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Full Length Research Paper

Effect of rotation of cowpea (*Vigna unguiculata*) with fonio (*Digitaria exilis*) and millet (*Pennisetum glaucum)* on *Macrophomina phaseolina* densities and cowpea yield

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***Macrophomina phaseolina*, the causal agent of charcoal rot, causes great damage to cowpea in the Sahel. One of the few options to manage the disease is by cropping nonhosts that may reduce the soil inoculum below a damage threshold level. To test this, fonio (*Digitaria exilis*) and millet (*Pennisetum glaucum*) were cropped continuously for 3 years in plots with a natural infestation of 24 - 53 microsclerotia g^{-1} soil at the onset of the experiment. Next, a susceptible cowpea variety was grown to quantify disease incidence and severity on these soils. Fonio and millet both reduced microsclerotial densities in soils from the first year onwards. Reductions under fonio (81% after the 2nd year; 86% after the 3rd year) were significantly stronger than under millet (56 and 66% for the 2nd and 3rd year respectively). Fonio was not infected by *M. phaseolina*, while the root systems of millet had low densities of microsclerotia. Cowpea yielded significantly more hay and pods after 3 years of fonio than of millet. Cowpea yields and disease incidence (dead plants) could be explained well by pre-planting microsclerotial densities. We conclude that rotation of cowpea with a gramineous crop may lead to a relatively fast decline of inoculum density. In the case of a high inoculum density, fonio can be grown for three years to reduce *M. phaseolina* densities in soil.**

Key words: Charcoal rot, fonio, millet cowpea, crop rotation, Sahel.

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid is a soilborne fungus causing charcoal rot and ashy stem blight on a wide range of plants in the world (Dhingra and Sinclair, 1978; Adam, 1986, 1995). In the Sahel, smallholder farmers grow cowpea and millet during the rainy season (June – September). Cowpea is host to *M. phaseolina*, but millet not. In the absence of hosts, the fungus survives in soil and host tissues in the form of microsclerotia, which are able to survive for 2–15 years in soil depending on environmental conditions, and whether or not the sclerotia are associated with host residue (Cook et al., 1973; Short et al., 1980). Many factors have been reported to adversely affect the persistence of these propagules, for example soil moisture content (Dinghra and Sinclair, 1975), soil structure and depth (Short et al., 1980; Sharma et al., 1995; Young and Alcorn, 1984). Factors that adversely affect the survival of these propagules include also repeated freezing and thawing of soil, low carbon:nitrogen ratios in soil, high soil moisture content Dhingra and Sinclair, 1975), high soil temperatures (Dhin-

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gra and Chagas, 1981; Lodha et al., 2002), and or-ganic matter amendment (Israel et al., 2005). Several characteristics of the Sahelian zone particularly favour development of *M. phaseolina*, including low soil organic matter content, its associated limited soil microbial activi-ty (Ouattara and Persaud, 1986), and low soil moisture content during the long off-season. In a preliminary study, we observed that under field conditions millet (*Pennise-tum glaucum*) cv. HKP was not infected by *M. phaseo-lina*. However, the fungus was able to colonize millet roots saprophytically after physiological maturity. To avoid inoculum build-up, it may be better to lift millet plants at harvest time, a practice that is common in Sine Saloum (Senegal) (Diouf, 1990) and Maradi (Niger). Roots exposed to the sun dry quickly and limit coloni-zation by the pathogen considerably.

A significant reduction in nematode populations of *Meloidogyne javanica* and *M. incognita* race B was observed in pots cropped with fonio (*Digitaria exilis*) (Sarr and Prot, 1985). The authors suggested its use as rotation crop to manage nematode pests. The effects of fonio on other diseases are not well documented. In particular its effect on survival of inoculum of *M. phaseolina* is not known. Fonio is a popular crop in southern Senegal, Mali, Burkina Faso, and Niger. In these countries, it is cultivated in rotation with millet and cowpea. The crop is less important than millet in the Sahel with respect to cultivated areas and production. However, it has recently received more interest thanks to its flavour and nutritional qualities and to growing demands in developed countries and urban towns (Vodouhe et al., 2003). It may therefore be acceptable as rotation crop.

This study aimed to investigate the susceptibility of millet and fonio to *M. phaseolina* and the effects of three years monocropping of millet (lifted from soil immediately after harvest) and of a local variety of fonio on soil propagules of *M. phaseolina* and disease incidence on cowpea in plots amended with compost.

