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Wild Rice Yield Losses Associated with Growth-Stage-Specific Fungal Brown Spot Epidemics

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ABSTRACT

Kohls, C. L., Percich, J. A., and Huot, C. M. 1987. Wild rice yield losses associated with growth-stage-specific fungal brown spot epidemics. Plant Disease 71:419-422.

The effects of fungal brown spot (FBS), caused by *Bipolaris oryzae*, on the yield of wild rice (*Zizania palustris*) during various specific growth stages were studied for 2 yr. When epidemics of FBS were initiated at boot, heading, early grain elongation, and milk stages, reductions of 67, 56, 42, and 0% resulted compared with the nondiseased control. Disease curtailment treatments, consisting of applying inoculum at the boot stage and allowing the disease to progress to mid grain elongation, milk, and grain ripening, resulted in yield reductions of 39, 74, and 74%, respectively, compared with the nondiseased control. A critical-point yield loss model derived from multiple linear regression analysis of disease severity data on the three uppermost leaves at the milk stage accounted for 87% of the variation in yield in the disease onset treatments.

Additional key words: *Cochliobolus mayabeanus*

Wild rice (*Zizania palustris* L.) is grown commercially on 10,522 ha in Minnesota and California (29). Fungal brown spot (FBS) of wild rice caused by *Bipolaris oryzae* (Breda de Haan) Shoem. (=*Cochliobolus miyabeanus* (Ito & Kurbayashi) ex Dastur)) is destructive in Minnesota (14) but not in California. Although heavy losses have occurred during severe epidemics in commercial stands in Minnesota (3), yield reductions associated with the disease have not been quantified previously.

B. oryzae caused the Bengal epidemic on rice in 1942 (22) and accounted for severe yield losses of rice in both regional crop loss assessments and in growth-stage-specific yield loss experiments (1,28).

In 1982 and 1983, studies were made at the University of Minnesota Rosemount Experiment Station to quantify yield losses associated with varying disease severities of FBS and to determine the efficacy of fungicides in curtailing an FBS epidemic at several stages of plant development. Initial accounts of these studies have been reported (17,18).

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Paper 14,831, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.

Accepted for publication 20 November 1986 (submitted for electronic processing).

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MATERIALS AND METHODS

Wild rice seed, cultivar K2, obtained from a commericial grower was stored submerged in a watery pit at 2 C for 6 mo and then sown during the first week of June. The experimental paddy was prepared by rototilling and thereby incorporating 33.7 kg of nitrogen, applied as urea. A second application of urea at 3.4 kg/ha was made during the boot stage of plant development. The seed was sown in plots 2.13 × 3.05 m. The plot density was adjusted to 23 plants per square meter. Most plants had about three tillers. The plots were sprayed with the insecticide diazinon at 1.12 kg/ha 30 days after planting to control leafminer (*Eribolus longulus* (Loew)), then sprayed with malathion at 1.12 kg/ha at the early milk stage of grain to control riceworm (*Apamea apamiformis* (Guenee)) (24).

Experimental design. Treatments in the disease onset and disease curtailment studies consisted of four and three disease progressions, respectively. A randomized complete block design was employed for the disease onset and curtailment studies with five and six replicates, respectively. Treatment plots were kept free of disease before epidemic initiation by timely applications of the protective fungicide mancozeb (Dithane M-45) at a rate of 2.25 kg/ha.

In the epidemic onset study, FBS was initiated at boot, heading, early grain elongation, and milk stages by inoculations with mixed isolates of *B. oryzae* (2.5 × 10^3 conidia per milliliter) (Table 1).

In the epidemic curtailment study, FBS was initiated in all treatments at the boot stage of development. As the growing season progressed, manocozeb was applied at 7- to 10-day intervals beginning at mid grain elongation, milk, and 50% darkened grain (ripe), respectively. The control plots were kept free of disease throughout the growing season by applying mancozeb at 2.24 kg a.i./ha every 7–10 days beginning at the boot stage and continuing up to harvest.

