

Resistance

**Some Components of Slow-Blasting Resistance in Rice**

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**ABSTRACT**

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Disease efficiency (DE), lesion size, and sporulation capacity (SC) of three isolates of *Pyricularia oryzae* were measured on rice cultivars Tetep, Gogowierie, Dourado Precose, IRAT 13, and 1021. The results showed that these cultivars possess different levels of slow-blasting resistance with high levels of resistance in IRAT 13 and Gogowierie. Among these cultivars, resistance was attributed to reduced DE, SC, and lesion size. Prediction models using multiple linear regression statistics indicate that

data obtained from phytotron experiments (eg, DE, sporulation rate, and total number of spores produced per lesion) could be used as predictors of apparent infection rates obtained in the field for each cultivar. The study also revealed an interaction between the components of host resistance and parasitic fitness, indicating that slow-blasting resistance could possibly erode to some extent over time.

*Additional key words:* *Oryza sativa*.

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Attempts to utilize single resistance genes to control the blast disease, which is induced in upland rice (*Oryza sativa* L.) by *Pyricularia oryzae* Cav., have met with little permanent success. Accordingly, other breeding strategies are being considered, including the use of slow-blasting resistance that reduces the apparent infection rate ( $r$ ). Research workers at the Institut de Recherches Agronomiques Tropicales et des Cultures Vivures

(IRAT) (3-5) and the International Rice Research Institute (IRRI) (6,7) report evidence of rate-reducing resistance in some cultivars. Several of those cultivars and breeding lines were evaluated at IRRI by Villareal (14) for slow-blasting qualities by determining their  $r$ -values and disease severities relative to susceptible check cultivars. Nine of the cultivars had significantly lower  $r$ -values and disease severities. The rate-reducing resistance of the five cultivars with the lowest  $r$ -values was confirmed in two additional field experiments by using additional isolates of *P. oryzae*. Analyses of rates of disease increase are useful in plant breeding for identifying

host genotypes that restrict the increase of disease. These analyses cannot, however, be applied to genotypes in segregating populations, because the apparent infection rate is determined by the population and not by an individual plant genotype. Evaluating some of the components of rate-reducing resistance is a possible solution to the problems inherent in working with segregating populations. Infection frequency, latent period, and sporulation rate and capacity are considered to be some of the controlling components of rate-reducing or rate-limiting resistance. A critical evaluation of these and other components should make it possible to identify the desired plant genotypes to be used in a breeding program.

The present research was designed to identify some of the components of rate-reducing resistance in the previously mentioned five rice cultivars.

## MATERIALS AND METHODS

All experiments were conducted in the phytotron of the IRRI at Los Baños, The Philippines. Components analysis of slow blasting was conducted for five cultivars, Tetep, Gogowierie, Dourado Precose, IRAT 13, and 1021, which were selected because of their low *r*-value determined in a previous study (14). IR 442-2-58 (IR 442) was selected as the susceptible check cultivar because of its high *r*-value (14).

Three highly virulent and stable isolates of *P. oryzae* were used, including isolate 78-116-2, collected from leaves of IR 4493-2-2 in Leyte, and isolates T-9 and T-27, collected from leaves of Tetep at IRRI. The isolates were grown on prune agar in petri plates and kept under continuous fluorescent lighting for 9–11 days before being used as inoculum. Inoculum was prepared by pouring 10 ml of sterile distilled water into each culture plate and gently stroking the surface of the colony with a camel's-hair brush. The resulting spore suspension was filtered through two layers of fine wire mesh to remove mycelial fragments and agar particles. Inoculum concentrations were adjusted to the desired level by using a hemacytometer.

**Disease efficiency.** Disease efficiency (DE) is defined herein as the relative number of sporulating lesions that developed from a given amount of inoculum. DE is relative in the sense that no true ratio of lesions to spores can be assigned because not all of the spores in the inoculum actually land or remain on the leaves. Four 14-day-old seedlings of each of the six cultivars, grown in a 5.5 × 15 × 10-cm-deep flat, were inoculated with a spore concentration adjusted to 7.5 × 10<sup>4</sup> spores per cubic centimeter of sterile distilled water and sprayed on leaves at the rate of 0.02 ml per square centimeter of leaf area; the length and width of each leaf was measured one day prior to inoculation and the computed area was expressed in square centimeters per flat. Inoculated plants were placed in dew chambers for 24 hr and then transferred to Koitotron KB-10D growth cabinets with programmed day/night temperatures of 29/21 C and a relative humidity of 90–95%. A split-plot design was used with isolates as main plots and cultivars as subplots. There were three replications in separate growth chambers for each experiment. Each growth chamber contained three flats, one flat inoculated with each of the three isolates, and each flat containing six cultivars. The numbers of lesions were recorded daily from the third through the ninth day after inoculation. The number of lesions per 10 cm<sup>2</sup> of leaf area for each cultivar × isolate combination after 9 days constituted DE.

