Charcoal Rot Screening Procedure and Virulence of *Macrophomina phaseolina* Isolates on Dry Edible Beans[1] *

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ABSTRACT

In greenhouse tests, dried sclerotia of *Macrophomina phaseolina* were the most effective form of inoculum to initiate a high and consistent incidence of charcoal rot needed to determine the reaction of bean accessions and the virulence of pathogen isolates. Sclerotia were produced in a liquid medium containing 10 g peptone, 15 g dextrose, 0.25 g $MgSO_4 \cdot 7H_2O$ and 0.5 g K_2HPO_4 in one liter of water. After a minimum of two weeks incubation at 30°C, mycelial mats with abundant sclerotia were homogenized in a mixer with distilled water, centrifuged, washed once and then dried for 48 hrs. Dried sclerotia were mixed into pasteurized soil at a rate of 2 g/kg soil. Inoculation consisted of covering bean seeds planted in pots or flats with 2-3 cm layer of the soil infested with *M phaseolina* Seedlings of susceptible materials such as A 70 and A 464 failed to emerge and/or exhibited severe disease symptoms and died within two weeks after planting. In contrast, seedlings of resistant accessions such as San Cristobal 83, BAT 477, BAT 332 and G 5059 (H6 Mulatinho) exhibited slight or no disease symptoms. Isolates of *M. phaseolina* obtained from bean tissues collected from Brazil, Colombia, and Peru differed significantly in their virulence to bean cultivars suceptible to *M phaseolina* For example, plants of A 70 inoculated with *M phaseolina* isolates Nos. 35 and 103, had a disease severity (1-9 scale) of 9.0 and 1.6, respectively.

COMPENDIO

En prueba de invernadero se encontró que esclerocios secos de *M phaseolina* son la forma de inóculo más efectivo para iniciar pudrición carbonosa (charcoal rot) con una incidencia alta y consistente. Este tipo de incidencia es necesaria para determinar la reacción de líneas de frijol y la virulencia de aislamientos del patógeno. Los esclerocios fueron producidos en medio líquido que contenía 10 g de peptona, 15 g de dextrosa, 0.25 g $MgSO_4 \cdot 7H_2O$ y 0.5 g K HPO por litro de agua. Después de un mínimo de dos semanas de incubación a 30°C, nudos de micelio con abundantes esclerocios fueron homogenizados en una mezcladora con agua destilada, cintrifugados, lavados una vez y secados por 48 horas. Esclerosios secos fueron mezclados con suelo pasteurizado en una proporción de 2 g/kg de suelo. Para la inoculación, las semillas de frijol fueron sembradas en materas o bandejas y cubiertas con una capa de 2 a 3 cm de suelo infestado con *M phaseolina* Las plántulas de líneas suceptibles, tales como A 70 y A 464, no emergieron y/o exhibieron severos síntomas de enfermedad y murieron después de dos semanas de sembradas. Por el contrario, semillas de material resistente como San Cristóbal 83, BAT 477, BAT 332 y G 5059 (H6 Mulatinho) presentaron ligeros o ningún síntoma de enfermedad. Aislamientos de *M phaseolina* obtenidos de tejidos de frijol colectados en Brasil, Colombia, y Perú difirieron significativamente en su virulencia a variedades de frijol suceptibles a *M phaseolina* Por ejemplo, plantas de A 70 inoculadas con los aislamientos 35 y 103 tuvieron una severidad de enfermedad de 9.0 y 1.6, respectivamente (evaluadas con la escala DSR, 1-9).

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid is the causal agent of charcoal rot (Ashy stem blight) of beans (*Phaseolus vulgaris* L.) (8, 10, 13, 17). The disease is prevalent in warmer bean-growing areas, especially those with drought periods which occur in the north-east region of Brazil, and certain regions in Colombia, Mexico, Peru, Venezuela and Kenya The pathogen is worldwide in distribution and has a wide host range, causing charcoal rot diseases on more than 500 plant species including soybean, maize, sorghum, cotton and edible legumes (7). Variation in morphology and virulence among isolates of *M phaseolina* has been reported on soybeans, sesame and other crops (5, 6, 11). The fungus survives in soil as sclerotia embedded in organic debris or free in soil (3, 5, 15).

