# A Technique to Screen for Resistance to Stem Rot caused by *Sclerotium rolfsii* in Groundnut under Greenhouse conditions

**S.Pande, J.Narayana Rao, M.V. Reddy, and D.McDonald**

*International Crops Research Institute for the Semi-Arid Tropics Asia Center (IAC), Patancheru 502 324, Andhra Pradesh.*

**Abstract**

A technique was standardized to screen for resistance to stem rot (*Sclerotium rolfsii* Sacc.) in groundnut (*Arachis hypogaea* L.). The technique involved inoculation of 10 day old groundnut plants raised in pots by spreading mycelial propagules of *S. rolfsii* grown on sorghum grain medium (SGM) on soil surface, and covering them with groundnut leaf debris collected form the field at the time of harvesting of the previous season's crop. Inoculated plants were placed in a greenhouse in which temperatures ranged between 16° C and 34°C. Pots were watered at 24 h intervals to ensure high soil moisture. Almost 100% stem rot was produced on a known susceptible cultivar Robut 33-1 within 10-12 days after inoculation. Stem rot developed at all plant growth stages (10 to 90 days), but disease development was slower in older (≥40 day-old) plants than in younger plants. Time between inoculation and stem rot expression also increased with an increase in the age of plants. None of the 350 groundnut genotypes screened using the greenhouse technique showed resistance when the inoculated plants were incubated at 23- 36° C but eleven of them showed less than 20% incidence at 16-31° C.

## Introduction

Stem rot caused by *Sclerotium rolfsii* Sacc. is an important disease of groundnut (*Arachis hypogaea* L.) in most groundnut- growing areas of the world. It is prevalent in areas where groundnuts are grown in warm and humid environments (Aycock, 1966). Availability of senescent plant tissues, and a warm moist environment near the base of the plant favor stem rot (Shew and Beute, 1984).

Host-plant resistance offers the most economical means of controlling plant diseases, but progress on transferring resistance into high yielding genotypes depends on the availability of an effective technique to identify resistant genotypes. Shew *et al.* (1987) evaluated groundnuts for resistance to stem rot, but high levels of resistance have not been identified. Earlier, several methods of inoculation have been tried to develop high levels of disease severity under greenhouse conditions (Patil and Rane, 1983; Patil *et al.*, 1977; Shew *et al.*, 1987). But, no technique has yet produced consistently high levels of disease in repeated tests (Patil and Rane, 1983; Patil *et al.*, 1977; Shew *et al.*, 1987). It is, therefore, desirable to develop a simple greenhouse screening technique for evaluation of groundnuts for stem rot resistance. In this paper, we describe a greenhouse screening technique utilizing locally available natural products for identifying groundnut genotypes resistant to stem rot.

## Materials and Methods

Ten-day-old plants of a susceptible groundnut cultivar, Robut 33- 1 grown in 15 cm diameter plastic pots filled with a 3:1 autoclaved mixture of Alfisol soil and sand were used to study the influence of different factors on stem rot development under greenhouse conditions. Five plants were grown in each pot. Stem rot incidence was recorded 30 days after inocula-

tion. Mean maximum and minimum temperatures in the greenhouse ranged between 28-33°C and 16-19°C, respectively. Ambient relative humidity ranged between 40 to 55%.

***Sclerotium rolfsii Isolate and its Maintenance***

The culture of *S. rolfsii* used in these studies was isolated form stem rot-infected groundnut plants (cv.ICGS 11) collected form the experimental farm of the International Crops Research Institute for the Semi-Arid Tropics Asia Center (IAC), Patancheru, A.P., India. The fungus was isolated on potato- dextrose agar (PDA), and stored in a refrigerator at 4°C.

***Culture Medium***

Sorghum grain medium (SGM), sorghum stalk medium (SSM), and groundnut shell medium (GSM) were evaluated as culture media for multiplication of *S. rolfsii*. These media were prepared as follows: 200 g sorghum grains soaked for 16 h (mixture of several cultivars), 100 g sorghum stalks (2cm long), and 100 g partially broken groundnut shells were filled separately in 1 L Erlenmeyer flasks and autoclaved at 121° C for 45 min. Each flask was seeded with a mycelial plug ($1cm^2$) from a 10 day old culture of *S. rolfsii* grown on PDA, and incubated at 25 ± 1° C under 12 h of cool white fluorescent light (40 W) for 20 days. Three flasks were used for each medium, and each flask was considered as a replicate. Ten grams of 20 day old fungus culture were taken as a sample from each flask to estimate sclerotical production. The sample was carefully washed in water in a petri dish, the debris removed, and sclerotia counted.

