Pakistan J. Sci. Ind. Res., Vol. 13, Nos. 1–2, August 1970

SYSTEMIC CONTROL OF RICE BLAST CAUSED BY PIRICULARIA ORYZAE CAV.

HASAN ASHRAFUZZAMAN

Department of Plant Pathology, Agricultural University, Mymensingh, East Pakistan

(Received March 3, 1969)

Thirteen fungicides were tested to evaluate their merits in systemic control of rice blast. Blasticidin–S and PMA (Phenyl Mercuric Acetate) were found to act systemically and they significantly reduced both the number and size of lesions found at sites relatively distant from the point of application.

Blasticidin–S, Du-Ter W–50 (Triphenyl tin hydroxide) and PMA acted as eradicants when they were applied at the pin-head stage of development of lesion. These fungicides did not kill the internal mycelium nor could they reduce the viability of the conidia but they retarded lesion development and controlled leaf blast by reducing sporulation nearly two fold. A new experimental product, Fungicide 1991 also found to control blast systemically.

Numerous attempts have been made to control blast. Extensive research on blast ecology, resistance and on methods of control, has been conducted in Japan. Control of disease by systemic fungicides is the most efficient and is certainly the most appealing. The systemic toxicant must be absorbed by the plant and translocated to different parts. Various greenhouse methods have been devised to evaluate the systemic action of candidate fungicides. Potted plants may be watered for a few days with a fungicide solution[1,2] or the seedlings may be allowed to stand in solution for 2 to 3 weeks prior to inoculation.[3]

Eradicative fungicides permit control of diseases after infection has taken place; this is highly desirable. Eradicative fungicides must be systemic. Keitt and Jones[4] were to report that distinct inhibition of scab development was possible when the mixture of lime-sulphur and arsenate of lead was applied 24 hr after the infection period began. The possibility of the use of so-called eradicative spray for scab control was thoroughly explored in the greenhouse studies on potted apple trees by Hamilton.[5] Since then various workers have done much to expedite the use of eradicative fungicides against many diseases.[6,7,8] In Japan, Misato *et al*[9] demonstrated that greenhouse grown rice plants when sprayed with a solution of Blasticidin–S 1 or 2 days after inoculation with the spore suspension of *Piricularia oryzae*, fewer lesions were detected on the leaves. He also reported that Blasticidin–S was more effective than PMA as a curative fungicide. Du–Ter W–50 is claimed to be an eradicant of several diseases by some workers in the United States (Chemical and Physical properties of Triphenyl Tin Hydroxide. Thopmson Hayward Chemical Co.). The relative merits of several fungicides in systemic control of blast are reported in the present paper.

Materials and Methods

The experiment was conducted at the Ricepasture Research and Extension Centre, Beaumont, Texas, U.S.A. The systemic effect of the fungicides was evaluated in two tests. In the preliminary experiment 13 materials were included. Gulfrose rice seedlings were grown in metal flats. The fungicides were applied at the 3–leaf stage until run-off occured. Five to six days later the seedlings in the 4–leaf stage were inoculated by spraying 10 ml of the standard spore suspension per flat. These were kept in the humidity chamber for 36–48 hr. Each treatment was replicated 3 times with a water control. One week after inoculation the numbers of typical blast lesions, on the fourth plant leaf, from different treatments were compared. The percentage reduction (R) in blast lesions with test fungicides was calculated as follows:

$$R = \frac{C-T}{C} \times 100$$

where, C=Average number of lesions in the control

T=Average number of lesions in the treatment

In the second test, 18–day old rice seedlings were dug up and washed in water. Seven seedlings were transplanted into a 1 pint Mason jar containing the fungicidal preparation. The seedlings were kept erect by 2 wire nets, one at the mouth and the other approximately 3″ lower. For a better estimation of systemic action of the candidate fungicides, the plants were grown in the aqueous fungicidal preparations for 6 days prior to inoculation inorder to maintain a continuous flow of the test materials into the plant tissue. The plants of each jar were inoculated by 10 ml of the standard spore suspension and then were incubated as in the previous experiment. The fungicidal preparation was changed prior to inoculation. This experiment was conducted with 3 replicates and repeated. Plants growing in water were served as a control. Seven days after inoculation the lengths of the lesions on the third and

fourth leaves of each seedling were recorded, thereby minimizing the effects of natural variation in lesion size due to plant aging.

