Peanut Science (1982) 9, 6-10

Resistance to Rust and Late Leafspot Diseases in Some Genotypes of *Arachis hypogaea*[1]

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ABSTRACT

Resistance to rust (*Puccinia arachidis* Speg.) and late leafspot (*Cercosporidium personatum* (Berk. & Curt.) Deighton) in some peanut genotypes was studied under field conditions. Late leafspot development was also assessed in the glasshouse and the parameters lesion diameter, defoliation percentage and sporulation gave highly significant correlations with the field disease scores. Several genotypes were found to be resistant to both rust and late leafspot and should be useful sources of multiple disease-resistance in a breeding program.

Key Words: groundnut, field screening, glasshouse screening

The leafspots, *Cercospora arachidicola* Hori and *Cercosporidium personatum* (Berk. & Curt.) Deighton, are serious diseases of peanuts on a world scale (6, 10). Rust of peanuts, caused by *Puccinia arachidis* Speg., has also become a worldwide problem since 1969 (8, 17). Losses in yields of around 10% have been estimated in the USA due to leafspots, where fungicide application is normally practiced (10). In the semi-arid tropics, where chemical control is rarely used, losses in excess of 50% are commonplace (6). Although the diseases can be controlled by certain fungicides, these are costly and are not readily available to small-scale farmers in the semi-arid tropics (6). Screening for resistance to leafspots and rust has been intensively carried out by many workers and a number of sources of resistance have been reported in both cultivated peanuts and wild *Arachis* species (1, 2, 7, 9, 11, 13, 14, 15, 16, 18). In recent years efforts have been extended to identify sources of resistance to more than one pathogen (3, 5, 12).

In this paper, the evaluation of some peanut genotypes for resistance to both rust and late leafspot (*C. personatum)* is reported.

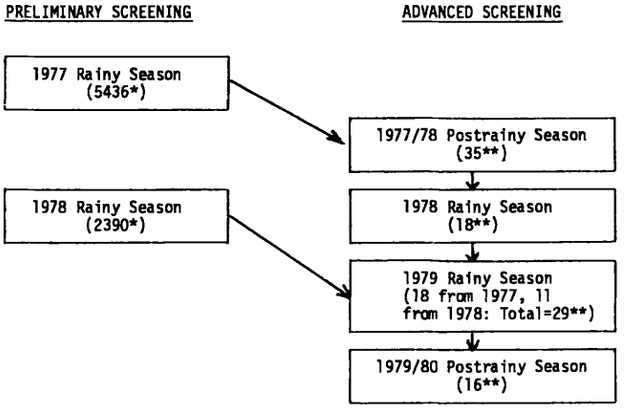
Materials and Methods

Field screening for resistance to rust and late leafspot:

Screening of a world collection of peanut germplasm for resistance to rust and late leafspot diseases was started at ICRISAT Center near Hyderabad, India, in the 1977 rainy season and continued in subsequent postrainy and rainy seasons. A total of 7,826 genotypes were screened in the first 3 years of the project as shown in Figure 1.

Preliminary screening was done on germplasm multiplication material in the rainy seasons. Genotypes were grown in unreplicated plots of 2 rows 75 cm apart and 5 m long. Plots of the cultivars, TMV 2 and Robut 33-1, known to be highly susceptible to rust and late leafspot, were arranged throughout the germplasm fields, one to every ten test genotypes. One week before harvest each genotype was scored for the development of both diseases using a 9-point scale (Table 1). Genotypes rated between scores 1 and 5 for either disease were selected for advanced screening (Table 2).

Advanced screening was done in both rainy and postrainy seasons. Plots were of the same size as in preliminary screening but there were two replications. Test plots were separated by single infector rows of a mixture of cultivars TMV 2 and Robut 33-1 which were sown 14 days before the test material. TMV 2 and Robut 33-1 were also sown in test plots to monitor disease spread from infector rows. To encourage the development of rust disease in the postrainy season irrigated crop, the infector rows were inoculated with a uredospore suspension at the time of peak flowering. The suspension (50,000-100,000 spores/mL) was made up in tap water to which a small amount of the wetting agent Tween 80 had been added. The inoculation was done in the evening following furrow irrigation. Potted "spreader" plants heavily infected with rust were placed systematically throughout the field to serve as additional sources of inoculum. Following inoculation, the field was irrigated with over-



* No. of genotypes screened in 1977 and 1978 rainy seasons.