***Greenhouse Inoculation Technique***

Inoculum produced on each of the three media (SGM, SSM, and GSM) was compared in six inoculation methods (Table 1). The experiment was laid out in a split plot design with media as main plots and inoculation methods as sub-plots with four replication.

***Inoculum Propagules***

Mycelial propagules and sclerotia of *S. rolfsii* were compared for their ability to infect groundnut by six inoculation methods (Table 1). To obtain mycelial propagules and sclerotia, autoclaved SGM and GSM in 500ml flasks were inoculated separately with *S. rolfsii* maintained on PDA. After 15 days of incubation at 25 ± 1° C,the flasks were stored at 4° C until used. Mature sclerotia (dark brown color) formed on GSM were harvested by gently tapping the flasks and groundnut shells and air-dried. The experiment was laid out in a split plot design with inoculation methods as main plots and fungal propagules as sub-plots. Each sub-plot was replicated four times.

***Inoculum Quantity***

Mycelial propagules grown on autoclaved SGM were used to determine the relationship between amount of inoculum and stem rot development. Inoculum quantities ranging form 1 to 15 g per pot were tested (Table 2). After inoculation, the soil surface was covered with a layer of 10 g of groundnut leaf debris. The experiment was arranged in a randomized complete block design with three replications.

***Temperature***

Different sets of plants grown in 15 cm pots were maintained at seven different temperatures (10,15 ,20, 25, 30, 35, and 40° C) in percival incubators after inoculation. Inoculated plants were incubated at 12 h photoperiod for 30 days (Table 3). The experiment was arranged in a randomized complete block design with three replications.

***Plant Debris***

Ten day old seedlings of groundnut cultivar, Robut 33-1 grown in pots were inoculated by

**Table 1. Effect of incoulation method and culture medium on stem rot incidence in groundnut in a greenhouse pot culture experiment.**

| Inoculation method | Mortality (%) | | | | | |
|---|---|---|---|---|---|---|
| | SGM | | SSM | | GSM | |
| | Test 1 | Test 2 | Test1 | Test2 | Test 1 | Test 2 |
| 1.Inoculum spread on soil surface | 81 (65) | 78 (62) | 31(30) | 34(36) | 9 (15) | 12 (21) |
| 2.Inoculum spred on soil surface and covered with groundnut debris | 100 (90) [a] | 100 (90) [a] | 47 (43) | 50 (45) | 22 (28) | 25 (30) |
| 3.Inoculum placed in plant collar region | 72 (58) | 7 5 (60) | 47 (43) | 41(39) | 9 (13) | 9 (15) |
| 4.Inoculum placed in plant collar region and covered with groundnut debris | 78 (63) | 81 (64) | 50 (45) | 47 (43) | 12 (21) | 16 (23) |
| 5.Inoculum mixed in the soil | 100 (90) [b] | 100 (90) b | 23 (25) | 31 (33) | 4 (6) | 3 (6) |
| 6.Inoculum mixed in the soil and covered with groundnut debris | 100 (90) [b] | 100 (90)b | 0 | 25 (22) | 0 | 0 |
| 7.No inoculation (control) | 0 | 0 | 0 | 0 | 0 | 0 |
| LSD at P 0.01 | | | | Test 1 | | Test 2 |
| Ioculation method | | | | 10.34 (9.45) | | 9.72 (8.21) |
| Culture media | | | | 6.77 (6.18) | | 6.36 (5.37) |
| Inoculation method x culture media | | | | 17.54 (16.37) | | 16.84 (14.23) |

a100% mortality occured 10 days of inoculation

b 100% pre-emergence rottig of seeds occured

In methods 1 and 2, fifteen grams inoculum was spread on soil surface in pots

In methods 3 and 4 , four to five grains/stem or shell pieces were placed around the collar of the plant

In methods 5 and 6, inoculum was mixed in soil @ 2.5% w/w

Ten grams of groundnut leaves were used to cover soil surface in methods 2, 4 and 6.

