

イネ病害用新浸透性防除剤フェリムゾン(TF-164)の作用機 作

いもち病菌(*Pyricularia oryzae*)の代謝に及ぼす影響

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Mode of Action of Ferimzone (TF-164), a Novel Systemic Fungicide for Rice Diseases: Effects on the General Metabolism of *Pyricularia oryzae*

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Abstract

Ferimzone (TF-164) did not significantly inhibit the syntheses of protein, DNA, RNA and cell walls in mycelia of *P. oryzae*. Ferimzone inhibited the uptake of ^{14}C -acetate and ^{14}C -pyruvate into cells and caused apparent incorporation inhibition of these precursors into lipids. Any individual components of lipids, however, were not found to be specifically inhibited by ferimzone in the incorporation of ^{14}C -acetate and ^3H -S-adenosylmethionine. Ferimzone did not inhibit the activity of fatty acid synthetase *in vitro* prepared from mycelia of *P. oryzae*. The incorporation of several amino acids including ^3H -leucine into acid insoluble fraction was rather enhanced by ferimzone. In the present study any general metabolic pathways were not revealed to be significantly and specifically affected by ferimzone. In consideration of the fact that ferimzone caused specific leakage of acidic electrolytes from mycelia of *P. oryzae*, the results presented in this study suggested that ferimzone could disturb membrane permeability for specific molecules or ions in influx as well as in efflux.

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Key words: fungicide, *Pyricularia oryzae*, membrane function, ferimzone, lipid synthesis, rice blast.

INTRODUCTION

Ferimzone (TF-164), (Z)-o-methylacetophenone 4,6-dimethyl-2-pyrimidinyl-hydrazone is a novel systemic fungicide developed for the control of rice diseases caused by *Pyricularia oryzae* and other pathogenic fungi such as *Helminthosporium oryzae*, *H. sigmoideum*, *Cercospora oryzae* and *Entyloma oryzae*¹²⁾. Furthermore, ferimzone exhibits strong antifungal activity against strains of *P. oryzae* resistant to organophosphorous fungicides, kasugamycin and blasticidin S (unpublished results). In a previous paper¹⁴⁾ we showed that the antifungal activity of ferimzone was most prominent against mycelial growth including appressorial germination of *P. oryzae* and that ferimzone caused specific leakage of acidic electrolytes from mycelia. The antifungal activity of ferimzone, however, is fungistatic and it does not give fatal damage to the fungus *in vitro*¹⁴⁾. In this paper the effects of ferimzone on the general metabolism of the fungus were studied and the mode of action was discussed in comparison with that of other fungicides and antifungal compounds.

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MATERIALS AND METHODS

Fungus. *Pyricularia oryzae* Cav., P-2 was cultured at 28 C for 5 to 7 days on a V-8 juice agar medium as described¹⁵⁾. Spores were incubated in Czapek medium containing 2% sucrose and 0.05% yeast extract (CSY) at 28 C for 16 to 20 hr without shaking in the dark to obtain mycelial suspension¹⁴⁾.

Chemicals. Ferimzone (Technical compound containing Z-form of more than 97.0%) was used throughout all experiments. Ferimzone was dissolved in ethanol and diluted with water to obtain the stock solution containing 200 µg/ml ferimzone and less than 0.4% ethanol. Cerulenin was purchased from Sigma Chemical Co. (USA). The solution of ferimzone was autoclaved at 105 C for 5 min. The solution of cerulenin was filtered through a millipore filter (0.22 µm) before use.

Labeled materials. All radioactive materials were purchased from Amersham International plc (England). [¹⁻¹⁴C] acetic acid, sodium salt (56 mCi/mmol), [¹⁻¹⁴C] acetyl CoA (58 mCi/mmol), [U-¹⁴C] alanine (30 mCi/mmol), [2-¹⁴C] pyruvic acid, sodium salt (11.4 mCi/mmol), [U-¹⁴C] aspartic acid (228 mCi/mmol), D-[U-¹⁴C] glucosamine (200 mCi/mmol), [U-¹⁴C] glycine (114 mCi/mmol), D-[U-¹⁴C] glucose (290 mCi/mmol), L-[2,3,4,5-³H] arginine (50 Ci/mmol), L-[4,5-³H] leucine (130 Ci/mmol), L-[methyl-³H] methionine (50 Ci/mmol), [5,6-³H] uridine (45 Ci/mmol), [methyl-³H] thymidine (75 Ci/mmol) and S-adenosyl-L-[methyl-³H] methionine (73 Ci/mmol) were used.

