Biobased USA PicoAg: An Organic Formula that Kills Rice Blast Fungus

By Sheng-Cheng Wu, Ph. D.

Complex Carbohydrate Research Center, the University of Georgia
315 Riverbend Road, Athens, GA 30605

Introduction

Rice blast is a devastating fungal disease that results in a global loss of about 20% in rice output annually. The food lost to the disease is enough to feed about 60 million people [1,2]. The causal agent, ***Magnaporthe grisea***, also imposes serious threat to other cereal crops and turf grasses. Disease prevention methods such as cleaning of seeds, fertilizer and water management, selection of resistant varieties and burning of diseased crop residues will reduce, but not eliminate, the impact of blast disease. The most effective treatment so far has been the application of chemical fungicides which are not friendly to the environment. The organic formula, PicoAg, being tested here consists of 100% natural ingredients, thus are considered environmentally safe.

Fungal Strain

Magnaporthe grisea (Hebert) Barr, strain CP987, a potent laboratory derivative, was used for all experiments described below. Conidia (spores) were harvested from 2-week-old culture on oatmeal agar plates [3], and suspended in 0.25% gelatin solution with a final density of 2 x 10^5 conidia per milliliter.

Culture Media

The liquid media consists of Vogel's basal salt, 5 μg/L of biotin, 1 mg/L of thiamin and 10 g/L of sucrose. Aliquots (100 mL) of the media in 500-mL flasks are autoclaved and cooled to room temperature prior to inoculation [3].

Rice Seedlings

Rice seedlings (*Oryza sativa L.* cultivar Sariceltik) susceptible to *M. grisea* strain CP987 were grown for 18 days in a vermiculite medium in a growth chamber (14 h light at 29 C, 10 h dark at 22 C and a relative humidity of 70%). Each 4-inch pot contain approximately 10 seedlings [3].

Test in Culture Media

Each medium flask with a designated **PicoAg** concentration was inoculate with 1 mL of the *M. grisea* conidial suspension, and incubated on a gyratory shaker (150 rpm) in darkroom at 22 C for 5 days. The mycelial "balls" were counted and their diameters and fresh weight measured. The results in Table 1 show, with the increase of **PicoAg** concentration, the total number of mycelial balls increases and their size decreases which indicates a clear pattern of inhibition in fungal aggregation and radial expansion. At 500-time dilution (0.2%, or 1.5 teaspoons per gallon) of **PicoAg**, the fresh weight dramatically decreases, and at 300-time dilution (0.3%, or 2.25 teaspoons per gallon) the growth of *M. grisea* in the culture is totally eliminated. Three independent tests were performed, and the results were similar to those shown in Table 1.

Test on Rice Seedlings

Rice seedlings were treated in pairs of two pots in 2-gallon Ziploc bags. The leaves of seedlings were sprayed evenly with 2 mL of the conidial suspension, a 10% **PicoAg** solution, or a 0.25% gelatin solution using a Paasche Airbrush type H-1 [3]. For pre-treatment, **PicoAg** was sprayed on the seedlings and air-dried for 10 min prior to conidial inoculation, and for post-treatment, PicoAg was

sprayed on the seedlings three days post-inoculation. As shown in Figures 1 and 2, treatment of 10% **PicoAg** does not appear to affect growth of the rice seedlings (Compare Pots #11 and #13). Both pre-treatment and post-treatment offered significant protection against the disease (Pots #15, 17, 20). In contrast, the non-treated seedlings were 100% colonized by the fungus, resulting in "blasted" leaves typically observed in the field (Pot #7). There are much fewer disease lesions on the **PicoAg** treated leaves than on the non-treated, and the size of the lesions is also smaller (observations over the course of 3-7 days post-inoculation; also in Fig. 1). It should be noted that, under the defined experimental conditions, the 10% PicoAg solution (a 10-time dilution) did not completely eliminate the blast disease. However, it is evident that the treated plants are healthy enough to recover from the impact, while the non-treated controls remain diseased 11 days post-inoculation (Fig. 2).

Table 1. Inhibition of *M. grisea* growth in liquid culture

Flask ID	Percentage PicoAg (V/V)	Number of Mycelial balls	Mycelial size in diameter (mm)	Fresh weight (g)*
Blank	0.4	0		
1	0.0	100	8.0	0.246
2	0.1	250	2.5	0.286
3	0.2	>1000	1.2	0.087
4	0.3	0		
5	0.4	0		

*The culture liquid was filtered through a piece of glassfiber membrane, and the fungal mycelia on the membrane were picked using a pair of sharp-pointed tweezers and immediately weighed.

Conclusion

1. A 300-time dilution (3%, or two and a quarter teaspoons per gallon water) of the organic formula, **PicoAg** , is sufficient to eliminate *M. grisea* growth in liquid culture. Application of this discovery possibly includes pretreatment of seeds and medium prior to germination, decontamination of diseased crop residues and soil, etc.

2. Spraying of a 10-time dilution (10%, or 12.8 fl oz per gallon water) PicoAg on rice seedlings pre- and post-inoculation sharply reduces the blast disease symptoms under growth chamber conditions, with no negative impact on healthy plants observed.

Therefore, PicoAg is an effective organic alternative for rice blast control.

References

[1]. Zeigler, R.S., S.A. Leong and P.S. Teng (ed.) 1994. Rice Blast Disease. C A B International Mycological Institute, Kew, UK.

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[3]. Wu, S.-C., Ham, K.-S., Darvill, A.G. and Albersheim, P. 1997. Deletion of two *endo*-β-1,4-xylanase genes reveal additional isozymes secreted by the rice blast fungus. Mol. plant-Microbe Interac. 10: 700-708.

