1997. Bars indicate standard deviations (SD) from the mean (*n*=3).

Results

1. Field tests

1.1. Wheat powdery mildew

Cyflufenamid was applied in 1 foliar spray to wheat powdery mildew in the field. The disease degree in the plot of the untreated control reached over 70 at the last assessment (Jun. 9, 1997), showing that natural infection pressure was heavy. Cyflufenamid at 25 ppm (w/v) showed excellent control of wheat powdery mildew, and the efficacy was equivalent or superior to reference fungicides (Fig. 2).

1.2. Strawberry powdery mildew

Cyflufenamid at 25 ppm was applied in a foliar spray to strawberry powdery mildew in the greenhouse. Natural infection pressure was heavy, and the disease degree in the plot of untreated control was over 75 at the last assessment (Jun. 16, 1998). In this test, the efficacy of cyflufenamid with 1 or 2 applications was compared with reference fungicides with 3 applications. The efficacy of cyflufenamid at 25 ppm with 1 application was excellent, and was equivalent to reference fungicides. Two applications of cyflufenamid showed excellent efficacy even at 29 days after the last application (Fig. 3).

1.3. Cucumber powdery mildew

Cyflufenamid at 25 ppm was applied in a foliar spray to cucumber powdery mildew in the greenhouse. The test was carried out by artificial inoculation with a strobirulin-resistant strain. Infection pressure was heavy, and disease degree was

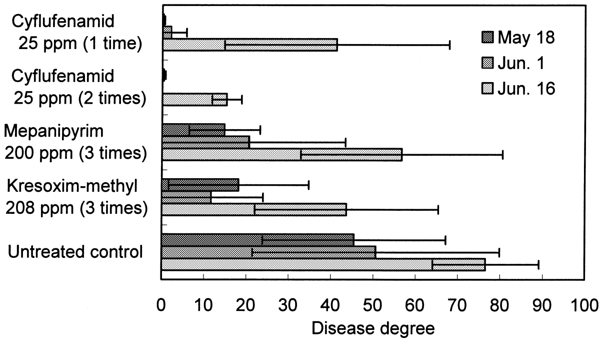

Fig. 3. Efficacy of cyflufenamid against strawberry powdery mildew. Crop: Strawberry (cv. Nyohou), 8 plants/plot, 3 replications. Application: May 8, (150 liters/10 a), May 8 and 18, or May 8, 15 and 22, 1998. Assessment: May 18, Jun. 1 and 16, 1998. Bars indicate standard deviations (SD) from the mean (n=3).

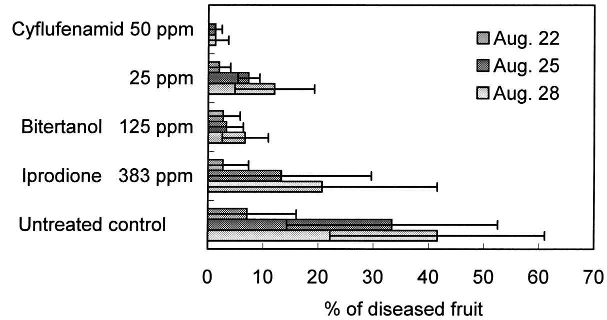

Fig. 5. Efficacy of cyflufenamid against peach brown rot. Crop: Peach (cv. Akatsuki), 1 tree/plot, 3 replications. Application: Jul. 18, 29 and Aug. 9, 1997 (400 liters/10 a). Assessment: Aug. 22, 25 and 28, 1997 (fruit harvested on Aug. 20). Bars indicate standard deviations (SD) from the mean (n=3).

over 60 at the last assessment (Oct. 25, 2000) in the plot of the untreated control. In this test, the efficacy of cyflufenamid with 1 or 2 applications was compared with reference fungicides with 2 applications. The efficacy of 1 application of cyflufenamid at 25 ppm was excellent, and was superior to that of tetraconazole at 39 ppm with 2 applications. Kresoxim-methyl at 208 ppm showed no effect against cucumber powdery mildew with 2 applications. Two applications of cyflufenamid showed excellent efficacy and cucumber powdery mildew lesions were hardly observed in the plot even at 22 days after the last application (Fig. 4).

