J. Gen. Plant Pathol. 66 : 353-359 (2000)

Sensitivity of *Fusarium moniliforme* Isolates to Ipconazole

Hideaki TATEISHI[1]* and Tsuneaki CHIDA[1]

ABSTRACT

To estimate the sensitivity of *Fusarium moniliforme* to ipconazole, a sterol biosynthesis inhibitor (SBI), minimum inhibitory concentrations (MIC) were determined for isolates which were collected before the launch of ipconazole as a rice seed disinfectant. Research institutes from various prefectures in Japan supplied 211 isolates (group I) from their collections, and 84 isolates (group II) were isolated from rice paddy fields in Iwaki, Fukushima Prefecture. In group I, the MIC ranged from 0.10 to 6.25 μg/ml with a peak at 0.39 μg/ml. In group II, MIC values had the same range as group I, but the main peak was at 0.20 μg/ml. Ipconazole sensitivity did not differ significantly among groups I and II. Though the ranges of MIC values for ipconazole, pefurazoate and triflumizole were different in 60 isolates randomly chosen from group I, positive correlations were observed in their sensitivities to SBIs, suggesting a common mechanism in *F. moniliforme* for lowering sensitivities to SBIs. Among the 14 isolates tested, isolates with MIC values lower than 0.78 μg/ml for ipconazole were pathogenic to rice seedlings, and all the isolates with MIC values higher than or equal to 1.56 μg/ml were not pathogenic in the nursery test. Good protection against isolates causing "Bakanae" disease was obtained by dipping seeds for 24 hr in ipconazole. The pathogenic isolates can be controlled by the seed treatment with the practical dosage of ipconazole because of the adequate margin between the highest MIC value for the pathogenic isolates and the treatment concentration. In addition, the low or lack of pathogenicity of the isolates less sensitive to ipconazole may also contribute to the stable efficacy of ipconazole.

(Received November 8, 1999 ; Accepted April 18, 2000)

Key words : ipconazole, seed disinfectant, rice "Bakanae" disease, *Fusarium moniliforme*, sensitivity.

INTRODUCTION

Ipconazole (code name : KNF-317, trade name : Techlead®, (1*RS*, 2*SR*, 5*RS* ; 1*RS*, 2*SR*, 5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-1-(1*H*-1, 2, 4-triazol-1-ylmethyl)-cyclopentanol), a sterol biosynthesis inhibitor (SBI), is used as a seed disinfectant for controlling important seed-borne diseases of rice such as "Bakanae" disease (caused by *Fusarium moniliforme*), blast (*Pyricularia oryzae*), and Helminthosporium leaf spot (*Cochliobolus miyabeanus*). It is also effective against rice seedling blight caused by *Rhizopus* spp. and *Trichoderma viride*. Antifungal properties of ipconazole against these diseases were reported previously[18].

Benzimidazole fungicides have been widely used to treat rice seeds in Japan but in the 1980s, strains of *F. moniliforme* resistant to benzimidazoles appeared[8,15,17]. Since 1986, SBIs, such as triflumizole, pefurazoate and prochloraz, have been introduced as rice seed disinfectants effective against benzimidazole-resistant isolates. However, weakly resistant isolates to triflumizole were found concomitantly with reduced efficacy of triflumizole[4,5]. The resistant isolates and decreased efficacy of ipconazole have not been reported until now.

For any specific fungus, resistance to a fungicide should be determined by comparing the baseline sensitivity of the fungus to the fungicide. Therefore, the importance of surveying the sensitivity of a pathogenic fungus before launching a fungicide has been emphasized[7]. Concerning the sensitivity to triflumizole in relation to the pathogenicity of *F. moniliforme* isolates, Hamamura *et al.*[3] reported that less sensitive isolates (MIC values more than 1000 μg/ml) of *F. moniliforme* were poor producers of gibberellin, which coincided with lower pathogenicity. Wada *et al.*[19] reported that the MIC values of pefurazoate ranged between 0.78 and 12.5 μg/ml with one main peak at 1.56 μg/ml. Isolates moderately sensitive to pefurazoate (MIC value more than 6.25 μg/ml) also produced less gibberellin. These studies imply that the sensitivity of *F. moniliforme* to SBIs is related to pathogenicity.

