Seed Transmission and Effect of Fungicide Seed Treatments Against *Macrophomina phaseolina* in Dry Edible Beans[1]

G S Abawi*, M.A Pastor-Corrales**

ABSTRACI

Macrophomina phaseolina was shown to be seedborne in beans (*Phaseolus vulgaris*) both internally and on seed-coat surfaces. The pathogen was isolated readily on acidified potato-dextrose agar from nontreated and surface disinfested whole seeds, seed coat pieces or cotyledonous tissues. Seeds obtained from severely infected pods exhibited typical symptoms of *M phaseolina* infections including discoloration and the presence of pycnidia and sclerotia. Severely infected seeds with *M phaseolina* generally failed to germinate or emerge. Bean seeds obtained from asymptomatic pods on infected plants revealed as high as 28% infection or contamination with *M. phaseolina*. Surface disinfestation of bean seeds for 2 min in 0.6% NaOCl, or their treatment with an effective fungicide, reduced the incidence of charcoal rot to about 3 to 5%. Seeds of six of 62 bean accessions grown in sterilized sand exhibited typical symptoms of charcoal rot on emerged seedlings that ranged from 5 to 30%. Six fungicides were evaluated as a slurry-seed treatment at 2.5 g formulation/kg seeds for their effectiveness in protecting seeds and seedlings from infection by *M phaseolina* in artificially-infested soil under greenhouse conditions. Benomyl (Benlate 50 wp) was the most effective, followed by carboxin (Vitavax 75 wp).

COMPENDIO

Se demostró que *Macrophomina phaseolina* está presente en las semillas de frijol (*Phaseolus vulgaris*), tanto en el interior como en la superficie de la semilla (testa). El patógeno fue aislado fácilmente en agar-papa-dextrosa acidificada (APDA) a partir de semillas enteras desinfectadas y no tratadas, de pedazos de cubierta de la semilla (testa) o de tejidos cotiledonares. Las semillas fueron obtenidas de vainas severamente infectadas que exhibían los síntomas típicos de la infección con *M. phaseolina*, entre ellos, descoloración y presencia de picnidios y esclerocios. En general, semillas severamente infectadas con *M phaseolina* no germinaron o no emergieron. Semillas de frijol obtenidas de vainas asintomáticas en plantas infectadas mostraron hasta 28% de infección con *M. phaseolina*. La desinfectación superficial de semillas de frijol con 0.6% NaOCl por 2 min así como distintos tratamientos con fungicidas efectivos, redujeron la incidencia de la pudrición carbonosa (charcoal rot) hasta aproximadamente 3 a 5%. Semillas de seis líneas de frijol, de un total de 62, fueron cultivadas en arena esterilizada y exhibieron los síntomas típicos de 12 pudrición carbonosa en las plántulas que emergieron, en un rango del 5 al 30%. Seis fungicidas fueron evaluados como tratamiento líquido (slurry), a una dosis de 2.5 g de fungicidas/kg de semillas, como protectores de semillas y plántulas contra la infección por *M phaseolina* en suelo artificialmente infectado, bajo condiciones de invernadero. Benomyl (Benlate 50 wp) fue el más efectivo seguido por Carboxin (Vitavax 75 wp).

INTRODUCIION

Macrophomina phaseolina (Tassi) Goid is an important pathogen of beans (*Phaseolus vulgaris* L.) causing charcoal rot (ashy stem blight) in the warmer, dry bean-growing areas of Latin America and other regions (1, 6, 11, 12). The pathogen survives in soil mainly in the form of sclerotia, the primary source of inoculum (5, 10). Sclerotia added to pasteurized soil was reported recently as an effective inoculum for inciting charcoal rot of beans under greenhouse conditions (1). Scerotial inoculum also was used to assess differences among bean germ plasm to infection by *M phaseolina* (11) and to determine the variability in virulence of geographical isolates of the pathogen (1). During these studies, considerable seedborne infections of *M phaseolina* were observed in seed sources of the bean accessions evaluated. Seed transmission of *M phaseolina* has been reported in beans (8, 14, 15) and many other crop plants (6, 9). However, detailed information on dry edible bean seed infection by *M phaseolina* and its importance as an inoculum source are limited.

