Rice Blast in Puerto Rico[1]

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ABSTRACT

Rice blast, which had not been observed during the last six decades in Puerto Rico, reappeared recently in Manatí and in the Gurabo Substation, affecting Brazos, a cultivar introduced from Texas. Pathogenicity tests, as well as studies on the conidial morphology, showed that the fungus isolated from typical blast lesions is *Pyricularia oryzae* Cav. The physiology of the fungus and its sporulation on various media also were studied. The optimum temperature range, both for mycelial growth and for germination of conidia, was found to be between 24 to 28° C. Sporulation occurred satisfactorily on potato-dextrose agar enriched with coconut milk, and on steamed corn and barley, and rice-straw-decoction agars. In vitro tests with fungicides benomyl, pentachloronitrobenzene, mancozeb, chloroneb, and captan indicated that the first two compounds are the most effective in inhibiting mycelial growth of *P. oryzae*.

INTRODUCTION

Rice blast, caused by *Pyricularia oryzae* Cav. is a disease of great economic importance in Asia, as well as in Latin America, where rice is a major source of carbohydrates in the diet. Blast is widely distributed in the rice-growing countries of the world. The disease has been reported in Argentina (12), Brazil (10), British Guiana (1), Colombia (2), Costa Rica (9), Cuba (3), the Dominican Republic (6), El Salvador (5), Guatemala (8), Mexico (19), Nicaragua (11), Panamá (13), and Perú (14). It occurs mostly under conditions of intensive cultivation and high levels of nitrogen fertilization.

Although rice blast was reported some 58 years ago in Puerto Rico by Stevenson (15) and Thomas (17), the incidence of the disease did not cause concern. Lately, new rice cultivars have been introduced into Puerto Rico from the United States and other rice-growing countries. Brazos, a cultivar newly released in Texas, was found severely infected by *Pyricularia* sp. in the Manatí area.

The information on isolation, pathogenicity, conidial morphology, physiology, as well as in vitro relative sensitivity of the Puerto Rican isolates of *P. oryzae* to various fungicides, is presented in this report.

MATERIALS AND METHODS

Plants of rice cultivars Brazos, Chontalpa, Blue Bonnet 50, Girona, and Bahía, grown in a 1:1 steam-sterilized mixture of soil and filter press cake,

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(12 pots per cultivar) were inoculated by spraying them, using a fine atomizer, with a water suspension of fresh collected conidia (about 10 conidia per 100 × microscope field). The inoculated plants were divided into two groups (six pots per group). One group was kept in a moist chamber with saturated air for 24 h and the other group was placed in a ventilated greenhouse. After incubation, the seedlings in the moist chamber were transferred to a ventilated greenhouse at 28 to 32° C. Blast symptoms in both groups of plants were recorded 10 days after inoculation.

In the studies on the effect of temperature on germination of *P. oryzae,* washed conidia were secured from a culture grown on potato-dextrose agar (PDA) enriched with coconut milk. The conidia were transferred to petri dishes containing double-distilled water. Three dishes containing the conidia were incubated at each of the following temperatures: 8, 12, 16, 20, 24, 28, 32, and 36° C.

In the studies on the effect of temperature on mycelial growth, a monoconidial isolate of *P. oryzae* was grown on PDA at 8, 12, 16, 20, 24, 28, 32, and 36° C. For each temperature, three petri dishes containing 15 ml of the PDA-coconut milk medium were seeded with a 2 mm culture disc of the isolate. The disc was cut with a sterile cork borer from the advancing margin of colonies grown on PDA. The dishes containing the inoculum were incubated at different temperatures for 8 days. The increment in the diameter of colonies was measured at the end of the incubation period.

