Evaluation of Peanut Genotypes for Resistance to Southern Stem Rot Using an Agar Disk Technique[1]

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ABSTRACT

Six tests were conducted at two locations over 3 yr using an agar disk technique. Multiple disease assessments were made with a 1 to 5 scale, allowing the use of areas under the disease progress curve for genotype comparisons. Of the 11 genotypes evaluated, four were commercial cultivars. The cultivar Florunner was highly susceptible, Southern Runner exhibited a moderate level of partial resistance, Early Bunch was slightly more resistant than Southern Runner, and Marc I was less resistant than Southern Runner. The runner line UF91108 and the virginia lines F79/4-6-2-1-1-Z1, F79/4-6-2-1-1-Z16, and F84/49-2-2 all appear to have similar levels of resistance to stem rot. The runner breeding lines UF85112 and UF86107 had less disease over the six tests than any other genotypes. The agar disk technique worked well in the field, allowing selection of breeding lines with resistance to stem rot. Lines that exhibited good resistance in the six tests will be useful germplasm resources for a cultivar improvement program.

Key Words: *Athelia rolfsii*, resistance screening, southern blight, white mold.

Southern stem rot caused by *Sclerotium rolfsii* Sacc. is a serious disease on peanut (*Arachis hypogaea* L.) in many areas of the world (1). The stem rot pathogen usually attacks the plant in the crown area (1, 6, 8) and may kill a portion or the entire plant. Under favorable conditions this fungus is an incitant of pod rot. The pathogen produces oxalic acid and cell wall degrading enzymes that kill the plant tissue (17). Sclerotia germinate under warm, moist conditions and may utilize decaying organic matter as a food base (12). This food base provides energy for mycelial growth after sclerotia have germinated and enhances the ability of the fungus to infect living plants. Sclerotia also may germinate eruptively in the presence of volatile compounds provided by degrading organic matter and infect plants without a food base (16). The peanut canopy provides a warm, moist environment that is often conducive to infection by the stem rot pathogen. Pod losses due to stem rot have been calculated to be as high as 2.9% per disease locus in peanut fields (2) and are estimated at 7 to 10% annually in the Southeastern United States (14).

Cultivars of peanut with resistance to stem rot are needed and differences among peanut genotypes in their response to the stem rot pathogen have been reported (3, 4, 5, 11, 13). Resistance of peanut plants to *S. rolfsii* may be phenological, associated with canopy type. It is not known whether this type of resistance has any effect on the underground infections that occur in the pod development zone under dryland conditions. Resistance to *S. rolfsii* also may be metabolic, associated with structural barriers to infection or with active plant responses to infection (20). Brenneman *et al.* (3) suggested that evaluating germplasm for resistance in field plots may be the best way to verify both types of resistance. Shokes *et al.* (22, 23) found that field screening was more consistent than greenhouse tests for evaluating genotype responses to stem rot. Although field tests work best for screening peanut for stem rot resistance, there are several factors that may hinder the process. The presence of natural antagonists in field soil can completely inhibit the growth of *S. rolfsii* (15). The nonuniform spatial distribution of natural inoculum can be a problem for evaluations. Inoculum in peanut fields is often aggregated (19), resulting in plants that escape disease by not coming into contact with the fungus. In some areas of a given field, whole plots may have low disease incidence due to the low pathogen populations in the respective plot. Amending soil with inoculum of *S. rolfsii* grown on

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sterilized oat seed has been used to increase the pathogen population and improve the uniformity of distribution of the fungus (3, 20, 23). However, even with uniform inoculum distribution, individual plants may escape the disease. Use of oat inoculum presents additional problems since disease responses may be due to inoculum density and independent of genotype (20). In practice, when testing peanut for resistance to *S. rolfsii* in field plots, many replications may be necessary to obtain significant differences between genotypes. This can cause a problem when evaluating early generation breeding lines where seed supply is limited.

To overcome difficulties in the screening process, a method for inoculating individual plants to prevent escape from the pathogen was developed to allow the use of small plots for genotypes with limited seed and to increase precision in the assessment of genotype response. This method, termed the 'agar disk technique', is very effective when compared to the use of oat inoculum and other methods of inoculation of peanut with *S. rolfsii* (21, 22, 23).