**Sporulation capacity, duration of sporulation, and lesion size.** These three studies were carried out simultaneously and comparable sets of experimental materials were used in each one. Two seedlings each of two randomly paired cultivars were planted side by side in a flat. When the seedlings were 14 days old, they were inoculated with a spore concentration of 3 × 10<sup>4</sup> spores per milliliter, a concentration that produced relatively few, usually discrete, lesions. The three isolates used in the DE studies were used in these studies.

One lesion on a leaf of each of the four seedlings (two seedlings per cultivar) was selected and the area of each lesion in square millimeters was calculated by measuring its length and width. With the leaf still attached to the plant, the part bearing a single lesion

was enclosed in a 5.5-cm-diameter petri plate containing water agar with the upper leaf surface facing the agar. Slots cut on opposite sides of the top and bottom of the petri plate allowed the leaf to be inserted without damage to it. The petri plates were placed on a wire mesh mounted several centimeters above the top of the flat.

The plates for spore collection were changed every morning and all of the spores released by each lesion were counted at ×100 with a 6 × 6 square grid in the microscope eyepiece. Mean counts of the spores on the two plates for each cultivar × isolate combination were recorded as the sporulation for that day. Lesion size was also measured daily. Spore counts and lesion-size measurements were continued until the lesion no longer produced spores and that time period was recorded. The experiments were repeated four times.

Data on sporulation were analyzed by using a nonlinear regression procedure to fit cumulative sporulation vs time to the logistic model (13). Data on lesion size were analyzed by using a two-way analysis of variance and pairwise comparisons of the means for each cultivar × isolate combination.

Multiple linear regression was used to determine whether sporulation rate, total number of spores produced per lesion, and disease efficiency could be used as predictor values for apparent infection rates obtained for each cultivar in field tests.

## RESULTS

**Disease efficiency.** Table 1 summarizes the data for the DE of the five slow-blasting cultivars relative to the blast-susceptible check cultivar IR 442. It is evident that a marked reduction in the number of successful infections contributes to the slow-blasting tendencies of the test cultivars.

**Lesion size.** Blast lesions commenced sporulation when they were 1–2 mm<sup>2</sup> in size. Lesions continued to enlarge until just before they ceased to sporulate. Table 2 summarizes the mean lesion size attained on the test cultivars at the last day of spore collection. Differences in maximum lesion size between IR 442 and the five slow-blasting cultivars were large and indicate that lesion size should be an important component contributing to a reduced *r*-value.

**Sporulation capacity.** Cumulative sporulation per lesion, the rate of sporulation per lesion, and the length of the sporulation period were used as criteria in an attempt to describe the overall sporulation capacity of the three isolates on the test cultivars (Table 3).

A nonlinear least squares regression produced a statistically significant fit for all sporulation data to the logistic model of Rouse et al (13): The logistic model used in this study is stated as:

$$S = S_m S_0 / [S_0 + (S_m - S_0) e^{-\rho t}]$$

in which *S<sub>m</sub>* = maximum cumulative sporulation, *S<sub>0</sub>* = initial sporulation, and *ρ* = sporulation rate. The analysis was calculated on means of four experiments because of high computer costs for

TABLE 1. Disease efficiency<sup>w</sup> of six rice cultivars expressed as the mean number of lesions per 10 cm<sup>2</sup> leaf area resulting from inoculations with three isolates of *Pyricularia oryzae*

Cultivar	Isolate		
	T-27	78-116-2	T-9
Tetep	1.18 d <sup>x</sup> 2 <sup>y</sup>	3.05 b 1	2.71 d 1
Gogowierie	2.04 c 3	1.30 d 2	4.04 c 1
Dourado Precose	3.82 b 3	1.37 d 2	6.58 b 1
IRAT 13	0.96 d 1	0.99 e 1	0.81 e 1
1021	3.81 b 2	2.61 c 1	3.00 d 1
IR 442-2-58 <sup>z</sup>	11.60 a 3	9.27 a 2	13.51 a 1

<sup>w</sup> Average of three replications and 10 experiments.

<sup>x</sup> In each column, cultivar means followed by a common letter are not significantly different (*P* = 0.05) according to Duncan's multiple range test.

<sup>y</sup> In each row, isolate means followed by a common number are not significantly different (*P* = 0.05) according to Duncan's multiple range test.

<sup>z</sup> IR 442-1-58 served as the blast-susceptible check cultivar.

the procedure.