Inoculation. Isolates of *B. oryzae* were collected from lesions on cultivated wild rice plants in Minnesota, maintained on potato-dextrose agar (PDA, Difco) slants at 24 C, and cycled through wild rice plants in the greenhouse to ensure pathogenicity. Cultures were reisolated from lesions on the infected plants and single-spored, then maintained on PDA in the dark. A grain culture medium consisting of 500 cm^3 each of oats, wheat, and rye (1:1:1) seed was placed in aluminum foil-lined galvanized trays (30 × 20 × 10 cm) and soaked for 12 hr in distilled water. After decanting the water, the trays were re-covered with aluminum foil and autoclaved at 121 C for 2 hr. Mycelial plugs from several *B. oryzae* isolates were added and the medium was incubated at 24 C for 3 wk, then spread to dry in the laboratory (24 C).

About 1 L of bulked grain culture inoculum containing from five to seven

Table 1. Epidemic onset study schedule for mancozeb application and *Bipolaris oryzae* inoculation at various stages of plant development

Days after each plant stage began	Inoculum or mancozeb applied per stage of plant development			
	Boot	Heading	One-fourth grain elongation	Milk
0	M[a]	M	M	M
7	I	...	M	M
14	I	I	...	M
21	I	I	I	...
28	I	I	I	I

[a] M = mancozeb and I = inoculation.

isolates of *B. oryzae* was mixed with 3 L of water immediately before inoculation. The resulting spore suspension was sieved through a 300-μm (U.S. Standard Sieve Series No. 50, W. S. Tyler Co., Mintor, OH) screen. This procedure was repeated four times. Gelatin (Knox) was suspended in boiling water and added to the inoculum suspension (2.5×10^3 conidia per milliliter) to give a final ratio of 0.05% (w/w). Each plot was inoculated using a backpack sprayer (Hudson Stainless Steel Suprema 67376, H.D. Hudson Mfg. Co., Chicago, IL) at 30 psi at a height of 30–40 cm above the plants. Inoculations were usually made by 0900 hours on a day free of precipitation and with expected temperatures of 27–32 C.

Immediately after inoculation, an overhead misting system was activated. The misting system was suspended by 3.2-mm-diameter aircraft cable strung over the paddy with height-adjustable cable hangers. Polyvinyl chloride (PVC) pipe, 1.9 cm in diameter, spaced 1.52 m apart, with Flora-Mist fogger deflector-type nozzles spaced 1.52 m apart on the pipe, provided intermittent moisture uniformly over the plots. Two timers, a 24- and a 1-hr in series, were used to control the misting. An electric motor and piston pump provided up to 33.8×35 kg/cm^2 (500 psi) to move the water from a large self-filling reservoir to the misters.

Disease assessment. In the epidemic onset and curtailment studies, disease severity ratings were recorded periodically for each plot. The standard area diagrams (9) used for glume blotch (key no. 1.6.1) was used to determine the percentage of leaf area diseased. In treatments more heavily diseased than a trace or 1% severity rating, the older, lower leaves had substantially greater disease severities than the younger, upper leaves of the plant. When such differences were apparent, disease ratings were recorded for the individual leaves and the amount of disease was expressed as the percentage of leaf area covered by lesions on the flag, second, and third leaves down from the flag.

The mean disease severities on the three uppermost leaves of each treatment at the milk stage in the disease onset study and the mean treatment yield data were used with a multiple linear regression analysis to derive a critical-point yield loss model (11). The model relates disease severities on the three leaves at milk stage to yield losses.

Yield analysis. At harvest, panicles from the inner plots (1.22×2.13 m) were cut individually, dried at 60 C, and threshed, then the grain was hulled, sized, and weighed. Kernels were sized by diameter into categories 4, 3, and 2 with minimum diameters of 1.60, 1.25, and 0.86 mm, respectively. The number proportions of kernels in each category was used to determine if a relationship existed between grain quality and the disease onset and disease curtailment treatments. Grain of sizes 4 and 3 was combined for determination of final weights in yield loss analysis. Kernels in category 2 are considered to be of noncommercial quality. Yield reductions are expressed as a comparison with control yields from each experiment.

Linear regression analysis is the preferred method for statistical analysis of experiments with treatments based on quantitative factors (13,20,23). The disease onset and curtailment studies were based on plant growth stage but may be analyzed on the basis of the number of days that disease was allowed to increase in severity.

RESULTS

The disease severity and yield (dry wt kg/ha) means for treatments in the epidemic onset and curtailment studies are summarized in Tables 2 and 3, respectively. In both the onset and curtailment studies, treatments had significant effects on yield, with $P < 0.01$.

