Note

Control of Cucumber Downy Mildew by Cyazofamid

Shigeru MITANI,* Ken KAMACHI, Koji SUGIMOTO, Satoshi ARAKI and Tomona YAMAGUCHI

Central Research Institute, Ishihara Sangyo Kaisha, Ltd., 2-3-1 Nishi-Shibukawa, Kusatsu, Shiga 525-0025, Japan

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INTRODUCTION

Cucumber downy mildew caused by *Pseudoperonospora cubensis* (Berkeley et Curtis), Rostowzew is one of the most important diseases affecting field and glasshouse cucumber production around the world.^{1,2)} Heavy infection of foliage by this pathogen interferes with photosynthesis and respiration, leading to reduced fruit quality and yield. Although there are fungicides available for control of downy mildew, some protective fungicides need a high use rate to obtain satisfactory effects. Furthermore, in spite of the availability of various fungicides belonging to different chemical families, restrictions on the use of several fungicides have resulted in a growing need for new products with a different mode of action.

Cyazofamid (ISO proposed common name, 4-chloro-2-cyano-*N,N*-dimethyl-5-*p*-tolylimidazole-1-sulfonamide, development code: IKF-916, trade name: Ranman[®]) is a novel fungicide developed for the control of plant diseases caused by Oomycetes and clubroot disease (Fig. 1).^{3–9)} Due to its activity against a restricted specific spectrum of fungal pathogens, cyazofamid will reduce pressure on non-target and beneficial organisms. It also has a good fungicidal and environmental profile^{3,6,9)} and can be used in integrated pest management (IPM) programs.

Biochemical studies on the mode of action of cyazofamid clarified that it acts on mitochondrial cytochrome *bc_L* at the *Q_o* site.⁵⁾ Since this mode of action differs from those of other currently registered and commonly used fungicides, there is no cross-resistance between cyazofamid and other fungicides including the strobilurin-type fungicides (*Q_o* inhibitors) and the phenylamides.^{3–10)} Cyazofamid can therefore be a useful tool in resistant management strategies.

We previously reported that cyazofamid at 80 g a.i./ha exhibited excellent activity against all downy mildews tested in the

field,^{3,6,8)} but there was no data concerning the field evaluation of cyazofamid at the recommended use rate of 50–100 µg/ml against cucumber downy mildew. The purpose of this study was to evaluate the activity of cyazofamid against cucumber downy mildew at different rates and to compare it with the standard fungicides mancozeb and chlorothalonil in Japan.

MATERIALS AND METHODS

1. Fungicides

Cyazofamid was synthesized and formulated as a 100 g/l suspension concentrate (the formulation for the Asian market), and as a 400 g/l suspension concentrate (the formulation for the European and the American markets). Unless otherwise stated, the formulation used for the field tests was 100 g/l SC. Dimethomorph was synthesized and formulated as a 150 g/l emulsifiable concentrate in our laboratory. Chlorothalonil (40% suspension concentrate, Daconil 1000[®]) was purchased from SDS Biotech. Mancozeb (75% wettable powder, Ziman-Dithane[®]) was purchased from Rohm & Haas.

2. Plants

Cucumber (*Cucumis sativus* L. variety: Tokiwa 3-go P type) seedlings at the three true leaf stage, grown in polyethylene pots (diameter: 7.5 cm, 1 plant/pot), were transplanted into the soil of an open field. Natural infection by *P. cubensis* readily occurred and artificial infection was not conducted in any of the trials.

3. Location

Seven field tests were conducted in Kusatsu, Shiga, Japan from 1993 to 1997 and in 1999 (1993: 2 tests, 1994–1997 and 1999: 1 test/year). The field tests were laid down as randomized plots (2 × 2.4 m), with 2 replicates. Cucumber seedlings were planted with 5 or 7 plants/plot (Table 1).

4. Application

The application was made using a small plot sprayer (Sakata KHZ-2, Japan), and was carried out by the same 3 or 4 persons. Spray suspensions were prepared immediately before application. The equivalent of 720–1440 ml water volume/plot (equal

Fig. 1. Chemical structure of cyazofamid.

