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Original Article

Mode of Rice Blast Control by Chlobenthiazone*

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Chlobenthiazone (S-1901), 4-chloro-3-methyl-2(3*H*)-benzothiazolone, is a new fungicide for the control of rice blast. Greenhouse studies indicated that the fungicide had high preventive and residual activities at low concentrations against the disease. On the contrary, curative activity was not obtained even at a concentration as high as 500 μg/ml. The fungicide was also effective in controlling the disease by submerged application, indicating systemic movements in plants. When ^{14}C-labeled chlobenthiazone was applied to the leaves or roots of rice seedlings, radioactivity was found to be rapidly translocated to the untreated parts of the plants, confirming the result in pot trials. Furthermore, the rice seedlings exposed to the fungitoxic vapor were protected from the disease. It is suggested that both the systemic and the vapor phase activities must contribute to the effective disease control in fields. Some of the field trials demonstrated that chlobenthiazone controlled rice blast by foliar or submerged application.

INTRODUCTION

Since fungicidal activities of benzothiazole derivatives were first described in the patent[1] which disclosed 1-hydroxy, 1-amino and 1-thiol derivatives in 1932, many analogous compounds have been tested for their fungitoxicities.[2–4]

Our recent investigations showed that the derivatives of benzothiazolone were effective in controlling rice blast disease caused by *Pyricularia oryzae* Cav., whereas their fungitoxicities in a nutrient medium were low. Among the derivatives, we found that the compounds substituted with alkyl or halogen groups at both 3 and 4-positions of a benzothiazole ring were generally more effective than those substituted only at 3 or 4-position, and 4-chloro-3-methyl-2(3*H*)-benzothiazolone (chlobenthiazone) was selected as one of the most effective compounds. Effective disease control of the fungicide could be achieved by foliar or submerged treatment in the field.[5] The objective of this study is to understand how the fungicide controls the disease.

MATERIALS AND METHODS

1. *Pot Tests*

Either a 10% wettable powder or a 10% emulsifiable concentrate of chlobenthiazone was diluted with water at appropriate concentrations and employed in the following tests.

Rice seedlings (*Oryza sativa* L., var. Kinki No. 33) were used as test plants which had been grown for 2 to 3 weeks in a greenhouse.

Inoculation of the pathogen was carried out by spraying spore suspension containing 10^5 spores/ml to the plants. For production of conidia, the blast fungus, *P. oryzae* (Ken 60-19) was cultured on an oatmeal agar medium in a petri dish at 28°C for 14 days. The aerial mycelium was removed with a brush by washing with tap water and then the culture was incubated for another 4 days under Black Light-Blue lamps.

* Mechanism of Rice Blast Control of Chlobenthiazone (Part I)

Inoculated plants were placed in an air-conditioned room maintained at 28°C under a relative humidity of more than 95% (described as the incubation room in the following). After 4 days incubation, disease severity was assessed by the observation of infected leaf area. The other procedures for the respective tests are as follows.

1.1 Preventive activity

The potted rice seedlings of the 2.5th leaf stage were sprayed with the fungicide suspension 4 hr before inoculation.

1.2 Curative activity

The fungicide suspension was sprayed onto the plants of the 2.8th leaf stage 18 hr after inoculation and then the treated plants were returned to the incubation room.

1.3 Residual activity

The fungicide suspension was sprayed onto the plants of the 2.8th leaf stage, which were then placed in a greenhouse. After 4 days, these plants were inoculated and kept in the incubation room.

1.4 Systemic activity

The pots (90 ml in volume), in which the seedlings of the 1.5th leaf stage were grown, were placed in a plastic cup (250 ml in volume) containing 100 ml of the fungicide suspension and incubated in a greenhouse. After 5, 10 or 15 days, the plants were inoculated and kept in the incubation room.

