Research on Fungal Diseases of Groundnut at ICRISAT

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Many fungal diseases of groundnut are known (Jackson and Bell 1969; Garren and Jackson 1973) and many fungi are reported to be closely associated with groundnut fruits and seeds. Some of the diseases are of restricted distribution but most are of common occurrence throughout the Semi-Arid Tropics (SAT). At ICRISAT the main concern is with those widespread diseases that cause economically important losses in yield, and in this paper investigations carried out during the past 4 years on important foliar and soilborne diseases are briefly reviewed.

Foliar Diseases

Rust *(Puccinia arachidis* Speg.)

Previously unimportant outside the Americas (Bromfield 1971), rust is now of economic importance in almost all groundnut growing areas of the world (Hammons 1977; Subrahmanyam et al. 1979). Yield losses from rust may be substantial and damage is particularly severe where the crop is attacked by both rust and the longer established *Mycosphaerella* leaf spots.

Investigations were carried out on the biology of the rust fungus so as to determine what factors were likely to influence perpetuation and spread of the disease. Biological data were also needed for development of methods for screening germplasm for resistance to the disease.

A wide range of crop and weed species were checked for possible collateral hosts of rust but none was found outside the genus *Arachis.*

The uredial stage only of the rust has been found although constant examination was made of many germplasm lines and some wild *Arachis* species at ICRISAT. Groundnut plants from various parts of India have also been examined at every opportunity. Attempts to induce teliospore formation by modification of environmental factors were not successful. It was concluded that uredosporeswerethe main, if not the only, means of rust carry-over and dissemination in India.

Laboratory experiments showed that uredospores could be stored for long periods at low temperature without loss of viability but that at high temperatures, they rapidly lost viability. For instance, when stored at 40°C they lost viability within 5 days. Uredospores on exposed crop debris lost all viability within 4 weeks under postharvest conditions at ICRISAT. Pods and seeds from rust affected crops are commonly surface-contaminated with uredospores. Tests on uredospores taken from surface-contaminated seeds stored at room temperature showed viability to decrease from an original 95% to zero after 45 days. Implications for disease carry-over and for plant quarantine are obvious. Rust is particularly severe in South India where groundnuts are grown in some areas at all times of the year.

Light was found to inhibit uredospore germination and germ-tube elongation, indicating that field inoculation might be more successful if carried out in the evening rather than through the day.

The presence of liquid water on the leaf surface was found to be necessary for uredospore germination and infection.

Preliminary rust resistance screening of the ICRISAT germplasm collection (now over 8000 entries) was carried out in the rainy seasons of 1977, 1978 and 1979 under natural disease pressure in the field. Infector rows and check plots of the highly susceptible cv TMV-2 were arranged systematically throughout the trials. Entries which were rated between 1 and 5 on a 9-point disease scale (where 1 = no rust, and 9 = 50-100% offoliagedestroyed by rust) were

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selected for advanced field screening. This was done either in the rainy season as described or in the postrainy season when artificial inoculation with uredospores and overhead irrigation to maintain high humidity were required to ensure good development of rust. Genotypes found to show good resistance to rust at ICRISAT are listed in Table 1 together with their mean rust scores on the 9-point scale. Scores for two susceptible cultivars are included for comparison.

Wild Arachis species being grown in the field in close juxtaposition with severely rust affected groundnuts were examined at intervals through the season for evidence of rust infection. Those species which showed no development of rust are listed in Table 2. Although rust did not develop on *Arachis stenocarpa,* some necrotic lesions were formed that may have resulted from arrested invasion by the pathogen.

Using artificial inoculation, potted plants and rooted detached leaves were used in screening trials in glasshouse and laboratory, respectively. The methods were effective in separating genotypes with large differences in resistance, e.g., highly resistant as opposed to susceptible, but were not suitable for showing any intermediate reactions.

In studies on components of resistance, it was found that neither size nor frequency of stomata was correlated with resistance. Infection frequency was lower in resistant than in susceptible genotypes and the incubation period was longer. Irrespective of whether genotypes were immune, resistant, or susceptible, uredospores germinated on the leaf surface and germ-tubes entered the leaf via stomata. In immune genotypes, the germ-tubes died without further development. Differences in resistance were manifest by differences in rate and degree of development of the rust mycelium in the substomatal cavities and in invasion of leaf tissues.

