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Short Communication

In vitro growth of four isolates of *Sclerotium rolfsii* Sacc in the humid tropics

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Investigation was carried out on *Sclerotium rolfsii* isolated from four plant parts on PDA media for differences in the mycelia and sclerotia growth, in the tropical humid lowlands of Southeastern Nigeria. The result reveals that there were variation in the number and size (in diameter) of the sclerotia produced by the isolates. The mycelia growth rate was same in all the isolates covering the 9.0 cm plates at the 7 and 8 day after inoculation. These sclerotia constitute the major source of inoculum for the organism in nature.

Key words: *Sclerotium rolfsii*, sclerotia, mycelia, inoculum, infection loci.

INTRODUCTION

Sclerotium rolfsii has a very extensive host range which includes more than 500 plants particularly in the tropical, subtropical and warm temperate areas (Mordue, 1974; Singh and Allen, 1979; Wydra, 1996). Although no statistical data are available, disease caused by this pathogen lead to heavy losses in vegetable crop yield especially during the wet season (May and October) when weather conditions are favorable for both crop production and for the growth and dissemination of the sclerotia of the pathogen (Wokocha et al., 1986). These sclerotia constitute the primary inoculum of the pathogen as well as its principle means of dispersal and the sole organs by which the fungus survives adverse environmental conditions, awaiting germination and infection of susceptible hosts when favorable conditions return (Wokocha, 1988; Okabe et al., 2000). They also often appear with the first rains soon after ridging (Arene and Okpala, 1981).

Studies aimed at evaluating the sclerotia production level of this pathogen on infected plant species under favorable condition are necessary. Information on this subject matter comes entirely from the northern Nigeria, thus the need for the study. The aim of the investigation therefore was to evaluate the differences in growth of *S. rolfsii* isolated from four economical crops on PDA media, for information that could prove useful in evaluating possible control measures.

MATERIALS AND METHODS

Isolation of *S. rolfsii*

S. rolfsii Sacc was isolated from four naturally infected plant parts; stems of cowpea and tomato and leaves of cocoyam and pawpaw in the research farm of the Michael Okpara University of Agriculture, Umudike, located at the tropical humid lowlands of Southeastern Nigeria, after the 2004/2005 cropping season. Infected plant materials brought back from the field were washed, cut into 5 mm segments including the advancing margins of infection. The segments were surface disinfected in 0.5% sodium hypochlorite solution for 5 min and rinsed in three changes of sterile water. The segments were separately dried in between sheets of sterile filter paper and plated (3 pieces per plate) on fresh potato dextrose agar (PDA) medium impregnated with streptomycin, and incubated for 7 days at 28°C. Pure culture was obtained by sub-culturing three times. Following the method as described by Okereke (2004), pathogenicity test on the crops were carried out and following this and subsequent isolation Koch's postulates were carried out. Pure cultures of the final isolates were maintained on PDA slants in McCartney bottles and kept in the refrigerator until required.

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Table 1. Number and size (in diameter) of sclerotia produced by *S. rolfsii* isolates incubated for 28 days on PDA media at 28°C.

Plant species	Days after inoculation							
	Number of sclerotia				Size of sclerotia (mm)			
	7	14	21	28	7	14	21	28
Cocoyam	-	42	112	136	-	1.0	1.2	1.4
Cowpea	-	72	136	178	-	1.2	1.5	1.8
Pawpaw	-	24	86	102	-	0.5	0.8	1.0
Tomato	-	52	122	144	-	0.8	1.2	1.4

Values are average of five replicates.