Incorporation studies with radioactive substrates. Mycelia were suspended in either Czapek medium containing 2% sucrose (CS) or CSY, pH 7.0 and aliquots (1~3 ml) were incubated in 20-ml flasks or 10-ml test tubes at 30 C in a reciprocal shaker. Radioactive substrates in one to 5 µl solution were added at appropriate times to give final concentrations of 0.05 to 0.5 µCi/ml. In some experiments non-labeled cold substrates were added at 0.1 to 0.5 mM to adjust the specific activity of radioactive substrates. Labeling was stopped by adding cold deionized water containing corresponding non-labeled substrates (DWNS) at 1 to 5 mM and labeled mycelia were washed three times with DWNS by centrifugation. The washed mycelia were suspended in cold 5% trichloroacetic acid (TCA), kept in ice for 2 to 3 hr or overnight. TCA insoluble materials were precipitated by centrifugation and an aliquot of the supernatant was taken as acid soluble materials incorporated into mycelia. TCA insoluble materials were washed twice with cold 5% TCA by centrifugation, collected and washed on a millipore filter (GS, 0.22 µm) or a glassfiber filter (GF/C) with vacuum filtration. TCA insoluble materials were regarded as acid insoluble molecular fraction. Further fractionation of acid insoluble materials was done basically according to the method of Schmidt and Thannhause modified by Schneider¹⁷⁾ in order of lipids, RNA, DNA, protein and cell walls. Lipids were extracted with ethanol:ether (1:1, v/v) at 50 C for 15 min. RNA was extracted with 0.3 N KOH at 37 C for 16 hr. DNA was extracted with 5% perchloric acid in boiling water for 15 min. Protein was extracted with 1 N NaOH in boiling water for 20 min. The resultant residue unextracted by the treatment described above was collected on a glassfiber filter, washed with cold 5% TCA with vacuum filtration and regarded as cell wall fraction.

Extraction and analysis of lipids. Total lipids were extracted with chloroform and methanol according to the method of Bligh and Dyer modified by Yung and Mudd²²⁾. Extracted lipids were dissolved in a small amount of chloroform in the presence of nitrogen gas. Separation of total lipids into components was done by a thin-layer chromatography (TLC) using the solvent system of heptane:isopropylether:acetic acid (60:40:4, v/v) (solvent system I) for neutral lipids and that of chloroform:methanol:28% ammonia (70:35:5, v/v) for polar lipids. TLC plates (Silica Gel 60 F₂₅₄ with concentration zone, 0.25 mm thick, 10×10 cm, Merck) were used.

Saponification of lipids was done by heating lipids in a 10% KOH solution of 60% ethanol at 70 C for 60 min. Unsaponifiable lipids were extracted with hexane, and separated by TLC

using a solvent system I. Separated lipid components on TLC were detected by an autoradiography by exposing TLC plate to X-ray film at -80°C for 5 to 14 days. Portions on TLC corresponding to spots on film were scraped off and the radioactivity was measured.

Further analysis of sterols. Spot corresponding to the fraction of desmethylsterols on TLC plate was scraped off and sterols were extracted with chloroform:methanol:ether (1:1:1, v/v). The sterols were acetylated with acetic anhydride and pyridine (1:1, v/v) at room temperature for 16 hr as described by Johnston *et al.*⁵⁾ Acetylated sterols were extracted with hexane and analyzed using the solvent system of benzene on an argentation TLC plate (18 g AgNO_3 , 0.1 g of Rhodamine 6G and 40 g Silica Gel G; Type 60, Merck, 0.25 mm thick) for an autoradiography⁸⁾.