This paper describes the sensitivity to ipconazole before its practical use, gibberellin productivity, pathogenicity of *F. moniliforme* field isolates and the efficacy of seed treatment with ipconazole against several isolates.

[1] Nishiki Research Laboratories, Kureha Chemical Industry Co., Ltd., Nishiki, Iwaki 974-8686, Japan
* Corresponding author (E-mail : h-tateishi@kureha.co.jp)

Relationships between sensitivities to ipconazole, pefurazoate and triflumizole are also discussed.

MATERIALS AND METHODS

Isolates Two-hundred eleven *F. moniliforme* isolates that had been collected from 22 prefectures in Japan (group I) were kindly supplied by various national institutes for agricultural research, prefectural agricultural experiment stations and the Life Science Research Institute of Kumiai Chemical Industry Co., Ltd. In addition, 84 isolates were isolated from rice plants with "Bakanae" disease collected from nine paddy fields in Iwaki city from June to August in 1993 (group II). In these fields thiram-benomyl or benomyl was used as a seed disinfectant but the application record of SBIs was not clear. The origins of these isolates are shown in Table 1. All the isolates were collected before the practical use of ipconazole in 1994.

Isolation of *F. moniliforme* from rice plant Stems of diseased rice plants were cut into 3 to 4 cm lengths starting about 5 cm above the water surface. The segments were sterilized with a 20-fold diluted sodium hypochlorite solution for 1 min and washed three times with sterilized water. Then they were placed on Komada's *Fusarium*-selective medium[9] and incubated at 25°C for 10 days. The mycelial mat was transferred onto potato dextrose agar (PDA, Nissui) medium and incubat-

Table 1. Origin of *F. moniliforme* isolates

Group and code of isolates	Number of isolates[a]	Prefecture	Cultivar	Isolated part of rice plant	Year of isolation
<Group I>					
HKD 1	1	Hokkaido	not identified	not specified	before 1991
AMR 93-1 to 5	5	Aomori	Mutsukaori	elongated stem	1993
AKT 1	1	Akita	not identified	not specified	before 1991
IWT 88-1, 2	2	Iwate	not identified	elongated stem	1988
IWT 92-1 to 17	17	Iwate	Akitakomachi *etc.*	elongated stem	1992
YMG 92-1 to 4	4	Yamagata	not identified	elongated stem	1992
MYG 92-1 to 19	19	Miyagi	Satohonami	elongated stem	1992
MYG 93-1	1	Miyagi	Satohonami	elongated stem	1993
IBR 1 to 10	10	Ibaraki	not identified	not specified	before 1989
TCG 91-1, 2	2	Tochigi	not identified	elongated stem	1991
TCG 92-1, 2	2	Tochigi	not identified	elongated stem	1992
SZK 93-1	1	Shizuoka	Harebare	seed	1993
NGN 92-1 to 15	15	Nagano	not identified	elongated stem	1992
NGT 89-1 to 3	3	Niigata	Niigatawase	seed	1989
TYM 91-1 to 10	10	Toyama	not identified	elongated stem	1991
GIF 89-1 to 3	3	Gifu	Takayamamochi *etc.*	elongated stem	1989
MIE 92-1 to 7	7	Mie	Koshihikari	elongated stem	1992
OKY 91-1 to 10	10	Okayama	Kibinohana *etc.*	elongated stem	1991
OKY 93-1 to 13	13	Okayama	Koshihikari *etc.*	elongated stem	1993
HRS 93-1 to 5	5	Hiroshima	Nakateshinsenbon *etc.*	elongated stem	1993
SMN 86-1, 2	2	Shimane	not identified	elongated stem	1986
SMN 91-1 to 16	16	Shimane	Yashiromochi	elongated stem	1991
TTR 88-1 to 20	20	Tottori	not identified	elongated stem	1988
KGW 91-1 to 4	4	Kagawa	not identified	seed	1991
KGW 1	1	Kagawa	not identified	not specified	before 1991
EHM 92-1, 2	2	Ehime	Nihonbare *etc.*	elongated stem	1992
EHM 93-1, 2	2	Ehime	Akitakomachi *etc.*	elongated stem	1993
FKK 81-1	1	Fukuoka	not identified	not specified	1981
KMM 92-1 to 8	8	Kumamoto	Reihou *etc.*	elongated stem	1992
KGS 93-1 to 24	24	Kagoshima	not identified	not specified	1993
(Group I total : 211 isolates)					
<Group II>					
IWK 93-1 to 84	84	Fukushima	Nihonbare etc.	elongated stem	1993

