Suppression and Elimination of *Rhynchosporium oryzae* by Benomyl in Rice Foliage and Seed in Liberia

MELVILLE D. THOMAS, UNV Assistant Professor, DAVID MAYANGO, Senior Laboratory Technician, and WILHELMETTA OBERLY, Teaching Assistant, Department of Agronomy, College of Agriculture and Forestry, University of Liberia, Monrovia, Liberia

ABSTRACT

Thomas, M. D., Mayango, D., and Oberly, W. 1985. Suppression and elimination of *Rhynchosporium oryzae* by benomyl in rice foliage and seed in Liberia. Plant Disease 69:884-886.

In field experiments, three to five applications of benomyl at 0.5 kg a.i./ha reduced foliar infection by *Rhynchosporium oryzae* in the rice cultivar Lac 23. In laboratory tests, *R. oryzae* was eliminated from seeds of Lac 23 soaked in 2,500 ppm a.i. of benomyl in water for 30 min. Lower concentrations of 1,200 and 600 ppm a.i. of benomyl did not eliminate the fungus from the seeds. Seed viability was not affected by soaking in 2,500 ppm a.i. of benomyl.

In 1972, leaf scald disease of rice (*Oryza sativa* L.) caused by the fungus *Rhynchosporium oryzae* Hashioka & Yokogi was reported to be widespread in West Africa (3) and later in Asia (1,2,5). In 1980, the disease was considered one of the more serious diseases of rice in Liberia (14). Although a secondary host has been reported for *R. oryzae* (6), rice seeds are probably the primary source of inoculum (4,7,10). At present, no control method specific for leaf scald is practiced in Liberia. Fungicide application to the foliage and seed could be one way of controlling the disease. Significant reductions of *R. oryzae* infection have been obtained by foliar application of the fungicide benomyl in a preliminary study in Liberia (11) and in India and Sierra Leone (8,13). This paper reports on the suppression of *R. oryzae* infection in rice leaves by benomyl and the efficacy of the fungicide in reducing seed colonization by the fungus.

Present address of first author: Principal Plant Pathologist, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), B.P. 4881, Ouagadougou, Burkina Faso (via Paris), West Africa.

Accepted for publication 14 January 1985.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

In 1982, seeds of Lac 23, a cultivar widely grown in Liberia, were sown on 9 June at the rate of six per hill, with 30 cm between hills in plots 4.5 × 2.4 m at the university farm, University of Liberia. The experimental design was a randomized complete block replicated four times. Two guard rows of Lac 23, 20 cm apart, were drilled 40 cm from each plot border. There was a total of four treatments. Two treatments involved foliar application of benomyl (50WP) in aqueous suspension. Benomyl was first applied to plants on 22 July at the four-leaf stage. Plants either received four applications until the boot stage (23 August) or five applications until the heading stage (8 September) at 0.5 kg a.i./ha per application. Each plot received 12 L of benomyl suspension from a knapsack sprayer. The other two treatments were control plants that were

either naturally infected or were inoculated with a conidial suspension of *R. oryzae* at flowering.

R. oryzae inoculum was prepared by washing 14-day-old colonies from V-8 juice agar (VA) (9) in several 125-ml Erlenmeyer flasks with sterile distilled water followed by straining through two layers of gauze cloth. The inoculum was applied as evenly as possible with a hand-operated sprayer along each row. Fourteen days before harvest, disease severity was assessed by percentage of leaf lamina with typical leaf scald symptoms. The flag and successive lower two leaves were assessed separately for each of 20 fertile tillers randomly selected from two center rows of each plot. Disease incidence was obtained by computing the percentage of each leaf type recorded as having leaf scald symptoms. The experiment was repeated on 3 June 1983 with two modifications. Plants either received two applications of benomyl until the boot stage (30 August) or three applications until the flowering stage (14 September). Disease severity was assessed on a scale of 0 = no infection to 5 = 100% infection.

Lots of 10-mo-old seeds from the naturally infected plants from the 1982 experiment were soaked in 2,500 ppm a.i. (w/v) of benomyl (25 g of seed per 250-ml suspension) for 10, 20, 25, and 30 min. The seeds were then thoroughly rinsed in water and air-dried at room temperature (26–28 C). Incidence of *R. oryzae* in the treated and untreated seeds was determined by surface-treating 2 g of seeds in 1% sodium hypochlorite containing a few drops of concentrated detergent for 2 min. Fifty seeds were selected and aseptically transferred to VA amended with 200 mg of penicillin per liter in 9-cm petri plates. One hundred seeds were examined from each treatment, and each plate received 10 seeds. Seeds were incubated at room temperature for 4 days, and the number of seeds with *R. oryzae* colonies was noted. *R. oryzae* colonies were distinguished from other fungal colonies by the mass of pink conidia, usually at the center of the colony, and by sparse, white aerial mycelium. Final identification of *R. oryzae* colonies was done with the aid of a light microscope. Percent germination of treated and untreated seeds was determined by placing 100 seeds from each lot on wet filter papers in 9-cm petri plates and in unsterilized field soil in 10-cm plastic pots. Ten seeds were placed in each plate and pot. This experiment was repeated twice.