In the studies on the effect of culture media on sporulation the following media were tested: potato-dextrose agar (PDA), coconut-milk-enriched potato-dextrose agar (CEPDA) (39 g PDA and 1000 ml of medium), steamed barley (SB) (12 g barley + 15 ml water), steamed corn (SC) (12 g corn + 15 ml water) and rice-straw-decoction agar (RSDA) (100 g of rice straw + 39 g PDA + 10 g sucrose per 1000 ml of medium). A 2 mm culture disc of the monoconidial isolate of *P. oryzae* was placed in the center of each plate, using 10 plates per medium. The seeded plates were then incubated at 28° C. The number of conidia per microscope field (100 ×) was recorded at the end of 10-day incubation period.

Fungicides benomyl, pentachlorobenzene (PCNB), mancozeb, chloroneb and captan were assayed in vitro in the laboratory, at 0.05, 0.25, 0.5, and 1.0% concentrations, for their toxicity against *P. oryzae.* The fungus was grown on PDA medium to which four 2-mm paper discs containing varying concentrations of the active ingredients of the five fungicides were added. Paper discs containing no fungicide were added to the plates used as controls. For each concentration, three petri dishes were used. The plates containing the paper discs were then incubated at 28° C. The diameter of the colonies was measured at the end of a 5-day incubation period.

RESULTS AND DISCUSSION

DESCRIPTION OF SYMPTOMS

In the early stage of disease development, the affected plants of cultivar Brazos showed circular to irregular, minute yellowish-brown spots on both sides of the leaf. These spots soon became dark brown with reddish borders and measured 2 to 3 mm long × 1 to 2 mm wide. Sometimes, these spots were confluent, forming stripes with straw-colored centers. In the advanced stage, the affected plants in the field showed typical

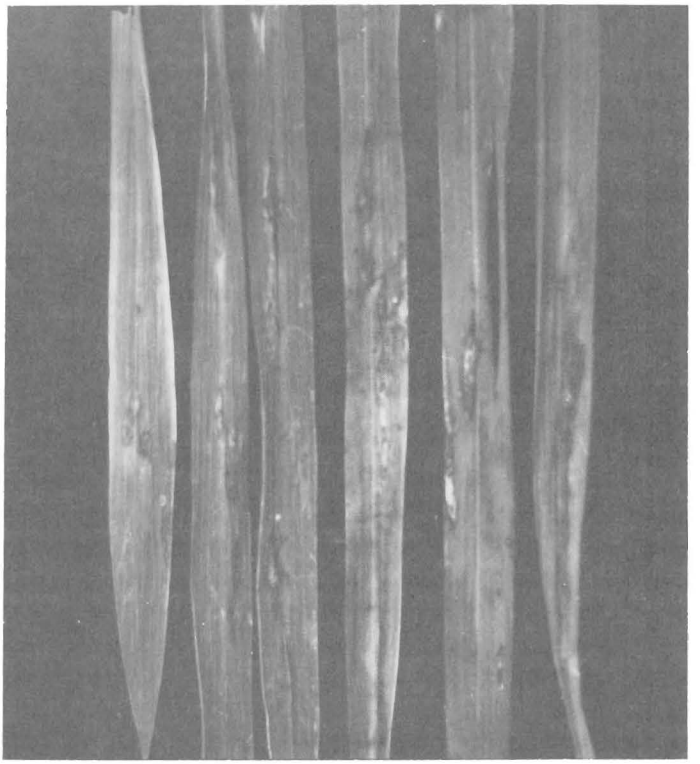

FIG. 1.—Symptoms of the blast disease on rice leaves in the field.

elliptical blast lesions which measured 3 to 40 mm long × 2 to 5 mm wide. These lesions were usually confined by two main veins and had large necrotic gray centers with brown or reddish-brown margins (fig. 1). The affected seedlings showed a general blasting effect resembling herbicide injury (fig. 2).

CULTURAL APPEARANCE AND MORPHOLOGY OF THE CAUSAL FUNGUS

The appearance of the fungus colony on PDA enriched with coconut milk was dark grayish-green with a light margin (fig. 3). Conidia were

pyriform and three-celled with a basal appendage (fig. 4). However, the size of the conidia varied considerably, depending upon the culture media, as well as age of the leaf lesions. Conidia formed on steamed-corn measured 21.2 to 34 μ long $\times$ 8.5 to 11.9 μ wide. Conidia produced on

FIG. 2.—Blasting effect of *Pyricularia oryzae* on seedlings in the Manatí area.

