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CHEMICAL CONTROL OF RICE BLAST CAUSED BY PIRICULARIA ORYZAE CAV

Part I.—Effect of Several Foliar Fungicides on P. Oryzae in Vitro

HASAN ASHRAFUZZAMAN

East Pakistan Agricultural University, Mymensingh

R. A. FREDERIKSEN

A & M University, College Station, Texas, U.S.A.

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The relative merits of several fungicides potentially effective against foliar diseases were evaluated in the laboratory for their effectiveness against *Piricularia oryzae*. All fungicides tested inhibited spore germination when used at a concentration of 200 ppm, but only four products were effective at 100 ppm or less: Blasticidin-S (an antibiotic from *Streptomyces griseochromogenes* Fukunaga), PMA (phenyl mercuric acetate), Actidione (cycloheximide) and Du-Ter W-50 (triphenyl tin hydroxide). Similarly, only these 4 materials were highly effective on the growth of *P. oryzae* and retarded mycelial growth at concentration as low as 50 ppm. Because of its selectivity, the growth-inhibition test is preferable to the spore-germination test as a primary means of selecting potential rice blast fungicides.

Introduction

Blast, caused by *Piricularia oryzae* Cav., is an internationally important disease of rice. In many countries it seriously reduces rice production.[1–3] Blast is characterized by brown lesions on the leaves and on the neck (top node of the panicle). Both of these phases can be highly destructive.[4] Severe leaf infection leads to total destruction of the foliage (Fig. 1). As a result of the neck infection, half filled or totally chaffy panicles are formed and the heads also tend to break over at the point of infection. The disease is known by a number of common names: rotten-neck, leaf blast, node blast, brusone, momi-imochi and many others.[5] The early records of rice blast were mainly from Italy and Japan. *P. oryzae* was first described in 1891 by Cavara in Italy.

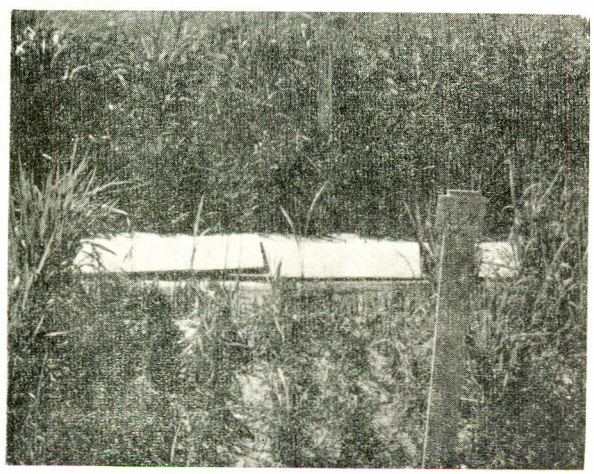

Fig. 1.—An epiphytotic of leaf blast: The plants in the center of the picture have severe leaf damage whereas those on the perimeter are not so severely affected.

Control of blast by means of resistant varieties is the most economical control method, but the task of replacing the present susceptible types with resistant ones is, in most cases, laborious and time consuming. Besides, the possible occurrence of new or more virulent pathogenic races of *P. oryzae* challenges the available sources of resistant germ plasm and may nullify prior breeding work. In addition, the blast pathogen has many pathogenic races which complicates breeding work. Frequently, however, susceptible varieties are grown when resistant varieties are unavailable or when certain qualities of rice are desired. These varieties can undergo even greater yield losses from rice blast when they are heavily fertilized, particularly with nitrogenous fertilizers.[6,7] Some of the more serious losses may be reduced by proper chemical control. The primary objective of the present investigation was to explore the possibility of chemical control of rice blast. Several fungicides potentially effective against foliar diseases were selected. The relative merits of these materials, based on their ability to retard mycelial growth and spore germination of *P. oryzae*, were evaluated in the laboratory.

