Comparison of Blotters and Guaiacol Agar for Detection of Helminthosporium oryzae and Trichoconis padwickii in Rice Seeds

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ABSTRACT

The blotter and guaiacol agar methods were compared for the detection of *Helminthosporium oryzae* and *Trichoconis padwickii* in rice seeds from 98 lots grown in the southeastern United States in 1972 and 1973. Correlation coefficients were 0.95 and 0.65 between the data from these two tests used to detect *H. oryzae* and *T. padwickii*, respectively. The guaiacol agar method appeared to be more sensitive than the blotter method for the detection of *H. oryzae*, and less sensitive for the detection of *T. padwickii*. The guaiacol agar method was much faster than the blotter method and did not require a microscope and "blacklight"-equipped incubator.

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Rice (*Oryza sativa* L.) is subject to attack by a large number of fungal diseases (8), at least 15 of which may be seed transmitted (7). One of the most important seed-borne diseases of rice is brown leaf spot, caused by *Helminthosporium oryzae* v. Br. de Haan [syn. *Drechslera oryzae* (v. Br. de Haan) Subram. & Jain; perf. st. *Cochliobolus miyabeanus* (Ito & Kuribay.) Drechsl. ex Dastur]. This ubiquitous pathogen is common in rice seeds produced throughout the rice-growing areas of the world. The stackburn disease, caused by *Trichoconis padwickii* Ganguly [syn. *Alternaria padwickii* (Ganguly) M. B. Ellis] also occurs widely, but this fungus is only weakly pathogenic on the foliage. However, heavily infected seeds may not emerge or may be weakened (8).

In the methods recommended by the Plant Disease Committee of the International Seed Testing Association for detection of these two seed-borne pathogens, seeds are placed either on blotters or on potato-dextrose agar to allow the development of characteristic fungal colonies and conidia (6). Both methods are time consuming and require equipment such as microscopes and incubators that are not always found in seed testing laboratories throughout the world. A new method for detection of *H. oryzae* and *T. padwickii* in rice seeds employs guaiacol agar as the detection medium, and can be used in most seed testing laboratories (3).

The purpose of this study was to compare the blotter and guaiacol agar methods for detection of *H. oryzae* and *T. padwickii* in rice seeds. The potato-dextrose agar method, which is not widely used for detecting these fungi, was not included.

MATERIALS AND METHODS.—Rice seeds, cultivar Starbonnet, 48 lots harvested in 1972 in Arkansas, Louisiana, Mississippi, and Texas, and 50 lots harvested in 1973 in Arkansas, Louisiana, and Texas, were used. The blotter method was modified to incorporate the freezing technique of Limonard (5). Two-hundred seeds from each lot were rinsed for 1 minute in 1% NaOCl, followed by a 1-minute rinse in sterile water. Seeds were placed on moist blotters in covered plastic containers at 22-25 C on a laboratory bench for 24 hours, then exposed to −15 C for 24 hours. Containers with seeds were then placed in an incubator at 22 C ± 0.5 C for 7 days under 12 hours of "blacklight" [emitted by 20W ultraviolet fluorescent lamps, average intensity at seed level (i.e. 13 cm) of 233 μW/cm^2, meter absolute accuracy of 15%] and 12 hours of darkness daily (4).

A binocular dissecting microscope was used at × 15-45 to examine each seed individually for the presence of conidia of *H. oryzae* and *T. padwickii*. A seed was counted as infected only if at least one conidium was present.

Prior to plating on guaiacol agar [guaiacol (*o*-methoxyphenol) 0.125 g, agar 5 g, and water 1 liter, plus 0.5 g streptomycin sulfate added after autoclaving], seeds were rinsed for 1 minute in 1% NaOCl, followed by 1 minute in sterile water, placed on moist blotters and frozen as described above. Seeds then were gently pressed onto guaiacol agar in petri dishes so that they became half-immersed in the soft agar. Freezing seeds on guaiacol agar results in mushy agar and is not recommended. The guaiacol agar plates were incubated in darkness at 22-28 C for 4-6 days to allow the development of colonies of *H. oryzae* and *T. padwickii*. Dishes were examined macroscopically after incubation.

Colonies of *H. oryzae* on guaiacol agar consist of submerged, red mycelium [approximating Ridgway's Brazil Red (9), Centroid Color 38 (1), Munsell renotation 9.3R 4.0/9.1 (1)] growing from infected seeds in a characteristically dendritic pattern (Fig. 1). Colonies of *T. padwickii* are generally much smaller in diameter and do not form a dendritic mycelial pattern (Fig. 1). Also, they often exhibit clusters of small, dark-red sclerotium-like structures submerged in the agar adjacent to an infected seed. Colonies of *T. padwickii* may sometimes have a pale bluish-red cast or be of the same color as *H. oryzae*. On guaiacol agar, the rice blast fungus *Pyricularia oryzae* Cav. produces a dark-red coloration or halo (not mycelium) in the agar around an infected seed. Unfortunately, the present guaiacol agar method is not sensitive enough to detect every seed in a given lot that is infected with *P. oryzae*. Although single-spore isolates of *Epicoccum purpurascens* Ehrenb. ex Schlecht. and *Nigrospora oryzae* (Berk. & Br.) Petch. may produce colored halos on guaiacol agar, when growing out of rice seeds, these, and other fungi commonly isolated from rice seeds, do not produce a color reaction or colored mycelium on this medium (2).

