Original Article

Biological Activity of Carpropamid (KTU 3616) : A New Fungicide for Rice Blast Disease

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Carpropamid ((1*R**, 3*S**)-2, 2-dichloro-*N*-[1-(4-chlorophenyl)ethyl]-1-ethyl-3-methylcyclopropane-carboxamide)) was found to be an effective rice blast fungicide with systemic properties. The basic activities of the compound were studied *in vitro* and *in vivo*. The compound did not inhibit mycelial growth of various plant pathogenic fungi tested except *Oomycetes* spp. In addition, carpropamid did not show direct activity on the mycelial growth, the spore germination and the appressorium formation of rice blast fungus. Inhibition of fungal melanin biosynthesis was observed, however, in the mycelial growth test with many fungi and in appressorium cells of rice blast fungus. The compound showed strong penetration inhibition into rice epidermal cells at very low concentrations but was less effective once the appressorial penetration had started.

INTRODUCTION

Carpropamid ((1*R**, 3*S**)-2, 2-dichloro-*N*-[1-(4-chlorophenyl)ethyl]-1-ethyl-3-methylcyclopropane-carboxamide)) belongs to a new class of fungicides for the control of rice blast. It is a safe compound with low mammalian and fish toxicity. With its low water solubility as well as low application rates per unit area, it could reduce the risk of water pollution in river or well water when used to control blast in rice paddy fields.

The compound provides a remarkable effect against rice blast with good plant compatibility by foliar spray, nursery box treatment, and water surface application. Especially in tests with nursery box treatment carried out by official experimental stations, carpropamid proved to be prominent in efficacy against leaf and panicle blast.

Its systemicity in rice plants is different from that of existing systemic rice blast fungicides. When these compounds are used for nursery box application, most of them show their effect promptly but the effect disappears within a relatively short period after transplanting. Carpropamid is, however, gradually absorbed during a very long period after transplanting, distributed uniformly in all parts of rice plants, and translocated even to the panicles as well as to the newly developing leaves.¹⁾ So the chemical can provide stable effect for a long period from leaf to panicle blast. These characteristics are

derived from biological, physical and chemical properties such as high activity against infection by *Pyricularia oryzae*, adequate water solubility and lipophilicity, and chemical stability in rice plants in the field.

Substituted cyclopropanes are reported to induce momilactones, kinds of phytoalexin in rice plants.²⁾ Carpropamid has a substituted cyclopropane moiety in the structure. However, it is far better than the former cyclopropanes in efficacy against rice blast and is different from the conventional ones in biological properties for plant pathogenic fungi.

This paper presents characteristic features of carpropamid with regard to biological properties on fungi and the action against rice blast disease.

MATERIALS AND METHODS

1. Chemicals

Carpropamid, NTN 33853 and 2, 2-dichloro-3, 3-dimethylcyclopropanecarboxylic acid were synthesized by Bayer and Nihon Bayer Agrochem. Tricyclazole, pyroquilon and fthalide were purchased from the market. Those compounds were dissolved with distilled water, small amounts of methanol and Tween 20 at the appropriate concentrations.

2. Rice Plants

Rice plants (*Oryza sativa* L., cultivar Kusabue) were

Fig. 1 Carpropamid (KTU 3616).

grown in 7 cm vinyl pots in the greenhouse at 22–33°C.

3. Rice Blast Fungus and the Other Plant Pathogenic Fungi

Pyricularia oryzae, TH 67-22 was kindly supplied by the National Institute of Tohoku Experimental Station and P2 was provided by The Institute of Physical and Chemical Research (RIKEN). Seventeen fungi and three bacteria used were maintained in the laboratory at the Yuki Research Centre.

4. Antimicrobial Activity

In order to evaluate antimicrobial activity *in vitro*, seventeen fungi and three bacteria were cultured on potato dextrose agar (abbreviated as PDA) medium amended with fungicides.

