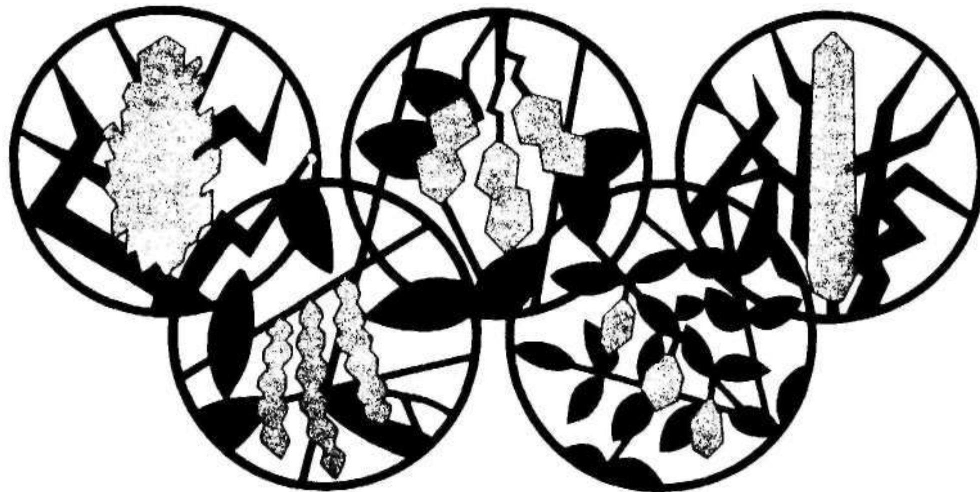
Major Diseases of Groundnut

Compiled by

Faujdar Singh and D.L. Oswalt



Skill Development Series no. 6

ICRISAT

Human Resource Development Program

International Crops Research Institute for the Semi-Arid Tropics
Patancheru, Andhra Pradesh 502 324, India

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Introduction

Disease is an alteration in one or more normal physiological processes, resulting in a loss in utilization of energy in plants. The concept of disease embraces any loss of a plant's ability to function normally or to coordinate the production and utilization of energy. Organisms causing diseases are fungi, bacteria, viruses, and nematodes.

Fungi

Fungi are nonchlorophyllous, nucleated, unicellular, or multicellular filamentous bodies that are reproduced by sexual or asexual spores. Plant pathogenic fungi survive in soil, seed, and weeds. These are dispersed by insects, wind, water, and animals. Fungi live as either saprophytes on dead tissue or as parasites on living tissue. Some fungi are biotrophs, i.e., active only in the living host. The fungal pathogens are classified into two major divisions, Myxomycota and Eumycota (Ainsworth et al. 1973).

Myxomycota. This group includes unicellular fungi that produce Plasmodium or pseudo Plasmodium. These fungi are mostly propagated by motile zoospores formed in Plasmodium. Resting spores are also produced.

Kumycota. This group consists of filamentous fungi. These are classified into five subdivisions based on sexual spores.

Mastigomycotina. They produce asexual spores in sporangia. They include downy mildews and many pathogenic species of the water mold genera. Their mycelium is mostly nonseptate, and the spores liberated from the sporangia may be motile in some cases. Many species cause seed decay and seedling failure of plants that are grown in wet soils. The examples are *Pythium* and *Phytophthora.*

Zygomycotina. These include *Rhizopus* spp. and *Mucor* spp. The sexual spores are zygospores.

Asconycotina. These fungi destroy foliage and parts of plants that are above ground by abundantly producing ascospores on infected foliage that spreads by wind and water. Filamentous ascomycotina produce several spores that spread with splashing rain or by air. It includes powdery mildew and sexual stages of fungi such as *Aspergillus flavus, Cerospora arachidicola,* and *Cercosporidium personatum.*

Basidioaycotina. These include rusts and smuts that are host specific pathogens, and often have a complex life cycle.

Deuteromycotina, or Fungi imperfecti. In these fungi the sexual reproductive stage is unknown or seldom found. This group includes leaf-spotting fungi such as species of *Alternaria, Cercospora, Phoma,* and *Colletotrichum.* The perfect stage, i.e., teleomorph, if found, are usually in ascomycotina, although some **are** in Basidiomycotina.

