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**on the**  
**Resistance**  
**to Soil-borne Diseases**  
**of Legumes**

**International Crops Research Institute for the Semi-Arid Tropics**

**Proceedings of the  
Consultants' Group Discussion  
on the  
Resistance to Soil-borne Diseases  
of Legumes**

**8–11 January 1979  
Patancheru, A.P., India**

**Y.L. Nene, Editor**



**ICRISAT**

**International Crops Research Institute for the Semi-Arid Tropics**

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## Foreword

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has a mandate to improve three legumes—pigeonpea, chickpea, and groundnut—in the semi-arid tropical regions of Asia, Africa, and Central and South America. These legumes, like others, are affected by diseases; and those diseases caused by soil-borne pathogens are widespread and devastating. In order to review the present knowledge of some of the major diseases of pigeonpea and chickpea, with special emphasis on disease resistance, and to develop recommendations for research priorities leading to better disease control, ten leading scientists from several countries were invited for an in-depth discussion with ICRISAT staff in early 1979.

Dr. Y.L. Nene, then Principal Plant Pathologist (Pulses) and now Leader, ICRISAT Pulse Program, presented a comprehensive review of the Institute's work on soil-borne diseases of pigeonpea and chickpea. Groundnut pathology work was not reviewed, since research on groundnut diseases at ICRISAT has been initiated only recently. Consultants presented papers based on their experience with soil-borne diseases mainly of beans and peas. Presentations and discussions pertained to topics such as epidemiology, host-parasite interactions, laboratory/glasshouse screening procedures, and field-screening procedures.

The Group Discussion was inaugurated by Dr. L.D. Swindale, Director-General of ICRISAT.

We are certain that these proceedings, along with the recommendations, will stimulate more research, not only at ICRISAT but also at other institutions.

August 1980

**J. S. Kanwar**  
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CONSULTANTS' GROUP DISCUSSION ON THE RESISTANCE  
TO SOIL-BORNE PATHOGENS OF LEGUMES  
JANUARY 8-11, 1979

ICRISAT, HYDRABAD, INDIA



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# **Review of ICRISAT Work**

# Review of the Work Done at ICRISAT on Soil-borne Diseases of Pigeonpea and Chickpea

Y. L. Nene, J. Kannaiyan, M. P. Haware, and M. V. Reddy

Work on the pathology of ICRISAT's two pulse crops, pigeonpea (*Cajanus cajan* (L.) Millsp.) and chickpea (*Cicer arietinum* L.) was initiated in September 1974. According to the requirements of the Institute, a seminar on the proposed plan of work was presented by Nene, Principal Pathologist, in November 1974. He stated that the objective of the pathology program should be to play an appropriate role in (1) the Crop Improvement Program, by providing assistance in breeding disease-resistant material, and (2) maintaining the gains made in the Crop Improvement Program. Subsequently we planned all our research projects with these two objectives in mind. While the first objective explains all our work on screening techniques and their application, the second objective explains our work on relevant aspects of biology and epidemiology of the pathogens concerned. The phrase "soil-borne diseases" can cover several diseases; we have, however, restricted ourselves to the more commonly accepted connotation of the phrase for the coverage in this review.

## Pigeonpea

### Wilt

A very large number of papers on various aspects of pigeonpea wilt have appeared in the literature since the disease was first described from India by Butler in 1906. In 1910 he described in detail pathogenicity experiments and also described the causal fungus as a new species of *Fusarium* *F. udum*. Although attempts have been made to change the name of the fungus to *F. oxysporum* f. sp. *udum* we agree with Booth (1971) and retain the name *F. udum*. It is fairly easy to distinguish *F. udum* from *F. oxysporum*

on the basis of spore morphology. An attempt to identify wilt-resistant lines was initiated as early as 1905 at Poona in India (Butler 1908, 1910).

### Occurrence

Wilt is widely prevalent in India (Butler 1906). It has consistently been reported to be more serious in central and northern India.

The disease has also been observed in Kenya, Tanzania, and Uganda in Africa; Thailand and Indonesia in south east Asia; and Trinidad in the Caribbean. Seriousness of the disease in these countries, however, is doubtful.

### ICRISAT surveys

There are no two opinions about the seriousness of wilt in India. Several workers have made general statements on the widespread occurrence of the disease and the serious losses that it causes. We have not, however, come across any report of a systematic survey of this disease. In 1975 we started roving surveys in cooperation with agricultural universities in different states in India. To date we have surveyed five states, covering over 18 000 km. Stops were made approximately every 30 to 40 km, except in areas that do not grow pigeonpea. At each stop the data were collected using a standard proforma which ensured uniformity in data collection. The results obtained so far are summarized in Table 1.

These surveys confirm the presence of the disease in every state surveyed so far, with relatively more in central India. We have yet to conduct surveys in the three major northern states of India.

**Table 1. Pigeonpea wilt survey (1975-1977).**

State	Distance covered km	Locations	Districts	Average % wilt	Range in farmers' fields (%)
Andhra Pradesh	4000	102	19	5.26	0-92
Maharashtra	4000	82	19	22.61	0-93
Karnataka	2000	37	14	1.12	0-17
Tamil Nadu	2100	46	11	1.36	0-65
Madhya Pradesh	6000	136	40	5.42	0-96

**Loss estimation**

It was generally presumed that every wilted plant represents total loss. Since we see (a) partial wilting in many plants and (b) more wilt incidence in flowering and podding stage, we

wanted to estimate the yield loss in relation to the stage at which wilt occurs. We now have 2-year data on loss in yield on a per plant basis (Table 2).

**Table 2. Grain yield loss in pigeonpea (cv Sharda) as influenced by the stage at which wilt occurred.<sup>a</sup>**

Stage at which plants wilted	Yield per plant (g)	Actual loss of yield (g)	Loss of yield (%)	Normal seed weight (%)	Wrinkled seed weight (%)
Prepod	0.05	57.05	99.92	-	-
Early pod	0.71	56.39	98.80	72.80	27.20
Pod-fill	6.35	50.75	88.85	86.01	13.99
Pod maturity	18.84	38.26	67.18	85.94	14.06
Preharvest	40.46	16.64	29.58	85.88	14.12
Healthy (check)	57.10	0.00	0.00	87.69	12.31

<sup>a</sup> Average grain yield from a total of 40 plants in 1976 and 1977 tests.

It is clear that loss was almost complete when wilt occurred at or prior to early pod stage. Even when pods were full and plants close to harvest, the loss was around 30% in wilted plants. It is interesting to note that wilted plants produced over 70% normal seed, and when the wilt was delayed, the percentage of normal seed produced was almost equal to the percentage produced on healthy plants. Only one cultivar Sharda, was tested and it is possible that other cultivars might show different loss patterns. However, we expect the general pattern to remain the same i.e., less yield loss with late wilting.

### Symptoms

When Butler published his paper in 1906, he described the symptoms fairly accurately and very little has been added to that description since then. The infected plants show symptoms of gradual chlorosis and wilting starting from 4 to 6 weeks after planting. However, more wilt is observed during the flowering and podding stage. Black streaks in the vascular region as well as under the bark are characteristic.

Partial wilting in affected plants is not uncommon. Many such plants show a dark purple band extending from the base to several feet above ground towards wilted branches. We could often trace the band to one of the two major lateral roots of such a plant. Infection of the tap root most commonly produced complete wilting, whereas infection starting and extending from one of the two lateral roots more often caused partial wilting; however, exceptions were observed.

The dried leaves on wilted plants do not drop off for a long time.

### Morphological variation in the fungus

We made hundreds of isolations from specimens collected at Hyderabad and a large number of other locations visited during surveys. This species, like

most other *Fusarium* spp., shows a great deal of variation in cultural characters. Based on characteristics such as type of growth, sporulation, color, and change in medium color, we have classified these into 12 distinct groups (A to L). We have single-spored the 12 isolates, had the identification confirmed by the Commonwealth Mycological Institute, and have preserved them on autoclaved sand. We are of course not the first to do this kind of work; Butler reported this type of work as far back as 1910, and many other workers have done so since then (Sarojini 1951; Subramanian 1955; Baldev and Amin 1974).

We have not yet made any attempt to ascertain existence of physiologic races. Baldev and Amin (1974) presented evidence to suggest the existence of races. Their work, however, suffers from certain weaknesses. For example, they do not clarify whether the three cultivars — NP (WR)-15, T-21, and C-11 — they used as differentials were homozygous for resistance to at least one isolate. It has been our experience that unless selfing is resorted to for several generations, the cultivars show considerable heterogeneity for different traits, including disease reaction as a result of natural cross-pollination. Also, the tests with different fungus isolates were carried out only once. In spite of this, we admit that the results presented by Baldev and Amin do point to the possibility of the existence of races.

### Stage of infection

At what stage are plants infected? As mentioned elsewhere, the disease incidence is very low in the first 2 months. More incidence is seen during flowering and podding stages. We therefore carried out a study to detect the fungus in the plants prior to the appearance of wilt symptoms. Plants of the susceptible cultivar, Sharda, grown in a wilt-sick plot, were used for this study. In the 1977-78 season ten plants were removed 15, 30, and 45 days after sowing. In the 1976-77 season, the fungus could

be detected from the collar region downwards in apparently healthy plants (3 to 5 plants only) collected 30 days after sowing, but not in those collected 15 days after sowing. However, in the 1977-78 season, the fungus could be detected in plants 15 days after sowing. The first wilted plant was noticed in the plot 45 days after sowing in 1976-77 and 30 days after sowing in 1977-78. This study shows that the plants are infected fairly early in the season and many plants apparently keep on fighting the fungus until flowering/podding.

While we were attempting to detect the infection prior to symptom appearance through fungus isolation, we came across a paper by Miller-Jones et al. (1977) reporting detection of infection of *Salix alba* var. *caerulea* (Cricket bat willow) by *Erwinia salicis*, before symptom appearance, by using an instrument called the Shigometer. Diseased tissues were distinguished from healthy by their low resistance to a pulsed electric current. We got ICRISAT Electronics Engineer (Instrumentation), Mr. S.K.V.K. Chari, interested in the pigeonpea wilt problem. He has developed a similar instrument, using direct current, which he tentatively calls a "wilt detector". Preliminary tests were carried out in pots as well as field. Plants were raised in sick soil. Electrical resistance was measured every 3 to 4 days. Plants showing a drop of more than  $0.4\text{K}\Omega$  between two readings ultimately showed wilt. Work is being continued.

### Systemicity of the fungus

The main purpose of this study was to confirm the findings of Mohanty (1949), who reported that the fungus was systemic. Five completely wilted plants of three cultivars (Sharda, BDN-1, ICP-6997) were selected; samples taken for isolation every 15 cm from the root tip to the top and included leaflets, petioles, rachis, pedicel, pod hulls, flowers, and seeds. The seed samples were collected after surface-sterilizing the pods with 0.1% mercuric chloride. The samples

from individual plants were plated on modified Czapek's-Dox agar selective medium (Sharma and Singh, 1973) after surface sterilization with mercuric chloride. The plates were incubated at  $28^{\circ}$  to  $30^{\circ}\text{C}$  for 15 days. *Fusarium udum* was isolated from tap root, lateral roots, collar region, main stem, branches, leaflets, petioles, rachis, pedicel, and pod hulls. However, it could not be isolated from flowers or seeds.

*Fusarium udum*, however, can be detected as a surface contaminant on seed.

### Survival

We have failed to find in the published literature any work done specifically to ascertain how long the fungus survives in wilted plant stubble. McRae and Shaw (1933) made the following statement:

"Exposed in the open, the fungus in many of the stems and roots dies but when kept in a cooler room in the shade most of it survives. The source of infection then exists in the uncut portions of roots below the ploughing-depth. From such parts of roots *in situ* the fungus has been isolated after two years though with difficulty, so even here it would appear that the fungus dies out though more slowly. Disinfected *arhar* (pigeonpea) seed sown in land free from a *arhar* crop for from eight to twenty years generally produces a crop with little or no wilt, while with a shorter interval the crop comes up more or less severely wilted according to the shortness of the interval."

This indicates that the fungus survives something less than 8 years. Agnihotrudu (1954) has shown that *F. udum* does not colonize plant debris in the soil but can survive only in tissues already invaded as a pathogen. It then follows that the stubble fragments may be enabling the fungus to survive in soil up to 8 years. To find out how long *F. udum* survives in pigeonpea stubble, an experiment was initiated in November 1974. Stubble

(root system with about 15-cm long stem base) of naturally infected plants was obtained, weighed and buried in 35-cm diameter earthen pots. Two sets were prepared; one with black soil (Vertisol) and the other with red soil (Alfisol) collected from the ICRISAT Center farm. Some properties of these two soils are indicated in Table 3.

Some studies by other workers need to be mentioned in connection with the survival of *F. udum*. Sarojini (1950) concluded through pot studies that application of zinc (20, 40, and 80 ppm) to soil in which infected stubble was buried resulted in the disappearance of the fungus in 5 to 6 weeks. Boron and manganese were less effective. Dey (1948)

**Table 3. Some properties of Vertisol and Alfisol used in the pigeonpea wilt fungus survival study.**

Soil type	pH (1:2)	E.C. mmho/cm	Organic carbon	Avai- lable P	Mechanical analysis		
					Sand (%)	Silt (%)	Clay (%)
Alfisol	5.90	0.10	0.20	2.10	59.60	7.20	33.2
Vertisol	7.85	0.15	0.38	1.60	38.80	20.00	41.2

Sixty pots, 30 with Vertisol and 30 with Alfisol, were prepared and buried in the ground so that the top of each pot was in line with the ground surface. Stubble from six pots (3 Vertisol + 3 Alfisol) was removed after every 6 months, weighed, then checked for the survival of *F. udum*. The experiment was planned for 5 years. Weather data (average maximum and minimum temperatures and rainfall) from the Meteorological Station of ICRISAT were noted. The identity of the fungus was verified through microscopic observations, and pathogenicity of some representative isolates was checked. In addition, assistance from the Commonwealth Mycological Institute was sought. The data obtained after every 6-month interval have been given in detail in ICRISAT Pulse Pathology reports of 1974-1978.

We were able to detect *F. udum* in stubble fragments from Vertisol up to 2½ years and from Alfisol up to 3 years. Based on this limited study, we are unable to understand how the fungus could survive up to 8 years, as suggested by McRae and Shaw (1933).

has claimed reduction in the wilt incidence when sorghum was grown as an intercrop. Bose (1938) made a chance observation of reduced wilt incidence in a field where tobacco was grown in the preceding season. McRae and Shaw (1933) through observations in permanent manurial and rotation experiments over several years reported that (1) manuring with superphosphate (7-23 lb P<sub>2</sub>O<sub>5</sub>/acre) and with cattle manure increased the wilt, (2) green manuring with *Crotalaria juncea* (60 lb seed/acre) decreased wilt, and (3) superphosphate and green manure together increased wilt.

#### Screening techniques

Since one of the major objectives of our program is to assist the breeders in developing disease-resistant varieties, we have spent a great deal of our time in working out efficient and simple techniques to screen germplasm and breeding material for resistance to different diseases, including pigeonpea wilt.

## Water culture

The technique essentially consists of transplanting pigeonpea seedlings, raised in autoclaved sand, into glass tubes containing aqueous suspension of *F. udum* conidia. Although we spent about 2 years in developing this technique, we gave it up subsequently because of the lack of correlation between the results obtained by this technique and those obtained by field screening. The water culture technique works well in the case of chickpea wilt and therefore we shall give more details elsewhere.

When we first developed this technique, we thought we had worked out something original. Subsequently we discovered that similar techniques had been described by Wensley and McKeen (1962) and Roberts and Kraft (1971), and we were surprised to note that the idea of such a technique had even occurred to Butler (1910). He used water culture (he called it so) for studying the site of root infection.

## Pot screening

This well-known technique — transplanting seedlings of which roots are injured and inoculated to autoclaved sand/soil in pots — gave us erratic results. On the other hand, we had good success in preliminary tests with the following procedure:

1. Alfisol, non-autoclaved, is filled in large (35 cm) earthen pots.
2. *Fusarium udum* is multiplied on sand-pigeonpea flour (9:1) medium (SPM) for 15 days.
3. Fungus on SPM (200 g) and autoclaved pigeonpea stem bits (200g) are mixed with the top 15 cm of soil in pots.
4. Susceptible cultivar ICP-6997 (approx. 50 seeds) is raised in each pot. All plants wilted within 60 days are chopped and incorporated in the same pot.

5. Step 3 is repeated.

6. Step 4 is repeated.

7. Step 4 is repeated once more.

After step 7 we get over 90% wilt in each pot. Currently we are developing 1000 such pots, mainly to have a screening procedure to support field screening.

## Sick plot

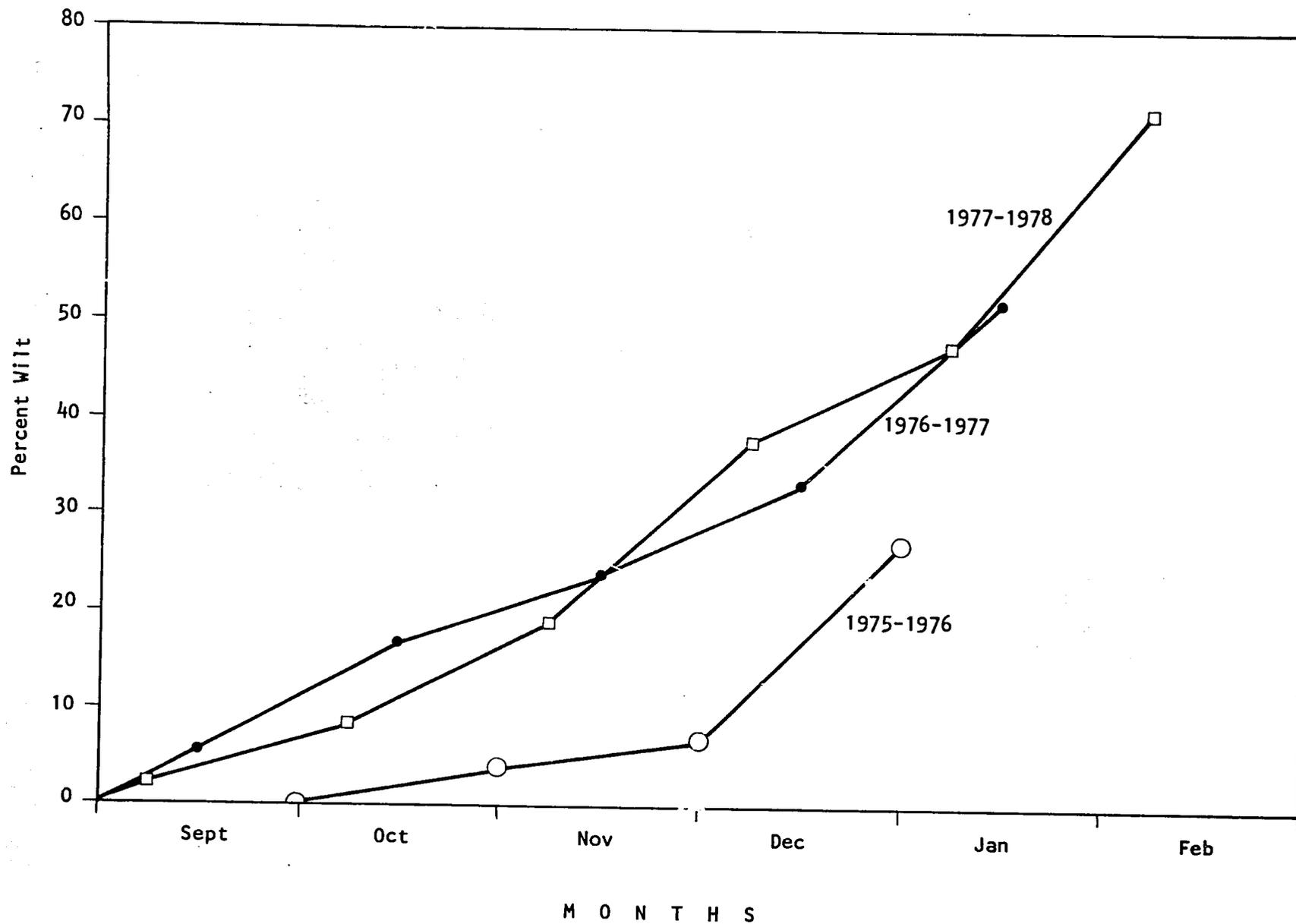
The idea of using a sick plot is well-known and this procedure has been used for a long time for screening against several vascular wilts. We have developed two sick plots in Vertisol (1.5 ha each) and two small sick plots in Alfisol (0.1 ha and 0.4 ha). Figure 1 shows how the sickness has developed in one of the Vertisol plots over three seasons. In our experience, the sickness develops more quickly in Alfisol than in Vertisol; also, wilt shows up earlier in Alfisol than in Vertisol. It is pertinent to point out here the pot studies of Shukla (1975), which revealed that the wilt incidence was high in sand alone (93.75%) and least in heavy black soil (18.18%). The disease increased with the decrease in the proportion of soil in a soil-sand mixture.

The procedures we followed in developing wilt-sick plots are given in Appendix 1. At first we multiplied the fungus on materials other than pigeonpea stubble, but later realized that the best way is to incorporate the stubble from diseased plants and grow wilt-susceptible cultivars in intermittent rows all over the field.

The planting pattern we follow for screening is one susceptible check row after every two test rows in plots that are in the process of becoming "sick" and one susceptible check row after every four test rows in plots that have already become "sick."

## Screening work to end of 1978

Screening work was initiated in India from the time the disease was described



6 Figure 1. Monthly pigeonpea (cv Sharda) wilt incidence in sick plot 'A' during 1975-1976, 1976-1977 and 1977-1978.

In 1906. Research centers where resistance work was or is being carried out are: Poona (Butler 1910), Pusa (McRae and Shaw 1933), Delhi (Deshpande et al. 1963), Kanpur (Dey 1948), Parbhani (Raut and Bhombe 1971), Sangareddy, Hyderabad (Vaheeduddin 1958), Patancheru (ICRISAT 1976 onwards). Several cultivars have been claimed resistant. When we tested many of these, we did not get uniformity in performance. It is possible that the seed we have in our germplasm collection came from outcrossed plants and therefore many plants show susceptibility. Some of the cultivars that consistently show low disease level are NP(WR)-15 (NP-24 x NP-51), 15-3-3, BDN-1, and 20-1. Another cultivar, NP-80, is mentioned repeatedly in the literature since 1933 (McRae and Shaw 1933) as a highly resistant one. The seed of NP-80 however, has not been available to us for testing.

Since it took some time to develop a good sick plot, we could initiate dependable field screening only in the 1976-77 season. As the first step we focused our attention on (1) cultivars already claimed resistant, and (2) lines identified as resistant to another important disease, sterility mosaic. We have been discarding the susceptible segregants and selfing individual resistant plants to fix wilt resistance in a homozygous condition. We now have some promising lines that come from both types of materials indicated above. Systematic screening of germplasm has been initiated, but has been given low priority at this time. Screening of breeding populations generated by ICRISAT breeders is being carried out. Multilocation testing of promising lines has been initiated. Table 4 summarizes ICRISAT's screening work.

#### Resistant/tolerant lines

At this stage we feel reasonably confident about the performance of the following lines when grown as annuals, with

no ratoon crop. Some of these are resistant to sterility mosaic also (marked\*)

ICP-8859, ICP-8860, ICP-8861\*  
 ICP-8862\*, ICP-8863, ICP-8864\*  
 ICP-8865, ICP-8867\*, ICP-8868,  
 and ICP-8869\*.

**Table 4. Screening for resistance to pigeonpea wilt at ICRISAT.**

Materials screened in 1976-77 and 1977-78	
Breeding materials	: 2000
Germplasm	: 194
Promising lines identified	: 19
Under multilocation test	: 12
Promising against wilt and sterility mosaic	: 10
Breeding materials being screened in 1978-79	: 4000

It should be pointed out that most of these are apparently still segregating, giving a very small percentage of susceptible segregants. We are continuing to self single plants and to advance their progenies to the next season.

Most pigeonpea cultivars have a tendency towards being perennials. Therefore, after the first harvest of pods, the plants produce new leaves and another flush of flowers/pods (ratoon crop). We find that all the promising lines indicated above show high wilt incidence even in the first ratoon and have been able to detect the presence of the fungus in many of these lines before the first harvest. Apparently, the fungus is held in check by these lines until the first harvest is over, after which the fungus dominates and kills the plants.



Pigeonpea wilt screening in a sick plot at Hyderabad.

## Phytophthora Blight

### Earlier work

A "stem rot of pigeonpea" was described for the first time from India by Mahendra Pal et al. in 1970, although its suspected occurrence was reported by Williams et al. (1968). These workers observed the disease in serious form in the 1968-69 season at certain locations in northern India. The causal fungus was identified as *Phytophthora drechsleri* Tucker var. *cajani* Pal, Grewal and Sarbhoy. Five years later a "Phytophthora stem blight" of pigeonpea was described from the same areas of northern India (Williams et al. 1975). The species was not identified at that time, but was later described by the same group of workers as *Phytophthora cajani* (Amin et al. 1978).

### Occurrence

The disease has been reported from the northern Indian states of Delhi and Uttar Pradesh. A similar disease was observed by us at ICRISAT Center in 1976 in severe form. Although we have not conducted extensive surveys, we suspect the disease occurs in most pigeonpea-growing areas, particularly during longer wet spells, which are common during the first 3 months of crop growth. Information on losses caused by this disease is not available, but there is no doubt that the disease has the potential to cause devastation in a susceptible cultivar. One of us (YLN) was told during a trip to central America in November 1977 that *Phytophthora* stem blight incidence is commonly observed in Puerto Rico, Dominican Republic, and Trinidad. *P. parasitica* was mentioned as the species affecting pigeonpea in Puerto Rico.

### Symptoms

The symptoms can be seen only on above-ground parts, and the root system as well as the portion of the stem below the soil surface are not affected. (Mahendra Pal

et al. 1970, Williams et al. 1975). Williams described the symptoms thus:

"Symptoms include rapid wilting of the plant parts above the invasion site; desiccation and upward rolling of leaflets, usually without chlorosis; withering of petioles and small stems; and dark-brown to black necrotic lesions encircling the stem at the base, or up to a meter or more above soil level. Lesions at the plant base often extend 15-20 cm up the stem. Lesions on the upper parts of the plant are on the main stem, branches, or petioles, usually have definite margins, and initially have a plain surface which later becomes slightly depressed. Lesions are often centered on a leaf scar, and extend several centimeters in each direction from the apparent invasion site. Longitudinal cuts into newly formed lesions show brown-to-black discoloration of the bark and cambium, but not the older xylem. Later, the older xylem tissue may become discolored and the stem may break at the lesion site. Gross symptoms resemble those of *Fusarium* wilt (caused by *Fusarium udum* Butler), and it is possible that *Phytophthora* stem blight has been confused with this disease in the past."

In addition to the above symptoms, we have observed at ICRISAT Center water-soaked lesions on leaves, from which the fungus can be isolated.

### Identification of species

Since we could not identify the species isolated at ICRISAT Center, we sought help from the Commonwealth Mycological Institute, UK, for expert opinion. Dr. D.J. Stamps identified the species as *Phytophthora vignae* (IMI-211490). When we attempted to obtain infection of cowpea (11 cultivars, var. 57, 1149, 1160, G.C.187, G.C.10-72, var.25/3/2, Sel.K-1, FS-68, New Era, Pale Green, and Pusa Dofasli) with the fungus, we failed in repeated tests. We, therefore, took up the question with Dr. Stamps, who

commented that:

"morphological features agreed more closely with those described for *P. vignae*, though we have no type culture here for comparison. However, in view of the difference in pathogenicity now known, identification with *P. vignae* should perhaps be reconsidered."

A comparison of our *Phytophthora* with other species was made by us in 1976-77 (Table 5, reproduced from the 1976-77 annual report of ICRISAT).

One of us (JK) worked (15 October to 15 December 1978) with Dr. D.C. Erwin at the University of California, Riverside, California, USA, and hopefully we should be able to know soon what species of *Phytophthora* is involved in causing blight at ICRISAT Center.

We must emphasize here that the symptoms we observe at ICRISAT Center are identical to those that are seen in diseased plants in Delhi and Uttar Pradesh states in northern India.

### Survival

There is no published material related to this topic, and we have yet to initiate extensive studies. However, we wish to record a few observations.

- We have seen the disease in fields where pigeonpea had not been cultivated at least for the preceding 4 years.
- In seed pathology studies, we have so far not observed any *Phytophthora*.
- Artificial inoculations of several plant species other than pigeonpea have been unsuccessful.
- In general, more disease is seen in pigeonpea grown in Alfisol than in Vertisol.
- More disease incidence is observed in low-lying patches. In poorly drained fields, an increase in the disease is seen

in successive pigeonpea crops, whereas the disease may not show at all in a similar cropping situation in well-drained soil.

- Infected stem bits when left on the surface of soil in pots (kept in the open) failed to provide inoculum to infect susceptible cv HY-3C after 4 months. (This was a preliminary study.)
- We have been able to detect oospores in diseased leaves.

### Screening techniques

#### Pot screening

We have been able to standardize a pot screening procedure. The steps followed are:

1. Isolate P<sub>2</sub> of *Phytophthora* sp. isolated at ICRISAT Center is grown on V-8 juice agar (V-8 juice 100 ml; CaCO<sub>3</sub> 2g; agar 20g; distilled water 900 ml) for 1 week (28°-30°C).
2. Five-mm discs of the culture are transferred to 100 ml autoclaved V-8 juice broth (as above, without agar) in 250-ml flasks. Incubation is at 28°-30°C for 15 days.
3. The mycelial mat from each flask is removed and washed twice with distilled water. It is then macerated in 100 ml distilled water in a Waring blender for 2 to 3 minutes. The suspension prepared this way serves as inoculum.
4. Five to 10-day old seedlings (25-30), raised in nonsterilized Alfisol (7.5 kg/pot) in 20-cm pots, are inoculated by pouring 50 ml inoculum (step 3) diluted further with 50 ml of tap water (i.e., 100 ml inoculum per pot).
5. Susceptible checks (cv HY-3C), both inoculated and non-inoculated, are kept with each batch of germplasm or breeding material.

**Table 5. Comparison of the characters of pigeonpea *Phytophthora*.**

Characters	<i>P. drechsleri</i> var. <i>cajani</i> (Mahendra Pal <i>et al.</i> 1970)	<i>Phytophthora</i> sp. (Williams <i>et al.</i> 1975)	<i>P. vignae</i> Purss <sup>a</sup> (1963)	<i>Phytophthora</i> sp. (ICRISAT 1976)	
	1	2	3	4	5
1. Hyphal swellings	Not mentioned	Not present	Present	Present	
2. Sporangia	Ovate to pyriform and very few spherical 9-33 x 4.7-13.9 $\mu$ Av. 17.4-22 x 8.0-11.6 $\mu$ with a minute papilla	Ovoid to obpyriform 49-82 $\mu$ (Av.60 $\mu$ ), terminal, persistent and non-papillate	Ellipsoid, ovoid or obpyriform often tapering somewhat to the base Av. 48 x 27 (Max. 72x54) $\mu$ non-papillate apical thickening inconspicuous	Ovate to pyriform 10.0-27.5 x 7.5-17.5 $\mu$ (18.4 x 11.0) $\mu$ mostly non-papillate	
3. Zoospores	8 to 20 in number in each sporangium, and sometimes they liberate out with an evanescent type of vesicle or proliferation of zoosporangium	Zoospores differentiated within the sporangium and were released one by one upon the dehiscence of sporangial apex	Not mentioned		
4. Sex organs	Oogonia spherical 23.4-37.0 $\mu$ , amphigynous antheridia nearly spherical (8.1-15.0 x 8-14 $\mu$ ) Av. (11.6 x 12.7 $\mu$ )	Oogonia with amphigynous antheridia were formed on the same hyphae	Oogonia spherical 32 (max.46) $\mu$ dia, antheridia all amphigynous, variable in size and shape Av. 16 x 15 (max. 27 x 18) $\mu$	Oogonia with amphigynous antheridia were formed.	

Table 5. (Continued).

	1	2	3	4	5
		Oospores spherical to globose 23.4-37 $\mu$ (Av. 30 $\mu$ ) in dia.	Oospores single, spherical, light brown, smooth, and plerotic. Because of intermediate exit pore, 6.6-10 $\mu$ m the present sp. does not fit into any of the six groups of Waterhouse.	Oospores loose in the oogonium 26 (max.32) $\mu$ dia.	Oospores spherical, 27.5-47.5 $\mu$ (37.3 $\mu$ ) in dia.
5. Optimum temperature for growth		30-32 $^{\circ}$ C	30 $^{\circ}$ C	28-30 $^{\circ}$ C	Approx. 30 $^{\circ}$ C
6. Host range		Not tested	Nonhosts: Green gram, Black gram, beans, soybean, cowpea, chickpea, safflower, <i>Xanthium</i> , <i>Cannabis</i> , <i>Croton</i> , and <i>Atylosia scarabaeoides</i>	Not given. However it has been reported on cowpea.	Nonhosts: Green gram, Black gram, French bean, Lima bean, cowpea (11 cvs.) chickpea
7. Chlamydo-spores		Present	Not present	Not present	Not present

<sup>a</sup>Commonwealth Mycological Institute, Mycol. Paper No. 92, p.17, 1963.



Pot screening for *Phytophthora* blight resistance. (Note some pots having no seedling mortality.)



Pigeonpea Phytophthora blight at ICRISAT (tolerant cultivar in background).

6. Pots are liberally watered three times a day.
7. Symptoms usually appear in 48 hours. Final observation is taken 10 days after inoculation.

The above procedure has worked extremely well and excellent correlation between pot and field screening has been observed.

#### Field screening

The steps followed are:

1. Isolate P<sub>2</sub> of *Phytophthora* sp. is grown in V-8 juice agar for 1 week (28°-30°C).
2. Inoculum is mixed well with medium after adding carborandum (600-mesh).
3. Individual plants (1 month old) are inoculated at the collar region by rubbing.
4. The field is flood-irrigated immediately afterwards and again if dry weather prevails 1 week later. Typical blight symptoms appear within 10 days.
5. Surviving plants are reinoculated as above.

The method has worked satisfactorily, but we do find a small percentage of escapes. Also, it is not the most convenient method. We are considering alternatives that will give us a simpler and more efficient technique.

#### Screening work

We initiated systematic screening work in the 1976-77 season. Table 6 summarizes the work.

#### Resistant lines

As mentioned in Table 6, we have identified 28 lines/cultivars resistant to the blight. These are: ICP-28, ICP-113, ICP-214, ICP-231, ICP-339, ICP-580, ICP-752, ICP-913, ICP-914, ICP-934,

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**Table 6. Summary of the work on screening pigeonpea for resistance to *Phytophthora* blight.**

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Germplasm screened	
Pot	: 1200
Field	: 343
Resistant lines identified	: 28
Breeding materials	
Screened	: 150
Being screened in 1978-79 season	: 1700

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ICP-1088, ICP-1090, ICP-1120, ICP-1123, ICP-1149, ICP-1150, ICP-1151, ICP-1258, ICP-1321, ICP-1529, ICP-1535, ICP-1570, ICP-1950, ICP-2376, ICP-3753, ICP-6974, ICP-7065, ICP-7182.

*Atylosia* is a wild relative of pigeonpea; two species *A. sericea* and *A. platycarpa*, have been found resistant.

#### Existence of physiologic races

When we subjected all 28 lines resistant to the ICRISAT isolate of *Phytophthora* to inoculations with an isolate from Kanpur, we found all of them to be susceptible. An isolate from New Delhi caused a certain percentage of mortality in each of the 28 lines. Once the identification of the *Phytophthora* species is settled, it should be possible to state whether the susceptibility of lines resistant to ICRISAT isolate to Kanpur and New Delhi isolates is due to a different species or due to a different race or races of the same species.

#### Chemical control

A newer fungicide Ridomil [N-(2, 6-dimethylphenyl)-N-(methoxyactyl)-alanine methylester] from CIBA - GEIGY, has been found extremely effective

against several diseases caused by phycomyces. We have initiated studies on the control of *Phytophthora* through seed dressing in pot culture. The results are awaited.

### Other Pathogens

Under certain situations we do find some other soil fungi causing problems in pigeonpea.

#### *Sclerotium rolfsii*

Seedling mortality caused by this fungus is fairly common in India and some other pigeonpea-growing countries. We have observed more mortality when undecomposed stubble of cereals (e.g. sorghum) is present in the soil. One of the common practices at ICRISAT (in spite of our protest) is to chop and incorporate cereal stubble only a few days before planting pigeonpea. This practice, we feel, is mainly responsible for more seedling mortality caused by *Sclerotium rolfsii*.

#### *Rhizoctonia bataticola*

Dry root rot has been reported so far only from India. It is a minor problem in the normal season (June-December/March) crop, but a major problem when an off-season summer crop is attempted, especially in black soils. One of the ways by which pigeonpea production in central/southern India can be increased is to have an extra crop between November and April. However, *R. bataticola* seriously hits this crop and we need to identify resistant genotypes if the idea of an extra crop is to succeed.

#### *Rhizoctonia solani*

Root rot in seedlings or aerial blight by this fungus has been reported/observed in India, Sierra Leone, Philippines, and Malaysia. One of us (YLN) has observed serious aerial blight in experimental plantings in Malaysia. On the

whole, however, it is a minor problem.

## Chickpea

### Wilt Complex

#### History

Chickpea wilt was first mentioned by Butler in his book in 1918. In 1923 McKerral, working in Burma, considered the disease to be soilborne. He sent to India specimens that yielded *Fusarium* sp. Narasimhan in 1929 reported association of *Fusarium* sp. and *Rhizoctonia* sp. with wilted plants. Later, Dastur (1935) found *Rhizoctonia bataticola* producing "wilted" plants and he called the disease "Rhizoctonia wilt". Although he isolated *Fusarium* from several wilted plants, he could not produce the disease artificially. Since his description of symptoms (he did not look for vascular discoloration) and field pattern of incidence is almost identical to those of typical wilt caused by *Fusarium oxysporum* f. sp. *ciceri*, his failure to prove pathogenicity of the *Fusarium* he isolated is a mystery to us. He concluded that the wilt was due to "physiological" reasons and called it "physiological wilt." In 1939 Prasad and Padwick published a detailed account of their studies and reported *Fusarium* sp. to be the cause of chickpea wilt. The fungus was later named by Padwick (1940) as *F. orthoceras* var. *ciceri*. Erwin (1958) from the USA reported *F. lateritium* f. *ciceri* to be the cause and questioned the name *F. orthoceras* var. *ciceri*. Following the classification of Snyder and Hansen (1940), Chattopadhyay and Sen Gupta (1967) renamed *F. orthoceras* var. *ciceri* as *F. oxysporum* f. sp. *ciceri*. This change has been accepted by Booth (1971).

