Research Note

CHARACTERIZATION AND COMPARATIVE PATHOGENICITY OF TWO MACROPHOMINA PHASEOLINA *ISOLATES FROM PUERTO RICO*[1]

Macrophomina phaseolina (Tassi) Goid is the causal agent of the ashy stem blight (charcoal rot) of common beans (*Phaseolus vulgaris* L.)[2,3]. The first symptom of ashy stem blight (ASB) on common bean seedlings is an irregular dark brown sunken lesion with a sharp margin on the hypocotyl at the bases of cotyledons. The fungus also attacks adult plants near senescence. The infection extends upward from near the cotyledonary node to the length of the stem with dark brown lesions. Symptoms are more pronounced on one side of the plant. In later stages the pathogen produces tiny fruiting bodies, pycnidia or microsclerotia, on a gray background of the surface of infected stems.[3] The disease is prevalent in warmer bean growing areas, especially in those with drought periods.[4] However, the disease incidence has also been severe in soils with high moisture.[5] The pathogen attacks more than 500 plant species, including soybean, cotton, corn, sorghum and edible legumes. The pathogenicity is obtained for most isolates near 30° C. The fungus affects the hypocotyl of seedlings or the base of cotyledons. Seeds may be affected by the fungus, resulting in a seed-borne inoculum.[6,7] *Macrophomina phaseolina* survives as microsclerotia in the soil and in organic debris in the soil.[8] Host variation in level of resistance to *M. phaseolina* exists among common bean genotypes.[9] Variation in morphology and virulence among isolates of *M. phaseolina* was also reported in soybean, common bean and other crops.[4,10] Soil inoculation techniques with microsclerotia or mycelia were reported by Dhingra and

[1]Manuscript submitted to Editorial Board 8 March 1991. We thank James Beaver for assistance in statistical analysis of the data.

[2]Kendrick, J. B., 1933. Seedling stem blight of field beans caused by *Rhizoctonia bataticola* at high temperatures. *Phytopathology* 23: 949-63.

[3]Schwartz, H. F., 1980. Miscellaneous fungal pathogens. *In*: Bean production problems: Disease, insect, soil and climatic constraints of *Phaseolus vulgaris*. Ed. by Schwartz, H. F., G. E. Gálvez. CIAT, Cali, Colombia. 424 p.

[4]Dhingra, O. D. and J. B. Sinclair, 1977. An annotated bibliography of *Macrophomina phaseolina*, 1905-1975. Brazil, Universidad Federal de Visçosa. 244 p.

[5]Anonymous, 1989. Annual Report AID-PSTC/UPR-RUM/Dom. Rep. "Biocontrol of Bean Ashy Stem Blight By Improved Rhizobium Biotechnology" Project.

[6]Andrus, C. F., 1938. Seed transmission of *Macrophomina phaseoli*. *Phyotopathology* 28: 620-34.

[7]Gangopadhyay, S, R. D. Wyllie and V. D. Luedders, 1970. Charcoal rot disease of soybean transmitted by seeds. *Plant Dis. Rep.* 54: 1088.

[8]Papavizas, G. C. and N. G. Klag, 1975. Isolation and quantitative determination of *Macrophomina phaseolina* from soil. *Phytopathology* 65: 182-87.

[9]Echávez-Badel, R. and J. S. Beaver, 1987. Resistance and susceptibility of beans, *Phaseolus vulgaris* L., to ashy stem blight, *Macrophomina phaseolina* (Tassi) Goid. *J. Agric. Univ. P. R.* 71(4): 403-05.

[10]Echávez-Badel, R. and A. Sánchez-Paniagua, 1991. Reaction of landrace Pompadour beans to *Macrophomina phaseolina* isolates. (Submitted for publication.)

Sinclair.[11] However, the toothpick inoculation has been an effective, rapid, economical technique for greenhouse screening of common bean germplasm.[9] The objectives of this study were to characterize and to determine the pathogenicity of two isolates of *M. phaseolina* causing the ashy stem blight of common beans in Puerto Rico.

