Preliminary Examination of Blast Thresholds for Seed Borne Rice Blast in Arkansas

D.O. TeBeest and M. Ditmore

ABSTRACT

Rice blast, caused by the fungus, *Magnaporthe grisea*, is a common disease of rice in Arkansas. The disease has reached epidemic levels previously in Arkansas on susceptible cultivars. Also, it has been established that artificially infected seeds placed on the soil surface could initiate disease in small plots. The research reported here was conducted in small plots to determine if naturally infected seeds planted beneath the soil surface were capable of producing significant levels of rice blast disease. In addition, experiments were conducted to determine if seed treatment with fungicides reduced or eliminated the presence of *M. grisea* on seeds. The results of field tests appeared to indicate that planting naturally infected seed subsurface leads to significant levels of rice blast disease in the cultivars tested. Preliminary investigations also suggest that fungicides can reduce the presence of blast on seeds.

INTRODUCTION

Rice blast is an economically important disease of rice and it can be an important disease in Arkansas on many high-yielding but blast-susceptible cultivars (Lamey, 1970; Long et al., 2001). The epidemiology of rice blast has been studied in tropical regions that differ from Arkansas in environmental conditions, host resistance, pathogen population, management strategies, and production techniques. A fundamental reason for studying the epidemiology of rice blast in Arkansas was to understand the development of the disease, the origin(s) of the inoculum in fields, and the spatial distribution of rice blast within individual fields in Arkansas (Lamey, 1970). Torres and Teng (1993) found that yield losses are most easily estimated or correlated to the
incidence of neck blast (one half percent loss for each one percent neck infection); too late for implementing a management strategy.

Previous work (Long et al., 2001) conducted in commercial fields and experimental plots illustrated several important points about rice blast in Arkansas: 1) that rice blast developed in a fairly consistent pattern across years and cultivars, 2) that blast can be found randomly distributed throughout many commercial fields early in the growing season, 3) that seed infested by *M. grisea* can initiate rice blast epidemics if placed on the soil surface, and 4) that the spread of rice blast from heavily infected areas within a field is often limited in fields with the most seriously blasted areas (neck blast) at harvest coinciding with the areas most severely blasted before internode elongation. Recently, it was reported that seed was the likely primary source of inoculum for rice blast epidemics in California although it was concluded that infested seed were not the likely source of inoculum for subsequent epidemics (Greer and Webster, 2001). Previous work (Lamey, 1970; Manandhar et al., 1998) suggested that infection of seedlings after planting infested seed beneath the soil surface was always low or inhibited the infection of seedlings grown from infested seed. For example, a seed sample with a 21% seed infection resulted in less than 4% seedlings with blast. However, Shetty et al. (1985) showed that planting finger millet seed infested with *M. grisea* clearly resulted in epidemics in the field. Similar field experiments have not been reported for rice blast.

Recently, several fungicides have been evaluated for effectiveness as blast control measures when applied to seed and plants in the field (Hayakaka et al., 2002; Serghat et al., 2002). These and similar studies have evaluated the effect of fungicides on seed germination, plant vigor, and the incidence of blast over time in the field but do not test the effectiveness of fungicides on the incidence of blast on treated seeds following treatment.

The objectives of this research were to determine if planting naturally infected seeds in small plots resulted in the subsequent development of rice blast in plots, and to determine if treating seeds infected by *M. grisea* with hypochlorite, azoxystrobin, or Quadris reduced or eliminated infection of rice seed by the fungus.

**PROCEDURES**

Sixteen plots of each of the rice cultivars ‘M201’ and ‘Wells’ were planted on 23 May 2002 with seeds harvested from field plots infected by blast in 2001. All plots were planted with 100 grams of seed in plots nine rows wide by 20 feet long. Treatments for each cultivar consisted of 0%, 1%, 2%, and 3% seed infected by blast and all treatments were planted in a randomized complete block design with four replications. The initial level of seed infestation for seeds that were planted was estimated to be 5.4% for Wells and 5.5% for M201 in 2001 based on microscopic examination of seeds incubated on moist blotter paper as described by Agarwahl (1989). The levels of seed infection described above were obtained by mixing non-infected seed with infected seed in the appropriate amounts to obtain the desired levels of seed infection. All plots of Wells
and M201 were separated by plots containing the blast resistant cultivar, ‘Drew’. All plots were visually inspected for seeds remaining on the surface 3 days after planting and again 7 days after planting, at emergence, and all seeds found on the surface of the plots were removed.