Data on yield reduction and economic losses resulting from infection of dry edible beans by *M phaseolina* are limited However, epidemic outbreaks and yield losses due to charcoal rot of bean have been recently observed in many bean-growing areas in Latin America where drought stress has prevailed during part of the growing season Depending on the severity and time of initial infection, damage to beans by *M phaseolina* may be in the form of reduced

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emergence, postemergence damping-off, reduced vigor, progressive wilting, defoliation and premature dying. The Centro Internacional de Agricultura Tropical (CIAT) has emphasized the identification and development of resistant bean germplasm as a relatively low input and lasting management practice for small farmers, who produce the majority of beans in Latin America. However, only limited information is available on screening methodologies and the reaction of dry edible bean germplasm to *M. phaseolina*. Thus, the objectives of this study were to develop a rapid, accurate and simple screening method for evaluating the reaction of bean accessions to *M. phaseolina*, and to determine the extent of variability in virulence among isolates of *M. phaseolina* to beans. A brief summary of this investigation has been published previously (1).

MATERIALS AND METHODS

All isolates of *M. phaseolina* used in this study were obtained from infected bean tissues. Isolates 1, 2, 33, 34, 37, 38, and 40 were collected in Colombia; 101, 102 and 103 from Peru and 35 and 36 from Brazil. Infected bean tissues were washed in running tap water, surface sterilized for 2 min in 1% NaOCl solution, and then plated onto acidified potato-dextrose agar (APDA) at 30°C. Isolates were maintained by periodic hyphal tip transfers onto APDA or were stored under mineral oil in screw-capped glass test tubes.

Initially, several inoculum sources of *M. phaseolina* such as sclerotia, colonized whole rice seeds and mycelial agar disks were compared for their efficiency in inducing charcoal rot on the snapbean cultivar 'Bush Blue Lake 47' and the dry bean cultivar 'California Light Red Kidney'. Sclerotial inoculum was produced on the soybean-seed extract broth (SSEB) (9), Difco potato-dextrose broth (PDB), and a synthetic liquid medium (SLM) consisting of 10 g peptone, 15 g dextrose, 0.25 g $MgSO_4$ $7H_2O$, and 0.5 g K_2HPO_4/l water. After 15 days incubation at 30°C, the mycelial-sclerotial mat was blended in a Vertis mixer with distilled water, centrifuged at 5 000 g, the pellet resuspended in distilled water and recentrifuged. Washed pellets were spread on filter papers and allowed to dry aseptically for 48 h at 30°C. The dried sclerotial masses were ground in a mortar and the preparation mixed into pasteurized soil (30 min at 60°C) at different rates (0.5-4.0 g/kg soil) for inoculation tests. The highest number of sclerotia was produced on SLM, which was used exclusively in subsequent tests unless otherwise stated. One gram of the sclerotial preparation produced approximately 6×10^5 growth forming units on APDA plates.

The rice inoculum was prepared by first autoclaving moistened whole rice seeds (1:1 g rice seeds: ml water). After cooling, the rice seeds received 7-day-old mycelial agar disks of *M. phaseolina* and the mixture was incubated at 30°C for 15 days. The mycelial agar disk preparation consisted of 6 mm diameter disks obtained from the margin of 7-day-old colonies of *M. phaseolina* on APDA.

Inoculation procedures consisted of i) placing a 2-3 cm layer of the sclerotial inoculum (approximately 150 ml/10 cm diameter pot); ii) placing one to three *M. phaseolina* colonized whole rice seeds; or iii) placing 6 mm diameter mycelial potato-dextrose agar (PDA) disks (one to three) of *M. phaseolina* on top of seeds or around lower stems of 7 to 10 day-old seedlings. All plants were maintained in a greenhouse at Cali, Colombia (about 1000 m above sea level) with a temperature fluctuating between 20°–33°C and relative humidity of 35–80%. Plants were watered daily as needed, and fertilized once a week with 50 ml solution/10 cm diameter pot of a complete fertilizer (15-15-15, NPK; 3 g/l).

Disease severity ratings (DSR) were recorded at weekly intervals using the CIAT adapted scale of 1-9 (4). For above-ground evaluations, a DSR of one refers to no visible symptoms, whereas a DSR of nine indicates that all stem tissue and the growing point are often affected, resulting in a dead plant. Ratings of three, five and seven refer to lesions that are limited to cotyledonary tissue, lesions that have progressed from cotyledons to approximately 2 cm of stem tissue, and to extensive lesions covering stem tissue and resulting in chlorosis and necrosis of the foliage, respectively. For below-ground evaluation, DSR of one indicates no visible symptoms and DSR of nine refers to 50% or more of lower stem tissue covered with lesions and the associated production of numerous fruiting structures. Ratings of three, five and seven for stem infections refer to approximately 1, 10 and 25% of lower stem tissue covered with lesions of *M. phaseolina*.