Preparation of crude fatty acid synthetase (FAS)⁴⁾. Mycelia of *P. oryzae* prepared from 48 hr-shake culture of spores in potato sucrose medium were collected on a glassfiber filter with vacuum filtration. The mycelia (2~3 g) after blotting extra water with sheets of filter paper were homogenized in 0.1 M phosphate buffer, pH 7.0 containing 3 mM 2-mercaptoethanol (PBM) together with sea sand (40~50 mesh) in an ice-chilled glass homogenizer for 5 min. The homogenate was centrifuged at $10,000\times g$ for 20 min and the clarified supernatant was centrifuged at $100,000\times g$ for 60 min. The pellet was suspended in 5 to 10 ml of PBM and used as crude fatty acid synthetase (FAS)⁶⁾.

Assay for FAS activity^{1,21)}. FAS activity was assessed by the incorporation of radioactive [$1\text{-}^{14}\text{C}$] acetate and [$1\text{-}^{14}\text{C}$] acetyl CoA into lipid fraction. A standard reaction mixture (600 μl) consisted of 490 μl of FAS solution, 50 μl of PBM containing 3 μmol ATP, 0.02 μmol CoA, 0.04 μmol malonyl CoA, 0.22 μmol NADPH, 0.2 μmol NADP, 0.17 μmol NADH, 6 μmol isocitrate, 0.1 μmol lipoic acid, 3 μmol glucose-1-phosphate, 0.02 μmol MnCl_2 , 0.86 μmol [$1\text{-}^{14}\text{C}$] acetyl CoA or [$1\text{-}^{14}\text{C}$] sodium acetate, and 60 μl of 200 $\mu\text{g/ml}$ ferimzone or 100 $\mu\text{g/ml}$ cerulenin or deionized water. The reaction was started by adding enzyme solution and incubation was made at 30°C in a reciprocal shaker. The reaction was stopped by adding 30 μl of 60% PCA and chilling in ice. After addition of 600 μl of ethanol, lipids were extracted twice with petroleum ether.

Measurement of radioactivity. Radioactivity measurement was made in a Packard liquid scintillation spectrometer in glass vials with 5 ml of emulsification type liquid scintillation cocktail (Univer-Gel II, Nacalai Tesque Co., Ltd., Kyoto) containing PPO, bis-MSB and nonionic surfactant in xylene. For alkaline sample solution, it was neutralized with HCl before measurement.

RESULTS

Effects on the incorporation of radioactive substrates

Mycelia were labeled with various radioactive precursors for 30 min after 3 hr of incubation with or without ferimzone and fractionated into several cellular constituents. A majority of radioactivity of ^{14}C -acetate, ^3H -uridine, ^3H -leucine, ^3H -thymidine and ^{14}C -glucosamine were incorporated into the fractions of lipids, RNA, DNA, protein and cell walls, respectively (Table 1). Ferimzone inhibited the incorporation of ^{14}C -acetate into lipid fraction most strikingly as well as that into acid soluble fraction. Milder inhibition also occurred in the incorporation of ^3H -uridine and ^{14}C -glucosamine into RNA and cell wall fractions, respectively. Ferimzone did not inhibit the incorporation of ^3H -leucine and ^3H -thymidine into protein and DNA fractions, respectively. Similar incorporation studies were conducted in periodical pulse labeling in CS and CSY media at 30°C (Tables 2 and 3). In a CS medium, the incorporation inhibition of ^{14}C -acetate into acid insoluble fraction was first detected and the inhibition rate increased with increase of time of incubation with ferimzone (Table 2). Ferimzone did not inhibit the incorporation of ^3H -uridine into acid insoluble fraction at early pulse periods and that of ^3H -thymidine at any pulse periods. The incorporation of ^3H -leucine into acid insoluble fraction

was enhanced by ferimzone especially at early pulse periods (Table 2). Similar results were obtained in a CSY medium except that the incorporation of ^3H -thymidine and ^{14}C -glucosamine into acid insoluble fraction was inhibited by about 30~45% at 6.0~7.5 hr pulse period (Table

Table 1. Effects of ferimzone on the incorporation of radioactive substrates into cellular macromolecules of mycelia of *Pyricularia oryzae* ^{a)}