The germination of fungus spores in presence of chemicals is an important method for determining the fungistatic action of the materials and is recommended by the American Phytopathological Society.[8] Many investigators [3,9] have used spore-germination tests for initial screening of fungicides against various pathogens. Terui

et al.[10] studied the inhibitory effect of several antibiotics and Fumiron, an organo-mercurial fungicide, on the conidial germination of *P. oryzae* by the drop culture method but only Actidione was found to be effective. Misato *et al.*[11] demonstrated that PMA is better than Blasticidin-S in inhibiting spore germination. Fukunaga,[12] on the contrary, stated that Blasticidin-S has the same effect as PMA in this respect. Hashioka *et al.*[13] reported that the germinability of conidia could be severely repressed by PMA at the concentration of 0.1 ppm. Misato *et al.*[14] obtained complete inhibition of conidial germination with PMA and Blasticidin-S at 50–100 ppm.

Many investigators[15,16] have used the paper-disk plate method with a high degree of precision for assaying fungicides and bacteriocides against various organisms but none are reported for tests with *P. oryzae*.

Materials and Methods

The investigation was conducted at the Rice-Pasture Research and Extension Center, Beaumont, Texas, U.S.A. The isolate of *P. oryzae* was supplied by Dr. John. G. Atkins. A rice-polish agar medium composed of rice polish (20 g) and agar (15 g) was used for growing the fungus. Twenty fungicides, listed below, were selected on the basis of their ability to control several foliar diseases.

Fungicide	*Source*	*Active ingredients*
Actidione	Upjohn Co.	85--100% cycloheximide
Blasticidin-S	Nihon Nohyaku Co. Ltd. (Japan)	2--4% Blasticidin-S benzyl amino benzene sulfonate
Brestan-60	American Hoechst Corporation	60% Triphenyl tin acetate wettable powder
Dithane M-22 Sp	Rohm & Haas Co.	80% manganese ethylene bisdithiocarbamate (maneb) plus zinc sulphate
Dithane M-45	Rohm & Haas Co.	A coordination product of zinc ion and manganese ethylene bisdithiocarbamate 80%
Dithane M-22	Rohm & Haas Co.	80% maneb (manganese ethylene bisdithiocarbamate)
Du-Ter W-50	Thompson-Hayward Chemical Co.	50% triphenyletin hydroxide
Difolatan	Chevron Chemical Co.	80% cis-*N* [(1, 1, 2, 2-tetrachloroethyl) thio]-4-cyclophexene-l, 2-dicarboximide
El-331	Eli Lilly & Co.	11.6% cyclohexyl-phenyl-3-pyridineme-thanol in emulsifiable concentration
Fungicide 328	E.I. DuPont de Nemours & Co. Inc.	75% 3,3-ethylenebis (tetrahydro 4,6-dimethyl-2H-1,3,5-thiodiazine-2-thione)
Fungicide 1991	E.I. DuPont de Nemours & Co. Inc.	50% 1-(butyl carbamoyl)-2-benzimidazole carbamic acid, methyl ester wettable powder
Manzate	E.I. DuPont de Nemours & Co. Inc.	80% maneb (manganese ethylene-bisdithiocarbamate)
Phygon-XL	U.S. Rubber Co.	50% 2,3-dichloro-l, 4-napthoquinone
Pipron 25 W	Eli Lilly & Co.	25% 3(2-piperidino) propyl-3, 4-dichlorobenzoate
Pipron E.C.8	Eli Lilly & Co.	24.4% 3(2-piperidino) propyl-3, 4-dichloro-benzoate in emulsifiable concentration
PMA	Buckman Laboratories Inc.	30% phenylmercuric acetate in emulsifiable concentration
Plantvax	U.S. Rubber Co.	75% 2,3-dihydro-5-carboxanilido-6-methyl-, 4-oxathiin-4,4-dioxide
TH 204-F	Thompson-Hayward Chemical Co.	50% Wettable powder Identity not known
TH 174-F	Thompson-Hayward Chemical Co.	50% wettable powder Identity not known
Vitavax	U.S. Rubber Co.	75% 2,3-dihydro-5-carboxanilido-6-methyl 1-1,4-Oxathiin