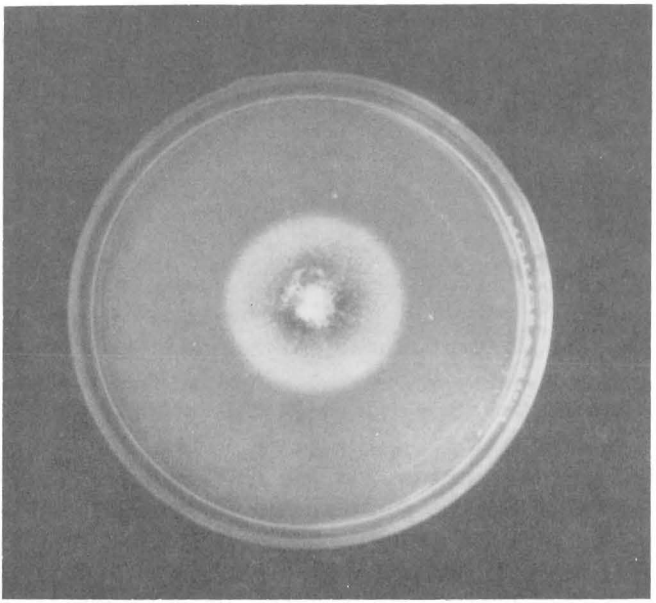

FIG. 3.—Cultural appearance of *Pyricularia oryzae* on coconut-enriched potato-dextrose agar after 5-day incubation at 28° C.