Macrophomina phaseolina was isolated from stems of common beans with ashy stem blight grown in Fortuna (PR Mp1) and Isabela (PR Mp2) substations. Affected bean tissues were washed under running tap water, surface-sterilized for 1 to 2 min in 0.05% NaOCl, then plated on potato dextrose agar (PDA) supplemented with streptomycin sulphate as medium. After 72 h of incubation at 28° C, hyphal tips were transferred to PDA slants at 28° C in order to maintain isolates in pure cultures.

Disks of active growing PR Mp1 and PR Mp2 isolates were cut and placed in the center of each PDA Petri dish and incubated at 28° C for 48 h. We determined radial growth at 12, 24, 36 and 48 h; morphology, color of the mycellium and microsclerotial formation. A completely randomized design with five replications was used. A rapid growing mycellium was characteristic of *M. phaseolina* isolates. The following tabulation and fig. 1 show that the size of microsclerotia and the radial growth rates were not significantly different. The isolates PR

Isolates	Sclerotia size (micron) 1/	F Test
Fortuna (Mp1)	83	NS
Isabela (Mp2)	79	NS

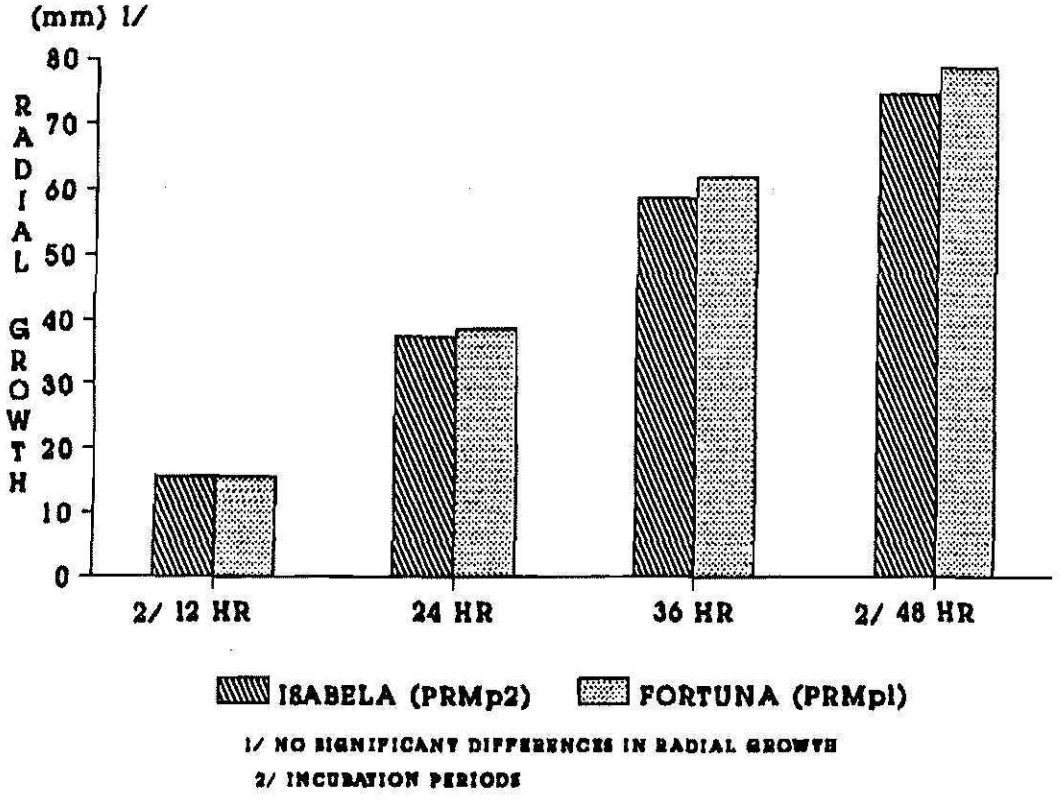

FIG. 1.—Radial growth of *Macrophomina phaseolina* isolates (Mp1, Mp2) on PDA medium at 28°C for 48-hr incubation.

