The Role of *Bipolaris oryzae* in Floral Abortion and Kernel Discoloration in Rice

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ABSTRACT

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Foliar symptoms of brown spot (caused by *Bipolaris oryzae*) were modified in field plots by the presence of a rice line that promotes spreading of the disease and by the use of fungicides. There was no correlation between brown spot symptoms and floral abortion, and kernel discoloration was moderately correlated with brown spot. In this study, panicle blight, a disease of unknown etiology, was the primary cause of floral abortion and rice stink bug feeding was the major factor in kernel discoloration. It was concluded that *B. oryzae* was a secondary invader of aborted florets, that it was possibly the primary cause of some kernel discoloration, and that it was just one of many microbes that colonized kernels through stink bug feeding wounds.

Additional key words: *Helminthosporium oryzae, Oebalus pugnax, Oryza sativa*, peck

Bipolaris oryzae (B. de Haan) Shoem. (=*Helminthosporium oryzae* B. de Haan) causes seedling blight and brown spot disease on foliage and panicles of rice (*Oryza sativa* L.), particularly when rice is grown in nutritionally deficient or otherwise unfavorable soils (1,4,16). Brown spot was considered a major contributor to the Bengal famine of 1942 (6). Reductions in grain weight and quality have been associated with this disease (2,11). However, several investigators believe that losses have been caused primarily by stress factors that predispose rice to infection by *B. oryzae* rather than by the pathogen itself (1,7,9,15).

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Rice producers in the southern United States are concerned about the potential role of *B. oryzae* in the grain's failure to fill completely and in its subsequent deterioration. They are also concerned about the incidence of kernel discoloration called pecky rice or peck. Peck has been attributed to both kernel-infecting fungi and feeding by the rice stink bug (*Oebalus pugnax* F.) (5). The amount of peck influences the selling price of a rice crop. Farmers have associated kernel discoloration and "sterility" with brown spot symptoms on foliage and floral glumes, but at present, there is no foliar fungicide available to farmers that is effective against *B. oryzae.*

The objective of this study was to determine whether *B. oryzae* was a direct cause of kernel discoloration and floral abortion and whether foliar brown spot severity was indicative of the incidence of kernel discoloration and floral abortion under field conditions. In a preliminary experiment in 1978, incidence of kernel discoloration did not correlate with severity of foliar symptoms of brown spot among 92 rice lines grown at Beaumont, TX (13). In a replicated experiment with eight rice lines in 1979, neither kernel discoloration nor floral abortion was correlated with foliar brown spot, but kernel discoloration was correlated with stink bug feeding ($r = 0.55$, $P < 0.01$) (*unpublished*).

MATERIALS AND METHODS

In the first of two experiments, eight rice lines that showed foliar brown spot and kernel discoloration in different combinations of severity in a preliminary experiment were selected for a study of the effects of brown spot and stink bug feeding on the incidence of floral abortion and kernel discoloration. We sought to broaden the range of brown spot symptoms with a susceptible line in spreader rows and with protective fungicides to enhance and inhibit, respectively, the presence and activity of *B. oryzae*.

Field plots six rows by 6.1 m with 18-cm row spacing were drill-seeded on 30 April 1980 at 90 kg/ha in two blocks 40 m apart. Each rice line was replicated four times in each block in a randomized block design. In the leeward (north) block, the windward row of each six-row plot was planted to a "Sekiguchi" mutant rice line (14) that functioned as a spreader for *B. oryzae*. The windward block had no spreader rows, and half of the plots of each line were treated with propiconazol (Tilt), an experimental fungicide recently proven effective against *B. oryzae* (17).

Plots were fertilized with ammonium sulfate (21% N) at 90 kg N/ha when the plants were at the four- to six-leaf stage, then flooded and maintained at a water depth of 3–10 cm. Weeds were controlled with propanil herbicide applied at 3.4 kg a.i./ha 2 days before flooding. Plots were top-dressed by hand with ammonium sulfate at 45 kg N/ha when the plants were at the 2-mm-panicle stage.

Fungicide-treated plots were sprayed on 9 July and every 2 wk for 6 wk with a 15% aqueous solution of commercially formulated propiconazol (about 27 ml/plot). Fungicide was applied with a Hudson-type sprayer shortly after sunrise, when the plants were covered with dew and the air was still. The earliest rice line flowered on the day of the first application; the latest flowered 4 days before the penultimate application.