Bacteria

Bacteria are unicellular, primitive, plant-like, procaryotic organisms, and lack an organized nucleus. Plant pathogenic bacteria are mostly rod shaped, nonmotile, or motile by means of one or more flagella on their body. Plant pathogenic bacteria are usually gram-negative. The bacteria are mainly dispersed through seed, soil, air, and water. Sometimes insects also transmit bacterial cells. Bacterial infection in plant causes specific diseases such as blight, soft rot, leaf spots, tumors, cankers, and vascular wilt.

Viruses

The word 'virus' means poison or the poisonous element by which infection is communicated. The virus can be defined as a transmissible parasite whose nucleic acid genome is less than 3×10^8 daltons in mass and that need ribosomes and other components of their host cells for multiplication (Gibbs and Harrison 1976). Plant viruses are submicroscopic entities showing obligate relationship with living cells of the host and ability to cause specific diseases. The majority of the plant viruses possess the following characteristics (Feakin 1973).

- They are composed of protein and nucleic acid.
- They contain one type of nucleic acid, either RNA, or DNA.
- They multiply only in the host cell (obligate pathogens).
- The RNA and protein subunits are formed separately in the host cell and combine to form intact virus particles.
- Viruses do not possess enzyme systems, required to perform metabolic processes.
- They are transmitted by graft inoculations, by sap, by insects, and by nematodes.

An agent that transmits a virus is called a vector. In many plants, viruses are transmitted under natural condition by insects. Some of them are also transmitted by mechanical sap inoculation. Viruses are also transmitted by seed, especially in the plants of *Leguminosae.*

Nematodes

Nematodes (Greek for thread) are elongate, tubular organisms that move like a snake. These are nonsegmented round worms, sometimes referred to as eelworms or simply nemas. Their body is long, narrow, and the internal organs consists of a set of tubes enclosed by the body wall. The alimentary canal runs directly back from the anterior mouth chamber (stoma) to the posterior anus. The excretory system is a long coiled tube or set of tubes that discharge to the exterior through a duct in the body wall in the anterior third of the body. There are no blood vessels. The nervous system consists of a limited number of nerve cells clustered in a group anteriorly and another posteriorly. Nerve fibers extend along

the body wall. Most, nematodes are circular in cross section. The dorsal (back) region differs from the ventral. The right and left lateral sectors are distinct from either the dorsal or ventral sector (Fig. 1).

The sexes are separate in most nematode species. Females are larger than males. The female gonad consists of one or two elongated tubes. The gonad wall is a single layer of flat cells forming a tube with many distinct regions. The ovary is at the distal end. The region of cell division (germinal zone) contains small cells, called oocytes. These enlarge and in the growth zone accumulate the cellular machinery for embryo formation. The gonad lining changes and the gonad narrows in the next section (oviduct). This connects the ovary to the uterus, a region of enlarged diameter. A pouch-like structure, the spermatheca is situated between the oviduct and uterus. The sperms are stored in spermatheca. From the uterus, a short muscular vagina leads to a ventral opening through the body wall, the vulva (Dropkin 1980). In some species males have lateral cuticular extensions in the tail region, the caudal alae, also called the bursae (Dropkin 1980).

Disease Assessment

To protect a crop from diseases, it is important to know the causal organisms, symptoms, method of infection, and the methods for recording observations and for correct interpretation of data. The quantitative study of damage to plants due to disease is called pathometry or phytopathometry. The pathometric methods are visual assessment, severity relationship (I-S), inoculum disease relationships (I-D), remote sensing or video image analysis (Mayee and Datar 1986). The former three are frequently used for groundnut disease assessment.

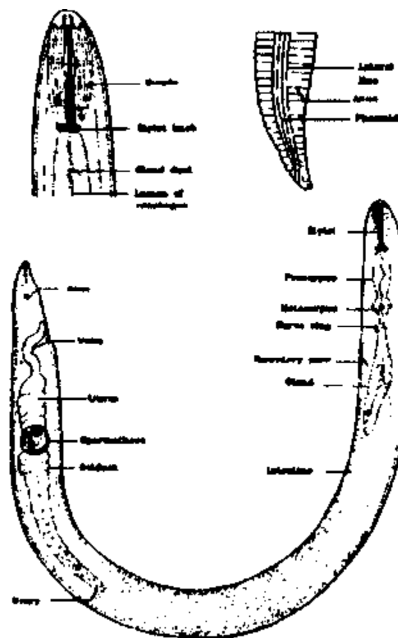

Figure 1. A plant parasitic nematode. (Source: Dropkin 1980)

Diseases of Groundnut

Fungal Diseases

In groundnut, fungi cause seed rots and seedling diseases such as root rot, stem rot, wilts, blight, .pod rot; and foliar diseases such as rust and early and late leaf spots.