The objectives of this study were to i) document the natural occurrence of seedborne infection of *M. phaseolina* in dry edible beans; and ii) to determine the efficacy of selected fungicide seed treatments against *M phaseolina* infections of bean seeds and seedlings. A brief summary of this investigation was published previously (2)

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* Professor, Department of Plant Pathology, N.Y.S. Agricultural Exp Station, Cornell University, Geneva, NY 14456, USA

** Plant Pathologist, Bean Program, CIAT, Apartado Aereo 6713, Cali, Colombia

MATERIALS AND METHODS

Bean seeds used in this investigation were produced near Palmira and Santander de Quilichao, Colombia. Dried pods of several bean accessions with and without typical symptoms of *M. phaseolina* infections were collected from research plots. In addition, seeds of many bean accessions from different sources available at CIAT were randomly selected and examined for infection or surface contamination by *M. phaseolina*.

The seedborne nature of *M. phaseolina* infection of beans was confirmed by isolations of the pathogen on acidified potato-dextrose agar (APDA) and by planting seeds in sterilized sand or pasteurized soil (treated with aerated steam for 30 min at 60°C), and determining charcoal rot symptom expression

Whole and decoated seeds were plated on APDA as nontreated or after surface disinfestation for 2 min in 0.6% NaOCl. Four seeds were placed on each culture plate and usually with five replications per treatment. For the grow-out test, nontreated or fungicide-treated bean seeds were planted in 10 cm pots or flats filled with sterilized sand or pasteurized soil. Carboxin (Vitavax 75 wp) or benomyl (Benlate 50 wp) were applied at a rate of 2.5 g/kg seeds as a slurry seed treatment with methocel (1.5% solution of methylcellulose using 95 ml/kg seeds). The test was maintained in a greenhouse with a fluctuating temperature of 20-33°C and relative humidity of 35-80%. Plants were watered and fertilized as needed. Incidence of charcoal rot was recorded at 1 or 2 wk after planting, and disease severity was recorded using the 1-9 scale reported previously (1)

The efficacy of Captan 50 wp; mancozeb (Dithane M-45, 80 wp), quintozene (PCNB, Terraclor 75 wp), benomyl (Benlate 50 wp), carboxin (Vitavax 75 wp), and thirram (Arasan 50 wp) in protecting seeds and seedlings against *M. phaseolina* was evaluated under artificial inoculation conditions in the greenhouse. All fungicides were applied at a rate of 2.5 g formulation/kg seeds as a slurry treatment. Four seeds of line A 464, susceptible to *M. phaseolina* (11), were placed in 10 cm diameter pots approximately three-fourths filled with pasteurized soil. The seeds were then covered with 150 ml of pasteurized soil infested with dry sclerotia of *M. phaseolina* isolate No. 34 (2 g sclerotia/kg soil) (1). Each fungicide treatment was replicated five times arranged in a randomized block design. In another test benomyl and quintozene were evaluated each at 1.5, 2.5 and 3.5 g formulations/kg soil on both A 464 and BAT 477, a bean line resistant to *M. phaseolina* (11). Both tests were evaluated after 2 wk of incubation as reported above and previously (1, 11).

RESULTS

Seedborne nature of *M. phaseolina*: Seeds obtained from severely infected pods (Fig. 1A) were discolored, deformed and showing extensive hyphal growth and reproductive structures of the fungus (Fig. 1B). Numerous sclerotia and pycnidia were visible on the seed coat with sclerotia also buried deep in the cotyledoneous tissues (Fig. 1D). *M. phaseolina* was always isolated from such seeds on APDA, regardless of the NaOCl surface disinfestation treatment, and from seeds with and without the seed-coat (Fig 1C and Table 1). Isolation frequency (70%) of *M. phaseolina* from symptomless seeds obtained from symptomless pods on infected plants was surprisingly high (Table 1). The pathogen was detected in surface sterilized, decoated symptomless seeds, suggesting deep infection sites of *M. phaseolina* in bean seeds. Similar results were obtained when isolation of *M. phaseolina* was attempted from a variety of seed sources available for evaluation at CIAT, although the recovery from symptomless seeds varied considerably from none to about 50%.

Table 1. *Macrophomina phaseolina* detected in bean seeds obtained from pods without symptoms and those with severe symptoms of charcoal rot.