Four millimeters mycelial agar discs prepared from the preculture of PDA plates were placed on the test agar plates and incubated at 25 or 20°C. Two to 7 days after inoculation the diameters of mycelium colonies were measured to see the effects of chemicals on fungal growth. Bacteria were grown in PDA slants and suspended in autoclaved distilled water at 10⁷/ml respectively and inoculated by streak. After 2 days the growth inhibition of bacterial cultures was evaluated as compared with the growth in untreated plates.

5. Spore Germination and Appressorium Formation of Rice Blast Fungus

Rice blast P2 was cultured on oatmeal agar plates under irradiation with BLB light. Formed spores were washed out into distilled water by a paint brush and filtrated through fourfold cheese cloth. The density of the spore suspension was adjusted to 10⁴/ml spores with distilled water and diluted again with the fungicide solution by two times. A few drops of the treated spore suspension were dropped on the cellophane membrane prepared on the glass slide and incubated in a moist chamber at 25°C. Spore germination and appressorium formation were assessed 24 hr after application of the droplets.

6. Effective Spray Timing of the Chemicals

Blast spore suspension (TH 67-22) was sprayed to young rice seedlings (cultivar Kusabue at 2.5 leaf stage) and incubated in the moist chamber at 25°C. Every 2 hr from 2 to 12 hr after inoculation, the rice seedlings were

sprayed with carpropamid or tricyclazole at 0.01 and 0.001% a.i., and incubated again.

7. Pot Test and Evaluation of Penetrating Activity

Test compound solutions at double rates of the desired concentrations were diluted with the same volume of blast spore suspension (TH 67-22). The mixture was sprayed to young rice seedlings grown in 7 cm vinyl pots and incubated in the moist chamber for 18 hr. The damage of blast infection was evaluated 7 days after inoculation.

The penetration inhibition of rice blast into cellophane membranes was examined by the improved cellophane membrane method developed by Araki and Miyagi.³⁾ Invasion of the pathogen into adaxial epidermal cells of leaf sheaths was tested by Takahashi's method.⁴⁾

RESULTS

1. Antimicrobial Activity of Carpropamid

The antimicrobial activity of carpropamid against seventeen plant pathogenic fungi and three bacteria was

Table 1 Growth inhibition and colony colour change of plant pathogenic microbes on the PDA medium amended with carpropamid.

Microbial tested	ED ₅₀	Colony colour change (- ~ + + +)
Ascomycetes		
<i>Botryosphaeria berengeriana</i>	>200	+++
f. sp. <i>piricola</i>		
<i>Cochliobolus miyabeanus</i>	>200	(+)
<i>Diaporthe citri</i>	>200	+(+)
<i>Gibberella fujikuroi</i>	>200	-
<i>Sclerotinia sclerotiorum</i>	>200	-
<i>Valsa ceratosperma</i>	>200	+++
Basidiomycetes		
<i>Thanatephorus cucumeris</i>	>200	+
<i>Verticillium dahliae</i>	>200	+++
Oomycetes		
<i>Phytophthora nicotianae</i>	=50	-
var. <i>parasitica</i>		
<i>Pythium</i> sp.	≅200	-
Fungi Imperfecti		
<i>Alternaria brassicicola</i>	>200	+(+)
<i>Botrytis cinerea</i>	>200	-
<i>Colletotrichum lagenarium</i>	>200	++
<i>Fusarium oxysporum</i>	>200	-
f. sp. <i>lycopersici</i>		
<i>Helminthosporium</i>		
<i>sigmoideum</i> var. <i>irregulae</i>	>200	+++
<i>Penicillium digitatum</i>	>200	-
<i>Pyricularia oryzae</i>	>200	+++
Bacteria		
<i>Erwinia carotovora</i>	>200	-
<i>Pseudomonas lachrymans</i>	>200	-
<i>Xanthomonas campestris</i>	>200	-
pv. <i>oryzae</i>		

+++ : very strong, ++ : strong, +(+) : medium, + : weak, (+) : very weak, - : no change.

Fig. 2 Spore germination and appressorium formation of *Pyricularia oryzae* in carpropamid solution.