The objective of the present study was to evaluate the response of peanut genotypes to *S. rolfsii* using the agar disk technique. The response of 11 genotypes to the stem rot pathogen is reported here. A portion of this research has been previously reported (22).

Materials and Methods

Over 120 different peanut genotypes were evaluated at two locations using the agar disk technique from 1991-1993. Eleven of those genotypes (Table 1) were common to each test. Peanut seed were planted with a cone planter (about 22 cm apart) at the North Florida Research and Education Center (NFREC), Quincy, and at the NFREC, Marianna.

Table 1. Characteristics of 11 peanut genotypes common to six field tests for resistance to *Sclerotium rolfsii* over 3 yr using an agar disk inoculation technique.

Genotype	Market type[a]	Growth habit[b]	Days to maturity
Florunner//Early Run./Florispan	R	D	135-145
Southern Runner//PI 203396/Florunner	R	D	≥145
Marc I//F439-17/F459B-3-2-4-6	R	D	125-130
UF85112//F439-17/F459B-3-2-4-6	R+	B+	125-130
UF86107//F70115/GK 19	R+	B+	125-130
UF81206-2-Z16//PI 203396/F427B4	R+	D	≥145
UF91108///South. Run.//Andru 93/81206	R+	D	≥145
Early Bunch//F406A/F420	V	B+	125-130
F79/4-6-2-1-1-Z1///NC Fla 14//72/93-9[c]	V-	D-	≥140
F79/4-6-2-1-1-Z16///NC Fla 14//72/93-9[c]	V-	D-	≥140
84/49-8-2-2///78114//South. Run./81206	V	D-	>145

[a]Runner types are designated by R; virginia types are designated by a V. A plus (+) indicates that the genotype is large-seeded for its market type and a minus (-) indicates that it is small-seeded for its market type.

[b]A decumbent or spreading growth habit is designated by a D and an upright bunching growth habit is indicated by a B. A plus (+) or minus (-) indicates that a line is somewhat intermediate in growth habit.

[c]Breeding lines F79/4-6-2-1-1-Z1 and -Z16 are sister lines.

One test per location was conducted in each of 3 yr from 1991-1993 for a total of six tests. The Quincy site had been planted to soybean, wheat (winter), and corn 2 yr prior to the peanut test. The Marianna site was in a 3-yr rotation of peanut, corn, and grain sorghum prior to the present study. Plot area locations at the test sites were changed each year. The soil for tests at the Quincy location was a Dothan loamy sand, pH 5.6 to 6.1, with < 2% organic matter. The soil at the Marianna site was a Chipola fine sandy loam, pH 5.8 to 6.2, with < 2% organic matter. Plots were 2.6 m long single rows that were planted on 0.91-m centers. Standard recommended practices were used for soil preparation, weed control, and insect management. Foliar diseases were controlled with the use of chlorothalonil (0.88 kg/ha in 233 L of water/ha) on a regular 14-d schedule beginning 30 to 35 d after planting and continuing until 2 wk before digging (*ca.* 135 d after planting each year) Each plot had 11 plants and every other plant in each plot (five plants/plot) was marked with a surveyor's flag to identify it for inoculation and assessment. Spotted wilt virus was only a minor problem at the test sites and plants with symptoms were removed before inoculation with *S. rolfsii*. When a plant had to be removed due to spotted wilt virus, adjacent plants were inoculated so that five inoculated plants were available for assessment. All tests were planted between 1 May and 22 May, except the 1993 test at Quincy. Planting of this test was delayed by the wet weather until 4 June. Either 53 or 56 genotypes were planted in a randomized complete block design with five or six replications in each test. Four of the 11 genotypes compared in this paper (Table 1) were the commercial cultivars Florunner (susceptible), Southern Runner [partial resistance (3)], Marc I (9) with unknown susceptibility, and Early Bunch [partial resistance (D. W. Gorbet, unpubl.)].