While on the one hand chickpea wilt was considered to be caused by *Fusarium*, on the other, several workers were not convinced. In addition to other fungi reportedly found associated with wilt, high temperatures at the time of sowing

and flowering, deficient soil moisture and "bad soil" were considered to be the causes (Bedi and Pracer 1952; Anonymous 1953). The state of Punjab in India had a project on chickpea wilt from 1947-54 (J.S. Chohan - personal communication) and it was concluded that soil and weather factors, and not fungi, were the cause. It seems that the use of the term "wilt complex" began after all these investigations and any dead/dried chickpea plant was considered wilted due to "wilt complex." A report on virus-induced wilts in chickpea from Iran (Kaiser and Danesh 1971) further contributed to the confusion in India. In the literature we find the term "wilt" used loosely for root rots and even blights. So much confusion has existed since then that it prompted Dr. H.K. Jain, now Director of the Indian Agricultural Research Institute, New Delhi, to organize a symposium in 1973 on "Problems of wilt and breeding for wilt resistance in Bengal gram." Several Indian pathologists and breeders participated, and a part of one of the conclusions reproduced below (Jain and Bahl 1977) pointed out the problem clearly:

"The participants concluded that considerable confusion exists with regard to the causation of the wilt disease of Bengal gram; most workers have tended to emphasize a wide variety of factors including those of physiological, agronomical, environmental and pathological nature, which in one way or the other contribute to the development of wilt symptoms."

This was the status of the problem when we initiated our investigations at ICRISAT. It was clear that various causal agents were responsible for the drying of plants and the foremost need was to understand the characteristic symptoms produced by each. Once the diagnosis of the cause based on host symptoms became possible, there would be no room for confusion.

We have gone into detail mainly to ensure a proper understanding of the problem and the reason why we have

devoted considerable time to investigate the so-called "wilt complex." Although the term "wilt complex" has been used mainly in India, we have noted through the literature similar situations in some other chickpea-growing countries, such as Pakistan and Iran.

#### ICRISAT work

We initiated a project in 1974 to understand the "wilt complex." After many critical observations of symptoms, hundreds of isolations of fungi in pure cultures, pathogenicity tests, and visits to research stations and farmers' fields in India and other chickpea-growing countries, we concluded that what has generally been referred as the "wilt complex" is actually a number of distinct diagnosable diseases. In order to assist workers in identifying the main disorders of chickpea, we have prepared a bulletin with colored plates (Nene et al. 1979), with a key for diagnosing the common, but confusing, disorders.

Key for the diagnosis of wilt-like disorders of chickpea\*

#### CHICKPEA PLANTS SHOWING PREMATURE WILTING/DRYING

- I. Wilting (drooping of petiole and rachis)
  - A. No external root rot
    1. Internal (xylem) .. *Fusarium oxysporum* f.sp. *ciceri* (WILT) discoloration
    2. No internal dis- .. Frost injury coloration; ir- (to be con-regular pattern- firmed th- of leaflet scor- rough wea- ching ther data)
  - B. External root rot (tap root not brittle)

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\*Source: Nene et al. 1979

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1. Rotting at collar region downwards; small (1 mm) brown, round, rape-seedlike sclerotia along with white mycelium visible at base. .. *Sclerotium rolfsii* (COLLAR ROT)
2. Dark brown lesion extending on stem above collar region; lesion can extend to lower branches; no sclerotia seen. .. *Rhizoctonia solani* (ROOT ROT)
3. Dark brown lesion at base; mycelium not visible; internal brown discoloration restricted to periphery of the wood. .. *Operculella padwickii* (FOOT ROT)
- C. External base/stem lesion; white mycelium on lesions with/without white mycelial knots developing into dark sclerotia. .. *Sclerotinia sclerotiorum* (STEM ROT)
- sis mild mottle clearly seen on broader leaflets of kabuli cultivars; no phloem necrosis.
- b) No proliferation of branches
- i) Browning of older leaflets in desi and yellowing in kabuli cultivars; younger leaflets remain green; no phloem browning. .. Salinity injury
- ii) Young foliage bright yellow; terminal bud necrosis; mottle at mid-height on a recovering plant .. Iron deficiency (CHLOROSIS)
- II. Drying without general wilting
- A. Stunting/discoloration
1. No external rotting of roots
- a) Proliferation of branches
- i) Browning of leaves in desi and yellowing in kabuli cultivars; phloem necrosis in the collar region .. Unidentified virus (STUNT)
- ii) Terminal bud necrosis .. Alfalfa Mosaic Virus
2. External rotting of roots; galls on roots quite distinct from *Rhizobium* nodules .. *Meloidogyne* spp. (ROOT-KNOT)
- B. No stunting/dyscoloration; only tops may show drooping; rotting of most roots; tap root brittle; minute sclerotia and/or sparse grey mycelium in pith cavity in the collar region, which can be seen with a 10X hand lens. .. *Rhizoctonia bataticola* (DRY ROOT ROT)

Also the sclerotia can be seen under the root bark, which peels off easily.

We wish to make a special mention of chickpea stunt. We feel that this particular disease, which is observed at most places in India and also many other chickpea-growing countries, contributed in a major way to the confusion in diagnosis. Very frequently, it is possible to isolate *Fusarium* spp. from the root system of the stunt-affected plants, but no one could produce typical stunt symptoms with any *Fusarium*. It is pertinent to cite here the observations made by Prasad and Padwick (1939). They divided the wilt-affected plants into three groups on the basis of symptoms. These were:

1. Those in which the first symptom was drooping of the upper leaves followed soon by the lower leaves. The plants withered and died within about a week.
2. Those in which the leaves gradually turned yellow and then began to drop, the remaining leaves rapidly withering and the plant dying.
3. Those in which the leaves became red. In the later stages these plants resembled those of group (2)."

Whereas the symptoms of first group above are of typical wilt (*Fusarium oxysporum* f. sp. *ciceri*), the symptoms in the second group can also be seen in the wilt in certain genotypes. The symptoms of the third group, however, are never seen in wilt and we feel certain that those are of stunt. Further Prasad and Padwick (1939) mentioned phloem browning as a symptom of wilt, but in the results of their pathogenicity tests they did not mention red leaves or phloem browning. Obviously, they were unable to produce those symptoms through inoculations with *Fusarium*. It seems, therefore, that

chickpea stunt was not identified earlier and was confusing the workers.

### **Wilt (*Fusarium oxysporum* f. sp. *Ciceri*)**

#### **Occurrence**

The disease is relatively more serious and has been reported from Burma, India, Mexico, Pakistan, Peru, and the USA. From several other countries, *Fusarium* species have been reported and we presume that the wilt fungus is also present in those countries. The disease is widely prevalent in India.

#### **Symptoms**

We have given a detailed description of symptoms in the bulletin for diagnosing wilt-like disorders of chickpea. The characteristic symptoms are (a) sudden drooping of leaves and petioles, (b) no external rotting of roots, and (c) black internal discoloration involving xylem and pith.

#### **Early/late wilt**

In northern India, wilt is often referred to as "early" or "late" wilt, depending upon the time of occurrence. Early wilt refers to seedling wilt (October-November) and late wilt refers to wilting at post-flowering stage (February-March). Generally the wilt incidence is negligible in the intervening period. We think it is possibly due to the cold winter in northern India that the wilt incidence is negligible during the vegetative stage. With moderate winter at Hyderabad, we have not noticed any clear-cut "early" or "late" wilt; in fact, wilt occurs here right from the seedling through the podding stage.

#### **Loss estimation**

As in several other diseases, no precise information on losses caused by this dis-

ease is available from any country.

According to a rough estimate, about 10% loss in yield due to wilt was considered to be a regular feature in chickpea-growing states of India (Singh and Dahiya, 1973). According to Grewal et al. (1974), 2 to 5% loss is caused every year in India, but it could go as high as 60%. In both these reports the term wilt was used in a general sense to include mortality due to various causes, and not due only to *F. oxysporum* f.sp. *ciceri*.

To get an idea about the loss on a per-plant basis in relation to the stage at which the wilt occurs, we conducted an experiment in the 1977-78 season. Wilting prior to the flowering stage, of course, results in total loss. We therefore selected stages after podding had begun. Four cultivars were included in the study. These were sown on 14 October 1977 in a wilt-sick plot and also in a nonwilt-sick plot. Healthy plants were obtained from the latter as most of the plants of these cultivars in the wilt-sick plot were affected. Thirty plants of cvs Chafa, P-436, JG-62 and 850-3/27 showing wilt at three stages (flowering/podding; full podding; preharvest) were tagged from 15 January onwards and harvested on 27 February 1978. Likewise, 30 healthy plants of each cultivar were also harvested for comparing yields and estimating losses. The data on grain yield loss and loss in 100-seed weight is presented in Table 7.

These data reveal that (a) earlier wilting caused more loss than late wilting, though even the latter resulted in substantial loss, (b) the 100-seed weight was adversely affected by wilt, and (c) loss in seed weight at all the three stages of wilting was much more in JG-62 and P-436 than in Chafa and 850-3/27.

Seeds harvested from diseased plants of chickpea were lighter, rough (wrinkled surface), and dull in color as compared to healthy ones.

Chauhan (1960) attempted to develop a loss estimation technique based on the time and amount of wilting. There was, however, no followup on that.

### Stage of infection

In 1977-78 season we conducted experiments to see at what stage plants are infected. Two cultivars, one highly susceptible (JG-62) and one moderately susceptible (850-3/27), were raised in heavily inoculated soil in pots. Whereas cv JG-62 was infected on the fourth day after sowing, cv 850-3/27 was infected on the seventh day. JG-62 showed 100% infection within 6 days, but 850-3/27 showed that much only after 20 days. Age of chickpea plants at the time of inoculation was found to influence infection. Cultivars JG-62 and 850-3/27 could not be infected after they reached the age of 70 and 63 days, respectively.

### Systemicity

In repeated studies we have confirmed that the fungus is systemic and can be isolated from all parts of an infected plant, including the seed.

### Seed transmission

Our studies have conclusively established that the fungus can be internally seed-borne and it is located mostly as chlamydospores in the hilum region of the seed. Cultivars show differences in seed transmission percentage.

We have further found a fungicidal seed treatment to eradicate the fungus. Benlate-T (30% benomyl + 30% thiram) at 0.15% rate eradicates the fungus completely.

We have adapted a seed-clearing technique (using NaOH) to directly observe the fungus in the hilum region of seed.

It may be pointed out here that Erwin and Snyder (1958) had suspected seed transmission of the wilt fungus, but Westerlund et al. (1974) failed to obtain evidence of such transmission. Westerlund et al. have not mentioned the cultivar from which they obtained the seeds; nor have they made clear

**Table 7. Influence of wilting at different stages on the grain yield of four chickpea cultivars.<sup>a</sup>**

Cultivar	Stage of plant <sup>b</sup>	Average number of seeds/plant	Average seed weight/plant (g)	Percent loss in seed weight/plant	100-seed weight (g)	Percent reduction in 100-seed weight
Chafa (Wilted)	S1	22	2.80	89.23	13.09	22.12
	S2	60	7.85	69.80	14.00	16.71
	S3	132	19.86	23.61	15.00	10.76
	(Healthy)	158	<u>26.00</u>		<u>16.81</u>	
P-436 (Wilted)	S1	25	2.08	91.40	9.44	35.16
	S2	56	5.66	76.61	10.37	28.77
	S3	121	12.16	49.75	11.17	23.28
	(Healthy)	161	<u>24.20</u>		<u>14.56</u>	
JG-62 (Wilted)	S1	15	1.44	94.26	8.44	44.51
	S2	42	4.36	82.65	9.62	36.75
	S3	133	14.76	41.26	12.18	19.92
	(Healthy)	166	<u>25.13</u>		<u>15.21</u>	
850-3/27 (Wilted)	S1	9	1.41	91.45	15.75	43.44
	S2	20	5.83	64.66	20.85	25.13
	S3	50	12.10	26.66	23.31	16.30
	(Healthy)	61	<u>16.50</u>		<u>27.85</u>	

<sup>a</sup>Data represent averages of 30 plants

<sup>b</sup>S1 - Flowering and podding

S2 - Full podding

S3 - Preharvest

whether seeds for their tests were obtained from wilted plants. As pointed out earlier, it is important to know the cultivar, as there seem to be clear differences between cultivars in percentage seed transmission. In our tests we found that the extent of seed transmission in cv Chafa was considerably less than in cvs JG-62 and P-436.

#### Survival/host range

We have not seen any published paper on this aspect. It is logically presumed

that the fungus survives in the dead plant debris in the soil. There are many questions on this aspect that need answers. As a first step we have initiated an experiment to find out how long the fungus can be detected in dead plant tissue buried in the soil. The experiment is continuing. The fungus could be detected in the buried roots after 6 months. In leaflets and stem pieces, it could not be detected after 2 and 4 months, respectively.

Since nonsusceptible plant species are known to be carriers of pathogenic

*Fusaria* (Armstrong and Armstrong, 1948) we wanted to know if such a situation exists in case of chickpea wilt *Fusarium*.

Plant species were sown in the chickpea wilt-sick plot in 5-m rows (50 seeds/row) along with the susceptible chickpea cv JG-62 on 28 October 1977. They were observed for wilt symptoms up to March 1978. Isolations of *Fusarium* were attempted from five plants of each crop at 30-day intervals during the season (Table 8).

From wilt-sick plots, naturally growing weeds were collected throughout the season and isolations of *Fusarium* were attempted on a selective medium, as shown in Table 9.

*Fusarium* isolates from crop plants grown in the wilt-sick plot as well as from weeds, were multiplied in the laboratory on potato-sucrose broth and tested for pathogenicity using the water culture technique and the susceptible JG-62 cultivar of chickpea. Although the results with regard to certain plant species tallied with those obtained through laboratory tests, the *Fusarium* (*Fusaria*) from field grown plants proved nonpathogenic. This is intriguing and will be investigated further.

## Screening techniques

### Water culture

The water culture technique is similar to the procedures described by Wensley and McKeen (1962) and Roberts and Kraft (1971). The steps are:

1. An isolate of *Fusarium oxysporum* f.sp. *ciceri*, most predominant in ICRISAT fields, is used for inoculations. The culture was originally single-spored and is being maintained.
2. Inoculum is multiplied on PD broth (100 ml) in flasks (250 ml) on a shaker for 10 days at room temperature (25°-30°C).
3. The inoculum (entire contents of

the flask) is diluted with sterilized distilled water to get a final inoculum concentration of 2.5% (spore concentration  $6.5 \times 10^5$ ).

4. Seedlings 14 to 18 days old, raised in autoclaved sand, are transferred to glass tubes containing 20 ml of inoculum. Seedlings are held in position by cotton plugs. Sterilized distilled water is filled in tubes after every 48 hours to make up the loss of water.
5. Ten seedlings are used for each line/cultivar. A susceptible check cultivar (JG-62) is likewise inoculated with each batch of test lines. Also for each line/cultivar, a noninoculated seedling is kept as check.
6. The susceptible check usually wilts between 7-10 days. Data are recorded 15 days after inoculations. Non-inoculated seedlings remain green for more than 3 weeks.

### Pot screening

The procedure we have followed is similar to that described under pigeonpea wilt. The only difference is that we use Vertisol instead of Alfisol. After incorporation of inoculum, susceptible cv JG-62 is grown and wilted plants are incorporated in the soil of those pots. Once more the same procedure is followed. After two such cycles, the pots are ready for use in screening. This procedure, like the water culture technique, is being used to supplement field screening and in assisting breeders in inheritance studies.

### Sick plot

In contrast to pigeonpea wilt-sick plots, we had an easy time in developing wilt-sick plots for chickpea. In 1975-76 season, wilt appeared in a corner of a 2-ha block. By the end of the 1976-77

**Table 8. Detection of *Fusarium* in the roots of different plant species grown in the wilt-sick plot (B-5).**

Crop	Isolation of <i>Fusarium</i> from 5 plants		
	16-11-1977	16-12-1977	25-1-1978
Mungbean	+ + + - -	+ - - - -	
Black gram	- - - - -	- - - - -	
Pea	+ + - - -	+ + - - -	
French bean ( <i>Phaseolus vulgaris</i> )	+ + + - -	+ + - - -	
Groundnut	+ + - - -	+ - - - -	+ - - - -
Lucern	- - - - -	- - - - -	
Lentil	- - - - -	- - - - -	
Soybean	- - - - -	- - - - -	
Cowpea	+ + + - -	+ + - - -	+ + - - -
Pigeonpea (ICP-6997)	+ + + + -	+ + + + +	+ + + + -
Pigeonpea (NP(WR)-15)	+ + + - -	+ + + - -	+ + + - -
Sorghum (CSH-1)	- - - - -	- - - - -	- - - - -
Climbing bean ( <i>Dolichos lablab</i> )	- - - - -	- - - - -	- - - - -
Chilli	- - - - -	- - - - -	- - - - -
Tomato	- - - - -	- - - - -	- - - - -
Pearl millet (NHB-3)	- - - - -	- - - - -	- - - - -
Pearl millet (HB-3)	- - - - -	- - - - -	- - - - -

+ Isolated  
- Not isolated

season, the whole plot developed into an almost uniformly sick plot. We incorporated all the dead plants in the same plot and had excellent screening in 1977-78. We made use of this information and have developed sick plots totaling about 4 ha by growing susceptible cultivars and incorporating dead plants.

One plot of about 1.0 ha has been developed as a multiple disease sick plot. Every year we add to it all dead plants from this as well as from other plots, regardless of the cause of death. We have thus obtained substantial infection not only from *F. oxysporum* f. sp.

*ciceri*—the most common fungus in our fields at ICRISAT Center—but also from *Sclerotium rolfsii*, *Rhizoctonia solani*, *Rhizoctonia bataticola*, etc.

Because we cannot exclude the build-up of other soil-borne pathogens in our wilt-sick plots, we expect that, as time passes, all our plots will become multiple disease sick plots.

#### Screening work

Table 10 summarizes our work up to the end of 1978.

**Table 9. Detection of *Fusarium* in the roots of several weed species found growing naturally in the chickpea wilt-sick plots.**

Weed	Isolation of <i>Fusarium</i> <sup>a</sup>				
	7-11-'77	11-11-'77	18-11-'77	28-12-'77	16-1-'78
<i>Amaranthus viridis</i>	+	-	-	x	x
<i>Hibiscus panduriformis</i>	+	-	-	x	x
<i>Phyllanthus niruri</i>	+	-	-	-	-
<i>P. medenaspatensis</i>	-	-	-	x	x
<i>Corchorus olitorius</i>	-	-	-	-	-
<i>Digera arvensis</i>	-	-	-	-	-
<i>Launea asplenifolia</i>	-	-	-	x	x
<i>Xanthium strumarium</i>	-	-	-	-	x
<i>Cyanotis axillaris</i>	-	-	x	x	x
<i>Euphorbia prostata</i>	-	-	-	-	-
<i>E. hirta</i>	-	-	-	-	-
<i>Indigofera</i> sp.	-	-	-	x	x
<i>Convolvulus</i> sp.	-	-	-	x	x
<i>Cassia</i> sp.	-	-	-	x	x
<i>Cyperus rotundus</i>	+	+	-	+	+
<i>Commelina bengalensis</i>	-	-	-	x	x
<i>Paspalum distichum</i>	-	-	x	x	x
<i>Eragrostis</i> sp.	-	-	x	x	x
<i>Desmodium triflorum</i>	-	-	-	x	-
<i>Heliotropium</i> sp.	-	-	-	x	-
<i>Tribulus terrestris</i>	+	-	+	+	x
<i>Cardiospermum halicacabum</i>	-	-	+	+	x
<i>Convolvulus arvensis</i>	-	-	+	+	+
<i>Lucas aspera</i>	x	x	x	-	-
<i>Argemone mexicana</i>	x	x	x	-	-

+ : Present

- : Absent

x : Not attempted

<sup>a</sup> Five plants were used. Even if a single plant yielded *Fusarium*, + sign has been indicated.



Chickpea *Fusarium oxysporum* wilt screening at ICRISAT.

**Table 10. Screening of chickpea for wilt/root rots resistance, 1976-1978.**

Germplasm screened in wilt-sick plot	:	6000
Promising against wilt	:	120
Germplasm screened in multiple disease nursery	:	1300
Promising against wilt/root rots	:	80
Lines being tested in International Chickpea Root Rots/Wilt Nursery (19 countries/37 locations)	:	63
Breeding materials		
Screened	:	3300
Promising	:	175

#### Resistant lines

We consider the following lines/cultivars to be resistant to *Fusarium* wilt:

ICC-202, ICC-391, ICC-658, ICC-858, ICC-1443, ICC-1450, ICC-1611, ICC-3439, ICC-4552, NEC-790, WR-315, CPS-1, JG-74, and BG-212.

Work on wilt resistance has been done mainly at Kanpur (Singh et al. 1974)—where the resistant line WR-315 was developed—and at Jabalpur (Sharma and Khare, 1969). The sick plot screening at Gurdaspur is mainly against *Operculella padwickii*, the foot rot organism (Singh and Bedi, 1974). Some work has also been done in Mexico (Lopez Garcia, 1974).

#### Existence of physiologic races

Chauhan (1962) seems to be the only worker who made attempts to study variation in this pathogen. He studied 22

isolates and grouped them into five groups on the basis of filtrate toxicity and percent mortality in pot inoculations however, he did not specify them as races

Preliminary studies have provided us evidence of the existence of races. The pot culture procedure was followed to study the pathogenicity of five isolates of *F. oxysporum* f.sp. *ciceri* collected from as many locations (Hyderabad, Hissar, Jabalpur, Kanpur, Gurdaspur). Ten genotypes, 4 resistant and 6 susceptible to the Hyderabad (ICRISAT) isolate, were used. The test was conducted three times and reactions in most cases were consistent. Table 11 summarizes the results.

A critical look at the results in Table 11 reveals that C-104 is resistant to the Gurdaspur isolate but susceptible to all others. JG-74 is resistant to all isolates except the Kanpur one. CPS-1 is resistant only to the ICRISAT isolate. WR-315 is resistant to all isolates except the Gurdaspur isolate. JG-62, Chafa, and L-550 are susceptible to all isolates and moderately susceptible to Gurdaspur isolate. 850-3/27 is susceptible to the ICRISAT isolate and moderately susceptible to all others.

The Gurdaspur isolate was differentiated from others through resistance of C-104 and susceptibility of WR-315. The Kanpur isolate was differentiated through susceptibility of JG-74. If the "R" and "M" categories are considered as not too distinct, the ICRISAT, Hissar, and Jabalpur isolates could be considered identical; on the other hand, if these categories are considered distinct, then only the Hissar and Jabalpur isolates could be considered identical and the ICRISAT isolate a distinct one. The data indicate that we may have three or four distinct races.

Before we draw conclusions on this aspect, however, we would like to verify how serious these isolates are in field conditions at respective locations. Kraft and Haglund (1978) have emphasized this aspect in their paper on *F. oxysporum* f. sp. *pisi*.

**Table 11. Reactions of chickpea cultivars to five isolates of *Fusarium oxysporum* f. sp. *ciceri*<sup>a, b</sup>**

Cultivars	Isolates from				
	ICRISAT Hyderabad	Hissar	Jabalpur	Kanpur	Gurdaspur
JG-62	S	S	S	S	M
C-104	S	S	S	S	R
BG-212	R	M	M	S	M
JG-74	R	R	R	S <sup>c</sup>	R
CPS-1	R	M	M <sup>d</sup>	S	S
WR-315	R	R	R	R	S <sup>c</sup>
Annigeri	S	S	S	S	S
Chafa	S	S	S	S	M <sup>d</sup>
L-550	S	S	S	S	M
850-3/27	S	M	M	M	M

<sup>a</sup>20 seedlings were used in each test and test was carried out three times.

<sup>b</sup>R = Resistant (less than 20% wilt)

M = Moderately susceptible (20-50% wilt)

S = Susceptible (more than 51% wilt)

<sup>c</sup>Showed 'S' reaction in two tests and 'M' in one.

<sup>d</sup>Showed 'M' reaction in two tests and 'S' in one.

## Other Pathogens

Most of the literature on other soil-borne fungi deals with disease identification and prevalence. Almost no work has been done on the epidemiology of these organisms in relation to the diseases they cause in chickpea and on host resistance.

We have learned from surveys in chickpea-growing countries that *Ascochyta* blight and stunt are widely prevalent, but these do not fall within the scope of our present review. As far as the soil-borne diseases are concerned, after wilt, dry root rot caused by *Rhizoctonia bataticola* is a relatively major problem, particularly where daytime temperatures rise to 30°C in the post-flowering stage.

All other fungi discussed below are generally present, but are more of local importance, the incidence varying from field to field.

In general, we observe more diseases at experiment stations than in farmers' fields. This we attribute to certain factors in farmers' fields such as rotations, mixed cropping pattern, and wide spacings because of broadcast sowings; many of these will change once high-yielding cultivars are available to farmers. There will be more monocropping of chickpea, which might mean more soil-borne diseases, unless resistant cultivars are made available right from the beginning. Our efforts to identify good lines under multiple disease and multi-location testing situations represent a step in that direction. For location-

specific diseases, the germplasm collection of ICRISAT will be made available to concerned pathologists for identifying resistance.

In the following paragraphs we discuss other soil-borne fungi. Symptoms have been mentioned earlier.

#### ***Rhizoctonia bataticola* (Dry root rot)**

This pathogen does cause substantial mortality and loss in a crop which gets caught in higher ambient temperatures (30°C and above) in the post-flowering stage. In the Indian situation, this occurs in central and southern India where we see more dry root rot; it is insignificant in northern India, where cooler temperatures extend through March and by the time temperatures rise, the crop is ready for harvest. Likewise, in Lebanon, Syria, Turkey and Iran, we have observed only a low incidence of dry root rot.

We are attempting to develop a laboratory screening procedure based on root lesion length as the criterion for comparing genotypes and hope to standardize a procedure in the near future.

Dry root rot in ICRISAT Center sick plots is common in the post-flowering stage. In both chickpea and pigeonpea, the disease is observed more in Vertisol, and we find (in the laboratory) that Vertisol extract medium supports more sclerotia production than Alfisol extract medium. Our screening does help us identify highly susceptible cultivars.

#### ***Rhizoctonia solani* (Root rot)**

Root rot has never been reported to be serious from any chickpea-growing area. Most of the incidence is in the seedling stage when soil moisture content is high, particularly in chickpea planted after the harvest of paddy. In irrigated chickpeas, the disease may occur any time. We have seen root rot occasionally in our multiple-disease nursery at ICRISAT Center.

#### ***Sclerotium rolfsii* (Collar rot)**

The incidence is related to high moisture content and presence of undecomposed organic matter near soil surface. It is a problem in the seedling stage, except in irrigated crops where the disease can occur at any stage, provided temperatures are not low. Chickpea following paddy shows more incidence.

Our multiple-disease sick plot shows some incidence of collar rot every year. At Jabalpur, where the crop in the sick plot is irrigated, the collar rot incidence is relatively higher.

#### ***Sclerotinia sclerotiorum* (Stem rot)**

The problem is seen in northern India where cool temperatures, relatively more rain in January, and heavy dew, which are favorable to the pathogen, occur. The disease does cause substantial damage if plantings are close and the crop canopy is thick. In seasons with heavy rain, when the vegetative growth of chickpea becomes excessive, this disease can become serious.

No attempt has been made to identify resistance to this disease. Besides India, the disease has been reported from Chile (Mujica 1955) and Iran (Kaiser 1972).

#### ***Operculella padwickii* (Foot rot)**

Kheswalla (1941) described this disease first from Punjab and Delhi in northern India. Although the fungus has been isolated from several locations in central and northern India, the disease seems to be location-specific; at Gurdaspur in northern India, it is the most dominant one in the sick plot. We think wet soil is conducive to this disease. From Gurdaspur, Singh and Bedi (1975) reported that G-543 is a resistant cultivar and F-61 is moderately resistant.

This fungus has been reported only from India.

### ***Fusarium solani* (Root rot)**

Kraft (1969) first reported that *F. solani* f.sp. *phaseoli* can infect chickpea. Westerlund et al. (1974) reported it to be one of the root rotting fungi of chickpea in California. The same year Grewal et al. (1974) reported it from northern India. Although the fungus has been isolated from diseased chickpea plants from different areas of India, it is restricted mainly to northern India. The chickpea plots at New Delhi usually show a higher incidence of *F. solani*, and screening against this pathogen should be possible there.

No specific resistance sources have yet been identified.

### ***Ozonium texanum* var. *parasiticum* (Wilt/Foot rot/Root rot?)**

Mishra (1955) first reported this pathogen from Bihar state of India. He called the disease wilt although the fungus causes rotting at the base as well as of roots. So far the disease has been reported from Bihar state and the adjacent area of eastern Uttar Pradesh state.

Again there is no information on resistance to the disease.

### **A sterile fungus (white seed and root rot)**

Haware and Nene (1976) have reported a sterile fungus responsible for causing seed rot as well as root rot. Thick white mycelial strands cover the seed, affecting germination, or cover the young roots of seedlings. The disease is observed only if the soil is too wet after sowing, which happens due to chance rains.

Since the disease is a minor problem we have not done any further work.

### ***Meloidogyne* spp. (Root-knot)**

The problem has been seen mainly in irrigated chickpeas; more incidence has been

noted in northern India. A good root-knot infested plot at Ludhiana offers an excellent opportunity to screen for resistance. After the problem was identified at Ludhiana, there has been increased interest in this problem amongst the nematologists in northern India.

One of the species identified is *M. incognita* (Ahmad Jamal 1976).

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## Appendix 1

### Development of Pigeonpea Wilt-sick Plots

#### Vertisol sick plot A (1.5 ha)

- |                    |  |                         |   |
|--------------------|--|-------------------------|---|
|                    |  |                         |   |
| March 11, 1975     | : Added 750 cu.ft. of compost (chopped stubble) of field-wilted pigeonpea, pod husk and sorghum heads after these were composted together for 1 or 2 months. | April 30, 1976          | : Scattered 7.50 q of <i>Fusarium</i> -colonized pigeonpea seeds.                             |
| May 1st week, 1975 | : Again added 750 cu.ft. of compost as described above.  | July 12, 1976           | : Incorporated 11.25 q of <i>Fusarium</i> multiplied on Alfisol + pigeonpea flour (9:1 w/w).  |
| June 26, 1975      | : Incorporated 1.87 q of sorghum grain colonized by pigeonpea <i>Fusarium</i> .  | July 29, 1976           | : Buried wilted pigeonpea stem pieces (15 cm) in every row (one piece after every two plants) |
| April 30, 1976     | : Scattered 7.50 q of <i>Fusarium</i> -colonized pigeonpea seeds.  | February and March 1977 | : Scattered 400 cu.ft. wilted pigeonpea stem bits.  |
| May 5, 1976        | : Chopped and incorporated into soil stubble of all wilted plants.   | April 6&7, 1977         | : chopped and incorporated all the wilted plant stubble of 1976-77.                           |
| July 12, 1976      | : Incorporated 11.25 q of <i>Fusarium</i> multiplied on Alfisol + pigeonpea flour (9:1 w/w).   | May 1978                | : Chopped and incorporated all the wilted plant stubble of 1977-78.                           |
| April 5, 1977      | : Chopped and incorporated into soil stubble of all wilted plants.   | May 1978                | : Scattered uniformly about 500 cu.ft. of wilted pigeonpea stem bits.                         |
| May 2, 1978        | : Chopped and incorporated into soil stubble of all wilted plants.   |                         |   |
| May 8, 1978        | : Scattered uniformly about 500 cu.ft. of wilted pigeonpea stem bits.  |                         |   |

#### Alfisol sick plot A (0.1 ha)

- This plot was used as pigeonpea sterility mosaic screening nursery for 3 years (1974-77) continuously. During that period increased wilt incidence was observed every year.
- In 1977-78 the plot was used to screen pigeonpea for wilt and sterility mosaic diseases. Wilt-susceptible check line (ICP-6997) showed 99.4% disease.

#### Vertisol sick plot B (1.5 ha)

April 19&20, 1976: Scattered 1,500 cu.ft.

- All wilted plant stubble of 1977-78 was chopped and incorporated into soil (April 24, 1978)

**Alfisol sick plot B (0.4 ha)**

- 1977-78 : Planted pigeonpea materials for sterility mosaic screening.
- January 1978 : Wilt incidence was observed in large patches.
- April 1978 : Chopped and incorporated into soil all the wilted plants.
- May 1978 : Scattered about 400 cu.ft. of pigeonpea wilted stem bits.

## Appendix 2

### Suggested Points for Discussion

#### Pigeonpea wilt

1. Have our studies on the survival of *Fusarium udum* in pigeonpea stubble been carried out adequately?
2. What could be the reasons for the failure of water culture screening technique in the case of pigeonpea but not chickpea?
3. The technique of transplanting seedlings, roots of which are injured and inoculated, to autoclaved sand/soil in pots gave us erratic results. What could be the reasons?
4. We would appreciate comments/criticism on the pot screening procedure we have developed.
5. We have developed two wilt-sick plots in Vertisol for resistance screening.
  - (a) Is it possible that the plots may contain "too much" inoculum as the years pass?
  - (b) Are we likely to face other problems?
  - (c) We are using mainly one susceptible check (ICP-6997) to monitor wilt sickness. Is that adequate?
  - (d) The susceptible check rows are planted after every 2 to 4 test rows also to ensure that inoculum multiplies every year. Is this adequate or should we follow the procedure of growing only a susceptible cultivar one year and test material in the next year (with a few check lines)? The two sick plots that we have developed can be used in such a way that when one has only the susceptible cultivar, the other would have the breeding material.
- (e) There are indications that continuous planting of pigeonpea is resulting in poorer growth in every succeeding season. This is likely to result in rejection of breeding material that may be resistant but shows poor growth in sick plot. What could be done to avoid such a situation?
6. Our experience tells us that wilt sickness can be developed more quickly and uniformly in Alfisol than in Vertisol. We have developed two large sick plots in Vertisol because farmers prefer this type of soil (i.e., deep soils) for cultivating pigeonpea. We find that some genotypes that show "resistance" in Vertisol get affected by wilt in Alfisol, but the reverse has never happened. Should we therefore develop sick plots in Alfisol and give up the existing sick plots in Vertisol? Or should we have large sick plots in both types of soil? We must mention here that to grow pigeonpea, irrigation is required in Alfisol but not in Vertisol.
7. We consider multilocation testing of promising lines desirable before using them in crosses. Is our thinking correct?
8. Since the wilt incidence increases considerably after ratooning, is it desirable to go by the post-ratoon reaction of lines?
9. What are the possibilities of developing a selective medium for *Fusarium udum*?

### Pigeonpea Phytophthora blight

1. Our observations concerning the survival of the fungus have been described on page 13. We need suggestions to plan research on this aspect.
2. We would appreciate comments/criticism on the pot screening procedure we have developed.
3. We need suggestions to improve upon our field screening procedure.

### Chickpea wilt/root rots

1. Many plant species grown in the wilt-sick plot yielded *Fusarium*, which morphologically looked similar to the isolate of *F. oxysporum* f. sp. *ciceri*. However, *Fusarium* isolates from all these plant species were nonpathogenic to chickpea. We would appreciate discussion on this point.
2. We would appreciate comments/criticism on water culture and pot culture screening techniques for wilt resistance.
3. Several soil-borne pathogens that can attack chickpea are present in most soils, even though one or two pathogens may dominate. In sick plots at ICRISAT *Fusarium oxysporum* f. sp. *ciceri* dominates, but other pathogens such as *Rhizoctonia bataticola* also kill many lines. Should we therefore encourage "multiple-disease sick plots" and identify lines which show least mortality for use in the breeding program? Or should we concentrate on working out procedures for identifying resistances to different soil-borne pathogens individually?
4. Pathogens other than *Fusarium oxysporum* f. sp. *ciceri* are important at other locations. For example *Operculella padwickii* is the dominant fungus at Gurdas-

pur. How should we conduct work to meet such situations?

5. Evidence indicates that physiologic races of *Fusarium oxysporum* f. sp. *ciceri* exist. Is multi-location testing of our promising lines the only answer to meet this situation?
6. Dry root rot caused by *Rhizoctonia bataticola* is another widely prevalent disease. We are making attempts to develop a laboratory screening procedure based on root lesion length. We invite your comments/criticism/suggestions.
7. We may have to work out techniques to screen for resistance to root rots caused by *Fusarium solani* and *Rhizoctonia solani*. We would appreciate suggestions.

## Discussion — Session 1

Chairman : R. K. S. Wood  
Rapporteur : M. V. Reddy

After Dr. Nene reviewed the work done at ICRISAT on the soil-borne diseases of pigeonpea and chickpea, he raised some points for discussion.

Nene : Have our studies on the survival of *Fusarium udum* in pigeonpea stubble been carried out adequately?

Chohan : In Punjab we have seen *F. udum* in groundnut field soil where pigeonpea was never grown. That means *Fusarium udum* can survive in any field for many years.

Wood : What species was it and who identified it?

Chohan : *Fusarium udum*, and it was identified by the Commonwealth Mycological Institute (CMI). However, pathogenicity was not tested.

Wood : It could be saprophytic!

Kraft : Pea wilt fungus can survive in the rhizosphere in the form of chlamydospores for long periods and can survive on weed hosts without actually penetrating them. Dr. Nene, what method did you follow for the detection of the fungus?

Nene : Root stubble after weighing was buried in big pots and isolations were made from root tissue after every 6 months. After 2½ years no root tissue could be detected in black and red soils. However, the soil around the buried stubble was sieved for any decomposed root bits and isolations were tried from these. After 3 years

no *Fusarium udum* could be isolated from red soil.

Kannaiyan : Soil around the buried roots was plated but *F. udum* was not isolated on selective medium; rather, *F. solani* was isolated.

Singh : The crop sequence can affect the survival of the chlamydospores. Following will result in the decrease of chlamydospores. In some parts of Uttar Pradesh a 3- to 4-year rotation was found to control *F. udum*; but in some other areas even a 7- to 8-year rotation did not help. This could be due to the sequence of different crops in the rotation. Some crops may help in formation of repeat chlamydospores without being hosts of the pathogen.