●: germination, ○: appressorium formation, ○: unmelanized appressorium.

tested and the results are shown in Table 1. ED_{50} values of carpropamid against 2 tested fungi (*Phytophthora nicotianae* var. *parasitica* and *Pythium* sp.) of Oomycetes were in the range of 50–200 ppm, suggesting no significant activity on their mycelial growth. All of the other plant pathogenic fungi belonging to Ascomycetes, Basidiomycetes and Fungi Imperfecti, and bacteria were not substantially inhibited in their mycelial growth or cell proliferation. Their ED_{50} values were more than 200 ppm.

Though carpropamid hardly affected mycelial growth or cell proliferation of fungi and bacteria tested except Oomycetes, colour change of the colonies of many fungi was recognized by the treatment with carpropamid. Especially in the colonies of *Pyricularia oryzae*, *Verticillium dahliae*, *Botryosphaeria berengeriana* and *Colletotrichum lagenarium* the colour clearly changed to brown and the change was supposed to be caused by fungal melanin biosynthesis inhibition (Table 1).

2. Effect on Spore Germination of Rice Blast Fungus

As shown in the Fig. 2, carpropamid hardly affected spore germination and appressorium formation of rice blast fungus at concentrations up to 40 ppm. Dark brown pigmentation in the appressorium cells formed on the cellophane membrane was observed in untreated drops, but not in the drops treated with carpropamid at more than 0.1 ppm. So transparent appressoria were formed by the treatment.

3. The Effect of Carpropamid and Tricyclazole Treated at Different Stages of Rice Blast Infection

Carpropamid or tricyclazole (0.01 and 0.001% a.i.) was sprayed onto the inoculated rice seedlings at every 2 hr from 2 to 12 hr after inoculation. Under microscopic observation, spore germination was observed 2 hr after inoculation and many young appressoria were formed on the rice leaves 6 hr after inoculation. Pigmentation of the appressoria was hardly observed. Eight hours after

Fig. 3 Effective period of carpropamid and tricyclazole against blast infection after artificial inoculation by spraying spore suspension.

Fig. 4 Basic activity of MBIs diluted with the spore suspension for rice blast infection.

inoculation, appressoria were grown to full size. Melanization in appressoria was recognized but this was incomplete up to 10 hr. Clear melanization was observed 12 hr after inoculation. Carpropamid showed perfect control of blast infection by treatment if it was sprayed within 6 hr after inoculation but was not perfect at 8 hr. On the other hand, tricyclazole could control blast infection 8 hr after inoculation. Ten hours after inoculation, both compounds did not work well against blast infection (Fig. 3).

4. Effect of Chemicals on Appressorial Penetration into Rice Epidermal Cells

Basic activity of carpropamid was compared with the other melanin biosynthesis inhibitors (MBI) by inoculation with spore suspension containing those fungicides. Carpropamid at more than about 0.3 ppm and tricyclazole at more than 0.1 ppm could well control blast infection on young rice seedlings. But pyroquilon and fthalide needed more than 1 and 30 ppm, respectively, to get sufficient inhibition of blast appressorial penetration by this method (Fig. 4).

The activity of carpropamid for blast appressorial penetration into cellophane membrane or rice leaf sheath cells was evaluated by the method of Araki & Miyagi³⁾ and Takahashi's method, respectively.⁴⁾ Carpropamid could prevent the appressorial penetration into the cello-

Table 2 Effect of carpropamid and tricyclazole on appressorial penetration into adaxial epidermal cells of rice leaf sheath and cellophane membrane.