The virulence to beans of several *M. phaseolina* isolates obtained from different growing areas in Brazil, Colombia and Peru were compared on susceptible (A 70) and resistant (BAT 477) breeding bean lines from CIAT. Sclerotia of all isolates were produced on each SLM and mixed into pasteurized soil at a rate of 2 g/kg soil. Seeds of each bean line were inoculated, maintained for three weeks in the greenhouse, and evaluated following the procedures described above.

All tests were conducted in randomized block designs, replicated at least four times. Unless other-

wise indicated, each experimental unit (replicate) consisted of four seeds or four seedlings per 10 cm diameter pot. Data from each test were subjected to analysis of variance and LSDs were calculated if F-tests indicated statistical significance.

RESULTS

Efficiency of inocula and method of inoculation. Since sclerotia are the primary surviving structure of *M. phaseolina*, the initial test compared different densities of sclerotia (0.1, 0.5, 1.0 and 2.0 g dry sclerotia/kg soil) in pasteurized soil placed around the stems of ten-day-old seedlings. Charcoal rot severity ratings generally increased as sclerotial density increased from 0.1 to 2.0 g/kg soil (Table 1). Also, disease severity was higher on the snap bean cultivar 'Bush Blue Lake 47' than on the dry bean cultivar 'California Light Red Kidney'. In addition, sclerotia produced on the SSEB induced greater charcoal rot severity than a comparable density of sclerotia produced on PDB. The analysis of variance showed significant effects ($P = 0.05$) due to cultivar, growth medium used for sclerotial production, and sclerotial density. Nevertheless, disease severity ratings from lower stem tissue were low with no apparent effect on plant growth even at the highest sclerotial density of 2 g/kg soil. However, a greater charcoal rot severity developed when bean seeds, as compared to seedlings, were inoculated with infested soil samples. All further tests therefore were conducted by inoculating seeds at planting time.

Table 1. Effect of growth medium and sclerotial density of *Macrophomina phaseolina* (isolate No. 33) on charcoal rot severity. Ten-day-old seedlings of Bush Blue Lake 47 (BBL 47) and California Light Red Kidney (CLRK) were inoculated with 150 ml of pasteurized soil infested with sclerotia of *M. phaseolina* placed around the lower stem tissue.

	DSR (1-9)[a] sclerotial density (g/kg soil)				
Media and cultivar	0.1	0.5	1.0	2.0	Mean
PDB[b]:					
BBL 47	1.3[d]	2.8	3.1	3.5	2.7
CLRK	1.1	2.1	2.0	2.6	2.0
SSEB[c]:					
BBL 47	2.0	2.6	3.4	3.6	2.9
CLRK	1.3	2.7	3.0	3.3	2.6

ANOVA revealed significant effects due to cultivar, medium, and inoculum density, $LSD_{0.5} = 0.9$

a) Disease severity ratings (DSR) were recorded three weeks after inoculation using a scale of one (no visible symptoms) to nine (50% or more of stem tissue covered with lesions).

b and c) Refers to potato-dextrose broth and soybean-seed extract broth produced sclerotia of *M. phaseolina*, respectively.

d) Each number is an average of five replicates (4 plants/replicate). Noninoculated controls remained free of disease (DSR = 1.0).

Charcoal rot severity was high when bean seeds were inoculated with sclerotial inoculum (2 g dry sclerotia/kg soil), colonized rice seed or a 6 mm mycelial agar disk of *M. phaseolina* at planting time (Table 2). Below-ground charcoal rot severity ratings of the same plants were comparable to those recorded on foliar parts with a modified scale. Disease severity ratings for the sclerotial, colonized rice seeds and mycelial agar disks of *M. phaseolina* isolate No. 34 were 7.6, 8.7 and 7.4, respectively. Dry weight of inoculated plants was significantly lower than noninoculated plants.

There were significant differences in the virulence of the three isolates of *M. phaseolina* used, with isolates 34 and one as the most and least virulent to beans respectively.

