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Is the recently described *Macrophomina pseudophaseolina* pathogenically different from *Macrophomina phaseolina*?

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Charcoal rot disease causes heavy yield losses to many hosts including cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogea*) in Senegal. The causal agent of the disease was a longtime considered to be *Macrophomina phaseolina*. However, a new *Macrophomina* species, *Macrophomina pseudophaseolina* was reported recently to also cause charcoal rot disease alone or in association with *M. phaseolina* on several hosts in Senegal. Since 1969, charcoal rot has become increasingly important in cowpea and other crops in the Sahel. It was not known if this status is correlated with the occurrence of the new *Macrophomina* species. This study therefore aimed to investigate the pathogenicity of *M. phaseolina* and *M. pseudophaseolina* on three varieties of cowpea under two temperature regimes. Ten *M. pseudophaseolina* and nine *M. phaseolina* isolates were tested on three cowpea varieties in a complete randomized design. Plants were grown in infected soil at two growth temperatures (24/34 and 26/36°C) in a climatic chamber. Disease incidence, level of tissue infection and potential primary inoculum produced in cowpea plants were determined 45 days after planting. By and large, the two *Macrophomina* species showed the same results, except that at 36°C, *M. pseudophaseolina* induced more disease development than *M. phaseolina* in the susceptible cv. Mouride. In conclusion, *M. pseudophaseolina* induces less disease incidence on cowpea at 34°C than at 36°C. At this temperature, it becomes as damageable as *M. phaseolina* on cowpea.

Key words: *Macrophomina phaseolina*, *Macrophomina pseudophaseolina*, growth temperature, disease incidence, potential primary inoculum.

INTRODUCTION

Cowpea and groundnut production is a significant economic activity in Senegal. Both crops are increasingly damaged by charcoal rot caused by *Macrophomina phaseolina*, which is supposed to be due to increased conditions of drought (Koenning and Wrather, 2010). In the Sahelian zone of West Africa, charcoal rot is estimated to cause a yield loss of 10%, equaling 30,000 tons of cowpea worth approximately US$ 146 million only for Niger and Senegal (Ndiaye, 2007). *M. phaseolina* is a soil- and seed-borne polyphagous pathogen causing various rots and blights in more than 500 crop species (Sinclair and Backman, 1989; Dhingra and Sinclair, 1977).

Since about 1987, charcoal rot is in the Sahel recognized as the most important diseases of legumes, including cowpea and groundnut (Paré, 1990; Adam, 1995; Gaïkwad and Sokhy, 1987). Characteristic disease symptoms include presence of black sclerotia on the lower part of the stem and wilting and drying of the leaves and subsequently the whole plant at the flowering and fruiting stage. *M. phaseolina* can also infect roots which show necrotic lesions, leading to pre- or post-emergence seedling damping off or bad plant growth (Bouhot, 1967; Adam, 1986). The disease development was reported to be affected by host growth stage and environment (Csöndes et al., 2007). High root infection was associated with hot and dry weather occurring early in the growing season (Cloud and Rupe, 1994). Furthermore Manici et al. (1995) reported that in Italy, isolates from cold area grew better at low temperature and showed a better adaptability to 40°C. Whereas, isolates from the Mediterranean climate grew fast at high temperature (30, 35 and 40°C) but showed the poorest adaptability at low temperature. In the Sahel, the 1970-1990 period is characterized by frequent drought spells during the period of crop growth and the temperatures during the hottest months of the dry season are high (30-45°C) (Morrel, 1992). The results of a study on the influence of temperature and soil humidity on the germination of microsclerotia of *M. phaseolina* in a sandy soil showed that the greatest percent of germination occurred at 30 and 33°C at -80, -300 and -1,500 kPa water tensions (Viana and Souza, 2002).

Recently, based on 189 isolates which were morphologically identified as *M. phaseolina*, Sarr et al. (2014) identified two well-defined clades on the basis of a multi gene DNA analysis of five loci. This led to the description of a novel *Macrophomina* species, *Macrophomina pseudophaseolina* Crous, Sarr & Ndiaye (Sarr et al., 2014). Up to now *M. pseudophaseolina* species is known only from Senegal where it has been isolated from *Abelmoschus esculentus*, *Arachis hypogaea*, *Hibiscus sabdarifa* and *Vigna unguiculata* and some time in a joint infection with *M. phaseolina*. Although the morphology of *M. pseudophaseolina* appears to be largely similar to that of *M. phaseolina*, its ecological attributes need further study.