Seven fungicides were tested; 2 new candidate fungicides reported to have systemic action were included in this test. The other materials were selected on the basis of their performance in the preliminary test. In addition, Du–Ter W–50 and Fungicide 328 were included for comparative purposes. Initially the plants in the aqueous preparation of fungicides developed phytotoxic symptoms such as yellowing, drying and rolling of leaves. The concentration of the fungicidal preparations were determined by diluting the solutions until they produced little or no toxicity.

To evaluate the eradicative properties of the test fungicides rice seedlings were grown in 4″ plastic pots and thinned to 10 plants per pot. Seedlings at the 4–leaf stage of growth were inoculated with a conidial suspension of *P. oryzae* as in the preceding tests. After inoculation the plants were incubated in the humid chamber for 24 hr at room temperature and then returned to the greenhouse. Seedlings with pinhead lesions (three days after inoculation) were sprayed with 20 ml of the fungicidal preparation per pot. This test included two experiments, each replicated 3 times with a water control.

One week after inoculation the plants were rated for disease severity. An index chart for evaluating the severity of disease in different treatments was devised as follows:

Index for the development of lesions (total mm length of lesions on each of 10 leaves)	Index for the number of lesions on each of 10 leaves
A= 1–25	I= 1–10
B=25–50	II=10–25
C=50–100	III=Above 25
D=Above 100	

The above infection score was converted into a numerical scale as follows:

Numerical index for the lesion size (lengthwise)		Numerical index for the number of lesions	
A	1		
B	2	I	1
C	4	II	2
D	8	III	3

The numerical score for the intensity of disease was then obtained by multiplying the two numerical index values. The numerical score was used in the analyses of varience.

Mode of Action of PMA, Du-Ter W-50 and Blasticidin-S on P. oryzae.—Because of the specific activity of PMA, Du–Ter W–50 and Blasticidin–S, as determined in earlier tests, three exploratory tests were carried out for the purpose of obtaining some informations as to the manner in which these chemicals act on the blast pathogen. Leaves with blast lesions were collected from the eradication test for this purpose.

To determine the effect of PMA, Du–Ter W–50 and Blasticidin-S on internal mycelium, leaves with blast lesions were collected and washed thoroughly with a brush in running water to remove the residual fungicide. Later, pieces of tissue from the margin of the lesions were sectioned off, surface-sterilized in 1:10 chlorox for a half-minute, and plated out on rice-polish agar. The plates were incubated at room temperature (23 to 24°C) for 72 hr. Later the test plates with fungal cultures were examined for blast pathogen.

In the second experiment an attempt was made to determine the effect of PMA, Du–Ter W–50 and Blasticidin-S on sporulation of *P. oryzae*. Several leaves with blast lesions were collected from different plants from each treatment and were incubated in moist chamber for 48 hr. Two groups of five lesions of approximately similar size were selected from different leaves from each treatment. Their upper surfaces were brushed with a fine camel hair brush into the depressions of a porcelain spot-plate containing 0.05 ml of water. Two suspensions were prepared from 2 groups of 5 lesions. One drop of spore suspension was placed on a clean slide and covered with a cover glass. Two slides were prepared from each suspension. The number of spores appearing in 10 fields was counted under the high power objective (430×).

In the last experiment, the effect of the above fungicides on the viability of conidia of *P. oryzae* was determined. Leaves with blast lesions from treated and untreated plants were transferred in a moist chamber with the humidity high enough to stimulate growth but not so high as to form a film of moisture on the sprayed surfaces. After a 48 hr incubation period, conidia produced on the lesions were collected on a small block of water agar that was held on the tip of a needle. The agar block was then placed on a slide with the spore-adhering surface up. Ten slides were prepared from 10 different lesions for each treatment. Assay slides were incubated in a moist chamber. After 16 hr a cover glass was put on the agar block. Fifty spores from each slide were checked for germination.