The effect of different soil densities of sclerotia on charcoal rot incidence and severity was evaluated on California Light Red Kidney. Analysis of variance showed a significant linear effect of sclerotial inoculum on charcoal rot severity and reduction of dry weight (Table 3). Again, differences were evident for isolate virulence to beans. Charcoal rot severity ratings from lower stem tissues of the same plants were remarkably similar and proportional to foliar ratings as reported in Table 2. For example, DSR of lower stem tissues for the 0.5, 1.0, 2.0 and 4.0 g sclerotia/kg soil for isolate 34 were 5.5, 6.4, 7.6 and 8.4, respectively. The virulence to beans of the four isolates of *M. phaseolina*, used in descending order, were isolate Nos. 34, 33, 1 and 2. Results of this test and others, have shown that the 2 g dry sclerotia/kg soil consistently incited a severe incidence of charcoal rot with considerable reduction of plant growth, and thus it was used in all future tests.

The effectiveness of sclerotial and colonized whole rice seed inocula to incite charcoal rot was further evaluated on susceptible (A 464) and resistant (BAT 477) bean lines (3, 16). Sclerotia of *M. phaseolina* isolate No. 34 were produced on SSEB or SLM and were used to infest soil at the 2 g/kg soil rate, or two rice seeds colonized by *M. phaseolina* were placed next to each bean seed prior to covering seeds

Table 2. Effect of three inocula sources on charcoal rot severity and plant growth in California Light Red Kidney exposed to three Colombian isolates of *Macrophomina phaseolina* (Mp).

	DSR (1-9)[a] and dry wt., (g)[b]/inoculum source[c]					
	Sclerotia		Colonized seeds		Mycelial Agar disks	
Isolate code	DSR	Dry wt	DSR	Dry wt	DSR	Dry wt
None (check)	1 0[d]	2.9				
Mp 1	3 6	2.1	4 8	2.0	2.7	2 2
Mp 33	6 7	1.1	5 0	1.9	5.7	1 7
Mp 34	7 4	0.6	8 5	0.1	7.3	0 7
$LSD_{0.5}$	DSR = 1 16			Dry weeks = 0 42		

a) Disease severity ratings (DSR) were recorded three weeks after inoculation using a scale of one (no visible symptoms) to nine (all stem tissues and the growing point are affected – dead plant)

b) Plants were dried at 95°C for 48 h

c) About 150 ml of pasteurized soil infested with sclerotia of *M. phaseolina* (2 g dry sclerotia/kg soil) were placed on top of bean seeds; one colonized rice seed, or one 6 mm mycelial agar disk from the margin of a five-day-old colony of *M. phaseolina*, was placed on top of each bean seed

d) Each number is an average of five replicates. Data for noninoculated checks were not included in the ANOVA.

(4/10 cm diameter pot) with 150 ml of pasteurized soil. Seeds were inoculated with the sclerotia inoculum by covering them with 150 ml of infested soil. All inocula differentiated the reaction of the two bean lines by one-two weeks after inoculation (Table 4). Sclerotial inoculum most consistently produced significantly greater disease severity, especially when sclerotia were produced on SLM. Analysis of variance revealed highly significant effects for inocula, cultivar and inocula x cultivar interactions. Based on these results others, it was concluded that the screening technique involving the use of sclerotia of *M. phaseolina* produced on SLM at a rate of 2 g/kg soil was the most appropriate for evaluating the reaction of dry edible bean germplasm to infection by *M. phaseolina* under greenhouse conditions (Fig. 1I).

Virulence of *M. phaseolina* isolates to beans. Isolate No. 34 of *M. phaseolina*, the most virulent isolate used to evaluate inocula form and inoculation methodologies (Tables 2, 3), was compared to nine other isolates of *M. phaseolina* obtained from infected bean tissue collected in Colombia, Peru and Brazil. Sclerotia of all isolates were produced on SLM and incorporated into pasteurized soil at 2 g/kg soil. The ten isolates of *M. phaseolina* varied significantly in their virulence on the susceptible bean breeding line A 70 when evaluated one, two or three weeks after inoculations (Table 5, Fig. 1L). However, only slight disease symptoms restricted to cotyledonary tissue were observed on the resistant breeding line BAT 477. Isolate No. 39 induced the highest disease severity ratings (a score of 2.2) on BAT 477. Bean germplasm with disease severity ratings of three or less are considered resistant (4).