1.4. Peach brown rot

Cyflufenamid at 25 ppm and 50 ppm was applied in a foliar spray to peach brown rot in the field. Diseased fruit was over 40% at the last assessment (Aug. 28, 1997) in the plot of the untreated control. Cyflufenamid at 25 ppm showed good control against peach brown rot. The efficacy of cyflufenamid was slightly inferior to bitertanol at 125 ppm, but was superior to iprodione at 383 ppm. The efficacy of cyflufenamid at 50 ppm was excellent, and was superior to the reference fungicides through the test period (Fig. 5).

1.5. Cherry brown rot

Cyflufenamid at 25 ppm and 50 ppm was applied in a foliar spray to cherry brown rot in the field. Infection pressure was heavy, and diseased fruit reached over 55% at the last assessment (Jun. 24 1997) in the plot of the untreated control. Cyflufenamid at 25 ppm showed good control against cherry brown rot. The efficacy of cyflufenamid was slightly inferior to bitertanol at 125 ppm, but was superior to iprodione at 400 ppm. The efficacy of cyflufenamid at 50 ppm was excellent, and was equivalent to that of bitertanol at 125 ppm throughout the test period (Fig. 6).

2. Pot tests

2.1. Preventive activity

Cyflufenamid exhibited excellent preventive activity against cucumber powdery mildew even at 0.2 ppm, which was markedly lower than that (3.1 ppm) of kresoxim-methyl (Table 1).

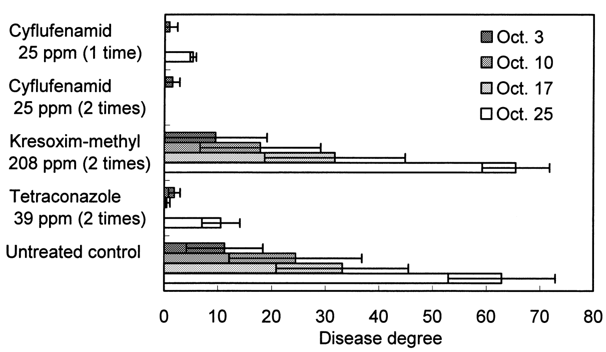

Fig. 4. Efficacy of cyflufenamid against cucumber powdery mildew. Crop: Cucumber (cv. Hokushin), 10 plants/plot, 3 replications. Plants were inoculated with a strobilurin-resistant strain, and symptoms appeared on Sep. 25, 2000. Application: Sep. 26, (200 liters/10 a) or Sep. 26 and Oct. 3, 2000. Assessment: Oct. 3, 10, 17 and 25, 2000. Bars indicate standard deviations (SD) from the mean (n=3).

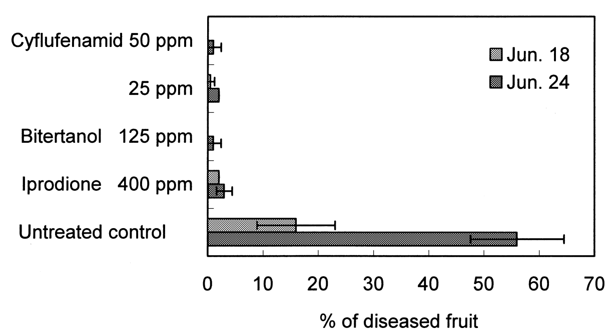

Fig. 6. Efficacy of cyflufenamid against cherry brown rot. Crop: Cherry (cv. Koukanishiki), 1 tree/plot, 2 replications. Application: Jun. 10 and 18, 1997 (400 liters/10 a). Assessment: Jun. 18 and 24, 1997. Bars indicate standard deviations (SD) from the mean (n=2).

Table 1. Preventive activity of cyflufenamid against cucumber powdery mildew[a)]

Chemical	Conc. (ppm)	Control value (%)
Cyflufenamid	3.1	100
	0.8	100
	0.2	99
	0.05	71
Kresoxim-methyl	12.5	100
	3.1	83
	0.8	62
	0.2	39
Untreated control		0

[a)] Inoculation was done immediately after the chemical solution was air-dried.

2.2. Curative activity
When cyflufenamid was applied 4 days after spore dust inoculation, cyflufenamid at 6.3 ppm showed excellent curative activity against cucumber powdery mildew. Kresoxim-methyl also showed curative activity at 6.3 ppm (Table 2).

2.3. Residual activity
To determine the residual activity, cucumber seedlings were kept for 10 days in a greenhouse after the application of cyflufenamid. Cyflufenamid at 6.3 ppm showed excellent residual activity against cucumber powdery mildew. Kresoxim-methyl showed residual activity at 12.5 ppm (Table 3).