To verify the identity of isolates from guaiacol agar, classified as *H. oryzae* or *T. padwickii* on the basis of macroscopic examination, 300 mycelial isolates considered to be *H. oryzae* were transferred from guaiacol agar to potato-dextrose agar (with 0.5 g of streptomycin sulfate per liter added after autoclaving). These isolates were incubated for 8 days at 22 C ± 0.5 C under 12 hours of "blacklight" and 12 hours of darkness daily. Colonies were examined under a binocular dissecting microscope at × 15-45. Likewise, 400 mycelial isolates considered to be *T. padwickii*, on the basis of

macroscopic examination, were transferred from guaiacol agar to rice polish agar (rice polish 3 g, agar 15 g, water 1 liter, 0.5 g streptomycin sulfate added after autoclaving) and incubated for 11 days under 12 hours of "blacklight" and 12 hours of darkness daily prior to microscopic examination.

RESULTS AND DISCUSSION.—For detection of *H. oryzae*, results from the blotter and guaiacol agar methods were significantly correlated (r = 0.95). However, there was a strong indication that the guaiacol agar method was more sensitive than the blotter method for detection of this fungus. The number of seeds identified as being infected with *H. oryzae* was larger in 92 out of the 98 lots tested on guaiacol agar than in the comparable blotter tests.

Correlation between these two methods was lower when they were used to detect *T. padwickii* (r = 0.65). In general, the blotter method appeared to be more sensitive for detection of this fungus although 17 seed lots showed a higher incidence of *T. padwickii* on guaiacol agar than on blotters. However, some evidence indicated the possibility that growth of *T. padwickii* on guaiacol agar might have been repressed by *H. oryzae* growing from *T. padwickii*-infected seeds. To test that possibility, correlations were run between the results from the blotter and guaiacol agar methods for 42 seed lots that had 20% or less *H. oryzae*, for 27 lots that had 10% or less *H. oryzae*, and for 20 lots that had 5% or less *H. oryzae*. The correlation coefficients were 0.64, 0.63, and 0.64, respectively, which indicated that the presence of *H. oryzae* in seed lots also infected with *T. padwickii* apparently had no influence on detection of the latter fungus on guaiacol agar.

Of the 300 mycelial isolates considered to be *H. oryzae* and transferred from guaiacol agar to potato-dextrose agar, 297 isolates (i.e., 99%) produced conidia typical of *H. oryzae* and three isolates proved to be nonviable. The 400 mycelial isolates identified as *T. padwickii* on guaiacol agar and transferred to rice polish agar produced 353 isolates (i.e., 88%) with conidia typical of *T. padwickii* and 47 isolates which turned out to be *Alternaria*, *Cladosporium*, or *Curvularia* spp. These latter fungi were probably present in some rice seeds also infected with *T. padwickii*, and may have been included in the inoculum of *T. padwickii* transferred to rice polish agar. They do not produce colored mycelium or halos on guaiacol agar (2).

The guaiacol agar method was found to have two advantages over the blotter method: (i) macroscopic examination of the 400 seeds per lot required in regulatory seed health testing takes only a fraction of the time required for microscopic examination of this number of seeds; and (ii) the guaiacol agar method can be used in laboratories that have only simple equipment; microscopes and "blacklight"-equipped incubators are not needed. This method would be particularly suitable for use in seed testing laboratories in developing nations where much of the world's rice is produced.

The guaiacol agar method is highly sensitive for detection of the important rice pathogen *H. oryzae*, but less sensitive than the blotter method for detection of *T. padwickii*. However, the method may be useful for rapid screening of seed lots that may have relatively large numbers of *T. padwickii*-infected seeds.

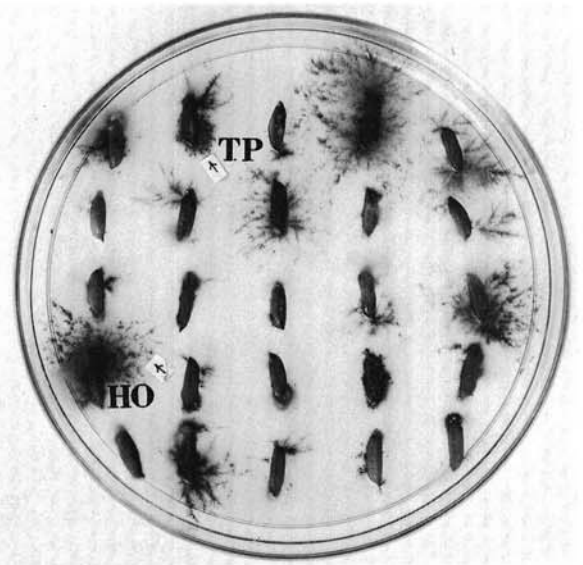

Fig. 1. *Helminthosporium oryzae* (HO) and *Trichoconis padwickii* (TP) growing from rice seeds on guaiacol agar.

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