Seed rots and seedling diseases. Many soil inhabiting fungi infect and damage the seed and germinating seedlings of groundnut (MP 1 and MP 2). They may be identified by fungal spores that give characteristic colorations to the seed, e.g., gray spores indicate *Rhizopus arrhizus,* black spores are Aspergillus *niger,* and green or blue spores are *Panicillium* sp. The fungus, *Aspergillus flavus,* produces aflatoxin and causes aflaroot of groundnut (MP 3 and MP 4).

Foliar diseases. The major foliar diseases of groundnut caused by fungi are rust *(Puccinia arachidia* Speg.) (MP's 5, 6, and 7), late leaf spot *(Cercosporidium personatum* recently renamed *Phaeoisariopsis peraonata* Berk & Curt), and early leaf spot (*Cercospora arachidicola* Hori) (MP's 8, 9, and 10). Rust and late leaf spot are important diseases in India and most of the semi-arid tropic (SAT) regions. Early leaf spot is an important disease in Africa, particularly in southern Africa.

Bacterial Disease

Bacterial wilt of groundnut caused by *Pseudomonas solanacearum* E.F. Sm. was first reported from Indonesia (1905) and later in Georgia, USA (1931). Presently, this disease is distributed worldwide in cultivated areas of groundnut including Asia and Africa (MP 15).

Virus Diseases

The major virus diseases of groundnut are bud necrosis, clump, rosette, peanut stripe, and peanut mottle (MP 11-14). Peanut clump virus is transmitted by the fungus *Polymyxa graminis;* chlorotic and green rosette viruses are transmitted by aphids; bud necrosis virus causing bud necrosis disease is transmitted by thrips. Peanut mottle and peanut stripe viruses are transmitted by aphids.

Nematode Diseases

Several nematodes are parasitic to groundnut. These are root-knot nematodes *(Meloidogyne arenaria, Meloidogyne hapla, Meloidogyne javanica),* root lesion nematode *(Pratylenchus brachyurus),* ring nematode *(Macroposthonia ornata),* sting nematode *(Belonolaimus longicaudatus),* and testa nematode *(Aphelenchoides arachidis)* in MP's 16—21.

MP 1. Seed Rots and Seedling Diseases of Groundnut

The important fungi-causing seed rots and seedling diseases, and their symptoms are given in Table 1 (Subrahmanyam and Ravindranath 1988).

Table 1. Pungi causing seed rots and seedling diseases of groundnut.

Fungi	Disease	Symptoms
Aspergillus flavus	Aflaroot or yellow mold	Affected seeds are shriveled and dried, covered by yellow or greenish spores. Cotyledons show necrotic lesions with reddish brown margins. Seedlings are highly stunted, leaf size greatly reduced, with pale to light green color.
Aspergillus niger	Crown rot or collar rot	Germinating seeds are covered with masses of black conidia, rapid drying of plants. Later, whole collar region becomes shaded and dark brown.
Fusarium solani and *Fusarium oxysporum*	Wilt	Lower end of tap root becomes brown to reddish brown. Secondary roots become brown and brittle. Leaves turn grayish green and plants dry.
Rhizopus arrhizus and *sclerotium rolfsii*	Seed and seedling rot, stem rot	Sudden wilting of lateral branches that are completely or partially in contact with soil. White coating of fungus mycelium on affected plants.
Rhizoctonia solani	Root rot, pod break down, wilt	Preemergence death of seedlings; shrunken, elongate dark brown areas on on the hypocotyl. The decayed areas are covered with light-brown mycelium.
Pythium ultimum and *Pythium myriotylum*	Damping off	Soft rot of the hypocotyl region causing damping off.
Verticillum alboatrum	Vascular wilt	Wilting of leaflets and petioles, leaflets are curled and chlorotic.