[11]Dhingra, O. D. and J. B. Sinclair, 1985. Basic plant pathology methods. CRC Press Inc., Boca Raton, Florida.

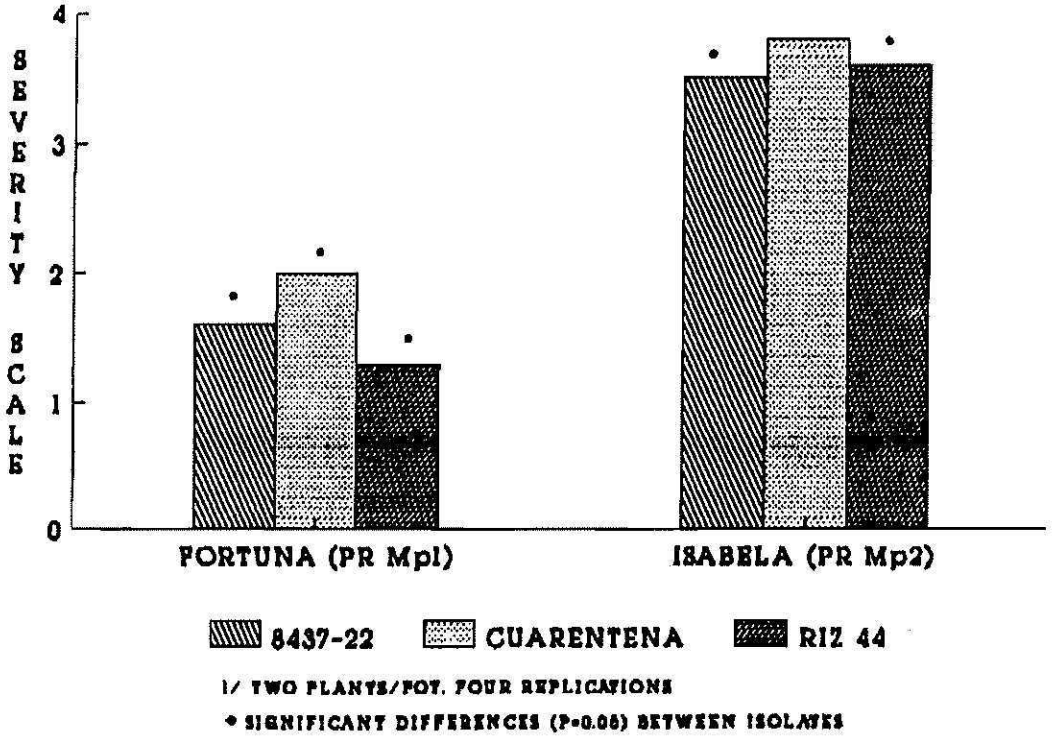

FIG. 2.—Pathogenicity of *Macrophomina phaseolina* isolates (PR Mp) to common bean (*Phaseolus vulgaris*) genotypes 1/.

Mp1 and PR Mp2 produced abundant white to gray aerial mycellium, which became darker with age. Both isolates produced on the surface of the colony black microsclerotia varying in shape from spherical to irregular. No pycnidia were observed on PDA medium.

To test the pathogenicity of the isolates, we planted two seeds per pot of three bean genotypes (8437-22, Cuarentena and RIZ 44) in 20-cm plastic pots containing pasteurized soil. Thirty days after planting, we inoculated the bean plants by inserting toothpicks with the fungus into the stem near the cotyledonary node. A visual rating scale (0=no symptoms to 4=plant death) was used to measure the disese severity at 10, 20 and 30 days after inoculation. Treatments were replicated four times and data were analyzed by a median test and chi-square analysis. Both isolates of *M. phaseolina* developed typical symptoms of ashy stem blight. Significant differences in virulence occurred between the two isolates (fig. 2). Similar results were obtained by Dhingra and Sinclair[4] and Echávez-Badel and Beaver[9] with varietal differences in charcoal rot in soybean and common bean, respectively. The Isabela PR Mp2 isolate caused significantly higher disease severity in beans than the Fortuna PR Mp1 isolate (fig. 2).

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