A System for Inducing Sporulation of *Bipolaris oryzae*

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ABSTRACT

HAU, F. C., and M. C. RUSH. 1980. A system for inducing sporulation of *Bipolaris oryzae*. Plant Disease 64:788-789.

Six media and five light treatments were used to obtain sporulation of *Bipolaris oryzae*. Commercial rabbit food agar induced abundant spore production. The optimum light regime for sporulation was short-cycle radiation of 12 hr of black light followed by 12 hr of complete darkness. Neither continuous illumination by black light nor complete darkness induced sporulation. Exposure of the fungus to fluorescent light after the black light treatment led to production of fewer conidia.

Additional key words: brown leaf spot, *Oryza sativa*

Spore production by species of *Helminthosporium* depends primarily on light (5,7,8,12), but nutritional elements are also considered important (3,9).

Many investigators have reported that *Bipolaris oryzae* (Breda de Haan) Shoem. (=*Helminthosporium oryzae* Breda de Haan), the incitant of the brown leaf spot of rice, failed to sporulate on artificial and natural media under various environmental and nutritional conditions (7). Because *B. oryzae* does not sporulate well in culture, a mixture of conidial and mycelial dust has been used as inoculum for screening for varietal resistance to brown leaf spot. However, conidia were 5–10 times more effective than mycelial dust in producing infection (4).

Chattopadhyay and Das Gupta (2) showed that production of conidia by *B. oryzae* was favored by media either rich in or made exclusively of plant parts. Sharma and Singh (11) reported that rice husk agar and near ultraviolet radiation were required for production of conidia. Various investigations in our laboratory that required production of large numbers of *B. oryzae* conidia were restricted by the failure of the fungus to sporulate readily in culture. In an attempt to solve this problem, the ability of *B. oryzae* to sporulate under five light treatments and on six different media was compared.

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0191-2917/80/08078802/$03.00/0

MATERIALS AND METHODS

Five isolates of *B. oryzae*, LR2312, LR2272, LR2172, LR2072a, and LR571, were used. All isolates were obtained from naturally infected rice in Louisiana and kept in stock culture on potato-dextrose agar (PDA).

The five light treatments tested were: 1) 8 hr of black light (310–410 nm, 20W lamp, Westinghouse Electric Corporation, Bloomfield, NJ) followed by 8 hr of fluorescent light (20W lamp, General Electric, Cleveland, OH), 2) 12 hr of black light followed by 12 hr of fluorescent light, 3) 12 hr of black light followed by complete darkness, 4) continuous black light, and 5) continuous dark.

The six media used included PDA, cornmeal agar (CMA), and Czapek agar (CA) prepared as described previously (13); rice polish agar (RPA) made of 15 g of rice polish and 20 g of agar in 1 L of distilled water; and rice hull agar (RHA) made of 50 g of rice hulls, 10 g of dextrose, and 15 g of agar in 1 L of distilled water. Rabbit food agar (RFA) was prepared as follows: 50 g of commercial rabbit food pellets (Rodent Laboratory Chow 5001, Ralston Purina Co., St. Louis, MO) was steeped in 500 ml of distilled water for 20 min and filtered through two layers of cheesecloth; and the filtrate was brought up to a total volume of 1 L with distilled water, and 15 g of agar was added. The final mixture was adjusted to pH 6.5 before autoclaving.

B. oryzae isolate LR2312 was grown on

Table 1. Sporulation of *Bipolaris oryzae* on six media under five light regimes

Light treatment[a]	Sporulation[b]					
	PDA	RFA	RPA	RHA	CMA	CA
8 hr BL + 8 hr FL	2	870	2	1	2	7
12 hr BL + 12 hr FL	3	530	9	2	94	4
12 hr BL + 12 hr DK	7	2300	23	7	24	29
24 hr BL	1	54	...[c]	...[c]	7	7
24 hr DK	9	23	1	1	1	...[c]

[a] BL = black light, FL = fluorescent light, and DK = complete darkness.

[b] Sporulation of the LR2312 isolate = number of conidia × 1,000 per petri dish of medium. Each value is the mean from five replicates. PDA = potato-dextrose agar, RFA = commercial rabbit food agar, RPA = rice polish agar, RHA = rice hull agar, CMA = cornmeal agar, CA = Czapek's agar.

[c] Less than 1,000 conidia per petri dish of medium.

Table 2. Sporulation of isolates of *Bipolaris oryzae* on rabbit food agar with 12 hr of near UV light followed by 12 hr of complete darkness

Isolate	Sporulation on RFA[a]
LR2172	9.2×10^5
LR2272	7.0×10^5
LR2072a	9.8×10^5
LR571	2.2×10^5

[a]Number of conidia produced per 100-mm-diameter petri plate of rabbit food agar.

PDA at 27 C for 15 days. A 4-mm-diameter cork borer was used to make mycelial agar plugs that were transferred to the freshly prepared media in 100 × 15 mm disposable polystyrene petri dishes.

Three inoculated plates of each medium were incubated at 27 C for 15 days under the different light treatments. The lamps were 35 cm from the top of petri plates. The tops of petri plates were left on during the incubation. After incubation, spores were scraped off with a rubber policeman into 10 ml of distilled water per plate, and the number of spores was determined with a Spencer hemacytometer. The number of spores per plate was the factor compared among treatments. The other *B. oryzae* isolates differed only in their ability to produce conidia on RFA.

RESULTS

Near UV irradiation induced sporulation of isolate LR2312 on all media tested (Table 1). Alternation of black light with dark periods induced the highest level of spore production, whereas alternation of black light with fluorescent light reduced sporulation (Table 1). These results agree with those reported by Leach (8) and Aragaki (1). Neither continuous black light nor continuous darkness gave satisfactory results (Table 1).

RPA, RHA, CMA, and CA media have been reported to give satisfactory conidial production by *B. oryzae* (2,11), but they did not support significant sporulation of the five isolates we tested (Table 2). *B. oryzae* sporulated abundantly on RFA with most of the light treatments. The 12-hr cycle of black light followed by dark was the most effective treatment.

When four additional isolates of *B. oryzae* were tested on RFA, they all consistently produced high numbers of conidia (Table 2).

DISCUSSION

Our study supports the findings of Leach (7,8) and Honda (5) that formation of conidia of *B. oryzae* is light dependent. However, nutritional elements such as vitamins, salts, and traces of metallic compounds may be critical for conidial development and maturation (6,7,10).

All the media tested except RFA were previously reported to be good for spore production by *B. oryzae* (2,11). Louisiana isolates of *B. oryzae* did not sporulate well on these media. This suggests that isolates from India may differ from Louisiana isolates in sporulation behavior.

RFA supported abundant sporulation in five randomly selected isolates of *B. oryzae* from Louisiana (Tables 1 and 2), suggesting that it may induce sporulation in most isolates of *B. oryzae*. RFA is a complex, undefined medium with alfalfa meal used as base plus four vitamins, traces of organic and metallic salts, and probably numerous other elements. The medium offers a tool for the study of the nutritional mechanisms controlling sporogenesis of *B. oryzae*. The RFA and black light-total darkness regime that we used should also be useful for providing inoculum for screening for varietal resistance and in pathogenicity studies.

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