Compound	Rate (a.i.) ppm	Adaxial epidermal cell		Cellophane membrane	
		Penetration (%)	Grade (0-4)	Penetration (%)	Melanization in appressorium
Untreated	—	100 (60.1)	2.28	100 (58.0)	+++
Carpropamid	0.03	75	0.91	6.4	±
	0.1	62	1.16	6.4	—~±
	0.3	17	0.11	2.4	—
	1.0	0	0	0	—
Tricyclazole	0.03	110	2.71	5.3	±
	0.1	64	0.97	5.0	±
	0.3	19	0.19	2.9	±
	1.0	50	0.73	4.3	±

Used blast strain : TH 67-22 for adaxial epidermal cell and Hoku-1 for cellophane membrane.

phane membrane or rice epidermal cells at around 0.3 ppm (Table 2).

DISCUSSION

Carpropamid hardly showed direct inhibitory effect on the growth of many plant pathogenic fungi and bacteria in spite of its high efficacy against rice blast disease. The property is, therefore, thought to resemble that of non-fungicidal blast controlling agents proposed by Yamaguchi *et al.*⁵⁾ and Woloshuk *et al.*⁶⁾ although carpropamid showed weak activity on mycelial growth of Oomycetes fungi and moderate activity in pot tests against *Phytophthora infestans* (data not shown). Several patents which cover compounds with a common moiety in the structure similar to carpropamid have been applied by Bayer,⁷⁾ Kumiai⁸⁾ and ACC.⁹⁾ The patents claimed that those compounds were effective against Oomycetes diseases. Thus, another mode of action of carpropamid is expected for the direct activity against Oomycetes fungi.

However, the most significant point of our findings is that many of the fungi treated with carpropamid changed in colony colour and the compound was supposed to affect fungal melanin biosynthesis. As the chemical structure of carpropamid is greatly different from that of existing MBIs and intermediates involved in the melanin biosynthesis,^{10, 11)} its melanin inhibition was hardly imaginable. But in our tests transparent appressoria were recognized when the spores were germinated in carpropamid solution at more than 0.1 ppm although spore germination and appressorium formation themselves were not inhibited. These actions have great resemblance to those of several MBIs reported so far.^{5, 11, 12)}

Melanization in the appressorium must be considerably correlated with appressorial penetration of rice blast fungus into rice epidermal cells. Woloshuk *et al.*¹³⁾ suggested that rigidity of the appressorium is necessary for the penetration by the pathogen, and melanization in

the cells contributes to their rigidity. Based on the fact that colony colour was changed and transparent appressoria of rice blast fungus were formed by treatment with carpropamid, the action is supposed to be largely correlated with the prevention of blast infection.

Carpropamid did not work well against blast infection after the start of melanization (8 hr) whereas tricyclazole worked at this stage. This difference in time may be based on the speed of penetration of each compound into the appressorium. Physical-chemical properties of both compounds are considerably different, *e.g.*, the solubility in water of carpropamid and tricyclazole is 4 and 700 ppm,¹⁴⁾ respectively. Inoue *et al.* mentioned in their report¹⁵⁾ tricyclazole was poor in efficacy when it was treated at 8 hr after inoculation. After sufficient melanization of appressoria, both compounds were poor in efficacy (12 hr). Thus, the action of both compounds on inhibition of blast infection is very similar in terms of penetration inhibitors.

Dewdrops formed on rice leaves are essential for blast infection. MBIs including carpropamid do not affect spore germination and appressorium formation but work at the infection sites. The dissolution of the active ingredient in dewdrops on rice leaves is one of important factors contributing to blast control. The basic activity of the MBI fungicides could be evaluated by inoculating rice seedlings with spore suspension containing these fungicides. At more than 0.3 ppm carpropamid provided almost perfect control of blast infection. The EC₉₅ values determined by this method for the individual MBI fungicides were estimated as follows: carpropamid = *ca.* 0.3 ppm, tricyclazole = 0.1–0.3 ppm, pyroquilon = *ca.* 1 ppm and fthalide = more than 30 ppm. The evaluation for carpropamid by this method yielded almost the same results as by the cellophane membrane method³⁾ and the leaf sheath penetration method.⁴⁾ Carpropamid at about 0.3 ppm could prevent leaf sheath cells or cellophane membrane from blast appressorial

penetration as well.