Table 1. Genotypes resistant to rust at ICRISAT.

Genotype	Rust Score[a]
NC Acc 17090	2.0
PI 414332	2.0
PI 405132	2.5
PI 341879	2.5
PI 393646	2.5
NC Acc 17133-RF	3.0
EC 76446 (292)	3.0
PI 259747	3.0
PI 350680	3.0
PI 390593	3.0
PI 381622	3.0
PI 393643	3.0
PI 407454	3.0
PI 315608	3.0
PI 215696	3.0
PI 393641	3.0
PI 314817	3.0
PI 393517	3.0
PI 414331	3.0
PI 393527-B	3.0
NC Acc 927	3.3
PI 390595	3.5
PI 393531	3.5
NC Acc 17127	3.8
PI 393526	4.0
NC Acc 17129	4.0
NC Acc 17132	4.0
NC Acc 17135	4.0
NC Acc 17124	4.0
PI 298115	4.0
PI 393516	4.5
NC Acc 17142	5.0
Krap Str 16	5.0
TMV-2[b]	9.0
Robut 33-1*	9.0

a. Rust score on 9-polnt disease scale.
b. Standard susceptible cultivars.

Table 2. Wild *Arachis* spp on which no rust developed in the field despite heavy disease inoculum.

Species	PI Number	Section	Source
A. duranensis	219823	*Arachis*	Argentina
A. correntina	331194	*Arachis*	Argentina
A. cardenasii	262141	*Arachis*	Bolivia
A. chacoense	276235	*Arachis*	Paraguay
A. chacoense x *A. cardenasii*	-	F_1 hybrid	USA
A. pusilla	338448	*Triseminalae*	Brazil
A. sp 9667	262848	*Rhizomatosae*	Brazil
A. sp 10596	276233	*Rhizomatosae*	Paraguay

Mycosphaerella or *"Carcospora"* Leaf Spots (Early Leaf Spot - Cercospora *arachldlcola* Hori; Late Leaf Spot — *Cercosporidium personatum* [Berk, and Curt.] Deighton)

The *Mycosphaerella* leaf spots are probably the most important diseases of groundnuts on a worldwide scale. Both are commonly present and their relative importance is determined by crop and environmental factors. At ICRISAT, the disease incited by *C. personatum* is of regular occurrence and reaches high levels on rainy season groundnuts but that incited by *C. arachidicola* is much less common and rarely reaches levels high enough to permit field resistance screening.

Field screening for resistance to the leaf spots was carried out simultaneously with the rust screening and a similar 9-point disease scale was used. Entries rated between 1 and 5 were selected for advanced field screening. All field screening utilized natural inoculum. The diseases developed more rapidly and screening was more effective in the rainy season than in the irrigated postrainy season crops. Genotypes found to have resistance to *C. personatum* at ICRISAT are listed in Table 3.

Genotypes with field resistance to *C. personatum* were further tested for resistance in glasshouse screening trials. Good correlations were found between field and glasshouse tests in respect of defoliation, lesion size and sporulation index. Laboratory screening in which rooted detached leaves were inoculated with *C. personatum* also proved useful. The latter method was also useful in the study of resistance mechanisms.

Both high resistance and immunity have been found among wild *Arachis* species (Table 4). The ICRISAT Groundnut Cytogeneticists have produced hybrids between some of the resistant wild species and the cultivated groundnut, and by backcrossing have obtained near tetraploid material which is being tested at all stages for resistance to leaf spots and to rust.

With leaf spots as with rust, germination of spores and entry into the leaf via stomata did not appear to be in any way inhibited in resistant genotypes. Resistance was again manifest in the postentry phase.

Table 3. Genotypes resistant to C. *parsonatum* at ICRISAT.

Genotype	Leaf spot score*
EC 76446 (292)	3.2
NC Acc 17133-RF	3.3
PI 259747	3.3
PI 350680	3.3
NC Acc 927	4.0
NC Acc 17127	4.3
Krap Str 16	4.3
RMP-91	4.7
NC Acc 17090	4.8
NC Acc 17130	4.8
NC Acc 17129	4.8
NCAcc 17132	4.8
NCAcc 17135	4.8
NC Acc 17124	4.8
RMP-12	5.0
TMV-2[b]	9.0

«. Leaf spot score on 9-polnt disease scale.
b. Standard susceptible cultlvar.