Substrate	Ferimzone ($\mu\text{g/ml}$)	Radioactivity (cpm)					
		Acid soluble	Lipids	RNA	DNA	Protein	Cell wall
Acetate	0	34970	71180	3544	1580	2952	770
	20	11630 (66.7) ^{b)}	18650 (73.8)	928	448	832	219
Uridine	0	316530	2715	100836	2984	660	43
	20	337320 (0)	3675	69616 (31.0)	5004	936	62
Leucine	0	17304	46550	— ^{d)}	—	170501	18944
	20	21984 (0) ^{c)}	44885 (3.6)	—	—	175784 (0)	19531
Thymidine	0	15015	1185	1612	3088	1476	40
	20	12805 (14.7)	975	1436	2920 (5.4)	1256	35
Glucosamine	0	60390	2855	5952	8340	7008	50466
	20	52110 (13.7)	2270	4280	8152	4524	38600 (23.5)

a) Mycelia were incubated with radioactive substrates for 30 min after 3 hr of incubation in Czapek medium containing 2% sucrose at 30 C. Cellular macromolecules were extracted in order of lipid, RNA, DNA, protein and cell walls as described in materials and methods. In this experimental condition, cycloheximide and cerulenin inhibited the incorporation of ^3H -leucine and ^{14}C -acetate into acid insoluble fraction by about 70 to 80% at the concentration of 10 $\mu\text{g/ml}$.

b) Figures in parenthesis are % inhibition.

c) Incorporation was enhanced significantly.

d) Extraction steps of RNA and DNA were omitted.

Table 2. Effects of ferimzone on the incorporation of radioactive substrates into acid insoluble materials of mycelia of *Pyricularia oryzae* ^{a)}

Substrate	Time for labeling (hr)	Radioactivity (cpm)		% inhibition
		Ferimzone ($\mu\text{g/ml}$)		
		0	20	
Acetate	1-2	22486	18726	16.7
	3-4	19745	15120	23.4
	5-6	16741	10102	39.7
Leucine	1-2	51992	86444	0 ^{b)}
	3-4	59146	65922	0 ^{b)}
	5-6	55469	54967	0.9
Uridine	1-2	3028	3102	0
	3-4	4787	4943	0
	5-6	9376	6510	30.6
Thymidine	1-2	4720	4479	5.1
	3-4	3479	3325	4.4
	5-6	8713	8384	3.8

a) Mycelia were incubated in Czapek medium containing 1% sucrose at 26 C. Radioactivity in 5% TCA insoluble fraction was measured. For other conditions, refer to the footnote of Table 1.

b) Incorporation was enhanced significantly.

Table 3. Effects of ferimzone on the incorporation of radioactive substrates into acid insoluble materials of mycelia of *Pyricularia oryzae* ^{a)}

Substrate	Time for labeling (hr)	Radioactivity (cpm)		% inhibition
		Ferimzone ($\mu\text{g/ml}$)		
		0	20	
Acetate	1.5-3.0	37209	31922	14.2
	3.0-4.5	58978	40523	31.3
	4.5-6.0	33371	18035	46.0
	6.0-7.5	60158	16664	72.6
Leucine	1.5-3.0	1391	2791	0 ^{b)}
	3.0-4.5	2298	2822	0 ^{b)}
	4.5-6.0	2470	2973	0 ^{b)}
	6.0-7.5	3521	3496	0.7
Uridine	1.5-3.0	9137	9341	0
	3.0-4.5	9533	6239	34.5
	4.5-6.0	7139	4704	43.1
	6.0-7.5	7784	6756	13.2
Thymidine	1.5-3.0	8831	8263	6.4
	3.0-4.5	7715	8793	0
	4.5-6.0	12750	10382	18.6
	6.0-7.5	6832	3770	44.8
Glucosamine	1.5-3.0	46079	40077	11.5
	3.0-4.5	37940	43512	0
	4.5-6.0	51785	53345	0
	6.0-7.5	55765	38162	31.6
Glycine	1.5-3.0	2796	3125	0
	3.0-4.5	3527	3047	13.6
	4.5-6.0	3613	3322	8.1
	6.0-7.5	3061	2871	6.2

a) Mycelia were incubated in Czapek medium containing 2% sucrose and 0.5% yeast extract at 30 C. For other conditions, refer to the footnotes of Tables 1 and 2.

b) Incorporation was enhanced significantly.

3). In another experiment the incorporation of ^3H -uridine into acid insoluble fraction was reexamined in continuous labeling. The inhibitory effect of ferimzone (20 $\mu\text{g/ml}$) on the incorporation of ^3H -uridine increased linearly during 12 hr of incubation with ferimzone (20 $\mu\text{g/ml}$) although the rate of incorporation gradually decreased 5 hr after incubation in comparison to that of the untreated control.