Agar Disk Inoculation. Individual plants were inoculated between 50-60 d after planting when it was determined that sufficient canopy was available to shade the crown area and provide conditions conducive to *S. rolfsii* infection. A pretested virulent isolate obtained from peanut at Marianna, FL, was used in all tests. Inoculum was prepared according to the method of Shokes *et al.* (23) by germinating sclerotia of the pathogen on potato dextrose agar (PDA). Disks of PDA (1 cm in diameter) with a germinated sclerotium and actively growing mycelium were used to inoculate plants (Fig. 1). One agar disk was placed within 2 cm of the soil surface on the central stem of each flagged plant. Unless natural rainfall occurred, plots were irrigated (1.25 cm) prior to inoculation and for 2 d after inoculation to ensure suitable conditions for infection by *S. rolfsii*.

Disease Assessments. Plants were checked within the week after inoculation to be sure that the agar disks had not been displaced. Any disks that were displaced were replaced. Disease was assessed beginning 2 wk after inoculation and at 2 wk intervals thereafter. Each test had four to six disease assessments. Individual plant assessments were made and a mean of five plants for each plot was used for all statistical analyses. Disease was assessed in 1991 using a 1 to 6 scale that was simplified in 1992 and 1993 to a 1 to 5 scale in which 1 = healthy plants, 2 = stem lesions only, 3 = ≤ 25% of the stems wilted or dead, 4 = 26-50% of stems wilted or dead, and 5 = > 50% of stems wilted or dead. The scale used in 1991 was readily converted to the final 1 to 5 scale by eliminating one descriptor which differentiated

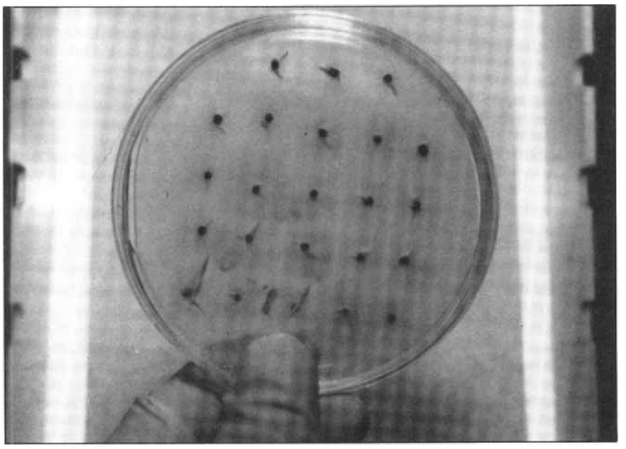

Fig. 1. Sclerotia of *S. rolfsii* germinated on potato dextrose agar. The 1-cm-disk, cut with a cork borer, was used to obtain uniform inoculum for placement on the central stem of a peanut plant.

lesion length. Since the plots were small, no attempt was made to determine pod yields; but observations of plants were made after inversion to note the effect of stem rot on underground plant parts.

Statistical Analysis. All data were subjected to analysis of variance. Fisher's protected LSD test was used for comparison of the genotypes at the $P \leq 0.05$ level. Areas under the disease progress curve (AUDPC) were computed using the method of Shaner and Finney (18). Correlation of the final disease rating was tested against the AUDPC. Since the interactions between years, locations, and genotypes were significant, separate comparisons were made for each test. In a separate analysis, each test was treated as an environment and the genotype-by-environment interaction was observed to determine whether genotype responses were stable across environments.

Results

Final Disease Rating. Because each test had to be analyzed separately, the differences in the means could not be compared statistically across all tests. However, it is helpful to consider these means to perceive the trends. Therefore, the means across years and locations and the means across genotypes within a given test are included in Table 2. Florunner, the susceptible standard cultivar, always had the highest disease ratings in each test. Stem rot killed a majority of the inoculated plants of Florunner by the last assessment, resulting in ratings > 4.0 in every test except at Quincy in 1991 (Table 2). Southern Runner had significantly lower disease ratings than Florunner in four of the six tests. Ratings for Early Bunch were similar to those for Southern Runner, and significantly lower than Florunner in all six tests. Marc I was variable in these studies, performing similarly to Southern Runner in four tests and worse in two tests. The seven breeding lines typically had lower ratings than Southern Runner although these differences were not significant.