2.4. Translaminar activity
The translaminar action (adaxial to abaxial) of cyflufenamid was evaluated. Cyflufenamid at 12.5 ppm exhibited excellent translaminar activity against cucumber powdery mildew. In contrast, kresoxim-methyl did not show translaminar activity even at 100 ppm (Table 4).

2.5. Translocative activity
The translocative action of cyflufenamid was evaluated using the root dipping method. Cyflufenamid scarcely showed translocative activity against cucumber powdery mildew even at 50 ppm. In contrast, triadimefon showed excellent translocative activity at 3.1 ppm (Table 5).

Table 2. Curative activity of cyflufenamid against cucumber powdery mildew[a)]

Chemical	Conc. (ppm)	Control value (%)
Cyflufenamid	12.5	100
	6.3	100
	3.1	69
Kresoxim-methyl	12.5	100
	6.3	87
	3.1	6
Untreated control		0

[a)] Chemical solutions were sprayed 4 days after inoculation.

Table 3. Residual activity of cyflufenamid against cucumber powdery mildew[a)]

Chemical	Conc. (ppm)	Control value (%)
Cyflufenamid	25	100
	12.5	100
	6.3	77
Kresoxim-methyl	25	100
	12.5	94
	6.3	0
Untreated control		0

[a)] Inoculation was done at 10 days after treatment with chemicals.

2.6. Vapor phase activity
Cyflufenamid showed excellent vapor phase activity against cucumber powdery mildew at 3.1 ppm, and was superior to that of kresoxim-methyl at 3.1 ppm (Table 6, Fig. 7).

Discussion

A novel fungicide, cyflufenamid, has excellent fungicidal activity against powdery mildews caused by various fungi and diseases caused by some limited fungi such as *M. fructicola* in the pot test or *in vitro* test as reported previously.[2–6] In this study, the field performance of cyflufenamid against powdery mildew and brown rot in stone fruits was investigated.

Cyflufenamid at 25 ppm showed excellent efficacy against wheat powdery mildew with 1 application, and the efficacy persisted even 27 days after application (Fig. 2). Cyflufenamid had long residual activity in the field at very low con-

Table 4. Translaminar activity of cyflufenamid against cucumber powdery mildew[a)]

Chemical	Conc. (ppm)	Control value (%)
Cyflufenamid	50	100
	25	100
	12.5	83
Kresoxim-methyl	100	0
	50	0
	25	0
Untreated control		0

[a)] Inoculation was done at 10 days after treatment with chemicals.

Table 5. Translocative activity of cyflufenamid from the root against cucumber powdery mildew[a)]

Chemical	Conc. (ppm)	Control value (%)
Cyflufenamid	50	25
	12.5	25
	3.1	13
Triadimefon	12.5	100
	3.1	81
	0.8	31
Untreated control		0

[a)] Inoculation was done at 5 days after treatment with chemicals.

Table 6. Vapor phase activity of cyflufenamid against cucumber powdery mildew[a)]

Chemical	Conc. (ppm)	Inhibition zone (ϕ, mm)
Cyflufenamid	50	41
	12.5	38
	3.1	33
Kresoxim-methyl	50	30
	12.5	29
	3.1	22
Untreated control		0

[a)] Inoculation was done at 10 days after treatment with chemicals.

Table 7. List of causal pathogens of powdery mildew controlled by cyflufenamid at 25 ppm in the field

Pathogen	Host
Blumeria graminis f. sp. *hordei*	Barley
B. graminis f. sp. *tritici*	Wheat
Erysiphe polygoni	Buckwheat
Microsphaera pulchra var. *japonica*	Dogwood
Oidiopsis sicula	Sweet pepper
Phyllactinia kakicola	Persimmon
P. moricola	Mulberry
Podosphaera leucotricha	Apple
Sphaerotheca aphanis var. *aphanis*	Strawberry
S. cucurbitae	Cucumber
S. fuliginea	Water melon
S. fuliginea	Eggplant
S. fuliginea	Melon
S. pannosa	Rose
Uncinula necator	Grape

centrations as compared with reference fungicides, triadimefon and tebuconazole. This result indicates that cyflufenamid might be effective for long interval application in the control of powdery mildews. This possibility was confirmed by field tests against strawberry and cucumber powdery mildews. The efficacy of cyflufenamid against strawberry powdery mildew with 1 and 2 applications was superior to reference fungicides with 3 applications (Fig. 3). One application of cyflufenamid also showed excellent control against cucumber powdery mildew surpassing 2 applications of reference fungicides (Fig. 4). This feature of cyflufenamid seems to be useful in agricultural production in the following 2 points, i) reducing