Figures in parenthesis are angular transformed values.

spreading the mycelial propagules (SGM) on the soil surface. Ten grams of leaf debris of chickpea (*Cicer arietinum* L.), groundnut (*Arachis hypogaea* L.), sorghum (*Sorghum bicolor* (L.) Moench), blue gum green leaves, blue gum dried leaves (*Eucalyptus* spp.), lawn grass (*Cynodon dactylon* Pers.), pearl millet (*Pennisetum glaucum* (L.) R. Br.) Jamun (*Syzygium cuminii* (L.) Skeels), neem dried leaves, neem green leaves (*Azadirachta indica* A. Juss), paddy (*Oryza sativa* Linno), and pigeonpea (*Cajanus cajan* (L.) Mill.) were spread on soil surface after inoculation. The quantity of inoculum used in this experiment was similar to experiments described above. The experiment was arranged in a randomized complete block design with three replication.

### *Sterilized and Unsterilized Soils*

Plants of the groundnut cultivar, Robut 33-1 were grown separately in sterilized and un-sterilized Alfisol soil in pots. Ten day old plants were inoculated with mycelial propagules (SGM) as described above. The experiment was conducted in a randomized complete block design with three replication.

**Table 2 . Effect of concentration of Sclerotium rolfsii inoculum on stem rot incidence in groundnut in a greenhouse pot culture experiment.**

| Quantity of inoculum (g) | Test 1 | | Test 2 | |
|---|---|---|---|---|
| | Days to mortality | Mortality (%) | Days to mortality | Mortality (%) |
| 0 (control) | 28 | 0 | 30 | 0 |
| 1.0 | 28 | 10 (13) | 30 | 13 (17) |
| 2.5 | 28 | 30 (32) | 30 | 20 (26) |
| 5.0 | 28 | 50 (45) | 30 | 46 (39) |
| 7.5 | 23 | 90 (76) | 30 | 86 (72) |
| 10.0 | 18 | 100 (90) | 24 | 93 (81) |
| 12.5 | 15 | 100 (90) | 16 | 100 (90) |
| 15.0 | 10 | 100 (90) | 10 | 100 (90) |
| LSD at P 0.01 | 3.7 | 15.7 (15.8) | 4.37 | 17.9 (23.8) |

Each value represents the mean of three replications and two runs

Each replication had 5 plants each

Figures in parenthesis are angular transformed values.

**Table 3 . Effect of ambient temperature on stem rot development in groundnut in incubators in pot culture experiment**

| Temperature °C± 1 | Test 1 | | Test 2 | |
|---|---|---|---|---|
| | Days to mortality | Mortality (%) | Days to mortality | Mortality (%) |
| 10 | - | - | - | - |
| 15 | 16 | 93 (81) | 15 | 100 (90) |
| 20 | 14 | 100 (90) | 13 | 100 (90) |
| 25 | 12 | 93 (81) | 12 | 100 (90) |
| 30 | 9 | 100 (90) | 9 | 100 (90) |
| 35 | 9 | 100 (90) | 9 | 100 (90) |
| 40[a] | - | - | - | - |
| LSD atP 0.01 | 1.83 | 14.98 (19.9) | 1.4 | -(-) |

Each value represents the mean of three replications and two runs.

Each replication had 5 plants each.

[a] Plants were not kept upto one month for observation as they suffered severe drooping at 10°C and scorching at 40°C.

Figures in parenthesis are angular tranformed values.

### *Depth of Inoculum*

Mature sclerotia (dark-brown) obtained form GSM and mycelial propagules grown on SGM were placed separately at five different depths (5,10,15, 20, and 25 cm) in sterilized Alfisol soils in 30 cm pots. Inoculum propagules spread on the soil surface served as a control. Seventy grams of sorghum grains containing mycelial propagules (SGM) and 9000 mature sclerotia (germination 95% on PDA) were placed at different depths in pots containing 9 kg of sterilized Alfisol soil. The inoculum was placed at different depths during sowing. In