This basic activity of carpropamid does not directly correlate with the efficacy of the compound in practical use as a matter of course. The effect in practical application is affected by various natural conditions and compound properties. Persistency, permeability and mobility in plants, and uptake and systemicity through rice roots when treated by nursery box application were very important factors influencing the efficacy. The basic activity inherent in MBIs, however, can be readily evaluated and compared with each other.

Carpropamid shows very high activity to control the rice blast penetration into rice epidermal cells. We believe that this biological property originates from melanin biosynthesis inhibition in appressoria of rice blast fungus. Since carpropamid is quite different in stereochemical structure from the former MBIs and melanin intermediates, it remains an interesting subject for further study to determine how it acts as a melanin biosynthesis inhibitor.

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REFERENCES

- 1) S. Kuroguchi, T. Takino, K. Ishikawa, C. Muraoka, M. Sato, I. Kobori, H. Yamamoto, I. Saito & S. Katsumata : Abstr. ; 21st Annu. Meet. Pestic. Sci. Soc. Jpn., Okinawa, p. 122, 1996
- 2) D. Cartwright, P. Langcake, R. J. Pryce, D. P. Leworthy & J. P. Ride : *Nature* **267**, 511 (1977)
- 3) F. Araki & Y. Miyagi : *J. Pesticide Sci.* **2**, 457 (1977)
- 4) Y. Takahashi : *Shokubutsu Boueki* **12**, 339 (1958) (in

Japanese)

- 5) I. Yamaguchi, S. Sekido & T. Misato : *J. Pesticide Sci.* **7**, 523 (1982)
- 6) C. P. Woloshuk, P. M. Wolkow & H. D. Sisler : *Pestic. Sci.* **12**, 86 (1981)
- 7) T. Zeitz, D. Wollweber & W. Brandes : Jpn. Kokai Tokkyo Koho JP-A 3-153657 (1992)
- 8) T. Shibata, S. Ito, J. Sakai & S. Hayashi : Jpn. Kokai Tokkyo Koho JP-A 6-279405 (1984)
- 9) D. A. Hunt, J. M. Lavanish, M. Asselin & M. Los : Jpn. Kokai Tokkyo Koho JP-A 3-308507 (1982)
- 10) A. A. Bell, R. D. Stipanovic & J. E. Puhalla : *Tetrahedron Lett.* **32**, 1353 (1976)
- 11) C. P. Woloshuk, H. D. Sisler, M. C. Tokousbalides & S. R. Dutky : *Pestic. Sci.* **14**, 256 (1980)
- 12) T. Okuno, K. Matsuura & I. Furusawa : *J. Pesticide Sci.* **8**, 357 (1983)
- 13) C. P. Woloshuk, H. D. Sisler & E. L. Vigil : *Physiol. Plant Pathol.* **22**, 245 (1983)
- 14) Japan Plant Protection Association (ed.) : "Nouyaku Handbook (Pesticide Handbook)," 9th ed., Japan Plant Protection Association, Tokyo, p. 583, 1994
- 15) S. Inoue, K. Maeda, T. Uematsu & T. Kato : *J. Pesticide Sci.* **9**, 731 (1984)

要 約

新規いもち病防除剤カルプロパミドの生物活性

倉橋良雄, 坂和慎二, 金原太郎, 田中桂子, 利部伸三
カルプロパミド (KTU 3616) は浸透性で優れたいもち病防除剤として見出された。その基礎活性と生物的特性について *in vitro* と *in vivo* で検討した。カルプロパミドは Oomycetes に属する菌類に弱い活性を示すものの、その他の植物病原菌類の菌糸生育にはほとんど抗菌力を示さない。さらにいもち病菌の胞子発芽, 付着器形成を阻害しない。しかしながら培養試験において多くの菌類のコロニーの色を変化させ、またいもち病菌の付着器のメラニン化を強く阻害した。そして本剤は非常に低い濃度でいもち病菌のイネへの付着器侵入を阻害するが、一度付着器侵入が始まった後にはその効果は劣った。