** No. of genotypes selected for further testing.

Fig. 1. Field screening of peanut genotypes for resistance to rust and late leafspot diseases at ICRISAT 1977-80.

Table 1. The 9-Point Field Scale for foliar disease assessment.

Late Leafspot	Score	Rust
No disease	1	No disease
Few, small necrotic spots on older leaves	2	Few, very small pustules on some older leaves
Small spots, mainly on older leaves, sparse sporulation	3	Few pustules, mainly on older leaves, some ruptured, poor sporulation
Many spots, mostly on lower and middle leaves, disease evident	4	Pustules small or large, mostly on lower and middle leaves, disease evident
Spots easily seen on lower and middle leaves, moderately sporulating, yellowing and defoliation of some lower leaves	5	Many pustules, mostly on lower and middle leaves, yellowing and necrosis of some lower and middle leaves, moderately sporulating
As rating 5 but spots heavily sporulating	6	As rating 5 but pustules heavily sporulating
Disease easily seen from a distance; spots present all over the plant; lower and middle leaves defoliating	7	Pustules all over the plant: lower and middle leaves withering
As rating 7 but defoliation is more severe	8	As rating 7 but withering is more severe
Plants severely affected, 50-100% defoliation	9	Plants severely affected, 50-100% leaves withering

[1]Contribution from the International Crops Research Institute for the Semi-Arid Tropics. ICRISAT Journal Article Approval No. 202.

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Table 2. Description of genotypes in advanced screening trials in the 1979-80 postrainy season at ICRISAT.

Genotypes	ICG No.*	Botanical type (var.)	Seed colour	Origin/Source
Selected for resistance				
NC Ac 17090	1675	*fastigiata*	tan	Peru
NC Ac 17133 (RF)	7013	*fastigiata*	purple	ICRISAT selection, originally from Peru
EC 76446 (292)	2716	*fastigiata*	purple	Uganda, probably recently introduced from South America
PI 259747	4747	*fastigiata*	purple	Peru
PI 350680	6340	*fastigiata*	purple	Honduras
NC Ac 927	6022	*fastigiata*	purple	Sudan
NC Ac 17127	1703	*fastigiata*	tan with purple stripes	Peru
NC Ac 17130	1705	*fastigiata*	tan	Peru
NC Ac 17129	1704	*fastigiata*	light tan	Peru
NC Ac 17132	1707	*fastigiata*	purple	Peru
NC Ac 17135	1710	*fastigiata*	purple	Peru
NC Ac 17124	6280	*fastigiata*	tan	Peru
NC Ac 17142	1712	*fastigiata*	dark tan	Brazil
Krapovikas Strain 16	4790	*fastigiata*	purple	Argentina
RMP 91	6323	*hypogaea*	tan	Upper Volta
NC Ac 15989	2379	*hypogaea*	purple	Bolivia
Susceptible 'checks'**				
TMV 2	221	*vulgaris*	tan	India
Robut 33-1	799	*hypogaea*	tan	India

* ICRISAT groundnut accession number; ** commercially grown cultivars in India

head sprinklers, on alternate days initially and then at irregular intervals until harvest.

No inoculation was made with the late leafspot pathogen, there being good development of the disease from natural inoculum sources in both seasons. the genotypes were rated for both rust and late leafspot development using the 9-point scale just before harvest.

Glasshouse screening for resistance to late leafspot:

The 16 resistant genotypes selected from advanced field screening and the 2 susceptible cultivars, TMV 2 and Robut 33-1, were tested for reactions to late leafspot disease in the glasshouse in March/April 1980. Plants were grown in a mixture of red sandy soil and farmyard manure (4:1 v/v) in 15 cm diameter plastic pots, 2 plants per pot.

Cercosporidium personatum conidia were collected from incubated, inoculated, detached leaves of the cultivar TMV 2, and suspended in sterile tap water containing a few drops of the wetting agent Tween 80. The inoculum was adjusted to approximately 50,000 conidia/mL.