1.5 Vapor phase activity

The fungicide dissolved in methanol was applied uniformly in an appropriate quantity to a filter paper (7 cm in diameter) (Toyo Roshi Co., Ltd. No. 2). The treated filter paper was hanged in a 5-liter glass beaker as drawn in Fig. 1 after the drying of methanol. The potted rice seedlings of the 2.5th leaf stage were put inside the beaker. The filter paper and the plants were kept apart to avoid direct contact. After exposure to the fungicide vapor for 24 hr, the plants were taken out from the beaker and then incubated in a greenhouse for 0, 24 or 48 hr, and inoculated.

2. *Field Trials*

Rice seeds (cv., Nihonbare) were sowed at a rate of 200 g/seedling flat (30×60×3 cm). The plants were cultivated in a greenhouse to the 2nd leaf stage and transplanted into an irrigated field by a transplanting machine. All trials were based on 3 replicates of 20 m^2 with randomized block design.

The rate of disease control was determined by counting the numbers of all diseased and healthy plants in 20 hills per plot. Severity of leaf blast was assessed by the estimation of the percentage of infected leaf area. Disease index of panicle blast was calculated from the rates of rotten neck and panicle branch blast incidence by the formula:

$$\text{Disease index (\%)} = \frac{(4A+3B+2C+D)\times 100}{4\times \text{total investigated numbers}}$$

A is the number panicles with rotten neck; B, C, and D are the numbers of panicles which have more than 2/3 of the spikeletes affected, between 2/3–1/3, and less than 1/3, respectively.

Foliar application of chlobenthiazone 2.5% dust was made 5 times in total, 3 times at 0.75 kg a.i./ha against leaf blast and 2 times

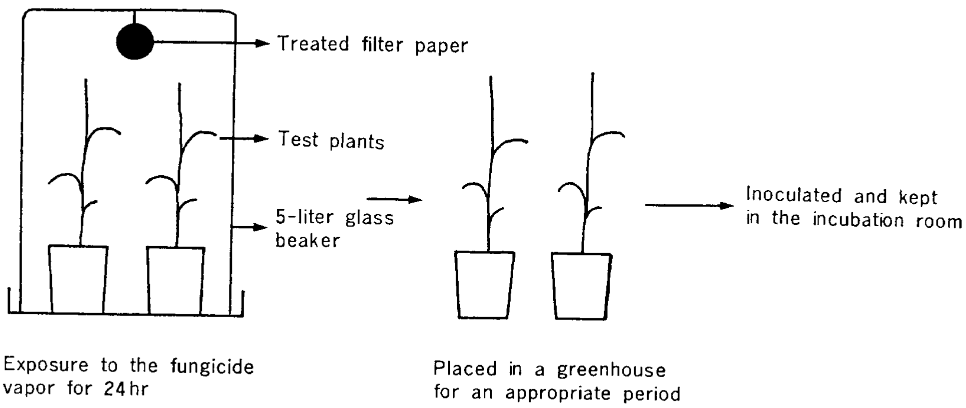

Fig. 1 Schematic representation for testing of vapor phase activity of chlobenthiazone.

at 1 kg a.i./ha against panicle blast, respectively. Edifenphos (*O*-ethyl *S,S*-diphenyl phosphorothiolate) and fthalide (4,5,6,7-tetrachlorophthalide), popular rice blast fungicides, were used as reference.

Submerged application was made by uniformly scattering chlobenthiazone 8% granule twice at 2.4 kg a.i./ha against leaf blast and 3.2 kg a.i./ha against panicle blast. Probenazole (3-allyloxy-1,2-benzisothiazole-1,1-dioxide), isoprothiolane (diisopropyl 1,3-dithiolan-2-ylidenmalonate) and IBP (*S*-benzyl *O,O*-diisopropyl phosphorothioate) were used as reference. All fungicides were used at the rates and intervals recommended by the manufacturers.

3. *Systemic Movement in Rice Plants*

Chlobenthiazone ^{14}C-labeled in the phenyl ring (19.2 mCi/mmol) was prepared as aqueous suspension at 500 μg a.i./ml. Foliar application of the labeled compound was made by spreading 50 μl on the upper surface of the 3rd leaf of the plant at the 3.5th leaf stage and then the plant was placed in a greenhouse for 2 days. With root application, roots of the plant at the 4th leaf stage, which had a primary tiller, were thoroughly washed with tap water and placed into Erlenmyer flasks with 100 ml of a Kasugai's nutrient solution containing 5 μg a.i./ml of ^{14}C-chlobenthiazone, and then the plant was incubated in a greenhouse for 2 or 4 hr.