Wood : Survival studies can be rather academic. Are present techniques adequate to study survival? Use of biological methods—e.g., growing of susceptible varieties to detect population levels—should be explored.

Abawi : Disease incidence does not always give a real estimate of the soil population level of the pathogen, as many other factors besides inoculum density affect disease incidence and severity. Also, survival of a pathogen in soil maintained fallow or under different cropping systems can be quite different and thus worth investigating.

Singh : If other leguminous crops

- follow pigeonpea, it can result in the formation of more chlamydospores. Grami-  
naceous crops can reduce chlamydospore formation.
- Purss : Dr. Nene, what was the reason for the survival study?
- Nene : Earlier reports on survival were vague, and workers in the Botany laboratory of Madras (India) felt that only fungus surviving in the host tissue is important. We felt that there is no point in studying this aspect further if the fungus survives for more than 5 years. Points made by Drs. Singh and Abawi need to be taken into consideration.
- Hubbeling : The pea wilt fungus survives in soil for at least 10 years. In case of tomato wilt the fungus survives in the remainders of roots at depths of 90 cm and below where even steam cannot penetrate.
- Wood : What is the ultimate use of studies along these lines?
- Nene : To recommend rotations, if possible.
- Kraft : Are there available techniques for assessing the inoculum threshold for economic cultivation of the crop?
- Nene : The technique of transplanting seedlings, roots of which are injured and inoculated, to autoclaved sand/soil in pots gave us erratic results. What could be the reasons?
- Kraft : Inoculum density and environmental conditions are very important.
- Hubbeling : Soil pH could also be an important factor.
- Hagedorn : In the case of pea wilt, where seedlings were transplanted in sand, moisture and temperature played an important role. Temperature is particularly important.
- Hubbeling : Autoclaved soil if used directly is toxic and damaging to the roots. Its use is not advisable, in particular if the soil contains organic substances.
- Nene : Would Dr. Kannaiyan comment on any toxicity problem?
- Kannaiyan : No such problem was encountered.
- Kraft : What type of erratic results were obtained?
- Kannaiyan : Results were not repeatable.
- Kraft : It could be due to lack of homozygosity in the seed.
- Nene : Such results were obtained with the same seed lots.
- Wood : Was autoclaved or non-autoclaved soil used?
- Kannaiyan : Autoclaved soil.
- Singh : Erratic results could be due to contamination of soil.
- Nene : How would you avoid contamination?
- Wood : Why use autoclaved soil?
- Singh : Using non-autoclaved soil is better.
- Nene : Since using autoclaved soil is a well-established procedure, it was used. Then we used non-autoclaved soil; by adding inoculum and repeatedly growing a susceptible genotype, the pot screening technique is being standardized.
- Kraft : The erratic results could be due to low inoculum density.
- Abawi : Variability in soil moisture and especially during the early part of the test may greatly influence disease incidence and severity. Thus, you might want to consider

- using plastic pots instead of clay pots. Plastic pots tend to maintain more uniform moisture and also dry up slower. Also, as Dr. Kraft suggested, inoculum density plays a major role, and the initial inoculum density used in the procedure should be standardized, if possible.
- Nene : We would appreciate comments/criticism on the pot screening procedure we have developed.
- Wood : Can a sick soil technique be developed?
- Nene : Yes. What are the chances of overloading with inoculum?
- Kannaiyan : Overloading is not likely. Growing of two successive susceptible lines gave only 90% wilt incidence.
- Singh : Use of heavily contaminated soil may eliminate moderately resistant lines.
- Kannaiyan : Pot tests supplement field screening. We are not ignoring the intermediate types.
- Singh : Pot tests need different levels of inoculum.
- Nene : We obtained 90% incidence of disease in highly susceptible check, and not in all genotypes. Cultivar 1258 from Bihar, resistant to sterility mosaic, is highly susceptible to wilt and is used for this purpose.
- Wood : The importance of inoculum level in relation to disease must be emphasized.
- Purss : What is the need for the pot technique?
- Nene : The pot technique supplements field screening. This crop is of long duration and wilt development also takes a long time. In pots the reaction can be obtained within 2 months.
- Hubbelling : One would get quicker results if the inoculum is mixed in the entire pot and not only in the top layers.
- Kannaiyan : The inoculum is first mixed in the top layer but this should not prevent any downward movement of the inoculum.
- Hagedorn : Why not mix the entire soil with inoculum and then fill the pots?
- Allen : Most important is the correlation between the laboratory and field screening techniques and stability of resistance. Intermediate resistant lines may represent horizontal resistance.
- Nene : What is an intermediate type? I would like to have clarifications from Allen and Singh.
- Allen : Multilocational testing can give information on the stability of resistance.
- Nene : This is already in progress and differential reactions are observed.
- Purss : The role of different environmental conditions needs to be studied.
- Nene : We have developed two wilt-sick plots in Vertisol for resistance screening:
- (a) Is it possible that the plots may contain too much inoculum as the years pass?
  - (b) Are we likely to face other problems?
  - (c) We are using one susceptible check (ICP-6997) to monitor wilt sickness. Is that adequate?
  - (d) The susceptible check rows are planted after

every two to four test rows also to ensure that inoculum multiplies every year. Is this adequate or should we follow the procedure of growing only a susceptible cultivar one year and test material in the next year (with a few check lines)? The two sick plots that we have developed can be used in such a way that when one has only the susceptible cultivar, the other would have the breeding material.

conditions (such as soil structure, compaction, etc.) may play a significant role on plant vigor and productivity in succeeding seasons.

- Nene : There are indications that continuous planting of pigeonpea is resulting in poorer growth in every succeeding season. This is likely to result in rejection of breeding material that may be resistant but is showing poor growth in the sick plot. What could be done to avoid such a situation?
- Hagedorn : Grow other crops for 2 to 3 years for balancing the soil texture.
- Purss : This may not be a good idea.
- Hubbeling : Certain levels of resistance may break down at certain high temperatures of the soil.
- Chohan : Poor growth may be due to production of toxins by the biomass in soil.
- Nene : In black soil we have experienced more inhibition in growth and plant physiologists suspected allelopathy and nematode buildup later on.
- Reddy : Red soils showed comparatively less inhibitory effect on growth.
- Kannaiyan : In heavy rainfall years, we observed poor plant growth and this may be due to the toxins released from host residues.
- Nene : Pigeonpea in general is sensitive to excess water.
- Purss : What is the effect of cropping on wilt?
- Nene : The first year there is no wilt; in subsequent years the wilt increases.
- Hagedorn : That is, crop growing increases disease incidence?
- Hubbeling : There is always a microbial balance under field conditions. This means there are always antagonists of different origin which keep soil pathogens in a "natural" balance.
- Hagedorn : Wilt sickness can be monitored by some adequate controls.
- Wood : Is there any monitoring from the beginning?
- Nene : After every two test rows one susceptible check is grown. The same genotype is used every year.
- Wood : Some indicators may be insensitive.
- Purss : Why not use a resistant line as a check?
- Saksena : Theoretically, as inoculum density increases, disease severity should also increase. In some instances as with *Rhizoctonia*, increasing the inoculum above a certain point results in decreased disease severity.
- Abawi : Monoculture has been shown to result in decline in disease incidence and severity on other crops such as wheat. Deteriorating soil

- Singh : Continuous growing of the same crop affecting growth in sick plot may be due to effect of decomposing residues and due to some nutrition problem.
- Kraft : What about interaction with other organisms such as nematodes?
- Hagedorn : Root exudates may be toxic to the next crop.
- Sharma : There is no information on causes of poor growth so far. Pigeonpea after pigeonpea is the practice in some areas without much effect. The sick plot situation may be different. Some cultivars such as NP(WR)-15 are growing well.
- Kannaiyan : I do not agree fully. NP(WR)-15 had better growth in the earlier years than now.
- Chohan : What is the effect of high doses of nitrogen?
- Sharma : We got no response even up to 200 kg N/ha. It only helps in the initial vegetative boost.
- Nene : We consider multilocation testing of promising lines desirable before using them in crosses. Is our thinking correct?
- Hubbeling : It is correct.  
(All other consultants agreed)
- Kraft : Progenies ( $F_3$ ) also should be tested at different locations.
- Sharma : We have not yet started this for diseases.
- Nene : Since the wilt incidence increases considerably after ratooning, is it desirable to go by the postratoon reaction of lines?
- Wood : Is it a common practice?
- Nene : In certain areas of India; mostly in other countries.
- Abawi : If you are interested in only immune lines or those with a very high level of tolerance, my answer would be "Yes."
- Kraft : Is it necessary to have postratoon immunity?
- Sharma : Physiologists looking for annual types based differential seed sizes at the base and top of the fruiting branches. Types that hold on after ratooning are looked for, because they give plasticity in the crossing system. We are interested in such types.
- Nene : What are the possibilities of developing a selective medium for *Fusarium udum*?
- Singh : It is possible but may take a lot of time. Temperature, pH, and some other factors need to be worked out.
- Sinclair : It is the type of work that should be undertaken at a university laboratory.
- Wood : It would be a good study for an enthusiastic M.Sc. student.
- Kraft : Would low pH media be suitable for *Fusarium oxysporum*?
- Nene : We had tried Komada's medium (pH 3.8±0.2) but did not find it promising. Let me raise another question. Several soil-borne pathogens that can attack chickpea are present in most soils, even though one or two pathogens may dominate. In sick plots at ICRISAT *Fusarium oxysporum* f. sp. *ciceri* dominates, but other pathogens such as *Rhizoctonia bataticola* also kill many lines. Should we therefore encourage "multiple-disease sick plots" and identify lines that show least mortality for use in the

breeding program? Or should we concentrate on working out procedures for identifying resistances to different soil-borne pathogens individually?

Wood : I suggest you start with *Fusarium oxysporum* and bring in others later.

Hubbeling : It is important to have notes on all the diseases. But it is frequently difficult to know what diseases are involved.

Haware : We have two sick plots. In the multiple-disease sick plot all the diseased plants, irrespective of the pathogen, are added. In the wilt-sick plot only wilted plants are added. In the multiple-disease plot irrigation is given to encourage the mortality due to *Rhizoctonia solani*, *Sclerotium rolfsii*, etc. After every 20 days, dead plants are collected and isolations made to follow the sequence of the pathogens involved during the crop season.

Kraft : We experienced similar problems. Screening germplasm for individual pathogens in the laboratory and screening in the field against all should be more ideal.

Allen : I agree with Dr. Kraft, but disagree with the screening of germplasm in the greenhouse. It is desirable to encourage as many pathogens as possible in the field by providing infector rows, etc. Where field screening is inappropriate, the gaps can be bridged by suitable laboratory screening. So both approaches have to be taken into consideration.

Haware : First, screening is done

against *F. oxysporum* as it is the major problem, then plants are subjected to other, comparatively minor, pathogens.

Hagedorn : In commercial production how important are the pathogens other than wilt?

Nene : Wilts caused by *F. oxysporum* f. sp. *ciceri* and *R. bataticola* are more common and widespread; others are localized and less important.

Wood : I wonder when multiple-disease plot screening should be introduced and should it be at early stages?

Allen : Yes, it should be introduced at an early stage in the program.

Kraft : That will be difficult.

Purss : Are "multiple diseases" a problem in farmers' fields?

Nene : Yes.

Allen : Do the problems occur together?

Nene : Yes; it depends on the stage at which you see the fields.

Singh : All the problems are common in the farmers' fields. They develop at particular stages of plant growth.

Purss : Is it necessary to have resistance to all?

Sinclair : We could take examples from other crops. Often breeders develop a cultivar resistant to one disease but the new cultivar is susceptible to another pathogen that was not important in the past. There is a need to screen for resistances to several pathogens at one time.

Wood : It may be easy to get resistance to *F. oxysporum*. But much more difficult to get it against *Sclerotium*. What do you do then?

Nene : After multilocation testing, lines with low mortality are selected, seed multiplied, and supplied to breeders. I agree it would be difficult to get lines resistant to all pathogens at all locations.

Kraft : Cultivars for specific areas or with broad tolerance should be considered.

Nene : After the initial disease surveys, resistance to wilt and *R. bataticola* was found necessary in the semi-arid crops. Resistance to Ascochyta blight is absolutely necessary in some West Asian countries and some other chickpea-growing countries. The main objective is resistance to the major pathogens and low or no susceptibility to others.

# **Epidemiology**

# Aspects of Epidemiology of Soil-borne Diseases

G. S. Purss

In his book *The Principles of Plant Pathology*, Tarr (1972) suggests that epidemiology "has a wide meaning and has come to include most field aspects of disease. In a sense it is the interaction of crop, pathogen and environment, populations of plants and pathogens rather than individuals being involved." This is a most appropriate way to approach epidemiology, particularly in the case of soil-borne diseases.

I have been asked to talk on the epidemiology of soil-borne diseases based on my experience. I will not attempt to review such a wide subject but will refer to aspects of epidemiology with which I have become familiar in my work and discuss in detail some examples that may have application in the work here at ICRISAT.

In soil-borne diseases such as root rots, basal stem rots, and vascular wilts, epidemiology has the three main components referred to by Tarr. There is the survival of the pathogen between crops or alternative hosts either in a saprophytic phase or as resting propagules. The concepts developed by Garrett are well known in this regard. There is the effect of the environment on the buildup of inoculum and its dissemination to produce an epidemic, then there is the crop, which is our ultimate concern. These three factors are all intimately related, as has been often stressed by authorities on epidemiology.

It is generally much easier to see these three components in airborne diseases. Indeed, when epidemiology is discussed at conferences and congresses, emphasis is placed on diseases such as rusts, foliage blights, and fruit spots. Inoculum levels can be monitored relatively easily, using equipment such as spore traps, and, as a result, forecasting services have been developed for diseases such as potato blight (*Phytophthora*

*infestans*) and black spot of apple (*Venturia inaequalis*). There are of course techniques available for the measurement of resting propagules and spore levels in the soil and new improvements are continually being made, particularly with respect to selective media. Techniques developed by Cook (1968) for *Fusarium* spp. and by Ledingham and Chinn (1955) for *Cochliobolus sativus* serve as examples. The range of specific fungicides now available should assist further development in this area. Dodman and Reinke (1978), for instance, have improved techniques for the recovery of *C. sativus* in this way. In our laboratory in Brisbane, K.G. Pegg has modified an elegant sieving technique of McCain et al. (1967) for counting chlamydospores of *Phytophthora cinnamomi* in the soil. These techniques are certainly improvements on the baiting techniques and have already helped in the understanding of the epidemiology of the relevant diseases.

The effect of environmental factors is infinitely more complex with soil-borne diseases than with airborne diseases. Apart from the obvious direct effects of moisture and temperature on the germination, dissemination, and pathogenicity of organisms, the complexity of soil itself is highly significant. Physical factors in the soil have been stressed by Griffin (1972), and chemical composition has been known to be particularly significant, a good example being browning root rot of wheat caused by *Pythium* spp. (Vanterpool 1952). The biological component of the soil environment is so variable as to produce effects that defy resolution. Obviously, all these factors interact, as has been hypothesised as an explanation for the severity of diseases such as Diplodia blight in peanuts (Purss 1962). There have been great advances in recent times in our understanding of antagonism and

biological control in soils (Baker and Cook 1974; Wildermuth and Rovira 1978). Ethylene levels have been shown to be related to the balance of organisms in the soil (Smith 1976). Management practices have been developed that exploit such environmental effects on pathogens for control. These can be simple measures, such as drainage affecting aeration and moisture relationships, to complex biological control systems, such as have been devised for root rot of avocado (Pegg 1977). While generally the mechanisms involved in such biological control systems are incompletely understood, sometimes there is a very simple explanation. An example of the latter is the direct effect of cultivation procedures on the germination of propagules of *Fusarium solani* for the control of root rot of beans (Cook and Snyder 1965). A pathogen such as *Fusarium* f. sp. *cubense* may be affected by flooding (Stover et al. 1953) or disease severity might be minimized by careful selection of time of planting. The latter is well-known for black shank of tobacco caused by *Phytophthora nicotianae* var. *nicotianae* (McCarter 1967) or crown rot of wheat caused by *Gibberella zeae* (Purss 1971) (Table 1).

Let us turn now to the crop factors. Immediately it must be recognized that these cannot be easily separated from the environmental factors. In some diseases, such as charcoal rot of sorghum and soybeans (*Macrophomina phaseoli*), the response of the crop to stress conditions is paramount in epidemiology. In this case the organism is generally abundant so inoculum levels are of lesser significance. Crops may have a direct effect on inoculum potential; thus crop rotation has become a classical means of control. There are the conflicting effects of reduced incidence of certain soil-borne diseases by continual cultivation to one crop, an example being take-all of wheat (Shipton 1975). Susceptibility of weeds is an important factor and some of the work on the effects of "nonhosts" on the inoculum potential of *Verticillium dahliae* are interesting in this regard (Evans 1971). Micro-

sclerotia production in crop residues has a significant effect on *Verticillium* wilt of cotton (Francis et al. 1975). Some interesting effects important in epidemiology are related to the reaction of the cultivars grown. These may have a direct bearing on the amount of inoculum produced both during the life of a crop and between crops. The effect of environmental factors on inoculum production, and hence the development of epidemics, can be quite different with cultivars possessing different levels of resistance. Some of the conclusions we have reached with stem rot of cowpea (*Phytophthora vignae*) in this regard will be referred to later.

For the control of soil-borne diseases in broadacre crops, we will fall back in the main on disease resistance of the polygenic type or management procedures. We will need to have an understanding of epidemiology in both instances if we are to achieve greatest effectiveness. This will allow us to attack the pathogen at the most vulnerable point in its life cycle or grow cultivars with a level of field resistance adequate for the environment.

I would like to refer now to specific examples in Queensland.

Stem rot of cowpea (*Vigna sinensis*) is caused by the fungus *Phytophthora vignae*. Recorded first in Australia in 1950 causing devastating losses in cowpea (Purss 1953), it remained undetected in other countries until recently. It was reported in Japan in 1977 causing a stem rot of small red beans (*Phaseolus radiatus* var. *aurea*) (Kodama personal communication). It will be of interest, therefore, to look at its epidemiology in Queensland.

*P. vignae* has only been found on cowpeas in Queensland. Pot experimentation has confirmed that it can survive in the soil for at least 12 months. It has been shown that the organism has poor saprophytic ability, with components such as mycelia and oogonia breaking down rapidly when diseased tissue is returned to the soil. Oospores survive and

**Table 1. Percentage wheat plants at maturity affected by crown rot (*Gibberella zeae*) from plots with different planting dates.**

Month	1963		1964		1966		1967	
May			19	63.3	3	59.5	5	54.3
June	17*	87.2	30	53.4	4	39.0		
July	2	73.8	29	47.3	4	28.5	7	14.4
	25	61.2						
August					4	12.5	16	8.1
September			1	12.7				

\*Day of the month planting occurred.

apparently provide initial inoculum. The level of disease increases with successive plantings of cowpeas (Table 2) and there is a differential effect on cultivars (Purss 1957, 1958). Environmental factors, particularly soil moisture, have a similar differential effect on the development of the disease in different cultivars (Table 3). Those cultivars that possess field resistance but are fully susceptible in glasshouse testing are most affected by variations in environmental conditions (Table 4). It is considered that this phenomenon may be related to inoculum potential, and this hypothesis is now being tested experimentally. The part played by free moisture in the dissemination of the pathogen is well illustrated by the movement of the disease in a front across a field or in tongues in low lying areas. Under high moisture conditions aerial lesions occur and it has been demonstrated that these are initiated by sporangia germinating directly. Although the organism has a wide temperature range (11-34°C) aggressive parasitism occurs within narrow limits (19-20°C). Plants growing in temperatures outside these latter limits will survive even under conditions of high inoculum potential. Dissemination of inoculum in seed is often important in soil-borne diseases, e.g., crown rot (*Gibberella zeae*) of wheat (Purss 1971),

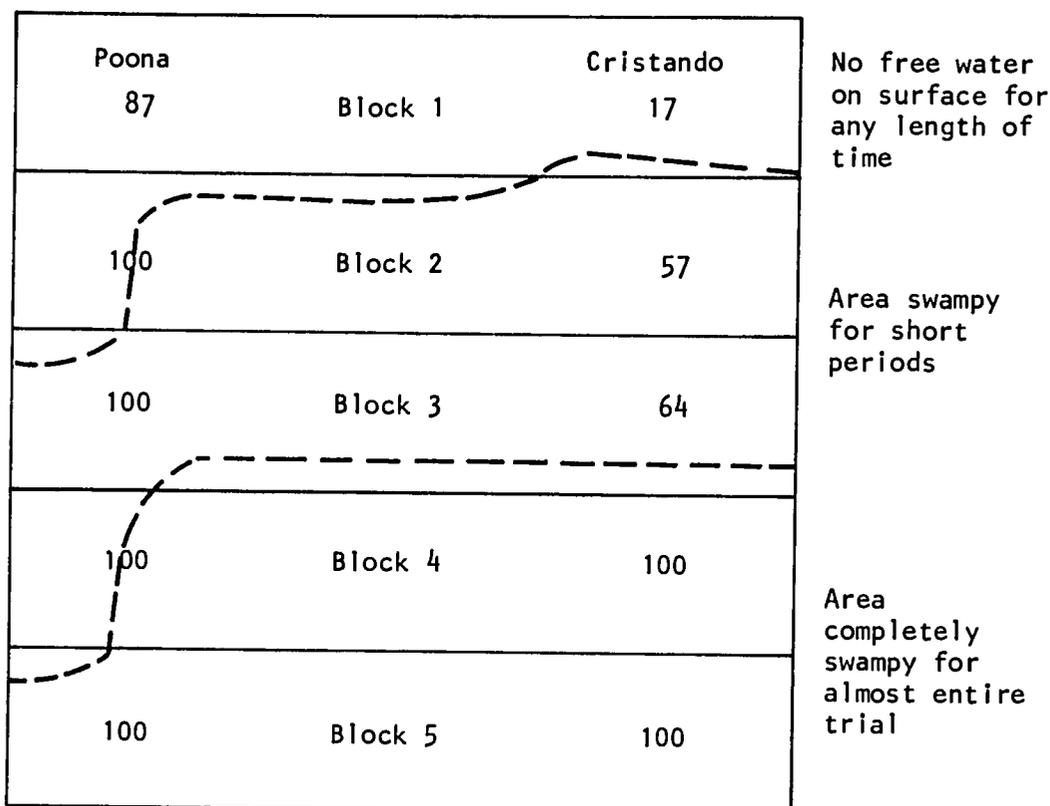
but while this is suspected with *P. vignae*, it has not been possible to demonstrate it experimentally.

**Table 2. Stem rot (*Phytophthora vignae*) incidence in cowpea during successive seasons at Boonah.**

Variety	% Infection		
	1952-53	1953-54	1955-56
Poona	60	90	100
Cristando	0	36	100

Wilt (*Verticillium dahliae*) has been known in peanuts in Queensland for many years. It was considered of little importance, occurring only in isolated patches (Morwood 1945). In the late 1950s the disease increased dramatically, with its distribution becoming comparatively uniform across fields, and it became a significant factor in yield loss (Purss 1961). What factors were involved in this sudden change? There was certainly no evidence of any dramatic change in the pathogen. There had, however, been a complete change in harvesting procedures. Previously the peanuts were stooked after

**Table 3. Percentage cowpea plants diseased with stem rot (*Phytophthora vignae*) in two cultivars under varying moisture conditions.**



"pulling" and transported when dry enough for threshing at a stationary site. Residues were either bailed and fed to stock or burnt off. Isolated patches of wilt in subsequent crops could be related to these threshing sites. The new methods involved windrowing and harvesting with a pick-up thresher, the residues being scattered all over the field. The change in disease distribution in subsequent years is considered to be due to this change in management. There is a conflict now between the need to retain these residues for soil conservation and dispose of them for disease control.

Crown rot of peanuts is caused by the unlikely pathogen *Aspergillus niger*. This disease was a limiting factor to production in Queensland for many years. Inoculum is widespread in peanut soils

(Purss, unpublished data) but its level appears to bear little relation to disease incidence. What is important is the effect of a management practice on the effectiveness of inoculum. Gibson (1953) in Africa found that organo-mercurials actually increased the effectiveness of this pathogen on seeds because it is more tolerant of mercury than other components of the seed and soil flora. Queensland work verified this and found that the devastating losses, much greater than had been reported elsewhere, could be reduced almost to zero by correct seed dressings (Purss 1960).

I would like to refer again to stem rot of cowpea and relate an experience that illustrates clearly the importance of the reaction of cultivars in the production of inoculum. This has long been

**Table 4. Percent plants diseased with stem rot (*Phytophthora vignae*) in a range of cowpea cultivars.**

Cultivar	Reaction type		Year and District					
	Glasshouse	Field Classification	1954-55 Brisbane	1955-56 Redlands	1955-56 Kumbia	1973-74* Brisbane	1974-75 Brisbane	1975-76* Brisbane
Chinese Red	Resistant all races	Resistant	-	-	-	9.7	0.0	12.6
Blackeye 5	Resistant to field races up until 1961	Some field resistance	1.8	1.5	0.9	35.2	61.8	90.7
Malabar	Fully susceptible	Some field resistance	16.5	1.5	13.4	24.9	43.4	93.2
Giant	Fully susceptible	Some field resistance	30.8	5.0	8.3	46.8	49.3	96.8
Andersons Early Giant	Fully susceptible	Good field resistance	30.4	6.0	34.6	33.6	74.3	22.4
Poona	Fully susceptible	Highly susceptible	100.0	91.0	99.5	99.1	99.2	95.5

\*Losses included *Rhizoctonia* stem rot as well as *Phytophthora* stem rot.

recognized in epidemiological studies of stem rust of wheat (*Puccinia graminis* var. *tritici*) but is just as applicable to soil-borne disorders. The cultivar Caloona was produced by incorporating a single gene for resistance to *P. vignae* into the popular but stem-rot-susceptible Poona (Purss 1963). Caloona withstood the disease in tests under a wide range of field conditions. I was called to observe a severe outbreak of stem rot in a planting of Poona. The farmer had Caloona growing immediately adjacent to it. The grower was very pleased because Caloona remained free of the disease. At one end of the field however, a small patch with a few diseased plants was noticed in the Caloona. It was thought this might be explained by mixed seed. Within a matter of a few days however, the disease spread in tongues from this small patch to devastate the planting. Research proved that a previously undescribed pathogenic race was responsible for this outbreak in Caloona (Purss 1972). Thus the epidemic in Caloona was a direct result of the cultivation of the highly susceptible Poona nearby. We cannot therefore ignore epidemiological factors even when growing highly resistant cultivars. We may unwittingly produce a very special type of inoculum by the way we use these cultivars.

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# Epidemiology of Diseases Caused by *Rhizoctonia* Species

H. K. Saksena

The form genus *Rhizoctonia* (from the Greek "death of roots") is characterized by the absence of any kind of fruiting bodies and the presence of sclerotia, cartilaginous to fleshy, and of uniform texture within. The vegetative mycelium has constriction of hyphal branches at their point of origin, and the septum is placed closed to the point of branching. The lateral growth of branches is given out often at right or acute angles. The number of species described is estimated to be 61. Most of the species have been placed in the genus in accordance with the above concept. A few species do not appear to have any of the generic characteristics other than the sterile mycelium.

The literature of *Rhizoctonia* diseases has grown enormously since Julius Kuehn in 1858 observed *R. solani* affecting potato tubers. Of the species described, *R. solani* and *R. bataticola* are the most widespread, destructive, and important plant pathogens. Diseases caused by them have received attention all over the world. The earlier work done on epidemiology of diseases caused by *R. solani* has been reviewed by Baker and Martinson (1970). Dhingra and Sinclair (1978) have discussed the epidemiology of *R. bataticola* diseases in their recent book. However, in spite of the voluminous literature on them, many aspects of the epidemiology of diseases they cause are still not properly understood. No attempt is made here at a complete literature review. Based on my experience, I intend to discuss some important aspects of epidemiology of major *Rhizoctonia* diseases.

## Important *Rhizoctonia* Diseases

The original concept that *Rhizoctonia* inhabits only soil and plant roots and causes rotting of roots and damping-off of seedlings has been modified over the

years. Several strains of *R. solani* and *R. bataticola* have been shown to occur on aerial plant parts, producing diseases such as leaf spots and blights, stem blight, stalk rot, bud rot, ear rot, and fruit rot. *R. solani* consists of a wide range of pathogenic strains varying from those specific to one host family to those that can attack a large number of families. The parasitic specialization is carried further in some isolates with respect to the part of the host they attack (Flentje and Saksena 1957).

The earlier work done in India was mainly with root rot and damping-off diseases caused by *Rhizoctonia* spp. With the intensification of agriculture that evolved changes in cropping patterns, crop husbandry, and cultivar types, many of the *Rhizoctonia* diseases have assumed severe proportions and some new diseases have become highly destructive and widespread.

## *Rhizoctonia solani*

Two distinct strains of *R. solani* in Indian soils—the root-attacking and the shoot-attacking—are mainly responsible for major diseases of crop plants. The root strain was known to be present in our cultivated soils, but the extent of damage it can cause became apparent only after the introduction and extensive cultivation of high-yielding dwarf varieties of wheat and paddy. In both these crops, the root strain of *R. solani* was found to cause extensive root rot of seedlings, leading to yellowing and death. In wheat it produced "no growth" patches by rapid successional killing of seedlings. The root rot strain has since been found to have a wide host range in nature, affecting diverse crops, such as cereals, millets, pulses, oilseeds, and a number of weed hosts to cause infection of immature root tissues. However,

differences exist in its parasitic behavior towards different hosts, ranging from production of minute lesions to extensive root rot (Kumar 1976). This root strain may not necessarily have been abundant in past years, and much of the root system may have escaped infection during the brief but critical period until its maturity. Under the present system of crop husbandry, multiple cropping, and crop rotation patterns, it is likely that the population of the pathogen has gradually built up to high levels, causing heavy damage under favorable conditions.

The aerial strain of *R. solani* has come into prominence with the appearance and spread of leaf sheath blight of paddy in the country. This disease was of no consequence on rice in India before the introduction of dwarf varieties; now it is known to occur in all the rice-growing states of the country. In other parts of the world, this disease is known to be soil-borne only and its attack confined mainly to the lower leaf sheath; under north Indian conditions, however, it severely attacks all aerial parts to cause banded blight symptoms (Saksena 1973). Work carried out in our laboratory (Chaubey 1976) has shown that the pathogen produces the otherwise rarely reported basidiomycetous perfect state of *Thanatephorus cucumeris* in abundance in nature. The basidiospores readily cause leaf and panicle infection and can be carried by wind to new infection courts to bring about rapid spread of the disease. The disease becomes seed-borne once panicle infection has taken place. Thus a disease which is known to be soil-borne only in other rice-growing areas has also been found to be seed-borne as well as airborne under our conditions. About 18 to 20% seed from affected plants carried the infection. Up to 14% plants raised from seed of affected plants developed disease symptoms in pot experiments. The disease can be readily reproduced by spraying basidiospore suspension.

First observed on rice, the aerial strain of *R. solani* was subsequently found to cause similar symptoms of banded

blight on maize, sorghum, and pearl millet also in nature. The pathogen has been observed in recent years to extend its host range further, to cause severe web blight in legume crops, including black gram, green gram, pigeonpea, cowpea, soybean, groundnut, and other beans (Saksena and Dwivedi 1973; Dwivedi and Saksena 1975). Under favorable conditions, there is abundant production of basidiospores in nature on many of the above hosts. It is our observation that the web blight now has become the most serious fungal disease of green gram, black gram, cowpea and soybean in north India, causing extensive damage. The disease becomes seed-borne once the floral parts get infected. Groundnut was also found seriously affected this season by aerial blight. The aerial blight is not so common on pigeonpea, mainly because of its growth habit and planting distance.

The aerial strain of *R. solani* has a very broad host range and has been found in nature on 45 crops and common weed hosts belonging to eight plant families—Gramineae, Leguminosae, Cruciferae, Cucurbitaceae, Cyperaceae, Comelinaceae, Convolvulaceae, and Euphorbiaceae. The pathogenicity and identity of the aerial strain was confirmed by numerous cross-inoculation tests. Basidiospores are produced in abundance in nature on many of these hosts. Under artificial inoculation, the aerial strain exhibited a much wider host range and was able to infect 68 plant species belonging to 14 families—the eight listed above, as well as Malvaceae, Solanaceae, Linaceae, Compositae, Chenopodiaceae, and Pedaliaceae.

The aerial strain of *R. solani* with *T. cucumeris* as its perfect state behaves like a classical foliar pathogen, with rapid production and dissemination of secondary inoculum, which are important for epiphytotics. The production of basidiospores on a large number of collateral hosts helps in maintaining inoculum potential and pathogen buildup. The web blight disease may pose a serious threat to cultivation of pulse crops, which are particularly susceptible to it.

## ***Rhizoctonia bataticola***

*R. bataticola* is the other important species that is also worldwide in distribution and can attack different plant parts to cause seed rot, root rot, collar rot, stalk rot, leaf spot and blight, and blossom and fruit rot. It is known to attack a wide variety of plants including pulses, oilseeds, sorghum, maize, jute, and vegetable and fruit plants. In legume crops, it causes root rot of pigeonpea, chickpea, black gram, green gram, and groundnut. There are reports from north India of *R. bataticola* causing blight and dieback of black and green gram. It is also reported to cause leaf spot and blight in pigeonpea in north India (Saksena et al. 1970). Some strains of *R. bataticola* produce the pycnidial state of *Macrophomina phaseolina*. Because of its extraordinary host range, geographical distribution, and environmental adaptability, *R. bataticola* has become one of the really important plant pathogens.

### **Inoculum—Source and Survival**

The inoculum of *Rhizoctonia* ordinarily consists of hyphae and sclerotia. The hyphae and sclerotia of *R. solani* can be found in soil up to a depth of 10 to 15 cm. The mycelium could grow in unsterilized soil for a relatively long distance without any energy source other than those present in natural soils. According to some workers, a certain "food base" may be necessary to initiate and sustain the growth of *R. solani* in soil. Growth velocities of 1 to 2.5 cm/day through soil have been recorded. The root strain was found to spread through steamed soil at the rate of 1.2 cm/day when wheat seedlings were planted as food base (Kumar 1976).

It is commonly thought that *Rhizoctonia* exists in the soil as sclerotia or thick-walled hyphae associated with plant debris. Dry sclerotia of *R. solani* are reported to survive up to 6 years when stored at room temperature. Longe-

vity of sclerotia is decreased by high temperature or high moisture conditions during storage. Strains differ markedly in their ability to survive in soil. The survival period is reported to vary from 4 or 5 months to more than a year for different strains. The root strain of *R. solani* has been shown to survive in infective state for more than a year in infected wheat roots buried in soil in pots. There was a gradual decline in recovery and parasitism with passage of time (Kumar 1976). Likewise, *R. solani* was isolated up to 11 months from rice and cowpea plant parts naturally infected with its aerial strain and buried in nonsterile soil (Chaubey 1976; Dwivedi 1977). The pathogen was obtained in culture from all the buried pieces used for isolation up to 4 months after burial. Thereafter, the percentage recovery gradually declined with increase in temperature and storage time.

*R. bataticola* is also known to survive for long periods in soil in the form of mycelia and sclerotia associated with plant debris. Meyer et al. (1974) found that inoculum from sclerotia or mycelium was almost equally effective in causing charcoal rot of soybean. Sclerotium populations of *M. phaseolina* are reported to decline rapidly under high soil moisture and in soils with high C:N ratio amendments. Reduction in number of viable sclerotia may be brought about in the field by keeping soil moisture at 60% moisture-holding capacity for 3 to 4 weeks at 30°C or above (Dhingra and Sinclair 1978).

Basidiospores have been shown to be an important source of inoculum and a major factor in dissemination of aerial strains of *R. solani* (Saksena and Dwivedi 1973). Maximum basidiospore production and discharge occurred during midnight and early morning hours before sunrise. Their production was favored by night temperatures below 24°C, relative humidity above 95%, and rate of evaporation below 1.5 mm per day. In nature, basidiospore production usually starts in mid-August in north India. Basidiospores germinated in 2 hours and leaf penetration

took place through intact surface by formation of infection cushions or by direct penetration through stomatal openings.

Basidiospores are reported to lose viability rapidly in dry storage. At temperatures above 30°C, viability was lost after 2 weeks, whereas at low temperatures the viability period varied from 4 to 6 weeks. Basidiospores lost viability after 6 hours of exposure of basidiospore-bearing plant tissue to direct sunlight.

Secondary spread through wind- and water-borne sclerotia is also reported for both the *Rhizoctonia* species. *R. solani* also grows from leaf to leaf as commonly observed in the web blight of legumes.

*R. solani* and *R. bataticola* are both carried on and in true seeds, as discussed earlier. This is also an important means of dissemination. Infected seed material can lead to spread of pathogens to areas where they are not known to occur.

Strains of *R. solani* and *R. bataticola* are known for their wide host range. They infect a large number of weeds and rotation crops which function as a source of inoculum and may help in the survival of the pathogens. This is very important in epidemiology. The aerial strain of *R. bataticola*, for example, survives parasitically on a very large variety of hosts, which not only provide nutrition for maintaining the inoculum potential but also serve as important means of buildup and survival of the pathogen.

Disease severity is commonly thought to be associated with the inoculum present. *Rhizoctonia* species have been widely used as a tool to determine inoculum-density relationships. The relative densities of *Rhizoctonias* in soil have been measured, mainly by noting the frequency of invasion of nonliving substrates and by taking counts of sclerotia or hyphae, and correlating with disease severity or incidence. Results confirmed that disease severity usually correlated with the population of *Rhizoctonia* (Baker

and Martinson 1970).

## Environment and *Rhizoctonia* Diseases

Much has been written about the effect of environment on *Rhizoctonia* diseases. Most of it is observational and few thorough studies have been made of the environment and disease development of *Rhizoctonia* spp.

Broadly speaking, deficiencies of potassium, nitrogen, or calcium or excess of nitrogen in cultivated soils increase the disease incidence. Application of calcium is reported to control *Rhizoctonia* root rot in some crops. The role of calcium in disease resistance is probably to form insoluble pectates in the plant cell wall, which are resistant to hydrolysis by *Rhizoctonia* polygalacturonases (Bateman 1970). In general, plants receiving unbalanced nutrition are predisposed rapidly and remain susceptible for a longer period than plants receiving balanced nutrition.