Control Measures

- o Follow a crop rotation, i.e., cereal-cereal-groundnut.
- o Sow good quality and disease-free seed.
- o Avoid damage to the seed testa and deep placement of seed at sowing.
- o Treat the seed with thiram e 3 g kg^{-1} seeds or with carbendazim % *2* g kg^{-1} seeds.

MP 2. Groundnut Pod Rots

Most of the pod rots are due to the combined attack of several fungi. Important pathogens of the pod rot complex are *Fusarium solani, F. oxysporum, Macrophomina phaseolina, Rhizoctonia solani, Sclerotium rolfsii,* and *Aspergillus niger.*

The occurrence of pod rots may be severe due to attacks of nematodes, termites, and millipedes on pods, thus predisposing the invasion by fungi and bacteria (Reddy and McDonald 1983).

Control Measures

- Use cereal-cereal-groundnut crop rotation and seed treatment with thiram.
- Avoid drought at pod formation and maturity.
- Avoid heavy irrigation before harvesting. It will lead to pod rot if harvesting is delayed.
- Grow varieties tolerant to stem and pod rots: ICGV 87157, or ICGV 86590.

MP 3. Groundnut Yellow Mold

The yellow mold fungus, *Aspergillus flavus,* is commonly found in the seed of both rotten and apparently healthy pods of groundnut. Many strains of this fungus are capable of producing aflatoxins that render the seed unacceptable due to high toxicity for human or animal consumption (Reddy and McDonald 1983). Aflatoxin contamination in groundnut can occur in the stems of seedlings, pods, and seeds. The fungus is capable of invading groundnut seeds before harvest, during postharvest drying, and during storage.

The A. *flavus* groups of fungi are facultative parasites. They invade plant tissues directly or attack tissues that have been predisposed by environmental stresses such as dry weather or damages caused by insects, nematodes, natural cracking, and harvest equipment (Pettit 1984).

Aflatoxins are carcinogenic and produced by the *Aspergillus flavus* group of fungi that have been identified as B_1, B_2, G_1, and G_2. The maximum aflatoxin level for groundnut acceptable in USA is 20 ppb (Pettit 1984).

Symptoms

Yellow mold first appears on groundnut cotyledons after the emergence of seedlings. Necrotic spots become covered with masses of yellow-green spore heads of the A. *flavus* group of fungi. Fungus toxins are

translocated throughout the seedling in the transpiration stream. Infected plants generally become stunted with symptoms of vein clearing chlorosis on the leaflets. Such seedlings lack a secondary root system, a condition known as "aflaroot." Yellow-green *Aspergillus* colonies develop on overmature and damaged seeds and pods.

Control Measures

- o Harvest at proper maturity and discard the wilted and dead plants as such plants are likely to have seeds infected by *Aspergillus flavus.*
- o Dry the groundnut pods to 6-8% moisture content immediately after harvesting and discard the infected pods and seeds.
- o Avoid damage to the testa while decorticating.
- o Prevent drought stress, and also prevent water logging (40-80% of field capacity) at late stages of growth.
- o Control nematode because they may predispose plants to attack by fungal pathogens.

MP 4. Determination of Seed Resistance to Colonization by *Aspergillus flavus*

To determine the invasion and colonization by yellow mold *(Aspergillus flavus)* in groundnut, Mehan and McDonald (1983) suggested the following procedure:

1. Take nondamaged, mature pods from each plot to provide:
   a. 50 g seeds for moisture determination, and
   b. 20 g seeds for the colonization test.

2. Use sample 'a' for moisture determination.

3. Place 20 g seeds (sample 'b') in a clean beaker with sufficient aqueous solution of sodium hypochlorite (0.5%) to rover the seeds. Soak seeds for 2 min, drain off excess solution, then rinse seeds in two changes of distilled water. Drain-off water.

4. Hydrate seeds to about 20% moisture content by soaking them for 10-15 min in distilled water. The exact time of soaking is determined by the initial moisture content of the seeds.

5. Place seeds aseptically in a sterile petri dish (9 cm diameter) and apply 1 mL of a suspension of spores of a toxigenic strain of *A. flavus* (4 x 10' conidia mL^{-1}) in distilled water. Now roll the seeds gently around the dish to spread the inoculum evenly over their surfaces. The spore suspension should be prepared from 8-10 day-old culture.

6. The petri dishes are arranged over water in semirigid plastic boxes provided with close fitting lids. Seal the lids with cellotape and place the boxes in an incubator at 25°C for 8 days.