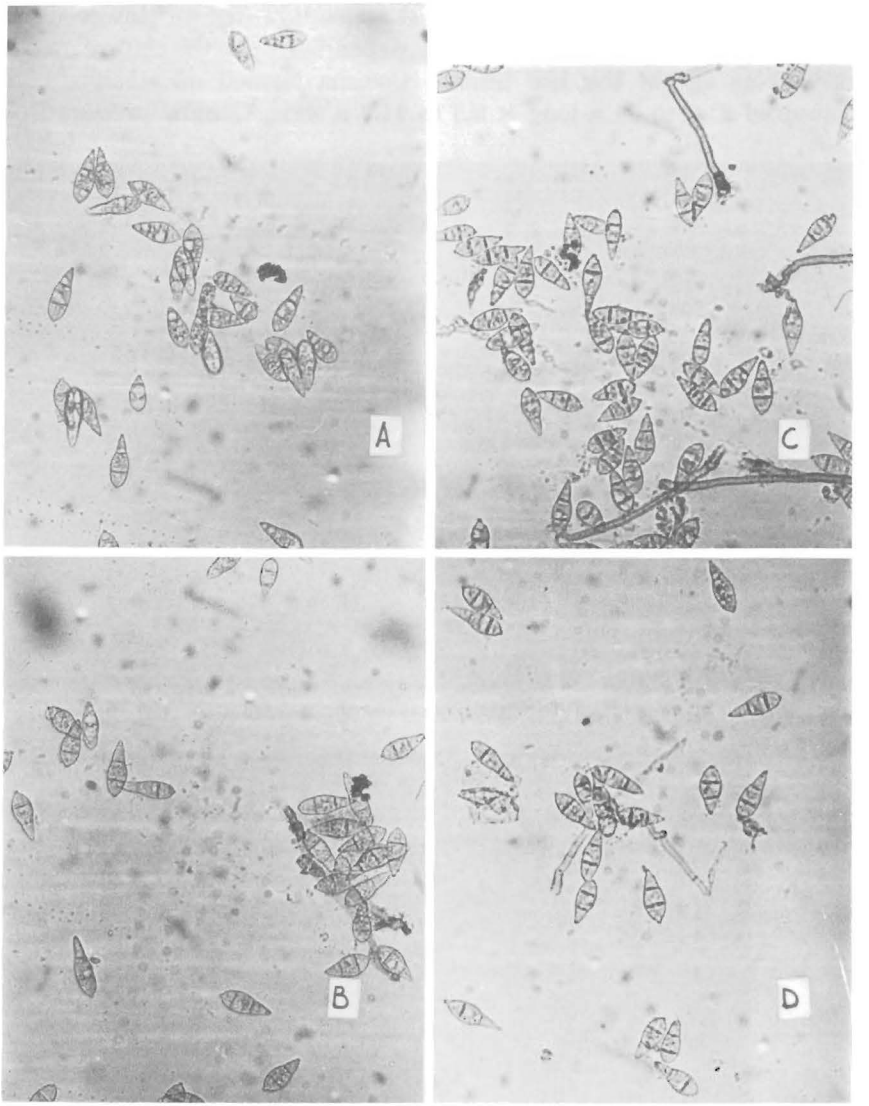

FIG. 4.—Conidia of *Pyricularia oryzae* isolated from rice plants showing symptoms of blast: A and B, conidia from young leaf lesions; C and D, conidia from old leaf lesions.

steamed-barley grains measured 25.5 to 29.7 μ long × 8.5 to 11.9 μ wide. Conidia from young leaf lesions measured 21.5 to 32 μ long × 9.5 to 12.5 μ wide and appeared normal and healthy (fig. 4 A and B). Conidia from the old-leaf lesions measured 22.5 to 34 μ long × 8.5 to 12.8 μ wide, and appeared to be devoid of cell contents (fig. 4 C and D). Generally

speaking, conidia from culture media such as coconut-milk-enriched PDA tended to be more slender than those from leaf lesions (fig. 5 A and B). Furthermore, as cultures aged, the conidia appeared fatter and the mycelium thicker (fig. 5 C and D).