In another experiment, 25-g lots of 10-mo-old seeds from plants artificially inoculated with *R. oryzae* from the 1982 experiment were soaked in 600, 1,200, and 2,500 ppm a.i. benomyl in water for 30 min, and the presence of *R. oryzae* in the treated and untreated seeds was determined as described before. This experiment was repeated twice. Seeds from the 1983 field experiment were not subjected to benomyl treatments because fewer than 3% of the seeds were colonized by *R. oryzae*.

Data based on field treatments were analyzed by multiple-comparison procedure using Duncan's new multiple range (DNMR) test. The log transformation of data from treatment of seeds with benomyl, which involved several levels of a quantitative factor, were subjected to regression analysis. In cases where the regression was not significant, the DNMR test was applied using the completely random design. All significance was determined at $P = 0.05$.

RESULTS

Results from plants inoculated artificially are not presented because they did not differ significantly from those obtained from naturally inoculated, unsprayed plants. In all cases but one, application of benomyl resulted in reductions in both disease severity and incidence. In 1982, the reduction in disease severity and incidence was significant in the three top leaves in plants that received benomyl until heading. Reduction of disease incidence in plants that received benomyl until the boot stage was significant only for the third leaves (Table 1). In 1983, the reduction in disease severity and incidence in plants that received benomyl until flowering was significant in the flag and third leaves and in the second and third leaves, respectively (Table 1). All other reductions of foliar infection for both years were not significant.

R. oryzae was completely eliminated from seeds soaked in 2,500 ppm a.i. benomyl for 30 min. Shorter periods of 10, 20, and 25 min significantly reduced but did not completely eliminate *R. oryzae* from the seeds (Fig. 1). Significant R^2 and F-ratio values from the log transformation for this experiment are 0.84 and 15.93, respectively. The regression equation is $Y = 1.48 - 1.15X$. Seed viability was not affected by

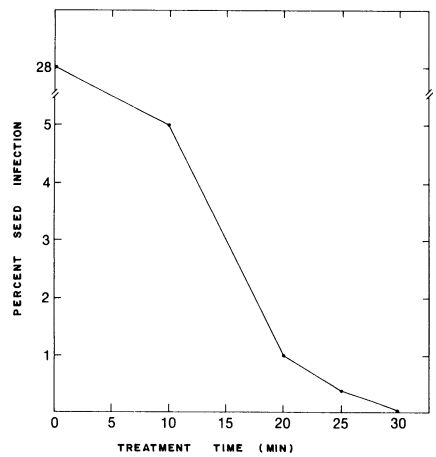

Fig. 1. Percent infection by *Rhynchosporium oryzae* of rice seeds soaked in 2,500 ppm a.i. (w/v) of benomyl in water for times indicated, based on the mean number of seeds with *R. oryzae* colonies per 100 seeds from three experiments. Critical regression values for log transformations are significant ($P = 0.05$).

Table 1. Effect of benomyl on disease severity and incidence in rice leaves infected by *Rhynchosporium oryzae*[y]

Treatment[z]	Disease severity			Disease incidence		
	Flag leaf	Second leaf	Third leaf	Flag leaf	Second leaf	Third leaf
			1982			
Unsprayed	1.06 a	3.35 a	8.41 a	30.0 a	70.0 a	93.8 a
Sprayed four times (until boot)	0.61 ab	3.19 a	5.73 a	15.0 ab	56.3 a	63.8 b
Sprayed five times (until heading)	0.0 bc	0.08 b	0.58 b	0.0 b	2.5 b	12.5 c
			1983			
Unsprayed	0.49 a	1.22 a	2.26 a	44.0 a	86.0 a	90.0 a
Sprayed twice (until boot)	0.44 a	0.89 a	1.81 a	36.0 a	69.0 a	93.0 a
Sprayed three times (until flowering)	0.19 b	0.62 a	1.37 b	20.0 a	44.0 b	75.0 b

[y] Based on visual assessment of leaf area infected (1982) and on a scale of 0 = no infection to 5 = 100% infection (1983) for severity and number of leaves with symptoms for incidence. For each year, means in a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

[z] Benomyl applied at 0.5 kg a.i. per spray.

benomyl. In the second seed treatment experiment, lower benomyl concentrations of 600 and 1,200 ppm a.i. for 30 min did not eliminate the fungus from the seeds. The mean percentage of seeds with *R. oryzae* from three experiments were 0, 1, 4, and 22 for seeds soaked for 30 min in 2,500, 1,200, and 600 ppm and untreated seeds, respectively. The regression analysis for this experiment was not significant. From the DNMR test 0, 1, and 4% were each significantly lower than 22%.