* To whom correspondence should be addressed.
E-mail: s-mitani@iskweb.co.jp

Table 1. Field trial conditions for evaluation of cyazofamid against cucumber downy mildew

Year	Application	Spray ^{a)} volume	Cyazofamid formulation (g/liter)	Duration ^{b)} of experiments	Number of plants/plot ^{c)}	Date of the first appearance of symptoms	Date of application	Fungicide application interval (days)	Date of evaluation
1993	Preventative	Standard	100	May 7- June 30	7	June 8	June 8, 15, 22	7	June 15, 22, 30
1994	Preventative	Standard	100	May 18- July 19	5	June 21	June 15, 22, 29, July 6, 13	7	June 28, July 5, 12, 19
1995	Preventative	Standard	100 and 400	May 9- June 28	7	May 30	May 30, June 6, 14, 21	7-8	June 6, 14, 21, 28
1996	Preventative	Standard	100	May 7- July 12	5	June 21	May 31, June 6, 13, 20, 27	6-7	July 5, 12
1997	Preventative	Standard	100	May 7- June 30	5	June 10	June 10, 17, 24 July 1	7-8	June 16, 23, 30
1999	Preventative	Low	400	May 12- June 25	5	June 15	June 4, 10, 21	6-11	June 21, 25
1993	Curative	Standard	100	May 7- July 15	7	June 8	June 17, 24, July 1, 9	7-8	June 24 July 2, 9, 15

^{a)} Standard: 1500-3000 l/ha, Low: 1000 l/ha.

^{b)} From the date cucumber seedlings (variety: Tokiwa 3-go P type) were transplanted to the date of final evaluation.

^{c)} Trials were laid down as randomized plots (2×2.4 m) with 2 replicates.

to 1500-3000 l/ha) was applied with a sprayer in the 1993-1997 tests. The water volume was adjusted to the growth stage of the plants. In the 1999 test, the equivalent of 480 ml water volume/plot (equal to 1000 l/ha) was applied to confirm the efficacy under low volume application. Details concerning field trial conditions are listed in Table 1.

5. Evaluation

An evaluation of fungicidal activity was made each time by the same 2 or 3 persons. The evaluation was conducted by observing the lesion area of all leaves visually. The disease intensity on a leaf was expressed in terms of a disease index (scale of 0 to 4), where 0: no disease, 1: 1-5% infested, 2: 6-25% infested, 3: 26-49% infested, 4: more than 50% infested. The disease severity of the plot was expressed by the following formula:

$$\text{Disease severity} = 100 \times (B + 2C + 3D + 4E) / 4(A + B + C + D + E)$$

where A, B, C, D and E represent the number of leaves rated at disease index 0, 1, 2, 3 and 4, respectively.

Crop tolerance was also assessed in all the trials before the application. Crop damage was rated on a scale of 0 (no damage) to 3 (complete crop destruction).

RESULTS AND DISCUSSION

1. Preventative Application Schedule

1.1. The standard spray volume application tests

1.1.1. Field test in 1993

Severe disease pressure occurred in 1993. The first symptoms

of downy mildew were observed on June 8 (disease severity: 1), thereafter the infestation spread rapidly. In the first, the second and the last evaluation (June 15, 22, and 30), disease severity in the untreated control was 21, 51 (data not shown in the tables) and 72 (Table 2), respectively. Even in the case of heavy infestation, cyazofamid at 50 and 100 µg/ml exhibited excellent control of downy mildew. The efficacy of cyazofamid at 50 and 100 µg/ml was superior to that of chlorothalonil at 500 µg/ml (Table 2).

1.1.2. Field test in 1994

The first symptoms of downy mildew appeared later than in the previous year, being observed on June 21 (disease severity: 1). In the first, the second, the third and the last evaluation (June 28, and July 5, 12 and 19), disease severity in the untreated control was 13, 36, 60 (data not shown in the tables) and 68 (Table 2), respectively. All the fungicides tested exhibited excellent control of downy mildew. The efficacy of cyazofamid at 50 and 100 µg/ml was equal to that of mancozeb at 1250 µg/ml. A dose-dependent effect of cyazofamid was not observed in the 1994 test (Table 2).