Effects on lipid metabolisms

In 30 min pulse labeling 4 hr after incubation, ferimzone inhibited the incorporation of ^{14}C -acetate into lipids by 5.5% at 1 $\mu\text{g/ml}$ and the inhibition rate increased by 54.5% with increase of the concentration up to 40 $\mu\text{g/ml}$. The inhibition was observed in both saponifiable and unsaponifiable fractions of extracted lipids.

Cerulenin is known to inhibit the synthesis of fatty acids in early steps of the metabolic pathway in various microorganisms^{13,16,20}. To make clear whether ferimzone has a site of action similar to cerulenin, the effect of ferimzone on lipid metabolism was compared with that of cerulenin both *in vitro* and *in vivo*. Both toxicants inhibited the incorporation of ^{14}C -acetate into lipids. Ferimzone, however, caused concomitant uptake inhibition of ^{14}C -acetate into acid soluble fraction of mycelia whereas cerulenin did not (Table 4). In the cerulenin-treated mycelia, free radioactive substrates seemed to be pooled in cells without being incorporated into lipid fraction. When ^{14}C -alanine was used as a precursor of lipids, no influence of ferimzone was observed both in the uptake and the incorporation into lipids.

Table 4. Comparison of ferimzone and cerulenin in the effects on ^{14}C -acetate incorporation into lipids and acid soluble fraction of mycelia of *Pyricularia oryzae*^{a)}

Chemical	Radioactivity (cpm)		
	Left in medium	Acid soluble	Lipids
Control	24192	29005	159347
Ferimzone	207368	12280 (67.7) ^{b)}	17399 (89.1)
Cerulenin	71170	71800 (0)	50286 (68.4)

a) Mycelia were incubated in Czapek medium containing 2% sucrose at 30 C for 30 min after 5 hr of incubation with ferimzone (20 $\mu\text{g/ml}$) and cerulenin (15 $\mu\text{g/ml}$).

b) Figures in parenthesis are % inhibition.

Effect on the fatty acid synthetase

Ferimzone did not inhibit the activity of the crude fatty acid synthetase *in vitro* extracted from mycelia of *P. oryzae* in the incorporation of ^{14}C -acetyl CoA into lipid fractions whereas cerulenin completely inhibited the enzyme activity (Fig. 1). Similar results were obtained using ^{14}C -acetate as a precursor for lipids in this assay system.

Effect on the synthesis of lipid components

Lipids extracted from mycelia of *P. oryzae* labeled with ^{14}C -acetate and ^3H -S-adenosyl-methionine (SAM) were analyzed. In neutral lipids a large amount of radioactivity of ^{14}C -acetate and ^3H -SAM was incorporated into triglycerides and desmethyl sterols (Table 5). Ferimzone rather uniformly inhibited the incorporation of both ^{14}C -acetate and ^3H -SAM into component of neutral lipids although in dimethylsterols the incorporation of ^3H -SAM was slightly stimulated with the ferimzone treatment (Table 5). The incorporation of both ^{14}C -acetate and ^3H -SAM into polar lipids was rather uniformly inhibited (Table 5).

Further analysis of desmethylsterols extracted from mycelia on an argentation TLC after acetylation did not show any difference in the separation pattern on an autoradiogram between the ferimzone-treated and the untreated.

Effects on the uptake of various substances

Ferimzone strongly inhibited the uptake of ^{14}C -acetate and slight mildly that of ^{14}C -pyruvate into mycelia among test chemicals including differently charged amino acids (Table 6).

Effects on the synthesis of purine and pyrimidine bases

[U- ^{14}C] glycine and [U- ^{14}C] aspartic acid were used as precursors for purine and pyrimidine bases, respectively, in the incorporation study. The incorporation of these precursors into nucleic acid fractions was not specifically inhibited by ferimzone compared with that into other cellular constituent molecules.