Rice blast was monitored visually within all plots beginning 14 days after emergence and ending at harvest. On 12 September, 25 to 35 heads (including the flag leaf) were collected from each plot, combined and placed in paper bags, one bag per plot, and taken to the laboratory. All plots were harvested on 1 October 2002. In the laboratory, the incidence of flag leaf, collar rot, neck blast, and panicle blast was determined for each individual head according to plot. Data were converted to the percentage of the total number of flag leaves, collars, neck, and heads infected by the blast fungus from each plot. Data presented here are the averages for four plots for each treatment and cultivar.

Seed infection levels were determined following the methods modified and adapted from the descriptions by Agarwah (1989). In the assessments of seed infection reported here, between 165 to 180 seeds from each seed lot were placed on four sheets of moistened filter paper and placed in glass 9-cm diameter petri plates. Fifteen seeds were placed in each plate in three rows of five. The plates were incubated at 22°C for 4 days and were re-moistened as needed to maintain a very thin film of water on the filter paper. After 4 days, each seed was examined microscopically for the presence of *M. grisea* by identifying sporulating colonies of the fungus. The identity of each colony was confirmed to be *M. grisea* by dipping the colony in a droplet of water and examining the droplet for spores of *M. grisea* at 100X. The incidence of rice blast on seeds was calculated as an average of three replications for each treatment.

Several seed treatments of infested seed of rice cultivar, ‘Francis’, were tested to determine if the level of infection of seed could be reduced. Treatments consisted of water (controls), hypochlorite, two levels of azoxystrobin 100FS, and two levels of Quadris SC (2.08 lb/gal). The water treatment was made by placing 15 g of seed in 50 mls of water for 20 minutes, removing the water, then air-drying overnight at 22°C. The hypochlorite treatment was made by placing 15 g of seed in 0.6% sodium hypochlorite for 20 minutes, washing seeds under running water briefly, then air-drying the seeds overnight. Seeds were treated with fungicides by using the plastic bag method to treat seed and in each treatment, 0.14 mls treatment were mixed with 15 g of seed. Fifteen grams of seeds were treated with azoxystrobin FS at 1 gm and 15 g active ingredient per 100 kg seed. Quadris SC was tested at two rates prepared to represent 0.06 g ai/ 100 kg seed and 0.9 g ai/100 kg seed. After treatment with the four fungicide solutions, seeds were mixed in a plastic bag for 1 minute, then air-dried as described above. Azoxystrobin 100FS and Quadris SC were provided by Syngenta, Inc.

**RESULTS**

Data summarizing the results of the field work conducted in 2002 are given in Tables 1 and 2. Rice blast remained at very low levels until August, when approximately
33% of the upper three leaves of cultivar M201 and about 1% of the upper three leaves of Wells were visibly infected with lesions (Table 1, Fig. 1). Assessments taken on 22 August showed that the amount of disease in the control plots of M201 was approximately 17% while the levels of disease in plots planted with 1% and 2% infected seed reached approximately 32%. The disease reached a level of approximately 46% in plants planted with the highest rate of infected seed. There were no clear differences in the amount of rice blast in Wells at that time. Blast developed very rapidly in all plots of M201 between 22 August and 12 September, about 3 weeks before harvest on 1 October 2002. On M201, the disease increased from levels of approximately 32% of the leaves being infected on 22 August to nearly 99% of the flag leaves, to nearly 78% of the collars, and about 89% of the necks being infected by blast on 12 September. On samples collected on 12 September, nearly 99% of the panicles had visible blast lesions. On Wells, the disease appeared to increase very rapidly between 22 August—when only 0.5% of the leaves were infected—and 12 September when head samples were collected. Of samples collected on 12 September and examined in the laboratory, about 99% of the flag leaves had one or more lesions while more than 61% of the collars were infected. On that date, an average of 69% of the necks and 72% of the panicles were visibly infected with one or more lesions.