Therefore, this study aimed to investigate the pathogenicity of *M. pseudophaseolina* on three varieties of cowpea under two temperature regimes.

MATERIALS AND METHODS

Collection of *Macrophomina* spp. isolates

From September to November 2011, charcoal rot-affected stems or roots of cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogaea*), sorrel (*Hibiscus sabdarifa*), sorghum (*Sorghum bicolor*) and okra (*Abelmoschus esculentus*) were collected from the major production areas of Senegal. 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 bags and dried in an oven at 37°C for 7 days. 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. 50 mg from each sample were mixed with 100 ml of PDA amended with 5 mg chloramphenicol and 225 mg PCNB, and plated as described by Ndiaye et al. (2007). From each sample, 1-3 colonies were transferred separately after 5-7 days incubation at 33°C in a fresh PDA medium. Each colony was then considered as an isolate. Isolates of *Macrophomina* were made at the CILSS/AGRHYMET Phytopathology Laboratory in Niamey, Niger and they were genetically characterized at the CBS-KNAW Fungal Biodiversity Centre (Utrecht, The Netherlands). Details regarding the geographical and host origin and *Macrophomina* species of the isolates used in this study are listed in Table 1.

Pathogenicity tests on three cowpea varieties

Cowpea varieties

Cowpea cv. Apagbaala (resistant), cv. IT93k-503-1 (moderately resistant) and cv. Mouride (susceptible) were provided by the Senegalese Institute for Research (ISRA). The cowpea varieties showed different levels of resistance towards *Macrophomina* in the field (ISRA, 2010, data not published).

Bioassay preparation

Sterilized soil amended with organic fertilizer (N-P-K, 1.5-1-1) was used to fill sterilized 0.5-L pots. After moistening the soil, three 2-cm deep planting holes were made at the pot surface. A 5-mm diameter disc was cut out from the margin of a 3-day-old fungal culture growing on PDA and one disc was placed in each hole. The discs were slightly covered with soil and a surface-disinfested (2.5% NaOCl for 5 min) seed was added, which again was covered with soil.

Climatic chamber experiments

The bioassay was carried out in a “SANYO Versatile Environmental Test chamber” model MLR -351 in a complete randomized design block with two factors (cowpea varieties (3) and *Macrophomina* species (3 treatments: *M. phaseolina*, *M. pseudophaseolina* and a non-inoculated control) with three repetitions per treatment combination. The pots were placed on trays in climatic chambers and plants grown at 34 and 24°C (climatic chamber temperature 1) alternatively for 12 h and at 36 and 26°C (climatic chamber temperature 2). These growing temperatures correspond to the mean maximal and minimal temperature for the period 1950-1959 (humid period) and 2000-2009 (dry period) in the Sahel, respectively (Alhassane et al., 2013). The lightening was insured by 15 20-mv-lamps, arranged along the 3 doors of the climatic chamber. The RH levels recorded were 90 ± 10% and 70 ± 20% for the climatic chamber temperatures 1 and 2, respectively.

The plants were watered two times and the number of stem

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Table 1. Isolates of *Macrophomina phaseolina* (Mp) *and M. pseudophaseolina* (Mps) collected from different crops and locations in the main production areas of Senegal used in this study.