7. Remove boxes and petri dishes after 8 days of incubation. Examine seeds under a stereoscopic-microscope for infection and colonization by *A. flavua.* This should be done with the material in a laminar flow hood or in a chamber with the air voided to the outside. It is necessary to wear a surgical mask and gloves while recording the data.

8. The observations recorded are:
   a. Number of seeds petri dish^{-1} (A)
   b. Number of seeds with sporulating growths of *A. flavus* on their surfaces (B)
   c. Percentage of seeds invaded = $\frac{B \times 100}{A}$

9. To classify the level of resistance to invasion and colonization by A. *flavus,* use the following criteria:
   a. Resistant = Sporulating growth on less than 15% of the seeds, with growth and sporulation sparse.
   b. Moderately resistant = Sporulating growth on 16-30% of seeds, sporulation moderate to dense.
   c. Susceptible = Sporulating growth on 31-50% of seeds, sporulation dense.
   d. Highly susceptible = Sporulating growth on over 50% of seeds with dense growth and sporulation.

MP 5. Groundnut Rust

Symptoms

Rust (*Puccinia arachidis)* is identified by the appearance of orange pustules (uredinia) on the abaxial (lower) surface of leaves and reddish-brown urediniospores (uredospores). Symptoms are mainly confined to leaflets but pustules can be seen on all the aerial parts of a plant except the flower. Brown to dark reddish-brown pustules appear on the lower surface with the upper surface developing yellow, chlorotic spots with necrotic brown areas in the center. At a late stage, the primary pustules are surrounded by secondary sori. The uredinias are usually circular, 0.3 mm– 1.0 mm in diameter, and can develop on all the aerial parts of the plant except flowers and pegs.

Control Measures

- A cereal-cereal-groundnut crop rotation and removal of volunteer groundnut plants from the field will help to check rust inoculum build-up.

- Adjust the sowing time to avoid the most conducive environmental condition for rust development (i.e., high humidity, cloudy weather) to help reduce damage caused by rust.

- Sprays of bordeaux mixture and dithiocarbamate have been found effective to control rust and late leaf spots. Chlorothalonil 0.2% spray has been found effective against rust and late leaf spot, when sprayed 30 days after germination till 15 days before harvesting at regular 10-15 day intervals. However, this schedule could be modified using a suitable disease forecast system based on temperature, humidity, cloudy weather, and rainfall pattern to save the fungicide and reduce the spray cost. Calixin is effective against rust but not against leaf spots, whereas benomyl is effective against leaf spots but not against rust (Subrahmanyam et al. 1984).

- Grow resistant cultivars: ICGV 87160 or ICGV 86590.

MP 6. Rust Screening in the Field

The screening of genotypes to assess rust resistance in the field is done at two stages (Subrahmanyam et al. 1980).

Preliminary screening. Preliminary screening is carried out in nonreplicated plots (1 row, spaced 60-cm apart and 4-m long) at ICRISAT Center. The highly susceptible standard checks are grown as infector rows throughout the field with every one to four rows of the test genotype. Each test genotype is scored using a 1-9 scale one week before harvesting. Genotypes scored 1 to 5 are selected for advanced screening and genotypes with scores between 6 to 9 are discarded. The description of the disease scale is in Table 2.

Table 2. Description of groundnut rust scoring scale (1 to 9).

Score	Description	Disease severity (%)
1	No disease.	0
2	Lesions sparsely distributed largely at lower leaves.	1- 5
3	Many lesions on lower leaves, necrosis evident; very few lesions on middle leaves.	6- 10
4	Numerous lesions present on lower and middle leaves; severe necrosis on lower leaves.	11- 20
5	Severe necrosis of lower and middle leaves; lesions may be on top leaves but less severe.	21- 30
6	Extensive damage to lower leaves. Lesions densely present on middle leaves with necrosis; lesions also on top leaves.	31- 40
7	Severe damage to lower and middle leaves; lesions densely distributed on top leaves.	41- 60
8	100% damage to lower and middle leaves; lesions on top leaves with severe necrosis.	61- 80
9	Almost all leaves withering; bare stems present.	81-100

Advanced screening. Advance screening is done by growing genotypes in replicated plots (at least three replications) of the same plot size as in preliminary screening plots. Each test plot is separated by an infector row that is a mixture of susceptible genotypes. The infector rows are sown 2 weeks before the test material. Infector rows are inoculated with a urediniospore suspension at flowering, using the artificially inoculated potted 'spreader' plants. Such potted plants are placed throughout the field to serve as an additional source of inoculum. After inoculation, the field is irrigated with a *purfo* irrigation system, on alternate days or as required until harvest. The observations are recorded using the 1–9 scale (Fig. 2).