Two screening trials were carried out, plants being inoculated in one when 30 days old and in the other when 50 days old. In the trial with 50 day old plants, 2 genotypes, NC Ac 927 and RMP 91, could not be included. For each test plant, all leaves on the main stem were tagged and then inoculated with the conidial suspension using an atomiser. The plants were then kept in a mist chamber for 48 hours after which time they were replaced on the glasshouse bench in a randomized complete block design with 5 replications of 2 plants (1 pot) or each genotype. Temperature in the glasshouse ranged from 25 to 35 C.

Disease development was determined in both trials at 28 and 42 days after inoculation. The parameters evaluated were:

a) **Percentage defoliation:** The total number of leaflets on the main stem and the number of abscised leaflets were counted on each plant and percentage defoliation was calculated.

b) **Percentage leaf area damaged:** The leaf area damaged was estimated for all leaves on each main stem by comparison with diagrams depicting leaves with known percentages of their areas affected (9).

c) **Infection frequency:** At 28 days after inoculation the total numbers of lesions on each main stem leaf were counted. Leaf areas were estimated by comparison with drawings of leaves of known areas. Infection frequencies were expressed as numbers of lesions/cm^2 leaf area.

d)**Lesion deameter:** For each main stem leaf the diameters of 5 lesions were measured.

e) **Sporulation:** At 42 days after inoculation, 5 leaflets were taken from the middle of each main stem and incubated on moist filter paper in petri dishes maintained at 25 C under continuous illumination in a Percival plant growth chamber for 5 days. Lesions were then examined under a stereo-microscope (x 20) and the degree of sporulation was scored on a 5-point scale (1 = no sporulation; 5 = extensive sporulation).

The percentage values were subjected to arcsine transformation and the trials were analysed separately. To study the effect of plant age, the 2 genotypes which were not included in the trial with 50 day old plants were deleted from the trial with 30 day old plants and a combined analysis was carried out.

Results and Discussion

Field screening: There was uniform disease development on infector rows and on susceptible cultivars in test plots. The mean and range of disease scores over seasons are shown in Table 3 for selected genotypes and the susceptible cultivars TMV 2 and Robut 33-1 for both rust and late leafspot. The genotypes NC Ac 17133 (RF), EC 76446

Table 3. Field disease scores for rust and late leafspot for selected genotypes grown for several seasons at ICRISAT.

Genotypes	Disease scores* over season for:			
	Rust		Late leafspot	
	Mean score	Range	Mean score	Range
NC Ac 17090	2.0 a	(2-2)	4.8 b	(4-5)
NC Ac 17133 (RF)	3.0 b	(3-3)	3.3 c	(3-4)
EC 76446 (292)	3.0 a	(3-3)	3.2 b	(3-4)
PI 259747	3.0 b	(3-3)	3.3 c	(3-4)
PI 350680	3.0 c	(3-3)	3.3 c	(3-4)
NC Ac 927	3.3 c	(3-4)	4.0 c	(4-4)
NC Ac 17127	3.8 a	(3-4)	4.3 b	(4-5)
NC Ac 17130	4.0 a	(4-4)	4.8 b	(4-5)
NC Ac 17129	4.0 a	(4-4)	4.8 b	(4-5)
NC Ac 17132	4.0 a	(4-4)	4.8 b	(4-5)
NC Ac 17135	4.0 a	(4-4)	4.8 b	(4-5)
NC Ac 17124	4.0 a	(4-4)	4.8 b	(4-5)
NC Ac 17142	5.0 a	(5-5)	4.8 b	(4-5)
Krapovikas Strain 16	5.0 c	(5-5)	4.3 b	(4-5)
RMP 91	7.0 c	(5-9)	4.7 c	(4-5)
NC Ac 15989	7.3 c	(5-9)	4.7 c	(4-5)
TMV 2	9.0 a	(9-9)	9.0 a	(9-9)
Robut 33-1	9.0 a	(9-9)	9.0 b	(9-9)

* Scored on 9-Point Scale a = mean score of 5 seasons

b = mean score of 4 seasons c = mean score of 3 seasons

(229), PI 259747 and PI 350680 showed good resistance to both rust and late leafspot in all field trials. The genotypes NC Ac 927, NC Ac 17127, NC Ac 17120, NC Ac 17129, NC Ac 17132, NC Ac 17135, NC Ac 17124, NC Ac 17142 and Krapovikas Strain 16 showed moderate resistance to both pathogens.