Isolate number	*Macrophomina* species	Host crop	Site	Geographical coordinates
Mp4	Mp	*Arachis hypogaea*	Kaolack	13° 45. 478 N; 15° 47. 085 W
Mp19	Mp	*Vigna unguiculata*	Thiès	15° 31. 547 N; 16° 18. 111 W
Mp41	Mp	*Vigna unguiculata*	Saint-Louis	16° 08. 395 N; 16° 19. 08 W
Mp47	Mp	*Vigna unguiculata*	Saint-Louis	16° 08. 395 N; 16° 19. 08 W
Mp48	Mp	*Arachis hypogaea*	Louga	15° 33. 638N ; 16° 16. 838 W
Mp70	Mp	*Vigna unguiculata*	Louga	15° 09. 754 N; 16° 15. 740 W
Mp73	Mp	*Vigna unguiculata*	Diourbel	14° 42. 634 N; 16° 28. 811 W
Mp110	Mp	*Arachis hypogaea*	Tambacounda	13° 31. 857 N; 13° 30. 020 W
Mp113	Mp	*Abelmochus esculentus*	Saint-Louis	15° 43. 173 N; 16° 28. 686 W
Mps18	Mps	*Arachis hypogaea*	Louga	15° 33. 638 N; 16° 16. 838 W
Mps22	Mps	*Arachis hypogaea*	Louga	15° 03. 754 N; 16° 15. 219 W
Mps23	Mps	*Arachis hypogaea*	Louga	15° 03. 754 N; 16° 15. 219 W
Mps42	Mps	*Vigna unguiculata*	Saint-Louis	16° 08. 395 N; 16° 19. 08 W
Mps84	Mps	*Vigna unguiculata*	Louga	15° 03. 754 N; 16° 15. 219 W
Mps127	Mps	*Arachis hypogaea*	Matam	14° 56. 902 N; 16° 38. 109 W
Mps174	Mps	*Hibiscus sabdarifa*	Matam	14° 56. 613 N; 16° 37. 796 W
Mps177	Mps	*Hibiscus sabdarifa*	Matam	14° 56. 613 N; 16° 37. 796 W
Mps191	Mps	*Hibiscus sabdarifa*	Matam	14° 56. 613 N; 16° 37. 796 W
Mps192	Mps	*Hibiscus sabdarifa*	Matam	14° 56. 613 N; 16° 37. 796 W

necrosis, withered and dead plants was recorded weekly for the incidence determination. For primary inoculum potential (total number of microsclerotia produced by plants of a pot) determination, the plants were uprooted 45 days after sowing. The root systems were washed with tap water, blotted dry with a paper towel and the roots and stems were air-dried in an oven at 37°C. Subsequently, the dried plants were weighed and milled, 150 mg of the powder was mixed with 100 ml of SS-medium and poured in 10 Petri dishes (Ndiaye et al., 2007). The plates were incubated at 30°C for 10 days, the number of germinated microsclerotia were counted.

Data analysis

Data were subjected to analysis of variance with Genstat® for Windows 12th Edition (IACR-Rothamsted, Harpenden, Hertfordshire, UK). Treatment means were separated by the Ducan's multiple range test (DMRT). To compare *Macrophomina* species in the different climatic chamber temperatures, an analysis was done on the collected data with a multifactorial design of 2x2x3 with 3 replicas where the factors are: Factor A: Climatic chamber temperature (34/24 and 36/26°C); Factor B: *Macrophomina* species (*M*. *phaseolina* and *M*. *pseudophaseolina*); Factors C: Cowpea variety (cvs. Apagbaala, IT93K-503-1 and Mouride).

RESULTS

Plant stand

In both bioassays, the plant stand depended only on cowpea varieties without *Macrophomina* species affecting it (Table 2). Cvs. IT93K-503-1 (98%) and Mouride (91%) germinated better than Apagbaala (73%). The interaction cowpea variety x climatic chamber temperature was also significant with plant stand of cv. IT93K-503-1 being higher (99%) at 34/24°C than at 36/26°C (73%). The mean germination rate of seeds in pots infested with *M. phaseolina* and *M. pseudophaseolina* was 88 and 87%, respectively, at 34/24°C and 82 and 88% at 36/37°C (Figure 1).

Charcoal incidence

In climatic chamber temperature 1 (34/24°C), average incidence of charcoal rot was 36 and 29% for *M. phaseolina* and *M*. *pseudophaseolina*, respectively and not different (P=0.218; Figure 2). However, the interaction cowpea variety x *Macrophomina* species was significant (P=0.003) with a low incidence on cv. Apagbaala when it is challenged with *M. pseudophaseolina*. Mouride was highly susceptible to both *Macrophomina* species with an average incidence of 57%. In climatic chamber temperature 2 (36/26°C), the incidence over the 3 cowpea varieties was high (53%), but there was no significant effect of *Macrophomina* species (P = 0.073). However, *M. pseudophaseolina* induced more disease incidence on cv. Mouride (64%) than *M*. *phaseolina* (36%) (Figure 2). The interactions climatic chamber temperature × *Macrophomina* species and climatic

Table 2. ANOVA table of a multiple experiments for plant stand, charcoal rot incidence, tissue population density of *Macrophomina*, plant dry weigh and potential primary inoculum 45 days after planting cowpea.