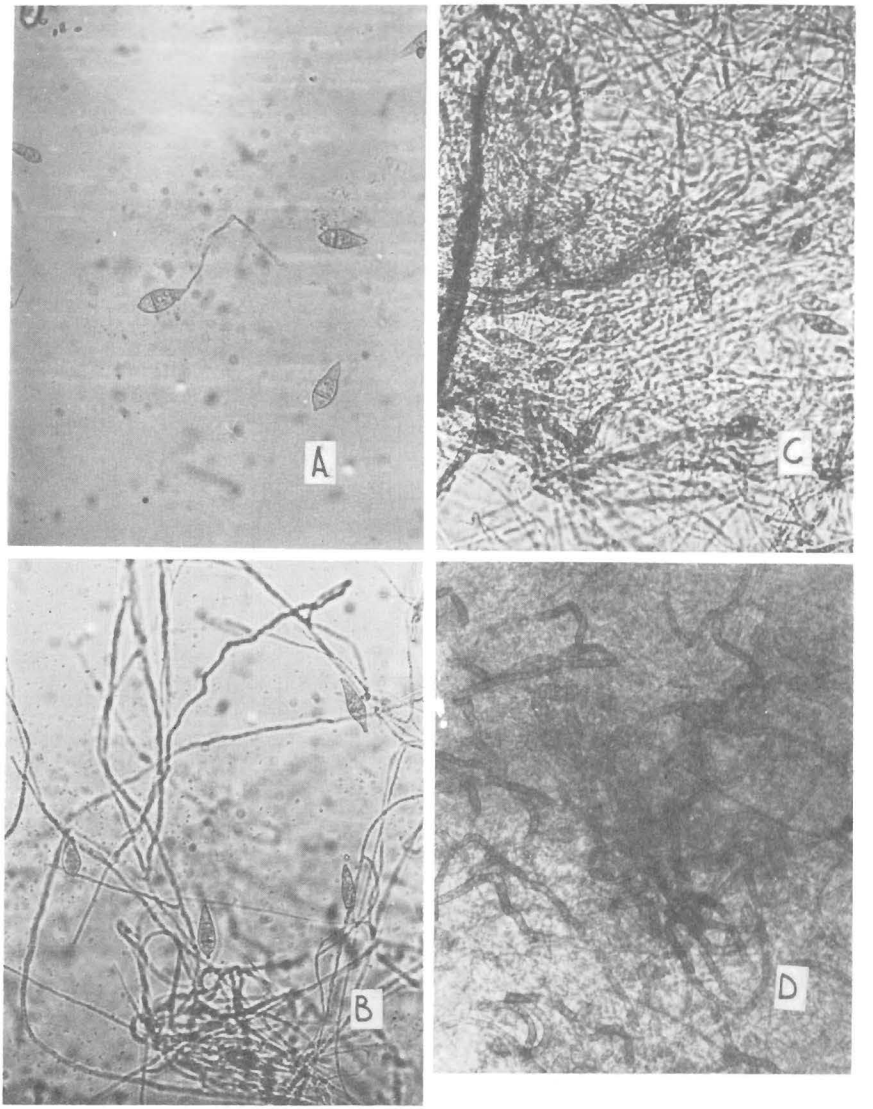

FIG. 5.—Conidia of *Pyricularia oryzae* on culture medium: A, germination of conidia on coconut-enriched potato-dextrose agar (CEPDA); B, sporulation of *P. oryzae* on 4-day old CEPDA; C, sporulation on 8-day old CEPDA; and D, sporulation on 12-day old CEPDA. Note the formation of thick-walled mycelium.

EFFECT OF TEMPERATURE ON GERMINATION

The optimum temperature range for germination of conidia from *P. oryzae* was found to be from 24° to 28° C (fig. 6). At 28° C, initiation of germination was observed in about 5 h. The process reached a maximum of 80% in 5 days. According to Sueda (16), the optimum temperature for germination of conidia was found to be 25° to 28° C. The results herein reported agree with Sueda's findings.

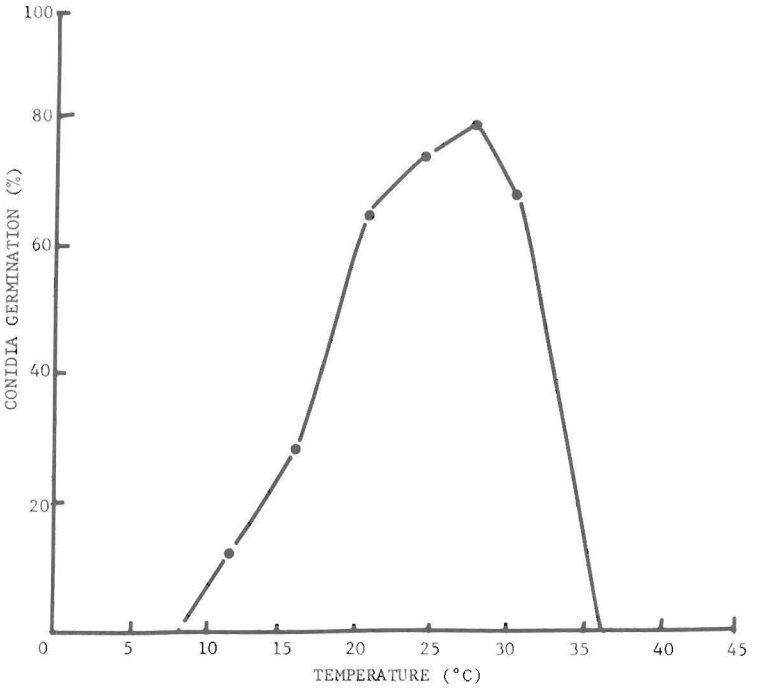

FIG. 6.—Percent of conidia germination of *Pyricularia oryzae* after a 2-day incubation in double-distilled water at different temperatures.

EFFECT OF TEMPERATURE ON MYCELIAL GROWTH

The optimum temperature range for mycelial growth of *P. oryzae* was found to be between 24° and 28° C. No growth was observed at 8° nor at 36° C (fig. 7). Yoshii (18), in his studies concerning effect of temperature on mycelial growth, observed that the optimum temperature for mycelial growth within a given length of time was 26° to 28° C.