For autoradiography, the treated plant was placed between several sheets of filter paper in an air-conditioned room maintained at 4°C until drying. The dried plant was exposed to an X-ray film for one week in the same room.

RESULTS

1. *Activities in Pot Tests*

The results in Table 1 show high preventive and residual activities of chlobenthiazone against rice blast disease. The fungicide at concentrations above 1.5 μg/ml showed preventive activity and completely controlled the disease at concentrations above 6.2 μg/ml. When the inoculation was made 4 days after the treatment, residual activity of the fungicide was recognized at concentrations above 50 μg/ml. On the contrary, curative treatment was not effective in controlling the disease even at a concentration as high as 500 μg/ml.

Systemic activity in the plants in relation to the time after treatment and to the dose by soil treatment of chlobenthiazone is shown in Table 2. When the inoculation was made 5 days after the treatmet, the disease was completely controlled at a concentration as low as 0.8 μg/ml. At concentrations above 3 μg/ml, the effectiveness of disease control persisted for 15 days but lower dosages were not enough to maintain the activity.

As shown in Table 3, chlobenthiazone gave protection against rice blast to the plants which were exposed to the fungicide vapor for 24 hr. The fungicide even at a quantity as small as 0.1 μmol per filter paper showed the effectiveness in the disease control. The plants which were exposed at rates above 10 μmol per filter paper were almost completely protected and the protection was maintained for 48 hr after the exposure to the vapor.

Table 1 Preventive, curative and residual activities of chlobenthiazone against blast disease.

Concentration (μg/ml)	Disease control activity (%)		
	Preventive	Curative	Residual
500	100	3	100
200	100	0	100
100	100	0	85
50	100	0	35
25	100	0	0
12.5	100	0	0
6.2	100	0	0
3.1	75	0	0
1.5	28	0	0

Table 2 Systemic activity of chlobenthiazone in relation to the time of treatment and the dose by soil treatment.

Concentration (μg/ml)	Disease control activity (%)		
	5 days	10 days	15 days
6	100	100	100
3	100	100	100
1.5	100	88	80
0.8	100	85	67

Table 3 Vapor phase activity of chlobenthiazone.

Chlobenthiazone (μmol/filter paper)	Disease control activity (%)[a)]		
	0	24	48
100	100	100	100
50	100	97	98
10	100	99	100
1	99	96	81
0.1	85	65	0

[a)] After exposure to the fungicide vapor for 24 hr, the exposed plants were placed in a greenhouse for indicated hr.

2. *Effectiveness in Field Trials*

When the application of chlobenthiazone 2.5% dust to the foliage began prior to the disease outbreak, leaf blast was effectively controlled. Panicle blast was also effectively controlled by the foliar application at both of booting and middle heading stages (Table 4).

With submerged application of the granule 7 days prior to the primary disease outbreak in controlling leaf blast, chlobenthiazone 8% granule provided leaf blast control as well as the reference fungicide, probenazole. When the fungicide was applied 14 days prior to the booting in controlling panicle blast, both rotten neck and panicle branch blast were the most effectively controlled among the fungicides tested (Table 5).

3. *Systemic Movement of ^{14}C-Chlobenthiazone*

The autoradiogram of the plant which was incubated in a greenhouse for 2 days after the treatment of the 3rd leaf with ^{14}C-chlobenthiazone showed that radioactivity from the labeled

Table 4 Leaf and panicle blast control by foliar application of chlobenthiazone 2.5% dust.

