Progress In Breeding Sclerotinia Blight-Resistant Runner-Type Peanut[1]

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ABSTRACT

Field screening tests were conducted in 1991, 1992, and 1993 to identify Sclerotinia blight-resistant runner-type peanuts. Selections were made from three populations, "backcross to runner," "backcross to Tamspan 90," and "single cross." Runner parents were chosen for agronomic qualities and resistance to other pathogens. Resistant (spanish) parents used were cultivar Tamspan 90 and TxAG-5 germplasm. Families within populations were planted as single row 3 x 0.9-m plots. Repeated plant-by-plant inspections (PBPI) were made for assessment of sequence of infection and subsequent calculation of an area under the disease progress curve (AUDPC). Most families in the backcross to Tamspan 90 population consistently had a disease reaction close to or lower than Tamspan 90 but also retained undesirable plant forms (i.e., upright). By 1993, the $F_{2:5}$ backcross to runner population had the highest percentage of runner-type families (88%) and a mean disease reaction close to Tamspan 90. Whole plot evaluation of Sclerotinia blight severity was compared with PBPI for effectiveness on a segregating population containing a total of 298 $F_{4:7}$ single cross and $F_{2:5}$ backcross entries. The whole plot system used a scale of 0 = no visible plot infection to 5 ≥80% infection in the plot. Correlation between area under disease progress curve values of both methods was high (>.85). Remnant seed of selections screened for resistance in the greenhouse were planted in the field and evaluated for resistance. Chi-square test for independence indicated a significant (α = .012) relationship between greenhouse and field performance. Field screening and limited greenhouse screening appears to be an effective way to develop resistant runner-type peanut lines.

Key Words: *Arachis hypogaea* L., *Sclerotinia minor*, groundnut, soil-borne pathogen, host plant resistance.

Sclerotinia blight of peanut (*Arachis hypogaea* L.) caused by *Sclerotinia minor* Jagger was first discovered in the United States in 1971 in Virginia (10). Symptoms of the disease were found in a peanut field in Mason County, Texas in the fall of 1981. Since that time, Sclerotinia blight has been observed in 10 commercial peanut-producing counties in Texas and *S. minor* now is considered to be a major soil-borne pathogen of peanut in the state (19). The pathogen can be disseminated by several methods and once established becomes difficult to eradicate. The fungus can survive at least 5 yr without a host and has several alternate weed hosts (9, 12, 13, 20). Currently registered fungicides have some efficacy against the disease (10), and effectiveness can be enhanced by using temperature threshold information to time chemical application (2).

Host plant resistance would provide the most effective solution to the Sclerotinia blight problem. Peanut cultivars with spanish ancestry appear to be more resistant to Sclerotinia blight than other cultivars or breeding lines (1). Crop morphology also plays an important role in host plant resistance (4). Plant canopies affect soil temperature, soil moisture, amount and duration of leaf wetness, canopy relative humidity, and canopy temperature (5, 14). When plant canopy was modified by thinning of foliage to allow air circulation that would decrease canopy humidity, significantly less disease resulted when compared to unmodified canopies (6).

Tamspan 90 is a spanish peanut cultivar with host plant resistance to both pod rot (*Pythium myriotylum* Drechs) and Sclerotinia blight (18). The mechanism of resistance is not due just to plant architecture since the canopies of spanish cultivars Pronto, Spanco, and Starr are similar to Tamspan 90 but do not contain resistance. Farmers in Texas and Oklahoma have reported that Tamspan 90 produced very acceptable yields of 4032-4144 kg ha^{-1} in areas seriously affected by *S. minor* in previous years. Field tests of TxAG-4 and TxAG-5 spanish peanut germplasm in infested soil in Oklahoma were conducted in 1982 and 1985 through 1988. The maximum percentage of diseased TxAG-4 and TxAG-5 plants in these tests was 16% as compared to 98% for cv. Florunner, a runner-type peanut (16).

Inheritance of resistance to *S. minor* is not clearly understood. Wildman *et al.* (19) reported that at least two loci were responsible for resistance in TxAG-5. However, variation among resistance groupings within $F_{2:3}$ and $BC_1F_{2:3}$ families interfered with distinct classifications for genetic determinations.