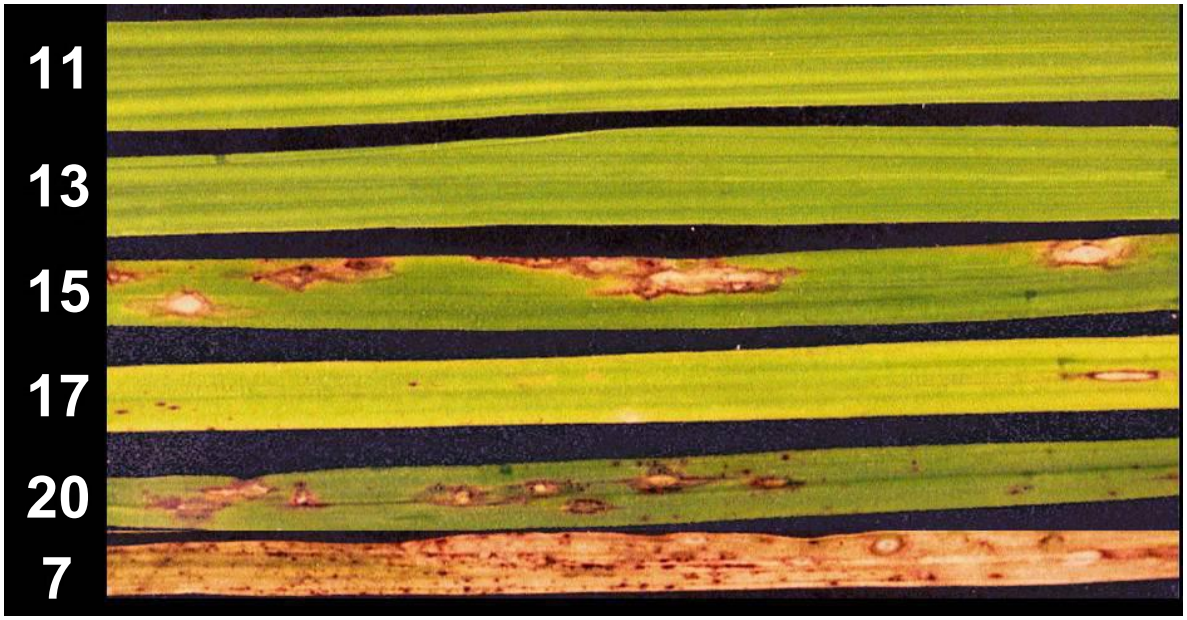

Fig. 1. Leaf Blast Lesions of Infected Rice Seedlings.
Representative fourth foliar leaves from 7 days post-inoculated seedlings were excised, softened under a wet paper tower for 5 min, and photographed.
Plant pots #7 & 8 were inoculated with the conidial suspension without any **PicoAg** treatment.
Plant pots #11 & 12 were not inoculated and not treated
Plant pots #13 & 14 were pre- and post-treated with **PicoAg** without inoculation.
Plant pots #15 & 16 were pretreated with **PicoAg** and inoculated.
Plant pots #17 & 18 were pretreated, inoculated and post-treated.
Plant pots #19 & 20 were inoculated and post-treated with **PicoAg.**

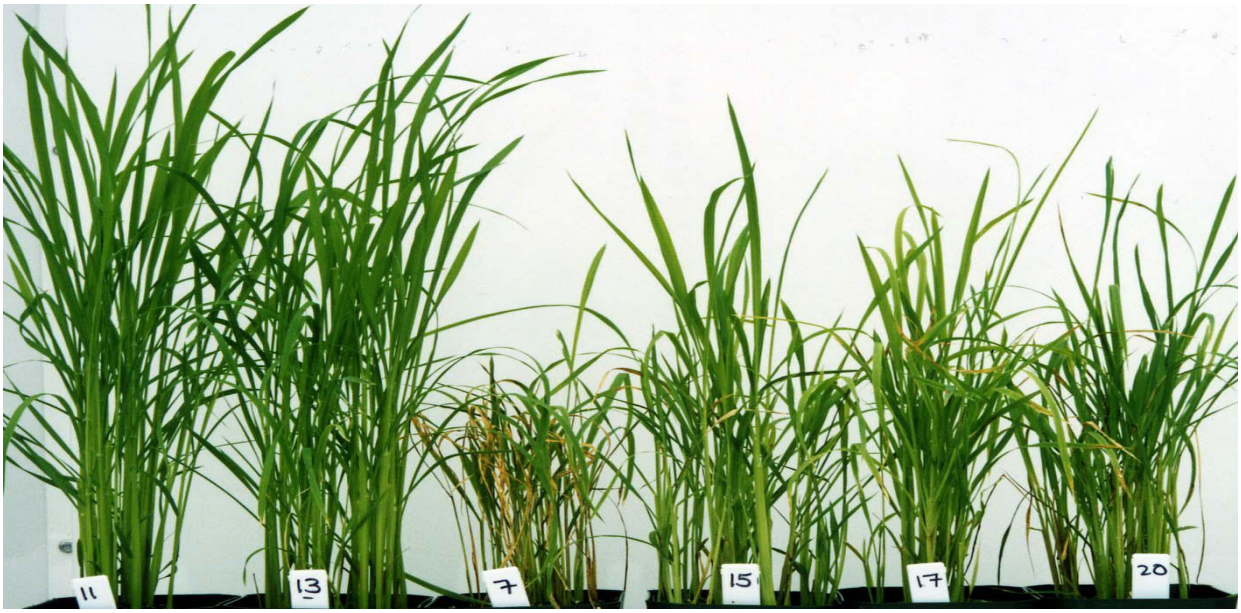

Fig. 2. Whole-Plant View of Disease Symptoms 11 Days Post-inoculation.
The numbering of seedling pots is the same as in Figure 1.