Although there were significant differences in the epidemics across years and locations, the trends for disease progress were very similar for each test (Fig. 2). Disease progressed rapidly for Florunner in all tests (Fig. 2a). Southern Runner had less disease than Florunner and final disease for Marc I was generally greater than that of Southern Runner but less than

Table 2. Final disease ratings on a 1 to 5 scale for 11 peanut genotypes inoculated with *Sclerotium rolfsii* using the agar disk technique.[a]

Market type and genotype	1991		1992		1993		Mean
	Quincy	Marianna	Quincy	Marianna	Quincy	Marianna	
Runner							
Florunner	3.6	4.4	4.5	4.6	4.3	4.8	4.4
Southern Runner	3.0	3.1	3.9	3.4	3.0	3.8	3.4
Marc I	3.1	3.0	3.8	4.0	3.6	4.8	3.7
UF85112	2.4	3.0	2.9	3.4	3.1	3.8	3.1
UF86107	2.6	3.1	3.1	3.2	2.7	3.5	3.0
UF81206-2-Z16	2.8	3.2	3.8	3.4	3.4	3.4	3.3
UF91108	3.0	2.9	3.3	3.2	2.8	3.4	3.1
Mean	2.9	3.2	3.6	3.6	3.3	3.9	3.4
Virginia							
Early Bunch	2.9	2.7	3.0	3.8	3.1	3.7	3.2
F79/4-6-2-1-1-Z1	2.8	3.0	3.7	3.2	3.0	3.9	3.3
F79/4-6-2-1-1-Z16	2.7	2.9	4.1	3.2	3.1	3.4	3.2
84/49-8-2-2	2.8	2.9	3.4	3.4	2.7	3.3	3.1
Mean	2.8	2.9	3.6	3.4	3.0	3.6	3.2
$LSD_{0.05}$	0.6	0.7	0.7	0.5	0.7	0.7	

[a]A 1 to 5 scale was used to measure stem rot severity in which 1 = healthy, 2 = stem lesions only, 3 = ≤ 25% of stems wilted or dead, 4 = 26 to 50% of stems wilted or dead, and 5 = > 50% of stems wilted or dead. Each year evaluations were made at the Quincy and Marianna locations. Tests were analyzed separately since genotype × location × year interactions were significant ($P \leq 0.05$).

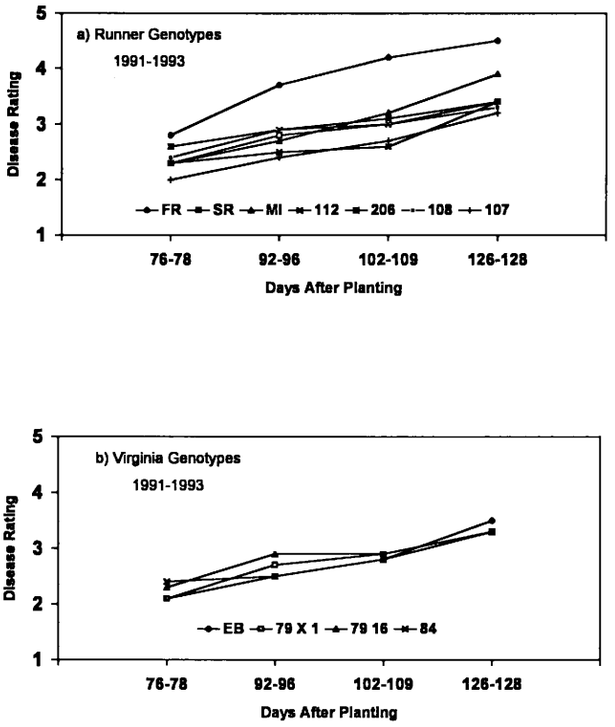

Fig. 2. Stem rot disease progress for 11 peanut genotypes inoculated with *Sclerotium rolfsii* using the agar disk technique: a) FR = Florunner, SR = Southern Runner, M1 = Marc I, 112 = UF85112, 206 = UF81206—2-Z16, 108 = UF91108, and 107 = UF86107; b) EB = Early Bunch, 791 = F79/4-6-2-1-1-Z1, 79 16 = F79/4-6-2-1-1-Z16, and 84 = 84/49-8-2-2. Values plotted are the disease rating means across years (1991-1993).