Each isolate produced sixfold to sevenfold more spores on IR 442 than on Gogowierie or IRAT 13 and three times more than on Tetep, Dourado Precose, and 1021. Spore production generally increased quite rapidly and then decreased gradually. The peak period varied among cultivar-isolate combinations. Highest mean daily sporulation was recorded for isolate T-9 on IR 442 with 2,052 spores on the sixth day after lesion appearance.

Surprisingly, only two of the five slow-blasting cultivars, Gogowierie and IRAT 13, had statistically significantly reduced sporulation rates relative to the susceptible cultivar IR 442. Sporulation rates ( $\rho$ ) for Tetep, Dourado Precose, and 1021 were not correlated with their apparent infection rates in the field, which were only 0.25–0.50 times that of IR 442; in the field, each cultivar sustained less than 3% disease compared to 88% for IR 442. The transformation needed to extract the sporulation rate requires a collapsing of all associated variances. The consequences of such transformation on statistical interpretation needs further elaboration before studies on rate of sporulation have any measure of biological usefulness.

The four experiments failed to show significant differences in sporulation period that could be attributed to isolates or to interactions between isolates and cultivars (Table 4). On the average, IR 442 has the longest sporulation period (26 days),

TABLE 2. Mean lesion size<sup>a</sup> (mm<sup>2</sup>) at last day of spore collection for six rice cultivars infected by three isolates of *Pyricularia oryzae*

Cultivar	Isolate		
	T-27	78-116-2	T-9
Experiment 1			
Tetep	9.67 b <sup>y</sup>	8.17 bc	9.50 bc
Gogowierie	4.67 bc	3.67 cd	5.00 cd
Dourado Precose	8.33 bc	8.67 b	10.33 b
IRAT 13	3.50 c	3.00 d	3.83 d
1021	9.33 b	10.00 b	8.00 bcd
IR 442-2-58	27.67 a	20.83 a	23.00 a
Experiment 2			
Tetep	9.17 bc	9.50 b	6.67 bc
Gogowierie	5.67 c	4.00 c	4.83 c
Dourado Precose	12.83 b	8.33 bc	10.00 b
IRAT 13	8.67 bc	3.83 c	3.83 c
1021	8.83 bc	8.83 bc	8.83 bc
IR 442-2-58	29.67 a	24.00 a	20.00 a
Experiment 3			
Tetep	9.33 b	9.67 b	8.67 bc
Gogowierie	6.00 b	5.00 bc	4.83 bc
Dourado Precose	10.00 b	8.33 b	9.67 b
IRAT 13	5.00 b	3.33 c	4.00 c
1021	8.33 b	9.50 b	9.67 b
IR 442-2-58 <sup>z</sup>	29.17 a	31.00 a	32.33 a

<sup>a</sup>Average of three replications.

<sup>y</sup>In each column under the same experiment, cultivar means followed by a common letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

<sup>z</sup>IR 442-2-58 served as the blast-susceptible check cultivar.

TABLE 3. Means for total number of spores produced per lesion<sup>a</sup> by three isolates of *Pyricularia oryzae* on six rice cultivars

Cultivar	Isolate			Means
	T-27	78-116-2	T-9	
Tetep	7896 bc <sup>y</sup>	12239 b	12149 b	10761 b
Gogowierie	5121 c	5326 c	5696 c	5381 c
Dourado Precose	11498 b	9015 b	14009 b	11507 b
IRAT 13	4566 c	5086 c	4760 c	4804 c
1021	12226 b	13585 b	12636 b	12816 b
IR 442-2-58 <sup>z</sup>	32258 a	31750 a	36161 a	33389 a

<sup>a</sup>Average of four experiments.

<sup>y</sup>In each column, cultivar means followed by a common letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

<sup>z</sup>IR 442-2-58 served as the blast-susceptible check cultivar.

followed by Dourado Precose (22 days), Tetep and 1021 (21 days), and Gogowierie and IRAT 13 (20 days).

Using multiple linear regression with  $r$ -values as the dependent variable, and with sporulation rate, total number of spores produced per lesion, and DE as independent variables, two prediction equations were derived, one for each of the two field experiments (14). The equations were:

$$\hat{Y} = 0.0229 + 0.00113 X_1 + 0.0000034 X_2 - 0.100 X_3 \quad (1)$$

$$\hat{Y} = 0.104 - 0.411 X_1 + 0.0000056 X_2 - 0.362 X_3 \quad (2)$$

in which  $\hat{Y}$  = predicted apparent infection rate,  $X_1$  = sporulation rate,  $X_2$  = total number of spores produced per lesion, and  $X_3$  = disease efficiency. The coefficients of determination were 87.3% for equation 1 and 92% for equation 2.

## DISCUSSION

The study of rice blast on six rice cultivars in the phytotron demonstrated that lesion size, DE, and sporulation capacity are components of slow blasting that are directly related to the rate of disease increase and are measurable by techniques adaptable to both field and greenhouse conditions.