Factors	DF	Wald statistic				
		Plant stand	Incidence	Microsclerotia g^{-1} tissue	Plant dry weight	Potential Inoculum
A: Temperature of climatic chamber	1	2.03 ns	25.65***[2]	5.29**	8.05**	1.52 ns
A: B: Macrophomina species[1]	1	0.42 ns	0.00 ns	0.01 ns	0.56 ns	0.73 ns
C: Cowpea variety	2	43.11***	22.87***	10.19***	0.56 ns	9.72***
AB	1	1.97 ns	4.43**	6.59 **	1.36 ns	2.13 ns
AC	2	21.31***	22.09***	20.41***	8.60**	9.07**
BC	2	0.17 ns	16.82***	3.06 ns	2.01 ns	0.62 ns
ABC	2	1.20 ns	0.65 ns	3.06 ns	3.34 ns	2.39 ns

[1]*Macrophomina phaseolina* or *M. pseuophaseolina*; [2]Significant levels: ***, p < 0.001; **, P < 0.01; ns = not significant at α = 5 %.

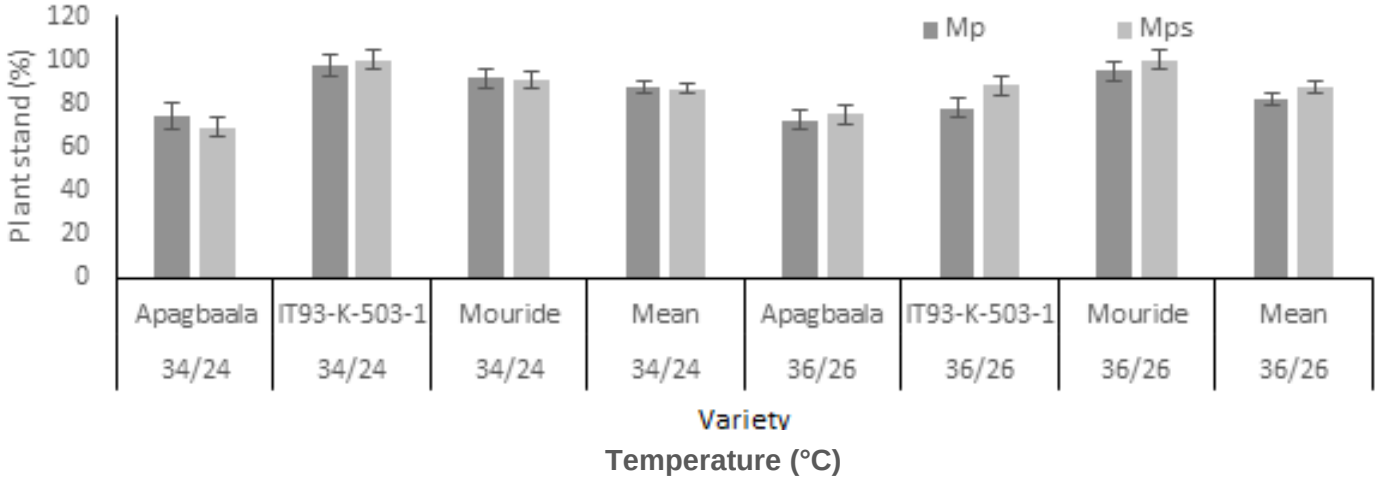

Figure 1. Plant stand of 3 cowpea varieties one week after planting in artificially infested soil and growing in a climatic chamber at 34/24 and 36/26°C. Bars = Standard error of means.