Florunner. Stem rot progress for the runner-type breeding lines was similar to that for Southern Runner and always less than for Florunner. Stem rot progress for the virginia-type breeding lines was similar to that of Early Bunch in all tests (Fig. 2b) and typically below that of Southern Runner. The genotype-by-environment interaction was not significant for the AUDPC, therefore no further analysis of genotype stability across environments was performed.

AUDPC. The mean for area under the disease progress curve in all tests is given in Table 3. The mean across years and locations and across genotypes within tests are included for comparison. The AUDPC for Florunner was significantly greater than for other genotypes, except for Marc I at Marianna in 1991. Marc I typically had slightly higher AUDPC than Southern Runner, but the difference was not significant. In contrast, Early Bunch had slightly lower AUDPC than Southern Runner each year, but the differences were only significant in the 1992 test at Quincy. Results for the seven breeding lines were variable, but two runner genotypes (UF85112 and UF86107) typically had lower AUDPC than Southern Runner. These differences were significant in both tests in 1992 and for UF86107 at Marianna in 1993. The means across years and locations for all of the breeding lines except 81206-2-Z16 are very similar to the AUDPC for Early Bunch. The AUDPC across all genotypes was highly and significantly correlated with the final disease ratings (R = 0.709).

Discussion

The agar-disk inoculation method used in this study induced various levels of stem rot in the peanut geno-

Table 3. Relative areas under the disease progress curves (AUDPC) for 11 peanut genotypes inoculated with *Sclerotium rolfsii* using the agar disk technique.[a]

Genotype	1991		1992		1993		Mean
	Quincy	Marianna	Quincy	Marianna	Quincy	Marianna	
Runner							
Florunner	142	226	169	174	212	291	202
Southern Runner	111	144	134	131	151	192	144
Marc I	123	151	124	136	168	226	155
UF85112	90	137	91	106	140	172	123
UF86107	92	148	89	97	135	148	118
UF81206-2-Z16	118	154	126	125	183	179	148
UF91108	111	143	106	123	146	173	134
Mean	112	158	120	127	162	197	146
Virginia							
Early Bunch	100	122	94	116	141	172	124
F79/4-6-2-1-1-Z1	99	145	126	113	142	174	133
F79/4-6-2-1-1-Z16	105	138	137	118	158	164	137
84/49-8-2-2	106	143	105	122	127	166	128
Mean	102	137	115	118	142	169	130
$LSD_{0.05}$	25.9	34.0	32.7	23.7	40.1	38.0	

[a]Data are based on disease assessments at the Quincy and Marianna locations. Tests were analyzed separately since genotype × location × year interactions were significant ($P \leq 0.05$).

types tested. It was clear that the inoculum placed on the plants was the source of the pathogen since adjacent noninoculated plants were generally healthy, although some disease was noted, particularly in the more susceptible genotypes late in the season. Most, but not all, of the plants of the highly susceptible genotypes were killed by stem rot by the end of each experiment.

The Agar Disk Inoculation Method. The agar-disk inoculation method worked very well for testing individual peanut genotypes for resistance to stem rot in the field. Advantages and disadvantages of this method have been discussed by Shokes *et al.* (23). The agar disk method was used in this study because it brings the pathogen into direct contact with the plant without bypassing any phenologic or metabolic resistance mechanisms, closely simulates natural inoculum, and allows little opportunity for plant escape. This technique is very useful for single-plant inoculations and for evaluations of limited numbers of plants. We evaluated up to 56 genotypes in a given test with as many as 1680 total plants.

By inoculating individual flagged plants, it was possible to use multiple disease assessments for resistance evaluations allowing genotype comparisons based on AUDPC. Multiple assessments allowed a more precise evaluation than the single assessment of stem rot that is usually used after the plants are inverted. Even when genotypes cannot be statistically separated within a given test, breeding lines with consistently lower AUDPC than susceptible checks in tests conducted at different locations and environments are likely to be superior to lines with higher values of AUDPC.