EFFECT OF CULTURE MEDIA ON SPORULATION

No conidia were formed on PDA. Only mycelial growth was observed. The maximum production of conidia per microscope field (9.5) occurred

on CEPDA, while the lowest number (5.0) was found on rice-straw-decoction agar (fig. 8).

PATHOGENICITY TESTS

Blast symptoms appeared on rice plants which were placed in the moist chamber with saturated air, whereas no symptoms were observed on

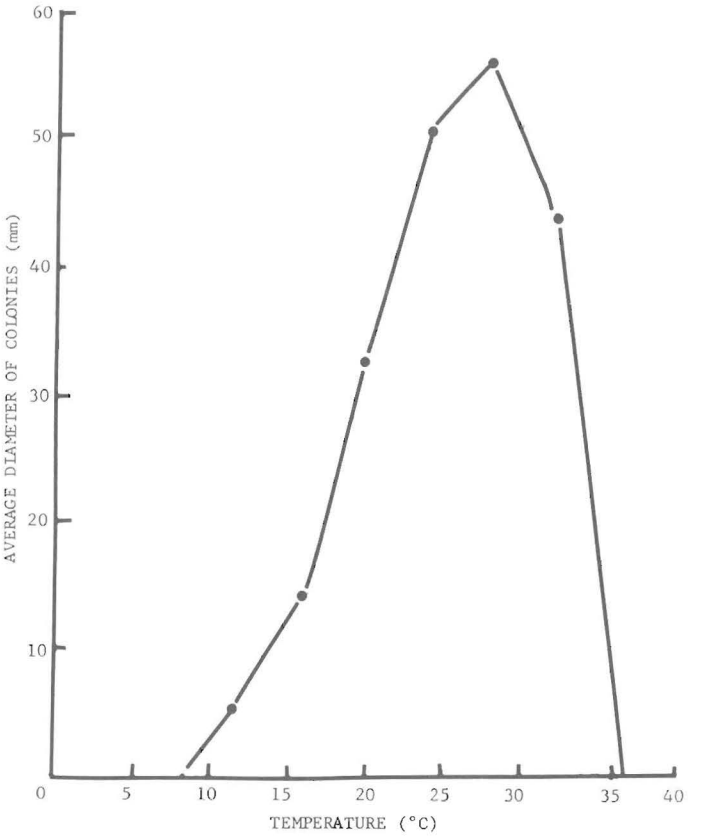

FIG. 7.—Size of colonies of monoconidial isolate of *Pyricularia oryzae* after an 8-day incubation on potato-dextrose agar at different temperatures.

plants which were left out of the chamber. A saturated air favors the infection of rice by *P. oryzae*. In the field, blast was observed more frequently in densely populated fields, in which, presumably, air humidity is higher.

Among the cultivars tested, Brazos seemed to be the most susceptible and Girona the least susceptible. The lesions on Brazos were elliptical (or lenticular), with large necrotic gray centers and with brown or reddish-

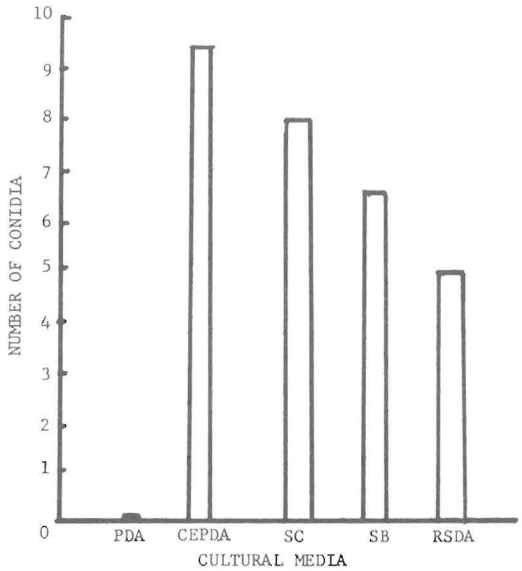

FIG. 8.—Number of conidia of *Pyricularia oryzae* observed after a 10-day incubation in a microscope field 100 × on various culture media (PDA = potato-dextrose agar; CEPDA = coconut-enriched potato-dextrose agar; SC = steamed corn; SB = steamed barley; RSDA = rice-straw decoction agar).