**Table 4 Reaction of groundnut genotypes to stem rot in greenhouse in pot culture experiment in four tests**

| ICG No. | Identity | Origin | Botanical type | Mortality (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Test 1 | Test 2 | Test 3 | Test 4 |
| 407 | Java PL | Indonesia | Spanish | 13 | 100 | 40 | 100 |
| 494 | Padegaon | India | Virginia Bunch | 7 | 100 | 8 | 100 |
| 500 | Virginia Red | Isreal | Virginia Bunch | 0 | 100 | 0 | 100 |
| 563 | E 16673 | China | Virginia Bunch | 13 | 100 | 0 | 100 |
| 605 | MPI 1 | Malawi | Virginia runner | 7 | 100 | 12 | 100 |
| 2279 | PI 138869 | Iran | Virginia runner | 7 | 100 | 12 | 100 |
| 2837 | Spanish Peanut | Argentina | Virginia runner | 13 | 100 | 0 | 100 |
| 4983 | PI 276235 | Paraguay | Arachis chacoense | 0 | 100 | 0 | 100 |
| 13172 | PI 497579 | Brazil | Arachis sp. (wild) | 0 | 100 | 0 | 100 |
| 13177 | PI 497260 | Argentina | - | 0 | 100 | 0 | 100 |
| ID No. 2256 | A. hypogaea x A. cardenosii | India | Virginia bunch | 0 | 67 | 60 | 87 |
| 799 (Susceptible check) | Robut 33-1 | India | Virginia bunch | 100 | 100 | 100 | 100 |
| LSD at P 0.05 | | | | 14.6 | 5.5 | 13.7 | 5.6 |

Each value represents the mean of three replications and two runs. Each replication had 5 plants each.
Test 1 was conducted during January 1993and temperature range was 16 to 18 (min.) and 28 to 31 C (maximum.)
Test 2 was conducted during May 1993 and Temperature range was 23 to 26 (minimum) and 27 to 36 C (maximum).
Test 3 was conducted during January 1994 and temperature range was 15 to 17 (minimum) and 27 to 32 C (maximum).
Test 4 was conducted during May 1994 and temperature range was 23 to 27 (maximum) and 30 to 37 C (minimum).

treatment where the propagules were placed on the soil surface, the plants were inoculated 10 days after seedling emergence. Inoculum placed at different depths was covered with 49 g of groundnut leaf debris. The experiment was arranged in a randomized complete block design, and these were in three replications. Disease incidence was recorded upto 120 days after sowing. Plants that did not show any wilting were uprooted and observed for pod, peg or root infection. Root, pod, and peg pieces were used for isolation of *S.rolfsii* on PDA.

### *Plant Age*

Plants were inoculated at nine growth stages (10-90 days after sowing, DAS). Plants were raised in 30 cm diameter pots. Sowing were taken up at 10 day intervals to obtain plants of different growth stage for inoculation at the same time. Potted plants were inoculated by spreading 70 g of mycelial propagules on the soil surface. All inoculated pots were covered with 49 g of groundnut leaf debris. The experiment was arranged in a randomized complete block design with three replications.

### *Evaluation of groundnuts for Resistance*

Groundnut genotypes (350), selected form diverse geographical regions, were evaluated for resistance to stem rot in the greenhouse in January 1993. Ambient temperature in the greenhouse in January ranged form 16 to 31°C.

Ten day old seedlings were inoculated with mycelial propagules of *S. rolfsii* as described in previous experiments. Inoculated plants were watered once a day. Stem rot incidence was recorded 30 days after inoculation. Eleven groundnut genotypes showing 20% mortality in 1993 were re-tested in May 1993 (at temperatures between 23 and 36° C), January 1994 (at temperatures between 15 and 32°C) and in May 1994 (at temperatures between 23 and 37°C) following the same procedure (Table 4).

*Disease Rating*

In all experiments, data on the number of plants killed and length of time that elapsed between inoculation and death of the plants were recorded.

Data were subjected to analysis of variance. Significant difference (LSD) was used to compare treatment means.

## Results

*Culture Medium*

All the three culture media tested supported the growth of *S. rolfsii*. SGM supported only mycelial growth, while the other two media (SSM and GSM) supported more sclerotial production than mycelial growth. GSM produced significantly more sclerotia (485 per 10 g of medium) than SSM (250 per 10 g of medium).

*Greenhouse Inoculation Technique*

Inoculum spread on soil surface and covered with groundnut leaf debris resulted in significantly more stem rot infected plants than the other methods (Table 1). However, mycelial propagules produced on autoclaved sorghum grains resulted in 100% stem rot infection within 10 days of inoculation. Methods in which inoculum was mixed with the soil before groundnuts were sown caused 100% per-emergence rotting of seeds (Table 1). Covering the soil with groundnut leaf debris after inoculation resulted in 1° C reduction in soil surface temperature compared to ambient temperature.

*Inoculum Propagules*

Mycelium grown on autoclaved sorghum grain resulted in 100% stem rot infection within 10 days of inoculation. However, 3 week old sclerotia obtained form GSM failed to produce stem rot by any of the inoculation methods.