a) Number of isolates includes isolates which were isolated from naturally infected seeds in our laboratory.

ed at 25°C for 7 days. Monospores, for which typical single-cell chains of conidia (anthophilum) and subglutinants were confirmed by microscopic observation, were isolated and identified as *F. moniliforme*[10,11].

Determination of MIC values MIC values were determined by the method of Irie and Inoue[6]. Formulations of ipconazole 6% wettable powder (WP), pefurazoate 20% WP and triflumizole 15% emulsifiable concentrate (EC) were each suspended in sterilized water and added to PDA medium to a 10% volume to obtain the predetermined concentrations. *F. moniliforme* isolates were pre-incubated on PDA medium at 25°C for 7 days. A mycelial disk (4 mm in diameter) of each isolate was placed on the PDA plate containing a fungicide, and mycelial growth was measured to determine the MIC after 5 days at 25°C. In this study, marginal growth of mycelia (less than 1 mm from the edge of disk) was regarded as no growth.

Determination of gibberellin activity Gibberellin activity in shake cultures of *F. moniliforme* isolates was determined by Nishijima's method[14] using dwarf rice cultivar, Tanginbozu, as described previously[18].

Evaluation of pathogenicity to rice plants and efficacy of seed treatments Two isolates were randomly chosen from each MIC class of group I for culturing in potato dextrose (PD) liquid medium at 28°C for 5 days. The conidia were collected and suspended in water to 5.0×10^6/ml. One-hundred ml of the conidial suspension was sprayed on two pots of rice plants (cultivar : Tanginbozu, 3 hills per 1/5000 a Wagner pot) at the flowering stage. The plants were then kept in a greenhouse until harvest. The infected seeds were dipped in the test solutions for 24 hr. Methods for evaluating pathogenicity and efficacy of the treatments were described previously[18].

RESULTS

Sensitivity of *F. moniliforme* isolates to ipconazole

The MIC-distribution profile of ipconazole for group I indicated the sensitivity in *F. moniliforme* (Fig. 1). MIC values ranged from 0.10 to 6.25 μg/ml with one peak at 0.39 μg/ml and a shoulder at 3.13 μg/ml.

MIC values for group II were distributed in the same range as group I, but with the main peak at 0.20 μg/ml and a minor peak at 6.25 μg/ml (Fig. 2). The main peak of group II indicated a slightly higher sensitivity than that of group I, but group II had almost the same profile as group I.

In groups I and II, the highest MIC value was 6.25 μg/ml, and none of the isolates had extremely low sensitivity.

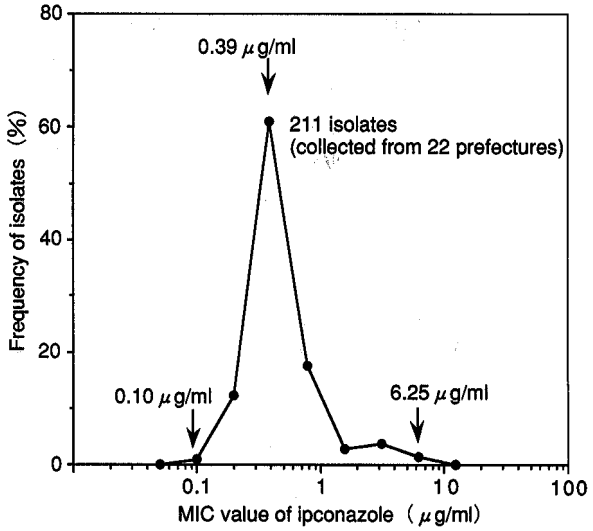

Fig. 1. Sensitivity distribution of *F. moniliforme* isolates from 22 prefectures (group I) to ipconazole.