MATERIAL AND METHODS

Effect of *M. phaseolina* on millet and fonio (pot experiment)

Three isolates of *M. phaseolina* (IS50, IS60 and IS100) isolated from soil (IS60 and IS100) and cowpea tissues (IS50) were multiplied on millet cv. HKP grains. 50 g of grains were soaked during 24 h in demineralized water, the water excess was discarded, the soaked grains were placed in a 250 ml flask and sterilized at 121°C for 30 min. After cooling, the grains in the flask were inoculated by placing 6 5-mm diam discs cut out from a 3-d old fungal culture grown on PDA. The inoculated flasks were then incubated for 15 d at 30°C. After air-drying in a laminar air-flow cabinet, the colonized grain inoculum was used to infest sterile soil at a rate of 5% (dry weight) (Mayek-Pérez et al., 2001). Plastic pots of 500 ml, having 5 drainage holes and a cotton layer at the base were filled with the infested soil or with non/infested soil (control). Pots were thereafter sown with 10 seeds of fonio (local variety) or 9 seeds of millet cv. HKP and incubated in a screenhouse in a randomized complete block design with three replicates. Pots planted with three cowpea cv. Mouride seeds were used as susceptible check. Optimal soil moisture for fonio, millet and cowpea growth was afforded for 7 d after sowing. Then, the pots were irrigated after the seedlings underwent a moisture stress for one day. During the experimentation temperatures in the screenhouse were 30 ± 7°C. When plants were 45-days old, they were uprooted carefully; roots were washed with tap water and air-dried. Plant stand and d.w. biomass, and number of sclerotia g^{-1} root and lower stem tissues were recorded.

Experimental design and cultural practices in the field

The study was conducted on an experimental field at the AGRHYMET centre (Niamey, Niger) that was naturally infested with *M. phaseolina*. The soil was a sandy soil (87% sand, 8.2% silt, 5.1% clay). The field was divided in 3 strata according to disease severity observed on cowpea at flowering in 1997 and soil inoculum density was recorded at planting in 1998: very high density (all plants dead and 53 microsclerotia g^{-1}soil), high (about 50% dead plants and 40 microsclerotia g^{-1} soil), and moderate (about 10% dead plants and 24 microsclerotia g^{-1} soil). In 1998, each stratum of about 12 m × 7 m was divided into four blocks, measuring 7 m × 3 m each; each of these blocks was divided into two plots of 10.5 m².

Yearly before planting cereals, all blocks were amended with 6 tons of compost ha^{-1} and the top 15 cm was plowed with the aid of a bull. In 1998, 1999 and 2000, fonio and millet were planted randomly in 2 plots per block, resulting in 4 plots per crop per stratum. Millet was planted in 4 rows (1 m wide, with 10 hills per row and a within-row planting distance of 0.5 m) per plot. Fonio was broadcast in 3 × 3.5 m plots. In December 2001, the whole field was sown with cowpea cv. TVx 3236, known to be susceptible to *M. phaseolina*. Cowpea row-distance was 0.5 m and hill distance within rows was 0.25 m. Each hill was sown with 2 seeds. Weeding was done when needed and drip irrigation was performed weekly.

Soil sampling and analyses

In 1998 and 1999, soil samples were collected before planting to estimate the density of *M. phaseolina*. Four soil subsamples (0 – 30 cm) were taken with an auger (diam. 2.4 cm) from each stratum within a randomly chosen surface of 1 m2 according to a diamond pathway pattern (Mihail and Alcorn, 1987) and the subsamples were pooled. Three such composite samples were randomly taken in each stratum corresponding to a particular soil infestation level. In 2000 and 2001, one composite sample per plot was collected since plots were small. After thoroughly mixing the composite soil samples, a 5-g subsample per composite sample was analyzed by mixing 5 replicates of 1 g of soil and 100 ml of a Potato Dextrose Agar (PDA) medium amended with pentachloronitrobenzene (PCNB) (225 mg) and chloramphenicol (5 mg). One hundred ml of this medium was divided over 10 petri dishes and incubated for 8 – 10 days at 30°C after which the number of propagules of *M. phaseolina* was determined (Ndiaye et al., 2007).

