A Detached Shoot Technique To Evaluate the Reaction of Peanut Genotypes to *Sclerotinia Minor*[1]

H. A. Melouk*[2], C. N. Akem[2], and C. Bowen[2]

ABSTRACT

The cut ends of 15-cm-long shoot tips from 15 peanut genotypes were immersed individually in 1 x 14 cm test tubes containing Hoagland's solution. Shoots were supported by foam plugs leaving about 12 cm extending above the foam plugs. All leaves were removed leaving about 1 cm of each petiole on the shoot. A 4-mm-mycelial plug of *Sclerotinia minor*, taken from the periphery of a 2-day old culture grown on potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA), was placed between the stem and a petiole in the middle of the shoot. Tubes with shoots were then placed in a polyethylene enclosure on a greenhouse bench where the day and night temperature were 29 ± 2C and 25 ± 2C, respectively. Relative humidity (RH) was maintained at 95 to 100% by lining the bottom of the enclosure with wet burlap. Lesions appeared on shoot tips 3 days after inoculation, and their length was measured at various times. Genotypes with the least percent of symptomatic stems also had the lowest rates of lesion expansion. Two weeks after inoculation, tubes were drained, and shoots remained in the chamber at about 60-70% RH to allow sclerotial production. Sclerotia from each shoot were removed, counted, and their viability determined by germination on SPDA at 25 ± 2C in darkness. This method was effective in differentiating the reaction of peanut genotypes to infection by *S. minor*.

Key Words: *Arachis hypogaea* L., groundnut, disease resistance.

Sclerotinia blight of peanut (*Arachis hypogaea* L.), caused by *Sclerotinia minor* Jagger (9), is a major problem in peanut-producing areas of the United States, especially Virginia (13), North Carolina (12), and Oklahoma (20). Symptoms of the disease included flagging, wilting, necrosis of one or more stems (21), and relatively "dry" lesions on stems, stalks, branches or twigs with demarcations between healthy and diseased tissue (3, 16). Under moist or humid conditions white, cottony, fluffy mycelium appear at the base of diseased stems. The pathogen produces numerous sclerotia on the surface and within infected stems, pegs, and roots. Sclerotia also form between the shell and seed of infected peanut pods. Sclerotinia blight, first observed in Oklahoma in 1972 (22), was widespread in most of the peanut-producing counties of the state by 1983 (23). In 1982, farm income losses in Virginia alone due to the disease

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[2]USDA, ARS, and Department of Plant Pathology, 110 NRC, Oklahoma State University, Stillwater, Oklahoma 74078-9947.

*Corresponding author.

were estimated at $8.6 million, and annual disease losses up to 13% are common in years with favorable disease development (4).

Such losses have resulted in the immediate need for effective, economical strategies for disease management. The disease, however, has not yet been controlled consistently and economically with commercial fungicides (17). In addition to economic considerations, repeated application of specific fungicides within a growing season or a succession of growing seasons may select for a fungicide-tolerant strain of *S. minor* (16). Although fungicide-tolerant strains of *S. minor* have not been noted under field conditions, *in vitro* development of resistance to dicarboximide fungicides by *S. minor* has been reported (2, 19). Variants of other fungi resistant to dicarboximide fungicides have developed under field conditions (4, 14). This could also happen in *Sclerotinia* species.

Porter *et al.* (12), the first to screen peanut germplasm for resistance to *S. minor*, showed that the cv. Florigiant was the most tolerant cultivar among 19 genotypes tested, although 100% infection was observed at harvest. Coffelt and Porter (6) reported on the existence of morphological and physiological resistance of peanut genotypes to *S. minor* under field conditions. Brenneman et al. (5) recently reported on an excised stem technique that could be adapted for rapid evaluation of physiological resistance, fungitoxicity of chemicals, and pathogenicity of isolates of *S. minor*.

Efforts are being directed to develop effective techniques to determine the reaction of genotypes and identify resistance in peanut germplasm to *S. minor*. This paper reports on a detached shoot technique for preliminary screening of peanut genotypes for their reaction to *S. minor* under controlled conditions using rate of lesion expansion, and sclerotial production and viability among genotypes. A preliminary report and a brief description of the method have been reported (10).

Materials and Methods

Fifteen-cm-long shoot tips from main stems of 8-week-old greenhouse-grown plants were used in this study to evaluate the reaction of 13 peanut genotypes to *S. minor*. All genotypes were obtained from Dr. Olin Smith, Department of Crop Science, Texas A&M University, College Station, TX 77843. The lines TX 804475, TX 798736 UF-73-4022, TX 798683, and TX 798731 were selected because they exhibited some resistance to *S. minor* in replicated field plots at Stillwater, OK in 1986 (11). Florunner was selected as a reference standard because of its susceptibility to *S. minor*. Other lines were included because of their varying susceptibility to *S. minor* as observed in field plots. The culture of *S. minor* used for inoculation was isolated from the peanut cv. Florunner, and maintained on potato dextrose agar containing 100 ug/mL of streptomycin sulfate (SPDA) at 25 ± 2C.