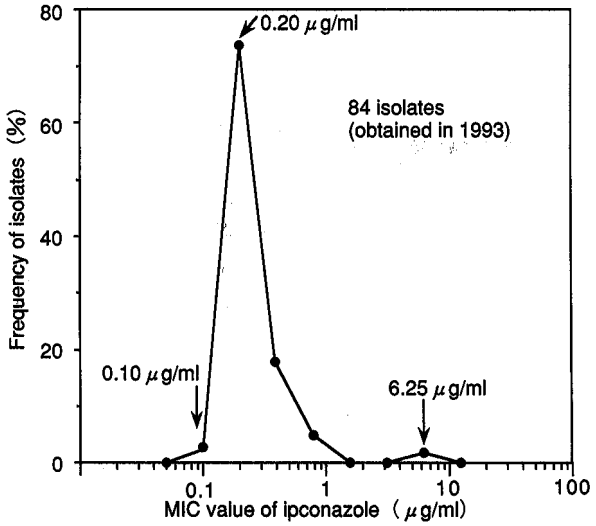

Fig. 2. Sensitivity distribution of *F. moniliforme* isolates from Iwaki city (group II) to ipconazole.

Relationship between sensitivities to ipconazole and triflumizole or pefurazoate

MIC values for triflumizole and pefurazoate were determined on 60 randomly chosen isolates from group I. Similar to ipconazole, the sensitivity distribution to pefurazoate had one main peak at 1.56 μg/ml with the lowest MIC value at 0.39 μg/ml and the highest at 50 μg/ml. This profile was identical to that reported by Wada *et al.*[19], except for a minor peak at 50 μg/ml. The sensitivity distribution to triflumizole had two or more peaks with a wider range of MIC values but the majority of MIC

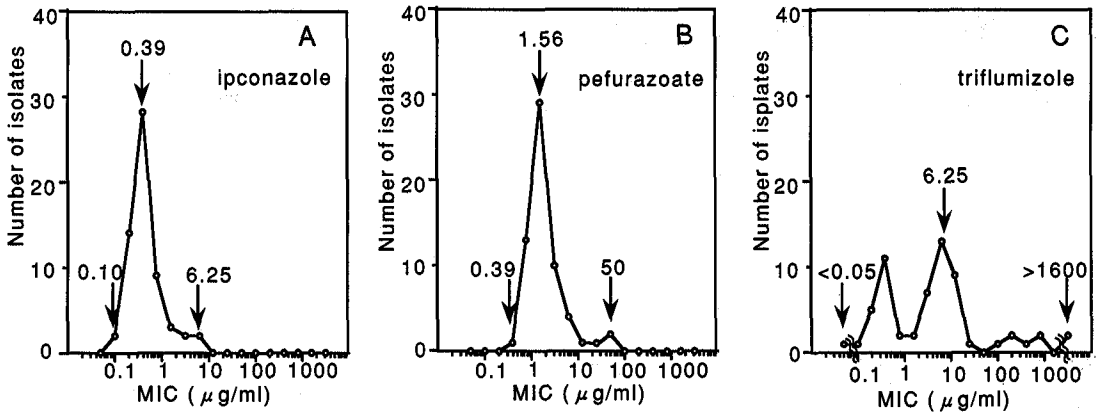

Fig. 3. Sensitivity distribution of *F. moniliforme* to three SBIs. Sensitivity distribution to (A) ipconazole, (B) pefurazoate and (C) triflumizole for 60 isolates randomly chosen from group I.

values were lower than 12.5 μg/ml. Sensitivity distributions to these three SBIs tended to cluster in a high sensitivity area, but the ranges of MIC values differed from each other (Fig. 3). Sensitivities (MIC values) to ipconazole and the other two SBIs were a significantly correlated. Correlation coefficients were 0.934 between ipconazole and pefurazoate, and 0.668 between ipconazole and triflumizole (Fig. 4). In the case of triflumizole, three isolates having MIC values lower than 0.05 μg/ml or higher than 1600 μg/ml for triflumizole were not included in the calculation, the correlation coefficients could be underestimated. The isolates having MIC values higher than 6.25 μg/ml for pefurazoate tended to have high MIC values for triflumizole.