Some genotypes showed different levels of resistance to the two pathogens. NC Ac 17090 was most resistant to rust but was only moderatly resistant to late leafspot. Genotypes RMP 91 and NC Ac 15989 showed greater resistance to late leafspot than to rust.

Glasshouse screening: In both trials, i. e., for plants inoculated with the late leafspot pathogen at 30 days or at 50 days old, statistically significant differences were found between genotypes for the parameters, percentage defoliation, percentage leaf area damaged, infection frequency, lesion diameter, and sporulation. From the combined analysis of trials with 30 day and 50 day old plants, significant interaction was observed between plant age and genotypes for all the parameters except sporulation.

Percentage defoliation figures are shown in Table 4. Defoliation increased with time from inoculation and was greater in some genotypes than in others. Genotypes NC Ac 17133 (RF), EC 76446 (292), PI 259747, PI 350680, NC

Table 4. Percentage defoliation of selected genotypes grown in the glasshouse and inoculated with *C. personatum* at 30 or 50 days after sowing.

Genotypes	Plant age when inoculated: 30 days		50 days	
	% defoliation at (days after inoculation): 28	42	% defoliation at (days after inoculation): 28	42
NC Ac 17090	10.2	31.8	15.6	59.1
NC Ac 17133 (RF)	5.4	16.6	2.8	23.3
EC 76446 (292)	2.3	18.0	0.6	23.7
PI 259747	2.5	17.8	2.1	13.0
PI 350680	2.9	23.8	0.4	19.8
NC Ac 927	1.8	16.4	NT	NT
NC Ac 17127	8.0	25.9	6.6	40.2
NC Ac 17130	24.2	49.7	28.1	50.5
NC Ac 17129	9.2	44.6	13.5	47.2
NC Ac 17132	5.3	25.4	2.7	42.7
NC Ac 17135	0.9	27.1	0	25.0
NC Ac 17124	21.3	53.0	28.2	59.4
NC Ac 17142	32.7	55.4	21.7	54.4
Krapovikas Strain 16	1.7	21.1	0.5	26.2
RMP 91	21.3	39.1	NT	NT
NC Ac 15989	25.5	62.3	21.9	60.6
TMV 2	29.0	72.2	46.5	79.8
Robut 33-1	21.9	61.2	27.0	73.6
LSD (5%)	6.82		4.99	
CV (%)	20.20		13.79	

NT = not tested

Ac 17135 and Krapovikas Strain 16 had significantly less defoliation than either TMV 2 or Robut 33-1.

Data on percentage leaf area damaged are shown in Table 5. In both trials the leaf area damaged increased for each genotype between 28 and 42 days after inoculation. On 30 day old plants, the increase in leaf area damage was consistant across the genotypes. However, in the case of 50 day old plants there was a significant interaction between genotype and days after inoculation. Some resistant genotypes had less and some had more leaf area damaged than the susceptible TMV 2 and Robut 33-1. However, the susceptible check cultivars had suffered considerable defoliation by 42 days after inoculation (Table 4).

Table 5. Percentage leaf area damage of selected genotypes grown in the glasshouse and inoculated with *C. personatum* at 30 or 50 days after sowing.

Genotypes	Plant age when inoculated: 30 days		50 days	
	% leaf area damaged at (days after inoculation): 28	42	% leaf area damaged at (days after inoculation): 28	42
NC Ac 17090	1.2	6.3	3.7	10.1
NC Ac 17133 (RF)	2.7	6.9	1.0	3.0
EC 76446 (292)	1.7	5.1	1.0	2.2
PI 259747	5.7	7.9	0.9	1.9
PI 350680	7.2	12.9	0.9	1.9
NC Ac 927	0.9	2.5	NT	NT
NC Ac 17127	3.9	6.8	4.0	7.4
NC Ac 17130	3.1	5.9	3.9	10.9
NC Ac 17129	4.7	6.4	3.2	6.4
NC Ac 17132	4.4	7.0	0.9	7.0
NC Ac 17135	3.6	6.8	0.2	1.5
NC Ac 17124	3.8	5.6	2.8	6.4
NC Ac 17142	4.8	8.6	4.6	7.3
Krapovikas Strain 16	5.3	10.2	0.7	1.7
RMP 91	1.5	3.3	NT	NT
NC Ac 15989	3.4	7.8	1.3	3.1
TMV 2	5.3	7.2	2.9	5.5
Robut 33-1	3.1	6.8	1.5	3.5
LSD (5%)	3.14		2.42	
CV (%)	19.99		19.65	