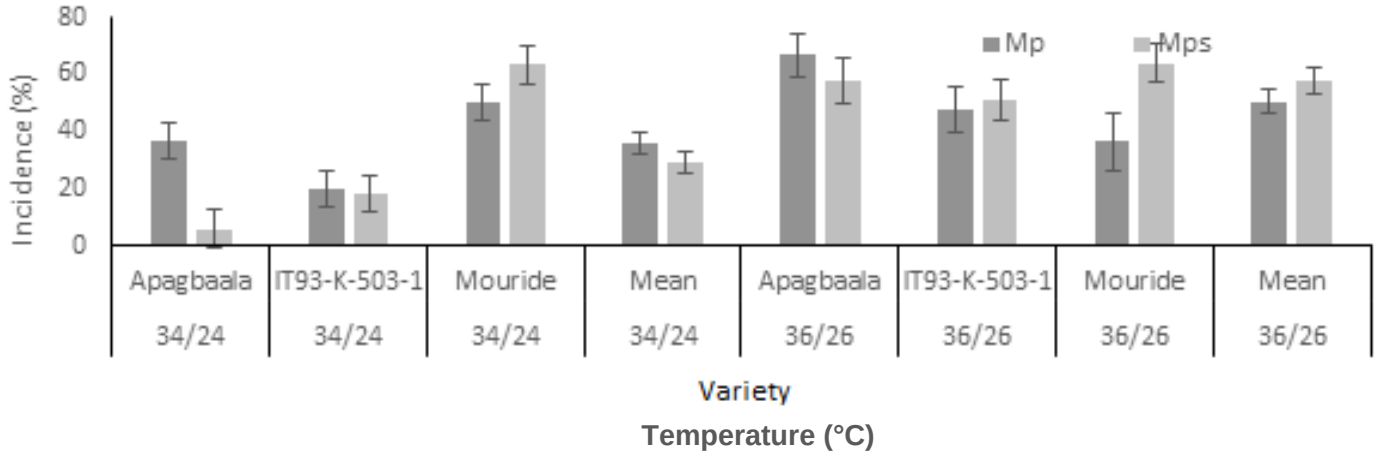

Figure 2. Effect of *Macrophomina phaseolina* (Mp) and *M. pseudophaseolina* (Mps) on charcoal rot incidence in cowpea 45 days after planting and growing in a climatic chamber at 34/24 and 36/26°C 12/12 h day/night temperature. Bars = Standard error of means.

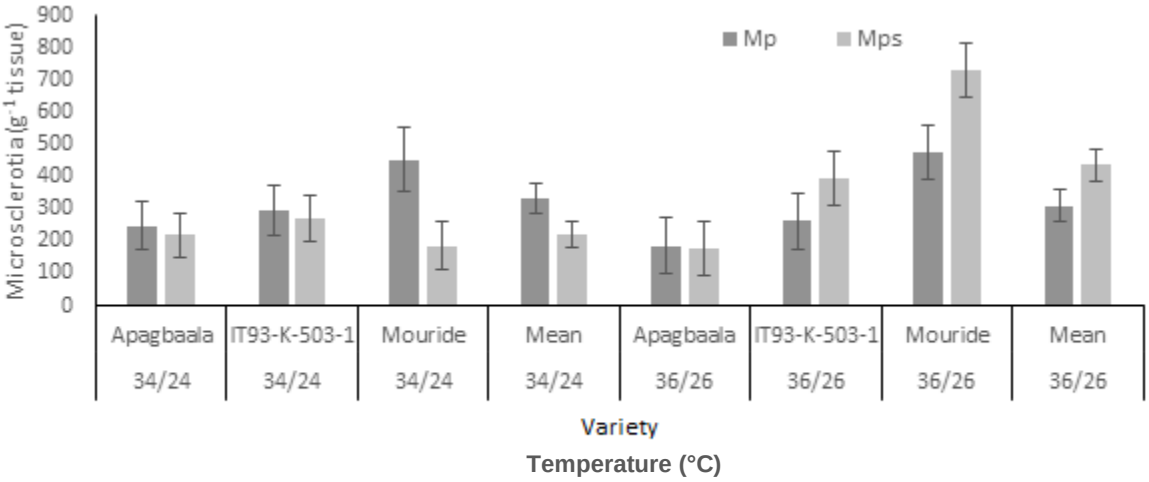

Figure 3. Effect of *Macrophomina phaseolina* (Mp) and *M. pseudophaseolina* (Mps) as affected by the temperature on the number of microsclerotia formed in cowpea tissue. Plants were grown in infested soil in pots in a climatic chamber for 45 days and roots and stems tissues assayed for microsclerotia content. Bars = Standard error of means in the x-axis indication.

chamber temperature × cowpea variety were also significant (Table 1) with *M*. *pseudophaseolina* more aggressive (58% incidence) and Apagbaala highly susceptible (62%) at 36/26°C.

Plant infection level as density of microsclerotia per gram tissue

The analysis of variance indicated significant differences in microsclerotia production among cowpea varieties and climatic chamber temperatures (growing temperatures) (Table 2). At growing temperatures 34/24°C, cv. Mouride produced more microsclerotia per gram tissue (454) when infected with *M. phaseolina* than with *M. pseudophaseolina* (186) (Figure 3). On the other hand, at 36/26°C, *M. pseudophaseolina* induced more microsclerotia (728) formation in cv. Mouride than *M. phaseolina* (476).