Many workers have studied the effect of incorporating organic manures, crop residues, and processed organic materials into soil on the development of *Rhizoctonia* diseases. These amendments affected the nutritional balance of the soil for the host and pathogen and for the other microbes in the soil. The organic amendments have been usually found to decrease the inoculum potential of the pathogen. There are also instances of increase in disease incidence and severity.

The influence of temperature and moisture on the development of *Rhizoctonia* diseases has been studied by many workers. The results cannot be generalized because of the differences in the behavior of the pathogenic strains under the influence of prevailing moisture and temperature. This can be illustrated by the requirements of temperature and moisture by root and aerial strains of *R. solani* for disease development.

The root rot strain of *R. solani* from wheat and paddy caused highest disease incidence in the lower soil moisture

range of 7 to 10% (on oven dry weight basis at 30°C. The disease incidence decreased with increase in soil moisture. At 10% moisture level the disease rating was lower at 27°C and 33°C than at 30°C (Kumar 1976). At high soil moisture levels, the pathogen instead of causing rotting of roots produced minute necrotic lesions scattered on the roots. Temperature around 30°C and low soil moisture have also been found to favor development of root rot by *R. solani* in chickpea and linseed. On the other hand, some *Rhizoctonia* diseases of roots are reported most severe under moist soil conditions.

Temperature and moisture greatly influenced the development of aerial blight caused by *R. solani* in rice and legumes (Chaubey 1976; Dwivedi 1977). Temperature in the range of 26° to 28°C and relative humidity near 100% were most suited for rapid development and spread of disease. With increase or decrease in the temperature and decrease in relative humidity, the incubation period was appreciably prolonged with less disease development.

Free moisture was essential for the germination of basidiospores and development of foliar epiphytotics in the suitable temperature range of 26° to 30°C. Four years' observations on the weather data and disease incidence in the field have shown that maximum disease development and spread occurred in the months of August and September in the mean temperature range of 20.5° to 30.75°C with relative humidity 86 to 100%. Disease development and spread was checked at temperatures above 35°C with 50 to 65% relative humidity.

Diseases caused by *R. bataticola* are generally favored by temperatures around 30°C and high soil moisture. In charcoal rot of sorghum and soybean caused by *R. bataticola* the response of crop to stress conditions has been found more significant in epidemiology than the inoculum level.

Host susceptibility is also important in disease incidence and severity. Ordinarily *Rhizoctonia* species are

successfully able to penetrate and invade the tissues during the seedling and early stages of growth. Plants become resistant as they grow. The increase in resistance with plant age has been linked with thickening of host cell wall, lignification, wound periderm formation, and calcium content of host tissue. In some cases mature tissues are attacked. Strain differences also lead to preferences in the age of plants attacked. With wheat and rice, 7- to 40-day old seedlings were found most susceptible to the root rot strain of *R. solani* (Kumar 1976). In banded blight of paddy, plants between 25 and 55 days old were most susceptible to infection by the aerial strain of *R. solani* (Chaubey 1976). The spread of the leaf spot formed on leaves of up to 15-day old plants was very slow and restricted. In case of web blight of legumes, the plants were susceptible to disease from the seedling stage until maturity. Disease severity was maximum in 30- to 70-day old plants. This was mainly due to rapid spread of the filamentous hyphae of the pathogen from leaf to leaf and then from plant to plant when in contact in a dense stand.

*R. bataticola* is also primarily pathogenic to young seedlings and immature tissues, particularly those devitalized by environmental conditions. In several cases the disease severity is found to increase in older plants as in root rot of soybean, cotton, and jute.

Thus, *Rhizoctonia* causes different types of diseases in a wide variety of plants under diverse environmental conditions. The main purpose of epidemiological studies is to obtain a good knowledge of the behavior of the diseases in the field for developing effective control measures. The predominantly subterranean habit of *Rhizoctonia* has made chemical control difficult, although several fungicides have been found effective to control the pathogen. PCNB is a good example for a chemical that has been widely used to control *Rhizoctonia* in the soil and on aerial parts. Isolates of *R. solani* differ in their tolerance to PCNB and continued use of this fungicide has led to development of biotypes with greater

tolerance (Shatla and Sinclair 1965).

The nature of parasitism of *Rhizoctonia* spp. (Saksena 1971), its extremely wide host range, and the presence of extremely variable strains has made the development of resistant host varieties a difficult task. Varietal resistance has been reported in beans, cabbage, lettuce, rice, and gladiolus. In general, varieties have been found to differ in their reaction to *Rhizoctonia* infection, but a high degree of resistance has rarely been found or produced by selection or breeding.

Information on the control of *Rhizoctonia* diseases is most extensive on the modification of cultural practices, including biological control. The pronounced saprophytic activity of *Rhizoctonia* spp. and its presence in most agricultural soils at various levels of infestation has weakened the effectiveness of cultural practices. Success can be achieved if the various methods of control are applied in a complementary manner.

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# Epidemiology of Soil-borne Diseases of Groundnut

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Soil-borne diseases of groundnut in India include (1) root rot and stem rot caused by *Corticium solani* (= *Rhizoctonia solani* Kuehn) and dry stem and root rot (ashy stem blight) caused by *Macrophomina phaseolina* (= *Rhizoctonia bataticola*); (2) Hypocotyl rot, collar rot, or *Aspergillus* crown rot caused by *Aspergillus niger*; (3) Aflaroot disease caused by *Aspergillus flavus*, and (4) *Fusarium* root, stem diseases, and *Fusarium* wilt; besides other root diseases.

## Root Diseases Caused by *Rhizoctonia* and *Macrophomina*

Both pathogens occur throughout the groundnut-growing areas of the world. *Rhizoctonia* and *Macrophomina* diseases occur in all groundnut-growing areas in India, but are particularly serious in Madras and Delhi, and occur sporadically in the Punjab (Mathur 1953; Chohan 1970; Vasudeva 1962). Both pathogens are seed- and soil-borne (Kang and Chohan 1966; Feakin 1973; Jackson and Bell 1969). Roots, stems, branches, pegs, and nuts are attacked, with the appearance of tiny black sclerotia of *M. phaseolina* studded in the tissues (Mathur 1953; Kang and Chohan 1966, Sunar and Chohan 1971a, 1971b). Strains of *M. phaseolina* are also reported to occur in nature (Sundararaman 1929). *M. phaseolina* from cotton is pathogenic to groundnut (Likhite 1936). The host range of *M. phaseolina* has been studied (Shaw and Ajrekar 1915). All the varieties so far tested have been found to be highly susceptible (Lewin et al. 1971), except Punjab-1 and TMV-3 which appear tolerant to the root rot diseases (Mathur 1953; Mathur et al. 1967). Lal and Mathur (1967) reported laboratory assay methods for detecting fungi such as *M. phaseolina*

from seeds. Raj and Prasad (1975) reported resistance of two varieties, namely, B-30 and B-31 to root rot of groundnut caused by *Rhizoctonia bataticola*.

## *Corticium solani*

*Corticium solani* is capable of infecting all principal organs of the groundnut plants. The pathogen persists for long periods in the soil, where, in the absence of living hosts, it lives saprophytically on bits of organic debris. The inoculum is also carried in groundnut seeds. Infection occurs through wounds or directly through intact surface tissue; cushion- or finger-like structures are typically produced by branches of hyphae in contact with the host surface and penetration of the host follows this development (Jackson and Bell 1969). Seeds planted too deep are more prone to infection, as their seedlings are weakened and etiolated.

Preemergence death of seed and emerging seedlings is caused by seed- and soil-borne pathogen in lighter soils. On emerging seedlings, lesions appear at ground level on hypocotyl as sunken, elongate dark brown areas 2 to 3 cm or less in length. Sometimes a dry rot develops and then the entire hypocotyl collapses and the plant dies very rapidly. Lesions also develop on seedling tap roots and spread to the lateral roots (Feakin 1973).

Plants in various stages of development are also affected by gradual disintegration of the roots. Sunken brown lesions appear on primary roots, leading to a progressive total browning of the secondary roots. Reddish-brown lesions also appear on the stem at soil level or

on branches (in spreading varieties) where they are in contact with the soil. Such areas on the stem and branches turn dark brown, sink into the tissue, affecting several centimeters along the stem and giving it a typical 'wire-stem' appearance. With the appearance of these symptoms the entire plant rapidly wilts and dies. The whole plant collapses rapidly in damp weather and in densely planted crops.

Infected pegs and young pods turn brown or black at their tips and wither. Infected nuts are a total loss. Lesions on pods are dark and angular. Many more pods are lost because their pegs rot and they are left in the soil at the time of harvest. Entry of secondary pathogens is also facilitated. Infected seeds are discolored, with stained or faded testas.

The pathogen persists in soil for a long time, living saprophytically or organic debris in the absence of a host. Soil inoculum can be reduced by burning the plant debris or by burying it by deep plowing. Planting of susceptible crops such as beans and soybeans increases soil inoculum before the groundnut crop is planted. Removal of weeds helps to reduce soil inoculum. There is little possibility of reducing inoculum through use of resistant varieties, since breeding for resistance is very difficult.

Lifting of the soil, use of pentachloro-*n*-benzene as a preplanting soil-incorporated treatment, or application of calcium sulfate in the form of gypsum as a preplanting soil-incorporated treatment reduces the inoculum and the incidence of *C. solani* infections and pod rot. Seed treatments also help in reducing inoculum.

### ***Macrophomina phaseolina***

*Macrophomina phaseolina* is soil- and seed-borne. The fungus persists in the soil for long periods as an actively growing mycelium or as dormant sclerotia. Growth and survival of *B. bataticola* in soil, on plant parts, and in mixed cultures are greatly influenced by other

microorganisms. The pathogen is spread by soil movement and on crop debris. Hydrating, mature, intact pods were invaded most rapidly at 26° to 32°C. Invasion at 38°C was initially very rapid but was soon limited at 32°C and 38°C by concomitant growth of *Aspergillus flavus* on and in the pod.

Seedlings were more rapidly and severely infected at 29°C and 35°C than at 18° or 24°C, plumules being invaded more frequently than roots. Irrigation reduces infection, since the fungus is known to spread rapidly in drier soils at high temperatures. Fungal growth in pods is increased by rain after harvest. Pods and kernels, if damaged during harvesting and shelling, are liable to more damage. Rotations do not help to reduce soil inoculum, but seed treatments do. Reduction of inoculum through use of resistant varieties is doubtful in this particular pathogen.

Red-brown water-soaked lesions appear on the stem just above soil level. The pathogen spreads upwards to the aerial parts of the plant and downwards into the roots. Lesions also sometimes girdle the stem, leading to wilting of plants, followed by rapid colonization of the branches, which turn brown, and the whole plant dies. The dead tissue is covered with abundant black sclerotia appearing as an ashy grey covering like a soot. Partial infection of a few branches of a plant can also occur in less serious cases. Along with stem rot and wilt, roots are also attacked. In rare cases, only roots are attacked, in which case the tap root turns black and later becomes rotten, shredded, and studded/covered with sclerotia (Jackson and Bell 1969; Feakin 1973).

Pegs and pods are also attacked and in some areas such infection by *M. phaseolina* is very serious. If pods are physically damaged, the pathogen rapidly infects the fruits, developing symptoms of blacknuts and leading also to concealed damage. Secondary parasites later attack such kernels.

## Diseases Caused by *Aspergillus*

### Collar Rot (Hypocotyl rot) Caused by *Aspergillus niger*

The disease is prevalent throughout the groundnut-growing areas of the world.

*Aspergillus niger* is soil- and seed-borne, thus the seed and the seedlings can be attacked at any stage from planting onwards. Preemergence rots cause a decrease in germination. The fungus also causes preemergence soft rot of the hypocotyl. Early postemergence seedling blight is very common. Young plants collapse and die soon after emergence, due to the rotting of the succulent elongating hypocotyl. The hypocotyl is attacked both by soil-borne *A. niger* and by the fungus growing from already infected cotyledons or from spores carried on the seed surface, which germinate after planting. The hypocotyl tissue becomes water-soaked and light brown. Lesions covered by the sporulating fungus also appear on stem at ground level. Tan or brown spots appear on the cotyledons. As the pathogen spreads from the cotyledons to the collar region, the whole region collapses, and the lower leaves of the plant become chlorotic. The infected tissue becomes dark brown and is covered with black spores. In emerging seedlings the first symptom is rapid wilting (partial or whole), especially after rainfall followed by a dry spell, during which period this fungal invasion of the collar region (cotyledonary node) causes shredding of the infected tissue, resulting in complete stem breakdown.

Plants that escape early infection (immediate postemergence phase) may later show crown rot symptoms. Large lesions develop on the stem and below the soil and spread upwards along the branches, causing wilting and death. The dead dried branches are easily detached from the disintegrated collar region and are blown by the wind through the crop, spreading spores as they go along. Injuries on the hypocotyl or

stem, or soil thrown over cotyledons or stem of growing plants are avenues of fungal invasion. Occasionally, rotting is confined to the lower portion of the main roots, in which case the plant may send out adventitious roots above the diseased area; such plants seldom thrive and usually die during dry weather (Feakin 1973).

*Aspergillus* species are found in almost every type of soil, being particularly prevalent in light tropical soils. *A. niger* can tolerate low soil moisture and develops best at temperatures between 30° and 35°C. The pathogen is saprophytic and produces numerous spores. The inoculum potential of *A. niger* in cultivated soils seems to increase after the growth of a diseased crop. The main carryover from season to season is in plant debris—not necessarily from a groundnut crop—rotting in the soil and on the seeds harvested from an infected field. Soil-borne inoculum is another important source of infection, but the pathogen is also carried on the seed surface in or under the testa. Superficial seed infection is more common than the deep-seated kind. Seeds become infected during the last days of maturation in the soil and during harvesting, handling, and particularly during shelling. If pods are harvested and dried promptly the numbers of infected kernels decrease but if pods are left in damp conditions after harvesting, *A. niger* may spread throughout the crop.

Infection takes place in most cases within 10 days of germination. The cotyledons are the primary infection sites, and most plants die less than 30 days after planting. The most serious crop losses are the result of infections occurring within 50 days of sowing. High soil and air temperatures predispose the plants to infection. Delays in emergence due to deep planting increase the risk of hypocotyl infections (Jain and Nema 1952; Chohan 1965, 1968; Kang and Chohan 1966; Gupta and Chohan 1970b, 1970c; Feakin 1973). Full symptomatology of the disease under field conditions in the Punjab has been described by Chohan (1965,

1972). The losses may amount to 40% in stand at the seedling stage. Infection in case of deep planting is common in India (Chohan 1971b, 1971c). The etiology, epidemiology, and physiological pathology of the disease have been fully worked out (Jain and Nema 1952; Chohan 1965; Chohan and Kapoor 1967; Chohan 1969a, 1969b; Bhatia and Chohan 1970; Gupta and Chohan 1970b, 1970c; Chohan 1971a, 1971b, 1971c). Antagonism of soil fungi, actinomycetes, and bacteria against *A. niger* has been reported (Chohan 1971a, 1971c; Chohan and Singh 1973). Kang and Chohan (1966), Chohan (1969a, 1969b) and Chohan et al. (1973) also reported the occurrence of a large number of seed- and soil-borne fungi and actinomycetes in the Punjab and also from rhizosphere of groundnut.

Chohan et al. (1970) tested 734 varieties of groundnut against the collar rot disease and demonstrated that one variety, namely EC-21115 (U-4-47-7), was immune in the Punjab. Some other varieties, namely B-4, B-21, B-60, B-76, B-101, B-181, and Asiriya mwitunde, are reported to be resistant (Nema and Jain 1955; Anonymous 1970; Aulakh and Sandhu 1970; Mathur and Sharma 1970; Verma 1971).

Ceresan (2%) and Agrosan GN (1%) increased the ultimate survival of groundnut plants by about 21% (Bedi et al. 1960; Jain and Nema 1962; Nema et al. 1955; Mathur and Sharma 1971). In the Punjab, however, the organo-mercury seed dressings later lost effectiveness under field conditions. Mercury-tolerant strains of *A. niger* are known to exist in nature. *A. niger* is protected from mercury poisoning by sulphhydryl compounds (glutathione and cysteine), which are present in abundance in the mycelia of the pathogen. Organic mercury fungicides partially sterilize the surrounding soil and help in promoting selective action (due to selection pressure), whereby the mercury-tolerant *A. niger* (also virulent strains) strains dominate the area during the germination period of the seed (Gibson 1953; Ashworth and Amin 1964; Ashworth et al. 1964). Di-

thiocarbamates such as thiram and heterocyclic compounds such as captan have proved highly efficacious in preventing seed rot and collar rot diseases of groundnut (Nema et al. 1955; Chohan et al. 1966; Feakin 1973; Chohan 1971c; Mathur and Sharma 1971; Gupta and Chohan 1970a).

### **Aflaroot Disease (Yellow mold) Caused by *Aspergillus flavus***

Infection by *A. flavus* soil- and seed-borne fungus causes undressed seeds to rot rapidly after planting. Seeds disintegrate in 4 to 8 days, resulting in low germination. Infected seeds are covered by secondary invading fungi particularly *Rhizopus arrhizus*. Dry soil conditions favor fungal spread, as seedling emergence is delayed. The cotyledons of emergent seedlings are often attacked and become necrotic, their infected surfaces covered by masses of yellow-green spores. After seedling emergence, new infections do not occur, but cotyledons already infected show necrosis of the central tissues and lesions with reddish brown margins, which may sporulate. This necrosis is always terminated at or near the cotyledonary axis.

Under field conditions, diseased plants are stunted, in poor condition, and show general chlorosis of the leaves due to the presence of aflatoxin, which is translocated from the cotyledons to other above-ground parts of the plant. The leaflets are reduced in size, with pointed tips, thick and leathery leaves, widely varied in shape and with veinal clearing. If affected plants are dug up, the radicle is found to be lacking in secondary root development (Aflaroot disease condition). *A. flavus* pathogen can also cause blue damage to groundnut seeds and discoloration, a form of concealed damage. *A. flavus* is a relatively weak pathogen on growing groundnut plants. Its main effect is on harvested pods (Aflatoxin problem in groundnut).

Aflaroot disease was reported in India by Chohan and Gupta (1968). The

effect of aflatoxin produced in the cotyledons continues on the groundnut plant for about a month, until the cotyledons are shed (Chohan and Gupta 1968; Kang 1970). *A. flavus* persists in senescent cotyledons at least 11 to 20 days after planting (Kang 1970; Kumar 1971).

Seed germination is reduced by 10 to 20% due to the seed-borne infection of *A. flavus* (Kang 1970; Aujla 1971); even the plumule becomes infected under humid conditions (Gupta and Chohan 1970a, 1970b). Under field conditions, about 5 to 10% of the seedlings showed the Aflaroot disease condition (Gupta and Chohan 1970b; Chohan et al. 1970; Aujla 1971). Visible damage caused by *A. flavus* in groundnut kernels in stored pods was up to 33.3% and concealed damage to the extent of 26.6% (Gupta and Chohan 1970b, 1970c). Once the seedlings have emerged and are healthy, chances of seedling infection thereafter by *A. flavus* become remote (Jackson and Bell 1969; Kang 1970). The inhibitory effect of aflatoxin on growth of seedlings of groundnut, wheat, and other crops is negated when aflatoxin-treated seedlings are incubated with gibberellic acid. The site of action of gibberellic acid in plants has been proposed to be a DNA-dependent RNA-level and it has been surmised that aflatoxin inhibits growth of the coleoptile of wheat by disorganizing the process of cell division dependent on nucleic acid multiplication (Rana 1971).

The control of *A. flavus* on a field scale revealed that fungicides such as Difolatan, Tecto-60, and Dithane M-45 (captafol) gave statistically significant increase over the check as compared with the remaining fungicides (Aujla 1971; Aujla et al. 1976).

## Diseases Caused by *Fusarium*

***Fusarium oxysporum*, *F. moniliforme*,  
*F. equiseti***

*Fusarium* spp. are cosmopolitan soil-

borne pathogens. The species that cause groundnut diseases are found in most groundnut-growing areas (Vasudeva 1962; Kang and Chohan 1966; Feakin 1973; Jackson and Bell 1969; Gupta and Chohan 1970b, 1970c).

Preemergence rots of seedlings and seeds are frequently the result of *Fusarium* infections in untreated seeds. Young seedlings are very susceptible if they are mechanically damaged or weakened by planting too deep. There is a general tissue disintegration and the surface of the seedling is covered with a sporulating mycelium, which varies in color depending on the species of *Fusarium* involved. *F. solani* may cause a dry rot of the tap root in slightly older seedlings. The end of the root becomes red-brown and shrivels or breaks off. The infection then spreads to secondary roots, which also turn brown and break easily. In India and Rhodesia, *F. oxysporum* is reported to cause a wilt of maturing plants. In dry weather when plants are about 2 months old, the leaves become yellow and plants wilt, dry up, and die. Roots and stem show internal vascular browning and discoloration. Mild sublethal attacks render the plants very susceptible to infection by other pathogens. Variety Namberquaire is moderately resistant. Seed dressings are very useful in controlling preemergence rots (Jackson and Bell 1969; Feakin 1973).

Kang and Chohan (1966) reported the occurrence of a few more seed-borne *Fusaria* on groundnut in the Punjab, namely, *F. solani*, *F. oxysporum*, *F. moniliforme*, *F. oxysporum* var. *redolens*, and *F. equiseti* along with several other fungi (Chohan and Sunar 1971; Sunar and Chohan 1970, 1971a, 1971b; Rao 1962). Gupta and Chohan (1970a, 1970c) recorded *F. oxysporum* in the first sampling of groundnut kernels after harvest.

The *Fusarium* wilt disease manifests itself in dry weather when the plants are 2 months old. The leaves turn yellow and plants dry up and die. The roots and stem show internal vascular browning and discoloration. Later, the roots are attacked by other fungi, which cause

secondary rot and the roots become brittle and easily broken (Mathur 1953; Vasudeva 1962).

Species of *Fusarium* persist for long periods in the soil as chlamydospores and as living hyphae in plant debris. Spores form readily on invaded plant parts and are a common inoculum source and a principal form in which the fungi are disseminated.

## Diseases Caused by Other Soil-borne Pathogens

- Damping off, preemergence rot, pod rot caused by *Pythium myriotylum*, *P. ultimum*, and *P. irregulare*.
- Stem rot caused by *Corticium rolfsii*.
- Seed and preemergence rots caused by *Rhizopus arrhizus*, *Pythium oryzae* and *Rhizopus stolonifer*.
- Black rot caused by *Cylindrocladium crotalariae*.
- Botrytis blight, grey mold caused by *Botrytis cinerea*.
- Sclerotinia rot, root and stem rots caused by *Sclerotinia* spp.
- Texas root rot caused by *Phymatotrichum omnivora*.
- Diplodia collar rot caused by *Phyalospora rhodina*, *P. zeicola*.
- Wilt, Verticillium wilt, pod rot caused by *Verticillium dahliae* and *V. albo-atrum*.
- Bacterial wilt caused by *Pseudomonas solanacearum*.

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# Techniques for Selective Isolation of Some Soil-borne Pathogens

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The environments in which soil-borne pathogens interact with the host roots are much more complex than the environments for pathogens of aerial parts. Although there is now some evidence to suggest that phyllosphere microflora play some determining role in the parasitism of foliar pathogens, the latter are not subject to as heavy biological pressure as the root pathogens. The complexities of biological environments create problems in the understanding of the ecology of the pathogens that is a prerequisite to their durable management. Population studies are a basic feature of ecology, and in the case of soil-borne pathogens, the population must be determined without introducing stimulants or inhibitors into the soil.

## The Need for Selective Media

The weakness in pathogenic forms of fungi due to their advanced evolutionary status results in their inability to compete successfully with the saprophytic or nonpathogenic forms of microbes that exist in abundance in the soil. Thus, in attempts to directly enumerate their population in soil on routine media, it is unlikely that the pathogenic fungi will express their full numerical strength. The obstacles could be overcome by using methods that favor their growth. Enrichment of soil with suitable substrates, use of baits, and host infection techniques have been employed with specific objectives (Durbin 1961; Menzies 1963; Banihashemi and Mitchell 1975; Grimm and Alexander 1973; Marks and Mitchell 1970; Pratt and Heather 1972). However, these methods fail to give a quantitative estimate of unstimulated population of pathogens in the soil, the figures only reveal the multiplied number of propagules. Although this helps in

detecting the possible level of infection, it is not quantitative determination for ecological purposes.

In direct enumeration, suppression of unwanted members of soil microflora is essential, and for this antibacterial and antifungal chemical agents have long been in use. These antimicrobial agents (oxgall, sodium propionate, sodium taurocholate, crystal or gentian violet, rose bengal, malachite green, etc.) helped in the determination of total fungi, bacteria, and actinomycetes, but failed to selectively isolate a given pathogen such as *Pythium*, *Phytophthora*, *Fusarium*, or *Rhizoctonia* (Butler and Hine 1958; Collins 1967; Durbin 1961; Goldberg 1959; Johnson 1957; Martin 1950; Papavizas and Davey 1959).

A selective medium picks out the desired organism from a group by completely eliminating or suppressing the other members of the group. The major thrust on this aspect of quantitative study of microflora was begun in 1960, and during the last 18 years, a number of fairly accurate media, easy to prepare, have been developed to determine the actual number of propagules in soil of such important root-infecting fungi as *Pythium*, *Phytophthora*, *Fusarium*, *Verticillium*, *Thielaviopsis*, *Macrophomina*, *Phoma* spp., *Cephalosporium gramineum*, and *Fomes annosus*. The subject has been extensively reviewed by Tsao (1970). In this paper only legume pathogens are considered.

## Principles and Materials for Selective Media

Selectivity of chemical inhibitors and stimulants is the main basis for developing a selective agar medium. In other words, differential response of micro-

organisms to toxicants helps in choosing inhibitors for incorporation in agar media. If this trait of microorganisms is further exploited, differential response to chemicals of species and different structures of fungi within a species can be utilized for formulation of agar media that could tell us which species, in what form, is present in the soil in a particular environment.

A selective medium has two components, the choice of each of which is important for best results. The first is the basal medium, which should be suitable for prompt germination of resting spores or other propagules and for growth of the pathogen. If media such as cornmeal agar are not being used, the synthetic medium must contain all the ingredients (trace elements, vitamins, most suitable sources of carbon and nitrogen) for best growth of the fungus. The medium should be compatible with inhibitors and should not react with them to produce a synergistic toxic effect on the pathogen. It should be well defined and easy to prepare. Testing of several media in different combinations with inhibitors is therefore necessary.

The second component of a selective medium is the inhibitor(s). In the selective isolation of fungi, three types of inhibitors are often used. Such agents as rose bengal, oxgall, etc., mainly help in restricting colony diameter to facilitate identification and counting, although many of them are toxic, in different situations, to microorganisms. The antibacterial agents are used to suppress bacteria and actinomycetes, and antifungal agents are employed to suppress unwanted groups of fungi. Obviously, mixture of such widely differing materials in a single substrate warrants caution in use to avoid erroneous results.

Among the inhibitors, the main agents are antibiotics such as penicillin and vancomycin for Gram-positive bacteria and streptomycin and polymyxin for Gram-negative bacteria. Combinations of these two groups of antibacterial antibiotics or broad-spectrum antibiotics

such as chloramphenicol are preferred. Chemicals such as pentachloronitrobenzene (PCNB) are often combined with these antibiotics to suppress actinomycetes and stimulate and differentiate between certain fungi. Among antifungal antibiotics, polyene antibiotics (pimaricin, endomycin, nystatin) are most commonly used. More recently, broad-spectrum systemic fungicides, such as benzimidazole, thiophanate methyl, and chloroneb have been successfully used for selective isolation of certain fungi (Follin 1971; Meyer et al. 1972, 1973; Papavizas and Klag 1975; Peethambaran and Singh 1977, 1978; Tichelaar 1974).

To achieve success in developing a selective medium for a given pathogen, up-to-date knowledge of physiological traits and specific nutrient requirements of the fungus, knowledge of toxicity spectrum or exhaustive spectrum tests of the inhibitory agents at different concentrations, their compatibility with other ingredients of the medium, use of mycelium as well as resting structures in the screening tests, and, finally, tests with different soils, autoclaved artificially infested and then natural soil, is essential.

## Methods of Plating

The conventional dilution plate and soil plate techniques are both used for plating soil, depending upon the nature of the pathogen and the study. The dilution plate technique is quite suitable when nonmycelial structures are to be enumerated. For mycelial structures, soil plate gives best results. With *Pythium*, better results have been obtained when the medium is poured over the soil in plates, while with *Fusarium*, better results are obtained by placing the suspension or the soil on the surface of the solidified medium. However, the exact procedure for each medium and organism should be determined before the technique is perfected, because oxygen demand and association with soil crumbs may vary from species to species.

## Limitations of Currently Used Selective Media

One of the main advantages of selective media should be to precisely determine the primary etiological agent of the disease in a complex biotic environment. It is not necessary that only one pathogen be present in the soil around diseased roots or in the decaying roots. In complexes where two wilt-inducing fungi are supposed to be involved, the selective media have been found very helpful. The wilt of sugarcane is believed to be caused by *Cephalosporium sacchari* (recently redesignated as *Acremonium furcatum* and *A. terricola* by Singh et al. (1975)). However, *Fusarium moniliforme* is always isolated from wilted canes. Thus, Singh and Singh (1975) using a selective medium developed by Sharma and Singh (1973) for *F. moniliforme* that does not permit growth of *Cephalosporium* or *Acremonium*, were able to demonstrate the presence of *Fusarium moniliforme* in canes showing very early symptoms of wilt, suggesting its definite association with the disease. Better frequency of appearance of this fungus in isolations made by Singh et al. (1975) and, more recently, proof of pathogenicity obtained by Singh (personal communication, 1978) support this finding.

Problems arise when several species or non-pathogenic forms of the same species are present in the environment from where detection is to be made. The presence of several forms of *Fusarium* in the rhizosphere of a diseased plant or in the decaying or diseased roots is one example. Most of the currently used selective media for this fungus fail to differentiate between species and between pathogenic and nonpathogenic forms, although claims to the contrary have been made (Komada 1976). Media commonly used for selective isolation of *Fusarium* (Nash and Snyder 1962; Papavizas 1967; Singh and Nene 1965b; Singh and Chaube 1970) determine the number of propagules of the fungus that is most predominant, but other species and nonpathogenic forms of the same species also appear in the soil plates. Standards based on growth

characters and pigmentation in pure culture on the same medium are used to overcome this limitation partly. Parmeter and Hood (1961) had made an attempt to selectively isolate species of *Fusarium* by incorporating culture filtrate of the species in the medium, but in our studies (Singh et al. 1975) the method failed to selectively isolate the form species culture filtrate of which was used in the medium. Most of the selective media for plant pathogenic fungi have been developed with only one pathogenic species in view, e.g., *F. solani* f. sp. *phaseoli*, *F. oxysporum*, *Fusarium culmorum*, etc., by workers studying diseases caused by them or interested in their ecology. There is hardly any reported work in which all pathogenic species or form species of a fungal pathogen (*Pythium*, *Phytophthora* or *Fusarium*) have been collectively studied for their preference for carbon and nitrogen sources, vitamins and trace elements, and response to temperature, pH, and toxicants, so that media and procedures differential for each species could be easily developed. Stray reports do indicate that such a study is possible and can be very useful. Riding et al. (1969) had reported that differentiation between *Pythium* and *Phytophthora* can be made in soil platings by using thiamin in the medium. Lumsden et al. (1975) have indicated that differentiation between species of *Pythium* is possible by means of selective media and differential temperature and pH responses of the species. Similar studies may help in isolation of species of *Phytophthora* and *Fusarium*. Such problems do not exist in the case of sclerotial fungi, where difference in size of sclerotia and use of proper sieves for processing the soil before plating make the isolation of species easy.

Plant pathogenic fungi usually occur in soil not in an active state but as dormant structures that vary from the mycelium in their response to toxicants. Most media fail to differentiate between these structures and hence the actual form in which the pathogen is surviving or is active during absence of the host but under differing conditions of the

cultivated field is not known. This information is important for length of survival studies that help in deciding about length of rotation and crops to be included in the rotation. Manipulations in concentration of inhibitors, increase in the period of study, and use of different temperatures sometimes help in overcoming this difficulty.

Most selective media have been tested for maximum recovery of propagules of the desired pathogen. Very little information is available on whether the recovery is perfect. According to our studies with *Fusarium*, none of the media effects 100% recovery of propagules added to the soil.

Obviously, more extensive and organized studies are needed to overcome the above limitations. It is not impossible, because each individual variety or species of pathogen and its structures possess some differential response to chemical and physical environments. Identification of these responses and their use is needed to further improve the selective media. At the moment, each medium developed by different workers seems to have its utility for specific soil type and conditions of study under which it was tested. Use of more than one selective technique may therefore be advisable to obtain better results.

## Selective Media for Pathogens of Legumes

### *Pythium* and *Phytophthora*

Due to sensitivity to antagonism and relatively low populations in soil, *Pythium* and *Phytophthora* are difficult to isolate on common agar media. Isolation from soil and quantitative population determination of *Phytophthora* had relied upon the use of susceptible host plants or fruits as bait (Tsao 1960; Waterhouse and Stamps 1969). In soil platings for direct enumeration, elimination or suppression of general soil microflora on media is necessary to

permit recovery of these fungi from soil and to prevent the masking effect of fast-growing soil microflora. Singh and Mitchell (1961) were the first to develop a selective agar medium for quantitative determination of *Pythium* spp. in natural soils. They compared potato dextrose agar, peptone dextrose rose bengal agar (Martin 1950) and Ohio Agricultural Experiment Station agar (Schmitthenner and Williams 1958) with and without different concentrations of PCNB (terraclor wettable powder, 75% a.i.) or pimaricin (original preparation from Lederle) for selective isolation and enumeration of *Pythium ultimum* from corn field soil in Wisconsin. Maximum recovery of the fungus was in peptone dextrose rose bengal agar containing 10 to 20 ppm pimaricin. In a concurrent but separate study, Eckert and Tsao (1960, 1962) had found pimaricin (100 ppm), penicillin (50 ppm) and polymyxin (50 ppm) effective in selective isolation of *Phytophthora* spp. from plant tissues. However, the medium was incapable of isolating *Phytophthora* from natural soils because the high concentration of pimaricin is toxic to spores and most *Phytophthora* spp. exist in soil as resistant spores (Ocana and Tsao 1965; Tsao 1969). Earlier, Haas (1964) had succeeded in recovering *P. megasperma* var *sojae* from soil by using only 2 ppm pimaricin. In subsequent selective media for *Phytophthora* spp., the concentration of pimaricin in corn meal agar was reduced to 10 ppm (Ocana and Tsao 1966; Tsao and Ocana 1969). Pimaricin and related antibiotics, nystatin (Mycostatin), and endomycin, still continue to be the main inhibitors in selective media for *Pythium* and *Phytophthora* (Burr 1973; Hendrix and Kuhlman 1965; Hine and Luna 1963; Holomon 1965; Flowers and Hendrix 1968, 1969; McCain 1967a; Mircetich 1970a, 1970b; Mircetich and Kraft 1973; Kerr 1963; Otrosina and Marx 1975; Peethambaran and Singh 1978; Schmitthenner 1962; Sneh 1972; Stanghelin and Hancock 1970; Vaartaja 1967, 1968; Vaartaja and Bumbieris 1964). Pimaricin stimulates actinomycetes (Tsao and Thieleke 1966). Pentachloronitrobenzene is suppressive for actinomycetes

as well as *Rhizopus*, it has been used (100 to 1000 ppm) alone or in combination with polyene antibiotics for enumeration of *Pythium* and *Phytophthora* in soil (Singh and Mitchell 1961; Takahashi and Ozaki 1965; Haas 1964; Hendrix and Kuhlman 1965; Tsao and Ocana 1969; McCain 1967; Flowers and Hendrix 1969; Vaartaja 1967).

Mircetich and Kraft (1973) made a comparative study of 15 selective media for isolation of *Pythium* and suggested that a full component of minor elements, thiamine, and sucrose in Difco cornmeal agar is a better basal medium than other synthetic and nonsynthetic basal media. Tsao and Ocana (1969) had suggested cornmeal agar containing 10 ppm pimaricin, 200 ppm vancomycin (Vancocin), and 100 ppm PCNB for selective enumeration of *Phytophthora* spp. Mitchell (1975) has used this medium with 5 ppm pimaricin and 300 ppm vancomycin hydrochloride for selective enumeration of oospores of *Pythium myriotylum*.

Nonavailability of pimaricin (Myprozine and Pimafucin) in India had prompted Singh (1962) to substitute it with easily available nystatin (Mycostatin); however, this failed to suppress *Rhizopus*, which overgrew colonies of *Pythium*. Nystatin was found inferior to pimaricin by Mircetich and Kraft (1973) also. A combination of Mycostatin with PCNB is, however, fairly satisfactory (Pandey 1965).

In a more recent study, Peethambaran and Singh (1977, 1978) have successfully used benomyl (Benlate) to modify the original pimaricin medium of Singh and Mitchell (1961). The modified medium consists of peptone dextrose agar (Martin 1950) with 50 ppm rose bengal, 500 ppm Dicrysticin, 20 ppm Benlate, 1000 ppm Mycostatin, and 500 ppm PCNB. This gives as satisfactory a result for *Pythium* as the pimaricin medium, it also selectively isolates *Phytophthora parasitica*, *P. palmivora*, and *P. colocasiae* from soil artificially infested with diseased debris. Work is in progress to adjust the concentration of inhibitors for maximum recovery.