Spore-germination Test.—In general the method of Abeygynawardena and Peiris[17] was followed. Solutions or suspensions of 4 different concentrations (5,10,50 and 100 ppm) of each fungicide were prepared. A circle with an internal dia of 12 mm was drawn with India ink on a clean slide. Two such circles were made on each 3″ × 1″ slide. A sample of the test fungicide (0.04 ml) was pipetted into each circular area on the reverse side of the slide to avoid mixing the ink and the test materials. The test sample was spread and dried at room temperature. Spore suspensions were prepared from 10-day old cultures. The concentration of spores was adjusted to approximately 10–15 per optical field (430X). A 0.04 ml sample of the spore suspension was put in each circular area and mixed with the deposit of the fungicides.

The slide with the spore suspension and fungicide was inverted over a Van Tieghem cell in a moist chamber. The moist chamber was prepared by adding sterile water to filter paper on the top and bottom of petri dishes. All spore suspensions and solutions or suspensions of fungicides were prepared in tap water.

Two hundred spores per treatment were examined for germination after an incubation period of 12 hr at room temperature (25–26°C). The absence of germ tube initiation after incubation period was considered inhibition of spore germination. The percentage inhibition (I) of spore germination with various concentrations of the test fungicide was calculated as follows:

$$I=\frac{c-t}{c}\times 100$$

where

c=average number of germinated spores in the control

t=average number of germinated spores in the treatment

Growth-inhibition Test.—Potato dextrose agar (PDA) was added to petri dishes in two operations. First, 10 ml of the molten medium without *P. oryzae* was poured. Next, 5 ml warm agar seeded with 0.5×10^5 to 1×10^5 spores of *P. oryzae* was poured over the surface of the solidified, non-seeded agar. The plate was rotated and levelled to assure even coverage before it solidified.

Test samples of 4 different concentrations of each fungicide were prepared with sterile water. Filter paper disks of 12.7 mm dia were evenly plated on the seeded agar. An 0.08 ml sample of fungicide preparation, which was slightly less than the amount necessary to saturate the disk, was applied immediately after the disks were plated on the agar. The fungicide preparations were measured and applied with sterilized 0.1 micropipettes fitted with a screw gauge.

The assay plates were stored at a low temperature (4°C) for 12 hr and later incubated at 25°C for 72 hr. Each treatment was replicated thrice with distilled water control. The filter paper disks were removed from the plates and the dia of the clear zone was measured to 0.5 mm accuracy by means of a metric ruler and a magnifier (Quebec bacteria colony counter). The zone of inhibition was obtained by substracting the dia of filter paper disk from that of the cleared zone.

Several fungicides not used before were tested in a separate experiment. The same general procedures of the first experiment were followed with few deviations. In this test 12×12 mm pieces of filter paper were used. Petri plates were prepared with rice-polish agar. The test plates were incubated directly in the laboratory for the development of the fungus, without pre-storing at a low temperature.

TABLE 1.—PERCENT INHIBITION OF SPORE GERMINATION OF *P. oryzae* BY DIFFERENT FUNGICIDES.

Fungicide	Inhibition%			
	150 ppm	100 ppm	50 ppm	10 ppm
Actidione	100.0	100.0	100.0	43.5
Blasticidin-S	100.0	100.0	90.0	41.2
Dithane M-22	100.0	89.2	37.2	21.9
Dithane M-22 Sp	100.0	83.2	37.4	25.4
Dithane M-45	100.0	100.0	66.3	29.3
Du-Ter W-50	100.0	100.0	78.2	32.5
Fungicide 328	75.5	49.3	21.5	15.5
Manzate	90.2	55.4	19.2	15.2
PMA	100.0	100.0	100.0	47.2
Plantvax	62.2	28.2	5.3	4.2
Phygon-XL	87.3	70.3	20.5	15.2
Pipron 25 W	91.3	61.3	17.3	14.3
Pipron E.C.	95.3	51.7	24.5	16·5
TH 174-F	100.0	74.5	49.2	19.5
TH 204-F	100.0	71.2	29.5	21.5
Vitavax	62.0	29.5	5.5	3.9

Results

Spore-germination Test.—The percentage of inhibition of spore germination are presented in Table 1. Spore germination was inhibited by all fungicides at higher concentrations. However, a marked difference in fungicidal activity in some materials at lower concentration was noticed. For example the PMA, Actidione, Blasticidin-S, Du-Ter W-50 and Dithane M-45 treatments resulted in maximum inhibition of spore germination at 10–50 ppm whereas other materials produced similar fungicidal activity at the relatively high concentration of 150 ppm. PMA, Blasticidin-S, Actidione, Du-Ter W-50 and Dithane M-45 were the most effective fungicides tested.