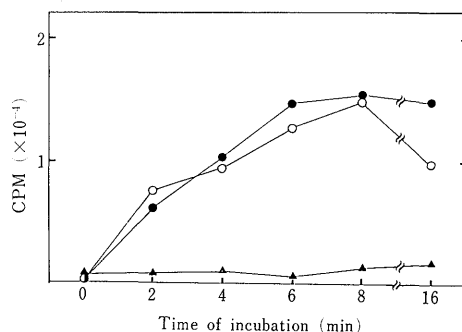


Fig. 1. Effects of ferimzone and cerulenin on the fatty acid synthetase activity of *Pyricularia oryzae* in the incorporation of ^{14}C -acetyl-CoA into lipids. Control: ○, ferimzone (20 $\mu\text{g/ml}$): ●, cerulenin (15 $\mu\text{g/ml}$): ▲.

Table 5. Effect of ferimzone on the incorporation of ^{14}C -acetate and ^3H -S-adenosylmethionine (SAM) into lipid components of mycelia of *Pyricularia oryzae* ^{a)}

Component ^{b)}	Radioactivity (cpm)					
	^{14}C -acetate			^3H -SAM		
	Ferimzone ($\mu\text{g/ml}$)			Ferimzone ($\mu\text{g/ml}$)		
	0	20		0	20	
Polar lipids						
CL	8807	7047	(17.5) ^{c)}	5894	4833	(18.0)
PEA	8590	7056	(17.9)	13007	10248	(21.2)
PC	15827	14055	(11.2)	26362	21967	(16.7)
PS and PI	5082	3689	(27.4)	6464	5187	(19.8)
PA	1392	1045	(24.9)	2127	1697	(20.0)
Neutral lipids						
SE	1166	1002	(14.1)	1505	1328	(11.8)
TG	20243	15681	(22.5)	29642	18965	(36.0)
FFA	3794	2590	(31.7)	2653	1768	(33.3)
DiMS	2654	2267	(14.6)	4802	5862	(-18.1)
MS	328	220	(33.0)	1079	1040	(3.6)
DesMS	7603	4799	(36.9)	35532	31002	(12.7)
DG	2269	1794	(20.9)	3510	2618	(25.4)

a) Mycelia were incubated in Czapek medium containing 2% sucrose at 28 C and labeled for 30 min after 3 hr of incubation.

b) CL: cardiolipin, PEA: phosphatidylethanolamine, PC: phosphatidylcholine, PS: phosphatidylserine, PI: phosphatidylinositol, PA: phosphatidate, SE: sterol esters, TG: triglycerides, FFA: free fatty acids, DiMS: dimethylsterol, MS: methylsterol, DesMS: desmethylsterol, DG: diglycerides.

c) Figures in parenthesis are % inhibition.

Table 6. Effect of ferimzone on the uptake of various substances into mycelia of *Pyricularia oryzae* ^{a)}

	Substance	Radioactivity (cpm)		% inhibition
		Control	Ferimzone	
Expt. 1	Leucine	38335	76293	0 ^{b)}
	Arginine	69254	65575	5.3
	Aspartate	12662	14827	0
	Acetate	37515	16815	55.2
	Pyruvate	14405	8333	42.2
Expt. 2 ^{c)}	Leucine	17304	21984	0 ^{b)}
	Uridine	31653	33732	0
	Thymidine	15015	12805	14.7
	Glucosamine	60390	52110	13.7
	Glucose	37730	26815	28.9
	Acetate	34970	11630	66.7
Expt. 3	Alanine	11316	11050	2.4
	Aspartate	17591	17557	0
	Methionine	39789	45609	0 ^{b)}
	Glycine	24150	33138	0 ^{b)}
	Acetate	37733	21205	43.8

a) Mycelia were labeled for 15 min after 5 hr of incubation (Expt. 1) and after 3 hr of incubation (Expts. 2 and 3) in the absence and the presence of 20 $\mu\text{g/ml}$ ferimzone. Incorporation of substances into 5%TCA soluble fraction was measured.

b) Uptake was enhanced significantly by ferimzone.

c) Data presented in Table 1 were used.

DISCUSSION

The results obtained from the incorporation studies with various radioactive precursors showed that ferimzone did not significantly affect the synthesis of protein, RNA, DNA, lipids and cell walls in mycelia of *P. oryzae*. The results may be consistent with the fungistatic property of ferimzone¹⁴. Apparent incorporation inhibition of ¹⁴C-acetate into lipids by ferimzone could be due to the inhibition in the uptake process of acetate into mycelial cells.