Treatment[a)]	Rate (kg a.i./ha)		Leaf blast (%)	Panicle blast		
	Leaf[b)] blast	Panicle[c)] blast		Rotten neck(%)	Panicle branch blast (%)	Disease index
Chlobenthiazone	0.75	1	3.9	8.1	16.3	18.5
Edifenphos	0.75	1	5.6	4.8	21.6	19.9
Fthalide	0.75	1	4.7	10.9	16.1	22.2
Untreated	—	—	23.0	28.5	14.9	39.0

[a)] 2.5% dust formulations of the test fungicides were applied.
[b)] The first application was made prior to the primary disease outbreak and the timing of the second and third applications depended on the disease development.
[c)] Applications were made at both of booting and middle heading (50% panicle emergence) stages.

Table 5 Leaf and panicle blast control by submerged application of chlobenthiazone 8% granule.

Treatment	Rate (kg a.i./ha)		Leaf blast (%)	Panicle blast		
	Leaf[a)] blast	Panicle[b)] blast		Rotten neck (%)	Panicle branch blast (%)	Disease index
Chlobenthiazone (8% G)	2.4	3.2	0.3	0.9	1.0	1.2
Isoprothiolane (12% G)	3.6	4.8	1.1	1.2	5.6	3.9
Probenazole (8% G)	2.4	3.2	0.2	1.4	7.1	4.9
IBP (17% G)	5.1	6.8	1.3	1.6	10.3	6.7
Untreated	—	—	1.5	2.8	19.4	10.2

[a)] Application was made 7 days prior to the primary disease outbreak.
[b)] Application was made 14 days prior to the booting stage.