Several screening techniques for identifying resistance have been documented (3, 8). These tests rely on rate of lesion growth and development. The age and/or developmental state of the lateral limbs of plants had a marked effect on lesion development (3). Melouk *et al.* (8) developed a detached shoot technique that was useful in assessing resistance to Sclerotinia blight in peanut genotypes and might be useful in screening populations segregating for resistance to the disease.

Because of a reduction in demand for spanish peanuts a breeding program targeted at development of an acceptable resistant runner-type peanut cultivar was initiated. The approach used included developing backcross populations with *S. minor*-susceptible runner or *S. minor*-resistant spanish parents and evaluation under field

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and greenhouse conditions. The purpose of the study reported herein was to evaluate the progress and some of the techniques used in that effort.

Materials and Methods

Field Testing. All field testing was performed at the Texas Agricultural Experiment Station, Stephenville, TX where Sclerotinia blight was first identified on peanut in research plots in 1986 and continues to proliferate. The soil type is a Windhorst fine sandy loam. Peanut was cultivated on the research site 1972 to 1986 with fallow in alternate years. Since 1986 the area has been in continuous peanut, managed as a disease nursery, and the inoculum level is high. Standard clean-till cultural practices were followed along with frequent irrigation to provide environments conducive for disease development. Three populations, "backcross to runner," using adapted runner types as the recurrent, susceptible parent; "backcross to Tamspan 90" using Tamspan 90 as the recurrent, resistant parent; and "single crosses" between runner and spanish were screened in the field for disease reaction to *S. minor* starting in 1991 (Table 1). The runner parents were Tamrun 88 (17), Southern Runner (7), TP107-11, and VP8140. Twelve families were evaluated for disease reaction and compared with Tamspan 90 (resistant); Florunner, a commercial runner cultivar having a low level of resistance; and Langley, a commercial runner cultivar that is extremely susceptible. Check plots were randomly scattered within populations.

Backcross $F_{2:3}$ and single cross $F_{4:5}$ families within populations were planted without replication in single-row plots 3 m long and spaced at 0.9 m apart. Plots were comprised of plants from 21 seed spaced 15 cm apart. Each plant was inspected visually—i.e., individual plant by plant inspection (PBPI), for branch terminal wilting and lesion formation. Weekly inspections were initiated 23 Aug. 1991 and 20 Aug. 1992, when maximum daily soil temperatures dropped below the critical temperature of 28 C (82 F) for disease development (21), and continued until 10 Oct. 1991 and 1 Oct. 1992. Colored flags, coded by date of inspection, were placed by each infected plant at the onset of appearance of symptoms. Selections were made on the basis of family resistance to infection as measured by disease incidence, with secondary consideration given to date of infection and important agronomic traits including growth habit and fruit characteristics. The families selected in 1991 were retested in 1992 using the same evaluation method as in 1991. The 1992 selections were re-evaluated in 1993 but, because of time and labor restrictions and preliminary results from this study, a single final visual rating (0-10) was used as follows:

Rating	Symptoms
0	No visible symptoms
1	1 or few wilting branches
3	30% of plants wilting or dead
5	50% of plants wilting or dead
9	90% of plants dead
10	All plants dead

An area under the disease progress curve (15) disease value for the season that accounts for different levels of disease at different dates was calculated as:

$$AUDPC = \sum_{i=1}^{n} [(X_i+X_{i+1})/2]\,[t_{i+1}-t_i] \qquad [Eq.1]$$

where:

X_i = rating at ith observation or total flags at ith observation.

t_i = time (days) after the i^{th} observation.

n = total number of observations.

Curves were standardized by dividing by number of days from first to final evaluation.

Disease reaction was compared among the three main populations for 1991, 1992, and 1993. Distribution of families within main populations was used to show which families were most frequently selected.

Comparison of Disease Assessment Methods. A disease evaluation method, based on whole plot infection, was compared with PBPI for effectiveness on a segregating population containing a total of 298 $F_{4:7}$ single cross and $F_{2:5}$ backcross families. The whole plot method (WPM) was based on percentage visible plot infection and used the following scale:

Rating	Symptoms
0	No visible symptoms
1	1-20% of plants wilting or dead
2	21-40% "
3	41-60% "
4	61-80% "
5	81-100% "

Both WPM and PBPI evaluations were made at weekly intervals seven times during the 1993 season beginning 9 Sept. The experimental design was completely random without replication. A Pearson correlation coefficient was calculated based on AUDPC estimates obtained from WPM and PBPI data.