Growth-inhibition Test.—The experiment, in general, confirmed the results of the spore germination inhibition test. However, this testw as more specific. Large inhibition zones were obtained only with PMA. Actidione, Du-Ter W-50, Dithane M-45, Dithane M-22 and Dithane M-22 Sp had smaller yet measurable inhibition zones (Fig. 2). PMA, Actidione, Du-Ter W-50 and Dithane M-45 checked the growth of *P. oryzae*

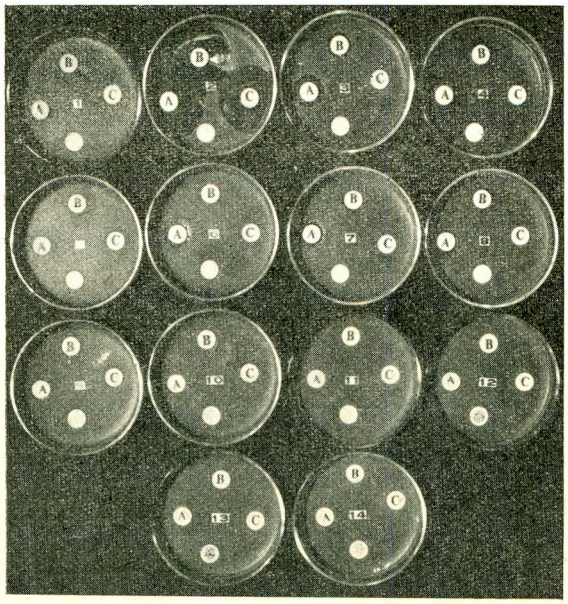

Fig. 2.—Inhibition of mycelial growth of *Piriculari i oryzae* by fungicides at various concentrations.

A—200 ppm; B—100 ppm; C—50 ppm; C—control

(1) Blasticidin-S; (2) Phenylmercuric acetate (3) Actidione (4) Du-Ter W-50 (5) Fungicide 328 (6) Dithane M-22 Sp (7) Dithane M-45 (8) Dithane M-22 (9) Phygon-XL (10) Manzate (11) Pipron E.C. (12) Pipron 25W (13) TH-174 F (14) TH-204 F.

TABLE 2.—DIAMETER OF THE GROWTH-INHIBITION ZONE OF *P. oryzae* PRODUCED BY VARIOUS FUNGICIDES.

Fungicide	Dia (mm) of inhibition zone*		
	200 ppm	100 ppm	50 ppm
Actidione	5.9	3.9	1.6
Blasticidin-S	0.0	0.0	0.0
Dithane M-22	1.3	0.0	0.0
Dithane M-22 Sp	3.6	0.3	0.0
Dithane M-45	4.3	2.0	0.0
Du-Ter W-50	5.8	2.6	0.2
Fungicide 328	0.0	0.0	0.0
Manzate	0.0	0.0	0.0
Pipron 25W	0.0	0.0	0.0
Pipron E.C.	0.0	0.0	0.0
Phygon-XL	0.0	0.0	0.0
PMA	40.0	35.0	31.9
TH 174-F	0.0	0.0	0.0
TH 204-F	0.0	0.0	0.0
Control	0.0	0.0	0.0

*Based on reading average of 3 replications after 72 hr of incubation.