Symptomatology. Seeds of highly susceptible bean germplasm such as A 70 and A 464 may become severely infected shortly after inoculation resulting in poor emergence and seedling establishment. The first observed symptoms on emerging seedlings of susceptible germplasm were dark, irregular lesions of different sizes on the cotyledons (Fig. 1 E). These lesions expanded rapidly in susceptible cultivars covering the whole cotyledonary tissues and progressed through the base of cotyledons into stem tissue (Fig. 1 H, K). Infected cotyledons always remained attached to stems, probably as a result of extensive hyphal growth of the fungus. A characteristic blight and systemic chlorosis appeared on young leaves of infected plants even when lesions were only small and arrested (Fig. 1 F, H, L). The systemic chlorosis can be confined to one side of the plant or even restricted to one-half of a leaf. The dark, sunken lesions continued to expand upward and downward in stem tissues (Fig. 1 F, K). The expanding lesions eventually reached the growing points, resulting in plant death, or the lesions weakened and broke the stem (Fig. 1 F, K, I). Similar symptoms and progression of disease on young seedlings also occurred under field conditions (Fig. 1 D). Occasionally, leaf tissues also were found infected and often appeared to occur in association with the veins of leaf tissues (Fig. 1 G). Diagnostic dark, sunken lesions also occurred on the lower stem tissues (Fig. 1 J). These lesions were initially longitudinal and narrow, but expanded and eventually