Productivity of gibberellin and efficacy of ipconazole in seed treatment

Fourteen isolates (two isolates from each MIC class of group I) were assayed for their productivity of gibberellin and pathogenicity (Table 2). Isolates with MIC values lower than 0.39 μg/ml for ipconazole, produced gibberellin, but isolates with MIC values higher than or equal to 1.56 μg/ml, produced little or no gibberellin. One of two isolates with an MIC of 0.78 μg/ml produced gibberellin, but the other isolate did not. The same result was also obtained using group II (data not shown).

When seeds infected with these 14 isolates were grown in nursery boxes, elongation, the typical symptom of "Bakanae" disease, and damping-off were observed only on the seedlings infected with isolates producing gibberellin. On the contrary, typical symptoms were not observed on seedlings infected with isolates producing little or no gibberellin. The 200-fold, 24-hr dip treatment with ipconazole 6% WP resulted in high efficacy against all pathogenic isolates, as did pefurazoate. The efficacy of triflumizole was reduced against the pathogenic isolates to which MIC values were 3.13 and 6.25 μg/ml (Table 2).

DISCUSSION

MIC values of ipconazole were determined for *F. moniliforme* isolates collected before the practical application of ipconazole. The MIC value ranged from 0.10 to 6.25 μg/ml in group I (collected from 22 prefectures). The most frequent MIC value was 0.39 μg/ml for more than 60% of isolates of group I. In group II (obtained from Iwaki city), the main MIC peak indicated a slightly higher sensitivity than in group I, but its profile was almost the same as that of group I.

Ipconazole was launched as a seed disinfectant in 1993 ; its practical use started in 1994. Because SBI was used as a rice-seed disinfectant in 1986[13] for the first time in Japan, some of the isolates used in this study might have come into contact with SBIs. In regard to the year the isolates were obtained, the sensitivity distribution demonstrated here may not be an exact baseline of sensitivity to ipconazole, but it could represent the sensitivity before the practical use of ipconazole.

Though the range of MIC values for ipconazole, pefurazoate and triflumizole differed, a majority of the isolates were in the high sensitivity area in the sensitivity profiles. Because of a shoulder or minor peak in the sensitivity profiles to ipconazole, isolates with MIC values of 1.56 to 6.25 μg/ml for ipconazole could be defined as less sensitive. Isolates with MIC values of 6.25 to 12.5 μg/ml for pefurazoate were defined as moderately sensitive[19], and isolates with MIC values higher than 1000 μg/ml for triflumizole were regarded as less sensitive[3]. Most of the isolates less sensitive to ipconazole were less sensitive to pefurazoate and triflumizole.

The sensitivities to ipconazole, pefurazoate and triflumizole were positively correlated. The relationship between the frequency of the less sensitive isolates and