		No. with *M. phaseolina*/no. tested[a]	
Seed source[b]	Seed coat[c]	Nontreated	NaOCl-treated[d]
Pods with symptoms	+	19/20	19/20
	−	19/20	19/20
Symptomless pods	+	14/20	14/20
	−	14/20	5/18

a) Refers to number of colonies of *M. phaseolina* identified per number of seeds placed on acidified potato-dextrose agar (APDA) plates (four seeds/plate)

b) *M. phaseolina*-infected and symptomless pods were collected from naturally infected plants in research evaluation nurseries near Santander de Quilichao, Colombia.

c) The shelled seeds were used whole or with seed coats removed under asceptic conditions

d) Seeds were soaked for 2 min in 0.6% NaOCl solution, rinsed in sterile distilled water and aseptically plated on APDA plates.

Seeds showing visible symptoms of infections by *M. phaseolina* obtained from severely infected pods generally failed to emerge when planted in pasteurized soil (Table 2). Only one seedling out of 296 symptomatic seeds of 15 different bean accessions germinated (0.3%) and it exhibited typical symptoms

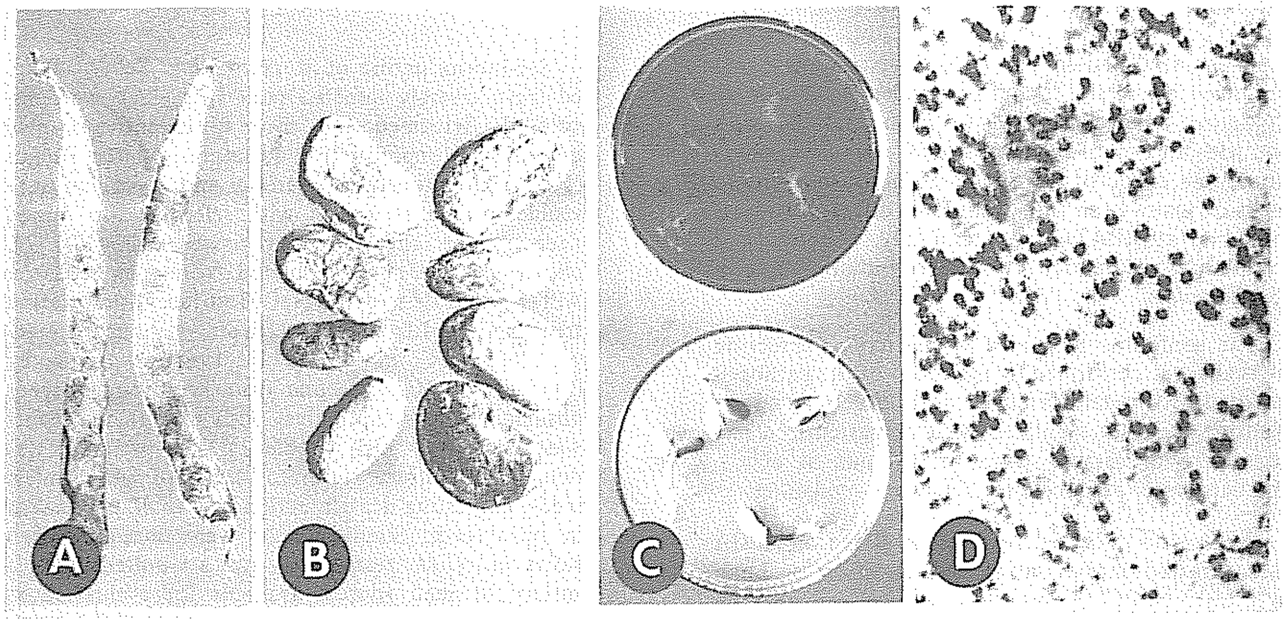

Fig 1 (A-D) Symptomatology of *Macrophomina phaseolina* (Mp) infection of bean pods and seeds A) Severely infected pods with typical Mp symptoms, B) Discolored and deformed infected seeds, C) Positive isolation of Mp on acidified potato-dextrose agar from infected whole seeds (above plate) and decoated seeds that also were surface disinfected with 0 6% NaOCl (bottom plate) and D) Close-up of an infected seed showing numerous sclerotia and pycnidia

Table 2. Emergence of selected bean lines originating from seeds obtained from pods, severely infected with *Macrophomina phaseolina*. pods collected from research evaluation nurseries near Santander de Quilichao, Colombia.