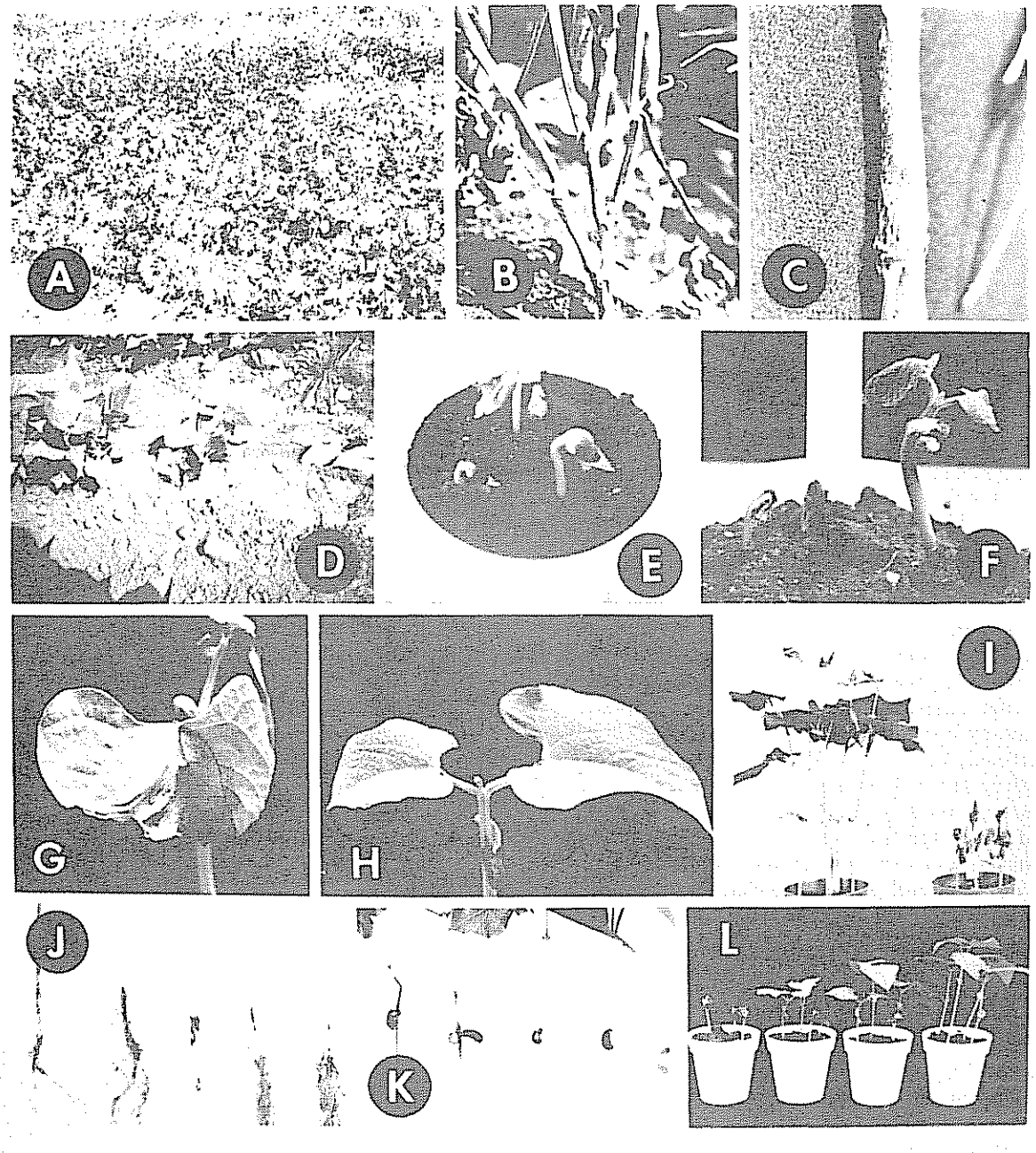

Fig. 1 (A-L) Symptomatology and host-parasite relationship of *Macrophomina phaseolina* to beans. A) Severely infected area in a field exhibiting wilting, chlorosis and necrosis on older plants. B) Close up of an infected plant showing large infected areas on stem and branches with the characteristic ashy color. C) Close up of an infected stem area with the diagnostic sclerotial production on and in the ashy colored tissue. D) Infected young plants in the field. E) Seedlings exhibiting lesions on cotyledons shortly after emergence. F) Young seedling severely infected with *M. phaseolina* resulting in death of two out of the three seedlings. G) Leaf tissues infected with *M. phaseolina*. H) Infection from the cotyledon is progressing into stem tissue with systemic chlorosis of the leaf on the left. I) Seedlings of resistant BAT 477 (left) and susceptible A 70 (right) inoculated with sclerotial inoculum of *M. phaseolina*. J) Lesions of *M. phaseolina* on lower stem tissue. K) Different stages of the progress of charcoal rot lesions from the cotyledons into stem tissue. L) Seedlings of A 70 inoculated with *M. phaseolina* isolate number (left to right) 35, 36, 102, and 103.

Table 3. Virulence of four Colombian isolates of *Macrophomina phaseolina* at four sclerotial densities on California Light Red Kidney beans.

	DSR (1-9)[a] and dry wt, (g)[b]/sclerotial density (g/kg soil)[c]							
	0.5		1.0		2.0		4.0	
Isolate code[d]	DSR	Dry wt	DSR	Dry wt	DSR	Dry wt	DSR	Dry wt
–(Check)	1.0[e]	2.9						
Mp 1	2.7	2.3	2.5	2.1	3.6	2.1	6.9	1.1
Mp 2	1.2	2.6	1.3	2.6	2.2	2.5	1.4	2.7
Mp 33	5.9	1.5	6.9	1.0	6.7	1.1	6.9	0.8
Mp 34	6.3	1.5	6.3	1.2	7.4	0.6	8.5	0.2
$LSD_{0.5}$	DSR = 1.75				Dry wt = 0.69			

ANOVA revealed a significant linear effect of sclerotial inoculum density

a) Disease severity ratings (DSR) were recorded three weeks after inoculation using a scale of one (no visible symptoms) to nine (all stem tissues and the growing point are affected – dead plant)

b) Plants were dried at 95°C for 48 h

c) About 150 ml of pasteurized soil infested with sclerotia of *M. phaseolina* (0.5-4.0 g dry sclerotia/kg soil) were placed on top of bean seeds.

d) Each number is an average of five replicates. The data for noninoculated checks were not included in the ANOVA

covered the entire stem. Expansion of these lesions was rather slow and rarely proceeded above the soil surface under greenhouse conditions. However, such expansion may occur under field conditions and may well be increased by water stress (Fig. 1 A, B). Infected stem and petiole tissues became bleached in advance of fungal growth and such areas eventually became covered with sclerotia and/or pycnidia under field or greenhouse conditions (Fig. 1 C).

DISCUSSION

Sclerotia of *M. phaseolina* were the most efficient and consistent form of inoculum for inciting charcoal rot of beans. Sclerotia were produced most abundantly on the simple synthetic liquid medium described in this study. These sclerotia can be produced in advance and stored dry until use without problems of contamination or loss of infectivity. Colonized whole rice seeds also were an effective form of inoculum. After 15 days incubation at 30°C, rice seeds became completely covered by mycelial growth of *M. phaseolina* with abundant sclerotia formed on the seed surface. It is possible to produce large amounts of this inoculum in a short time, and with a minimum cost. Pastor-Corrales and Abawi (16) used colonized whole rice seeds (4 g/2 m row) for evaluating bean germplasm in replicated trials under field conditions. Mycelial agar disks of *M. phaseolina* are also an effective source of inoculum, but are cumbersome and require considerable time to apply. Disease incidence was generally low and variable when naturally infected bean tissues were chopped into small pieces (1 cm long) or ground and used to inoculate bean seeds (unpublished data).