## **Fusarium**

The genus *Fusarium* has drawn maximum attention from plant pathologists because of its economic importance and the need for an effective means of quantitative enumeration of soil populations (Tsao 1970). The first semiselective medium for this group appears to be that of Snyder et al. (1959), who used streptomycin, rose bengal, and sodium taurocholate in Martin's peptone dextrose agar for isolation of *F. solani* f. sp. *phaseoli*. Since then, more than 15 media, both selective and semiselective, have been reported (Tsao 1970). The medium of Snyder et al. (1959) was modified by Wensley and McKeen (1962), who added 500 ppm oxgall for selective isolation of *F. oxysporum*, including *F. oxysporum* f. sp. *melonis*. Use of PCNB, nontoxic to most species of *Fusarium*, was first introduced by Nash and Snyder (1962), who substituted sodium taurocholate or oxgall with 750 ppm PCNB in a medium consisting of 15 g peptone, 1 g potassium dihydrogen phosphate, 0.5 g magnesium sulfate, and 20 g agar/liter. This medium, with occasional reduction in the concentration of PCNB, continues to be the most widely used for selective isolation of *Fusarium* spp. from soil. Kerr (1963) developed a basal medium containing sodium nitrate, potassium phosphate, potassium chloride, magnesium sulfate, sucrose, and yeast extract for spore production of *Fusarium* in shake cultures and used the same medium with 100 ppm PCNB, 50 ppm streptomycin and 60 ppm rose bengal for selective isolation of *F. solani* f. *pisi* and *F. oxysporum* f. *pisi*. Addition of phytoactin (10 ppm) to this medium was claimed to reduce the number of *Fusarium* colonies other than *F. roseum* (Stoner and Cook 1967). Smith and Snyder (1975) have suggested that hydrolysis of agar and peptone in the peptone-PCNB medium can be prevented by autoclaving only water and 2% agar together. The other ingredients, peptone, salts, and inhibitors are added to the water agar shortly after it is removed from the autoclave (temperature about 90°C). Plates are poured at 60°C, after

which they are held for 5 to 6 days and then the soil is plated. Abawi and Lorbeer (1971) have used Martin's peptone dextrose agar with 2 ppm chlortetracycline HCL, 1 ppm thiram, 100 ppm Dexon and 100 ppm PCNB for selective isolation of *F. oxysporum* f. sp. *capae* from organic soils.

Media mainly based on preferential utilization of carbon sources by *Fusarium* spp. have also been reported. Park (1963), Komada (1976), and Vaartaja (1967) used 1 to 2% galactose, and Bouhot and Billotte (1964) used 0.5 to 2% inulin for selective enhancement of *Fusarium* spp. in the medium. Ethanol (Messiaen et al. 1961), potassium metabisulphite (Park 1963; Vaartaja 1967), sodium azide (Denis et al. 1966) and the surfactant Tergitol, TMN (Banihashemi and deZeeuw 1969, 1973, 1975) have been used as other inhibitors.

Singh and Nene (1965a, 1965b) modified the Czapek-Dox agar medium by incorporating 0.75 g/liter Dicrysticin (mixture of streptomycin and procaine and sodium penicillin G), 50 mg/l malachite green, and 100 mg/l captan for exclusive isolation of hyphal fragments of *Fusarium* spp. from plant tissues and natural soil. The medium was highly toxic to conidia (Singh and Nene 1965b; Papavizas 1967). Because this fungus mainly exists as spores in soil free from stimulatory effects of host roots or other substrates, the recovery of colonies on the malachite green medium is far less than that on the peptone-PCNB medium of Nash and Snyder (1962). The same medium (Singh and Nene 1965a, 1965b) was later modified by Malalasekera and Colhoun (1969) to contain only 6.25 ppm malachite green and 3.12 ppm captan to eliminate its toxicity to spores of *F. culmorum* (*F. roseum* 'Culmorum').

Papavizas (1967) compared many of the above media and suggested that use of 100 ppm PCNB, 1000 ppm oxgall, 100 ppm streptomycin, and 50 ppm chlortetracycline (aureomycin) gave as good results for nine formae speciales of *F. oxysporum* and *F. solani* as the medium of Nash and Snyder (1962) and was better than other

media tested. Roberts and Kraft (1973) also compared 24 media and reported peptone-PCNB agar best. Komada (1976) has used a basal medium consisting of potassium phosphate, potassium chloride, magnesium sulfate, Fe-Na-EDTA, D-galactose, and L-asparagine, with Difco Bacto-agar. He used PCNB (750 ppm),  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$  (1000 ppm), oxgall (500 ppm) and streptomycin sulfate (300 ppm) as inhibitors. These were added after autoclaving the basal medium and adjusting the pH of the medium to 3.8 to 4.0 with 10% phosphoric acid.

Singh (1975) continued studies on the malachite green medium with a view to using it as a differential medium for estimating hyphal growth and sporulation of *Fusarium* spp. in the soil (in conjunction with the peptone-PCNB medium.) He modified the medium by reducing the concentration of malachite green to 15 to 20 ppm. By using soil infested with conidia and hyphae separately and by washing infested soil to remove conidia, Singh and Chaube (1970) and Singh et al. (1975) observed that the malachite green medium is capable of enumerating hyphal propagules better than the peptone-PCNB medium. By using soil dilutions in plain water, instead of water agar as suggested by Nash and Snyder and by not agitating the soil suspension during dilutions, they observed that in a 1:1000 serial dilution, the suspension was free from hyphal fragments. Thus, when this dilution was plated on peptone-PCNB medium, the colonies recovered represented those originating from conidia or free chlamydo-spores. In population studies of *Fusarium* from soil under different chemical and physical treatments and in rhizosphere studies, the two media and procedures have been used with success (Chaube 1975; Chaube and Singh 1969; Joshi et al. 1975; Khanna and Singh 1974, 1975; Singh and Singh 1970; Singh and Singh 1970; Singh and Chaube 1975; Singh and Khanna 1977).

In an attempt to differentiate *F. moniliforme* from other *Fusarium* spp. in soil plates using the malachite green medium, Sharma and Singh (1973) studied

colony characters, including pigmentation of *F. moniliforme*, *F. solani*, *F. vasinfectum* (cotton and okra isolates), *F. oxysporum* f. *betae*, *F. oxysporum* f. *lentis*, and some unidentified isolates of *Fusarium* from wheat and sunnhemp on a modified malachite green medium. They were able to quantitatively determine the populations of *F. moniliforme* in soil on the basis of nature of growth and pigmentation of the colony and the substrate. The modified medium contained 500 ppm PCNB in place of captan and only 25 ppm malachite green. Yeast extract (0.2%) was added as energy source and for characteristic pigmentation.

### ***Macrophomina phaseolina* (*Rhizoctonia bataticola*)**

Two selective media and procedures have recently been described for selective quantitative determination of this fungus from soil. These are an advancement over the procedure described by McCain and Smith (1972), who had used processed soil to concentrate sclerotia for plating on potato dextrose agar containing streptomycin and sodium hypochlorite.

1. Meyer et al. (1972, 1973) described the following two rice agar media for direct plating of soil and enumeration of mycelial and sclerotial populations of *Rhizoctonia bataticola*:

- a. Rice agar—prepared by boiling 10 g polished rice in a liter of water for 5 minutes, filtering through cheese cloth, and adding 20 g Difco agar to the filtrate before autoclaving—is amended with 150 ppm chloroneb (Demosan 65 WP) a.i., 25 ppm actual mercury as methoxyethyl mercury chloride, 40 ppm streptomycin sulfate, and 60 ppm potassium penicillin.
- b. Rice agar, prepared as above, is amended with 300 ppm chloroneb and 7 ppm mercury as methoxyethyl mercury chloride; 90 ppm rose bengal is also added.

Both media are highly selective, giving about 100% recovery. The plates are incubated in the dark at 30°C for 7 days.

2. Papavizas and Klag (1975) have reported a procedure and selective medium for quantitative determination of inoculum density of *Macrophomina phaseoli*. A 10 g soil sample is blended for 5 seconds and passed through 177 µm and then 44 µm mesh sieves. Residue retained on the 44 µm mesh sieve is washed in tap water for 1 minute and exposed to 100 ml of 0.25% NaClO solution for 8 minutes. After washing in water for 1 minute, again, the material is resuspended, washed on 44 µm sieve, and blended in 100 ml water. One ml is pipetted onto plates containing 2-day-old medium consisting of commercial potato dextrose agar, 25 mg/l chlortetracycline hydrochloride, 100 mg/l streptomycin sulfate, 50 mg/l Dexon, 2000 mg/l oxgall, and 100 mg/l PCNB. The last ingredient can be replaced with 150 mg/l rose bengal after reducing concentration of oxgall to 1500 mg/l. The plates are incubated at 30°C in the dark.

### ***Rhizoctonia solani***

In spite of advances in development of selective media, *R. solani* continues to be difficult to isolate from natural soil for accurate quantitative determinations. A number of semiselective media using antibacterial agents have been reported by Boosalis and Scharen (1959), Papavizas and Davey (1959, 1962), Martinson and Baker (1962), and Sneh et al. (1966). Ko and Hora (1971) have described a selective medium for determining populations of *R. solani* in soil. It consists of per liter water 1 g K<sub>2</sub>HPO<sub>4</sub>, 0.5 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g KCl, 10 mg FeSO<sub>4</sub>, 0.2 g NaNO<sub>2</sub>, 0.4 g gallic acid, 90 mg dexon, 50 mg chloramphenicol, 50 mg streptomycin sulfate and 20 g agar. One g naturally infested soil is spread evenly in 10 clumps on the medium and microscopically examined after 24 to 48 hours. We have tried to use this medium but with little success, perhaps because of differences in races that respond differently to toxic chemicals.

## ***Sclerotium rolfsii***

This has not been considered a fungus that needs much effort to isolate on routine media from plant tissues. Quantitative determination of its inoculum density in soil is also not difficult because of the size of its sclerotia, which are the only survival structures. These sclerotia germinate in soil under suitable environments and, due to microbial action, produce replacement sclerotia quickly while the hyphae are lysing. This trait of the fungus is an advantage in estimation, as it is possible to separate sclerotia from soil, either by sieving (Leach and Davey 1939) or by flotation-sieving (Rodriguez-Kabana et al. 1973) and then determine their number in soil. Baiting methods have been used to estimate the number of viable sclerotia (Avizohar-Hershenzon and Shacked 1968; Pal and Singh 1973).

Backman and Rodriguez-Kabana (1972) had reported a basal salt agar medium with 30 g/l glucose, 130 mg/l gallic acid and an oxalate (0.54 M, pH 4.2) selective for the fungus with few contaminants in the form of *Aspergillus* and *Penicillium* spp. in soil plates. Later, Backman and Rodriguez-Kabana (1976) described a procedure for isolation of the fungus. The following selective medium was used by them for isolations from organic debris:

1. Potassium dihydrogen phosphate 1.0 g, magnesium sulfate 0.5 g, potassium nitrate 2.0 g, thiamine HCl 1.0 mg, minor element solution 10.0 ml, gallic acid 160 mg, potassium oxalate 10 g in 250 ml water. The mineral element solution contains ferrous and zinc sulfate 1 g each and manganese sulfate 0.6 g in 1000 ml water.
2. Agar 20 g in 750 ml distilled water, steam-sterilized for 15 minutes at 121°C and cooled to 60°C.
3. Combine (1) with (2) and pour plates immediately.

The above medium works well with

small debris particles. When large particles are plated, *Rhizopus* overgrows the growth of *Sclerotium*. Obviously, the medium is not highly selective, and if there is direct soil plating it may not work well. However, it could be combined with sieving/flotation technique (Rodriguez-Kabana et al. 1973, 1974) to plate sclerotia and determine the viable number per g soil without interference by microorganisms present on sclerotial surfaces.

Several similar procedures have been described for *Sclerotium oryzae* (Krause and Webster 1972) and *S. cepivorum* (McCain 1967a; Papavizas 1972).

## **Trends in Uses of Selective Media**

The literature available on population studies of pathogenic fungi is full of references showing the varied uses to which selective media have been put. Besides being used in determining the primary etiological cause of disease, selective media have been used for surveying soils in geographic regions for relative abundance of pathogens, for determining the effect of organic amendments (Khanna and Singh 1974, 1975; Pandey 1965; Singh and Chaube 1975; Singh and Khanna 1977; Singh and Singh 1970), and for chemical treatments of soil and seed (Chaube 1975, 1978; Singh and Singh 1970; Singh and Nene 1965b, and Singh et al. 1971). They have also been used for seed health testing to detect internally seed-borne pathogens that normally escape detection (Agarwal and Singh 1974; Sharma and Singh 1973). Soil treatments affect pathogens differently in the root-free soil and in the rhizosphere; the use of selective media has helped in such studies (Chaube 1978; Khanna and Singh 1974, 1975; Singh and Khanna 1977).

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## Discussion — Session 2

Chairman : J. B. Sinclair  
Rapporteur : K. N. Rao

- Saksena : Is there any objection to the use of the phrase "soil-borne diseases" instead of "soil-borne pathogens?"
- Wood : I feel it is imprecise to use "soil-borne diseases." We have been using "soil-borne pathogens." We have more serious objections to "seed-borne diseases" than to "soil-borne diseases." There are seed-borne diseases that may be contaminants.
- Kraft : There are distinct anastomosing groups worked out in *Rhizoctonia* that one group out of three can be seed-borne; the others cannot. How can we deal with this? It affects the whole procedure and needs further investigation.
- Wood : When we talk about *Rhizoctonia solani*, we should at least state to which anastomotic group it belongs.
- Kraft : Groups of one area may not be present in other areas.
- Nene : Would you please elaborate on this?
- Kraft : I feel that fairly distinct groups exist within this genus. One group is not seed-borne. We talk about wide host ranges within the genus.
- Abawi : Four anastomosis groups are recognized at the present time; however, there are isolates that cannot be placed in any of these groups. Isolates of *R. solani* associated with bean roots and soils in New York belong largely to group 4, but a few isolates have been placed in groups 1 and 2. Even within each anastomosis group, isolates of *R. solani* differ considerably in their growth rate, pathogenicity, and production of sclerotia. The anastomosis grouping of *R. solani* isolates can be determined by pairing with known tester strains.
- Sinclair : Can you tell us where these clones can be obtained?
- Abawi : American Type Culture Collection maintains a set. Researchers in the USA who might be able to provide a set are Dr. Anderson at the University of Minnesota, Dr. Butler at the University of California, Davis, and Dr. Parmeter at the University of California, Berkeley.
- Wood : Is the aerial strain of *R. solani* a new strain?
- Saksena : It is difficult to say. I feel sheath blight of paddy was known 10 to 15 years ago and therefore do not consider it a new strain.
- Wood : Why do you think that it is not a new strain? Is there any way of finding out?
- Saksena : The disease was recorded, but not much work was done on *Rhizoctonia* previously and there was not much familiarity with the perfect stage. The introduction of exotic, highly susceptible varieties and excessive use of fertilizers and irrigation may be responsible for high disease incidence and new strains.

- Nene : A major factor contributing to high disease incidence may be high plant density in a new package of practices, something which does not happen in normal farmers' fields. My own experience in black gram at Pantnagar is that in the erect types the incidence was lower compared with plants that produced a lot of vegetative growth, leaf canopy, leaf contact, etc. This is true even for the sexual phase of the pathogen.
- Hubbeling : In Holland, with increase in fertilizer on short varieties of wheat stembase attack was more and I suppose the same is true with short paddy varieties. Ammonium compounds, particularly Ammonium nitrate or phosphate fertilizers, were stimulating infection; effecting a decrease in pH of the soil, they might easily induce a more dramatic attack of plants.
- Wood : Is it the aerial phase or root infection you are talking about?
- Hubbeling : In wheat, several fungi increased with this short-stem situation, but not *Rhizoctonia*. I am not talking about *Rhizoctonia* in different crops as Dr. Saksena was.
- Wood : I feel it is not increase in severity of the pathogen but perhaps the result of a new host-parasite relationship.
- Abawi : Web blight of beans (aerial infection by *R. solani*) is common in South America, and the perfect stage of the fungus has been reported to occur readily. The aerial strains have been reported to be high sclerotia producers, generally more sensitive to CO<sub>2</sub> and faster growing than the subterranean strains. High plant density and certain plant architecture are known to increase *Rhizoctonia* infection as well as other pathogens such as *Sclerotinia*, the causal agent of white mold of beans.
- Purss : We have *Rhizoctonia* web blight in northern parts of Queensland but we have never seen the perfect stage.
- Abawi : Similarly, we have not been able to observe the perfect stage of *R. solani* in New York, although we have obtained several isolates that very closely resemble the aerial strains and belong to the same anastomosis group.
- Saksena : In sheath blight of tall paddy, lesions are occasionally produced on the leaf, but no perfect stage. In legumes also, under certain conditions, only lesions may be produced without formation of the perfect state.
- Haware : *R. solani* causes root rot in chickpea, but we never observed the perfect stage. Factors such as soil moisture and temperature play a major role in disease development.
- Saksena : The root strain of *Rhizoctonia* never produces the perfect stage. In chickpea, the disease is more severe in early stages of growth and delay in sowing may reduce disease incidence.
- Chohan : Sheath blight of paddy caused by *R. solani* was not a problem on local varieties in the Punjab but as Dr. Nene observed, it is a problem on the new high-yielding varieties. In the Punjab we do not find the perfect state sclerotia. The selectivity of the pathogen causing aerial

- infection may be due to survival or perpetuation of the pathogen on rice kernels.
- Nene : Dr. Saksena, you have indicated a long list of hosts. Did you test chickpea at all? If yes, what was the reaction? I want to know because in northern India and parts of West Asia, chickpea makes a lot of vegetative growth in cool temperatures and this may be congenial to aerial blight.
- Saksena : I do not remember testing of chickpea against aerial strains. Chickpea is a rabi (postrainy season) crop and all other hosts listed are kharif (rainy season) crops. We have not come across aerial blight in rabi crops.
- Kraft : In the USA, when two crops of beans are grown, second crop of snap bean is devastated. Is that true here?
- Abawi : Our experience with *R. solani* on beans suggests that the disease is most severe under relatively dry and warm soil conditions.
- Kraft : Could Dr. Singh tell me—what is the statistical significance in soil sampling of large fields, such as 60 acres. I think in terms of dilution, the end point of inoculum in soils is important.
- Singh : I agree with Dr. Kraft that nondetection does not mean that no fungus is present. There is a limit up to which it can be detected. For example, we have encountered a problem in *Pythium* detection. No *Pythium* could be detected in the December to February period, but it started appearing in samples taken after March. Therefore,
- the number of samples and replications should be increased. These selective media are useful with large sample size and high inoculum populations, or when we artificially create conditions for detection.
- Kraft : In the case of bean and pea root rots, disease levels were low even when inoculum population levels were high, because of other factors such as soil compaction, stress, pH, etc.
- Wood : What techniques do you use for ensuring reasonable estimation of efficiency of selective media?
- Singh : First we isolate pure cultures of the fungus. Then we inoculate autoclaved soils. A known quantity of inoculum is added to soils and immediately plated for recovery. The recovery was only 36% for *Fusarium*, but was high for other organisms. When actually using the method we normally do not keep a check for recovery.
- Abawi : It is important to initially determine the efficiency and applicability of the technique to be used for estimating soil population of plant pathogens to the different soils involved in the investigation. Generally, none of the media and methods available will give an accurate estimate of the actual number of fungal propagules in the soil. However, these techniques give estimates of the relative abundance of fungal propagules and thus are especially suitable for comparing the differences between treatments.
- Dr. Purss, in your presentation you mentioned that

- stress factors play a role in the epidemiology of many diseases. Will you please elaborate on some of these stress factors and how they can be measured?
- Purss : I am not an expert on measuring moisture stress. Jim Cook in Washington did use a pressure bomb technique to measure the capacity of the plant to withstand moisture stress. We cannot talk of root and stalk diseases without actually talking about various stresses.
- Abawi : I agree with this and would like to add that factors such as soil compaction, herbicide use, and fertilizer affect the incidence and severity of many soil-borne pathogens, and thus the epidemiology of disease.
- Hagedorn : What crop can be rotated with the main crop for a deleterious effect on *Rhizoctonia* propagules in the soil?
- Chohan : A rice-potato-rice rotation can be followed to control the aerial strain of web blight. Rice isolate does not cause black scurf on potato and vice versa.
- Nene : Dr. Purss, I would like to clarify the position on *Phytophthora vignae*. This was the earlier identification from CMI and the latest information was given in my text yesterday. In our studies, pigeonpea roots are not attacked by *Phytophthora* and symptoms are seen only on the stem. Are there three different strains, that is, root, stem, and foliar strains?
- Purss : *P. vignae* was always first observed on the stem; infection goes to the roots later and is rarely detected primarily on roots. Foliar infection occurs, but there is no evidence of strain differentiation for this capacity.
- Kraft : Dr. Nene, was your observation with inoculations?
- Nene : No, with natural infection in the field.
- Kraft : I was wondering whether it would be possible to study this in glasshouse inoculations of roots and shoots. You may have root resistance but not stem resistance.
- Kannaiyan : Recently root inoculation of a known highly susceptible line showed no symptoms up to 10 days. But there was severe stunting of inoculated plants. Previous workers reported that they inoculated roots and symptoms were observed on the stem. I feel watering the plants may cause inoculum from the root to show symptoms on the stem.
- Nene : Are there any lesions observed on the roots?
- Kannaiyan : There was only stunting and no lesions on the roots.
- Nene : How is the stunting explained?
- Kannaiyan : Toxins may be playing a role in the stunting of plants.
- Hubbeling : Under high temperature or high N dressing, cells become bigger. If a linear enlargement of two times is effected, cells may become four times in volume. Water balance may be disturbed with this new situation, causing stress.
- Allen : Dr. Purss said *Phytophthora vignae* is aggressive particularly at 19 to 28°C. Can you explain the species' geographical distribution in terms of temperature?
- Purss : Temperature response could

- be explained based on *P. vignae* distribution in Australia and in Queensland particularly.
- Allen : Where has the *P. vignae* come from?
- Purss : I do not know, but it was not noticed until 1950 in Australia, when it appeared in epidemic form on the high susceptible cv Poona. I presume that the disease did not occur before because it was unlikely to have gone undetected. Earlier *P. cinnamomi* was observed in natural form but not *P. vignae*. In cross-protection studies, prior inoculations with *P. megasperma* var. *sojae* protected against *P. vignae*. *P. megasperma* is more widespread than *P. vignae*.
- Allen : Did you test cv Cristado against all races?
- Purss : Yes, Cristado was tested against all four identified races. All the "field-resistant" cultivars tested were fully susceptible, with the exception of cv Black Eye 5.
- Wood : Dr. Purss reported 95% infection or surface covered, what does it mean?
- Purss : I think it would be more correct to record 95% disease instead of infection. Since only one lesion is produced on each plant, the number of plants are counted for disease severity.
- Wood : What I am interested in is the size of the lesion rather than the number of plants infected.
- Purss : I am speaking about field resistance. How far the lesion gets up to the stem is not important. If a lesion is produced, no yield is generally obtained from the plant.
- Kannaiyan : In Dr. Purss' paper, much variation of disease incidence in the same variety in different years was indicated. What are the reasons for such variations?
- Purss : Variation in rainfall may be exclusively responsible for variation in the disease.
- Nene : In pigeonpea genotypic differences are such that some genotypes may not break even after lesion formation; that is, the plants may live with the disease.
- Hagedorn : Are the lesions superficial?
- Nene : They are essentially superficial, unless the weather continues to be favorable.
- Hubbeling : Dr. Chohan, could you explain which disease you are controlling with wheat-groundnut-wheat rotation or by seed treatment?
- Chohan : With this rotation, I have observed indication of low rhizosphere microflora, not complete control of collar rot disease caused by *Aspergillus niger*.
- Hubbeling : Is gypsum recommended in cultivation of groundnut?
- Chohan : Yes, it is recommended as per the Package of Practices by my university for the rainy season crops, particularly to correct sulfur-deficient soils.
- Kraft : We have observed gypsum and lime correlations with *Fusarium* incidence in the soil in dryland farming. In sandy alluvial soils, silica migration to the upper layers was observed due to low pH. We are running some liming studies.

- Wood : One of the recommendations from this group might be to explore the possibilities of cultural control of disease caused by *R. solani*, as obtaining genetic resistance may be remote for this group of diseases. This sort of research might well be justified and practiced on a much larger scale as cultural practices could well be productive.
- Singh : In traditional potato cultivation Margosa cake or sawdust was used as a cultural control against *Rhizoctonia* disease. It also keeps the tubers clean.
- Chohan : Some of the cultural practices may be very effective. For example, *Sclerotium oryzae* causing stem rot in paddy in the Punjab was completely controlled when farmers started cultivating rice fields after harvest with tractor-mounted furrow-turning plows.
- Wood : Any cultural method should be very practical. A lot of work on biological control turned out to be nonsense. I have a Ph.D. student who suggested application of 40 tons per acre of chitin for the control of *Fusarium oxysporum* var. *pisi*.
- Singh : Chitin of course is very costly, but there are a lot of common materials such as wood sawdust, along with urea as supplemental nitrogen, which may be useful. There should be different recommendations for different soils.
- Gibbons : Cultural control in peanuts in the USA is still working very well. Any control of foliar diseases such as *Cercospora* and rust might work for Fusarial root rots also.
- Sinclair : Yesterday we observed in the field a virus and fungus interaction. This type of interaction was also observed in soybean. There may be interactions between foliar and root pathogens. ICRISAT scientists might well consider the study of the interaction between pathogens occurring on the same host. This area needs more study.
- Allen : I am glad that host-pathogen and pathogen-pathogen interactions were brought out in the discussion. Cowpeas are generally intercropped. At IITA, we have measured effects of cropping system on disease and have observed reduced levels of some diseases (e.g. *Rhizoctonia* web blight; cowpea yellow mosaic) and increased levels of others (e.g. powdery mildew) in cowpea. We need more information on this.

# **Host-Parasite Interactions**

# Host-Parasite Relationships with Special Reference to Root-infecting Fungi

R. K. S. Wood

My brief is to talk about interactions that affect the incidence and severity of diseases of higher plants caused by soil-borne microorganisms. The great majority of these microorganisms are facultative parasites that grow well on simple media and would therefore be expected to grow readily on tissues of higher plants once their cells have been killed. *Prima facie*, there are no obvious reasons why such pathogens should not grow and multiply extensively on plant debris of host or of other plants on or in the soil. Some certainly do, at least for some time; hence the importance of the debris in their perennation. But increasingly the evidence suggests that many, if not most, do not compete well with saprophytes in the soil and that after limited vegetative growth on dead plant tissue, they disappear or persist only as dormant propagules of one type or another. A few facultative parasites may persist vegetatively at low levels by growing in association with roots of plants, but not doing sufficient damage to be regarded as pathogens.

The comparatively few soil-borne pathogens that are obligate parasites or facultative saprophytes, if I may be allowed to use such old-fashioned terms, must be presumed to persist in soil almost wholly as dormant propagules, unless they have subsidiary life cycles on other than their main hosts, of which we are unaware.

Before proceeding, I must qualify my use of "dormant." Whereas some of the propagules I have referred to are dormant in that they will not readily germinate if they are taken from soil and placed in the usual conditions of moisture, temperature, and so on, others do germinate and grow readily under these conditions. In the first group

endogenous, in the second group, exogenous factors control dormancy. There is now abundant evidence of exogenous factors in soil that suppress germination of propagules, the well-known and well-studied phenomenon of mycostasis. For both types of propagules and factors, something has to happen before they start to grow in soil as a preliminary to penetration and infection of plants. Here is a first and important role for the underground parts of plants in host-parasite relations. The propagules of a few pathogens produce zoospores, but most pathogens produce germ tubes and hyphae. Both types of propagules may be influenced by the host in the prepenetration stage, either at a distance from or at the host surface, so that the probability of the host being challenged by the pathogen may be influenced.

Also, and probably more importantly, the host will interact with the pathogen during penetration, infection, and colonization in ways that will determine the growth of the pathogen and its capacity to cause disease.

I shall now review these aspects of host-parasite relationships and speculate lightly on their possible significance in disease control.

## Effects Outside Plants

Subaerial parts of plants alter the soil, *inter alia*, by taking up water and oxygen, by producing carbon dioxide, by losing other substances in solution or in vapor, and by releasing cellular material as various high molecular weight structural polymers. Roots are also usually surrounded by a layer of mucilage, mucigel, 1-10  $\mu\text{m}$  thick, mainly polysaccharide, which separates epidermal cells from the soil. Each of these substances, or

groups of substances, undoubtedly will affect pathogens at a distance from the plant but most of what we know about such effects relates to substances in solution as exudates from roots and hypocotyls. I shall confine myself largely to such substances as are very varied in type, with sugars, amino acids, and inorganic ions as dominant, but including—usually at much lower concentrations—organic acids, glycosides, nucleotides, various growth factors, and enzymes. The substances come mainly from root tips, zones of elongation, and breaks in the epidermis elsewhere.

### Germination of Dormant Propagules

Dormant propagules remain alive for varying periods that depend on type of propagule and species. Propagules may be stimulated to germinate, usually non-specifically, as they are approached by roots of plants that the pathogen can or cannot parasitize. It seems that exudates from the roots nullify that part of mycostasis that depends on nutrient levels, which, in soil away from roots, are too low to allow germination of the propagules. Less certainly, exudates may counteract the toxicity of certain substances in soil solutions.

Specific effects of exudates have been reported much less frequently. Some of the claims made for specificity have not been confirmed by later work. But it has been established that exudates from roots of *Allium* spp., but not of many other plants, do cause sclerotia of *Sclerotium cepivorum* to germinate in soil with alkyl cysteine sulfoxides as the active compounds. Sclerotia of *Stromatinia gladioli* respond similarly to exudates from roots of many, though not all, species of the *Iridaceae* but not to exudates from species of various other families. For *S. cepivorum* there is the difficulty that sclerotia do germinate when removed from soil. How then do the alkyl cysteine sulfoxides nullify the factors that suppress germination in soil?

Substances from host plants may also act specifically on resting spores of pathogens such as *Plasmodiophora brassicae* and *Synchytrium endobioticum*, highly specialized parasites that persist in soil for very long periods in the absence of susceptible host plants.

The general lack of specificity in response to exudates is surprising, perhaps, considering the advantages to pathogens that could respond only to exudates from plants that could then be parasitized.

### Tactic Effects

Exudation from subaerial parts of plants will, presumably, establish gradients from the surfaces through the soil which could last long enough to effect activity or growth of pathogens away from the surfaces. Chemotactic movement of zoospores along gradients of increasing concentrations of substances of types found in exudates are, in vitro, striking and well documented, particularly for sugars and amino acids. But there is a lack of much firm data on whether chemotaxis increases the frequency of challenge of subaerial parts by pathogens. Again, most of the effects so far described are nonspecific, with exudates from resistant plants being as effective as, or more effective than, those from susceptible plants. Specific effects claimed for certain pathogens were again not confirmed in later work.

### Stimulation of Growth

There is little doubt that nutrients in root and hypocotyl exudates can increase growth of pathogens. But they also increase growth of other microorganisms. This is an extensively studied subject, but it has been difficult to separate these two effects in vivo and still more difficult to assess the consequences for growth of the pathogen near or at the surface as a prelude to penetration. Thus, increased growth of other microorganisms usually would be expected to

decrease growth of the pathogen and disease. But it can also have the reverse effect; and perhaps unexpectedly, increased growth of a pathogen in response to exudates may be associated with less infection and disease. A reasonably well-established example of increased infection is that seed of pea cultivars susceptible to *Pythium debaryanum* release significantly more sucrose during germination than do seed of resistant cultivars; resistant seed became susceptible after treatment with exudates from susceptible seed. Susceptibility of cultivars of *Phaseolus vulgaris* to *Rhizoctonia solani* and *Pythium* spp. is also related to increasing amounts of ninhydrin-positive, silver nitrate-positive substances exuding from seeds. These effects were assayed by germination of chlamydospores, but it seems likely that there were corresponding effects on growth of hyphae.

### Inhibition of Growth

There are many reports that substances in exudates are toxic to soil-borne pathogens, often with the claim or implication that the toxicity decreases significantly the chances that a pathogen will infect subaerial parts of plants. An early and much-quoted example is the role of the cyanogenic  $\beta$ -glucoside linamarin and its aglycone HCN in decreasing wilt of flax caused by *Fusarium oxysporum* f. sp. *lini*. Similar work on the toxicity of exudates from roots of pea cultivars to conidia of races of *F. oxysporum* f. sp. *pisi* in a pattern corresponding to disease caused by the races also suggested that toxicity is important in specificity. Unfortunately, later studies along various but similar lines strongly suggest that toxicity of exudates to pathogens is not important either in resistance or in specificity. There is little work with exudates and other diseases that suggests otherwise. This lack of good evidence for activity against fungal pathogens is again somewhat surprising, particularly in view of claims that exudates from roots of a

wide variety of plants contain substances toxic to bacteria and in view of the persuasive evidence that exudates from roots of certain higher plants decrease root growth of other higher plants—the well-studied allelopathic effects.

### Formative Effects

Certain soil-borne pathogens infect plants from specialized structures such as the infection cushions of *Rhizoctonia solani*. There is some evidence that formation of such structures is a prerequisite for infection. It has been claimed that infection cushions of some strains of *R. solani* are produced in response to exudates from susceptible, but not from resistant, plants. But it is also reported that although exudates do stimulate formation of infection cushions, they do so nonspecifically. Substances from roots also promote formation of infection cushions by *Helicobasidium purpureum*. The production of appressoria by *Colletotrichum* spp. is also influenced by nutrients, though not always in ways that would be expected. Thus, penetration of membranes by *C. phomoides* may be increased by added nutrients, though formation of appressoria is depressed, and we found some years ago that adding nutrients to suspensions of conidia of *C. lindemuthianum* greatly increased growth in and from inoculation drops on hypocotyls but completely suppressed penetration and infection. But we did not advocate this as a method of disease control!

### Effects Inside Plants

Let us assume that a soil-borne pathogen has started to grow and to penetrate a plant. What now are the main factors that determine whether it will continue to grow and cause disease? Again, almost all the information—and there is a great deal of it—concerns fungi that are facultative parasites. It is best considered under two main headings: (a) preformed substances that decrease growth, and (b) fungitoxic substances that are

newly synthesized in response to infection or to other stimuli, and that will be referred to somewhat loosely as phytoalexins. I shall not have time to refer to other substances, preformed or newly synthesized, such as lignin, which, though not toxic as such, may limit or prevent growth of pathogens in other ways.

## Preformed Substances

Subaerial parts of plants, as other parts, often contain substances toxic to microorganisms. Resistance to soil-borne pathogens based on this toxicity is easy to conceive and has been the basis of much research and many claims. But except for a few diseases, the evidence is not conclusive, because it has not satisfied the conditions that must be met before a significant role for a toxic substance in resistance can be accepted. These conditions are as follows:

- The substance must be present in those tissues of subaerial parts of plants that the pathogen would infect and colonize.
- The substance must be present in concentrations in which it will decrease growth of the pathogen sufficiently to explain resistance, though concentrations active in vitro and in vivo may differ considerably. Substances usually, though not always, will be more active in vitro.
- The substance must be present in a form in which it is available or becomes available to the pathogen. Extraction may make available substances that otherwise would not be, or cause in these substances changes significant to toxicity. Usually toxicity is increased by extraction, but in some circumstances it could be decreased.
- For plants with different levels of resistance, there should be a good relation between these levels

and concentrations of available substances. If not, then why not?

These exacting conditions explain why it has been so difficult to show convincingly that particular substances do indeed function in resistance.

I shall now refer briefly to a few examples in which reasonable or substantial progress has been made in establishing a role for preformed toxic substances in disease resistance.

Phenols and phenolic glycosides have been well studied in vascular wilts caused by *Verticillium* spp. and *Fusarium oxysporum* and in common scab of potato tubers caused by *Streptomyces scabies*. There is also the early and much-quoted example of *Colletotrichum circinans*, outer scale leaves of onions, and their content of fungitoxic catechol and protocatechuic acid, though these substances probably act against this pathogen mainly in surface films of water.

One of the best-studied diseases from this point of view is take-all of wheat and oats, caused by *Gaeumannomyces graminis*, the triterpenes avenacin A and B found in oats, and the enzyme avenacinase, which inactivates avenacins. Oats are resistant to *G. graminis*, which does parasitize wheat and does not produce the enzyme. In contrast, var. *avenae*, which parasitizes oats, does produce the enzyme, so that the avenacin in roots is made ineffective, presumably to the extent that growth of var. *avenae* is sufficient to cause disease.

The alkaloids  $\alpha$ -solanine and  $\alpha$ -chaconine may be significant in the resistance of potato tubers to *Fusarium* spp. Unsaturated lactones have been implicated in a number of diseases, notably as the tuliposides in tulip bulbs against *F. oxysporum* f. sp. *tulipae* and *Botrytis cinerea*. The cyanogenic glycoside linamarin in flax, referred to earlier, has also been studied as a preformed inhibitor, but with inconclusive results.

Diallyl-disulfide derived from alliin (S-allyl-L-cysteine-sulfoxide), well-known for its high fungitoxicity,

may account for resistance of garlic bulbs to many species of *Penicillium* that do not produce alliin-lyase. This enzyme is produced by *P. corymbiferum*, which does attack garlic; its growth is not inhibited by alliin or the products of its degradation.

For some time the mustard oils of the Cruciferae as esters of isothiocyanic acid, occurring mainly as glucosides, attracted a lot of attention because of the high toxicity of the aglycones, the isothiocyanates. Earlier claims of a role for mustard oils in resistance to club root caused by *Plasmodiophora brassicae* were discounted by later work, but there is some evidence that isothiocyanates are significant in resistance of roots to *F. oxysporum*.

A point of general interest is that many of the fungitoxic substances studied in relation to resistance occur as glycosides, which are less toxic than the aglycones. Glycosidases in host cells or, indeed, from the pathogen, may therefore be important in controlling the amounts of aglycone released during infection.

This leads to the next section in which pathogens and possibly other microorganisms induce the synthesis by host cells of toxic compounds.

## Phytoalexins

Much of the research on phytoalexins has been based on hypocotyls of various plants. Hypocotyls are very convenient to use because they are easily inoculated to produce well-defined lesions in relatively uniform tissue. Lesion development can be readily studied, and extraction made from tissue from lesions and from surrounding tissue, both chlorophyll-free. This means that much of the literature on phytoalexins is relevant to the subject of this lecture, because pathogens that attack hypocotyls are often either soil-borne or seed-borne so that they have to function in soil.

Surprisingly, much less work has

been done with roots, which probably produce phytoalexins as readily as do other parts of plants. Roots could be even better models than hypocotyls or other parts of plants, because a given volume of roots presents many more living cells that could react to microorganisms as inducers of synthesis. Thus it has been estimated that roots of a 4-month-old rye plant are about 600 km long in total with a surface of about 240 m<sup>2</sup>, additionally with about 14 x 10<sup>9</sup> root hairs, with a total length of 4700 km and a surface of 400 m<sup>2</sup>. It seems likely that roots as they move through soil will stimulate activity leading to growth of a proportion of the many microorganisms, including the pathogens that they encounter. Therefore, the high frequency of this association between living cells of roots and soil microorganisms would lead repeatedly to the synthesis of phytoalexins. But, though the prospect is enticing, we have little information as to whether this does happen.