Disease onset study. Varied severities of disease during boot (fully swollen), inflorescence (anthers emerged), and grain elongation (initiated) stages had significant effects on yield reduction (Table 2). Disease-free plots had a mean yield of 904 kg/ha (oven-dry wt). Disease initiated at the late milk stage did not cause significant yield differences from the nondiseased control. However, when FBS was initiated 12 days earlier than the milk stage (at early grain elongation), 20 days earlier at emergence of inflorescence, and 24 days earlier at the boot stage, yield losses were 33, 56, and 67% of the nondiseased control, respectively.

Contrasts were used with unequal spacing to divide the treatment sum of squares. Spacing used the number of days before harvest that disease was initiated in the treatment. The linear and quadratic contrasts for treatments were significant at $P = 0.05$. The resulting linear regression analysis had an r value of 0.94, which accounted for 88% of the variation in the treatment sum of squares. The residuals were evenly distributed. Thus, the number of days before harvest that FBS was initiated was related in a linear fashion between boot and grain ripening (one-half of panicle darkened) stages. Disease initiated at the earlier stages of plant growth caused greater yield reductions than disease initiated in later stages of maturity (Table 2).

Disease curtailment study. Disease associated yield losses was reduced when mancozeb was applied during mid grain elongation (9 days before midmilk), resulting in a 35% gain in yield compared with the noncurtailed disease treatment.

Yield was related linearly to the length of time that the disease progressed before curtailment. However, increasing the time that disease progressed beyond the milk stage did not result in a comcomitant

Table 2. Effects of four fungal brown spot (FBS) epidemics on disease severity and subsequent yield loss when epidemics initiated on wild rice at progressively later stages of plant development

Disease initiation growth stage	Percent disease severity during			Yield[w] (kg/ha)	Yield loss (%)
	Appearance of inflorescence	Grain elongation	Milk stage		
Boot	0/1/1[x]	Tr[y]/2.5/7	6/20/70	290 a[z]	67 ±10
Heading	Tr/1.5/50	4.2/14/82	4.0/14/82	385 b	56 ±6
Grain elongation	0	Tr/Tr/Tr	Tr/Tr/Tr	589 c	0 ±10
Milk	0	Tr/Tr/Tr	Tr/Tr/Tr	870 d	0
Control	0	Tr/Tr/Tr	Tr/Tr/Tr	904 d	0

[w] Yield does not include kernels of size category 2.
[x] Top/second/third leaf (from top down).
[y] Tr = trace (<1%).
[z] Linear regression analysis based on the number of days before harvest that FBS was initiated. Mean yields followed by the same letter within a column do not differ significantly ($P = 0.05$).

Table 3. Effects of three fungal brown spot (FBS) epidemics on disease severity and subsequent yield loss when epidemics were initiated on wild rice at the boot stage and curtailed at later stages

Disease curtailment growth stage	Percent disease severity during			Yield[w] (kg/ha)	Yield loss (%)
	Boot stage	Grain elongation	Milk stage		
Grain elongation	0	2/5/60[x]	2/7/60	340 c[y]	39 ± 14
Milk	0	2/5/60	6/16/97	147 b	74 ± 24
Ripe	0	2/5/60	5/18/100	143 a	74 ± 27
Control	Tr[z]	Tr	Tr	561 d	0 ± 8

[w] Yield does not include kernels of size category 2.
[x] Top/second/third leaf (from top down).
[y] Linear regression analysis based on days before FBS was initiated and then curtailed before harvest. Mean values followed by the same letter within a column do not differ significantly ($P = 0.05$).
[z] Tr = trace (<1%).

increase in yield loss. This was determined by using polynomial contrasts with unequal spacing to divide the treatment sum of squares. Contrast spacing was based on the number of days that the disease progressed from initiation at the boot stage to curtailment with mancozeb. The linear and quadratic contrasts had significant F tests ($P = 0.05$), and the cubic contrast was also significant ($P = 0.01$). A linear regression analysis resulted in an r value of 0.91, which accounted for 83% of the variability.