Most of ergosterol biosynthesis inhibitors (EBIs) having heteroaromatic rings in their chemical structure are known to specifically inhibit the oxidative removal of sterol C(14) methyl groups by the cytochrome P-450 enzyme^{18,19}. The inhibition leads to the accumulation of several ergosterol intermediates such as 4 α -methyl sterols and 4,4-dimethylsterols^{7,9}. On the other hand, some EBIs having morpholine derivatives in their chemical structure reduce the formation of episterol and ergosterol with the concomitant accumulation of fecosterol, ergosta-8, 22,24(28)-trien-3 β -ol and ergosta-8,22-dien-3 β -ol by blocking Δ^8 — Δ^7 isomerization without a striking effect on the amount of 4-desmethylsterols, 4,4-dimethylsterols and 4 α -methylsterols in their biosynthesis^{8,18}. Qualitative analysis of lipids did not show any specific inhibition and accumulation by ferimzone among the components of sterols and phospholipids. Therefore, the results indicate that ferimzone did not have the sites of action of either type of EBIs, nor those of organophosphorous fungicides like IBP and EDDP which were reported to affect phosphatidylcholine biosynthesis by inhibiting methylation of phosphatidylethanolamine^{10,11}. These results also suggest that the influence of ferimzone on membrane permeability might be caused by the direct interaction of ferimzone with membrane and/or cell walls of mycelia of *P. oryzae*.

Ferimzone has a pyrimidine ring in the chemical structure. Fungicides known to have a pyrimidine ring in their chemical structure are divided into two groups in the mode of action. One group is EBIs such as fenarimol and nuarimol which also have triazole ring in their chemical structure¹⁸. On the other hand dimethirimol and ethirimol categorized into another group are known to interfere with adenine metabolism and to inhibit the incorporation of ¹⁴C-glycine into RNA, resulting in the inhibition of RNA synthesis^{2,3}. The results obtained by using [U-¹⁴C] glycine and [U-¹⁴C] aspartic acid as precursors did not show any specific and significant incorporation inhibition in RNA and DNA fractions. Thus any metabolic pathways which were affected by ferimzone could not be revealed by the present study.

We reported that ferimzone caused specific leakage of acidic electrolytes from mycelia of *P. oryzae* at relatively early time after incubation¹⁴. The uptake inhibition of acetate and pyruvate by ferimzone was observed at the corresponding time when the specific leakage occurred. These results suggest that ferimzone could disturb membrane permeability for specific molecules or ions in influx as well as in efflux and that the disruption of membrane function is likely responsible for the antifungal activity of ferimzone.

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和 文 摘 要

奥野哲郎・古澤 巖・松浦一穂・獅山慈孝：イネ病害用新浸透性防除剤フェリムゾン (TF-164) の作用機作：いもち病菌 (*Pyricularia oryzae*) の代謝に及ぼす影響

フェリムゾン (TF-164) は *Pyricularia oryzae* や *Helminthosporium oryzae* などによるイネ病害防除のために開発された新しい浸透性防除剤である。フェリムゾンはいもち病菌におけるタンパク質、DNA、RNA、細胞壁合成を阻害しなかった。フェリムゾンは ^{14}C -酢酸や ^{14}C -ピルビン酸の菌糸細胞内への取込みを阻害し、これらの前駆物質の脂質画分への取込みを見掛け上阻害した。その取込み阻害は脂質のいずれの成分においても均一に認められた。フェリムゾンはいもち病菌菌糸より調製した脂肪酸合成酵素活性をまったく阻害しなかった。一方、 ^3H -ロイシンなどのいくつかのアミノ酸の酸不溶性画分への取込みは、フェリムゾンにより促進される傾向にあった。このように、フェリムゾンの作用点が、いもち病菌の生体構成成分の生合成系でないことが示唆された。フェリムゾンが、いもち病菌菌糸からの酸性電解質の漏出を起こすこと（既報）および、酢酸やピルビン酸の細胞内への取込みを特異的に阻害したことより、イオンを含む酸性電解質の透過性に関与する膜機能に影響を及ぼすことが、フェリムゾンの作用点の一つであることが示唆された。