Plant dry weight

The plant dry weight was only affected significantly by the growing temperature and the cowpea varieties (Table 2). It was higher at 34/24°C than at 36/26°C and at this last temperature, cv. Apagbaala produced lesser dry biomass than the others cowpea varieties (Figure 4).

Potential primary inoculum

The potential primary inoculum was estimated as the number of microsclerotia produced by plants of each treatment unit. It was significantly low in cvs. Apagbaala and IT93K-501-1 as compared to cv. Mouride in both climatic chamber temperatures. However, the mean potential primary inoculum was higher at 34/24 than at 36/26°C (Figure 5).

DISCUSSION

The recent discovery on *M. pseudophaseolina* raises questions about its pathogenicity which is relative to *M. phaseolina.* Here, for the first time, the pathogenicity to three varieties of cowpea at two temperature regimes were compared. In general, differences between the two *Macrophomina* species were limited. The interactions *Macrophomina* species × growth temperature and *Macrophomina* species × cowpea varieties were however significant for the disease incidence and intensity as microsclerotia^{-1} g tissues (Table 2). In cv. Mouride, *M. pseudophaseolina* induced higher incidence and more microsclerotia g^{-1} tissue production at 36/26°C than *M. phaseolina* (Figures 2 and 3), but induced less disease development in Apabaala.

Temperature strongly affected the incidence and the density of microsclerotia in plant tissue being higher at 36/26°C than at 34/24°C. The observed difference in disease incidence may be due to the variation in pot soil humidity more which is pronounced at 36°C than the direct effect of the temperature on the pathogen. Indeed, pots were watered once every four days, and the 4°C higher growth conditions in the climatic chamber temperature 2 lead to a more rapid reduction of the pot soil moisture suitable for charcoal rot symptom expression (Meyer et al., 1974; Ali and Ghaffar, 1991; Sheikh and Ghaffar, 1979; Kending et al., 2000). Such effects of temperature on *Macrophomina* development

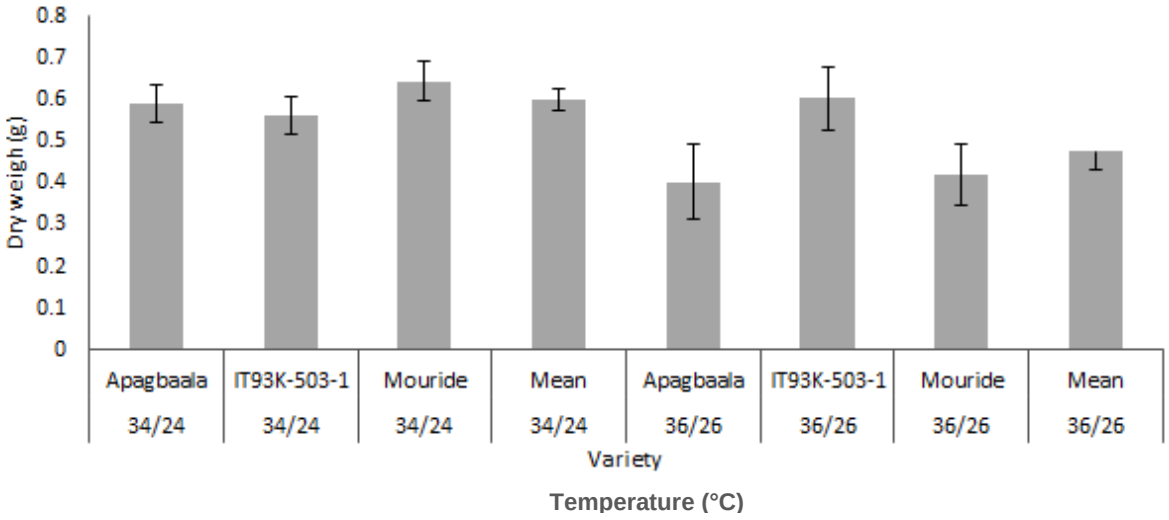

Figure 4. Effect of cowpea varieties as affected by growing temperature on the plant dry weight, 45 days after planting and growing in a climatic chamber. Bars = Standard error of means.