For a few diseases of hypocotyls, there is now quite good evidence that accumulation of one or more phytoalexins at sites of resistant reactions can explain why a pathogen causing the reaction does not continue to grow, although it may remain alive. Even so, some plant pathologists see no role for phytoalexins in disease resistance. Assuming a role, many problems still remain. I shall mention only a few of the more important. First, where are phytoalexins synthesized in relation to the position of inducing microorganisms in the plant or even outside the plant? In almost all cases studied so far, a few host cells are usually killed, and phytoalexins localized at the infection site. Are they produced only by the killed cells, in which they are certainly found, are they produced only by adjacent living cells, from which they then move into the killed cells, or are they produced by both? Considerable technical difficulties are involved in resolving these questions, but for a few diseases it now seems very likely that phytoalexins are synthesized by living cells close to cells infected and killed by pathogens.

For roots, therefore, and, to a lesser extent for hypocotyls, we should ask how often nonvirulent pathogens cause very restricted lesions such as are associated with synthesis of phytoalexins. If this happens frequently enough, what are the effects on later infection by pathogens? And, of course, we must also ask whether there are similar reactions to the microorganisms we class as saprophytes, particularly if these pass into xylem passively at wounds or other sites.

A related point of interest is as follows: in lesions on hypocotyls, accumulation of phytoalexins is largely confined to the lesion and contiguous tissue, at least in some diseases. In small roots are conditions so different that phytoalexins would be carried from lesions to act systemically elsewhere? Again, this is an intriguing prospect.

Another set of problems, even more difficult, concerns the early interactions between pathogen and host cells, the triggering or enhancement of pathways involved in the synthesis of phytoalexins, and then their accumulation and breakdown. Here there is the perplexing fact that in some higher plants synthesis is induced by a wide variety of unrelated substances, with a lack of specificity that contrasts strikingly with the specificity of most host-parasite reactions, though I suppose one could mention in passing that this specificity may be somewhat less pronounced for soil-borne than for other pathogens. In spite of much work, no one, to my knowledge, has yet obtained from pathogens with different capacities to cause disease in a range of plants, substances, or even cell-free preparations, that unequivocally cause disease symptoms and the accumulation of phytoalexins in patterns similar to those caused by the pathogens. But this problem is now being studied intensively, so we may not have to wait much longer for such substances. Recent work in this field has, however, led to the isolation from fungi and other microorganisms of the substances somewhat loosely called elicitors, which induce synthesis of phytoalexins in very low

concentrations. The best characterized are the glucans from *Phytophthora megasperma* var. *sojae*. Unfortunately, they are nonspecific inducers of synthesis, so that their role in disease caused by this pathogen is uncertain. However, the biological activity of the glucans is so high that one must ask whether they have a more general role in the induction of resistance. Even if they do not, they certainly demand attention as agents in the practical control of disease.

Earlier, I referred to the possibility that the repeated association between roots and microorganisms leads to the accumulation of phytoalexins that would increase resistance, especially if the phytoalexins moved some distance from sites of synthesis. But another, and possibly more likely, mechanism of induced resistance is as follows. Infection localized to a few cells may cause changes in nearby cells that lead not so much to synthesis of phytoalexins as to changes such that these cells will now react to later infection by virulent pathogens as they would to avirulent pathogens. The changes cause genetically susceptible host cells to react as do genetically resistant cells. Again there is the prospect that the factors that induce such changes may move from sites of production in roots to act systemically elsewhere and that we may be able to stimulate this activity with other substances.

The last point is one that is made repeatedly, but justifiably, because of its importance. Only a few of the many potential pathogens that constantly challenge plants actually infect and cause disease. This may be even more significant for subaerial parts of plants, especially roots, than for other parts of plants, because conditions in soil may increase greatly the frequency of such encounters. But infection sufficiently serious to cause what we recognize as disease is rare. This can only mean that apart from the specific mechanisms of disease resistance, which are by far the more studied, there are non-specific mechanisms that act constantly,

almost certainly are induced, and are therefore the most important form of resistance in plants. These mechanisms deserve much more study because, presumably, disease occurs only when a pathogen fails to invoke this resistance in a plant which we consider susceptible. We need to determine how this happens. Then we may be able to alter a plant so that it becomes able to act against the virulent as it does to the multitude of avirulent pathogens.

# Host-Parasite Relationships with Special Reference to *Rhizoctonia* Spp.: A Review

J. B. Sinclair

*Rhizoctonia solani* Kuehn (*Thanatephorus cucumeris* (Frank) Donk.) and *Rhizoctonia bataticola* (Taub.) Butler (*Macrophomina phaseolina* (Tassi) Goid.) are diverse, omnipresent plant pathogens, usually soil-borne, but also seed-borne in many crops.

*Rhizoctonia solani* is an excellent saprophyte, exists primarily in the vegetative state, and overseasons primarily as sclerotia. It is active over a wide range of temperatures, but high moisture levels are necessary for its penetration and colonization. *Macrophomina phaseolina* is a poor competitor in the soil and exists primarily as sclerotia. Warm, dry conditions are favorable for development of the charcoal rot it causes.

A report on the state of the knowledge of *R. solani* was published in 1970 (Parmeter 1970), and an annotated bibliography (1977) and review of the literature on *R. bataticola* (1978) were published by Dhingra and Sinclair. In developing methods of screening for resistance to these pathogens, a number of factors must be taken into consideration, including the host-parasite relationship.

## Activity Before Penetration

This subject has been reviewed for *R. solani* by Dodman and Flentje (see Parmeter 1970), who concluded that plant exudates influence the development of *R. solani* before penetration and probably provide better opportunities for penetration and infection. The activity of *M. phaseolina* was summarized by Dhingra and Sinclair (1978) who wrote that various compounds stimulate sclerotia germination. Crude root exudates and sugar fractions from okra roots were shown to stimulate sclerotial germination and mycelial growth of *M. phaseolina*,

and amino acids were found to be inhibitory (Goel 1975). An unidentified substance from dry whole pea seeds partially inhibited germination of sclerotia of *R. solani* (Pfleger and Harman 1975).

The use of various herbicides and nematicides can affect the incidence and severity of *Rhizoctonia* seedling diseases. The herbicide diphenamid enhanced colonization of bean stem segments by *R. solani* in natural soil and suppressed soil microorganism respiration and glucose utilization in glucose-amended soil, but it slowed down the decrease in time of *R. solani* colonization and disease incidence (Katan and Eshel 1974). Trifluralin and dinoseb reduced hypocotyl cellulose content, methylated pectin and reduced resistance to penetration, and reduced levels of phytoalexins of snap beans (Romig and Sasser 1972). Neubauer and Avizohar-Hershenson (1973) showed that although trifluralin could suppress the growth of *R. solani* in vitro, it increased the incidence of seedling disease in cotton; Grinstein, Katan, and Eshel (1976) claimed that resistance to *R. solani* in bean was not affected by trifluralin, nitratin, or butralin, but was decreased by dinitramine. Growth of *R. solani* in vitro, colonization of bean stems and of sugar beet seeds were less in the presence of the herbicide cycloate (Campbell and Altman 1977). The nematicide aldicarb increased damping-off of sugar beet but limited the growth of *R. solani* in vitro (Tisserat et al. 1977).

Other factors that can affect disease incidence are the isolates used, cultivar, soil moisture (should be above 70% moisture-holding capacity), pH (should be above 6.6), and organic matter content used as inoculum (Lewis and Papavizas 1977). Warren (1973, 1975) found that low concentrations of inoculum were sufficient

for test selection of lima beans for resistance to *R. solani* and that hypocotyl rot of lima bean was highest when soil was inoculated with diseased lima bean leaf tissue and lowest with root tissue. The highest inoculation density of *R. solani* was found after tissue of the first bean crop was plowed under, with root rot being more pronounced in the second planting (Papavizas et al. 1975).

The use of rice hulls was better than sawdust in reducing seedling disease caused by *R. solani* of bean and tomato (Mian and Khan 1974), as was the use of *Trichoderma viride* on lima bean and peas (Mall 1976). The survival of bean plants grown in soil infected with *R. solani* was increased with added N and P (Sirry, Higazy, and Faranat 1974).

Combined infection of *R. solani* and the root-knot nematode was more severe on cotton seedlings than either alone (Carter 1975).

## Penetration

The mode of penetration into plants by *R. solani* was reviewed in detail by Dodman and Flentje (see Parmeter 1970); penetration by *M. phaseolina*, by Dhingra and Sinclair (1978). Khadga et al. (1963) detailed the penetration of cotton seedling hypocotyl by *R. solani* (Fig. 1). Entry by both fungi may occur directly through the cuticle and epidermis, with or without complex organized infection cushions and infection pegs (Fig. 2); entry may also occur through wounds and natural openings. Some isolates may vary in the preferred mode of penetration, others may penetrate the same host in several different ways.

## Cutinase

It has been shown that *R. solani* secretes cutinase (Baker and Bateman 1978), but it is not clear whether *M. phaseolina* secretes cutinase.

## Enzymes

Both fungi have been shown to produce polygalacturonase, pectin methylesterase, and cellulytic enzymes. Absolute proof is lacking, but evidence suggests that *R. solani* produces proteases. Ramasami and Shanmugam (1976) showed that *M. phaseolina* produced pectolytic and cellulytic enzymes in vitro and in infected cotton seedling hypocotyl tissues.

## Toxins

A number of reports of nonenzymatic phytoalexins in culture filtrates of *R. solani* have been made by Bateman (see Parmeter 1970). Dhingra and Sinclair (1978) reviewed the role of toxins in disease development of *M. phaseolina*, which is not yet well understood.

## Establishment and Host Response

### Establishment

The establishment of both *Rhizoctonia* spp. is similar and has been summarized by Parmeter (1970) for *R. solani* and for *M. phaseolina* by Dhingra and Sinclair (1978). Both fungi may penetrate either inter- or intracellularly, depending upon isolate used and host tissue. Penetration may proceed rapidly or slowly, but it is usually accompanied by tissue disorganization and cell lysis in advance of the hyphae (Fig. 1). *M. phaseolina* will form sclerotia in xylem vessels (Fig. 3). This has not been reported for *R. solani*.

### Host Response

Phytoalexin type of resistance mechanisms have been studied only recently. Smith et al. (1975) isolated four phytoalexins from *R. solani*-infected bean hypocotyls bearing lesions of different ages, and studied accumulation with time and concluded that phytoalexins played a role

in disease resistance. One of the phytoalexins studied by several workers is kievitone, which occurs in the hypocotyls of bean infected with *R. solani* (Smith et al. 1973, 1975; Smith 1976, 1978). They found that it can be induced with  $\text{CuCl}_2$  in bean pods, inhibits *R. solani* in vitro, and has a role in localizing the fungus in lesions. Puepke and Van Etten (1975) found that concentrations of pisotin increased in *R. solani*-infected tissues up to 6 days after inoculation and that the concentration was great enough after 2 days to inhibit the test fungus in vitro. The concentration of phaseolin and phenylalanine ammonia-lyase increased in bean hypocotyls and roots of *R. solani*-resistant cultivars in response to infection by the fungus and both compounds inhibited the growth of the test fungus in vitro (Prasad and Weigle 1975). Other compounds that increased in cotton seedlings in response to infection by *R. solani* were catechin, which inactivated pectic enzymes (Hunter 1974) and seven terpenoid compounds (Hunter et al. 1978).

Khoury and Alcorn (1973) showed that carbohydrate concentration increased in cotton seedlings infected with *R. solani* up to the first leaf stage and may have a role in pathogenesis as well as increasing the susceptibility of infected plants to other fungi.

### Host Resistance

It has been shown that black-seeded cultivars of bean are resistant to *R. solani*, while white-seeded cultivars are susceptible. Prasad and Weigle (1975) showed that extracts of black-seeded types contained phenols that inhibited *R. solani*. Wyatt (1977) showed that white-seeded cultivars had larger cells with greater porosity than pigmented-seeded ones and that the former absorbed water faster than the latter.

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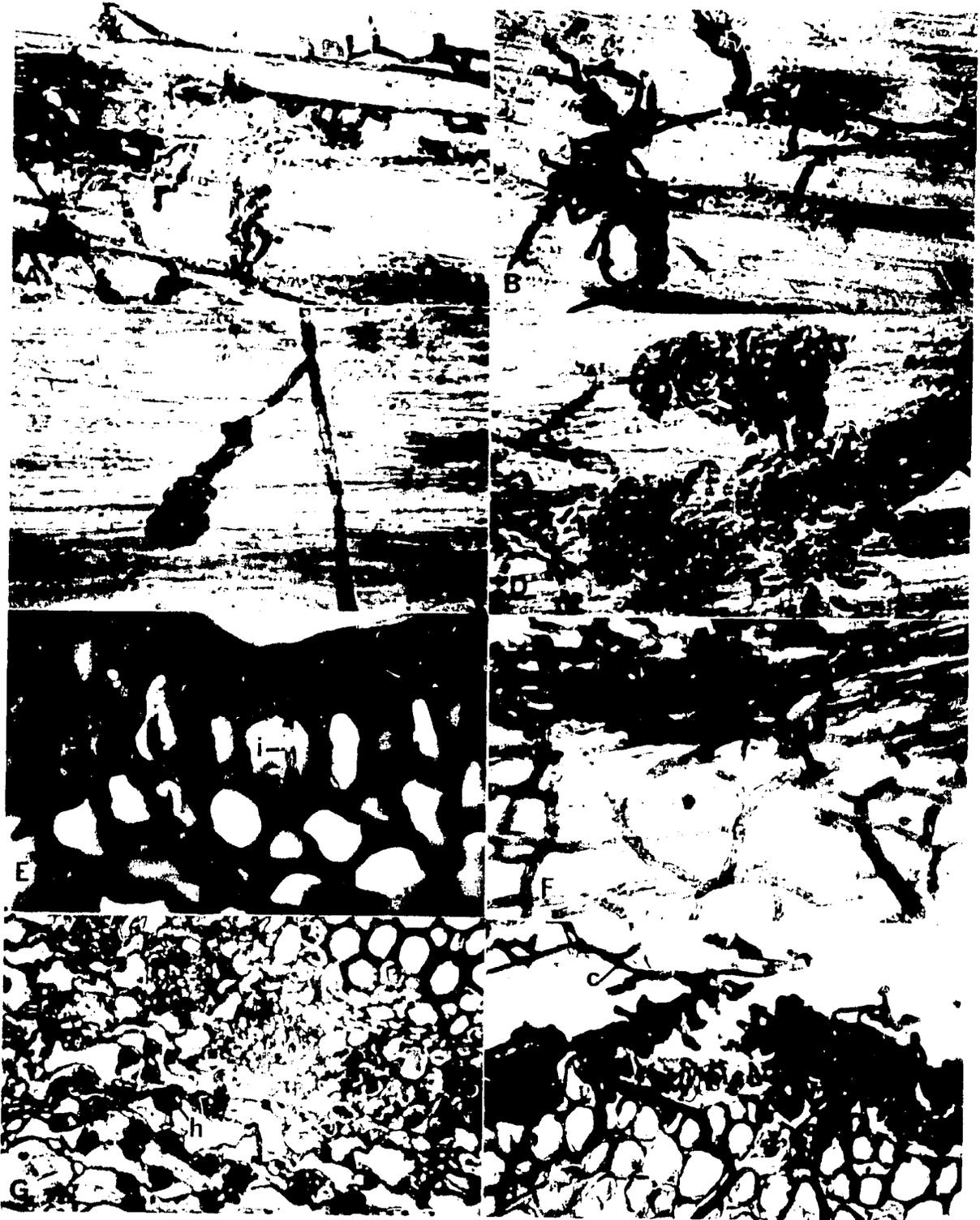


Figure 1. Penetration and colonization of cotton seedling hypocotyl tissues by *Rhizoctonia solani*: (A) aggregation of hyphal side branches; (B) aggregation of hyphal terminal branches; (C) knottin of a single hypha and (D) aggregation of several hyphae forming an infection cushion (all x340). (E-H) Transverse and cross-sections of cotton seedling hypocotyl tissues showing: (E) infection peg (i) of *R. solani* in epidermal cell lumen (x770); (F) growth of invading hyphae in cortical cells; (G) cross-section of infected cortical cells showing cross-section of invading hyphae (h) (x340); and (H) cuticle (c) separated from epidermal cells; note initial lesion formation (x340). (source Khadga et al. 1963)

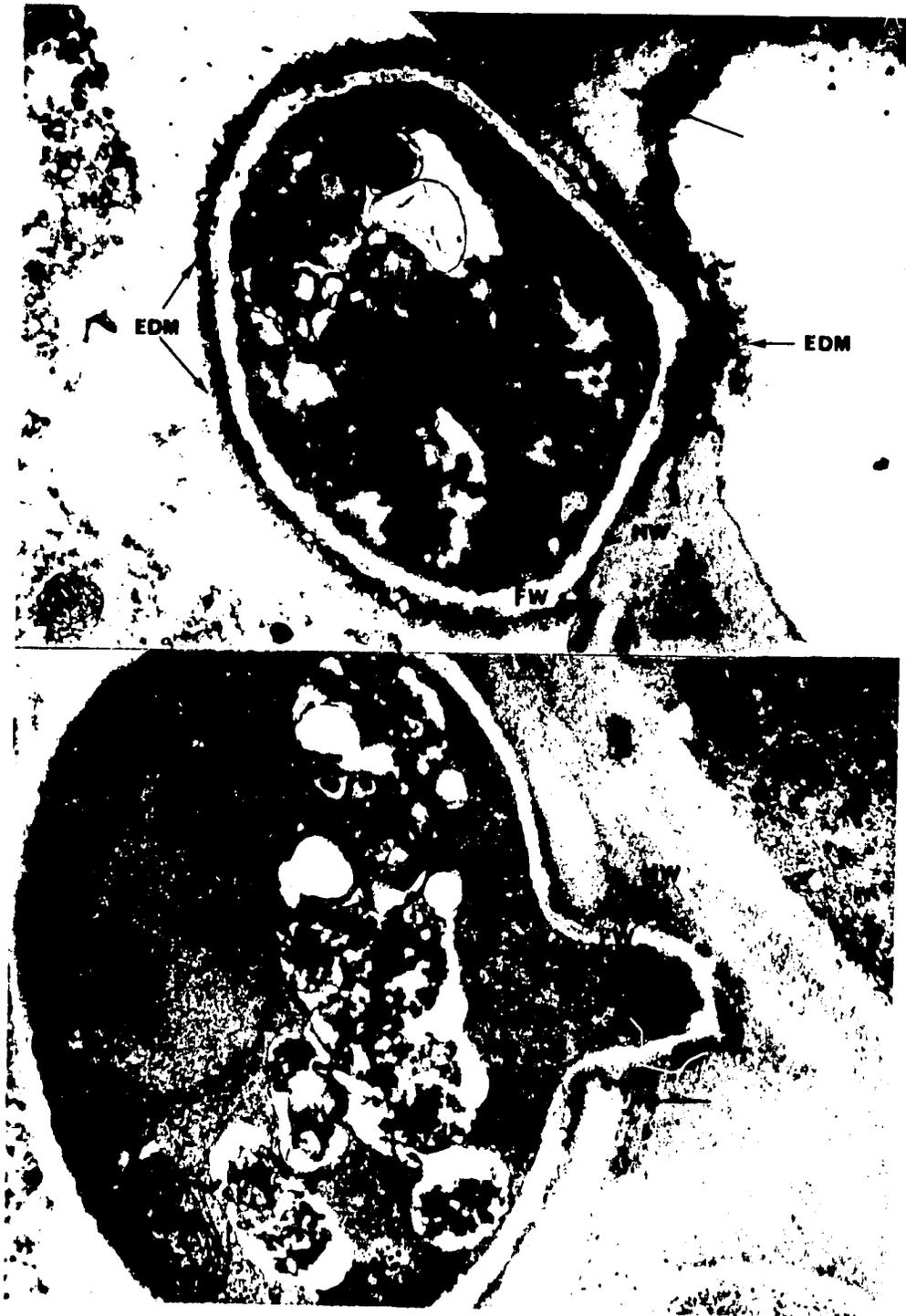


Figure 2. Soybean root tissue infected with *Macrophomina phaseolina*: above penetration of the primary cell walls (HW) separating two cortical cells [Note the distinction of the host cell wall (HW) and the middle lamella (ML) (arrow) and the collection of electron dense material (EDM) at the point of wall penetration and on the fungal strand (F)]. The host cytoplasm (HC) in the fungal colonized cells is disorganized (x 54,000); below penetration through the thickened portion of a host cell wall (HW) by the production of a narrow infection peg. [Note the lack of an inward bending of the laminated areas in the host wall (HW) (x 54,000)]. (Source: Ammon et al. 1974).

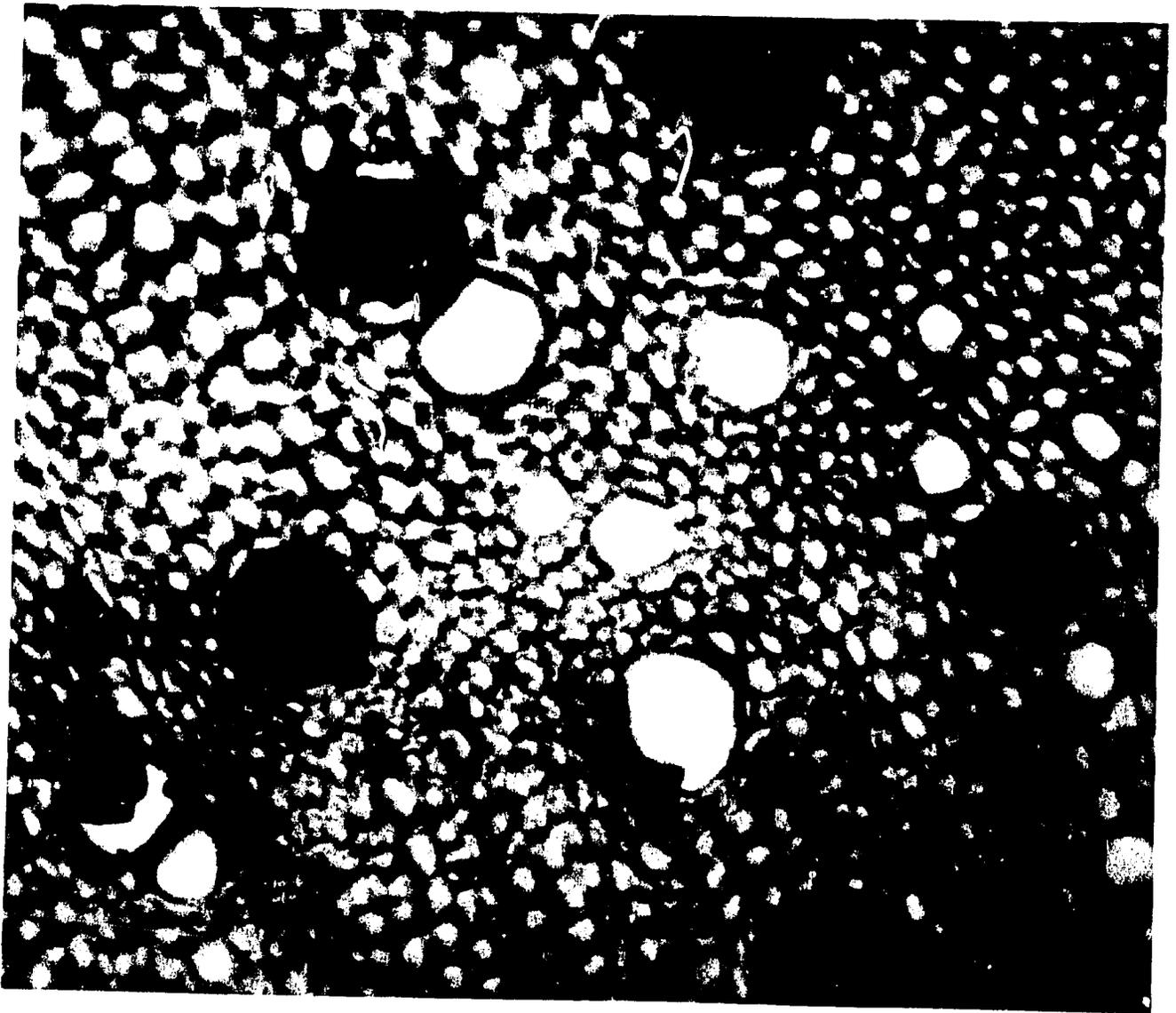


Figure 3. Photomicrograph of a cross-section of a soybean stem infected with *Macrophomina phaseolina* showing sclerotia of the fungus in xylem vessels (x320) (Source: Ilyas and Sinclair 1974).

## Discussion — Session 3

Chairman : H. K. Saksena  
Rapporteur : P. Subramanyam

- Wood : Much work has been done on root exudates and their influence on pathogens; has anybody demonstrated the specific effects of root exudates on the pathogen?
- Hubbeling : What about the work done at Rothamsted?
- Wood : Well, I don't think that they have demonstrated any specific effects of root exudates on pathogens. Dr. Zentmeyer from California published a lot of work on root exudates and their specific effects on *Phytophthora cinnamomi*, but now he has withdrawn his claim.
- Abawi : Potato root exudates stimulate eggs of the golden nematode to hatch.
- Wood : Well, it is difficult to say. The resting spores of *Plasmodiophora brassicae* do germinate when these come into contact with root exudates of the members of family Brassicaceae. But is it a specific response? I do not know.
- Sinclair : It is known that soybean root exudates stimulate the germination of sclerotia of *Macrophomina phaseolina*.
- Wood : I agree: Cole-Smith and his coworkers also demonstrated that exudates of *Allium* spp. stimulate the germination of sclerotia, but do not have any specific effect.
- Kraft : What about Flentje's work with *Rhizoctonia solani*?
- Wood : Flentje has undoubtedly shown that root exudates have a stimulatory effect, but it is not specific.
- Saksena : Yes.
- Haware : We have some evidence to show that root exudates show specificity. The root exudates collected from a wilt-susceptible chickpea cultivar showed a stimulatory effect on conidia; the exudates collected from the resistant cultivar showed inhibitory effect on spore germination.
- Wood : On chlamydo spores?
- Haware : Not on chlamydo spores. We have tested only micro and macroconidia.
- Wood : Are the conidia important in the initial infection by *Fusarium oxysporum*? Do they survive as such in soil?
- Abawi : Most conidia of *F. oxysporum* when added to the soil become shortly converted to chlamydo spores.
- Wood : Can you get conidia from the soil?
- Haware : Yes.
- Abawi : I do not think that conidia can survive for long periods in natural soil; chlamydo spores are probably the surviving unit.
- Wood : Do you get chlamydo spores also?
- Haware : We do get chlamydo spores from the soil.
- Wood : Have you published this?
- Haware : This is just a preliminary observation.

- Wood : What is a saprophyte? This may be an academic question. Take, for example, *Penicillium*: there are several hundred species but only a few of them are pathogens—for instance, *P. digitatum* on lemon; *P. expansum* on apples. Another interesting fungus is *Aspergillus*; *A. niger* is the classical example. To my knowledge it is a pathogen only on groundnut.
- Chohan : I doubt that. *Aspergillus niger* is a pathogen on several fruits. In Punjab, we have observed several fruit rots caused by *A. niger*.
- Allen : It also causes bulb rot in onions.
- Chohan : Yes, it can.
- Hubbeling : I would like to comment on *A. niger*. With reference to the work on *Pyrenochaeta lycopersici*, no rotting was observed when mycelial mat was added to the soil, but when the liquid culture which still contains lots of sugar was added, it favored *A. niger* growth in soil, causing rotting of young tomato seedlings.
- Wood : I would like to know how the living cells of roots respond to the saprophytes in the soil. Do they trigger phytoalexin formation in the roots?
- Chohan : Yes, that is why we have less root rot in groundnut, because the saprophytes trigger phytoalexin production in roots, as also on immature pods in soil. Our researches do indicate this type of mechanism in nature.
- Wood : Can you tell me why pea cotyledons are attacked rapidly and destroyed by microorganisms?
- Kraft : Well, seed is a good source of food for fungi. Hypogeous seeds like pea are more rapidly attacked and destroyed by fungi because they are under the soil.
- Wood : I think you are right.
- Abawi : I have one comment on Prof. Wood's presentation: It seems to me that some of the research efforts on physiology of disease might well lead to information that can be used in a practical manner; e.g., if a phytoalexin can be shown to be the factor responsible for disease resistance in a host plant, wouldn't it be possible that a screening procedure can be developed, based on determining the amount and speed of accumulation of such a phytoalexin?
- Wood : Better put the pathogen on the host and test the reaction rather than searching for phytoalexins.
- Abawi : I am only suggesting that some of the side benefits of such research efforts can be practical and feasible under certain conditions.
- Wood : Take a genetically susceptible plant and induce resistance; I hope that is one of the approaches to get resistance.
- Nene : Could Dr. Sinclair throw some light on Ridomil? These seed treatments with minute quantities can give control of downy mildew and other phycomycetous fungi for a long period. Does this little quantity trigger the mechanism within the plant system that makes the plant almost immune? Has some work been done on this? I think it is a more logical way of thinking because of

- the minute quantity involved and the time span for which it remains effective.
- Hagedorn : The amount is very small and plant is big.
- Kraft : Why should we take a susceptible plant and induce resistance rather than studying the resistant plant directly, which is simpler? Another interesting line is toxins: I think we have to pursue the toxin aspects also.
- Allen : I think there are many other ways of mechanisms of resistance.
- Wood : I agree with you Dr. Allen. In many cases, plant pathologists neglect the study of virulence. Take, for example, cabbage leaf, which is resistant to many microorganisms. Even if you inoculate the leaves with conidia, I do not know whether the conidia germinate or not. But the same conidia germinate in a drop of water on glass slides. What is the mechanism? We do not know. If they do not germinate on cabbage leaves I want to know why they have not germinated. I suggest we start with a genetically susceptible cultivar and induce resistance, which lasts longer.
- Allen : Induced resistance is easy to demonstrate in the glass-house but its role in the field is harder to measure.
- Hubbeling : The specificity of phytoalexins in host-parasite relationships is not true. For instance, in peas, even a little mechanical damage to host tissues stimulates phytoalexin production.
- Wood : Can you give me an example for horizontal resistance that is functional? I know
- Dr. van der Plank quotes this but he would not give any examples. On chickpea, you get heavy hairs that prevent the germination of spores of *Ascochyta*.
- Nene : Dr. M.V. Reddy did some work with chickpea leaf exudates.
- Reddy : There is no specificity: the exudates both from resistant and susceptible cultivars behaved similarly.
- Nene : That is, we could not confirm earlier claims.
- Sinclair : Soybean cultivars with slight pubescence on the pods showed fewer lesions and less seed decay due to *Phomopsis sojae* than cultivars with pods heavily covered with hairs.
- Saksena : Phytoalexin production has been shown only in a very small number of host-pathogen combinations. What is the possibility that phytoalexin is being produced in other host-pathogen combinations?
- Wood : Work is going on in various laboratories on the response of cereals to avirulent pathogens, which leads to very rapid lignification. This is not directly related to phytoalexins, but is a stimulation of host physiology that makes host cell walls more resistant to penetration.
- Williams : I would like to make two comments: First, Dr. Nene mentioned that Ridomil protects the plant for a long period. I think in the case of seed dressing to control pearl millet downy mildew the control is probably during the first 15 to 20 days as this is the period that the plant is vulnerable to infection. I do not think that there is any induced resistance.

- Nene : What about necrotic lesions later in the season? particularly actinomycetes, but there was no significant difference.
- Williams : We have not observed any: so far no information is available. My second comment is I think that it is wrong to screen for a mechanism of resistance, because we will miss some good cultivars that have resistance by other means. We should challenge the host with the pathogen and let the host integrate all factors contributing to resistance.
- Allen : Has anybody looked into the synthesis of phytoalexins in the roots in response to *Rhizobium* infection?
- Sinclair : I think that the presence of red pigments in soybean roots is related to the accumulation of phytoalexins in the roots.
- Abawi : In my previous comment, I was not suggesting that the mechanism of resistance, if known, should be used as an alternative screening method. I was only referring to the possible practical benefit and use.
- Wood : Phytoalexins are very interesting organic compounds in the sense that they have a very strong fungicidal action and complex organic molecules. I do not think any firm can take up the manufacture of phytoalexins in the near future. But there is some remote possibility of phytoalexin production by tissue cultures commercially.
- Singh : Can we expect the roots to exude phytoalexins into the surrounding soil?
- Wood : Possible! I do not know.
- Singh : For example, Vitavax is not effective against *Pythium* or any other phycomycete fungi: but when we apply Vitavax as seed dressing we do find reduced populations of *Pythium* for some time around the seed and damping-off is also considerably reduced. We tried to correlate with rhizosphere microflora,

# **Laboratory/Glasshouse Screening**

# Laboratory/Glasshouse Screening for Identifying Resistance to Soil-borne Diseases in Beans

N. Hubbell

Many soil-borne diseases, occurring all over the world where beans are grown, attack also some other crops. The causal fungi of such widespread diseases are, *Pythium* spp., *Rhizoctonia solani*, *Thielaviopsis basicola*, and *Sclerotinia sclerotiorum*. Soil-borne fungi with a worldwide distribution, adapted to *Phaseolus* beans only, are *Fusarium solani* f. sp. *phaseoli* and *Fusarium oxysporum* f. sp. *phaseoli*. Two soil-borne fungi, occurring exclusively in the tropics and attacking many other crops, such as sweet potato, maize, and several grasses, are *Macrophomina phaseoli* and *Sclerotium rolfsii*. Development of resistance to these tropical fungi in beans seems to be very difficult, even impossible. Much research has been devoted to resistance in beans to *Fusarium solani* f. sp. *phaseoli*. Also the resistance to some less specialized fungi, such as *Rhizoctonia solani* and *Pythium* received the attention of bean breeders and pathologists. In most cases, selection is carried out in heavily infested fields or in naturally infested soil in a greenhouse. Often several fungi are involved in the "natural" root-rot complex, and identifying resistance to each component separately is difficult. For this purpose, laboratory or greenhouse screening with pure cultures of the different fungi is necessary. This implies a study of the genetic variation of plants and fungi, and control of several environmental conditions after inoculation of seedlings. The aim of such screening is to enable handling of thousands of seedlings in a short time and a limited space to identify resistance efficiently at an early stage. The possibilities and difficulties of this "artificial testing" will be discussed in detail.

## Genetics of Plant and Parasite

Resistance in a plant should involve an

inheritable character that prevents serious attack by a parasite and consequent yield losses. In other words, the genetic base of resistance has to be proven within the genetic variation of the parasite. Accessions of wild material of primitive origin often exhibit a wide variation in reaction to a parasite; segregation of resistant and susceptible plants might be expected. In this case, the population mainly consists of homozygous resistant and homozygous susceptible plants. In populations from crosses between resistant and susceptible plants, many heterozygous plants can be expected. Whether the resistance can be called dominant or recessive depends on the nature of reaction of hybrid plants. If the resistance is dominant, heterozygous plants are also resistant, and selection can be made using any arbitrary breeding scheme.

If resistance is recessive, hybrids between resistant and susceptible plants are susceptible, and segregation of resistant plants in the progeny is still possible. Sometimes hybrids exhibit intermediate reactions, indicating incomplete dominance or quantitative inheritance of resistance.

In general, the genetic variation of soil-borne pathogens seems to be rather wide; considerable differences in virulence are quite common. Consequently, resistance is mostly relatively stable. The occurrence of very virulent races of a parasite and breakdown of resistance is uncommon. However, in pure culture, soil fungi often decline after some time, necessitating many precautions to keep the pathogenicity at a high level.

## Environmental Conditions

Several environmental conditions play an important role in infection and expres-

sion of symptoms. Reactions are strongly influenced by temperature, humidity, and aeration of the soil or the substrate in which the roots are developing. The pH and chemical composition of the soil, as well as quality and duration of light, also affect the reactions.

### Physiology and Relation of Host and Parasite

The reaction of the plant changes, depending on different phases of growth. Germinating seeds and young seedlings are often attacked before emergence by fungi such as *Pythium* spp., *Thielaviopsis basicola*, or *Rhizoctonia basicola* under unfavorable conditions. In this very first vegetative phase the development of the roots and the hypocotyl has to be fully supported by reserve food in the cotyledons; consequently, attack of the cotyledons means a general weakening of the seedling. Not until development of the primary leaves does this critical phase end. The formation of the root system fairly stops as soon as the plant starts to flower. The beginning of the generative phase is at the same time as the end of root nodulation by nitrogen-fixing bacteria. The activity of these bacteria is only possible as long as air is penetrating sufficiently into the soil around the roots and assimilates are transported to the roots. Attack of the root nodules results in a further weakening of the plants. Moreover, latent infections of tap roots and stem base, for example, by *Fusarium solani*, might lead to decay as soon as defense mechanisms stop functioning due to lack of assimilates. It is possible that the longer the vegetative phase continues, the longer the regeneration of roots occurs by adventitious rooting of the hypocotyl. Therefore it is important to distinguish between the genotypic and phenotypic resistance mechanisms of the host-parasite relation. Whether early-ripening bean cultivars are able to build up a well-functioning defense mechanism against soil-borne parasites in the short vegetative phase, and how

well such a mechanism might operate in the generative phase, particularly in the pod-setting stage, are points for discussion.

### Interaction of Parasites

Interaction of parasites does often result in synergistic effects. Also insects, nematodes, bacteria, and even viruses may interact with soil-borne fungi in such a way that rapid yellowing or wilting of plants results from simultaneous root attack. Some wilting of resistant pea cultivars was caused by activity of black flies of a *Meosciara* sp. in tests with *Fusarium oxysporum* f. sp. *pisi* races. Larvae of these small black-winged insects, developing from deposited eggs, cause decaying of cotyledons; thence, proceeding into the cortex of the epicotyl, the larvae girdle the stem base. Consequently, even resistant plants may exhibit wilting. Attack of untreated plants by *Fusarium oxysporum* f. sp. *pisi* in greenhouse trials prompted further research with black flies. A female fly, caught in a sterile tube with agar, pricking with the ovipositor into the agar medium, gave rise to a culture of *Fusarium oxysporum* f. sp. race 2! In contrast, several male black flies were unable to transmit this fungus into tubes of sterile agar. Labruyere, Den Ouden, and Seinhorst (1959) demonstrated the synergistic effect of the nematode *Rotylenchus robustus* on *Fusarium oxysporum* f. sp. *pisi* race 3 in peas. Severe attack could not be induced by the parasites separately; however, by interaction with the nematodes considerable yellowing and wilting was incited. Hubbeling (1974), establishing race 3 to be identical with race 2 by comparing the spectrum of attack of resistant and susceptible cultivars, could identify the black-fly-transmitted *Fusarium* as well as the pea near-wilt caused by race 2. It is yet unknown whether other soil-borne fungi may be insect-transmitted. Bean flies, *Hylemia cilicrura*, disturbing normal emergence of bean seedlings, often drastically, may perhaps be able to transmit

rotting bacteria. In any case the maggots, developing from the eggs deposited near the germinating seeds, cause many wounds by invading cotyledons and hypocotyls.