Soil pH was measured yearly before planting using a pH-meter (Hi 8519N, Hanna Instrument, Inc., USA). Distilled water (25 ml) was added to 10 g soil of each subsample in a cup, stirred vigorously for 5 s and let stand for 10 min. The pH was read immediately (Soltner, 1990). The pH_{H20} was 6.8.

Total N and total P contents of soil samples (0–30 cm) were determined using the methods described by Novozamsky et al. (1983; 1984). Three hundred milligrams air-dried, finely ground soil was digested using a mixture of H_2SO_4, Se and salicylic acid, and H_2O_2. After digestion, N and P were measured using segmented-flow analysis spectrometry. Bioavailable nutrients were determined using the methods described in Houba et al. (2000). Air-dried, gro-und soil samples were extracted for 2 h in 0.01 M $CaCl_2$ using a 1:10 extraction ratio (w/v). In the extract NO_3-N, NH_4-N, total soluble N ($N_{tsoluble}$) and PO_4-P were determined using segmented-flow ana-

Table 1. Soil nutrient content of experimental plots.

Nutrient content (mg/kg soil)								
$Nt_{soluble}$	$N_{org.}$	$P\text{-}PO_4$	Na	K	Nt	Ptot	OM (%)	C/N
12.3	1.8	0.8	4.2	39.4	129.5	173.2	0.35	11.6

Table 2. Microsclerotial density in roots and stems of 90 fonio and 81 millet plants growing in artificially inoculated soil in a screen house. 8 plants of the susceptible check (cowpea) were analyzed for microsclerotia tissue content.

Microsclerotia density (cfu g^{-1} tissue)		
Species	**Root**	**Stem**
Fonio	3.0 ± 3.7[1]c[2]	1.0 ± 2.3c
Millet	42 ± 21b	15 ± 5.9b
Cowpea	965 ± 280a	787 ±149a

[1] Standard deviation.
[2] Means within a column followed by the same letter are not significantly different at α= 0.05.

lysis spectrometry; Na and K were determined using Flame Atomic Emission Spectrometry (Flame-AES).

Total N and P content of the experimental field were 130 and 173 mg/kg soil respectively, which are relatively low concentrations for these elements. Plots were similarly low in concentrations of organic and inorganic elements (Table 1). The average organic matter content was 0.35% far less than the optimal content of 3 – 3.5% for this type of soil. The C/N ratio was also low.

Estimation of millet and fonio colonization

Colonization of millet and fonio tissue by *M. phaseolina* was assessed by destructively sampling 5 plants 30 and 60 d after planting (at the seedling stage and at physiological maturity, respectively). In addition, two months after harvesting, roots and stem residues of fonio and roots of millet that had been lifted onto the soil surface were randomly sampled and assayed for the presence of microsclerotia. The sampled plant tissues were washed with tap water, cut into small pieces, surface-sterilized in 0.8% NaOCl for 1 min, blotted dry with paper towels, placed in a paper bag and dried in an oven at 37°C for 7 d. Dried tissues were ground in a mixer mill (Retsch, GmbH and Co. Type MM2) for 4 min at 600 rotations min^{-1} and sieved through 180 and 45 µm screens. Three subsamples of 150 mg from each block were mixed with 100 ml of PDA amended with 5 mg chloramphenicol and 225 mg PCNB, and plated as described above. *M. phaseolina* colonies were counted after 5 – 7 days incubation at 33°C.

Proportions of emerged and dead cowpea plants were recorded 10 d after planting and at harvest. Dry pod and hay yields were measured at harvest. Evaluation of emergence, disease incidence and yield were based on the two central rows of each plot.

Statistical analysis

Data were analyzed by means of the computer program Genstat® for Windows 8th Edition (IACR-Rothamsted, Harpenden, Hertfordshire, UK). The data were subjected to analysis of variance. Treatment means were separated by the DMRT test. Numbers of microsclerotia in plant tissues were analyzed after log transformation if necessary.

RESULTS

Pot experiment

Screening for resistance of fonio and millet: There were no significant differences in total and root biomass (d.w.) between inoculated and non-inoculated plants for fonio and millet. The plant stand was also not affected by the pathogen (data not shown). For cowpea the weight of roots and stem of inoculated plants was significantly ($P < 0.001$) lower (0.11 and 0.095 g /d.w. plant, respectively) than roots and stem of non-inoculated plant (0.28 and 0.16 g /d.w. plant, respectively). Species significantly ($P < 0.001$) affected the number of microsclerotia in tissues (Table 2). However, mean microsclerotia population was low in both fonio and millet tissues.