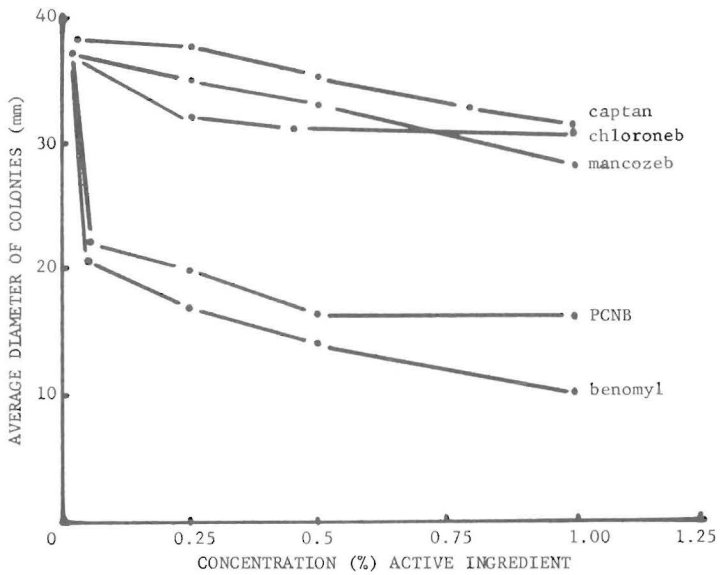

FIG. 9.—Effect of various fungicides (benomyl, PCNB, mancozeb, chloroneb, and captan) on mycelial growth of *Pyricularia oryzae* from rice.

brown margins. Approximately 3% of the leaf area was damaged. The lesions on Blue Bonnet 50 were small, roundish with necrotic gray centers surrounded by brown margins. On Chontalpa the lesions were restricted to brown specks. On both Bahía and Girona, small brown specks of pinhead size were evident. In addition, the blasting effect, i.e., killing or yellowing of the leaves, occurred on Brazos, Blue Bonnet 50, and Chontalpa, but not on Bahía and Girona. The fungus was isolated from the inoculated seedlings.

IN VITRO FUNGICIDE TESTS

Benomyl and PCNB appeared to be the most effective fungicides for inhibiting mycelial growth of *P. oryzae* (fig. 9). This finding agrees with that of Gálvez and Castaño, who in 1972 observed that benomyl (Benlate)[3] was effective in the control of blast in Colombia. In Costa Rica, Carrera (4) reported better blast control with benomyl than with mancozeb (Dithane M-45).

RESUMEN

La pudricíon de la base de la panoja del arroz, enfermedad conocida también por quema del arroz y brusone, que no había aparecido en Puerto Rico en las útimas seis décadas, reapareció recientemente en Manatí y en la Subestación Experimental de Gurabo, atacando al cultivar Brazos, introducido de Texas. Pruebas de patogenicidad, así como estudios morfológicos de los conidios, indicaron que el hongo aislado de lesiones típicas de esta enfermedad es *Pyricularia oryzae* Cav. Se estudiaron la fisiología del hongo y su esporulacíon en varios medios de cultivo. La temperatura óptima, tanto para el desarrollo filamentoso como para la germinación de los conidios, osciló entre 24 y 28° C. La esporulación fue satisfactoria en agar con dextrosa y papa, enriquecido con leche de coco, en maíz y cebada cocidos al vapor y en agar con una decocción de paja de arroz. Pruebas *in vitro* con los fungicidas benomyl, pentacloronitrobenzeno, mancozeb, chloroneb y captan, indicaron que los primeros dos son los más eficaces para inhibir el desarrollo filamentoso de *P. oryzae*.

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[3] Trade names are used in this publication solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico or an endorsement over other equipment or materials not mentioned.

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