The soil fungus, *Oplidium brassicae*, often attacking roots without causing visible damage, is able to transmit tobacco necrosis virus to roots of many plants. The strip-bleed virus, a strain of tobacco necrosis virus causing systemic necrosis between 15° and 25°C in beans, can be transmitted exclusively if the fungus itself carries the virus. Sometimes the fungus and the virus occur in peat moss, used as a constituent of potting soil in Europe. Oospores of *Pythium* or *Phytophthora*, being resistant to temperatures over 100°C, may also be present in potting compost.

## Screening Methods

Methods will be described for screening resistance to *Fusarium*, in particular to *Fusarium oxysporum* and *Fusarium solani*. Most experience has been obtained with the former one.

In analyzing screening methods for identifying resistance to soil-borne fungi, attention must be given to:

1. Isolation of fungi in pure culture.
2. Multiplication of fungi on or in artificial media.
3. Preparation of inoculum.
4. Inoculation methods.
5. Assessment of susceptibility.
6. Identification of disease resistance.

## Isolation of Fungi in Pure Culture

Several soil-borne fungi are able to withstand ethanol 96%, although most bacteria are killed by this chemical. Therefore isolation of *Fusarium* in pure culture appeared to be possible without antibiotics, using ethanol 96%. One-centimeter portions from infected roots

or stems were cleaned with tap water and dried on blotting paper. Pith and outer cortex were removed if exhibiting decay, and in such a case parts of the xylem ring were used exclusively. The stem and root pieces of xylem parts were sterilized by dipping in ethanol 96% for a few seconds and dried immediately on sterilized blotting paper. From the treated pieces on sterile paper, 1- or 2-mm lengths were cut with a sterilized scalpel and transferred to tubes with sterile cherry or prune agar. From each attacked plant part at least five tubes with two or three such lengths each were utilized. Other agar media are also possible, such as water agar, or somewhat acid media on which bacteria do not develop abundantly. After 3 days of incubation at about 25°C, *Fusarium* colonies started to develop from the cut pieces. Pure cultures can be obtained easily by transferring pieces of agar with mycelium from the outside of the colonies to tubes with potato dextrose agar. From these, monospore cultures can be produced. Often the tubes with the small particles are not contaminated by bacteria or other contaminants and thus in fact are already pure cultures. When petri dishes are used for isolation of fungi, even under optimal sterile conditions, contamination can hardly be avoided after some days. Moreover it is more expensive, laborious, and time-consuming to use petri dishes, even if made from plastic, and always special precautions are necessary. In contrast when tubes are used to isolate fungi, the narrow openings limit contamination by bacteria or spores from the air. It is quite possible to use simple laboratory facilities without special equipment for sterilization of the air.

## Multiplication of Fungi on or in Artificial Media

Petri dishes are not efficient for multiplication of fungi in my opinion, both for the reasons described above and because the quantities of spores developed are low. In liquid media such as

Czapek Dox solution, high concentrations of microconidia can be obtained in a few days, using pure cultures, absolutely free from bacteria. We mostly used 3-liter flasks with Czapek Dox solution. The old method of shaking the flasks by machine, after addition of some pieces of agar with mycelium of a *Fusarium* culture, often wets the cotton plugs, resulting in contamination of the liquid by bacteria, since cotton wool, even after autoclaving contains some heat-resistant bacterial spores. These multiply abundantly, rapidly exhausting the available nutrients meant for the soil-borne fungi. Often bacteria produce gas, inciting foam development in the solution.

This experience led me to develop another system of movement of the liquid that avoids wetting the cotton plugs. A narrow glass tube, penetrates the cotton plug, ending in the liquid. An aquarium pump is used to blow a stream of air, sterilized by a small, dry cotton plug, through this tube, providing a gentle moving of the liquid, thus preventing formation of a mycelium mat and stimulating the production of huge numbers of microconidia within 5 days at 25°C. Solid media, other than agar, are sometimes used, for instance, rice, straw, or a mixture of maize meal and sand or soil. These must be carefully sterilized in order to exclude bacteria. *Fusarium* cultures in sand- or soil-containing media remain viable and virulent for many months due to development of chlamydospores. In liquid media chlamydospores are normally not produced within 5 days.

### Preparation of Inoculum

At times in liquid cultures some concentrations of mycelia may result from irregular aeration. In that case the cultures have to be homogenized in a mixer before using them as inoculum. Moreover, standardization of the number of propagules might be necessary by addition of certain amounts of tap water. Often agar cultures are used as agar slants,

prepared by mixing with tap water. Cultures in soil or sand can be utilized directly as inoculum.

### Inoculation Methods

In principle there are two completely different methods of inoculation in screening for *Fusarium* resistance. The first method involves sowing of seeds in artificially infested soil; the second, dipping roots of seedlings in a spore suspension and planting in sterilized soil. The first method corresponds very well with sowing in infested soil in the field and is not laborious. The second method is rather time-consuming since the roots of the seedlings must be cleaned before they are dipped in a spore suspension. Consequently the roots will be wounded seriously. It is uncertain whether some field resistance is lost in this way. A modification of the first method, testing under sterile conditions, cannot be considered useful because of the many laborious precautions to be taken to avoid contamination. Moreover, it may be doubtful whether plants are exhibiting reliable reactions under these unnatural conditions.

Huge numbers of seedlings can be screened in a greenhouse with temperature conditioning using sterilized soil as a substrate in which the inoculum is mixed. In order to avoid problems inherent to soil sterilization, some requirements have to be fulfilled. Steaming organic material produces volatile compounds such as ammonia, which are harmful to seedlings and sometimes also to fungi. There are two solutions to this problem. First, using steam-sterilized soil, it is necessary to wait a few weeks, until the disappearance of all volatile products, before adding the inoculum. During that time a recolonization is realized of saprophytic bacteria and fungi, originating from the air. Secondly, a soil may be used from semisterile constituents, such as river sand vermiculite, and peat moss, free from plant pathogens.

For screening purposes I used shallow plastic boxes, 10-15 cm deep as containers

in which the infested soil or soil mixtures was prepared. The limited depth is essential to effect root competition and consequently a rapid symptom development. For artificial infestation 10 parts by volume of soil were mixed with 1 part of inoculum. When mixing somewhat dry soil with the liquid inoculum, good moisture conditions can be achieved.

In many trials I could demonstrate how important pH and calcium relations in the soil are to the degree of attack on the plants. Always at poor calcium nutrition conditions and low pH, serious attack, sometimes even of resistant plants, has been obtained. Using mixtures of 3 parts by volume of peat moss (pH + 4.5) and 1 part by volume river sand (pH + 7), containing a little bit of calcium, a soil mixture of about pH 5.5 was reached. In this mixture the pea cultivars New Era, New Season, and New Wales exhibited severe wilt symptoms with *Fusarium oxysporum* f. sp. *pisi* race 1, although they have been developed as resistant to race 1 and race 2. However, under the same pH conditions and likewise direct sowing of the seeds in the infested soil mixture, no wilt occurred with race 2. In contrast, the cultivars Cobri and Koroza appeared to be resistant to both races at pH 5.5. New Season and New Wales did not wilt when tested in river sand of pH 7, or in the peat sand mixture adjusted to pH 7 with CaCO<sub>3</sub>, with *Fusarium oxysporum* f. *pisi* race 1.

### Assessment of Susceptibility

The example of difference in behavior at pH 5.5 and pH 7 illustrates the difficulty of assessing susceptibility and resistance. I am quite sure similar observations can be obtained with other leguminous crops, for instance, *Phaseolus* beans depends on the degree of resistance available and on what symptoms must be considered as an expression of susceptibility under certain (pH) conditions. Since *Fusarium oxysporum* occurs in a high concentration in all parts of wilting susceptible plants, even stem tips,

the degree of wilting of stems and leaves may be directly proportional to root attack. The degree of root attack by other soil-borne fungi can likewise be proportional to overground symptoms, such as stunting and yellowing of plants. However, direct assessment is possible only by uprooting infected plants. If roots or stem base do not exhibit distinct symptoms, plants have to be judged as resistant. Some infection of underground parts of resistant plants usually occurs, because the pathogens can be reisolated from these parts. Therefore the presence of pathogen cannot be a criterion of susceptibility, in the sense of lacking in resistance. Uprooting of screened seedlings is not desirable, unless distinction of overground symptoms of susceptible plants is questionable and the method of screening is therefore inefficient.

After the screening procedures the resistant seedlings have to be multiplied. The effects of uprooting when transplanting cannot be completely avoided after selection of the resistant plants. First, the pathogen(s) are distributed to the place to which the plants are transplanted. Second, transplanting means some mechanical damage to the roots, affecting new root development of resistant plants, confirming the correctness of screening. Third, adverse conditions such as drought and high temperatures, particularly when planting is in the open, may cause an apparent breakdown of resistance. Often some difference in degree of resistance can be discovered by measuring total stem length and/or length of attacked stem tissue. Quantitative assessment can be achieved in this way.

### Identification of Disease Resistance

Zaumeyer and Meiners (1975) reviewed recent research on disease resistance in beans. They mentioned several wild or primitive plant introductions as sources of resistance to some soil-borne diseases. Not until accessions of such material became available did screening for

resistance become possible. Zaumeyer and Meiners (1975) also reviewed research on the genetics of resistance to soil-borne diseases. In most cases a polygenic inheritance of the resistance has been shown. Often a quantitative inheritance pattern occurred, excluding normal backcrossing or pedigree selection, and leading to the procedure of recurrent selection. The screening methods described enable adequate identifying of a high degree of resistance. They also facilitate research on inheritance of resistance under controlled conditions, excluding escape of infection.

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## Discussion and Conclusions

The laboratory/greenhouse methods described in this paper for screening resistance to soil-borne diseases are mainly based on experience with *Fusarium oxysporum* and *Fusarium solani* of beans and peas. Nevertheless, these methods might also be useful in breeding for resistance to other fungi and in modifying the preparation of inoculum.

Adequate screening is only possible under controlled temperature conditions in artificially infested soil, generally of a low pH and, to some extent, deficient in calcium. Under such conditions a high degree of resistance can be identified. Depending on the available sources of resistance, the pH of the soil has to be changed somewhat and the concentration of the inoculum reduced in order to avoid a "breakdown" of the resistance.

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# Laboratory/Glasshouse Screening Procedures for Identifying Resistance to Soil-borne Diseases of Peas

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The USDA pea-breeding program at the Irrigated Agriculture Research and Extension Center, Prosser, Washington, is primarily directed at combining resistance and/or tolerance to *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (Jones) Snyder & Hans., *Pythium ultimum* Trow, *Aphanomyces euteiches* Drechs., and *Fusarium oxysporum* Schl. f. sp. *pisi* (van Hall) Snyder & Hans. races 1, 2, 5, and 6 (new strain yet to be named).

## *Fusarium solani* f. sp. *pisi*

The most important component of the root rot complex of peas in the Pacific Northwest is *Fusarium solani* f. sp. *pisi* (Burke and Kraft 1974; Kraft and Roberts 1969). In a 1966 report of the USDA Plant Introduction Service (Braverman et al. 1966), 132 accessions were listed as being resistant or tolerant to this pathogen. Of these, 86 were classified as having definite root and epicotyl resistance. In previous studies, we had found that resistance to *F. solani* f. sp. *pisi* in several P.I. accessions was not affected by high soil-inoculum levels (Kraft 1974; Kraft and Roberts 1970), that resistance in epicotyl and root tissue was qualitative (Kraft 1977; Kraft and Roberts 1970), and that it could be observed in as little as 7 days after emergence (Kraft 1974). Based on this information, a technique was devised for rapidly screening and evaluating pea accessions or early genetic crosses for resistance (Kraft 1975).

The inoculum is grown in Kerr's liquid medium (Kerr 1963) for 5 days in a rotary shaker set to make 60 cycles/minute. The inoculum is strained through cheesecloth, centrifuged to remove excess nutrients and staling products, resuspended in distilled water

and, through an atomizer, added directly to soil in a cement mixer. The inoculum in soil is allowed to air-dry for 2 weeks (Burke and Kraft 1974), when conidia convert to chlamyospores, then sieved through a 10-mesh screen and adjusted to 40,000 propagules/gm of air-dry soil.

Seed of test lines is surface disinfested with a 10% solution of florox and rinsed in sterile water; 25 seeds of each line are planted in flats with removable sides. Two lines are planted in each flat, which is filled with an uncropped, Warden fine sandy silt loam soil, artificially infested with *F. solani* f. sp. *pisi* at an inoculum concentration of about 40,000 vp/gm of air-dry soil. The dilution plate technique, first devised by Nash and Snyder (1962), is used to determine inoculum concentration.

The seeded flats are then placed in a growth chamber with a 16-hr day, a 6,480 lux maximum illumination, and a 24° day and an 18 ± 1°C night temperature. The flats are watered with millipore filtered tap water as necessary for disease expression (i.e., flats are allowed to dry to 5-15 atm before re-watering to 1/3 atm) (Kraft and Roberts 1969).

Resultant seedlings are harvested 10-14 days after emergence by removing the sides of each flat, carefully removing each seedling, and washing out each root system. Seedlings are rated using a 0-5 disease index, with 5 indicating a completely rotted root. In the case of early generation crosses, plants with the more healthy root systems (i.e. less epicotyl and root necrosis than Dark Skin Perfection) are transplanted into a fumigated potting mixture consisting of 1 part soil, 1 part sand, and 1 part peat moss. The transplanted seedlings

that survive are grown to seed set. I believe that some of the advantages of this procedure over other screening techniques are: (1) that lines can be evaluated for resistance to *F. solani* f. sp. *pisi* at an early age, which results in a higher percentage of transplants surviving to set seed, (2) the entire root system of a test plant is exposed to a high inoculum level of *F. solani* f. sp. *pisi*, and (3) results are reproducible.

### ***Pythium ultimum***

Diseases of peas caused by *Pythium* spp. are most often referred to as damping-off and seed rot. However, in the Pacific Northwest, *Pythium ultimum* was found to play a role in the root rot complex affecting peas during the entire growing season (Kraft and Burke 1971).

For disease resistance screening trials, inoculum of *P. ultimum* is prepared by growing the fungus on sterile vermiculite to which a complete nutrient solution is added (Kraft and Roberts 1969). The fungus is grown in 500-ml Erlenmeyer flasks with 400 ml vermiculite, autoclaved 4 hrs; to this is added 200 ml nutrient solution and the vermiculite then autoclaved an additional 2 hrs. The inoculum is incubated 7 days in the dark at 20°C. The resultant inoculum is fragmented by forcing through a 10-mesh screen and added to uncropped field soil in a revolving drum. The infested soil is thoroughly mixed and air-dried for 14 days before assaying. Inoculum density of *P. ultimum* is determined by the surface dilution plate technique and selective medium (Mircetich 1970; Mircetich and Kraft 1973). Inoculum level of soil infested with *P. ultimum* is adjusted before each test to equal 1000 propagules/gm air-dried soil.

To eliminate preemergence damping-off and seed rot, all seed is treated with Captan prior to planting. Enough millipore-filtered water to adjust soil moisture tension to 1/3 atmosphere is added at planting time.

Test lines are harvested 2-3 weeks after emergence by carefully removing each plant, washing the root system, and scoring for disease severity using the 0-5 disease index scale. The more resistant segregants in an early generation cross are transplanted into a sterile potting mix and saved for seed.

### ***Aphanomyces euteiches***

Root rot of peas is caused by one or more of several pathogens, of which *Aphanomyces euteiches* Drechs. is perhaps the most important and difficult to control (Hagedorn 1976; Marx et al. 1972). No satisfactory control measures are known. Chemical control is too costly and breeding efforts have been stymied by the obvious lack of clear-cut resistance. Consequently, we have developed our screening and testing procedures for *A. euteiches* to detect low levels of resistance.

All isolates of phycomycetous fungi used in our laboratory, including *A. euteiches* and *P. ultimum*, are maintained on V8 juice agar slants (200 ml V8 juice plus 2 g CaCO<sub>3</sub>/l), covered with sterile mineral oil and kept in the refrigerator.

For disease screening and pathogenicity tests, all test isolates of *A. euteiches* are grown in liquid, still culture, in 50 ml of maltose-pentone broth (Carmen and Lockwood 1960) for 5 days at 28°C. After 5 days, the medium is decanted and replaced with 60 ml of tap water. This is replaced after 2 hrs by 40 ml of distilled water and each flask is then aerated at the rate of about 6 bubbles/second. After 12 hrs, the mycelial mats are removed and zoospore numbers/ml are determined using a haemocytometer. Six-day-old test plants (planted 10 days previously), growing in flats of coarse perlite are inoculated by pipetting 10 ml of zoospores/25 plants/row. Zoospore inoculum concentration is usually in the range of  $1 \times 10^5$ /ml.

Inoculated flats are then incubated at greenhouse temperatures (24 - 26° C)

until symptoms are evident on the susceptible control, Dark Skin Perfection. At this time, each row is carefully removed from the perlite, roots are washed, plants are read for disease severity (0-5 scale), and fresh weights of tops and roots determined. The main criterion for measuring resistance in our tests is the percent loss of fresh weight of roots and tops when compared to the uninoculated control for each test line. Disease severity ratings are usually meaningless as most lines have a high disease severity rating (4-5 class).

### ***Fusarium oxysporum* f. sp. *pisi*** **Races 1, 2, 5, and 6**

Wilt of pea, which is caused by *F. oxysporum* f. sp. *pisi*, race 1, was first described in 1925 (Linford 1928). Resistance to race 1 was attributed to a single, dominant gene factor in the host (Wade 1929). Race 2 was recognized and described when race 1 resistant cultivars were developed and grown to the exclusion of race 1 susceptible cultivars (Snyder 1933). Host resistance to race 2 was again attributed to a separate, dominant gene factor in the host (Hare et al. 1949). Race 3, described in 1951 in the Netherlands, caused wilt on cultivars resistant to races 1 and 2 (Schreuder 1951). However, no description of the differential cultivars used to define race 3 is available, and unavailability of an isolate of the original culture leaves the validity of this race in doubt (Hubbeling 1974). Race 4 was described in 1966 in Canada and was determined on the basis that New Era (resistant to races 1 and 2) was susceptible, and New Wales (resistant to race 1 and 2) was resistant (Bolton et al. 1966). However, it is likely that races 3 and 4 are more virulent cultures of race 2 (Hubbeling 1974). Further, the genetic basis for resistance in the host to races 3 and 4 was not defined. Race 5 was described in 1970, whereby all commercial cultivars known to be resistant to races 1 and 2 were susceptible (Haglund and Kraft 1970).

Resistance again was attributed to yet another single dominant gene (Haglund 1976; Kraft and Giles 1976).

For all pathogenicity and progeny tests using isolates of *F. oxysporum* f. sp. *pisi* at Prosser, cultures are derived from single-spore isolates (Tousson and Nelson 1976), which are increased on fresh PDA under artificial light with a 12-hr photoperiod. Only colonies representative of the wild type (white, restricted aerial mycelial types for races 1, 5, and 6) and colonies forming sporodochia for race 2 are used. For inoculum increase, isolates to be used are hyphal tipped and increased in Kerr's liquid medium; 2 ml of the resultant conidial suspension is placed in 10 g of a sterile soil mix in a test tube, air-dried to induce a dormant chlamydospore state, and stored in the refrigerator.

To produce inoculum of a test isolate, a small amount of infested soil is sprinkled on a PCNB plate (Nash and Snyder 1962) and a resulting colony is selected which is representative of the wild type for that isolate. A small agar plug is cut from the colony margin, after a 5-day incubation period, is placed in 50 ml of sterile Kerr's medium, and incubated an additional 5 days in a rotary shaker (1 cycle/sec.) with 16 hr of fluorescent light at 6,480 lux at  $24 \pm 1^\circ\text{C}$ . At that time, spore concentrations of the test isolate are determined by use of a haemocytometer and adjusted to  $1 \times 10^6$  conidia/ml.

Seed of each test line is surface disinfested with a 10% solution of clorox before planting in coarse, autoclaved perlite. Seedlings are inoculated in the third- to fourth-node stage by carefully removing each plant and pruning the root system—using a razor blade—while it is immersed in a conidial suspension of each isolate. Inoculated seedlings are transplanted back into the perlite and incubated on a greenhouse bench until wilt symptoms are evident and/or known susceptible inoculated controls are dead. Greenhouse temperatures are in the  $18-24^\circ\text{C}$  range. Wilt symptoms consist of stunting, yellowing, dying of

lower leaves, downward curling of leaf margins, and usually death of the plant.

## Summary

Our approach at Prosser has been one of first identifying sources of resistance to the pathogens listed above, using artificially infested soils in the greenhouse and naturally infested field soils, combining these sources of resistance, then rescreening the F<sub>3</sub> - F<sub>4</sub> progeny and saving the more resistant segregants. These segregants are then increased and evaluated in naturally infested field soils and the highest yielding lines, approaching commercial types, are saved to repeat the cycle.

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## Discussion — Session 4

Chairman : D. J. Hagedorn  
Rapporteur : M. P. Haware

- Hagedorn : Dr. Hubbeling, in the greenhouse testing of beans, you were mixing sand and inoculum. Did you not water afterwards?
- Hubbeling : By mixing sand or soil with liquid inoculum, good moisture conditions can be achieved. A little water was added on the top of soil for the first 4 weeks. Let me again emphasize the point I made in my talk about leaf size. In wild types of peas and beans, there are many with small leaves. Breeders too like to have plants with smaller leaves, and shorter internodes as an additional possibility. Such plants need four times more leaves of smaller size (half the length) in order to get the same assimilating surface.
- Wood : Does the leaf area matter? Small or big?
- Hubbeling : That does not matter, but in the field, there is the possibility of stress. It is good to have small leaf types that will not suffer as much stress as bigger leaf types.
- Hagedorn : Pea breeders in the USA have small leaf or leaflet breeding programs. Because of the higher leaf index there is a problem in green pea production. Development of a leaf canopy leads to difficulty in harvesting. There is a problem of *Fusarium* root rot disease. Do we need a leaf canopy to have more moisture? Small-leaflet peas with or without petioles with all kinds of combination are employed for good light penetration. We have similar problems in beans; a leaf canopy developing over the plant causes *Sclerotinia* and *Pythium* problems.
- Wood : What about yield?
- Hagedorn : The yield is quite good with small leaf types. Data are quite comparable.
- Abawi : Dr. Hubbeling, would you like to comment on the procedure that was developed by Wallace and Wilkinson for the evaluation of bean germplasm for resistance to *F. solani* f. sp. *phaseoli*? We are using this procedure at present for screening bean germplasm for resistance to *Rhizoctonia solani*.
- Hubbeling : I would not like to use the combination of two different fungi. In *F. solani*, the leaf area is important. Most resistant bean plants are of very small leaf type with a good root system, able to withstand stress situations for a long time.
- Wood : I see the extensive use of ethanol for isolation. Liquid media are good for *Fusarium* multiplication, for production of maximum spores.
- Kraft : It is a personal choice. For *Pythium*, a liquid medium is good because root surfaces have more sporangia and oospores. I also agree with Dr. Hubbeling that cotton plugs will easily become more contaminated if growth medium

splashes on them when used on incubator flasks in an incubator-shaker. In shake culture, *F. solani* f. sp. *pisi* produces microconidia that give a high percentage of kill.

- Hubbeling : Use of peat moss-sand mixture with pH 5.5 and 1 part of inoculum to 10 parts of such mixture gives 100% kill of susceptible check of peas with *F. oxysporum* f. sp. *pisi*. Our breeders always like to use this low pH in order to prevent escape of susceptibility in the tests.
- Kumar : Dr. Kraft mentioned that race 3 and 4 of *F. oxysporum* f. sp. *pisi* are more virulent cultures of race 2. What criteria are used for determining more virulent cultures?
- Kraft : Race 1 of Fusarium wilt in peas was first described in 1925 in Wisconsin, and resistance was attributed to a single, dominant gene factor in the host. Snyder in 1933 described race 2, when race 1 resistant lines wilted with near-wilt symptoms. Race 3 was described from the Netherlands; however, no type cultures are available. Race 4 was described in 1966 from Canada on the basis that New Era, resistant to races 1 and 2, was susceptible, and New Wales, resistant to race 1 and 2, was resistant. Dr. Hubbeling mentioned that, based on host reaction, races 3 and 4 are more virulent cultures of race 2. Race 5 was described in 1970 from Wisconsin, affecting about 30% acreage. Dr. Haglund and I defined it as a single dominant gene, resembling race 1. The Armstrongs took all cul-  
tures and claimed 11 different races on 27 different hosts, primarily based on degree of virulence. We used their technique, with pure-line and selfed seed and we could not repeat the results.
- Hubbeling : What is the situation in farmers' fields?
- Kraft : It is an interesting story too. We know race 2 is on sandy warm soil, race 1 on heavy clay soil.
- Haware : Are you not getting two to three races from the same locality or field?
- Kraft : Race 2 is aggressive in sandy soils, races 1, 5, and 6 in heavier clay soil. There is one situation where all races are found, but race 6 predominates.
- Haware : Are you screening peas separately against all races or together in the laboratory?
- Kraft : Separately. I like to work with individual cultures. When peas are screened against several isolates, simultaneously, we may end up with inoculum density problems. Also there may be interactions. *Fusarium oxysporum* f. sp. *pisi*, race 1 and 5 are unstable in culture.
- Hagedorn : In the mid-west (Wisconsin) all varieties are resistant to race 1, but race 2 is a problem.
- Hubbeling : In the Netherlands we mostly get less virulent race 2. Race 3 was established as identical to race 2, while race 4 was considered as a virulent race 2. The Armstrongs, with whom I co-operated for some years, did not agree to these races. Their classification of race 3 as race 4 is doubtful.

- Starr : In all this discussion on races, how will Dr. Nene and his group reassemble races, say, in pigeonpea *Phytophthora* or in Fusarium wilt of chickpea; that is, how will they describe races in India?
- Hubbeling : If we have four races, then we need to have a set of differentials that give reaction - (resistant) or + (susceptible) to these races in a clearcut way with any method of testing.
- Hagedorn : This is a very good point. We as a group, having this in mind, recommend studies on races.
- Wood : *F. oxysporum* is a notorious and variable vascular fungus. What steps do we take to ensure stability of isolates? How do you characterize race 2 from 4? Based on host reactions?
- Hubbeling : I called race 4 virulent isolate of race 2, because with race 2 we got only good infection with root damage; good wilting resulted also with race 4, without damage of roots.
- Wood : I suggest you standardize all your conditions to study races.
- Kraft : This suggestion has been made several times. We make isolates from diseased plants; these are single-spored and put in sterile soil for preservation. One has to be careful in detecting colonies of wild types.
- Hubbeling : Why do the new races show up in that part of the world—around northern Washington State? Do you think cultural practices or soil conditions have to do with it?
- Kraft : The pacific Northwest is a hotspot *Fusarium*. Farmers insist on planting peas for several years in succession. They never rotate peas with other crops. Addition of dead plants and heavy clay soil contribute to disease. Disease expression of race 2 is different from race 1, which is seen in patches. Race 5 is the same as race 1. Race 2 culture is a sporodochial type, stable. It is a high-temperature organism, while race 1 has a low temperature optimum, it is not stable and is a mycelial type.
- Abawi : Dr. Hubbeling, would you like to comment on how races might evolve? Is it by mutation or by other means? Also, do you refer to isolates with a different degree of aggressiveness as races?
- Hubbeling : Races are based on differentials. Aggressive types show different degrees of aggressiveness but no difference in differential attack.
- Abawi : This is the point I wanted to clarify. Can the isolates that exhibit a different degree of aggressiveness on the same hosts be appropriately referred to as races?
- Kannaiyan : Dr. Kraft, did you screen in glasshouse in controlled conditions and how did this material perform in the field?
- Kraft : All our lines go to the field from laboratory, greenhouse and/or growth chamber. Usually these lines in the field are tolerant or resistant. In general we have to find tolerance to diseases other than those we are screening against because tolerance in the field means yield in spite of disease.

- Hubbelling : Our plant breeders do not transplant mostly because of unfortunate experiences with transplanting seedlings in the field. This is not correct perhaps. Screening is usually done in glass-house tests with a reserved part of seed of F<sub>3</sub> or further developed lines. Only the homozygous resistant lines are shown in the field.
- Kraft : At 10 to 15 days the root system of pea plants is quite developed, and inoculum is mixed in the soil. Seedling resistance in peas is stable; that is, they are also resistant in the field. After combining these sources of resistance, we rescreen F<sub>3</sub> and F<sub>4</sub> progenies and segregants go in the field for yield trials.
- Kumar : What is the population size you are using for *F. solani* and *F. oxysporum* to identify resistance?
- Kraft : For *F. solani* 25 seeds in F<sub>3</sub> and for *F. oxysporum* 100 seeds in F<sub>4</sub>.
- Kumar : Is resistance quantitative?
- Kraft : One or two seedlings usually are saved.
- Kumar : It has to be dominant.
- Reddy : Dr. Hubbelling mentioned in his text that in soil-borne pathogens genetic variation is rather wide; consequently, resistance is relatively stable. Would you comment please?
- Hubbelling : Variation in declining virulence, so resistance is stable.
- Nene : I will refer to pigeonpea, where most of the lines that stand in the field till the first harvest get infected. Fungus can be detected in the root. Are such genotypes that restrict the fungus in the plant and stand in the field until the first harvest more stable? Are such genotypes better than immune genotypes?
- Hubbelling : We have a population in the soil of avirulent isolates of *Verticillium* in cotton. Penetration of avirulent isolates in the roots prevents infection with virulent isolates. Presence of pathogen in the root cannot be a criterion of susceptibility.
- Haware : How should we define resistance in chickpea wilt: lines that get infected but do not wilt?
- Kraft : Tolerant.
- Purss : If the plant is standing in the field without showing any symptoms, then it is resistant. It happens with *Phytophthora* also.
- Wood : It depends when it gets infected. It does not mean that plant is heavily colonized.
- Nene : In certain lines of pigeonpea no wilt is seen until the first harvest. When the plant is chopped, new growth starts, and we get wilt. That shows that the plant was colonized by the pathogen. I do not like to use vertical or horizontal resistance. What we need is material that will stand in farmers' fields. Nowadays it is called "durable resistance."
- Wood : Yes, it is fashionable to talk about vertical resistance. Many years ago pathogenicity was defined as capacity of a particular group of pathogens to parasitize the host. You can have

groups of isolates, pathogenic or nonpathogenic.

- Abawi : I would agree to the grouping of isolates as to pathogenic and nonpathogenic. Pathogenic isolates still may differ in their level of virulence (or aggressiveness) to the host plant.
- Chohan : About black flies? Are they very common in field condition?
- Hubbeling : Not common in field, but in greenhouse at high temperature 25°C and above, there is a high population of black flies.
- Chohan : We are getting maggots of black flies.
- Kraft : When we grow peas in a high organic potting mix, such as peat moss, black flies are a problem.
- Nene : Our physiologists found that defoliation aggravates the wilt incidence in pigeonpea. Deflowering leads to less wilt incidence. This supplements Dr. Hubbeling's statement on the physiology of the plant and the parasite. Leaf spot diseases in pigeonpea are of low priority, as our physiologists have told us that plants shed 50% leaves to provide normal yield without any adverse effect.
- Kraft : In a leaf disease of wheat, powdery mildew, it has been shown that mildew reduces root growth. Foliar diseases can drastically affect the physiology of the plant.

# **Field Screening**

# Field Screening Procedures for Identifying Resistance to Soil-borne Diseases of Peas

D. J. Hagedorn

One of the most important scientific procedures for the identification of resistance to plant diseases is the use of properly designed field plots. This is true of many studies conducted over the last 50 years on the diseases of peas, *Pisum sativum* L. Many of these experiments have been concerned with soil-borne diseases of this important food crop. This paper will examine this experimentation, especially with regard to the most important root diseases of peas.

## Ascochyta Foot Rot

Early studies on *Ascochyta* foot rot (*Ascochyta pinodella* Jones) were made in New York by L.K. Jones (1927) and in Wisconsin by Linford and Sprague (1927). Jones described the pathogen as a new species at that time and considered that it was seed-borne and could live in the soil for at least 2 years after it was found in a pea crop.

When he studied the reaction of pea cultivars to the disease in the field, three 5-foot-square plots were planted with eight pea cultivars resulting in 25 to 50 plants per plot. Pycnospore inoculum was produced on oat agar slants and washed from 10- 14-day-old cultures that had been incubated at 18°C. Pycnospore-sterile water inoculum was atomized onto the young pea plants in two plots; the third control plot was atomized with water only. The plots were covered with pyramidal canvas covers, which were sprayed continuously for 24 hours to maintain a high moisture content inside. Results showed that the cultivars Advancer, Horsford, Perfection, and Rice's 13 were more tolerant than Green Admiral, Alaska, Carter's Premium Gem, and Surprise.

Gould (1949) reported on screening procedures, including use of field plots, to study the reaction of about 500 pea strains to *A. pinodella*. Macerated cultures grown on agar or barley were made into suspensions of inoculum, which was poured on the pea seeds at planting time. Duplicate or quadruplicate tests were made and repeated at least once for all varieties. Plants were dug up at blossom stage and graded for foot rot severity; an index of infection was then calculated for each pea strain.

Results of these tests made in western Washington indicated that none of the peas could be considered immune, although one of the Shoemaker strains (Acc. No. 27625-2) showed little or no disease. However, too few plants were available for definitive conclusions. Dwarf Gray Sugar was the most promising pea cultivar tested. Other peas that showed some resistance were: VA2, a selection from Weimer's H251-11-E, D'Hollandia, DeGrace White Dwarf, Peulen de Grace, Chang, Horsford Market Garden, and Famous.

Weimer (1947) researched the development of winter peas for disease resistance (including resistance to *A. pinodella*) in the southern U.S. Out of 160 pea strains in the fields studied, Austrian Winter was the most resistant.

## Aphanomyces Root Rot

The most important pea disease in several major production areas is the root rot caused by *Aphanomyces euteiches* Drechs. It was described by Jones and Drechsler (1925) after 5 years of study, mostly in Wisconsin. Practical control measures of the common type have not been discovered. However, Sherwood and Hagedorn (1958) described how the disease could

be avoided through the determination of the root-rot potential of fields to be used for peas. The search for root-rot resistance has been long and thorough, but unsuccessful, even though such studies have been made under a relatively wide range of conditions.

Many of these studies have been made in the field, generally in naturally infested soil. One of the first reports of such investigations was made by Haenseler (1924) working in New Jersey as early as 1922. Forty pea cultivars were planted in a plot where root rot had never been found. Half of each 100-ft. row was inoculated with highly infested soil from a field where severe root rot had been observed. The infested soil was placed in a furrow before planting at a rate of 1 ton per acre. Above-ground symptoms began to show when the peas were at blossom stage, and by pod-fill many plants in inoculated plots were almost dead, while those in uninoculated plots were green. In the inoculated plots 90 to 100% of the roots were slightly to severely rotted. No resistance was found, although the cultivar Acquisition showed slight tolerance.

In another study, Haenseler (1928) inoculated disease-free field soil in the furrow with infested soil at the rate of one-half lb. per ft. before planting, with very good results.

The first known paper on resistance of peas to *A. euteiches* was published by F.R. Jones in 1926. He used three naturally infested field plots to study disease severity on six pea cultivars; one "resistant" and one susceptible cultivar in each of the maturity classes early, medium, and late. The late Black-Eyed Marrowfat cultivar displayed the most "resistance," although this character was considered to be a very small factor in pea production (probably because wilt resistance was a large factor).

Johnson (1953) developed a procedure for preparing artificially infested soil to be used for inoculum in the field. Flats of soil were autoclaved and planted thickly with peas. When the

plants were 2 to 3 inches (5 to 7.5 cm) tall, a zoospore suspension was poured on the soil surface. After 2 weeks, the soil was ready to be used as inoculum.

The zoospore suspension was prepared by first growing the fungus on concentrated cornmeal agar made with coarse meal. A decoction of 12 yellow corn kernels in 100 ml water was prepared, sterilized, cooled, and inoculated with pieces of fungus-supporting agar. This seeded decoction medium was grown 3 to 5 days at about 15 to 22°C before the liquid was poured off the fungus mat and sterile tap water was added. The flasks were then shaken to remove nutrients and the liquid poured off again before fresh sterile water was added. After 6 hours zoospores began to form and in 24 to 30 hours numerous zoospores could be easily found. The process of pouring off the water containing zoospores and adding fresh sterile water could be repeated for several days. Field inoculation with the infested soil from the flats was accomplished by opening furrows, planting the pea seed, and covering the seed in the furrow with a measured amount of soil inoculum—one-third gallon per 18 ft. of row.

Johnson (1953) tested 22 plant introductions (PIs) in such inoculated plots. Disease reaction was based on the degree of root rot in inoculated and noninoculated plants in comparison with the control commercial pea cultivar. Twelve PIs were considered tolerant: 162693, 162910, 164568, 164838, 167205, 167250, 174321, 174322, 174923, 174924, 175227, and 175228. Johnson also tested 285 pea cultivars, breeding stocks, and PIs in an infested field. Two-year studies gave no helpful data because weather conditions were not conducive to disease development.

## Fusarium Root Rot

Fusarium root rot of peas was described by F.R. Jones (1923) as a bothersome disease, caused by the pathogen now known as *Fusarium solani* f. sp. *pisi* (F.R. Jones)

Snyder and Hansen, and occurring in many of the pea-growing areas of the U.S.

In 1960, King et al. reported on their efforts to develop lines of peas resistant to *Fusarium* root rot and wilt. They used two Minnesota field plots: the LeSueur plot was naturally infested; the St. Paul plot was artificially infested by repeatedly inoculating the soil in an undescribed manner. One hundred and fifty pea cultivars, breeding lines, and PIs were grown in both nurseries for 2 years. Each pea line was replicated four times, 25 seeds per replicate. Selections were made on the basis of the number of emerged seedlings that survived to produce seed, as compared with the control cultivar, Perfected Wales. All cultivars were found to be as susceptible as or more susceptible than Perfected Wales. Eight of the 391 PIs showed some resistance and were selected for use as parents in a breeding program; these were: 164417, 164837, 164971, 165577, 165965, 169606, 171816, and 173057. Subsequent testing and retesting of the progenies under these disease pressures indicated that it was possible to develop new peas with a general resistance to *Fusarium* root rot and wilt.