NT = not tested

There were highly significant differences between genotypes in numbers of lesions per unit of leaf area in both trials, but ranking of genotypes differed (Table 6). Numbers of lesions per unit of leaf area are highly influenced by environmental factors (high coefficient of variation) and this parameter is of limited usefulness in disease resistance screening in the glasshouse.

Table 6. Infection frequency on selected genotypes grown in the glasshouse and inoculated with *C. personatum* at 30 or 50 days after sowing.

Genotypes	Plant age when inoculated: 30 days	50 days
	Mean lesion number/cm^2 of leaf at 28 days after inoculation	Mean lesion number/cm^2 of leaf at 28 days after inoculation
NC Ac 17090	0.19	0.85
NC Ac 17133 (RF)	0.31	0.31
EC 76446 (292)	0.24	0.23
PI 259747	1.07	0.45
PI 350680	1.25	0.38
NC Ac 927	0.28	NT
NC Ac 17127	0.38	0.68
NC Ac 17130	0.28	0.56
NC Ac 17129	0.49	0.61
NC Ac 17132	0.43	0.34
NC Ac 17135	0.42	0.05
NC Ac 17124	0.46	0.50
NC Ac 17142	0.64	0.73
Krapovikas Strain 16	0.36	0.22
RMP 91	0.17	NT
NC Ac 15989	0.39	0.36
TMV 2	0.33	0.48
Robut 33-1	0.32	0.34
LSD (5%)	0.17	0.19
CV (%)	30.95	34.17

NT = Not tested

Mean lesion diameters are shown in Table 7 for all genotypes, in both trials, measured at 28 and 42 days after inoculation. Genotypes differed significantly in mean lesion diameter. The susceptible TMV 2 had the largest lesions followed by the moderately field resistant genotype NC Ac 17142.

Differences in sporulation between genotypes were significant (Table 8) in both trials. Genotype PI 259747 had the lowest sporulation followed by NC Ac 17132 while the two susceptible cultivars, TMV 2 and Robut 33-1 had the most.

There was a significant correlation between the parameters percentage defoliation, lesion diameter and sporulation, determined in glasshouse tests and field disease scores for late leafspot (Tables 9 and 10). These parameters may be useful in identifying acceptable parental sources of late leafspot resistance and in screening of segregating populations in a breeding program. The parameters percentage leaf area damaged and lesion numbers per unit area of leaf measured in the glasshouse were not correlated with field disease scores and were therefore not useful indicators of disease resistance in the glasshouse. This was probably because of the interaction between pathogen development and defoliation. In suscep-

Table 7. Leafspot lesion diameters on selected genotypes grown in the glasshouse and inoculated with *C. personatum* at 30 or 50 days after sowing.

Genotypes	Plant age when inoculated: 30 days		50 days	
	Mean lesion diameters (mm) at (days after inoculation): 28	42	Mean lesion diameters (mm) at (days after inoculation): 28	42
NC Ac 17090	2.9	4.1	3.3	4.4
NC Ac 17133 (RF)	1.2	2.0	1.4	2.4
EC 76446 (292)	0.9	2.3	1.0	2.4
PI 259747	1.3	1.6	0.8	1.3
PI 350680	2.0	3.0	0.6	3.0
NC Ac 927	1.1	2.0	NT	NT
NC Ac 17127	1.7	2.5	1.5	2.4
NC Ac 17130	2.1	4.5	2.3	3.9
NC Ac 17129	1.8	2.6	3.0	3.3
NC Ac 17132	2.0	2.2	2.5	3.0
NC Ac 17135	1.5	2.2	0.6	1.0
NC Ac 17124	1.5	2.5	2.4	3.0
NC Ac 17142	4.2	5.0	5.7	7.0
Krapovikas Strain 16	1.5	3.6	1.4	3.4
RMP 91	1.7	3.0	NT	NT
NC Ac 15989	3.0	4.7	3.2	4.5
TMV 2	4.5	7.5	4.1	7.9
Robut 33-1	2.1	4.3	2.5	4.6
LSD (5%)	0.60		0.34	
CV (%)	18.20		9.05	

NT = not tested

Table 8. Sporulation of leafspot lesions on selected genotypes growing in the glasshouse and inoculated with *C. personatum* at 30 or 50 days after sowing.