Field experiments

1998 – 2000 crops. Millet and fonio emergence, plant stand, and growth were not affected by microsclerotial densities of *M. phaseolina* in the three strata, confirming that these crops are nonhosts. The density of microsclerotia in soil declined during the three years these nonhosts were grown. In all years, the reduction was significantly greater for fonio than for millet ($P < 0.01$). Millet did not significantly reduce the density of microsclerotia in soil for stratum 3 with a high initial inoculum density (Figure 1).

There were significant linear relationships between the density of microsclerotia in soil and the number of years cropped with fonio or millet (Table 3). However, at very high soil inoculum density, there was no linear relation between microsclerotial density and the number of years of millet cultivation.

Tissue and residue colonization

No propagules of *M. phaseolina* were found in fonio tissues and residues. Living tissues of millet were also not infected. However, two months after harvesting, millet roots showed a slight colonization that increased with soil

Table 3. Regression equations for changes in soil microsclerotia over time (years) during cultivation of millet and fonio at 3 different levels of soil infestation. Soil inocula were recorded yearly before planting crops from 1998 to 2001.

Soil infestation level	Crop species	Regression equations	Constant($P > t$) [1]	Year($P > t$)	Regression($P > F$)
Moderate	Fonio	27.38-6.79x	< 0.001	< 0.001	< 0.001
	Millet	30.12-6.13x	< 0.001	< 0.001	< 0.001
High	Fonio	44.35-11.29x	< 0.001	< 0.001	< 0.001
	Millet	52.00-12.52x	< 0.001	< 0.001	< 0.001
Very high	Fonio	61.85-13.56x	< 0.001	< 0.001	< 0.001
	Millet	52.10-3.27x	< 0.001	0.28	0.28

[1]Probability of a larger t or F value than expected under the nul hypothesis.

Table 4. Mean squares from the ANOVA table for soil density of *Macrophomina phaseolina* (number / g dry soil) at planting of cowpea, germination (%), dead plants (%), and pod and hay yield of cowpea (kg / ha), in 2001 after 3 years of non-host cropping with fonio or millet.

Source of variation	DF	Mean squares				
		Soil microsclerotia	Germination	Dead plants	Grain yield	Hay yield
Block	3	9.90 ns	13.72 ns	18.30 ns	22308 ns	187082
Crop	1	624.24***	363.48***	593.02***	336382***	3195346***
Stratum	2	1167.66***	1324.10***	267.04*	206834*	1933889**
Crop x Stratum	2	315.72**	137.71**	315.36***	139160**	1102759*
Residual	15	30.68	18.71	23.02	20823	202236

Significance levels: ***, p < 0.001; **, P < 0.01; *, P < 0.05; ns = not significant at α = 5 %

inoculum density: 16 ± 13, 32 ± 12, and 40 ± 5 microsclerotia g^{-1} of dry root tissue in the moderately, highly and very highly infested soil, respectively.

Cowpea crop in 2001

Analysis of variance for soil sclerotia of *M. phaseolina* at the beginning of the season, plant survival, and yield of cowpea indicated that effects of previous crops and the interaction between Crop and Stratum were significant (Table 4). The overall population density of *M. phaseolina* at planting in 2001 was significantly lower in moderately and highly infested soils (Strata I and II) (5 microsclerotia^{-1} dry soil) than in very highly infested soil (26 microsclerotia g^{-1} dry soil). However, soil microsclerotia were three times more in plots cropped with millet than in plots with fonio at the stratum III (Table 5).

There was a clear effect of initial inoculum density on disease incidence and yield of cowpea (Table 5). The effect of millet and fonio on soil inoculum density (Figure 1) was clearly reflected in cowpea yields and pre-cowpea soil inoculum density. There was no effect of cropping history on emergence of cowpea, but a highly significant negative correlation between microsclerotial density and the cowpea plant stand (r = –0.90; Figure 2a) and a positive correlation between plant stand and grain yield (r = 0.68; Figure 2b).