All leaves on shoot tips except the primordial leaves were excised leaving about 1 cm of each petiole on the shoot. Individual shoots were supported by a foam plug, and the cut ends were immersed individually in 1x14 cm test tubes containing Hoagland's solution (8), leaving about 12 cm extending above the foam plugs. Each shoot was inoculated by placing a 4-mm-mycelial plug of *S. minor* from the periphery of 2-day old cultures on SPDA at the axil between the stem and petiole at about mid portion of the shoot. Test tubes with inoculated stems were placed on wooden racks in fabricated clear polyethylene chambers (60 x 60 x 75 cm), the bottom of which was lined with wet burlap, and placed on greenhouse benches. The wet burlap maintained the relative humidity in the chamber between 95 and 100%. Temperature in the chambers were 25 ± 2C and 29 ± 2C during the night and day, respectively. Ten shoots of each peanut genotype were inoculated with *S. minor* in each test, and shoots inoculated with plain SPDA plugs served as controls.

Lesion Expansion

Lesion lengths (cm) were measured as the distance from the site of inoculation to the farthest macroscopically visible edge of the lesion. This was done daily from day 3 after inoculation through day 7 when some of the shoots were completely colonized with mycelia of *S. minor*. Mean lesion lengths of each genotype in each test, were calculated as the sum of individual lesion lengths divided by the total number of inoculated shoots whether infected or not. Length of lesions were linearly regressed against time after inoculation to determine the rate of lesion expansion, where the slope of the line represented the rate of lesion expansion (cm/day) on each genotype.

Inoculum Production

Upon conclusion of lesion measurements, one end of the chamber was opened to lower the relative humidity to 60-70%. Hoagland solution was then drained from test tubes. Tubes with infected shoots were left in the chambers for 2 weeks during which time sclerotia formed on the surface and in pith cavities of stems. Numbers of sclerotia per shoot both on the surface and within the pith tissue were counted at this time or 3 weeks after inoculation.

Sclerotial Viability

Sclerotia collected from all genotypes were tested for viability. Sclerotia were washed under running tap water and surface sterilized in an aqueous solution of 0.5% sodium hypochlorite for 3 min. Five samples, each consisting of 10 sclerotia randomly picked from each infected peanut genotype, were plated on SPDA. Plates were incubated at 25 ± 2C in darkness. The number of germinated sclerotia in each plate was recorded daily from day 2 to day 5 when most of the plates were covered with mycelial growth of *S. minor* from germinating sclerotia.

Results

Lesion Development

The following range of lesion types were observed on inoculated shoots: 1) small superficial lesions (Less than .2 cm in length) generally restricted to the point of contact of inoculum and stem; 2) rapidly expanding lesions restricted to one side of the stem; and 3) rapidly expanding lesions that completely girdled a stem and the fungus was actively colonizing the entire shoot. Table 2 shows the average length of lesions on the various peanut genotypes at various times after inoculation with *S. minor*.

In the most susceptible reaction the first symptoms on infected shoots were watersoaked lesions that started forming at the points of contact of *S. minor* and the stem 2 days following inoculation. These lesions expanded rapidly in the susceptible lines and completely girdled stems within 72 hrs. Following complete stem girdling the shoots began to wilt. Stem girdling was observed on TP 107-3-8, TX 833841, TX 771174, TX 835841, TX 833829, TP 107-11-14 and Florunner. Genotypes that showed moderate susceptibility were girdled slowly, starting with infection on one side and wilting was accordingly delayed. This was observed on TX 771108, TX 798731, TX 798683 and UF 73-4022 (Table 1). Lesions did not develop beyond points of contact of inoculum and stems on some genotypes. These points were restricted or confined and no further lesion expansion was observed. These reactions were noticed on some stems of TX 804475 and TX 798736 (Table 1).

The rates of lesion expansion as determined by slopes of regression lines for all the genotypes were compared (Table 3). The average lesion expansion rate on shoot tips was less for genotypes TX 798683, TX 804475, and TX 798731, all of which were identified as having some resistance to *S. minor* in field screening tests (11) as compared with the susceptible genotypes TP107-3-8, Florunner, and TX 741174. Other genotypes had varying lesion expansion rates, corresponding to their varying degrees of resistance to *S. minor* demonstrated previously in field studies. Genotypes with the least fraction of stems infected, also had the lowest rates of lesion

Table 1. Reaction of peanut genotypes to Sclerotinia minor at three days after inoculation[1].