Genotypes	Plant age when inoculated: 30 days	50 days
	Sporulation score* at 42 days after inoculation	Sporulation score* at 42 days after inoculation
NC Ac 17090	3.0	3.2
NC Ac 17133 (RF)	2.7	2.8
EC 76446 (292)	3.0	2.8
PI 259747	2.0	2.0
PI 350680	3.0	2.8
NC Ac 927	2.2	NT
NC Ac 17127	3.0	3.1
NC Ac 17130	3.0	3.0
NC Ac 17129	3.0	3.0
NC Ac 17132	2.4	2.6
NC Ac 17135	3.0	3.0
NC Ac 17124	3.0	3.1
NC Ac 17142	3.0	3.0
Krapovikas Strain 16	3.0	3.0
RMP 91	3.2	NT
NC Ac 15989	3.0	3.2
TMV 2	5.0	5.0
Robut 33-1	5.0	5.0
LSD (5%)	0.19	0.29
CV (%)	4.89	7.17

*Extent of sporulation scored on a 5-point scale

NT = not tested

tiable cultivars, the most severely diseased leaves were soon lost, and therefore a smaller percentage leaf area damaged was subsequently recorded on the retained leaves. In resistant genotypes, there was less defoliation and more damage was observed on retained leaves. Glasshouse screening may be useful in areas where the presence of rust and early leafspot complicate field screening for late leafspot resistance. However, it is important not to rely on any one characteristic for evaluation of disease resistance as suggested by Hassan and Beute (9). For example, genotypes that develop small lesions may not be useful if they suffer extensive defoliation.

Table 9. Correlations between field disease scores for late leafspot and measurements of disease parameters made on plants of the same genotypes grown in the glasshouse and inoculated with *C. personatum* when 30 days old.

Character	Correlation coefficient: Days after inoculation: 28	42
Percentage defoliation (Arcsine transformed)	0.513**	0.645**
Percentage leaf area damaged (Arcsine transformed)	0.108	-0.122
Lesion number	-0.291	-
Lesion diameter	0.515**	0.700**
Sporulation	-	0.829**

** Significant at 0.01% level.

Table 10. Correlations between field disease scores for late leafspot and measurements of disease parameters made on plants of the same genotypes grown in the glasshouse and inoculated with *C. personatum* when 50 days old.

Character	Correlation coefficient: Days after inoculation: 28	42
Percentage defoliation (Arcsine transformed)	0.637**	0.739**
Percentage leaf area damaged (Arcsine transformed)	0.312	0.049
Lesion number	0.190	-
Lesion diameter	0.495**	0.675**
Sporulation	-	0.837**

**Significant at 0.01% level.

In this study, the genotypes EC 76446 (292), PI 259747, PI 350680 and NC Ac 17133 (RF), were found to be resistant to late leafspot in both glasshouse and field tests and were resistant to rust in the field. The genotypes PI 259747 and PI 350680 were reported to be resistant to late leafspot and also to scab (*Sphaceloma arachidis* Bit. & Jenk.) in Brazil, but were susceptible to early leafspot (5, 12). They were, however, reported to be resistant to early leafspot in the USA (16). The genotypes NC Ac 927, NC Ac 17127, NC Ac 17130, NC Ac 17129, NC Ac 17132, NC Ac 17135, NC Ac 17124, NC Ac 17142 and Krapovikas Strain 16, were moderately resistant to both pathogens. The genotype NC Ac 17090, was most resistant to rust but was only moderately resistant to late leafspot. The genotypes RMP 91 and NC Ac 15989 were moderately resistant to late leafspot but susceptibel to rust; RMP 91 is resistant to rosette virus in West Africa (4). These results indicate that there are genotypes having resistance to more than one disease. Such source material would be very useful in a multiple disease-resistance breeding program.

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Accepted April 12, 1982