DISCUSSION

The field used for the present study contained very low concentrations of mineral nutrients and organic matter (0.35%) with a C/N ratio equal to 12. It is well documented that environmental stresses including nutrition affect severity of *M. phaseolina* (Cloud and Rupe, 1994) by weakening the host plant or altering its natural resistance. Moreover, low microbial activity in soils low in organic matter may contribute to survival of microsclerotia in soil (Soltner, 1990). A C/N ratio of 30 – 50 is optimal for soil microorganism activities (Soltner, 1990). This suggests that there was low biological activity in the soils used in this study.

Rotations are among the oldest methods to manage soilborne plant pathogens. *M. phaseolina* is in this respect difficult to manage given its wide host range (Adam, 1986) and the high microsclerotial densities in soil that are often encountered after growing hosts such as bean (17 – 47 microsclerotia g^{-1} soil), cowpea (15 – 45), sorghum (13 – 37), and maize (11–45) (Adam, 1995; Songa and Hillocks, 1996).

Table 5. Viable sclerotia, plant emergence, percentage of dead plants at harvest, and dry pod and hay yield of cowpea in fields cropped three consecutive growing seasons with fonio or millet. The soil infestation levels in 1998 were 24, 40 and 53 microsclerotia per gram dry soil in moderately, highly and very highly infested soils respectively.

CropSoil infestation	Sclerotia/g soil before cowpea		Emergence (%)		Dead plant (%)		Grain yield (kg/ha)		Hay yield (kg/ha)	
	Millet	Fonio	Millet	Fonio	Millet	Fonio	Millet	Fonio	Millet	Fonio
Moderate	7b[1]c	3c	76b	85a	18c	5d	455bc	855a	1797bc	2643a
High	5bc	5bc	76b	7 9ab	19c	19c	357bc	527b	1473cd	278ab
Very high	39a	13b	64c	75b	45a	27b	270c	409bc	982c	519cd

[1]Means within two adjacent treatments (millet-fonio) followed by the same letter are not significantly different at α = 5%.

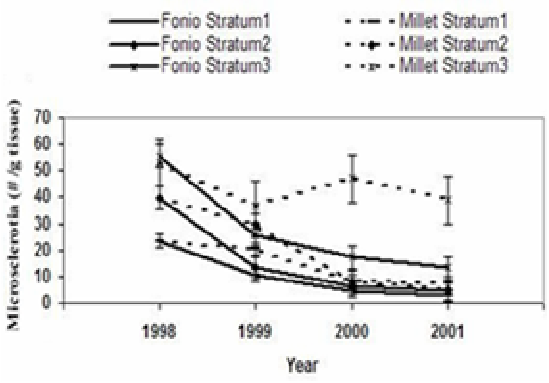

Figure 1. Evolution of microsclerotial soil inoculum density of *Macrophomina phaseolina* over 4 years in three plots with moderate (Stratum1), high (Stratum2), and very high (Stratum 3) inoculum density after cropping cowpea in 1997 (before planting cereals in 1998), and after cropping the nonhosts fonio and millet in 1998, 1999, and 2000. Vertical lines indicate the standard error (to be improved).

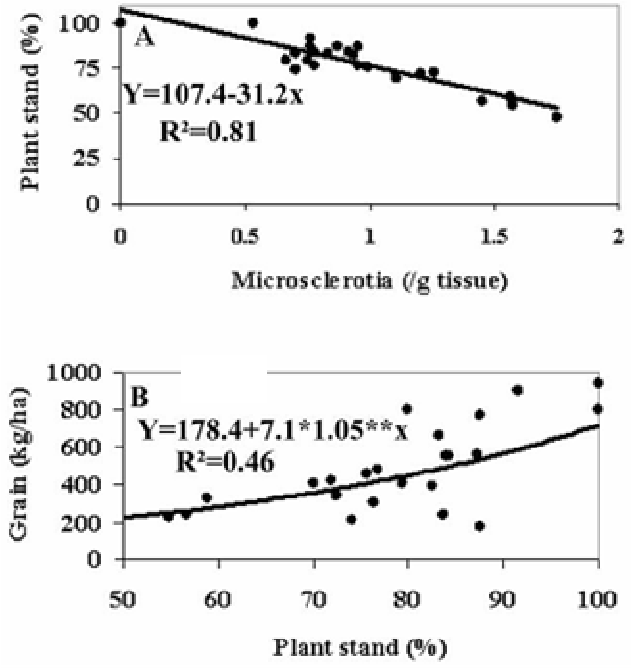

Figure 2. Relationship between plant stand (%) and soil microsclerotia (cfu g^{-1} soil) (A) and cowpea grain yield (kg ha^{-1}) and plant stand (%) (B). Actual data for the soil microsclerotia were transformed by log10 (x + 1) before plotting.