Compared with the nondiseased check plots, yield losses of 39, 74, and 74% were found in the treatments when disease initiated at the boot stage was curtailed at mid grain elongation, midmilk, and one-half of panicle darkened, respectively. Yield comparisons between the disease onset and curtailment studies cannot be made directly. The mean yield of the nondiseased control treatment in the disease curtailment study (561 kg/ha) was lower than yields from similar plots in the disease onset study of the previous year.

Critical-point yield loss model. Both the epidemic onset and curtailment studies indicated that disease progress during or after the milk stage does not decrease yield. Because natural FBS epidemics are similar year after year, our 2 yr of data are representative of most epidemics. Therefore, disease severity ratings at this critical stage of plant development should give an indication of the yield reduction caused by FBS (25). The following critical-point model was developed using final yields and disease severities observed on each of the upper three leaves at the midmilk stage of grain development.

Multiple linear regression analysis was used with the epidemic onset disease severity data and yields on an individual plot basis to derive the coefficients of yield loss for each leaf in equation 1. The items in the equation accounted for 87% of the variation in yields harvested from the experimental plots of the disease onset study.

$$YD = YH - 12.9 \times DS\,(L1) - 13.5 \times DS\,(L2) - 2.5 \times DS\,(L3), \quad (1)$$

where Y = yield loss estimate (kg dry wt/ha) (YD = diseased and YH = healthy) from disease severities (DS) on the three uppermost leaves (L1 = flag, L2 = penultimate, and L3 = third leaf from the top) at milk.

Grain size. Disease onset and curtailment treatments affected kernel size (Table 4). The nondiseased controls in both studies produced the greatest quantity of wild rice in the largest size category, category 4. Earlier disease onset resulted in greater proportions of kernels of sizes 2 and 3. Similarly, later disease curtailment up to the milk stage resulted in greater numbers of kernels of small diameters.

Table 4. Effects of fungal brown spot treatments in the disease onset and curtailment studies on wild rice grain quality as measured by kernel diameter

Treatment	Mean percent grain yield by weight		
	4[y]	3	2
Disease onset study			
Boot	61.3 a[z]	34.2 d	4.5 c
Heading	65.7 b	30.6 c	3.7 b
Grain elongation	69.9 c	27.1 b	3.0 ab
Milk	74.3 d	22.9 a	2.8 a
Control	77.1 d	20.2 a	2.7 a
Disease curtailment study			
Control	65.5 d	30.6 a	3.9 a
Boot–grain elongation	53.3 c	40.4 b	6.3 b
Boot–milk	45.0 b	46.4 c	8.6 c
Boot–ripe	44.8 c	45.9 c	9.3 c

[y] Kernels of categories 4 = 1.6 mm, 3 = 1.25 mm, and 2 = 0.86 mm.
[z] Means in each column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

DISCUSSION

The timing of an aerially applied protectant fungicide to wild rice should be related to expected gains against the risk of disease-related yield reductions. As with other cereal diseases, yield losses are dependent on epidemic progress, mediated by environmental conditions and inoculum concentrations and growth stage (5,8,12,15). Because weather conditions and inoculum concentrations are usually favorable to FBS increase late in the growing season (16), scheduling of the last fungicide application should be related primarily to growth stage and disease severity.

During the milk stage, neither disease initiation nor epidemic curtailment caused any variation in yield in healthy or diseased controls, respectively. However, initiating disease 12 days before the milk stage resulted in a 32% loss in yield over the control, and in an on-going epidemic, a single fungicide application 9 days before the milk stage resulted in a 35% gain in yield. Therefore, protective fungicide applications are not recommended during or after the milk stage of plant development. If environmental conditions and inoculum concentrations favor increase in disease severity, fungicide applications are highly recommended during mid grain elongation, about 10–12 days before the milk stage.

Rating disease severity by the three-leaf method has an advantage over a system using only one overall rating for the entire plant (7). Dissimilar readings on the upper three leaves of a wild rice plant were typical of fungal brown spot symptoms found in commercial fields (15). This may be due to microenvironmental differences found vertically in the foliage (16).