Comparison of Greenhouse and Field Screening. Remnant seed of 118 $F_{2:5}$ backcross and $F_{2:6}$ single cross families screened for disease reaction in a greenhouse were field evaluated in the disease nursery at Stephenville in 1993. Field tests were planted in a randomized complete block design with two replications. Disease assessments

Table 1. Composition of populations screened starting in 1991.

Population	Family pedigree	Plot no.
	$F_{2:3}$ generation	
Backcross to runner using TxAG-5 as nonrecurrent parent	So. Runner//So. Runner/TxAG-5	136
	Tamrun 88// Tamrun 88/TxAG-5	160
	TP107-11//TP107-11/TxAG-5	229
	$F_{2:3}$ generation	
Backcross to Tamspan 90 using runner type as nonrecurrent parent	Tamspan 90//So. Runner/Tamspan 90	150
	Tamspan 90//Tamrun 88/Tamspan 90	171
	Tamspan 90//TP107-11/Tamspan 90	202
	$F_{4:5}$ generation	
Single cross	So. Runner/Tamspan 90	110
	Tamspan 90/VP8140	35
	Tamspan 90/TP107-11	30
	VP8140/So. Runner	76
	VP8140/Tamrun 88	92
	VP8140/TP107-11	84
Check cultivars	Tamspan 90	
	Florunner	
	Langley	

were made using the PBPI system. Greenhouse evaluation of all 118 families was performed at Stillwater, OK. Ten random plants of each family were grown in individual pots (10 cm dia.) in a mixture of sand, soil, and shredded peat . Each plant was inoculated when 6 wk old and containing four to five nodes, by placing a 4-mm dia. mycelial plug from the periphery of a 2-d-old culture of *S. minor* at the axil between the main stem and leaf petiole at the first node. Inoculated plants were incubated in fabricated plastic boxes kept at 100% relative humidity with day and night temperatures ≅ 29 and ≅ 23 C, respectively. Five to 7 d after inoculation, disease reaction was measured as percent lesion severity on a scale of 1 to 5 as follows:

Rating	Symptoms
1	None
2	1-25%
3	26-50%
4	51-75%
5	>75%

Family disease assessments were derived by summation of disease reaction scores for all plants; hence a maximum score of 50 was possible. A Chi-square test for independence was used to determine if greenhouse results and 1993 field performance were associated. Families in the greenhouse that had a mean rating of ≤ 30 on a scale from 0-50, and families in the field with a AUDPC ≤ 67 (not standardized) were considered to contain some level of resistance.

Results

Tamspan 90 had significantly less disease injury than either Florunner or Langley all 3 yr. During 1991 and 1993, all checks differed significantly in disease ratings, with Tamspan 90 lowest and Langley highest, but in 1992 Florunner and Langley means were not significantly different (Table 2).

Table 2. Disease ratings for check cultivars in field plots at Stephenville, TX.[a]

	1991			1992			1993		
Cultivar	Mean	S.E.	no.	Mean	S.E.	no.	Mean	S.E.	no.
Tamspan 90	3.0 a	0.40	62	2.4 a	0.33	31	3.0 a	0.11	98
Florunner	5.1 b	0.45	63	5.0 b	0.61	31	5.5 b	0.19	98
Langley	7.5 c	0.58	63	4.6 b	0.54	31	7.5 c	0.20	98

[a]1991 and 1992 values based on AUDPC. 1993 disease rating based on average single visual observation. Means in columns followed by the same letter are not significantly different (P=0.05) as determined by the Waller-Duncan K-ratio T Test.

Selection for spreading vine form was most effective in the "single cross" and "backcross to runner" populations. The number and percentage of spreading-type families retained from the "backcross to Tamspan 90" population consistently were lower than in the other populations, and from 1991 to 1992 the percentage of plots with spreading vine growth decreased (Table 3). By 1993, the "backcross to runner" population had the highest percentage of spreading families (88%) but, because of different population sizes, the "single cross" population had slightly more spreading vine-type plots.

In all 3 yr, the "backcross to Tamspan 90" population either had the lowest disease value or was not significantly different (α = .05) from the lowest rated group. During 1991 and 1992 the "backcross to runner" population had the highest disease values compared to the other two populations, but in 1993 the mean rank was not significantly different (α = .05) from the lowest disease ranked population, "backcross to Tamspan 90." The "single cross" population showed the most variability for the 3 yr. In 1991 it had the lowest disease value, in 1992 it fell between the "backcross to Tamspan 90" population and the "backcross to runner" population, and by 1993 it was ranked with a significantly higher (α = .05) disease rating than the other populations (Fig. 1).