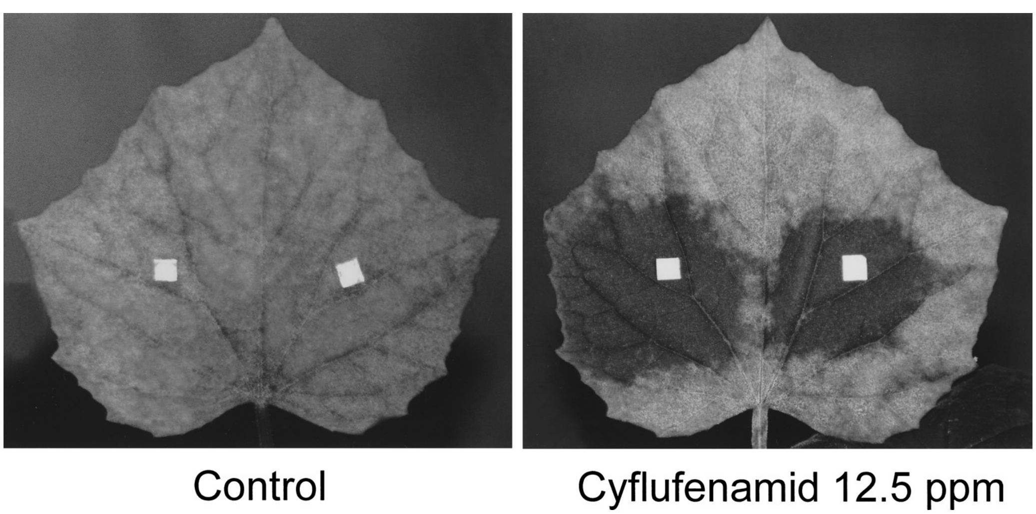

Fig. 7. Inhibition zone of lesions formed on cucumber leaves in the vapor phase activity test of cyflufenamid against cucumber powdery mildew.

the amount of fungicide used in the field and ii) saving the labor of farmers in controlling diseases.

In the cucumber powdery mildew trial, a strobilurin-resistant strain of *S. cucurbitae* was used as the inoculum. When kresoxim-methyl could not control cucumber powdery mildew, cyflufenamid showed excellent control. This result suggests that there is no cross resistance between cyflufenamid and strobilurins.

We had already confirmed the fungicidal activity of cyflufenamid against powdery mildews of various crops in the field (data not shown). As listed in Table 7, cyflufenamid at 25 ppm showed excellent control against powdery mildew caused by almost all genera of pathogens in agricultural production. Cyflufenamid at 25 ppm also exhibit excellent control against brown rot in stone fruits in the field (Figs. 5 and 6). The efficacy was almost equivalent to reference fungicides, bitertanol and iprodione. These results suggest that cyflufenamid is a highly effective fungicide against not only powdery mildew but also brown rot in stone fruits in practical use.

To elucidate the high performance of cyflufenamid in the field, the fungicidal properties of cyflufenamid were examined by pot tests against cucumber powdery mildew. Cyflufenamid showed excellent preventive, curative and long residual activities at low concentrations (Tables 1, 2 and 3), which are basically important for disease control. Cyflufenamid exhibited good translaminar activity in cucumber plants (Table 4). This property may participate in the excellent field performance, because chemical solutions are not always uniformly sprayed onto plants.[8] On the other hand, cyflufenamid did not show translocative activity from the root in contrast with the good translocative activity of triadimefon[9] (Table 5). Briggs *et al.*[10] reported that although chemical uptake by the root is greater in more lipophilic chemicals, translocation to the shoots is more efficient for compounds of intermediate polarity having a log Po/w between 1.5 and 2.0. The log Po/w value 4.70 of cyflufenamid[6] might be too high for translocation in plants. Cyflufenamid also showed vapor phase activity in spite of its low vapor pressure (3.54×10^{-5} Pa at 20°C)[6] (Table 6, Fig. 7). Small quantities of vaporizing cyflufenamid may be effective for control, because cyflufenamid has strong preventive activity at low concentrations. This property may also contribute to the excellent field performance.

In conclusion, the fungicidal activities of cyflufenamid are characterized by its preventive, curative, long residual, translaminar and vapor phase activities which lead to excellent disease control in the field. Therefore, cyflufenamid is a novel fungicide effective for the control of powdery mildew and brown rot in stone fruits.

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