*Inoculum Quantity*

The highest incidence of stem rot (100%) was observed on tenth day after inoculation when 15 g of mycelial propagules grown on sorghum grain were used for inoculation. In general, a linear relationship was observed between the quantity of inoculum applied and stem rot incidence (Table 2). With reduction in the inoculum quantity, there was an increase in the number of days taken for stem rot expression.

*Temperature*

Stem rot incidence was, 90% at 15, 20, 25, 30, and 35°C. Disease incidence at 10° C and 40°C was not considered, as the plant growth was not normal. Groundnut plants showed drooping and scorching when maintained at 10°C and 40°C. Nearly cent per cent stem rot occurred at 30°C and 35°C within 9 days after inoculation (Table 3). Disease incidence was nearly 100% between 12 and 16 days after incubation at 15, 20, and 25°C.

*Different Plant Debris*

Covering the inoculum with dried leaf-debris of all the 10 plant species tested, resulted in 100% stem rot infection. However, in pots covered with chickpea and groundnut debris,

the fungal colonization was faster and caused 100% mortality within 9 days of inoculation. Mycelial colonization was slow in pots covered with green leaves of *Eucalyptus* sp. and *Azadiracta indica*.

***Sterilized and Unsterilized Soil***

Stem rot development was similar in sterilized and unsterilized soils. However, all plants died within 10days after inoculation in sterilized soils whereas it took up to 17 days to obtain 100% mortality in unsterilized soil.

***Depth of Inoculum***

Mycelial propagules produced on sorghum grains, spread on soil surface, and covered with groundnut leaf debris caused 100% stem rot infection within 10 days after inoculation. Three week old sclerotia placed on the soil surface failed to induce stem rot even when covered with groundnut leaf debris. Both mycelial and sclerotial propagules buried 5-25 cm deep in the soil did not infect pegs, roots,or pods even at 120 days after sowing. Isolations from pegs, roots, and pods on PDA failed to recover *S. rolfsii*.

***Plant Age***

All the nine growth stages (10-90 DAS) tested developed severe stem rot. Ten day old plants showed 100% stem rot infection between 9 and 10 days after inoculation, and infection was 100% in 20 and 30 day old plants at 15 to 17 days after inoculation. Forty day to 90 day old plants took 19 to 24 days to develop 100% infection.

***Evaluation of Groundnut Germplasm for Resistance***

Of the 350 genotypes tested, 11 lines showed ≤ 20% stem rot infection in January 1993 (Table 4). However, all these lines showed about 100% disease when screened again in May 1993 and in May 1994. In January 1994, nine lines showed ≤ 20% mortality.

***Discussion***

Results of the present study clearly indicate that the type of inoculum, availability of organic substrate on soil surface, inoculum quantity, culture media, temperature, and depth of inoculum placement were critical for stem rot development in groundnut. Other factors such as plant debris, soil sterilization, and plant age played relatively a minor role. *Sclerotium rolfsii* has been reported to grow on a variety of natural, synthetic and semi-synthetic culture media (Backman and Rodriguez-Kabana, 1976; Patil and Rane, 1983; Punja and Grogan, 1981).

The ability of SSM and GSM to support abundant sclerotial production compared to SGM may be attributed to the lower weight (volume to weight ratio) of these two substrates compared to sorghum grain as observed by Boyle (1961).

Several studies have shown that volatiles form dried and moistened plant tissue stimulate germination of sclerotia (Beute and Rodriguez-Kabana, 1979a; Beuteand Rodriguez-Kabana 1979b). Mycelia from germinated sclerotia usually colonize dead or senescent plant tissue on soil surface, and bridge the distance between germinating sclerotia and the host. However, in the present study, sclerotia with 95% viability neither germinated in the presence of moistened groundnut leaf debris nor did they germinate eruptively in repeated tests as reported by Punja and Grogan (1981). The results of the present study support earlier observations (Bogle, 1961; Garren, 1964) that mycelium applied to the soil surface infect the groundnut plants more effectively in the presence of organic matter. There was no decrease in

groundnut susceptibility to stem rot with plant age as reported by Patil and Rane (1983).

Groundnut genotypes showed variation in susceptibility to stem rot depending on temperature. Some genotypes that were susceptible at 23-36°C, showed resistance at 16-31°C. This indicates the possible temperature sensitivity of stem rot resistance gene in groundnuts. Resistance genes are known to be temperature sensitive in several crop-disease combinations (Lewellen *et at.*, 1967), but this is probably the first demonstration of such a phenomenon in groundnut resistance to stem rot.

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