Evaluation of S. *rolfsii* isolates on PDA media

Growth of S. *rolfsii* isolated from the four plant parts were evaluated on the PDA media for differences in growth. 15 ml of molten potato dextrose agar were dispensed into each of 9.0 cm sterile Petri dishes. Discs (5 mm in diameter) taken from the advancing margins of 4 - 5day-old cultures of the isolates of S. *rolfsii* by the aid of a cork borer were separately placed each at the centre of the dishes. The inoculated dishes were replicated five times and incubated at 28°C for 28 days. Dishes were examined daily for the presence of the characteristic mycelium and sclerotia. Data on the average number of sclerotia produced by each of the isolates were evaluated 7, 14, 21, 28 days after inoculation. The average sizes of the sclerotia produced by each of the isolates were also determined using a transparent ruler. Separate experiments were maintained for the four inoculation dates in all the isolates

RESULTS AND DISCUSSION

S. rolfsii exhibits white cottony mycelia growth with ropy strands. The agar media were completely covered by the mycelia at the 7 - 8 days after inoculation in all the isolates. These mycelia were silky white at early stage of growth but after 10 days of inoculation, the pathogen lost its luster and become dull in appearance. Sclerotia were formed by the fungus at the edges of the plates from 11 days after inoculation at 28°C when the agar media were completely covered by the mycelia. The sizes of the sclerotia in diameter vary considerably with the isolates ranging from 0.5 mm in pawpaw isolate to 1.2 mm in cowpea isolate at 14 days after inoculation (Table 1). At 21 and 28 days after inoculation, same trend was observed given sclerotia sizes of 0.8 -1.5 and 1.0-1.8 mm, respectively. The number of sclerotia produced by the isolates followed same trend as above; cowpea isolate>tomato isolate>cocoyam isolate>pawpaw isolate in all the inoculation dates. The number ranged from 24 – 42, 86 - 136 and 102 - 178, at 14, 21, 28 days after inoculation, respectively (Table 1). No sclerotia were produced in all the isolates at 7 days after inoculation. The findings differ slightly from the observation made by Wokocha (1984) in Zaria, northern Nigeria on the size and number of sclerotia produced by *S. rolfsii* isolates. The author observed that *S. rolfsii* in the northern savanna produced sclerotia of 1.5 - 2.5 mm in diameter at the rate of 160 - 240/isolate, 28 days after inoculation and most covered the 9.0 cm Petri-dish in 3 – 4 days at 25 - 28°C. Though the *S. rolfsii* isolates from the two zones may be morphologically similar, the variation in growth could be due to differences in ecology (the northern savanna and the rainforest), genetic differences or the nutrient level of the soil. This fills a gap in our knowledge of *S. rolfsii* growth in the southeastern Nigeria. It has also shown that *S. rolfsii*, an economically important pathogen of most Nigerian crops was widespread throughout the agro-ecological zones of the country, though there could be differences in growth across locations as revealed in the study.

The fungus is best known as a parasite of stem (Aycock, 1966), because moisture, oxygen and perhaps nutritional needs of the fungus are more easily satisfied and conditions are often ideal for infection and disease development. The luxuriant growth of the cowpea and tomato isolates as recorded in the size and number of the sclerotia in the PDA media within the short incubation period may be attributed to this fact, though the pathogen apparently can grow well on any part of a susceptible plant under favorable conditions as in the case of cocoyam and pawpaw isolates which were isolated from the leaves. It can be deduced from the study that these crops when infected with *S. rolfsii* under favorable condition can aid in the sclerotia build-up in the soil. Control measures should therefore be preventive, in order to prevent infection. It should be applied as soil drenches/treatments before cropping. Fumigation of the stems should also be encouraged after crop emergence since the pathogen thrives very well on the stem.

Yam, *Eupatorium* sp. (Maduewesi, 1975) and cereals (Wokocha, 2001) have been reported to show resistant to *S. rolfsii* and some of these crops have also been reported to secrete exudates that are antagonistic to the pathogen at the root zone (Arene and Okpala, 1981). Crop management practices where more susceptible crops like tomato, cocoyam or cowpea follow less susceptible crops like yam or cereals could probably reduce the sclerotia population in the soil. Intercropping is

also recommended, where land is scare. Since these sclerotia can remain in the soil for 2 – 10 years (Messiaen, 1994), the management practices, which can easily be adopted by farmers, should be continuous.

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