Inoculation of bean seeds in the greenhouse with pasteurized soil infested with 2 g sclerotia/kg soil accurately differentiated susceptible and resistant bean breeding lines and cultivars such as BAT 477, San Cristobal 83, A 70 and A 464 (3, 16). This method facilitated the screening of large numbers of bean accessions under controlled conditions (16). In addition, this inoculation procedure successfully documented the variability in virulence among *M. phaseolina* isolates to beans. It was interesting to find that although *M. phaseolina* isolates varied greatly in their virulence on the susceptible breeding bean line A 70, only minor differences occurred on the resistant line BAT 477 (Table 5). Thus, there is no basis from the results obtained in this study to suggest that physiological races exist among the population of *M. phaseolina* attacking beans. However, the evaluation of a larger group of isolates on a wider range of bean germplasm may reveal different results and is warranted. Khare *et al.* (12) reported that a soil isolate of *M. phaseolina* was more pathogenic to Urd bean (*Vigna mungo* (L.) Hepper), than isolates obtained from plant parts such as seeds, roots and foliage. Variability exists among *M. phaseolina* iso-

Table 4. Influence of inocula sources of *Macrophomina phaseolina* (isolate No. 34) on severity of charcoal rot development in the CIAT breeding lines BAT 477 (resistant) and A 464 (susceptible).

	Disease severity ratings (1-9)[a]			
	2 weeks		4 weeks	
Inoculum source	BAT 477	A 464	BAT 477	A 464
None (Check)	1.0[e]	1.0	1.0	1.0
Sclerotia/SSEB[b]	2.4	6.5	2.4	7.5
Sclerotia/SLM[c]	2.5	9.0	2.6	9.0
Colonized seeds[d]	1.0	4.9	1.0	5.8
$LSD_{0.5}$	1.85		1.93	

ANOVA revealed highly significant effect of inocula, cultivar and inocula x cultivar interactions

a) Disease severity ratings two and four weeks after inoculation were recorded using a scale of one (no visible symptoms) to nine (all stem tissues and growing tip affected – dead plant)

b, c) Refers to the soybean-seed extract broth and the synthetic liquid medium, respectively, used for sclerotial production. Bean seeds were covered with the sclerotia infested soil inoculum (2 g/kg soil).

d) Three whole rice seeds colonized by *M. phaseolina* were placed in contact with each bean seed

e) Each number is an average of four replicates. Data of non-inoculated checks were not included in the ANOVA.

Table 5. Virulence of ten isolates of *Macrophomina phaseolina* to susceptible (A 70) and resistant (BAT 477) bean germplasm, evaluated at one, two and three weeks after planting.

	Disease severity rating (1-9)[a]					
	BAT 477			A 70		
Isolate	1 wk	2	3	1 wk	2	3
Mp 34[b]	1.0[e]	1.2	1.2	3.9	8.0	8.4
Mp 37[b]	1.1	1.3	1.6	2.8	4.6	4.6
Mp 38[b]	1.3	1.4	1.5	4.0	7.7	8.1
Mp 39[b]	1.3	2.2	2.0	4.3	8.2	8.7
Mp 40[b]	1.0	1.4	1.2	2.3	7.3	8.3
Mp 101[c]	1.0	1.2	1.2	2.7	5.2	6.3
Mp 102[c]	1.0	1.1	1.2	2.0	3.3	3.9
Mp 103[c]	1.0	1.1	1.0	1.2	1.4	1.6
Mp 35[d]	1.2	1.0	1.6	5.2	9.0	9.0
Mp 36[d]	1.2	1.5	1.5	4.1	8.0	8.2
None	1.0	1.0	1.0	1.0	1.0	1.0
$LSD_{0.5}$	1 = 0.81; 2 = 1.13; 3 = 1.13					

ANOVA revealed significantly different effects due to isolates, cultivar, and isolate x cultivar interactions.