1.1.3. Field test in 1995

The first symptoms of downy mildew appeared earlier than in the previous two years (May 30, disease severity: 5). In the first, the second, the third and the last evaluation (June 6, 14, 21 and 28), disease severity in the untreated control was 24, 33, 58 (data not shown in the tables) and 65 (Table 2), respectively. All the fungicides tested exhibited some degree of fungicidal activity

Table 2. Effect of preventative treatment with cyazofamid on cucumber downy mildew at standard water volume applications^{a)} in 1993 to 1997

Compounds	$\mu\text{g/ml}$	Disease severity ^{b)}				
		1993 June 30	1994 July 19	1995 June 28	1996 July 12	1997 June 30
Cyazofamid (100 g/liter)	100	15 d	8 b	17 d	17 c	-
	50	32 c	8 b	30 c	25 bc	12 c
Mancozeb	1250	-	7 b	33 c	30 b	36 b
Chlorothalonil	500	53 b	-	43 b	-	-
Untreated control	-	72 a	68 a	65 a	76 a	75 a

^{a)} 1500–3000 l/ha.^{b)} Values followed by different letters in the same column are significantly different according to Duncan's multiple range test ($p=0.05$).

— : not tested.

against downy mildew (Table 2). Among them, cyazofamid at 100 $\mu\text{g/ml}$ gave the best control of the disease. The efficacy of cyazofamid at 50 $\mu\text{g/ml}$ was equal to that of mancozeb at 1250 $\mu\text{g/ml}$ and superior to that of chlorothalonil at 500 $\mu\text{g/ml}$ (Table 2).

In 1995, we compared the efficacy of the 100 g/l formulation with the 400 g/l formulation of cyazofamid. The 400 g/l formulation also gave excellent disease control. Disease severity with this formulation at 50 and 100 $\mu\text{g/ml}$ was 30 and 15, respectively (data not shown in the tables). There was no significant difference in fungicidal activity between the 100 g/l (Table 2) and the 400 g/l formulation at the same application rate of active ingredient.

1.1.4. Field test in 1996

Severe disease pressure occurred in 1996, however the disease severity on June 21 was not so high (disease severity: 1). The infestation spread rapidly thereafter. In the first and the last evaluation (July 5 and 12), disease severity in the untreated control was 58 (data not shown in the tables) and 76 (Table 2), respectively. All the fungicides tested exhibited excellent control of downy mildew (Table 2). Among them, cyazofamid at 100 $\mu\text{g/ml}$ provided the best control. The efficacy of cyazofamid at 50 $\mu\text{g/ml}$ was superior or equal to that of mancozeb at 1250 $\mu\text{g/ml}$.

1.1.5. Field test in 1997

In 1997, severe disease pressure was observed as in 1996. The first symptoms of downy mildew were observed on June 10 (disease severity: 1), thereafter the infestation spread rapidly. In the first, the second and the last evaluation (June 16, 23 and 30), disease severity in the untreated control was 2, 49 (data not shown in the tables) and 75 (Table 2), respectively. Cyazofamid at 50 $\mu\text{g/ml}$ exhibited excellent control of downy mildew, which was superior to that of mancozeb at 1250 $\mu\text{g/ml}$ (Table 2).

1.1.6. Overview of the tests in 1993–1997

Throughout the field tests from 1993 to 1997, cyazofamid at 50 and 100 $\mu\text{g/ml}$ exhibited excellent activity in comparison with the standard fungicides, mancozeb at 1250 $\mu\text{g/ml}$ and chlorothalonil at 500 $\mu\text{g/ml}$, against cucumber downy mildew (Table 2). These cyazofamid concentrations (50–100 $\mu\text{g/ml}$) are the registered use rates in Japan,^{4,6,8,9)} and are 12.5 to 25 times lower than that of mancozeb and 5 to 10 times lower than that of chlorothalonil.

1.2. The low volume water application test

1.2.1. Field test in 1999

Generally, growers apply pesticides onto vegetables (including cucumbers), with lower spray volumes in Europe and the Americas (water volume: ca. 500–1000 l/ha), than in Japan (water volume: ca. 1500–3000 l/ha).^{11,12)} Therefore, we evaluated cucumber downy mildew control with cyazofamid using a low volume foliar application (water volume: 1000 l/ha). A 400 g/l formulation of cyazofamid was used in the test under severe disease pressure.