Line[2]	No. seedling emerged[1] /no. seeds planted	
	Infected pods	Clean checks[3]
RIZ 13	0/80	20/20
RAB 143	0/18	19/20
NAG 44	1[4]/13	20/20
EMP 133	0/11	15/20
Others[5]	0/174	–

1) Shelled seeds of each line were planted in pasteurized soil in trays and observed for emergence and charcoal rot symptom development

2) All the bean lines were included in disease and adaptation nurseries where high incidence of charcoal rot occurred

3) Seeds of these lines were increased near Palmira, Colombia under strict clean seed production recommendations (5)

4) This seedlings exhibited typical symptoms of charcoal rot

5) Included the bean lines VABRA 337, VABRA 449, VABRA 125, VABRA 120, VABRA 188, VABRA 270; RIZ 13; RAB 1433; EMP 133; NAG 44 and the cultivar Rio Tibagi.

of charcoal rot The majority of the nonemerged seeds decayed prior to germination A small proportion of them, however, were found with a short radicle that was infected with *M phaseolina*. In contrast, seeds of four of the same bean accessions increased at different locations under a strict clean seed production program (5) had a 94% germination and showed no sign of charcoal rot incidence. The soils with the decayed seeds from infection by *M phaseolina* were re-mixed and replanted with 60 seeds of A 464. Ten of the emerged seedlings were infected with *M phaseolina*, indicating that infected seeds in soil can function as a source of primary inoculum of *M. phaseolina* to bean seedlings

Clean seeds obtained from asymptomatic pods had germination in excess of 80% (Table 3) However, up to 28% of these seeds were found infected with *M. phaseolina* (Table 3). Treatment of such seeds with an effective fungicide such as carboxin, resulted in considerable reduction in the amount of infection by *M phaseolina* (Table 3). In another test, charcoal-rot incidence of two-week-old seedlings from A 464 seed samples that were nontreated, NaOCl, benomyl or carboxin-treated was 58.0, 0.0, 5 5, and 0.0, respectively The disease severity rating of the emerged seedlings in the nontreated seed treatment was 4.3 at 2 wk after planting. In addition, the level of seedborne *M. phaseolina* was high in a

Table 3. Incidence of charcoal rot in seedlings originating from non-treated and carboxin-treated seeds obtained from symptomless or severely infected pods by *Macrophomina phaseolina*

Seed Source[a]	Seed treatment[b]	No. seeds tested	No. plants	
			Emerged	Infected seedlings
Pods with symptoms	None	40	0	–
	Carboxin	40	0	–
Symptomless pods	None	40	32	9
	Carboxin	40	34	1

a) Pods were collected from research evaluation nurseries being conducted near Santander de Quilichao, Colombia Severely infected pods and symptomless pods were collected from the same or nearby plants

b) Shelled seeds were left nontreated or treated with 2 5 g carboxin 75 wp/kg seeds as a slurry treatment with Methocel.

sample of symptomless pods collected from an angular leaf spot nursery located near Santander de Quilichao, Colombia, that exhibited severe incidence of charcoal rot. Nontreated seeds of this sample plated on APDA revealed 19% recovery of *M. phaseolina*. Also, germination of seeds of this sample in pasteurized soil was 83%, with 7% of the emerged seedlings exhibiting typical charcoal rot symptoms.

A total of 62 bean accessions, that were recently increased in areas where charcoal rot was present, were randomly selected and planted in sterilized sand. Two weeks after planting, six of these accessions (A 295, BAT 477, XAN 112, BBL 274, MCD 254, Rosinha G2) had seedlings exhibiting typical symptoms of charcoal rot ranging from 5 to 30%.

Efficacy of fungicide seed treatments: All fungicides, evaluated as slurry seed treatments, significantly reduced the incidence and severity of charcoal rot on seedlings of A 464 (Table 4). Benomyl seed treatments appeared the most effective, followed by those treated with carboxin. In another test, benomyl and quintozene were evaluated each at 1.5, 2 5 and 3.5 g formulation/kg seeds on both A 464 and BAT 477. The nontreated seeds (received only

Table 4. Efficacy of selected fungicides as slurry seed treatments against *Macrophomina phaseolina* (Mp) infection of beans.