DISCUSSION

The suppression of foliar infection from *R. oryzae* by benomyl as reported here is in good agreement with earlier reports (8,11,13). The data for 1982 indicate that benomyl at 0.5 kg a.i./ha as a foliar application resulted in significant levels of control only when five applications were carried out until heading. Percent control was between 93 and 100 for disease severity and 86 and 100 for disease incidence in plants sprayed until heading.

In 1983, a cheaper schedule of benomyl application was carried out in which the highest number of sprays was three applied until flowering. Reductions in disease severity and incidence from this spray schedule were not significant in the second leaves and in the flag leaves, respectively. In all cases, the level of control did not exceed 61%. Thus, for a given concentration of benomyl, the number of applications is an important factor, especially in rain-fed rice.

Benomyl at 2,500 ppm a.i. in aqueous suspension applied for 30 min was effective in eliminating *R. oryzae* from the seeds. Apparently at 2,500 ppm, an organic carrier was not necessary for effective infusion of benomyl into the seeds. Since the next benomyl concentration tested was 1,200 ppm, it is not known whether concentrations between 1,200 and 2,500 ppm would completely eliminate *R. oryzae* from seeds of Lac 23.

R. oryzae survived in seeds of Lac 23 stored at room temperature for up to 9 mo (12). In this study, the fungus was isolated from 10-mo-old seeds. The time between harvest and planting is usually 7–8 mo. Thus seeds of Lac 23 could be important sources of *R. oryzae* inoculum. Planting seeds either from samples in which *R. oryzae* has been eliminated or from samples with low natural levels of colonization, as in the 1983 seeds, should result in reduced foliar infection.

ACKNOWLEDGMENTS

We thank M. Gweama, H. Beape, A. Johnson, and D. Duo for assisting in the field. This research was supported in part by funds from the Institute of Research of the University of Liberia and by the West Africa Rice Development Association (WARDA).

LITERATURE CITED

1. Bakr, M. A., and Miah, S. A. 1975. Leaf scald of rice, a new disease in Bangladesh. Plant Dis. Rep. 59:909.
2. D'Souza, T. F., and Venkataramanan, M. N. 1976. Note on the occurrence and distribution of leaf-scald disease of rice in Maharashtra. Indian J. Agric. Sci. 46:386-387.
3. Lamey, H. A., and Williams, R. J. 1972. Leaf scald of rice in West Africa. Plant Dis. Rep. 56:106-107.
4. Sanchez, L. M., Estrada, B. A., and Nuque, F. L. 1979. Seed transmission of *Rhynchosporium oryzae*. Int. Rice Res. Newsl. 4(6):9-10.
5. Shanmughom, S. N., Copalan, N., George, K. M., and Gopalakrishnan, R. 1973. Leaf scald of rice (*Rhynchosporium oryzae* Hashioka & Yokogi). Curr. Sci. 42:582-583.
6. Singh, S. A., and Gupta, S. P. K. 1980. *Echinochloa crusgalli* (L.) Beauv.—a new host for *Rhynchosporium oryzae* Hashioka & Yokogi. Int. Rice Res. Newsl. 5(5):17.
7. Singh, S. A., and Gupta, S. P. K. 1981. Transmission of *Rhynchosporium oryzae* Hashioka & Yokogi by seed. Int. Rice Res. Newsl. 6(2):11.
8. Singh, S. A., and Gupta, S. P. K. 1982. Efficacy of some fungicides against leaf scald disease of rice. Trop. Pest Manage. 28:412-415.
9. Stevens, R. B., ed. 1974. Mycological Guidebook. University of Washington Press, Seattle. 703 pp.
10. Thomas, M. D. 1981. Preliminary studies on isolation and means of spread of the rice leaf scald fungus in Sierra Leone. Int. Rice Res. Newsl. 6(3):13-14.
11. Thomas, M. D. 1983. Initial studies on the severity of infection and yield loss from leaf scald disease of rice under Liberian conditions. Univ. Liberia J. January: 71-78.
12. Thomas, M. D. 1984. Dry-season survival of *Rhynchosporium oryzae* in rice leaves and stored seeds. Mycologia 76:1111-1113.
13. Thomas, M. D., and Raymundo, S. A. 1983. Response of two rice varieties to *Rhynchosporium oryzae* infection. Int. Rice Res. Newsl. 8(4):11-12.
14. Virmani, S. S. 1980. Varietal resistance to rice diseases and insects in Liberia. Int. Rice. Res. Newsl. 5(2):3-4.