Table 4. Wild *Arachls* spp — reaction to *Carcospora arachidicola* and *Cercosporidium paraonatum.*

Species	PI Number	Reaction to	
		C. arachidicola	*C. personatum*
A. chacoense	276325	Highly resistant	Highly resistant
A. cardenasii	262141	Susceptible	Immune
A. sp 10596	276233	Immune	Immune
A. stenosperma	338280	Highly resistant	Highly resistant

Yield Losses from Rust and Leaf Spots and Multiple Resistance

Rust and leaf spots normally occur together and it is difficult to allocate individual responsibility for the resulting damage to the crop. In the 1979 rainy season we attempted to estimate yield losses by applying fungicides to susceptible and disease resistant genotypes; Daconil to control leaf spots and rust, Bavistin to control only leaf spots, and Calixin to control only rust. Loss estimates are shown in Table 5. Losses were less in the resistant than in the susceptible genotypes.

Comparison of Tables 1 and 3 will show that some of the genotypes resistant to rust are also resistant to *C. personatum* leaf spot. Also, some new sources of resistance to both diseases have recently been found in Federal Experiment Research Station — Puerto Rico (FESR) breeding lines (Table 6). These lines originated from a natural hybrid selected for resistance to rust in Puerto Rico by USDA scientists.

Some of the resistant genotypes can outyield established Indian cultivars when grown without protective fungicide treatment at ICRISAT. Further work is required of breeders to incorporate higher yields and better agronomic characters into the resistant materials.

Other Foliar Diseases

Some preliminary investigations have been made on what are at present regarded as minor foliar pathogens. These include diseases incited by *Leptosphaerulina crassiasca* (Sechet) Jackson and Bell, *Alternaria alternata* (Fr.) Keissler, and *Myrothecium roridum* Tode ex. Fr.

Table 5. Yield losses from rust and leaf spots at ICRISAT.

Genotype	Mean percentage loss of pod yield from		
	Leaf spots	Rust	Leaf spots and rust
Robut 33-1[a]	59	52	70
PI 259747	30	23	37
EC 76446 (292)	10	12	30
NC Acc 17090	18	14	29

a. Standard auscaptlble cultivar.

Table 6. Genotypes resistant to rust and laaf spot — FESR lines tested at ICRISAT.

Genotype	Mean disease scores (9-point scale)	
	Rust	Leaf spot
FESR 5-P2-B1	2.0	3.0
FESR 5-P17-B1	2.0	3.0
FESR 7-P13-B1	2.0	3.0
FESR 9-P3-B1	2.0	3.0
FESR 9-P4-B1	2.0	4.3
FESR 9-P7-B1	2.7	3.3
FESR 9-P7-B2	2.7	4.3
FESR 9-P8-B2	2.0	3.0
FESR 9-P12-B1	2.0	2.7
FESR 11-P11-B2	2.3	2.7
FESR 12-P4-B1	2.0	2.0
FESR 12-P5-B1	2.0	2.7
FESR 12-P6-B1	2.7	3.7
FESR 12-P14-B1	2.0	3.3
FESR 13-P12-B1	2.0	2.7
TMV-2[a]	9.0	9.0

a. Standard susceptible cultivar.

Soilborne Diseases

Seed and Seedling Rots

Seed rots and seedling diseases of groundnut are of common occurrence in the SAT and may cause serious losses in yield. The diseases may develop from fungi already established in the seeds before sowing, or may result from direct invasion of seeds or seedlings by soil fungi. Many species of fungi have been reported to cause seed rots and several are known to cause diseases of seedlings. Some fungi causing diseases at ICRISAT are listed in Table 7.

Two important diseases of groundnut seedlings are Crown Rot which is caused *by Aspergillus niger* van Tiegh and Aflaroot which is caused by toxigenic strains of *Aspergillus flavus* Link, ex Fr. Initial screening of the ICRISAT germplasm collection has indicated

Table 7. Fungi associated with seed and seedling diseases at ICRISAT.

Aspergillus flavus Link, ex Fr.
Aspergillus niger van Tiegh.
Botryodiplodia theobromae Pat.
Fusariurn spp
Macrophomina phaseolina (Tassi) Goid.
Penicillium spp
Rhizoctonia solani Kuehn
Sclerotium rolfsii Sacc.

that some genotypes may possess resistance to these diseases.