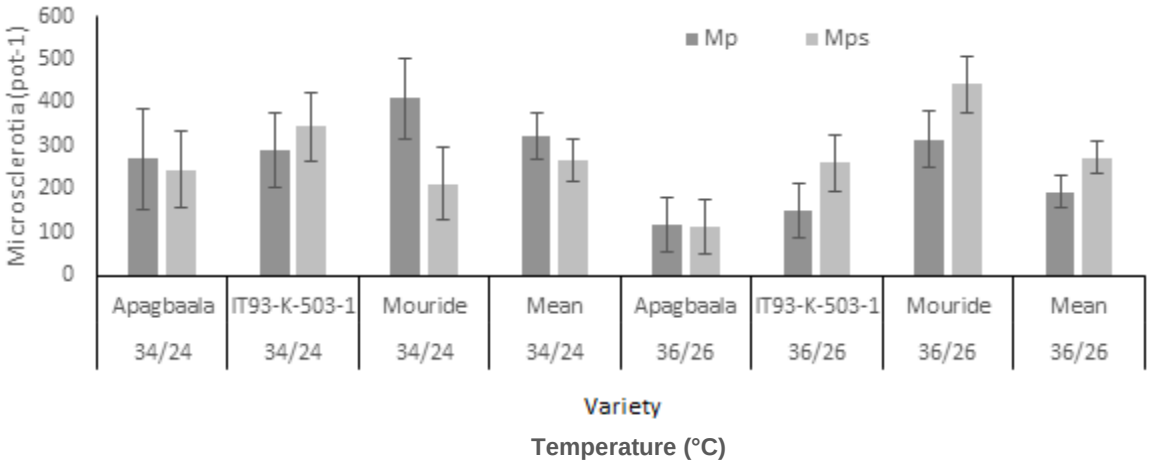

Figure 5. Effect of cowpea varieties, growing temperature and *Macrophomina* sp. on the potential primary inoculum of charcoal rot expressed as total microsclerotia formed per pot (experimental unit). Bars = Standard error of means.

have been reported repeatedly (Odvody and Dunkle, 1979; Mihail, 1989; Iqbal and Mukhtar, 2014) and cowpea varieties (Ndiaye, 2007) on charcoal rot development.

Among the cowpea varieties, cv. Mouride showed the highest incidence and density of microsclerotia in plant tissue in both growth temperatures. These results supported the field observations reported by ISRA (data no published) but also raised the question relative to its role in the rapid progress of the charcoal rot disease. Indeed cv. Mouride is one of the largest distributed varieties in the cowpea production zone of Senegal (Louga, Thiès and Diourbel), where it was introduced as response to the shortening and variation of the rainfall. The variety is resistant to drought, cowpea viruses and bacterial blight (Cisse al., 1995). Despite these evident positive characteristics of this cowpea variety, planting in *Macrophomina* affected areas should be discouraged.

In spite of the small differences in pathogenicity between *M. phaseolina* and *M. pseudophaseolina*, the latter *Macrophomina* species seems somewhat more aggressive in the susceptible variety (more microsclerotia/g tissue and less biomass production in the susceptible variety) at the higher temperature tested (36/26°C). This effect is however probably of lesser importance for management of the disease. So, based on the current results, it is phytopathologically not important to discern between the two *Macrophomina* species for charcoal rot management in cowpea. More pathogenicity

studies on other host crops should be run in order to see if there is a preference for other hosts and to understand why *M. pseudophaseolina* is less distributed than *M. phaseolina* (less than 1% in 180 isolates collected from Senegal and Niger) (Sarr et al., 2014).

Conflict of interests

The author(s) have not declared any conflict of interest.

REFERENCES

Adam T (1986). Contribution à la connaissance des maladies du niébé au Niger avec mention spéciale au *Macrophomina phaseolina* (Tassi) Goid. Thèse de Docteur Ingénieur. Université de Rennes I. 128p.

Adam T (1995). Etude de deux parasites d'origine tellurique sur niébé : *Macrophomina phaseolina* (Tassi) Goid et *Striga gesneroïdes* (Willd) Vatke. Thèse de Docteur-Es- Sciences Naturelles. Université Abdou Moumouni, Niamey, Niger. 102p.

Alhassane A, Salack S, Ly M, Lona I, Traore SB, Sarr B (2013). Evolution des risques agro climatiques associés aux tendances récentes du régime pluviométrique en Afrique de l'Ouest soudano-sahélienne. Sécheresse 24:282-93.