It is well known that cultivars differ in the number of lesions that result from inoculation with a given number of spores. Cultivars with low DE develop fewer lesions than susceptible cultivars. Plants with this type of resistance would normally develop little disease even though neighboring susceptible plants were already heavily blasted. Because the number of lesions that developed in the susceptible cultivar IR 442 was greater than that in cultivars IRAT 13, Gogowierie, Tetep, Dourado Precose, and 1021, reduced DE apparently contributed to the resistance of the latter cultivars.

Reduced lesion size as a form of host resistance includes the appearance of only small lesions on resistant plants as well as a decreased growth rate of the lesions over time. In effect, fewer spores are formed per lesion in the resistant type, compared to the susceptible types, simply because the total lesion surface available for sporulation is reduced. In this study, the cultivars Gogowierie and IRAT 13 had smaller lesions than IR 442 from the first to the last days of spore collection.

In oats, resistance may be expressed as a reduction in the sporulation of the pathogen even though the lesion size is similar to that observed in a susceptible cultivar (9). A greater frequency of lesions with low spore production potential on the host could mean a slower rate of disease spread in the field. Comparisons of the cumulative sporulation, length of sporulation period, and rate of sporulation suggest that reduced sporulation capacity is another component of slow-leaf-blast infection in cultivars Tetep, Gogowierie, Dourado Precose, IRAT 13, and 1021. This is clearly demonstrated by the reduced sporulation on cultivars IRAT 13 and Gogowierie, which was only 0.14 to 0.16 times that on the susceptible cultivar IR 442. In addition to the low spore yields on IRAT 13 and Gogowierie, the shortened sporulation period of the pathogen on these two cultivars may contribute to their resistance.

TABLE 4. Means of sporulation period (days)<sup>a</sup> for three isolates of *Pyricularia oryzae* on six rice cultivars

Cultivar	Isolate		
	T-27	78-116-2	T-9
Tetep	21 c <sup>y</sup>	22 b	21 c
Gogowierie	20 c	20 c	20 c
Dourado Precose	22 b	22 b	22 b
IRAT 13	20 c	20 c	20 c
1021	20 c	20 c	21 c
IR 442-2-58 <sup>z</sup>	26 a	27 a	26 a

<sup>a</sup>Average of three replications and four experiments.

<sup>y</sup>In each column, cultivar means followed by a common letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

<sup>z</sup>IR 442-1-58 served as the blast-susceptible check cultivar.

Lesions on IRAT 13 and Gogowierie stopped producing spores 6 days earlier than those on IR 442. Rates of sporulation on IRAT 13 and Gogowierie also were statistically significantly less than on IR 442, which indicates that this factor also may contribute to their resistance.

The prediction models using the multiple linear regression statistics indicate that the data from the phytotron experiments (eg, DE, sporulation rate, and total number of spores produced per lesion) could be used as predictors for apparent infection rates in the field.

The overall analyses show that IRAT 13, Gogowierie, Tetep, 1021, and Dourado Precose differ in levels of slow-blasting resistance, with highest levels expressed by IRAT 13 and Gogowierie. The major rate-reducing factors among these cultivars were their capacities to reduce DE and SC. Other cultivar differences in the components of slow blasting also were observed. The nature of complementation of the components to produce the total effect on resistance needs further study, but the information obtained so far should be of practical use. For instance, rice genotypes with one resistance mechanism (such as reduced DE) could be combined with other genotypes with other mechanisms (such as reduced SC) to achieve even higher levels of resistance. In actual practice, it is often found that the components of rate-reducing resistance tend to be associated with each other; eg, restricted lesion size is associated with fewer lesions per unit area, as reported for other host-pathogen systems (1,2,11).

Another important aspect revealed by the study is the occurrence of differential interaction in relationships involving rice and *P. oryzae*. The interaction of the components of slow-blasting resistance and the parasitic fitness (sensu MacKenzie [10]) indicated that slow-blasting resistance could possibly erode to some extent over time. Johnson and Taylor (8) provided evidence suggesting that factors determining components of slow-rusting resistance and epidemiological fitness are inherited quantitatively. This implies that if the resistance eroded it would occur slowly, rather than as a complete breakdown. Statistically significant interactions between the elements of rate-reducing resistance and epidemiological fitness have been demonstrated within other host-pathogen systems (8,12,13).

Although no rice breeding program is currently applying the techniques used here, they can be considered as one approach in the application of epidemiological theory to breeding disease-

resistant crops that restrict the rate of epidemic buildup of disease. Whether slow-blasting resistance will remain durable over long periods of time in cultivars that are monocropped remains to be seen.

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