Genotypes with[2]		
Girdled stem lesion[3]	Unilateral stem lesion[4]	Lesion at inoculation point[5]
TP 107-3-8	TX 771108	TX 804475
TX 833841	TX 798731	TX 798736
TX 771174	TX 798683	
TX 835841	UF 73-4022	
TX 833829		
TP 107-11-14		
Florunner		

[1]Inoculation was accomplished by placing a plug of actively growing mycelia of S. minor on the leaf axil of a detached shoot. Shoots were incubated in 95 to 100% relative humidity in polyethylene chambers.

[2]More than 50% of inoculated shoots in each of the categories showed the typical response of the group.

[3]Rapidly expanding lesions that completely girdled a stem and the fungus was actively colonizing the entire shoot.

[4]Rapidly expanding lesions restricted to one side of the stem.

[5]Small lesions generally restricted to the point of contact of inoculum and stem.

Table 2. Average lesion length (cm) per shoot of peanut genotypes in a 7-day period following inoculation with *Sclerotinia minor*.

	Days after inoculation[a]				
Genotype	3	4	5	6	7
TX 798683	.21	.58	1.18	1.75	2.23
TX 804475	.23	.60	1.25	1.70	2.25
TX 798731	.13	.60	1.35	1.98	2.85
TX 798736	.34	1.16	2.08	2.75	3.45
UF 73-4022	.34	1.16	2.05	3.20	4.30
TX 771174	.63	1.78	3.20	4.78	5.58
TX 771108	.50	1.05	2.13	3.30	4.33
TP 107-3-8	.83	2.18	3.68	5.03	6.15
TP 107-3-4	.48	1.23	2.28	3.60	4.60
TX 833829	.40	1.33	2.60	3.95	5.08
TX 835841	.57	.85	1.73	2.83	3.87
TX 833841	.88	1.58	2.90	3.88	4.90
Florunner	.58	1.60	2.83	4.38	5.85
$LSD_{(.05)}$	.37	.81	1.24	1.12	1.42

[a]Averages were calculated from two separate tests, each using 10 shoots per genotype.

expansion, while those with more stems infected had higher rates of lesion expansion (Tables 3). The data in Table 3 on the rate of lesion expansion in Tests 1 and 2 were significantly ($p \leq 0.01$) correlated, r=0.80.

Inoculum Production

Sclerotia were collected from both the surface of stems and inside of pith cavities. Not all infected stems produced sclerotia (Table 4). Among those that did, some produced sclerotia only on the surface of the stems. Genotypes TX 804475 an TX 798683, with lower rates of lesion expansion produced the lowest numbers of sclerotia on/in stems (Tables 3, 4). The other genotypes produced varying numbers of sclerotia corresponding to their varying rates of lesion expansion. A positive and significant ($p \leq 0.01$) correlation

Table 3. Infection of peanut shoots and rate of lesion expansion (cm/day) after inoculation with *Sclerotinia minor*.

	Percent of shoots		Rate of lesion expansion[a] (cm/day)	
Genotype	Symptomatic[b]	W/sclerotia	Test 1	Test 2
TX 798736	60	50	0.72	0.93
TX 804475	50	20	0.64	0.38
TX 798731	50	40	0.83	0.53
TX 798683	50	50	0.45	0.59
UF 73-4022	80	70	0.96	1.03
TX 771174	90	80	1.32	1.25
TX 771108	60	50	1.07	0.78
TP 107-3-8	80	80	1.42	1.27
TP 107-11-4	70	70	0.97	1.16
TX 833829	80	70	1.33	1.06
TX 835841	60	40	0.91	0.70
TX 833841	60	50	1.30	0.71
Florunner	90	90	1.32	1.34
$LSD_{(.05)}$	-	-	0.47	0.51

[a]Length of lesions (cm) was measured at 3, 4, 5, 6, and 7 days after inoculation.

[b]Symptomatic was determined by the formation of measurable lesions. Shoots with point infections were not considered symptomatic.

coefficient of 0.71 was obtained between the rate of lesion expansion and the total number of sclerotia produced on the genotypes tested.

Sclerotial Viability

The percent viability of sclerotia collected from the surface and pith tissue of peanut genotypes as determined by germination of SPDA medium ranged from 54% on TX 804475 to 74% on TP107-3-8 (Table 4). Sclerotia collected from the susceptible genotypes TX 835841, Florunner, and TP 107-3-8 were significantly ($P \leq 0.05$) more viable than sclerotia from the other genotypes evaluated (Table 4).

Table 4. Production and viability of sclerotia of *Sclerotinia minor* on infected peanut shoots.