Ali F, Ghaffar A (1991). Effect of water stress on rhizosphere mycoflora and root infection of soybean. Pak. J. Bot. 23:135-139.

Bouhot D (1967). Etude du *Macrophomina phaseolina* sur arachide. Agric. Trop. 22:1165-1171.

Cisse N, Thiaw S, Ndiaye M, Hall AE (1995). Registration of 'Mouride' Cowpea. Crop Sci. 35:1215-1216.

Cloud GL, Rupe JC (1994). Influence of nitrogen, plant growth stage, and environment on charcoal rot of grain sorghum caused by *Macrophomina phaseolina* (Tassi) Goid. Plant Soil 158:203-210.

Csöndes I, Kadlicskó S, Gáborjányi R (2007). Effect of different temperature and culture media on the growth of *Macrophomina phaseolina*. Commun. Agric. Appl. Biol. Sci. 72: 839-48.

Dhingra OD, Sinclair JB (1977). An annotated bibliography of *Macrophomina phaseoli* (1905-1975). Universidade Federal de Viçosa, Minas Gerais, Viçosa, Brazil.

Gaikwad DG, Sokhy SS (1987). Detection of seed rot, root rot and seedling infection in naturally infected cowpea seeds in Senegal and their control. Plant Dis. Res. 2:127-128.

Iqbal U, Mukhtar T (2014). Morphological and Pathogenic Variability among *Macrophomina phaseolina* Isolates Associated with Mungbean (*Vigna radiata* L.) Wilczek from Pakistan. Sci. World J. 2014:950175.

Kending SR, Rupe JC, Scott HD (2000). Effect of irrigation and soil water stress on densities of *Macrophomina phaseolina* in soil and roots of two soybean cultivars. Plant Dis. 84:895-900.

Koenning SR, Wrather, JA (2010). Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. Plant Health Prog. doi:10.1094/PHP-2010-1122-01-RS.

Manici LM, Caputo R, Cerato C (1995). Temperature responses of isolates of *Macrophomina phaseolina* from different climatic regions of sunflower production in Italy. Plant Dis. 79:834-838.

Meyer, WA, Sinclair, JB, Khare, MN (1974). Factors affecting charcoal rot of soybean seedlings. Phytopathology 64:845-849.

Mihail DJ (1989). *Macrophomina phaseolina*: Spatio-temporal dynamics of inoculum and of disease in a highly susceptible crop. Phytopathology 79:848-855.

Morrel M (1992). La pluviométrie, période 1968-1985. Pp Nig. 1-79 et Sen. 1-80 in Atlas agroclimatologique des pays du CILSS. CILSS, OMM, PNUD, CF/Centre AGRHYMET. Niamey.

Ndiaye M, Termorshuizen AJ, Van Bruggen AHC (2007). Combined effects of solarization and organic amendment on charcoal rot caused by *Macrophomina phaseolina* in the Sahel. Phytoparasitica 35:392-400.

Ndiaye, M (2007). Ecology and management of charcoal rot *(Macrophomina phaseolina)* on cowpea in the Sahel. Ph.D. thesis Wageningen University and Research Centre, Wageningen, The Netherlands.

Odvody GN, Dunkle LD (1979). Charcoal stalk rot of sorghum: Effect of environment on host-parasite relation. Phytopathology 69:250-254.

Paré D (1990). Technique de quantification de l'inoculum et distribution géographique de *Macrophomina phaseolina* (Tassi) Goid. au Burkina Faso. Deuxième Séminaire sur la Lutte Intégrée contre les Ennemis des Cultures Vivrières dans le Sahel, 4-9 Janvier 1990. Bamako - Mali. CILSS, INSA, UCTR/PV. 12p.

Sarr MP, Ndiaye M, Groenewald JZ, Crous PW (2014). Genetic diversity in *Macrophomina phaseolina*, the causal agent of charcoal rot. Phytopathol. Mediterr. 53:163-173.

Sheikh A, Ghaffar A (1979). Relation of sclerotial inoculum density and soil moisture to infection of field crops by *Macrophomina phaseolina*. Pak. J. Bot. 11:185-189.

Sinclair JB, Backman PA (1989). Compendium of soybean diseases. 3rd ed. American Phytopathological Society, St. Paul, MN. 106p.

Viana FMP, De Souza NL (2002). Effect of temperature-water tension interaction on germination of *Macrophomina phaseolina* microsclerotia. Fitopatol. Bras. 27:268-272.