More recently, Kraft and Berry (1972) described the artificial infestation of large field plots with *F. solani* f. sp. *pisi*. Inoculum was prepared by growing the fungus on Kerr's medium in shake culture employing a New Brunswick gyro-rotary shaker, modified to allow preparation of 18 l of inoculum at a time. Inoculum was grown for 1 week at  $24 \pm 1^{\circ}\text{C}$ , and the concentrated conidial suspension that developed was strained through double layers of cheesecloth and stored at  $5^{\circ}\text{C}$  until 90 l had been prepared. Before field application, the inoculum was diluted 1:1 with tap water, the final spore counts being  $6 \times 10^6/\text{ml}$  in 1969 and  $8 \times 10^6/\text{ml}$  in 1971. Seven and a half liters of inoculum were sprayed with a backpack sprayer on the soil surface of each of 24 plots and incorporated to 15 cm with a rototiller. Uninoculated control plots were rototilled first.

Two fields were used, neither of which had grown peas—the first (1969) in Prosser, Wash., and the second (1971) in Uthello, Wash. The 1969 field of Warden silt loam soil had a pH of 6.8; the 1971 field of Shano silt loam had a pH of 7.4. Before inoculation, each field was treated with trifluralin herbicide at the rate of 841 g ai/ha. Incorporation was by cross-discing. *Fusarium*-inoculated and uninoculated plots were replicated four times, with six subplots, each 3.05 X 9.14 m, per replication. Plots were separated by noncropped areas 1.5 m wide.

Planting was done with a grain drill that sowed Perfected Freezer peas at the rate of 283.5 kg/ha in rows 17.8 cm apart. Uninoculated plots were sown first. Ten days after seeding, all plots were ditched for rill irrigation, uninoculated plots first.

Random soil samples were taken before and after inoculation to determine populations of *F. solani* f. sp. *pisi*. Rhizosphere samples were taken 10 days after plant emergence and again at full bloom. These soil samples were air-dried and assayed in dilution plates of the Nash-Snyder medium.

High population levels of the pathogen were found in both inoculated fields. In 1969, plants grown in inoculated plots were severely enough diseased to reduce yields by 30% over uninoculated plants. In 1971, the high levels of inoculum in inoculated plots were insufficient to reduce yields because of unusually favorable conditions for pea production.

## Fusarium Wilt

Fusarium wilt of pea, *Fusarium oxysporum* f. sp. *pisi* (Linf.) race 1 Snyd. and Hans., was first described by Linford (1928) and he subsequently (1929) found that it was widespread in the U.S. It has since been found to be an important disease in many of the pea-growing areas of the world.

Wade (1929) used field plots artificially inoculated with many different cultures of the pathogen to study inheritance of resistance. Resistant plants were entirely resistant and susceptible plants entirely susceptible. No intermediates were found. Horal was the resistant control, and other resistant peas were Green Admiral, Resistant Alaska, Improved Surprise, and Fasciated Sweet.

Walker (1931) exercised an intelligent precaution before using a reportedly highly infested field for important disease reaction studies. Before using the field, he planted the entire plot area with a susceptible pea cultivar, using a grain drill. When the plants grew and disease developed, he could determine with precision disease location and severity. He tested 243 seed samples of the Alaska type, 199 Perfection samples, and 320 other types of peas in duplicate plantings. Thirty-three Alaskas, no Perfections, and 108 other cultivars were classified as resistant. Some cultivars had both resistant and susceptible seed stocks, depending on seed source.

Wade et al. (1938) tested the reaction of 1024 strains of peas (from worldwide sources) to Fusarium wilt in an infested field near Fairfield, Wash., in 1931. Duplicate plantings were made, and 10 seeds of appropriate resistant and susceptible controls were planted every 21st plot. Most varietal reactions were based on the reaction of about 25 plants per pea strain. Resistance was about five times more common than susceptibility, which was more common among cultivars from England than from other countries. No susceptibility was found in cultivars from Ethiopia, believed by some to be the place of origin for *P. sativum*. The wide distribution of resistance and its occurrence in presumably primitive types and in old cultivars (up to 150 years old) led to the conclusion that the gene for resistance is probably older than the gene for susceptibility. Nearly all of the important American cultivars were susceptible,

and resistant biotypes found in susceptible strains were often not typical of other plants in that strain. Dwarf early market garden cultivars with short internodes were all completely susceptible.

Cruickshank (1952) reported on the reaction of peas to Fusarium wilt in infested fields in New Zealand. He classified as resistant: 19 garden cultivars, 10 field peas, 14 canning cultivars, and three sugar peas.

In The Netherlands, Hubbeling (1956) tested the reaction of a substantial number of pea cultivars to Fusarium wilt in a uniformly infested field. His 1956 report listed 101 cultivars as resistant and 97 as susceptible. He later wrote that there was an increase of wilt at low soil pH of 4.8 (1966).

Buxton and Perry (1959) reported on a similar study made in wilt-infested fields in England the year before. Forty-four of the 90 cultivars tested were resistant. In general, their results agreed quite well with those obtained by Hubbeling (1956) and by Cruickshank (1952). However, some striking differences were obtained. For instance, in The Netherlands the following cultivars were very susceptible but were resistant in England: Daisy, Early Perfection, Senator, and Victory Freezer. In addition, Hubbeling found that Caractacus was resistant, but Buxton and Perry considered it to be very susceptible. Similarly, Early Perfection and Kelvedon Wonder were very susceptible in New Zealand but resistant in England, while Kelvedon Standby and Laxton's Progress were resistant in New Zealand but very susceptible in England. Buxton et al. (1960) reported the wilt reaction of 35 additional pea cultivars in infested soil in England: 23 were resistant, four susceptible, and eight very susceptible.

### Near-wilt

The near-wilt disease of pea was described by Snyder and Walker in 1935.

They called it near-wilt because it closely resembled *Fusarium* wilt. The causal fungus is *Fusarium oxysporum* f. *pisi* (Snyder) race 2 Snyder and Hansen. Virgin and Walker (1938) found that practically all pea cultivars that were resistant to wilt were susceptible to near-wilt. Although Rogers K and Horal showed some tolerance, it could not be fixed by inbreeding and was greatly influenced by environment. Happily, one breeding line was found showing good field resistance. It was later called Delwiche Commando and served as a parent in a breeding program designed to incorporate both wilt and near-wilt resistance into a range of canning pea types.

Hare et al. (1949) reported on experimentation that incorporated near-wilt resistance into several types of wilt-resistant canning peas. The inheritance of resistance to near-wilt was also studied in detail. From 1943 through 1946 the evaluation of the breeding lines and their parents was made in a highly infested field near Waupun, Wis. This field had previously been carefully mapped for severity of infection when a commercial pea crop was attempted there. Peas were planted in rows 4 feet apart, about eight seeds per foot of row. In each row, 6 feet of the wilt-resistant, near-wilt susceptible control was planted every 18 feet—the 18 feet containing the test hybrids, strains, and cultivars. Good results were obtained, and the breeding program moved ahead quite well. However, during cool growing seasons, it was difficult to make precise disease evaluations in the field, so a greenhouse technique for determining disease reactions was developed. Hagedorn (1953, 1959) used both the greenhouse technique and field testing in infested soil in the development of wilt and near-wilt resistant cultivars New Era, New Season, and New Wales.

## Race 5 Wilt

In 1970, Haglund and Kraft found a locally important wilt disease of can-

ning and freezing peas being grown in the Skagit valley in the state of Washington. It attacked all of the commonly grown pea cultivars, including those that were resistant to both wilt and near-wilt. The disease was called "race 5 wilt" because the causal fungus was *Fusarium oxysporum* f. *pisi* race 5. Highly infested fields were used for large-scale screening of many pea lines in a search for resistance. (Care was exercised not to use an infested field which also had a high soluble salt problem because wilt symptoms were complicated in such fields.) The search for resistance was successful, and now several new resistant pea lines and even cultivars are available for use in that area. The repeated use of field trials in highly infested soil has been an essential part of this resistance development research.

## Discussion and Conclusions

Considering that soil-borne diseases of pea have been studied by a number of very capable research scientists since the early 1920s, it was surprising to find such a small number of thorough papers on techniques for screening for disease resistance in the field. Most of the authors wrote something like "the tests were made in a highly infested field." Sometimes the number of replicates was not mentioned and often the plot layout with regard to randomization was not indicated. Thus, it is not surprising that a statistical analysis of the data was quite rare. Even so, important discoveries of very meaningful disease resistance in peas have been made; in the case of pea *Fusarium* wilt, for instance, the timely and widespread use of this resistance has led to practical control of this important disease.

We still need research on the screening of peas for disease resistance, because there are several diseases, especially the root rots, that are not being controlled by this means since no high level of resistance has been dis-

covered. Innovative approaches to the research involving field screening for disease resistance would be most welcome. With continued persistent research efforts along these lines, by more well-supported scientists, it seems only logical to believe that in the not too distant future, we will control more important seed-borne diseases of pea through the development of new disease-resistant cultivars.

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# Field Screening Procedures for Identifying Resistance to Soil-borne Diseases of Beans

G. S. Abawi

Over the years, monoculture of beans (*Phaseolus vulgaris* L.) has generally increased the prevalence and severity of diseases caused by soil-borne pathogens. The bean root-rot complex is the major disease of dry and snap beans. It occurs annually wherever the crop is grown throughout the United States and the world. However, considerable yearly variation in the incidence and severity of root rot often is observed within and between fields with a known history of the disease. Many soil and environmental factors—such as soil compaction, moisture, temperature, and plant spacing—are known to influence the incidence and severity of root rots (Burke 1964, 1965a, 1965b, 1968; Burke et al. 1972; Miller and Burke 1975, 1977; Pieczarka and Abawi 1978b). Burke and coworkers (Burke 1968; Burke et al. 1972; Miller and Burke 1975, 1977) have suggested that any soil condition that is unfavorable to vigorous root growth increases *Fusarium* root rot of beans, whereas soil conditions favorable for vigorous root growth tend to reduce root rot damage. Accurate figures on yield losses of beans due to root rot pathogens are generally lacking; estimated annual economic loss reports have ranged from a few percentage points to 100% loss for any bean-growing area, especially for central and western New York State.

Bean root rot can be incited by the fungi *Fusarium solani* (Mart.) Appel and Wr. f. sp. *phaseoli* (Burk.) Snyder and Hans., *Rhizoctonia solani* Kuehn, *Thielaviopsis basicola* (Berk. & Br.) Ferr. and several *Pythium* species. Root knot (*Meloidogyne* spp.), lesion (*Pratylenchus* spp.), and other soil-borne nematodes are known to attack and at times cause severe damage to beans (Zanmeyer and Thomas 1957).

Depending on soil and environmental

conditions, these pathogens may act independently or as a complex in any possible combination. Interactions between soil-borne plant pathogens have been demonstrated to greatly influence disease incidence and severity on many crops (Hendrix and Campbell 1973; Powell 1971), but most reports on bean root rot are concerned primarily with damage caused by individual pathogens (Zanmeyer and Thomas 1957). For example, *Fusarium solani* f. sp. *phaseoli* for many years has been considered the major causal agent of bean root rot in New York State. However, recent research results, field observations, and tests with selective fungicides for the control of bean root rot showed that *Pythium ultimum* Trow plays a major role in the complex causing root rot of beans (Pieczarka and Abawi 1978c). Similar information was earlier reported from Wisconsin (Hoch et al. 1975). In addition, synergism was demonstrated to exist between *Fusarium* and *Pythium* (Pieczarka and Abawi 1978a). Such information is important in the identification of resistant bean germplasm and in breeding resistant cultivars.

In the past, single measures to control soil-borne diseases have been attempted, but with inconsistent or limited success. Seed and soil treatment with certain pesticides, crop rotation, soil amendments, and other treatments have at times improved yield or reduced root rot severity. However, none of these methods has been consistently economical, effective, or adequately understood. Identifying sources of resistance to root rot pathogens has received considerable attention and bean germplasm tolerant to root rot—usually to single pathogen—is available (Dickson and Boettger 1977; Boomstra et al. 1977; Hagedorn and Rand 1975; York et al. 1977; Zanmeyer and Meiners 1975). However, due to many factors, including the low

level of tolerance in parental germplasm and its low heritability of the tolerance—the development of commercially acceptable tolerant cultivars has been difficult and very slow. Root rot tolerant snap bean varieties have not been developed yet and only a few tolerant dry bean varieties, principally to *Fusarium* root rot, have been released recently in the U.S. (Zanmeyer and Meiners 1975). Research aimed at the development of root rot resistant bean cultivars needs to be expanded and intensified, as this appears to be the most promising and lasting measure for reducing damage by root rot pathogens. However, control of soil-borne disease complexes of beans may be most effectively and economically attained only by employing integrated control programs, especially if cultivars with high levels of tolerance to root rot pathogens cannot be developed.

Considerable progress has been made in identifying bean germplasm tolerant to single root rot pathogens under controlled greenhouse or growth chamber conditions. Detailed information on the methodology and procedures employed in determining resistance or susceptibility is available in the literature. The following is a brief summary of the information available on field screening procedures utilized in evaluating bean germplasm for resistance to soil-borne pathogens. Only a few selected references are given in this paper, as no attempt is made here to fully cover the published literature.

## Field Screening Procedures

Field evaluation is the ultimate test in determining the level of tolerance of bean germplasm to soil-borne pathogens. Field testing provides screening under natural fluctuating environmental conditions and in the presence of a variety of interacting microorganisms, both pathogens and nonpathogens. Only under commercial field conditions can the influence of root rot incidence and

severity on the quantity and quality of marketable yield be accurately determined. The extent of hypocotyl or root discoloration and/or reduction of plant growth as obtained in most greenhouse screening procedures may not necessarily correlate with a similar reduction in marketable yield. In addition, field evaluations provide natural plant growth and thus selections for commercially acceptable horticultural characteristics from promising germplasms. Yet detailed information on field screening procedures and test conditions when conducted is lacking. The majority of reports dealing with field evaluation of the response of beans to soil-borne pathogens state that the trial was conducted in commercial or experimental bean fields where severe disease incidence had occurred in recent years. Often, no mention is made of the experimental design, number of replicates, statistical analysis, level and kind of soil-borne pathogens at planting time, soil and environmental conditions during the growing season, cultural practices performed, or soil type. Data on the quantity and quality of marketable yield are rarely provided. However, considerable progress has been made recently, and several excellent individual and institutional programs are now actively involved in extensive field screening and breeding for resistance of beans to soil-borne pathogens. Undoubtedly these programs will improve our field screening procedures and generate the much-needed information and materials in the development of resistant bean cultivars.

Field screening procedures can be divided into four general types according to soil infestation or method of inoculations. These are: (1) naturally infested commercial or experimental plots, (2) artificially infested commercial plots, (3) permanent root rot nurseries, and (4) root rot field microplots, usually of a short duration.

### Naturally Infested Plots

These are the most commonly used field

plots for evaluating bean germplasm tolerance to soil-borne pathogens as well as for studying the effect of cultural practices and chemical treatments on root rot incidence and severity (Burke et al. 1972; Prasad and Weigle 1970; Wallace and Wilkinson 1965). Usually, a commercial bean field where root rot epidemics have repeatedly occurred in recent years is selected. Often, plowing and seedbed preparation is done by the grower or according to commercial recommendations. Seeds of each selection are then hand- or machine-planted, usually in single short rows (1-2 m long) and, when replication is possible, a completely randomized block design is utilized. Data collected include any combination of the following: emergence and stand counts, root rot ratings at different intervals, growth ratings, and yield per plot or per plant. To determine root rot severity ratings, usually 10 to 25 plants per selection are dug up and hypocotyl or root discoloration and rotting is recorded once or twice during the growing season. A variety of schemes have been used to describe root rot severity levels such as slight, moderate, severe, and very severe; often a scale ranging from 0 to 10 is used. The latter severity rating scales are often converted to a Weighted Disease Index score from 0 to 100.

Naturally infested plots provide ideal testing conditions utilizing commercially acceptable practices. They also provide evaluation under naturally fluctuating environmental conditions and in the presence of the natural interacting complexes of soil microorganisms. These plots are the ultimate testing ground for all acceptable bean cultivars and are most appropriate and effective for evaluation of advanced materials with adequate seed supply to plant multiple large-scale plots. Unfortunately, these plots may produce considerable nongenetic variations due to nonuniform distribution of soil-borne pathogens within the plot area and also due to seasonal variation of environmental conditions. Thus, identification of sources of resistance to single soil-borne patho-

gens is difficult in naturally infested fields when several pathogens are operating independently or as a complex. Evaluation in naturally infested fields is especially difficult during the early stages of the screening program, particularly when a single plant selection is desired. Burke and Silbernagel (1965) demonstrated that increasing the seeding rate results in increased severity and uniformity of *Fusarium* root rot in naturally infested fields. They suggested a method where seeds of the desired germplasm are mixed with seeds of a susceptible check variety, 1:4, then planted in rows 55 cm apart with 2.5-5 cm between seeds in the row. The susceptible check variety used had a different seedcoat color; thus it was easy to separate the two cultivars, especially at harvest time. Burke and Silbernagel compared root rot ratings and yields of plants of the test germplasm with those of adjacent interplanted susceptible check plants. They postulated that this method of crowding and interplanting largely removes the effects of field variability.

### **Artificially Infested Field Plots**

This field screening procedure differs from the use of naturally infested plots in only two major points. First, bean fields with no known history or with only light root rot incidence can be used as field plots as well as those with known previous history of severe root rot epidemics. Secondly, it involves annual artificial soil infestation or plant inoculation with a single, or a combination of several root rot pathogens. Each pathogen is grown on an appropriate solid or liquid synthetic medium or on natural (generally autoclaved) host parts. Root rot pathogens have also been increased in steam-treated or in pasteurized soil mixes under controlled conditions. Occasionally, soil heavily infested naturally with bean root rot has been used as a source of inoculum. Solid forms of inoculum are usually added either as a broadcast or band treatment and incorporated in the top 5 to 10 cm

of soil just prior to planting or added to the open furrow after seed drop. Liquid forms of inoculum are usually added as an in-furrow spray at planting time or occasionally after emergence to the hypocotyl tissues near the soil line. *Fusarium* root rot was successfully established in relatively clean soil by coating seeds with blended agar plates of *F. solani* f. sp. *phaseoli* (Baggett and Frazier 1973). After overnight drying, the seeds were planted in rows 1.8 m apart with 4-8 cm between seeds in the row, utilizing a V-belt planter. With this technique, it was possible to follow the development of root rot and demonstrate differences between bean cultivars susceptible and resistant to *Fusarium*.

The advantage of the artificial inoculation procedure is that it provides uniform inoculum of the desired pathogen and undoubtedly reduces escape and field variability. However, the form of inoculum added is, at times, different from the naturally surviving propagules in field soils; this may lead to a severe disease incidence and development and consequent loss of bean germplasm with good field tolerance. Artificial inoculation procedures are also prone to seasonal environmental variation in the introduced pathogen and the resident soil microorganisms.

### Permanent Root Rot Nurseries

This is the most effective and practical field screening procedure, which permits testing of a large number of materials annually in the same complex soil environment. It also allows evaluation and a better correlation of yield potential of promising lines under a variety of fluctuating environmental conditions within a season as well as over several growing seasons. The sick plots at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) are a good example of such nurseries. Wallace and Wilkinson (1965) and coworkers (Bravo et al. 1969; Hassan et al. 1971) have done all their field screening of

beans in a nursery plot that was artificially infested with *F. solani* f. sp. *phaseoli* in 1918. Since then this field has been essentially in a continuous monoculture of beans. Initiation of nursery plots generally involves the introduction of a single or a combination of soil-borne pathogens, often in the form of colonized host tissues. Infected tissues are plowed under and the plot area is repeatedly planted to a highly susceptible variety until a high level of disease incidence is reached. Double cropping in one season is very effective in the rapid buildup of the pathogen(s), which often results in a corresponding increase in disease incidence and severity. Again, disease severity in root rot nurseries is influenced by prevailing weather parameters, especially at planting time and during the early part of the growing season. Yearly variation in root rot severity in the nurseries can be reduced if irrigation water is available for use when needed and if it is possible to adjust planting time to periods favorable for root rot incidence.

### Root Rot Field Microplots

The use of field microplots has been known for a long time; however, their extensive use in elucidating the relationship between population densities of soil-borne pathogens and yield losses has been only recently emphasized (Barker and Olthof 1976; Burke 1968). Field microplots can be of any size or shape. At Geneva, we use unglazed drainage clay tiles 30 cm long and 25 cm in diameter. These tiles are inserted in annually fumigated field soil using a post-hold digger mounted on a tractor. Each tile is then filled with pasteurized or non-treated bean field soil (about 15 kg soil/tile) to which one or more root rot pathogens at the desired population level(s) have been added. Each treatment is replicated a minimum of 10 times in a randomized block design. Root rot incidence and development has been uniform and predictable. Field microplots provide all the advantages of root rot

nurseries and also enable a better understanding and more accurate measurement of the reaction of bean lines with different yield potentials to soil-borne pathogens. This is possible because each line can be grown both in non-infested soil and in soil infested with different levels of the pathogen(s) under the same conditions; thus yields can be indirectly compared with those of the susceptible check cultivars. However, setting up and maintenance of field microplots is highly time-consuming and labor-demanding, and only limited numbers of germplasms can be adequately evaluated annually. Microplots are most suited to determine economic loss threshold levels, interactions between root rot pathogens, effect of soil type on root rot severity, etc.

## Summary

Several field-screening procedures for identifying resistance to soil-borne diseases of beans are available, although they have not been standardized or studied in detail. Establishment of permanent root rot nurseries appears to be the most effective and practical procedure for this purpose. Many soil and environmental factors as well as cultural practices are known to influence the damage potential of soil-borne pathogens to beans. Whenever possible, these factors should be used or manipulated in order to maintain adequate root rot severity and also to reduce seasonal variation in root rot incidence. The ultimate measure of tolerance of any bean germplasm to the soil-borne diseases should be the quantity and quality of marketable yield under commercial growing conditions in infested fields.

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## Discussion — Session 5

Chairman : N. Hubbeling  
Rapporteur : J. Kannaiyan

- Saksena : Dr. Hagedorn, you said that you got good results in the field; could you explain what you meant?
- Hagedorn : We got a good amount of disease in the field, from which we could differentiate clearly between resistant and susceptible cultivars. Both greenhouse and field tests are important for any screening program.
- Starr : Movement of plant parasitic nematodes within the field is very slow. One can expect lot of variations under those circumstances.
- Hagedorn : That is correct, Dr. Starr. It is very important to have more susceptible and resistant controls in appropriate places in the screening nursery. It is better to replicate the treatments at least six or eight times to get good results and be sure, by the end of the season, whether the disease was severe or mild.
- Abawi : Generally, the distribution of soil-borne plant pathogens is uneven and the number of propagules of these pathogens vary significantly within naturally infested fields. Thus, in my opinion, it is difficult to do the initial screening for disease resistance in such plots.
- Chohan : Of all the methods you have mentioned, the permanent root rot nursery is the best, especially when there is so much variation from place to place within a plot. We should standardize this procedure to get uniform and comparable results.
- Abawi : Yes, it is important to establish a uniformly infested sick plot. This might be accomplished by even incorporation of infected plant materials, followed by two to four successive plantings of a highly susceptible variety. Such nurseries should be sampled often to monitor disease incidence and development through the growing season. Also, it might be advisable not to use the nursery for yearly evaluation of different germ-plasms as this may lead to an uneven shift in the population or virulence of the pathogens.
- Wood : I would be concerned about these permanent nurseries. In the first place, you are assuming that they are the same, but if you use those nurseries continuously they can vary from year to year. Secondly, and what concerns me more, is that if you are dealing with a single-gene resistance it does not matter, but if you are dealing with a polygenic resistance, the environment plays an important role.
- Abawi : Your concerns are well justified and point out the importance and the need for continuous monitoring of the population of the introduced pathogens and of the incidence and severity of the diseases that they cause in such nurseries.

- Wood : Why not add inoculum every year?
- Abawi : One advantage of permanent nurseries is that they provide a natural source of initial inoculum, which consists mostly of naturally surviving propagules.
- Nene : In a crop such as pigeonpea it may not be easy to develop these nurseries every year, because it has taken us 4 years to get a plot that we consider as satisfactorily sick. In chickpea we have been fortunate, but 2 years would still be required, so an annual exercise, at least in the two crops that we are dealing with, would not be feasible.
- Abawi : We have had a similar experience in developing a 1-hectare field as a multiple disease nursery for bean root rot. This nursery is in its fifth year, and root rot severity is still only moderate.
- Allen : I wonder what evidence you have with *Fusarium oxysporum* that wilt is caused by mixtures of races under natural conditions? What degree of correspondence is there between field results based on one test with a single race and results from long-term permanent plots?
- Hubbeling : If you are talking about the *Fusarium oxysporum* of peas?
- Wood : Anything.
- Allen : What is happening in the commercial fields? Is there a case for inoculating a mixture of races of *Fusarium*? If not, why not? If you are using the permanent type of plot as George (Abawi) was advocating, to what extent is that a mixture?
- Hagedorn : As I said before, we do not have any evidence that we have more than one race of *Fusarium oxysporum* f. sp. *pisi* in any given field.
- Wood : What is the crucial thing for any screening procedure? You must know what you are screening against.
- Kraft : I have several points in response to Dr. Allen's question. We really never found a mixture of races in the field; one dominates when there is mixed inoculum, especially races of wilt, I am really worried about the procedure, because you can end up with so many infection sites in one particular plant. And are you screening against one pathogen or both pathogens or interaction? I have some indication that you end up with synergistic effects that can break down some resistances.
- Nene : I would like to share our experience on this point. Initially, when we started work in the 1974-75 season in chickpea, we made several isolations from wilted plants in and around this area. Then we compared the pathogenicity of the different isolates. Most of them were pathogenic and the one that appeared to us to be most aggressive in the laboratory conditions was the one we decided to use in the field. So we inoculated the pots with this but we had difficulty in getting pathogenicity. Then we also tried inoculations in the field and we didn't get any disease at all. The point is that when we isolated something that looked very aggressive under natural conditions and spread rapidly,

and used that particular isolate for inoculating the soil, we got excellent results. So what I am saying is we may get pathogenic cultures but one may survive and may have the capacity to survive in that particular agroclimate and may show its effects. Now, even at ICRISAT, in hundreds of isolations that we make every year, we get virtually the same pathogen. We check the resistance in a susceptible cultivar for its identity. So, perhaps it is a competition under local agroclimate that only one isolate may survive.

Abawi : Similar observations have been made with certain isolates of *Verticillium*. Non-pigmented pathogenic variants often are obtained under laboratory conditions; however, they are rarely isolated from soil or from naturally infected plants.

Nene : We had experience with one culture. We were excited at the results we got in the laboratory test with that culture, but results with it in the field were disappointing.

Kraft : It might have lost its capacity to survive in field conditions.

Hubbeling : Could we continue with this *F. oxysporum*?

Abawi : I could comment on our experience concerning the evaluation of bean germplasm for resistance to soil-borne fungal pathogens. We have found that isolates of *Fusarium* and *Rhizoctonia* obtained from naturally infected plants differ considerably in their pathogenicity to beans. Since we are inter-

ested in identifying the sources of highest possible resistance, we usually use the most virulent isolates in our screening. Initial screening is done under greenhouse conditions in pasteurized soils, each infested with a single organism. The second step is to screen in soils infested with multiple disease organisms. Seeds of promising germplasm are increased in the greenhouse or the field, and only then are evaluated under field conditions. Thus far, we have done very limited field testing.

Hubbeling : What fungi are you talking about?

Abawi : *Rhizoctonia solani*, *Fusarium solani*, *Pythium ultimum*, and *Thielaviopsis basicola*.

Hubbeling : I would like to come to *F. oxysporum* first because *F. solani* and *F. oxysporum* are not the same. I think they are quite different in reactions, in the field as well in the laboratory. I think we must discuss *Fusarium oxysporum* first.

Allen : I think it is extremely interesting that you found no susceptibility in peas from Ethiopia. Could you say something about the resistance in those?

Hagedorn : Well, I can quote you from that survey paper. Wade established the fact that resistance in peas to *Fusarium* common wilt was governed by one gene. Now what are the lines used in Ethiopia? It is also interesting to know that the world collection of peas has been studied for other diseases, including *Fusarium solani*, and the Ethiopian lines didn't

show up resistance to those because there was no resistance at all. Now, it may present no problem in Ethiopia, because it is a disease of high-moisture, high-temperature conditions.

Allen : There appears to be no record of *F. oxysporum* in peas from Ethiopia; it is not listed by Stewart and Yirgon (1967).

Hubbeling : Do you know anything about time of the year peas are grown in Ethiopia? Are they grown in winter time at low temperature? I think wilt must show up very much in summer.

Allen : I saw extensive intercropping of peas with *Vicia* bean in Ethiopia in September 1976. It is grown at high altitudes.

Nene : September would be cool; they grow chickpea at that time.

Wood : Why should you assume it is a permanent major gene resistance?

Allen : I am not saying it is major gene. I am saying if you get a situation in which pathogen and host evolve together and the net result is resistance, it is likely to be a durable sort of resistance.

Purss : Let us talk about the durability of single-gene resistance to race 1.

Hagedorn : In the United States, I think the resistance does quite well, especially in the mid-west. I think it does quite well in Europe also.

Allen : Do you have any evidence of race shifts? When I asked you about race mixtures, you

said no. If one race is low in proportions, what effect would alternating genotypes have on prevalence?

Hubbeling : Don't you have evidence that race 1 is still scattered all over the U.S.?

Kraft : We find it all over the place.

Hubbeling : So you should have race 1 and race 5 together?

Kraft : That's not the way it happens, race 1 being the very problem. We find it in the eastern parts of the Washington state and in dryland conditions, whereas the problem back in early 1930 was quite severe. They are still growing some small areas with M-163 type, which is susceptible to race 1 wilt. Last year race 1 showed up again in four or five fields, so it is around, but race 5 has never been found in Eastern Washington. On the other hand, over on the coast of Western Washington, race 1 was the problem back in the 1930. It disappeared because the resistant varieties were grown until race 5 appeared. Now in fields race 5 is a problem. We can grow race 5 resistant lines which are race 1 susceptible for several years and still survive. So the predominant race in the field is race 5. What we don't know is what will happen if we grow a variety resistant to race 5 as a sole crop for 5 to 10 years. Now in Canada, right across the border at Vancouver, British Columbia, the Canadians were importing pea silage from western Washington and the disease has already spread and race 5 is now established there.

- Hagedorn : This MI-63 was susceptible this year, even on my brother's farm. This variety is very widespread; in 4, 10, 12, may be 15 fields in the Mid-west and may be in New York. We haven't heard any complaints about disease in this variety in the Mid-west.
- Kraft : The variety Mini is being grown in Europe and it is very susceptible to race 1.
- Hubbeling : What variety do you grow?
- Kraft : Mini.
- Hubbeling : Calcium-rich soils have not showed up any *Fusarium oxysporum* until now. All calcium-rich soils in Holland never showed wilt. We have race 2 on acid sandy soils only.
- Nene : Am I right, therefore, in concluding that this race prevalence is dependent upon the environment? That is, a certain race prevails at a certain time, depending upon the genotypes and the environment and the other races remain suppressed.
- Wood : Are you talking about pathogens?
- Nene : Yes, *Fusarium oxysporum* as a pathogen.
- Allen : Has anybody actually looked at other variations, such as high temperature tolerance, between two races? This could offer explanation.
- Kraft : I have done this in the greenhouse accidentally, inoculating soil with race 1, and I had two or three crops with good race 1 test and race 2 started showing up. This particular soil is light sandy soil. If you plant a crop in warmer temperature you will find more race 2 eventually. There is a shift from race 1 to race 2.
- Sinclair : I have had this experience in developing varieties resistant to certain fungi. Where do these races come from? We have concluded that resistance is in the population. I just wonder if resistance races are in this population of *Fusarium* spp. rather than being transferred from other areas.
- Hubbeling : Let me comment on this important point: the initiation of new races and the possibility of mutation. I was working one year with Dr. Basu Chaudhary. He is now working in Varanasi, India, and we published together that herbicide used in tomatoes induced a lot of mutation in *Verticillium*. This was quite interesting. Does anyone else know of chemicals used in fields evidently giving rise to alterations in fungi?
- Sinclair : Herbicides will alter host tissue and may change its resistance or susceptibility to fungi by changing the sugar content, by some physiological process or by breaking down the cell wall. Thus a strain of the pathogen that will infect the host under such conditions could become dominant.
- Kraft : On beans the herbicide Eptam was shown to dissolve the cuticle on the hypocotyl and the host becomes susceptible to *Fusarium solani* f. sp. *phaseoli*.
- Sinclair : Do the ICRISAT scientists routinely use herbicides on their plots?
- Nene : Except in plots where herbicide experiments are done, we are using manual labor for weeding.

- Hagedorn : In the case of *Aphanomyces*, the herbicide effect is directly on fungus, because the zoospore flagellum are not developed.
- Kraft : At rates where phytotoxicity occurs (i.e., root pruning) Treflan will protect a race 1 susceptible plant when grown in race 1 infested soil.
- Sinclair : This is a warning to use this herbicide cautiously in screening plots.
- Abawi : At present, we are evaluating the effect of the commercially used bean herbicides (Treflan, Eptam, and Premerge) on the incidence and severity of bean root rot. In 1978, no significant effect on root rot severity or yield was observed when these herbicides were used singly or in combination at the recommended rates.
- Hubbeling : Could we come now to the root rot problem, in particular, *F. solani*?
- Wood : There was a complaint about suppression of nodulation in beans when there was *F. solani* infection.
- Abawi : We rarely observe any nodules on bean plants that show severe root rot symptoms. A graduate student in the Department of Agronomy at Cornell is currently studying the interaction between *Rhizobium* and, I believe, *Pythium* root rot.
- Singh : I think the antagonism between *Fusarium* and *Rhizobium* has already been reported. There is a strong antagonism between these two organisms.
- Wood : This is specific under the conditions I am describing, when the lesions are confined to hypocotyls, with a suppression in nodulation. There might be antagonism in the soil?
- Abawi : I have not observed nor am I sure about the relationship between infection of hypocotyl tissues alone and nodulation.
- Kraft : Burke has shown that a disease-free hypocotyl does not equate to increased yields because *Fusarium* can affect the entire root system.
- Hubbeling : This year we had reasonably high temperatures at the beginning of the season. So we started seeing a lot of *Fusarium solani* in our trials. Later on we got heavy rains and the attack was slowed down. But at the end of the season in September we got hot weather; even most resistant plants showed the disease. Small leaved and late cultivars are better than big leaved and early ones.
- Abawi : Root rot of dry and snap beans is most severe when wet conditions prevail during the early part of the season and then are followed by a dry period. Dry conditions appear to prevent the formation of adventitious roots and thus increase stress on the plant. Data on yield losses in beans due to root rot are generally lacking as there are no standardized procedures for estimating loss. In addition, bean plants that escape infection or show only moderate levels of infection appear to compensate in yield for severely infected plants, and thus make it more difficult to assess losses due to root rot. Actually, in the last few seasons, we have

not been able to correlate final stand counts with yield using commercial seeding rates. Under our conditions, early losses due to seed decay and preemergence damping-off are caused mainly by *Pythium ultimum*.

- Allen : Host ability to compensate for damage, such as we have observed in cowpea in response to *Pythium*, is not strictly a "resistance."
- Kraft : Lot of adventitious roots is advantage; plant can outgrow disease in beans.
- Abawi : It appears that plants with thick stems and vigorous root systems generally show higher levels of tolerance to root rot under field conditions.
- Reddy : That could be due to genotypic superiority. This is perhaps what a breeder experiences when screening efficiently. Our basal knowledge of durable resistance, strong genes for resistance, horizontal resistance, etc. needs to be further developed in order to define the genetic and physiological implications. In fact any farmer without any knowledge is able to see well the superiority of healthy plants in an infested field.

# **Recommendations**

## Recommendations

The program of research on soil-borne pathogens of pigeonpea and chickpea has made remarkably good progress since 1974. The initial decision to concentrate on the use of host resistance as a control measure made it imperative that emphasis be placed on screening techniques. The disease plot and screenhouse work is impressive, both in extent and in its obvious success in developing high disease pressure. The level of resistance evident is encouraging. Dr. Y.L. Nene and his colleagues are to be commended for their efforts, which, in our opinion, have resulted in a well thought out and executed program.

It is important that this screening work be continued both on the Alfisol and Vertisol. It is recommended that both individual and multiple disease nurseries be maintained. Attention will need to be given now to the development of race identification techniques. Multilocational testing is, we understand, currently being developed. This should be encouraged and expanded to represent the diversity of the environments and the pathogens in the semi-arid tropics.

With the successful development of the screening phase of the work, the Consultants group feels that a broader approach should be encouraged. The longterm effective control of soil-borne pathogens requires a detailed understanding of the ecology of the pathogen and the epidemiology of the diseases they cause in different environments. Thus we strongly recommend that ICRISAT initiate work in these areas with the major diseases of pulse crops.

With the development of resistant material from the program it is important that work be initiated on the "whole systems" approach of crop management to minimize disease incidence.

It is important to study stress physiology, especially moisture stress, in relation to disease, and it is considered this work should have a high pri-

ority for the semi-arid tropics.

The need for the ICRISAT program to relate to the small farmer offers an opportunity to study the effects of intercropping and other cropping patterns on disease incidence. Such work should be encouraged.

It is important that studies be initiated on the interactions of the various diseases within the root disease complex in both chickpea and pigeonpea. This has important implications, both in resistance and management control practices.

Several of the pathogens, such as *Rhizoctonia* spp., have an extremely wide host range. Resistance is generally difficult to find for such organisms; consequently studies on the effects of cultural practices on the incidence of disease they cause should be encouraged.

It is inevitable that as this program develops, basic studies will be required from time to time. Personnel from universities should be encouraged to visit ICRISAT to carry out such studies. In other situations it may be appropriate for universities to carry out related studies on behalf of ICRISAT at their own establishments.