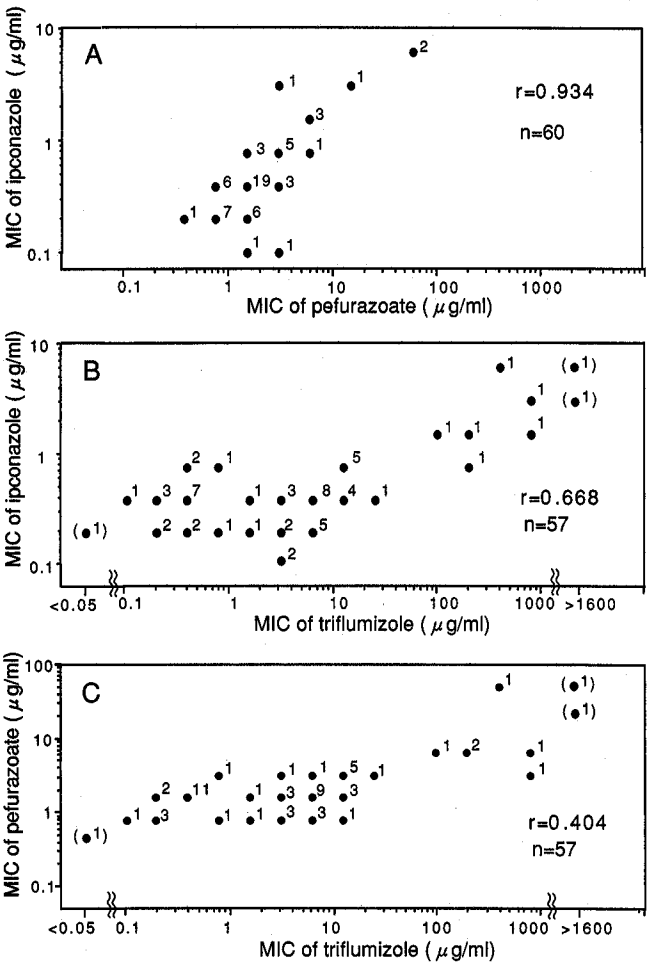

Fig. 4. Correlation of sensitivities of *F. moniliforme* between three SBIs. MIC correlations for 60 isolates randomly chosen from group I were plotted for (A) ipconazole and pefurazoate, (B) ipconazole and triflumizole, and (C) pefurazoate and triflumizole. Three isolates with MIC values lower than 0.05 μg/ml or higher than 1600 μg/ml (bracketed dots) for triflumizole were not included in the calculation. Number next to each dot is the number of isolates and r is linear correlation coefficient.

their origin was not clear in this study. Periodic monitoring of less sensitive isolates combined with the application records of SBIs may be necessary.

Some mechanisms to reduce sensitivity to sterol 14α-demethylation inhibitors have been compiled[1]. Recently, molecular mechanisms of multidrug resistance in *Penicillium digitatum*[2,12] have also been reported. Although mechanism to reduce sensitivity in *F. moniliforme* to SBIs has not been reported yet, a similar mechanism may be involved in lowering sensitivity of *F. moniliforme* to SBIs.

Among the 14 isolates tested, isolates with MIC values lower than 0.78 μg/ml for ipconazole were pathogenic and produced gibberellin. Isolates with MIC values higher than 1.56 μg/ml were not pathogenic and did not produce gibberellin. *F. moniliforme* isolates less sensitive to ipconazole also produced less gibberellin and were less pathogenic. Isolates less sensitive to triflumizole and insensitive mutants to pefurazoate were also less pathogenic to rice, and their gibberellin productivity was also low[3,16]. Takenaka *et al.*[16] suggested that an enzyme component or factor for the 14α-demethylation of 24-methylenedihydrolanosterol in the pathway to ergosterol and the oxidation of kaurene in the pathway to gibberellins were common or closely related in their study of mutants insensitive to pefurazoate. In this regard, the isolates less sensitive to SBIs with poor gibberellin productivity may also produce less ergosterol or be insensitive to the demethylation step as a result of a mutation. Isolates less sensitive to ipconazole were not reduced in their mycelial growth compared with sensitive isolates in the absence of SBIs. In addition, ipconazole strongly inhibited gibberellin production at relatively lower concentrations than that which inhibited mycelial growth of *F. moniliforme* in our previous experiment[18]. Perhaps the kaurene oxidation step is more sensitive to mutation or SBIs than the 14α-demethylation of sterol. Further investigation may be necessary on less sensitive isolates to SBIs.

Although the efficacy of 24-hr dip treatments with triflumizole against isolates to which MIC values of triflumizole showed 3.13 or 6.25 μg/ml was slightly reduced, all the tested pathogenic isolates were controlled by the practical dosage of ipconazole or pefurazoate.

Five years have passed since the launch of ipconazole, but decreased efficacy of ipconazole against "Bakanae" disease or the appearance of resistant isolates has not been reported. The narrow range of MIC values for ipconazole against *F. moniliforme* isolates may result in an adequate margin between the highest MIC value to pathogenic isolates and the practical dosage for seed treatment and could be one reason for stable effectiveness of ipconazole to "Bakanae" disease. Presumably, the lack of or reduced pathogenicity of the isolates less sensitive to SBIs may partially contribute to the efficacy of ipconazole.