The use of a single disease severity value would result in different ratings depending on the way averaging was done among the ratings of the three leaves. Measurements of the areas of the four uppermost leaves of many wild rice plants at the boot stage (fully swollen) through panicle formation (stamens emerged) indicated that the third and fourth leaves from the top were about the same in area and that the second from the topmost leaf and the flag leaf were about 0.75 and 0.25 the area of the third leaf, respectively. A single disease severity mean, weighted for leaf area, from the disease severities from the three uppermost leaves would be different from a simple arithmatic mean of the three ratings. Therefore, the three or four uppermost leaves were rated for disease severity individually rather than collectively. The relative importance of these leaves to wild rice grain filling has not been determined.

If wild rice is similar to other cereals, most of the carbohydrates in the grain are produced by the flag leaf (8,26) after the boot or inflorescence stage (complete anther dehiscence) (27), although compensation through photosynthesis in the penultimate leaf, mobilization of stored carbohydrates, and photosynthesis in the culm may occur under conditions of disease stress (6,7,24). Rating disease intensity by the three-leaf method does not allow the simple calculation of area under the disease progress curve because this requires a single disease intensity scale. However, disease rating by the three-leaf method has advantages in estimating yield reduction attributable to FBS as demonstrated in the critical-point yield loss model (equation 1). Single disease severity ratings have been used in critical-point models for southern corn leaf blight (2), leaf rust (4), powdery mildew (19), yellow rust (21), and stem rust of wheat (25). Just as the precision of the critical-point model can be improved by increasing the number of assessments (4,10), the partitioning of disease severities to their respective leaves may improve a critical-point model.

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Accuracy of Methods to Monitor Sensitivity of *Phytophthora infestans* to Phenylamide Fungicides

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ABSTRACT

Sozzi, D., and Staub, T. 1987. Accuracy of methods to monitor sensitivity of *Phytophthora infestans* to phenylamide fungicides. Plant Disease 71:422-425.

Sensitivity of *Phytophthora infestans* to metalaxyl was monitored on potato leaf disks, detached leaves, or whole plants. Only minor differences between the various experimental systems were found. Values obtained on amended agar showed similar resistance factors. The less differentiating slopes of the dose response curves from agar tests, however, leave more space for interpretation of sensitivity of test isolates. The drawback of all methods is that they are not sufficiently quantitative. Mixed populations of sensitive and resistant sporangia may give either a completely sensitive or resistant reaction, depending on the ratio of the two types of sporangia in the mixture. Levels of resistance lower than 0.1%, which occur at an early phase in the development of resistance, remain undetected with these methods. Lower levels of resistance down to 0.001% were detected only when large numbers of sporangia were bulked and subjected to preselection of metalaxyl-treated potato plants. Sensitivity testing was carried out subsequently with sporangia recovered from lesions developing on the treated plants. The methods described, therefore, are well suited for monitoring programs aimed at detecting fungicide resistance at higher levels late in the overall selection process. Other methods have to be developed to detect early selection with resistance frequencies lower than 10^{-3}.

The introduction of site-specific fungicides has led to an increasing number of fungicide-resistant plant pathogens during the last 10 yr (6). A typical example is the use of the highly active phenylamides (13), which interfere specifically with RNA polymerases of fungi in the order of the Peronosporales (4). Cases of resistance were found soon after the introduction of these fungicides as single products on various crops (3,5,7,9,11), and cross-resistance was invariably confirmed among various representatives of this chemical group (2,8). A strategy of using these products in mixtures with protective multisite fungicides has been successful in slowing down the development of resistance (11,12). In this context, various methods are used to measure fungicide sensitivity and to detect resistance in target populations. Such methods have not only allowed detection of resistance but have contributed to designing and validating antiresistance strategies or helped to clarify suspected cases of resistance. The methods do not allow precise quantitative descriptions of target populations, because their power to detect low frequencies of resistant spores is not elucidated. The purpose of this paper is to review the strengths and limitations of these resistance detection methods.

Accepted for publication 24 October 1986.

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MATERIALS AND METHODS

Potato test material. Experiments on whole plants were carried out on 4-wk-old, single-stemmed potato plants (cultivar Bintje) grown in the greenhouse, from eye cuttings on peat in 5-cm pots. For tests on detached leaves or leaf disks, fully expanded leaves of plants grown similarly from whole tubers in 5-L pots were used.

Fungicide. Metalaxyl was used in all trials either as active ingredient (a.i.) or formulated as Ridomil 25WP. Fresh fungicide suspensions were made with tap water for each experiment.