The first symptoms of downy mildew were observed on June 15 (disease severity: 1), and thereafter the infestation spread rapidly. In the first and the last evaluation (June 21 and 25), disease severity in the untreated control was 57 (data not shown in the tables) and 81 (Table 3), respectively. Even in the case of heavy infestation, cyazofamid at 80 g a.i./ha (80 $\mu\text{g/ml}$) exhibited excellent preventative efficacy (Table 3), which was superior to that of chlorothalonil at 1125 g a.i./ha (1125 $\mu\text{g/ml}$). The efficacy of cyazofamid was remarkably enhanced when the compound was tank-mixed with 150 ml/ha of an organosilicone surfactant. It is well known that some organosilicone surfactants such as Makupika (KF-640) and Silwet L-77 have excellent wetting properties.^{13,14)} We tentatively conclude that this property results in an improved coverage leading to a higher level of control by cyazofamid.

Table 3. Effect of preventative treatment with cyazofamid on cucumber downy mildew at low water volume applications^{a)} in 1999

Compounds	g a.i./ha	$\mu\text{g/ml}$	Disease severity ^{b)}
			June 25, 1999
Cyazofamid (400 g/liter)	80	80	28 c
Cyazofamid (400 g/liter) + surfactant ^{c)} (150 ml/ha)	80	80	14 d
Chlorothalonil	1125	1125	34 b
Untreated control	-	-	81 a

^{a)} 1000 l/ha.^{b)} Values followed by different letters are significantly different according to Duncan's multiple range test ($p=0.05$).^{c)} Organosilicone surfactant, Silwet L-77.

2. Curative Application Schedule

2.1. Field trial in 1993

Severe disease pressure occurred in 1993. The first symptoms of downy mildew were observed on June 8 (disease severity: 1), and thereafter the infestation spread rapidly. Fungicide applications started on June 17 when disease severities reached 33–41, at 9 days after the first appearance of the symptoms of downy mildew. Cyazofamid at 50 $\mu\text{g/ml}$ clearly suppressed the development of the disease compared with the untreated control and the protective fungicide chlorothalonil (Fig. 2). Although the efficacy of the curative fungicide dimethomorph^{15,16)} was similar to that of cyazofamid in the 1st and the 2nd assessment (June 24

and July 2), the activity was inferior to that of cyazofamid in the last assessment (July 15). This result is consistent with our previous report on pot tests.⁸⁾ However, in order to fully benefit from the very strong preventative activity of cyazofamid,^{3,8,9)} it should be applied before the pathogen enters the crop.⁸⁾

3. Crop Tolerance in 1993–1997 and 1999

Beside the assessment for efficacy, all the aforementioned field tests were also used to assess crop tolerance to the applications. In none of these trials were any adverse effects on leaves or fruits observed with repeated application at up to 100 $\mu\text{g/ml}$ cyazofamid (data not shown in the tables). We also reported that no adverse effects from cyazofamid at 400 $\mu\text{g/ml}$ (single application, 4 times higher than the practical use rate) were seen on cucumbers, potatoes, tomatoes, grapevines and onions.³⁾ Furthermore, no adverse effects from cyazofamid at 100 $\mu\text{g/ml}$ have been seen on any adjacent crops among a range of 39 different crops tested.⁶⁾ Cyazofamid can be tank-mixed with most commonly used insecticides, fungicides and acaricides, and therefore, will be a useful tool in pest management systems.^{3,6,8)}

4. Conclusion

Cyazofamid is highly effective against cucumber downy mildew at very low doses (50–100 $\mu\text{g/ml}$). Its low, effective use rates will reduce the total amount of active ingredient applied to cucumbers. Cyazofamid will be utilized as part of an IPM program that will provide for total disease control as well as disease resistance management strategies in cucumber fields.

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Fig. 2. Effect of curative application of cyazofamid on cucumber downy mildew.

Fungicide: —●— cyazofamid (50 $\mu\text{g/ml}$), —■— dimethomorph (250 $\mu\text{g/ml}$), —▲— chlorothalonil (500 $\mu\text{g/ml}$), —○— None (untreated control).

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