Treatment[b]	%Inf[c]	DSR (1–9)[a] incubation time	
		10 days	17 days
Nontreated	100 0[d]	7 6	8.2
Methocel alone	100 0	8.1	7 8
Captan 50 wp	75.0	3 6	4 6
Dithane M 45, 80 wp	37.5	2 0	3 3
Terraclor 75 wp	87.5	3.6	4.9
Benlate 50 wp	12 5	1.0	1.2
Vitavax 75 wp	25.0	1.3	2.0
Arasan 50 wp	81.3	2.1	3.4
LSD.05		1.76	2.56

a) Disease severity ratings were recorded at 10 and 17 days after planting (inoculation) using the CIAT scale (1) of 1 (no visible symptoms) to 9 (all stem tissues and growing point are affected-dead plants).

b) Seeds of the bean line A 464 were treated with 2 5 g formulation of the fungicides using the sticker methocel and dried. Four seeds were planted in 10 cm pots and covered with 150 ml of pasteurized soil infested with 28 dry sclerotia of Mp isolate No. 34/kg soil. All non-inoculated controls regardless of the fungicide treatment remained free of Mp infection.

c) Seedlings with any detectable symptoms of charcoal rot were considered infected with Mp

d) Each number is an average of four replicates with four seedlings/replicate.

the sticker methocel) of A 464 and BAT 477 had a disease severity rating of 7.8 and 1.3, respectively, at 2 wk after inoculation Benomyl, even at the lowest rate of 1.5 g/kg seeds, gave 100% protection against *M. phaseolina* as the disease severity rating was 1.0. In contrast, quintozene was less effective as the disease severity ratings for the 1.5, 2.5, and 3.5 g formulation/kg seeds of the susceptible A 464, were 4.2, 4.2, and 2.6, respectively. No disease symptoms developed on seedlings of the resistant line BAT 477 treated with even the lowest rates of quintozene (1.5 g formulation/kg seed)

DISCUSSION

Results of this study demonstrated the seedborne nature of *M. phaseolina* in field-grown bean seeds. The high incidence of seedborne *M. phaseolina* found was probably due to the collection of bean seeds from a production area where charcoal rot had occurred in epiphytotic proportions. Seed transmission of *M. phaseolina* may have a significant impact on the dissemination and introduction of this pathogen into distant bean-producing areas. The use of infested or infected seeds may also have an important influence on the incidence and epidemiology of charcoal rot and its management. These findings confirmed earlier reports on the seed transmission of *M. phaseolina* in beans (8, 14, 15) and other crops (6, 9).

Seeds obtained from severely infected pods exhibited typical symptoms of infections by *M. phaseolina* and were often with numerous sclerotia and pycnidia These seeds generally failed to germinate or emerge and those that emerged were often infected and died shortly thereafter. This finding agrees with that of Basu Chandhary (3) who reported that seeds of Sannhemp (*Crotalaria juncea* L.) with either pycnidia or sclerotia of *M. phaseolina* failed to germinate

The high incidence of *M. phaseolina* detected in asymptomatic bean seeds obtained from apparently normal-looking pods was not expected Kunwar *et al* (9) found that *M. phaseolina* was capable of infecting soybean seed tissues without producing any symptoms. They reported that examining thin sections of such seeds revealed sparse hyphae of the pathogen in the hourglass cell layer of the seedcoats

Benomyl and carboxin were the most effective fungicides evaluated as slurry seed treatments in protecting bean seedlings against *M. phaseolina* In addition, treating infected or infested bean seeds with these fungicides resulted in reduced charcoal rot incidence in the emerged seedlings Ellis *et al.* (8) reported that benomyl, carboxin and thiram improved emergence and stand count of low quality bean seeds (with 88% internally-seedborne fungi of which 6% were *M. phaseolina*), but not those of high quality seeds of the bean cv. Tui. Many other reports are available in the literature documenting the effectiveness of seed treatments on other crops (6, 7, 13)

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