Results of microscopically examining seeds from the cultivar Francis incubated on moistened filter papers showed that seed treatments with fungicides can effectively reduce infection of rice seeds (Table 2). In these experiments, controls (seeds treated with water) were found to have an infection level of 4.25% (Fig. 2). In comparison, hypochlorite appeared to be ineffective in reducing blast infection since treatment with hypochlorite reduced the level of infection to only 3.94%. Treatment with azoxystrobin at 1 g and 15 g active ingredient per 100 kg significantly reduced the level of infection from 4.25% to levels below the level of detection in our tests in which 495 seeds were examined across three replications (Table 2). Quadris applied at 0.06 g ai/100 kg reduced infection from 4.25% to 0.2%. Only one seed was infected in the sample of 495 observed in our blotter tests. A commercial formulation of azoxystrobin, Quadris SC, applied at 0.95 g ai/100 kg reduced the incidence of seed infection to a level equivalent to the application of azoxystrobin and below the level of detection in our tests.

Treatment of rice seeds with hypochlorite, Quadris, or Azoxystrobin in our tests did not appear to affect germination of seed as measured by germination on moistened blotters for four days.

**SUMMARY**

The field data show that significant levels of rice blast appeared in replicated plots of two cultivars in which infected seeds were planted beneath the soil surface. It is unlikely that blast originated from external inoculum since nearby rice plots planted with non-infected seeds of the same cultivars did not have significant levels of the disease. Rice blast appeared in control plots planted with seed lots in which blast was not detected in our seed assays. However, the lowest limit of detection was only 0.5%
and this may have been too high to assure that blast was not present in the sample and it may be too high to significantly reduce the incidence of disease. Shetty et al. (1985) estimated that one infected seed in ten thousand (0.01%) was sufficient to cause an epidemic of blast on pearl millet. Thresholds have not been established for rice blast in Arkansas. On the other hand, Lamey (1970) suggested that planting infected rice seed beneath the soil surface inhibited infection of rice by the rice blast fungus. The data also suggest that significant yield losses are possible as a result of planting infected seed since nearly 50% of the necks were infected with blast and the incidence of neck blast is considered indicative of yield reductions amounting to 0.5% loss for each 1% neck infection (Torres and Teng, 1993).

In 2002, the rapid increase of rice blast occurred later in the growing season than previously reported by Long et al. (2001) when larger amounts of inoculum were placed on the soil surface but was consistent with their results when reduced amounts of inoculum were applied. The delay in the appearance of the epidemic in 2002 may have been caused by a seasonal effect or it may have resulted from the very low levels of inoculum in this study as compared to earlier reports. Similarly, temporal delays in the appearance of significant levels of disease caused by reduced inoculum levels on seeds have been reported for other diseases (Gabrielson, 1987). This question remains to be investigated more completely for rice blast. It is also interesting to note that the disease appeared later and was not as severe on Wells as on M201. Wells is more resistant to rice blast in Arkansas than is M201.

Results of the laboratory assays of the effect of seed treatments on the level of infection by rice blast suggest that azoxystrobin is effective in reducing the level of rice blast to a level less than 0.2%, the limit of detection in our tests. However, treatment with insufficient levels of this fungicide resulted in the detection of rice blast on 0.2% of the seeds. Most of the infected seeds in our tests did not produce viable seedlings; however, in one test, an infected seed germinated and produced a viable seedling that survived for at least 4 weeks in greenhouse tests. Previously, TeBeest and Guerber (1999) observed the blast fungus on senescent coleoptiles and primary leaves of germinated rice seedlings that did not have blast lesions in tests conducted in field soil in the greenhouse.