Results

In the preliminary test the first sign of infection on the leaves was observed after 48 hr. Within a week the lesions attained a prominent size. The lesions varied in size from minute flecks to large spots. Blasticidin-S and PMA considerably reduced the number of lesions on the newly-developed leaf (fourth leaf) compared to the other treatments (Table 1). This indicates that there had been some translocation of the materials from the treated lower leaves to the untreated fourth leaf.

In many cases lesions developed slowly in treated plants whereas in the control and in some treatments development of lesions was rapid. In order to avoid overlapping of the lesions, data was collected one week after inoculation of the plants. As a result, fewer lesions were recorded in some cases because it was possible to count only those typical lesions that were large enough to be observed. The number of lesions depend on the number of viable spores in the spray preparation and also on the number of spores which actually come in contact with the host test, therefore, has some disadvantages when used to evaluate systemic fungicides.

In the second test several chemicals caused substantial suppression of blast lesion development suggesting that the chemicals had been translocated (Table 2). At least 60 percent reduction in lesion size was obtained with Blasticidin–S, PMA and Fungicide 1991 whereas other materials caused little or no reduction of lesion size. Statistically these fungicides were superior to all other fungicides and significantly reduced the lesion size.

TABLE 1.—CHEMOTHERAPEUTIC REDUCTION OF LESION NUMBER ON GULFROSE RICE SEEDLINGS GROWN IN THE GREENHOUSE.*

Fungicide	Concentration (ppm)	Percentage reduction of blast lesions**
PMA	30	29.14
Blasticidin–S	30	28.25
Dithane M–45	2400	17.93
Du–Ter W–50	100	17.93
Fungicide 328	2250	16.36
TH 204–F	500	9.64
Dithane M–22 Sp.	400	7.39
Phygon–XL	250	7.39
TH 174–F	500	7.39
Manzate	2400	5.15
Dithane M–22	400	4.48
Pipron E.C.	740	4.48
Pipron 25–W	750	4.48

*Plants were sprayed at 3–leaf stage and inoculated at 4–leaf stages of development.

**Based on the lesion counts from fourth leaf of each plant.

In eradication test disease intensity was reduced by 96.5, 92.0 and 87.5 percent with Blasticidin–S PMA and Du–Ter W–50 respectively (Table 3). Fungicide 1991, Dithane M–45 and EL–331 retarded lesion growth moderately while little or no control was obtained with Fungicide 328 and Manzate. The extent to which the plants were affected was estimated by calculating a disease index based on the number and size of lesions on a particular leaf. This method of estimation is more accurate since the index reflects the 'actual' disease, not merely the number of lesions. Statistically the most effective fungicides are Blasticidin-S, PMA, Du–Ter W–50 and Fungicide 1991, respectively. Dithane M–45, EL–331 are moderately effective while the others are ineffective.

Mode of Action of PMA, Du–Ter W–50 and Blasticidin–S on P. oryzae. The pathogenic fungus was easily isolated from the culture lesions plated on rice-polish agar medium. It also formed spores. This indicates that the mycelium within the tissue of the host was not killed by the fungicidal sprays.

Concerning the effect of the above fungicides on sporulation, all treatments reduced the number of spores as compared to the untreated leaves (Table 4). Blasticidin-S treatment significantly inhibited spore production in affected lesions.

The third experiment revealed that conidial germination was not affected by the spray materials. This indicates that the chemotherapeutic action of these materials does not carry over into the second generation of conidia, although the lesions from which these conidia developed were restricted in growth.

TABLE 2.—DEVELOPMENT OF BLAST LESIONS WHEN THE SEEDLINGS WERE GROWN IN VARIOUS AQUEOUS FUNGICIDAL PREPARATION.