Genotype	Avg sclerotia/[a] stem surface	Avg sclerotia/ pith tissue	Percent germination[b]
TX 798736	12	8	56
TX 804475	13	2	54
TX 798731	12	8	60
TX 798683	16	0	62
UF 73-4022	14	11	64
TX 771174	23	11	68
TX 771108	21	13	60
TP 107-3-8	16	11	74
TP 107-11-4	13	8	56
TX 833829	24	0	55
TX 835841	18	8	70
TX 833841	22	14	68
Florunner	25	17	71
$LSD_{(.05)}$	8	4	11

[a]Average number of sclerotia per shoot, determined from a total of 20 shoots in 2 tests with 10 shoots per test.

[b]Sclerotia were plated on potato dextrose agar medium containing 100 ug/ml streptomycin sulfate in five replications of 10 sclerotia per plate. Germination counts were made 3 days after incubation at 25 ± 2C in darkness.

Discussion

The detached shoot technique described in this paper provides a rapid evaluation procedure for preliminary screening of peanut genotypes for resistance to *S. minor* under greenhouse conditions. Actively growing mycelia from the periphery of *S. minor* culture plates provided inoculum in its optimum aggressive form to infect peanut stems. The high relative humidity provided by the wet burlap and favorable temperatures within the polyethylene enclosures provided the proper conditions for infection by *S. minor* (1).

Induction of lesions on some plants under optimum greenhouse conditions that are not normal in the field could be advantageous in screening genotypes for resistance. Genotypes that exhibit resistance under optimum conditions are likely to have high levels of resistance in field conditions in which their actual fitness under disease pressure is evaluated. Even genotypes classified as moderately resistant under artificial conditions exhibit useful resistance in the less favorable field conditions.

Most stem inoculations are performed through wounds (5, 7) which facilitate penetration of the host by the pathogen. Extrapolations of results from such laboratory wound inoculations to field conditions could be misleading. In this research, we were able to induce infection on shoots of the peanut genotypes without wounding.

Notes should be made concerning the response of stems when challenged by the pathogen. Point infections on some of our peanut genotypes suggest a form of hypersensitive response. We suspect that such a response may be initiated by a reaction of the pathogen to structural components of the cell wall. This still needs to be determined.

The rate of lesion expansion appears to be a simple and effective method of screening peanut genotypes for resistance to *S. minor* under controlled conditions. The rate of stem lesion expansion can be used to rank peanut genotypes for resistance to *S. minor*. A significant ($p \leq 0.01$) correlation coefficient of +0.64 was obtained between the rank of rate of lesion expansion and maximum disease incidence under field conditions (11). This technique, however, should not be used as a substitute for field evaluations because some genotypes may react differently under controlled and field conditions. For example, the genotype TX 835841, a susceptible genotype to *S. minor* under field conditions, showed some resistance to *S. minor* using this technique. This difference in reaction could be attributed to several factors such as the effect of plant canopy which could render the stems more susceptible to infection by the pathogen. Also, in this technique, a single inoculation was performed on the shoot, whereas under field conditions multiple infections occur throughout the growing season which may affect the susceptibility of the plants to infection by *S. minor*.

The method described was useful in assessing resistance to Sclerotinia blight in peanut genotypes and it may be used in screening populations segregating for resistance to the disease in a breeding program. It can be effectively adapted as a useful tool for rapid evaluation of plant genotypes before whole plant evaluations in the greenhouse and field. It has several added advantages over evaluation of intact plants. There is an economy of labor as an experiment requires only 8-9 wk, 7-8 wk to grow the plants and 1 wk for disease development. In addition this technique requires a minimum of laboratory and greenhouse space. It can provide reproducible results within a limited period of time without having to wait on seasonal field evaluations of whole plants. The technique could be adapted for other uses including evaluation of efficacy of fungicides and fungicide resistance. Another adaptation of this technique would be the use of several shoots from a single plant to maximize the utility and savings of plant material.

Sclerotia of *S. minor* collected from some stems of less susceptible genotypes were not fully developed. They had a whitish appearance and were not as dark as fully formed sclerotia on the susceptible genotypes. Low viability counts were a characteristic of sclerotia from these genotypes. It appears viability of sclerotia can be affected by the genotype's degree of resistance and the developmental state of sclerotia.

In summary, our results showed that the genotype TX 771174, TP 107-3-8, TP 107-11-4, TX 833829, TX 833841, TX 771108, (UF 73-4022 and cv. Florunner, are very susceptible to *S. minor*, while the genotypes TX 798736, TX 804475, TX 798731, TX 798683 and TX 835841 have some resistance to *S. minor*, if we consider all the parameters evaluated. These results highly correlate with field results for most of the genotypes as previously described. Further field testing of the breeding line TX 798736 led to the release of Tamspan 90, a spanish peanut cultivar with resistance to *S. minor* (18).

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