Selection for resistant runner-type families in the three populations consistently reduced population size in all years (Table 4), but at least one plot from all families within main populations was maintained until 1993. Backcross to Tamrun 88—i.e., TxAG-5/Tamrun 88// Tamrun 88—had the most entries left by 1993 and phenotypically appeared to be the best family. Other families with relatively low disease values but not as acceptable agronomically as backcross to Tamrun 88 included TxAG-5/TP107-11//TP107-11 and the single crosses VP8140/TP107-11 and Southern Runner/ Tamspan 90.

The coefficient of correlation between whole plot screening and PBPI partial AUDPCs was only 0.29 for the first inspection, but rapidly increased from the second inspection (r = 0.67) until the fourth inspection (r = 0.86). The correlation (r ≥ 0.88) remained high from inspection four to the end.

The Chi-square test indicated a significant relationship (α = .012) between field and greenhouse results. A 2x2 contingency table was generated and conditional probabilities were estimated. Based on this data set, the probability of an entry performing well in the field after having performed well in the greenhouse was 33%, and the probability of an entry performing poorly in the field

Table 3. Proportion of plants with spreading vine form in families grown in 1991, 1992, and 1993.

	1991			1992			1993		
Population	Spreading	Total	%	Spreading	Total	%	Spreading	Total	%
	--- no. ---			--- no. ---			--- no. ---		
Backcross to Tamspan 90	200	523	38	21	60	35	13	22	59
Single cross	223	441	51	75	129	58	33	42	78
Backcross to runner	--	525	--	74	120	62	31	35	88

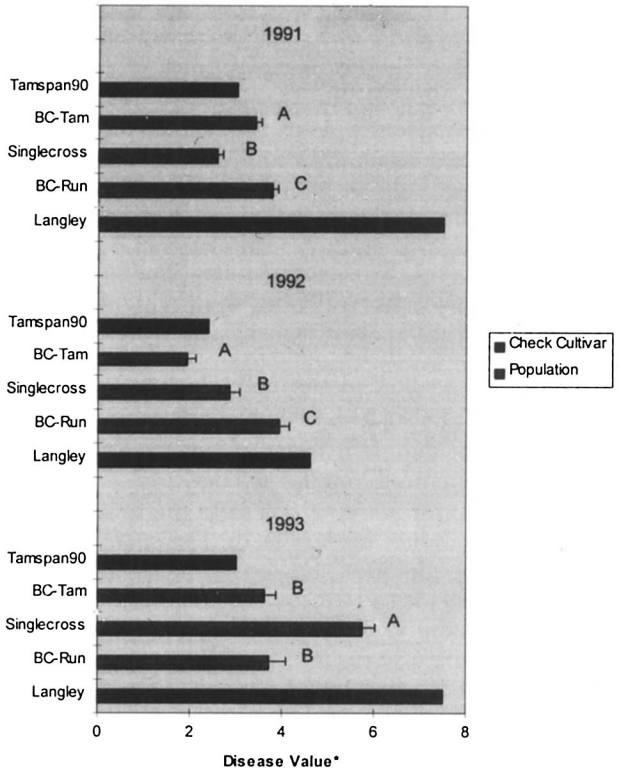

Fig. 1. Comparative reaction to disease of populations evaluated in 1991, 1992, and 1993. Values for 1991 and 1992 are from standardized area under disease progress curves. The 1993 values are late season, single date visual scores where 0 = no disease and 10 = plant death. BC-Tam = Backcross to Tamspan 90 population, BC-Run = Backcross to Runner population. Populations with the same capital letter within years are not significantly different (P=0.05) as determined by the Waller-Duncan K-ratio T Test.

Table 4. Distribution after 2 yr of selection for resistant runner-type families.