**SIGNIFICANCE OF FINDINGS**

The work reported here was done to determine if rice blast could develop from planting naturally infected seed and if fungicides could effectively reduce the incidence of disease on seeds. The data from this work with naturally infected rice seeds appear to suggest that infected or infested rice seed is a viable source of the inoculum for rice blast disease in Arkansas. Also, the data show that effective fungicides, such as Quadris, can reduce seed infestation levels by the blast fungus, but the work also shows that the fungus can survive on treated rice seeds presumably if treatment levels are too low or if treatments are inefficient in treating seeds.
ACKNOWLEDGMENTS

The authors gratefully acknowledge the cooperation of the Arkansas rice producers, the support of the Arkansas Rice Research and Promotion Board, and the support and donation of fungicide materials by Syngenta, Inc. The authors are also grateful for the cooperation of Dr. R. Cartwright for his assistance with this research and preparation of this report. The authors also appreciate the cooperation of Mr. Roger Eason and Mr. S. Clark for their invaluable assistance with the work at the Pine Tree Experiment Station, Colt, AR.

LITERATURE CITED

Table 1. Incidence as percentage of leaves infected by rice blast during the growing season, and on flag leaves, collars, necks, and panicles near harvest on two cultivars, M201 and Wells, resulting from planting infected seeds at different levels of seed infection at the Pine Tree Experiment Station, Colt, AR, in 2002.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Seed infected (%)</th>
<th>Vegetative (%)</th>
<th>Incidence near harvest (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16 July</td>
<td>22 August</td>
<td>Flag</td>
</tr>
<tr>
<td>M-201</td>
<td>0</td>
<td>16 a</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>32 b</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>36 b</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>46 b</td>
<td>97</td>
</tr>
<tr>
<td>Wells</td>
<td>0</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.75</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.5</td>
<td>100</td>
</tr>
</tbody>
</table>

* The percentage of seeds infected was determined by incubating 200 seeds from an infected seedlot on moistened filter paper at 22°C under 12 hr lighting for 4 days. The controls (0%) consisted of seeds from a seedlot in which blast was not detected. Individual levels of infection were prepared by diluting the infected seedlot with the control seedlot in the appropriate amount.

y The incidence of rice blast on 16 July and 22 August 2002 was determined by estimating the number of leaves from 100 to 200 plants per plot exhibiting typical blast lesions. The incidence of rice blast near harvest was determined by collecting 25 to 35 heads (including the flag leaf) and determining how many flag leaves, collars, necks, and panicles from within each sample were infected by blast. All data are the averages of four replications of each treatment.

x Numbers in a column within each cultivar followed by the same letter are not significantly different at P=0.05 using SAS GLM ANOVA tests. Numbers in columns within each cultivar not followed by any letters are not significantly different using SAS GLM ANOVA tests.

Table 2. Germination and incidence of *M. grisea* on seeds of rice cultivar Francis following treatment with distilled water, hypochlorite, Quadris, and azoxystrobin, and incubation on moistened filter paper disks at 22°C for 4 days in a 12 photoperiod.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Seeds germinated (%)</th>
<th>Seeds infected with <em>M. grisea</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>65.7</td>
<td>4.25</td>
</tr>
<tr>
<td>Hypochlorite</td>
<td>59.0</td>
<td>3.94</td>
</tr>
<tr>
<td>Azoxystrobin (1 g ai/100 kg)</td>
<td>65.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Azoxystrobin (15 g ai/100 kg)</td>
<td>64.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Quadris (0.06 g ai/100 kg)</td>
<td>67.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Quadris 2 (0.94 g ai/100 kg)</td>
<td>66.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* The treatments consisted of sterile distilled water (controls), treatment with sodium hypochlorite (0.06%) for 20 minutes, and Azoxystrobin 100FS and Quadris SC (2.08 lb ai/gal). Chemicals were added to the seed by adding seed to a plastic bag containing the appropriate amount of chemical.

y The percentage of seeds germinated and infected presented are averages of three replications of 165 seeds per replicate for each treatment. Seeds from each treatment were placed on moistened filter paper and incubated at 22°C for 4 days with a 12-hour photoperiod.
Fig. 1. Rice blast developed in 2002 in our field plots planted with infected seeds. The figure shows a panicle and neck infected by rice blast two weeks before harvest.

Fig. 2. Spores of *M. grisea* were found on infected seeds incubated for 4 days at 22°C under lights (12-hour photoperiod). In most cases, spores were found at the base of the seed where the seed joins the seed stalk. Occasionally, spores were found on the seed coat in a discolored area.