Fingicide	Concentratin (ppm)	Percentage reduction in lesion size*	Average length of lesions* (mm)	Statistical significance**
Blasticidin–S	5	87.3	4.8	a
PMA	10	73.4	10.0	ab
Fungicide 1991	500	60.8	15.0	b
EL–331	30	17.9	31.3	c
Dithane M–45	100	14.7	32.6	c
Du–Ter W–50	60	3.5	36.8	c
Fungicide 328	94	1.4	37.6	c
Control	0	0.0	38.1	c

*Based on lesions from 42 leaves.

**Means not followed by the same letter differ significantly at the 1% level (Duncan's multiple range test).

TABLE 3.—RELATIVE THERAPEUTIC EFFECT OF FUNGICIDES AGAINST LEAF BLAST ON GULFROSE RICE SEEDLINGS GROWN IN THE GREENHOUSE.*

Fungicide	Concentration (ppm)	Percentage disease control**	Average blast intensity	Statistical significance‡
Blasticidin–S	30	96.5	1.0	a
PMA	30	92.0	2.3	a
Du–Ter W–50	100	87.5	3.6	a
Fungicide 1991	1000	75.0	7.3	ab
Dithane M–45	2400	50	14.6	bc
EL–331	330	45.0	16.6	c
Fungicide 328	2250	26.6	21.3	cd
Manzate	2400	0.0	29.3	d
Control	0	0.0	29.3	d

*Based on the lesions from 60 leaves (10 leaves per replication) per treatment.
**Plants were inoculated at the 4 leaf stages of growth and sprayed with fungicide preparation 3 days later.
‡Means not followed by the same letter differ significantly at the 1% level (Duncan's multiple range test).

TABLE 4.—AVERAGE NUMBER OF SPORES DEVELOPED ON LESIONS OF EQUAL SIZE ON GULFROSE RICE SEEDLINGS SPRAYED WITH VARIOUS FUNGICIDES.*

Fungicide	Concentration (ppm)	Average number of spores	Statistical significance**
Blasticidin–S	30	10.0	a
PMA	30	13.2	ab
Du–Ter W–50	100	15.0	ab
Control	0	20.7	b

*Seedlings were sprayed 3 days after inoculation.
**Means not followed by same letter differ significantly at the 1% level (Duncan's multiple range test).

Discussion

A systemic fungicide is one that is translocated within a given plant or from one part of the plant to other parts and minimizes the importance of precise timing and sequences of spray required for disease control. Blasticidin–S and PMA were systemic fungicides although the preventive action of Blasticidin–S on plant parts not treated with fungicides was much weaker than that produced by PMA.[10] In the present experiment there was a gap of 5 to 7 days between the application of fungicide and inoculation. Blasticidin–S is unstable and decomposes rapidly in sunlight.[11] In addition, since Blasticidin–S is translocated upward in the leaves and stems, it remains only one third of its original activity after 4 days and none after 7 days.[12] This loss in activity probably accounted for the reduced effectiveness of Blasticidin–S in the translocation study.

In subsequent experiments leaf blast was assessed by measuring the lesion length. This method is slower but more sensitive than merely counting lesions. The assessment of average lesion size tended to avoid minor variations in the inoculation technique as pointed out in the results section. In liquid culture, there was a continuous transportation of fungicides into the plant tissues when the plant roots were submerged. The continuous exposure of the plants to the test materials may account for the better fungicidal activity and retarded lesion development. Roots are adapted for rapid absorption and, in liquid culture, almost anything which is presented to them in solution will enter. Movement from the root is apparently passive in the translocation stream. If this water movement can be maintained, accumulation in the leaves will continue. Systemic control of disease *via* the roots, then, depends more on findings chemicals which are nonphytotoxic (and which remain available in the soil) than on overcoming major difficulties in translocation.