a) Disease severity ratings were recorded one, two and three weeks after planting using a scale of one (no visible symptoms) to nine (all stem tissues and the growing point are affected – dead plant).

b, c, d) These isolates were obtained from infected bean tissues collected from Colombia, Peru and Brazil, respectively. Sclerotia of all isolates were produced on the synthetic liquid medium and mixed with pasteurized soil at a rate of 2 g/kg soil. Inoculations were made by placing 150 ml infested soil on bean seeds.

e) Each number is an average of four replicates. Data of the noninoculated checks were not included in the ANOVA

virulence (5, 6, 7, 11). Tompkins and Gardner (18) reported that strains (= isolates) of *M. phaseolina* obtained from sugar beet, cowpea, sweet potato, begonia, strawberry and cotton attacked beans at temperatures ranging from 20 to 40°C. They also reported that a strain from citrus and two others were not pathogenic to beans.

The high affinity of *M. phaseolina* to cotyledonary tissue under both greenhouse and field conditions as observed in this study is of significance and is well documented in the literature (5, 7). Lesions on cotyledonary tissue of susceptible bean germplasm enlarge rapidly and infect stem tissue in a few days, which eventually results in plant death. In contrast, cotyledonary tissue of resistant germplasm either escapes infection or established lesions expand slowly. Often, slightly infected cotyledons of resistant germplasm fall off and this response may be an expression of the resistant mechanism.

Lesion incidence on lower stem tissue was proportional to the disease severity observed on the foliage. However, these lesions remained rather restricted as compared to the rapidly expanding lesions on the cotyledons and upper stem tissue. The maturity and physiology of different plant parts may be responsible for the observed differences in lesion expansion.

Nevertheless, restriction in the expansion of below-ground lesions from upward movement may be influenced by high soil moisture and relative humidity conditions which prevailed during the greenhouse tests. Incidence of *M. phaseolina* on older bean plants under field conditions has been considered to be most prevalent and damaging under high temperatures and drought stress (3, 7, 10, 14, 18).

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Notas y Comentarios

Alga que se esconde de noche para sobrevivir

Las plantas han desarrollado toda clase de defensas contra los animales que se alimentan de ellas, sustancias químicas de sabor desagradable, espinas, refugios para albergar a agresivas hormigas guardianas, y muchas formas más Pero, una de las tácticas más brillantes ha sido descubierta recientemente en una alga que usa la oscuridad de la noche como una defensa contra los peces

Una alga tropical, *Halimeda*, crece en lujuriantes y suculentas alfombras sobre los arreciles de coral, donde viven muchos peces herbívoros Mark Hay, de la Universidad de North Carolina, y un equipo de biólogos británicos y norteamericanos notaron que la *Halimeda* era dejada sola mientras otras plantas marinas eran devoradas

Previamente, los científicos pensaban que, debido a que *Halimeda* está fuertemente encostrada con carbonato de calcio y sustancias químicas defensivas tenía una armadura propia contra los peces

Hay y sus colegas encontraron que, al revés de otras plantas terrestres o marinas, *Halimeda* no crecía

más rápido durante el día. Midieron los números y longitudes de algunas frondas seleccionadas (el equivalente algal de una hoja), cada cuatro horas durante 56 horas. Encontraron que los nuevos segmentos de fronda sólo aparecían durante la noche, cuando los peces estaban menos activos

Pero, al siguiente día, los nuevos segmentos se habían expandido, endurecido con el calcio, cambiado de color y comenzado a llenarse de sustancias químicas que disuaden a los peces a que las comiesen.

Los investigadores concluyeron que la etapa más vulnerable de desarrollo ocurre bajo la cubierta de la noche (**Oecologia**, Vol. 75, p. 233).

Quizás, el aspecto más notable de este proceso es el repentino cambio de color de las frondas jóvenes, de transparentes a verde oscuro, justamente antes de la salida del sol. Sólo cuando aparece la aurora, la fronda necesita comenzar a fotosintetizar y a proveer a la planta con nuevos azúcares. Los investigadores construyeron un modelo de arrecife de coral en su laboratorio, para estudiar los efectos sobre *Halimeda* de los cambios de luz o de sustancias en el agua de mar. Encontraron que el cambio repentino de color es provocado por la salida del sol

Ahora que el crecimiento nocturno ha sido descubierto en *Halimeda*, la búsqueda está ahora dirigida a casos de otras variedades de algas que se comportan de esta manera. **A.G.**