Severity of brown spot on the top three leaves was rated 15–20 days after flowering on a scale of 0–9, where 1 = <1% affected with lesions, 3 = 1–5%, 5 = 5–25%, 7 = 25–50%, and 9 = >50% (8). Lesion size was estimated on a scale of 0–9, where 1 = pinpoint necrotic lesions to 9 = lesions 1–2 mm × 4–6 mm with ashen centers. Panicle blight, a disease of unknown etiology, was rated at the same time on a scale of 0–9, where 1 = <1% affected to 9 = >90% affected. Panicle blight is characterized by abortion of filling kernels, which are subsequently colonized by saprophytes (12). In addition, brown spot lesions were counted on 10 flag leaves sampled randomly from each plot.

A few days before harvest, four samples totaling 1.2 row-meters were cut from the fifth row leeward of each plot to estimate the number of panicles per square meter. Samples were treated as gently as possible to prevent dislodging of stink bug stylet sheaths. Ten randomly selected panicles were exposed to acid fuchsin solution that stained the proteinaceous stylet sheaths left by the stink bugs during feeding (3). Each panicle was examined, floret by floret, under ×2.3 magnification to determine the numbers of clear, discolored, and unfilled kernels with and without stink bug stylet sheaths on their glumes. More than 65,000 florets were examined.

Yields were calculated from the weight (12% moisture) of rough (unhulled) rice harvested from 4.9 m of the center two rows of each plot. Twenty-five milled, discolored kernels from each yield sample were incubated at room temperature on wet filter paper in petri plates under alternate 12-hr cycles of light supplemented by near-ultraviolet (black light) and darkness for 4 days (10). On the fourth day, the numbers of kernels colonized by *B. oryzae, Alternaria (Trichoconis) padwickii* (Ganguly) M. B. Ellis, and *Curvularia* spp. were recorded.

The second experiment, planted on 13 April 1981, focused solely on the effects of *B. oryzae* on floral abortion and kernel discoloration. Two rice lines were selected: Labelle, a long-grain cultivar grown on more than 90% of the Texas rice acreage; and RU7802030, a medium-grain line that had shown severe foliar brown spot symptoms in previous tests. Two nitrogen-fertilizer rates and three fungicide treatments were superimposed in a completely randomized design. Each nitrogen-fungicide-cultivar combination was replicated six times.

All plots received a basal application of 112 kg N/ha when plants were at the four- to six-leaf stage. Half of the plots were top-dressed with 45 kg N/ha when the plants were at the 2-mm-panicle stage. Fungicides were applied about 4 days before flowering and again 15 days later. One-third of the plots were protected with benomyl (Benlate) at the rate of 1.1 kg a.i. in 40 L of water per hectare (about 30 ml/plot/application) and another third with propiconazol as described previously. The remainder were not treated with fungicide. Benomyl is effective against all major foliar pathogens of rice, except *B. oryzae*; propiconazol is effective against all, including *B. oryzae*. Theoretically, differences between untreated and benomyl-treated plots should be attributable to pathogens other than *B. oryzae* and differences between benomyl- and propiconazol-treated plots should be attributible to *B. oryzae*.

Brown spot severity, flag leaf lesions, and panicle blight were evaluated as described previously. In addition, panicle discoloration was rated a few days before harvest on a scale of 0–9, where 0 = no discoloration to 9 = >90% of the florets discolored. Panicles were collected as described previously for determining filled and unfilled grains. There was no stink bug determination; the numbers of discolored grains per 1,000 filled grains were determined from a hulled subsample of the yield sample. Yields were estimated and discolored milled grains assayed for microflora as described previously.

RESULTS

Data from the first experiment are summarized in Table 1. Brown spot severity ratings and flag leaf lesions were significantly greater ($P = 0.05$) in plots with spreaders than in plots without spreaders, and both lesion numbers and lesion size were reduced significantly by

Table 1. Effects of brown spot, stink bug feeding, and panicle blight on floral abortion and kernel discoloration in rice exposed to natural populations of *Bipolaris oryzae* (cause of brown spot) and *Oebalus pugnax* (rice stink bug) in plots with a spreader to increase disease severity, no spreader or protective fungicide, and propiconazol fungicide but no spreader, Beaumont, TX, 1980