In the semi-arid areas of the Sahel, commonly grown hosts include cowpea, sorghum, maize, peanut and other legume crops. Alternate cropping of cowpea and millet is common practice in the Sahel. This type of rotation is favorable to rapid build up of *M. phaseolina*, except when millet plants are lifted, including their root systems, which is common practice in the peanut basin of Senegal (Diouf, 1990). Although sorghum and maize were less susceptible than leguminous hosts, these gramineous crops still resulted in increase in density of *M. phaseolina* (Songa and Hillocks, 1996). A considerable reduction of 75 – 95% in the counts of *M. phaseolina* was achieved by biofumigation of irrigated soil at 46 – 53°C for 15 days after amending with cruciferous residues (Lodha et al., 1997). This rapid decline in soil inoculum was attributed to the combined effect of sub-lethal soil temperatures and toxic cruciferous residues. However, cruciferous crops are not suited to the Sahelian region. In the present study, cropping of fonio and millet both resulted in lower inoculum densities, decreased numbers of dead cowpea plants, and increased pod and hay yields. The effect of fonio was clearer than that of millet, which was likely due to the partial colonization of dead organic matter of millet. Higher colonization of millet root systems with microsclerotia of *M. phaseolina* has been observed when the roots were left in soil. In the present study we achieved 87 – 94% reduction in soil populations of *M. phaseolina* after three years monoculture of fonio. Fonio clearly is a nonhost to *M. phaseolina* under field conditions, so, it would be advisable to include it in the rotation with cowpea to prevent build up of populations of *M. phaseolina*.

Survival of *M. phaseolina* in uncropped soil was inve-

stigated by Short et al. (1980) and Songa and Hillocks (1996). After burial of soybean residues with microsclerotia in a soil in Missouri, densities of *M. phaseolina* first increased for about half a year in fallow soil, and then decreased in the next half year (Short et al., 1980). In Eastern Kenya, densities of *M. phaseolina* also first increased and then decreased in fallow soil after addition of a mixture of infected residues of various crops to soil. The increase lasted only 3 months and the decrease 3–6 months (Songa and Hillocks, 1996). The persistence of the inoculum of *M. phaseolina* was attributed to the presence of weed hosts in the fallow plots. So, an additional aspect of cropping fonio is the suppression of weeds that can serve as host for *M. phaseolina* (Young and Alcorn, 1984; Songa and Hillocks, 1996).

Cowpea grains and hay yields were significantly affected by preceding crop and soil infestation level of *M. phaseolina*. Yield advantages due to the treatments were, however, modest in comparison with the 1.2 t/ha potential grain yield of the variety used (INRAN, 1986), but acceptable as far as yield under farm conditions (200 – 400 kg/ha) is concerned. Heavy yield losses owing to plant death at flowering compelled farmers to abandon growing of cowpea in the Louga, Bambey regions of Senegal and Gabougoura in Niger (Adam, 1995). Monocropping of cowpea or biannual rotation of cowpea and millet, and intercropping of cowpea and millet contributed to the damage caused by *M. phaseolina* in these areas. Our data suggest that acceptable control of charcoal rot of cowpea at high inoculum densities in adverse Sahelian environments, can be achieved partly through use of non-hosts as fonio and (to a lesser degree) millet. Farmers could plant fonio or millet continuously for three years to reduce soil inoculum of *M. phaseolina* to a level safe for cowpea production under conditions of moderate and high soil infestation (Figure 1). In case of very high soil infestation, millet rotation is not efficient for a rapid reduction of soil inoculum, but fonio can reduce inoculum to acceptable levels within four years of monocropping. However, monocropping with these grain crops may invoke other disease problems. Thus, more research is needed to find additional crops that are non hosts to *M. phaseolina*. In addition, alternative management strategies, such as increasing soil organic matter contents with compost, will need to be developed, as charcoal rot can only be controlled by an integrated management approach.

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