Population/family	Total plots/yr 1991	1992	1993
	--------- no. ---------		
Backcross to runner			
So. Runner // So. Runner / TxAG-5	136	20	2
Tamrun 88 // Tamrun 88 / TxAG-5	160	39	17
TP107-11 // TP107-11 / TxAG-5	229	61	14
Total	525	120	35
Backcross to Tamspan 90			
Tamspan 90 // So. Runner / Tamspan 90	150	18	5
Tamspan 90 // Tamrun 88 / Tamspan 90	171	23	9
Tamspan 90 // TP107-11 / Tamspan 90	202	19	8
Total	523	60	22
Single cross			
So. Runner / Tamspan 90	110	30	13
Tamspan 90 / VP8140	35	1	1
Tamspan 90 / TP107-11	30	19	10
VP8140 / So. Runner	76	7	1
VP8140 / Tamrun 88	92	45	3
VP8140 / TP107-11	84	27	14
Total	427	129	42

after having performed poorly in the greenhouse was 87%.

Discussion

Selection for spreading vine form with resistance to Sclerotinia blight was effective in this study. Initial concern that the resistance displayed in the erect spanish-type lines was due primarily to their open canopy, and not some physiological factor, was not verified. This was most easily seen in the backcross to runner and single cross populations, where the percentage of spreading vine form families consistently increased (Table 3) and selections were almost always lower in disease value than Tamspan 90 (data not shown).

A considerable amount of variability for disease reaction among and within populations was seen throughout all 3 yr. Although the "backcross to Tamspan 90" population consistently had a disease reaction close to or lower than Tamspan 90, it also had the most families with undesirable vine types. This is reflected in the lower percentage of "backcross to Tamspan 90" selections retained from the first year of selection. These results indicate that plant architecture is a factor, but not the only factor, that determines plant reaction to the disease. The "single cross" and "backcross to runner" populations showed similar reactions in 1991 and 1992, with AUDPC for the "single cross" population significantly lower than that for the "backcross to runner" populations (Fig. 1). In 1993, however, mean rating for the "backcross to runner" population was lower than the "single cross" population (Fig. 1). This might be explained by the effect of selection over 2 yr; it was not until 1993 that the elite families had a noticeable effect on the population mean. Other sources of variability could be the use of different disease evaluators within and between years, changes in evaluation method, different number of disease inspections among populations within and among years, relatively small population sizes by 1993, and the inherent unpredictability of epidemics caused by soil-borne pathogens.

Correlations between the two field screening methods by the end of the 1993 season were high. The low correlation for the first two inspections was because of the thoroughness of the PBPI system. When visible symptoms of the disease were not readily observed in the WPM, a score of zero was recorded. In contrast, with careful inspection of all plants in the plot (PBPI), infected plants were labelled in early stages of disease development. After the epidemic was well under way, the correlations improved. Individual plant response to infection was not accounted for using the PBPI system. If a plant displayed an initial infection symptom—i.e., small lesion, but contained enough resistance to survive to harvest—it was flagged the same as a plant that died within a few days of initial infection. Two different plots could, therefore, have 21 infected plants and 21 flags, but have very different levels of resistance. The whole plot inspection system did not rely on individual plant infection as a means of disease evaluation. If the plants in the plot were infected, but displayed minimal visual plant damage, a low rating would be given that would reflect

restricted movement of disease within plants. Correlation between the two methods was high probably because they were based on AUDPC estimates which account for disease progression over time and not a single rating.

An advantage of the PBPI system is the ability to discern among pathogens displaying symptoms similar to *S. minor* and avoid mislabeling. The main disadvantage of the PBPI system is the time it takes for implementation. It required two people almost 3 hr to flag approximately 300 plots on one inspection date. With the use of a small hand-held computer containing a spreadsheet with field maps, the whole plot system, although not as meticulous as PBPI, required about 45 min for a single inspector to rate 300 plots. For large field screening experiments the whole plot system is more practical, but for small field experiments that are known to have other pathogens present the more tedious PBPI system combined with a single final visual rating is more applicable.

Results from the greenhouse screening and field performance tests show that greenhouse screening can be used to supplement field studies. With these particular crosses, greenhouse screening would have been effective for the elimination of highly susceptible families before they were tested in the field. Other criteria could be used in greenhouse evaluations that might strengthen the field and greenhouse relationship, such as the number of plants per family that displayed a complete vascular wilt of the main stem at the inoculation site.

Field and greenhouse screening are valid tools for developing runner-type cultivars with resistance to *S. minor*. Field testing has the advantages of creating a natural disease environment and the ability to evaluate large sample sizes. Future advancements in greenhouse screening might strengthen the relationship with field results making greenhouse selection more useful in a breeding program.

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