ACKNOWLEDGMENTS

We would like to express our sincere gratitude to the staff of the national and prefectural research institutes of agriculture for supplying *F. moniliforme* isolates, the personnel of JA ZEN-NOH and Kumiai Chemical Industry for supporting the collection of the isolates, Mr. T. Kobayashi (former Technical

Table 2. Relationships between sensitivity to SBIs, productivity of gibberellin, pathogenicity of isolates and efficacy of seed treatment

Isolates	MIC value (μg/ml)			Gibberellin production (mg/g dry wt.)[a]	Percentage of elongated or damping off of seedlings[b]			
	Ipconazole	Pefurazoate	Triflumizole		Untreated	Ipconazole[e]	Pefurazoate[f]	Triflumizole[g]
KMM 92-6	0.10	3.13	3.13	12.6	49.3	0.2	0.2	11.7
NGN 92-15	0.10	1.56	3.13	4.1	98.0	0.2	n.t.[d]	n.t.
OKY 93-4	0.20	0.39	<0.10	5.2	99.0	0.2	n.t.	n.t.
KMM 92-8	0.20	0.78	0.20	6.8	99.5	0.6	n.t.	n.t.
KGS 93-10	0.39	1.56	6.25	14.1	86.6	0.0	0.0	14.0
MYG 92-10	0.39	0.39	0.39	26.2	82.3	0.0	0.0	0.0
AOM 93-4	0.78	1.56	12.5	6.5	90.6	0.0	1.1	2.7
IBR 9	0.78	6.25	200	n.d.[c]	0.0	0.0	n.t.	n.t.
TTR 88-17	1.56	6.25	100	n.d.	0.0	0.0	0.0	0.0
TCG 92-2	1.56	6.25	200	n.d.	0.0	0.0	0.0	0.0
GIF 89-2	3.13	12.5	>1600	n.d.	0.0	0.0	0.0	0.0
IBR 2	3.13	3.13	800	n.d.	0.0	0.0	n.t.	n.t.
NGN 92-1	6.25	50	400	n.d.	0.0	0.0	0.0	0.0
KGW 91-3	6.25	25	1600	n.d.	0.0	0.0	n.t.	n.t.

a) Gibberellin activity was determined by Nishijima's modified micro-drop method and productivity was expressed as GA_3.
b) Number of elongated seedlings or seedlings with damping off per total number of seedlings.
c) n.d., Not detected.
d) n.t., Not tested.
e) Dip in 200-fold dilution of ipconazole 6% WP (300 μg/ml) for 24 hr.
f) Dip in 200-fold dilution of pefurazoate 20% WP (1000 μg/ml) for 24 hr.
g) Dip in 300-fold dilution of triflumizole 15% EC (500 μg/ml) for 24 hr.

Adviser of Kureha Chemical Ind. Co., Ltd.) for valuable advice and Mrs. K. Amai for assistance with the culture of isolates.