Individual Plant Assessment. Seed were space-planted about 22 cm apart to allow individual plants to grow with minimal interference from neighboring plants. This also allowed easy identification of individual plants for inoculation and assessment. This is a tedious process because each plant must be individually observed. Such examination reveals differences in genotype response to the pathogen. In the case of susceptible plants, evaluation becomes easier as the season progresses and more of the plant exhibits disease. Lines with a moderate level of resistance are more difficult to evaluate. Sometimes lesions develop and the disease stops. On other plants of the same genotype, stem rot may progress and kill one or several lateral branches and then stop. In other cases the disease may continue and kill an entire plant while others of the same genotype survive. The disease response of given genotypes varies as stem rot progresses and is affected by environmental factors such as temperature and moisture. The microenvironment may vary somewhat depending on the canopy structure or the growth habit of a particular genotype. These growth characteristics may be a function of individual plant vigor, genetic potential, and competition with nearby plants.

By using the disease assessment scale that we developed it was possible to assess a number of plants fairly rapidly. It took approximately 8 hr to assess a test with 1680 inoculated plants. The 1 to 5 scale allowed a breakdown of plant responses that accounted for most of the variables we encountered. There were some variables which could not be accurately assessed. For example, occasionally the central stem of a given genotype died whereas the lateral branches survived. When this occurred, yield potential was drastically affected and the assessment depended on the judgment of the rater. Plants which had the central stems killed usually received a high score on the 1 to 5 scale and these plants produced very few pods (based on observations of inverted plants).

Genotype Comparisons. As expected, Florunner suffered greater damage from stem rot than the other genotypes discussed in this paper. Southern Runner, which has been noted to have partial resistance to stem rot in other field trials (3), had considerably less disease than Florunner, but higher ratings than Early Bunch. Southern Runner is a late maturing (145 to 150 d) cultivar that was released by the Florida Agricultural Experiment Station in 1985 mainly because of its partial resistance to late leaf spot caused by *Cercosporidium personatum* (Berk. & Curt.) Deighton (10). Southern Runner was known to have partial resistance to rust (*Puccinia arachidis* Speg.) and web blotch (*Phoma arachidicola* Marasas, G. D. Paur, & Boerema). Southern Runner also has resistance to tomato spotted wilt virus and stem rot (3, 7). The virginia cultivar Early Bunch consistently exhibited a slightly greater resistance to stem rot than Southern Runner in our study. However, Early Bunch is seldom grown commercially because of its high susceptibility to Pythium pod rot, poor oil chemistry, and blanching characteristics (E. B. Whitty, pers. commun.).

Marc I was released by the Florida Agric. Exp. Sta. in 1992 to provide growers a high yielding, early maturing (125 to 130 d) runner cultivar (9). Little was known of the response of Marc I to stem rot disease at the beginning of this study. It usually ranked between Florunner and Southern Runner in response to stem rot and exhibited a low level of resistance in some tests. This was evidenced by a slightly higher, but generally similar, AUDPC for Marc I compared to Southern Runner in five of the six tests.

By assessing multiple plants (five per plot or 30 per genotype), we were able to arrive at a genotype mean that accurately described the above-ground response to stem rot. Although genotype performance varied in different environments and locations, some genotypes exhibited reasonably good resistance across environments. For example, the runner-type breeding line UF85112 had the lowest or second lowest AUDPC in four of the six tests (Table 3). Breeding line UF86107 had the lowest or second lowest AUDPC in five of the six tests. Therefore, these two genotypes are expected to do well when exposed to *S. rolfsii* in different environments. Both lines have Early Bunch in their pedigree. The runner breeding line UF91108 and the three virginia lines F79/4-6-2-1-1-Z1, F79/4-6-2-1-1-Z16, and F84/49-8-2-2 all appear to have similar resistance to stem rot. The breeding line UF91108 has exhibited some resistance to late leaf spot and TSWV in other tests (Gorbet, unpubl. data). This line has both Southern Runner and Early Bunch in its pedigree (Table 1). As a group, the virginia

genotypes had slightly less disease than the runner types (Tables 2 and 3). In general, the resistant genotypes performed similarly across environments.

From this study promising genotypes were identified with resistance to stem rot caused by *S. rolfsii*. Testing genotypes with attention to individual plant responses, though tedious, allowed disease progress to be followed and genotypes to be selected for determination of yield responses. Genotypes which have resistance to stem rot need to be tested for other important diseases as well. It is simpler to test for one major disease at a time, as we did in these studies, since multiple disease effects may confound results and make selection more difficult. Several genotypes from the tests discussed here are undergoing further development and one line, UF91108, has been released as FL MDR98.

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