		No spreader	
Measurements	Spreader (n = 32)	Unprotected (n = 16)	Propiconazol n = 16)
Symptoms and signs			
Brown spot			
Severity[a]	6.9 x[b]	4.6 y	1.1 z
Lesion size[c]	4.2 x	4.4 x	1.5 y
Lesions/10 flag leaves	>1,500.0 x	446.0 y	52.0 z
B. oryzae in 25 discolored kernels (%)	29.0 x	6.0 y	2.0 z
Florets with stink bug stylet sheaths (%)[d]	2.7 y	3.7 xy	4.6 x
Panicle blight (%)	21.4 x	6.3 y	5.6 y
Effects			
Aborted florets (%)	36.5 x	24.0 y	23.8 y
Discolored grains (%)	2.9 xy	2.0 y	3.6 x
Aborted kernels among grains with stylet sheaths (%)	29.2 x	28.5 x	28.7 x
Discolored kernels among grains with stylet sheaths (%)	33.0 y	25.8 y	42.9 x
Yield (kg/ha)	4,124.0 y	4,544.0 xy	4,933.0 x

[a] Top three leaves rated 18 days after flowering on a scale of 0–9, where 1 = <1% affected by lesions, 3 = 1–5%, 5 = 5–25%, 7 = 25–50%, and 9 = >50%.
[b] Values in the same line followed by the same letter are not significantly different ($P = 0.05$).
[c] Rated 18 days after flowering on a scale of 0–9, where 1 = pinpoint necrotic lesions to 9 = lesions with cross-dimensions of 1–2 × 4–6 mm and with ashen centers.
[d] Florets from 10 panicles per plot were examined. Average numbers of florets per panicle ranged from 99.3 in propiconazol-treated plots to 103.8 in plots with spreaders.

applications of propiconazol. Differences in kernel discoloration, however, did not coincide with differences in foliar brown spot. Kernel discoloration was greatest in plots with the least brown spot, the propiconazol-treated plots. Even though propiconazol did not reduce the number of discolored kernels, it significantly reduced the number colonized by *B. oryzae*. The numbers colonized by *A. padwickii* and *Curvularia* spp. were reduced significantly from 11 to 4% and from 12 to 3%, respectively, by propiconazol, indicating that all three fungi were secondary invaders. Almost all assayed kernels yielded colonies of unidentified yeasts and bacteria.

There were two confounding factors in assessing the role of *B. oryzae* in kernel discoloration and floral abortion: interblock differences in severity of panicle blight (a physiological disease unrelated to the experimental treatments) and in rice stink bug feeding (as indicated by stylet sheaths left by feeding bugs). The block with spreaders sustained significantly more severe panicle blight than the nonspreader block, and the effects of panicle blight were reflected in the percentage of aborted grains and in yields of the plots.

Within the nonspreader block, there was no significant difference in panicle blight ratings between propiconazol-treated and untreated plots. Propiconazol effectively controlled brown spot without reducing floral abortions, indicating that panicle blight, not *B. oryzae*, was the major cause of floral abortion.

The percentage of discolored kernels among florets with stink bug stylet sheaths was more than 10 times the overall incidence of kernel discoloration—evidence that stink bugs were a major factor in the pecky rice complex. Across all plots, the correlation between stink bug feeding and kernel discoloration was highly significant ($r = 0.70$, $P < 0.01$). The stink bug population was greater, or feeding more actively, in the nonspreader block than in the spreader block. Stink bugs seemed to prefer feeding on healthy rice. When hulling grains with stylet sheaths, we noted that some kernels had been fed upon by the insect without any occurrence of discoloration. Those kernels showed a red dot, indicating the presence of proteinaceous secretion of the insect stained by acid-fuchsin, surrounded by a chalky zone in an otherwise translucent endosperm.

The percentage of discolored kernels tended to increase, both with greater brown spot in the spreader plots and with greater stink bug feeding in the propiconazol-treated plots (Table 1), as though brown spot and stink bug feeding were independent, primary causes of kernel discoloration. In the nonspreader block, where panicle blight was more or less uniform in severity, control of brown spot had no effect on kernel discoloration. Therefore, we speculate that panicle blight predisposed florets, including some that would have been considered filled, to invasion by *B. oryzae* as well as by other microbes. This would contribute to the trend toward a higher than expected percentage of discolored grains, based on stink bug stylet sheath counts, in the spreader plots.

Plots protected with propiconazol yielded an average of 389 kg/ha (8.5%) more rough (unhulled) rice than unprotected plots. About 25% of the increase was accounted for by an increase in 1,000-grain weight (22.2 g in unprotected plots vs. 22.6 g in propiconazol-treated plots). The rest could not be accounted for by differences in panicles per square meter or percentage of filled grains, probably because of sampling error.

In the second experiment, conducted in 1981 and concerned with the effects of brown spot only on floral abortion and kernel discoloration, rate of nitrogen fertilization had no effect on any parameter, including yield. The two test rice lines, Labelle and RU7802030, differed significantly in susceptibility to brown spot and panicle blight but showed no significant cultivar-fungicide interaction. Therefore, data were combined to present the effects of brown spot as modified by fungicides on floral abortion and kernel discoloration (Table 2).