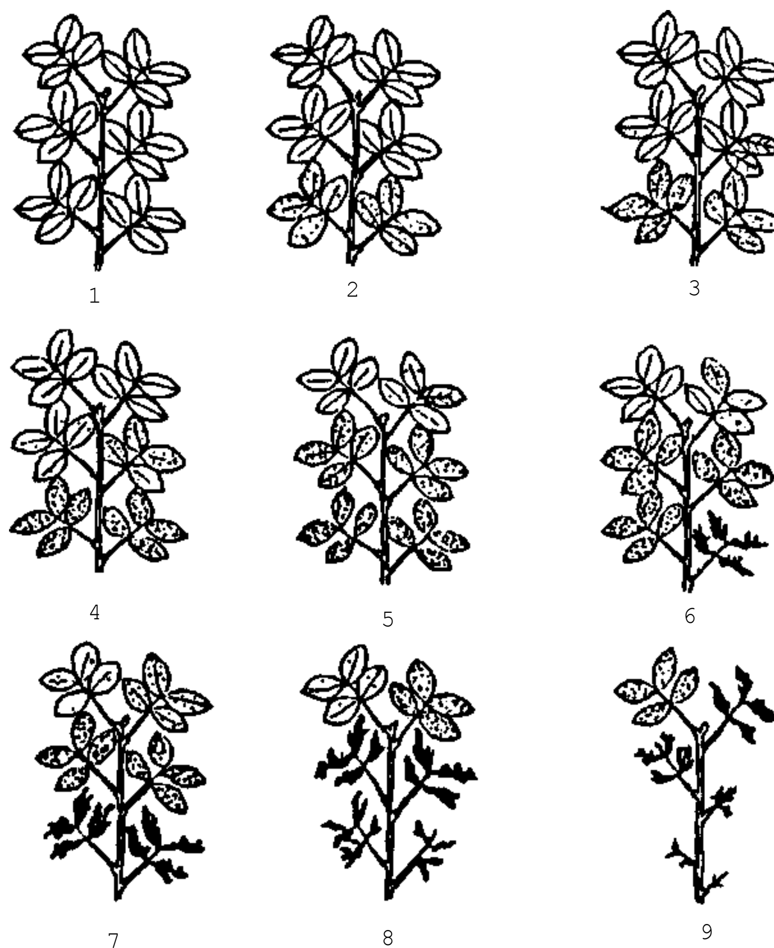

Figure 2. Diagrams of rust damages (1–9 scale) on groundnut plants. (Source: P. Subrahmanyam, ICRISAT, personal communication 1990)

MP 7. Rust Inoculation in the Laboratory

Rust inoculation in the laboratory is done using the following procedures:

1. Rust spores from infected leaves are collected in a glass tube with the help of a 'cyclone spore collector'.

2. The collected spores are suspended into distilled water with a few drops (1 mL lot^{-1}) of wetting agent (Tween 80). Complete suspension is made either by magnetic stirring or by manual shaking.

3. Inoculum is adjusted to approximately 50 000 spores mL^{-1} of solution. The spores are counted with the help of a 'Hemocytometer'.

4. The inoculum is sprayed on the lower side of leaves using a plastic atomizer.

5. The inoculated leaves are arranged with their petioles buried in moist river sand in a plastic tray covered with a polyethylene bag with sufficient moisture. They are kept in an incubator in the dark initially and later for a 12 h photoperiod at 25°C. The disease symptoms will start developing after 7 days.

This procedure can be carried out on single plants grown in small pots as well as on rooted detached leaves. The detached leaves are arranged in a tray with river sand supplemented with Hogland solution. The percentage infection could be estimated using the leaf diagrams depicting known percentages of damage caused by rust (Fig. 3).