LITERATURE CITED

1. De Waad, M.A. (1994). Resistance to fungicides which inhibit sterol 14α-demethylation, an historical perspective. *In* Fungicide Resistance (Heaney, S., Slawson, D., Hollomon, D.W., Smith, M., Russell, P.E. and Parry, D. W., eds.). pp. 3-10, The British Crop Protection Council, Farnham.
2. Hamamoto, H., Hasegawa, K., Nakaune, R., Lee, Y., Makizumi, Y., Akutsu, K. and Hibi, T. (1999). Molecular mechanism of DMI-resistance in *Penicillium digitatum*. Ann. Phytopathol. Soc. Jpn. 65 : 654-655 (Abstr. in Japanese).
3. Hamamura, H., Kawahara, M. and Shimoda, S. (1989). Some characteristics of *Gibberella fujikuroi* (*Fusarium moniliforme*) isolates less sensitive to triflumizole. Ann. Phytopathol. Soc. Jpn. 55 : 275-280.
4. Inoue, K., Date, H., Kasuyama, S. and Okamoto, Y. (1991). Sensitivity of "Bakanae" fungus to triflumizole and effect of seed treatment against seeds infested with isolates which different sensitivity to triflumizole. Ann. Phytopathol. Soc. Jpn. 57 : 432 (Abstr. in Japanese).
5. Inoue, K., Kasuyama, S. and Hatamoto, M. (1992). Effect of seed treatment with triflumizole against sensitive and less sensitive strains of *Fusarium moniliforme* ("Bakanae" disease) to triflumizole in paddy field. Ann. Phytopathol. Soc. Jpn. 58 : 581 (Abstr. in Japanese).
6. Irie, K. and Inoue, K. (1993). Methods for monitoring fungicide resistance—rice "Bakanae" disease (*Gibberella fujikuroi*). Plant Prot. 47 : 376-380 (in Japanese).
7. Ishii, H. (1993). Methods for monitoring fungicide resistance—general principles. Plant Prot. 47 : 279-281 (in Japanese).
8. Kitamura, Y., Hozumi, T. and Tanaka, N. (1982). Occurrence of benomyl-resistant strains of *Fusarium moniliforme*, the causal fungus of rice "Bakanae" disease. Ann. Phytopathol. Soc. Jpn. 48 : 380 (Abstr. in Japanese).
9. Komada, H. and Ogawa, K. (1980). Methods for evaluating activity of *Fusarium*. *In* Fusarium Disease of Cultivated Plants (Matuo, T., Komada, H. and Matsuda, A., eds.). pp. 201-220, Zenkoku Noson Kyoiku Kyokai Publishing Co., Ltd., Tokyo (in Japanese).
10. Matuo, T. (1969). Identification of *Fusarium*. Plant Prot. 23 : 473-480 (in Japanese).
11. Matuo, T., Endo, T. and Yoshii, Y. (1976). Morphological and physiological characters of Japanese isolates of *Fusarium moniliforme* Sheldon. Trans. Mycol.

Soc. Jpn. 17 : 295-305 (in Japanese with English summary).
12. Nakaune, R., Akutsu, K. and Hibi, T. (1998). Mechanisms of multidrug resistance of plant pathogenic fungi. p.45. *In* Abstr., Proc. 8th Symposium of Japan Fungicide Resistance Committee, Sapporo, Japan (in Japanese).
13. Nakazawa, Y. (1994). Techlead wettable powder, a novel seed disinfectant. Noyaku Tsushin 136 : 13-17 (in Japanese).
14. Nishijima, T. and Katsura, N. (1989). A modified micro-drop bioassay using dwarf rice for detection of femtomol quantities of gibberellins. Plant Cell Physiol. 30 : 623-627.
15. Ogawa, K., Suwa, M. and Watanabe, S. (1982). Sensitivity of *Fusarium moniliforme*, the causal fungus of rice "Bakanae" disease to benzimidazoles. Ann. Phytopathol. Soc. Jpn. 48 : 379-380 (Abstr. in Japanese).
16. Takenaka, M., Hayashi, K., Ogawa, T., Kimura, S. and Tanaka, T. (1992). Lowered virulence to rice plants and decreased biosynthesis of gibberellins in mutants of *Gibberella fujikuroi* selected with pefurazoate. J. Pesticide Sci. 17 : 213-220.
17. Takuda, T. and Mishima, T. (1985). Occurrence of benomyl-resistant strains of *Fusarium moniliforme*, the causal fungus of rice "Bakanae" disease in Shimane prefecture. Ann. Phytopathol. Soc. Jpn. 51 : 74-75 (Abstr. in Japanese).
18. Tateishi, H., Saishouji, T., Suzuki, T. and Chida, T. (1998). Antifungal properties of seed disinfectant ipconazole and its protection against "Bakanae" and other diseases of rice. Ann. Phytopathol. Soc. Jpn. 64 : 443-450.
19. Wada, T., Kuzuma, S. and Takenaka, M. (1990). Sensitivity of *Fusarium moniliforme* isolates to pefurazoate. Ann. Phytopathol. Soc. Jpn. 56 : 449-456.