The concept of systemic control of plant diseases is still too vaguely defined to permit definite conclusions regarding the type of compound which is likely to be active and the way in which it acts. Most of the chemicals tested are not necessarily specific to a particular type of disease; some of those selected for effectiveness against a variety of diseases may also reduce the spread of blast disease. When the results of different tests are compared, the only compound which appeared to give systemic control is Blasticidin–S. Although the effect of the remaining materials is relatively small, the test illustrated that a variety of compounds, such as PMA and Fungicide 1991, may be expected to act internally against plant diseases.

Preventive practices are more difficult to justify chemical control since blast varies in intensity from year to year. Repeated applications of protective fungicides during blast free years is needlessly expensive and discourages usage in other years. Consequently, an eradicative fungicide that can be applied as needed would be more desirable. Besides, rice plants are difficult to cover adequately with protective sprays. So, it would be very advantageous if the disease was controlled by carrying the therapeutant to the infection court. For this, the therapeautant must be systemic so that it can be absorbed by the plant and translocated. Blasticidin–S, PMA, Fungicide 1991 and Du–Ter W–50 were eradicative fungicides (Tables 3). Blasticidin–S, PMA and Fungicide 1991 were also systemic (Table 2). These findings suggest the possibility of reducing blast by spraying the fungicides after the infection has been initiated.

Blasticidin–S, PMA and Du–Ter W–50 did not kill the blast fungus within the tissue, nor could they reduce the viability of the conidia. The effectiveness of these chemicals against leaf blast lies in their ability to reduce the formation of conidia on the sporogenous hyphae. Sporulation on the treated infected leaves was significantly reduced by Blasticidin–S. Inhibition of protein synthesis has been suggested as the primary effect of Blasticidin–S on the metabolism of P. oryzae.[13] Whatever the effect of Blasticidin–S is on the physiology of the pathogen, the following conclusions can be made regarding the mode of action of Blasticidin–S in controlling blast: (1) The spray materials are absorbed by the host tissue and retarded lesion expression; (2) it inhibits sporulation on the infected parts. This reduces considerably the inoculum potential for secondary infection. Blasticidin–S is an eradicative fungicide and is much more effective than PMA or Du–Ter W–50 when applied after infection by the pathogen.

Acknowledgements.—The author wishes to express his grateful appreciation to Dr. Richard A. Frederiksen, Assistant Professor, A and M University, Texas, U.S.A. and Dr. John G. Atkins, Plant Pathologist, Rice-Pasture Research and Extension Centre, Beaumont, Texas, U.S.A. for their advice and assistance to the completion of this study.

References

1. G.A. Carter and R.L. Wain, Ann. Appl. Biol, **53,** 291 (1964).
2. J. Stubbs, Ann. Appl. Biol., **39,** 439 (1952).
3. S.H. Crowdy and R.L. Wain, Appl. Biol., **38,** 318 (1951).
4. G.W. Keitt and L.K. Jones, Wisconsin Agr. Exp. Sta. Res. Bull., **73,** 104(1926).
5. J.M. Hamilton, Phytopathology, **21,** 445 (1931).
6. J.M. Hamilton and M.Szkolnik, Plant Dis. Reptr., **41,** 293 (1957).
7. H.H. Hoston, Phytopathology, **43,** 659 (1953).
8. J.E. Livingston, Phytopathology, **43,** 496 (1953).
9. T. Misato, I. Ishii, M. Asakawa, Y. Okimoto and K. Fukunaga, Phytopath. Soc. Japan, Ann., **24,** 301 (1959).
10. H. Okamoto, *Chemical Control of Rice Blast in Japan. The Rice Blast Disease* (John Hopkins Press, Baltimore, Maryland, U.S.A. 1963).
11. T. Misato, I. Ishii, M. Asakawa and K. Fukunaga, Phytopath. Soc. Japan, Ann., **23,** 219 (1958).
12. T. Misato, M. Asakawa and K. Fukunaga, Phytopath. Soc. Japan, Ann., **23,** 181 (1958). (abtr. Rev. Appl. Mycol., **39,** 281 (1960).
13. T. Misato, Natl. Inst. Agr. Sci. Tokyo, Japan, Memo. (unnumbered), (1958).