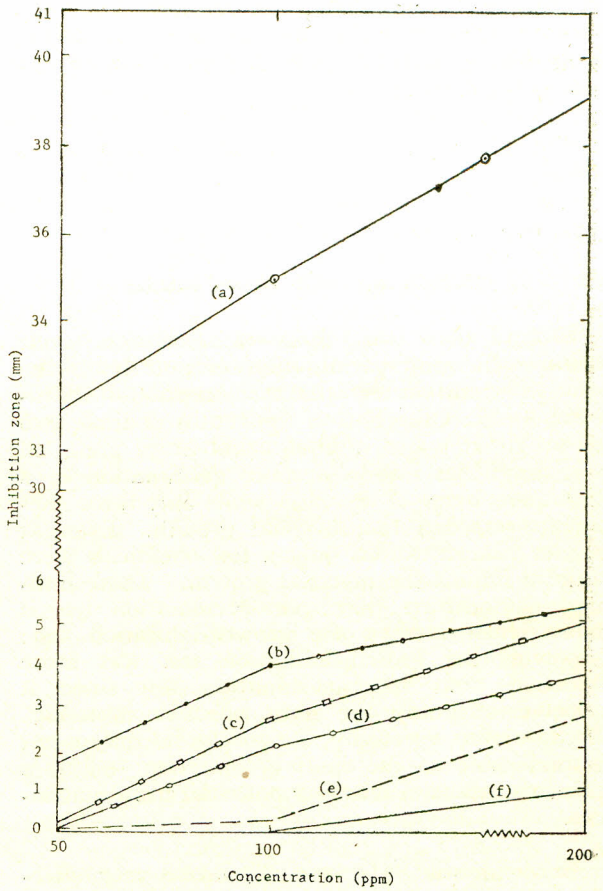

Fig. 3.—The relationship between the inhibition zone diameter and the concentrations of effective fungicides.

(a)—Phenylmercuric acetate (b)—Actidione (c)—Du-Ter W-50 (d)—Dithane M-45 (e)—Dithane M-22 Sp (f)—Dithane M-22.

TABLE 3.—DIAMETER OF THE GROWTH-INHIBITION ZONE OF *P. oryzae* PRODUCED BY FOUR FUNGICIDES.

Fungicide	Dia (mm) of inhibition zone*	
	200 ppm	100 ppm
Brestan 60	0.0	0.0
Difolatan	0.0	0.0
EL-331	29.6	20.0
Fungicide 1991	29.3	21.6
Control	0.0	0.0

*Based on average reading of three replications after 72 hr of incubation.

better than the other materials (Table 2). This method may be more suitable for screening than the spore germination method since it appears to be more selective.

The relation of the inhibition zone to the fungicide concentrations was plotted on a graph paper with the concentrations(ppm) as the abscissa and the zone of inhibition as the ordinate (Fig. 3). The

coordinate, in most cases, was a straight -line relationship indicating that the size of the inhibition zone was directly proportional to the fungicide concentrations.

The results of the second experiment are presented in Table 3. The growth of *P. oryzae* was markedly inhibited by fungicide 1991 and EL-331, two new experimental products.

Discussion and Conclusions

Overall, there was a close relationship in results between the spore-germination test and that of the growth-inhibition test with the exception of Blasticidin-S. A comparison of the results of these two tests (Table 1 and 2) shows that at 50 ppm, all test fungicides inhibited spore germination in a high percentage of the cases while only three chemicals retarded the mycelial growth. Even at higher concentrations only a few chemicals were able to inhibit the mycelial growth. These findings demonstrate that spore-germination test is more sensitive than the growth-inhibition test: however, the growth-inhibition test was more selective than spore-germination test since it selected out only the most effective materials. Because of its selectivity, the growth-inhibition test is preferable to the spore-germination test as a primary means of selecting potential blast fungicides.

Blasticidin-S, PMA and Actidione very effectively inhibited the germination of *P. oryzae* (Table 1). These results are in agreement with the previous findings.[10–14] Blasticidin-S did not inhibit growth (Fig. 2). There could be two possible reasons for this deviation: First, this experiment was conducted in 1967 and the material used was over one year old. Blasticidin-S is an unstable compound and loses its activity rapidly.[18] Second, much of the material in water suspension was absorbed on the upper surface of the filter paper disk.

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