Panicle blight was severe in this test, with a high percentage of aborted florets and poor yields. Benomyl, as expected, had no effect on symptoms of brown spot. Diseases controlled by benomyl were of no consequence in this experiment. Propiconazol did reduce brown spot symptoms significantly and also reduced the percentage of discolored kernels from 2.7 to 1.7%. Propiconazol did not reduce the number of discolored kernels from which *B. oryzae* was recovered. This finding could be explained by the presence of a spreader row in every plot and by the close proximity between the propiconazol-protected and unprotected plots, with the latter plots functioning as sources of inoculum for all plots. Reduced brown spot did not result in reduced floral abortion. Panicle blight and floral abortions were fairly uniform across fungicide treatments.

Brown spot and kernel discoloration were moderately correlated ($r = 0.58$, $P = 0.01$). Panicle blight clearly had the greater effect on floral abortion, masking any effect of brown spot in this experiment. Panicle blight becomes evident shortly after flowering, before there is much discoloration. Because panicle blight and panicle discoloration (much of which was surface-sporulation of *B. oryzae*) were highly correlated ($r = 0.70$, $P = 0.01$), we concluded that panicle blight predisposed florets to colonization by *B. oryzae*.

Table 2. Effects of brown spot and panicle blight on floral abortion and kernel discoloration in rice exposed to spreader-enhanced natural populations of *Bipolaris oryzae* (cause of brown spot) in plots protected with benomyl or propiconazol fungicide or not protected with fungicide, Beaumont, TX, 1981

Measurements	Check (n = 24)	Benomyl (n = 24)	Propiconazol (n = 24)
Symptoms and signs			
Brown spot			
Severity[a]	4.6 x[b]	5.0 x	1.9 y
Panicle discoloration[c]	4.5 x	4.5 x	3.0 y
Lesions/10 flags	972.0 x	960.0 x	365.0 y
B. oryzae in 25 discolored kernels (%)	43.0 x	46.0 x	45.0 x
Panicle blight (%)	22.3 x	18.9 x	21.1 x
Effects			
Aborted florets (%)	65.1 x	66.3 x	64.4 x
Discolored kernels (%)	2.7 x	2.7 x	1.7 y
Yield (kg/ha)	3,037.0 xy	2,912.0 x	3,197.0 y

[a] Top three leaves rated 20 days after flowering on a scale of 0–9, where 1 = <1% affected by lesions, 3 = 1–5%, 5 = 5–25%, 7 = 25–50%, and 9 = >50%.
[b] Values in the same line followed by the same letter are not significantly different ($P = 0.05$).
[c] Rated 20 days after flowering on a scale of 0–9, where 1 = <10% discolored to 9 = >90% discolored panicle.

DISCUSSION

In this study, floral abortion was caused mainly by panicle blight, a disease of unknown etiology. When brown spot symptoms were effectively controlled with propiconazol, there was no corresponding increase in the percentage of filled grains. Therefore, we concluded that *B. oryzae* was not a major cause of floral abortion but rather that the fungus colonized aborted florets.

In 1981, *B. oryzae* contributed to the incidence of kernel discoloration in that the percentage of discolored grains was reduced from 2.7 to 1.7% when brown spot symptoms were reduced by propiconazol (Table 2). However, the 10-fold increase in the percentage of discolored kernels among grains with stink bug stylet sheaths over the overall percentage of discolored kernels (Table 1) was evidence that the rice stink bug was the primary agent in kernel discoloration.

Some kernels, though punctured by the stink bug stylet, showed no discoloration; therefore, the insect itself did not cause the discoloration but provided favorable infection courts for microbes. The percentage of discolored kernels colonized by *B. oryzae* in the 1980 experiment (Table 1) corresponded with brown spot symptoms rather than with the percentage of discolored grains. In the 1981 experiment, in which all plots had spreader rows and fungicide-protected and unprotected plots were not spatially isolated, the percentage of discolored kernels colonized by *B. oryzae* was practically the same across all treatments, even though the percentage of discolored kernels was reduced significantly in propiconazol-treated plots (Table 2). Because of the inconsistent association of *B. oryzae* with kernel discoloration, we concluded that *B. oryzae* was just one of many microbes that colonized kernels damaged by insect feeding or other causes.

On the basis of this study, we would be reluctant to recommend fungicidal control of *B. oryzae* as an effective means to reduce floral abortion or kernel discoloration in rice.

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