Pod Rot

Pod rot diseases are widespread in the SAT and are known to cause severe damage in a number of countries (Abdou and Khadr 1974; Frank 1972; Mercer 1977; Porter et al. 1975). High levels of pod rot were observed in the 1978-79 postrainy season crop at ICRISAT and screening of germplasm for resistance was initiated. Some 2000 genotypes have now been screened under natural field disease conditions. Standard local cultivars had 20-25% of pods rotted while disease levels in germplasm lines ranged from 4 to 72%. Genotypes with pod rot scores of 10% or lower were selected for advanced screening in disease sick plots.

The etiology of the disease is still being investigated. Fungi commonly isolated from rotted pods at ICRISAT are listed in Table 8.

Table 8. Fungi Isolated from rotted pods a ICRISAT.

Dominant species	*Fusarium solani* (Mart.) Sacc. *Fusarium oxysporum* Schlecht
Subdominant species	*Macrophomina phaseolina* (Tassi) Goid. *Rhizoctonia solani* Kuehn
Associate species	*Aspergillus flavus* Link, ex Fr. *Aspergillus niger* van Tiegh. *Fusarium acuminatum* Ell. & Ev. *Fusarium equiseti* (Corda) Sacc. *Fusarium fusaroides* (Frag. & Cif.) Booth *Gliocladium roseum* Bain. *Trichoderma viride* Pers. ex Fr.

The Aflatoxin Problem

Contamination of groundnuts with aflatoxins is a serious problem in many parts of the SAT. The ubiquitous *Aspergillus flavus* which produces these toxic and carcinogenic substances may invade groundnut seeds before harvest, during postharvest drying, and during storage if the seeds are wetted. From the continued appearance of reports of aflatoxin contamination of produce it would appear that SAT farmers have not adopted the crop handling and storage methods designed to reduce aflatoxin contamination in groundnuts. It has therefore become necessary to investigate the possibilities of genetic resistance in the hope of developing cultivars with pods or seeds which *A. flavus* cannot invade, or which if invaded, do not support aflatoxin production.

Workers in the USA (Mixon and Rogers 1973; Bartz et al. 1978) have shown some genotypes to have high levels of resistance to A *flavus* invasion and colonization of dry seeds. This dry seed resistance is dependent upon the testa being entire and undamaged. The test is a simple one. Mature undamaged seeds that have been dried and stored for several weeks are placed in a petri dish and hydrated to 20-25% water content. A suspension of *A. flavus* spores is added to them, and they are incubated for about 8 days. The percentage of seeds which are colonized by the fungus indicates the degree of dry seed resistance possessed. The ICRISAT germplasm collection is now being screened. The reactions of three genotypes reported resistant in the USA and some Indian cultivars are given in Table 9.

Table 9. Dry seed resistance to *A. flavus* colonization.

Genotype	Percentage of seeds colonized by *A. flavus* and disease testing
Resistant lines from USA	
UF 71513	7.0 Resistant
PI 337394 F	9.1
PI 337409	9.2
Indian cultivars	
Junagadh 11	11.6
TMV-2	35.0 Susceptible
OG 43-4-1	96.0 Highly susceptible

There is no evidence th at the genotypes so far found with dry seed resistance have any special degree of resistance to invasion of pods or seeds before harvest or during postharvest drying. Investigations have started into possible resistance during these phases, and particular attention is being given to genotypes which have shown resistance to pod rots.

Some early research (Tulpule 1967; Kulkarni et al. 1967) indicated that certain cultivars had resistance to the production of aflatoxin. However, these findings were not confirmed by further research (Doupnik etal. 1969; Aujla et al. 1978), although there were indications that slight differences might exist between cultivars in their ability to support aflatoxin production. In dry seed resistance testing at ICRISAT, toxigenic strains of *A. flavus* are used and genotypes are being checked for possible differences in efficiency, as substrates for anatoxins production.

Other Soilbome Diseases

A number of soilbome diseases occur regularly at ICRISAT but at low incidence. These include wilt and root rot caused by species of *Fusarium;* a black root rot caused by *Macrophomina phaseolina* (Tassi) Goid; a root rot caused by *Rhizoctonia solani* Kuehn; and stem rot caused by *Sclerotium rolfsii* Sacc. Disease sick plots are being established to allow screening of the germplasm collection for possible resistance to these diseases.

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