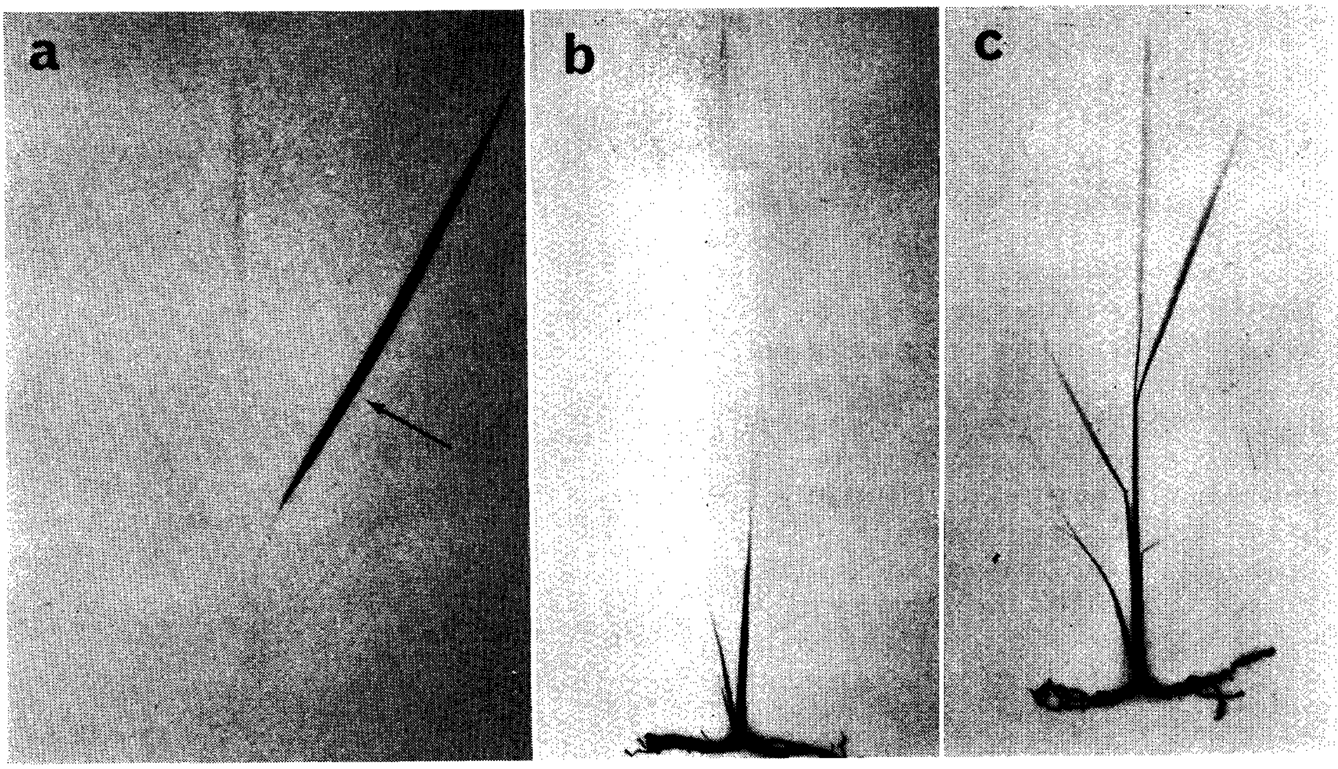

Fig. 2 Autoradiograms of rice plants which were treated with ^{14}C-chlobenthiazone.
a: 2 days after foliar treatment. (The arrow indicated the treated leaf)
b: 2 hr after root treatment.
c: 4 hr after root treatment.

fungicide was translocated to mature leaves and also leaves newly developed during 2 days incubation in a greenhouse (Fig. 2, a). Furthermore, ^{14}C-chlobenthiazone was rapidly absorbed by the roots. The autoradiograms (Fig. 2, b and c) of the plants, of which the roots were immersed in a Kasugai's nutrient solution containing the fungicide, indicated a rapid distribution of radioactivity during 2 to 4 hr incubation periods.

DISCUSSION

Chlobenthiazone was remarkably effective in controlling rice blast caused by *P. oryzae.* The fungicide is classified not only as a preventive fungicide like fthalide[6] of foliar blast fungicides[7-9] but also as a systemic fungicide like isoprothiolane,[10] probenazole[11] or IBP,[12] because of their preventive and residual activities without curative activity by foliar treatment and its systemic activity by root treatment. These properties were also confirmed in field trials.

In order to exert a fungicidal effect, fungicides must directly contact the pathogens attached to plant surface. However, in foliar application, fungicides do not form a uniform cover on the leaf. Therefore, systemic movement and vapor action of fungicides in or on plants must contribute to an efficient disease control in fields.

The autoradiograms of ^{14}C-chlobenthiazone treated plants (Fig. 2, b and c) showed that the fungicide was rapidly absorbed by rice seedlings through the roots from a nutrient solution containing the fungicide and translocated into all parts of the leaves. The fungicide accumulated in the leaves in a sufficient amount to control rice blast as demonstrated by pot trials. Similarly, when the labeled fungicide was applied to the leaves, the autoradiogram (Fig. 2, a) indicated that the fungicide penetrated translaminarly into the leaves and moved not only into mature leaves but also into leaves newly developed during 2 days incubation in a greenhouse after foliar treatment. Therefore it is concluded that the fungitoxic compound moves upward and downward in rice plants, although it is not clear whether the translocated substance is chlobenthiazone itself or a metabolized fungitoxic product.

Chlobenthiazone has also vapor phase activity which may contribute to a distribution of the fungicide. The fungicide gave protection to the plants which were exposed to the fungicide vapor. The exposed plants at rates above 10 μmol per filter paper in a 5-liter beaker were almost completely protected from the disease and the protection was maintained at least for 48 hr after the exposure to the vapor.

From the properties of chlobenthiazone described above, it is suggested that the upward and downward movements, and vapor phase activity of the fungicide must increase the chance of its contact with many spores on plant surfaces, and this contributes to efficient disease control in practice.

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要　　約

Chlobenthiazoneのイネいもち病への作用特性*

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新規なイネいもち病防除剤, chlobenthiazone(S-1901), 4-chloro-3-methyl-2(3*H*)-benzothiazolone の作用特性について検討した．本薬剤は低濃度の茎葉処理で予防，残効効果を示すが，治療効果は 500 μg/ml の高濃度でも示さなかった．土壌処理は低濃度で高い防除効果を示した．また本薬剤は，すぐれた浸透移行性および揮散性を有することが ^{14}C-chlobenthiazone を用いた実験およびポット試験結果から判明し，これらの性質は，圃場での茎葉処理または水面処理による防除効果に寄与しているものと思われる．

* Chlobenthiazone のイネいもち病防除機構（第1報）