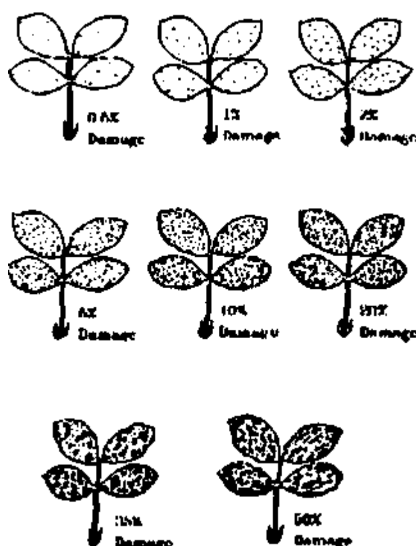

Figure 3. Rust reaction (%) on groundnut leaves.
(Source: P. Subrahmanyam, ICRISAT, personal communication 1990)

MP 8. Early and Late Leaf Spots of Groundnut

Symptoms

Early leaf spot. It is caused by *Cercospora arachidicola* Hori. It develops small necrotic flecks, that usually have light to dark-brown centers, and a yellow halo. The spots may range from 1 mm—10 mm in diameter. Sporulation is on the adaxial (upper) surface of leaflets.

Late leaf spot. It is caused by *Phaeoisariopsis personata* (Berk & Curt) V. Arx. It develops small necrotic flecks that enlarge and become light to dark brown. The yellow halo is either absent or less conspicuous in late leaf spot. Sporulation is common on the abaxial (lower) surface of leaves. Comparisons of early and late leaf spots are in Table 3.

Table 3. The comparisons of early and lata leaf spot of groundnut.

Character	Early leaf spot	Late leaf spot
Stage of occurrence	Early infection	Usually late infection
Shape of spot	Circular to irregular	Usually circular
Leaf surface on which most spores are produced and their arrangement '	Upper surface, random	Lower surface, in concentric rings
Color of spot on upper leaf surface	Light brown to black, tending towards brown with some yellow halo	Brown to black, tending towards black
Color of spot on lower leaf surface[1]	Brown	Black

' Important distinguishing features.

Control Measures

- A crop rotation of cereal-cereal-groundnut and burying all groundnut crop residues by deep plowing will reduce initial inoculum. Adjust the date of sowing to avoid conditions favorable for rapid disease development.
- Multiple applications of a fungicide such as benomyl, captafol, chlorothalonil, copper hydroxide, mancozeb, or sulphur fungicides may control early and late leaf spot (Smith 1984). However, carbendazim (0.05%) controls both leaf spots very effectively.
- Three sprays of 0.2% chlorothalonil at intervals of 10—15 days starting 40 days after germination up to 90 days provides effective control to early and late leaf spots, and rust.
- Grow cultivars tolerant to late leaf spot: ICGV 87160 or ICGV 86590.

The fungicidal control of diseases is more effective if a disease forecasting system based on temperature and relative humidity during the growing season, as has been developed in Georgia, is used. The fungicides are applied when the temperature and leaf wetness conditions are favorable for disease development. Indiscriminate application of fungicides to control early and late leaf spots results in nondesirable effects. For example, use of excessive chlorothalonil for control of foliar diseases increases the severity of *Sclerotinia* blight (Smith 1984).

MP 9. Screening for Early and Late Leaf Spots

The preliminary and advanced screening methodology discussed for rust is also applicable for screening of leaf spots. Normally, leaf spots are scored on the 1 to 9 scale (Table 4 and Fig. 3).

Table 4. Description of leaf spots scale (1–9).

Leaf spot score	Description	Disease severity (%)
1	No disease	0
2	Lesions largely on lower leaves; no defoliation.	1- 5
3	Lesions largely on lower leaves; very few lesions on middle leaves; defoliation of some leaflets evident on lower leaves.	6- 10
4	Lesions on lower and middle leaves, but severe on lower leaves; defoliation of some leaflets evident on lower leaves.	11- 20
5	Lesions on all lower and middle leaves; over 50% defoliation of lower leaves.	21- 30
6	Lesions severe on lower and middle leaves; lesions on top leaves but less severe; extensive defoliation of lower leaves; defoliation of some leaflets evident on middle leaves.	31- 40
7	Lesions on all leaves but less severe on top leaves; defoliation of all lower and some middle leaves.	41- 60
8	Defoliation of all lower and middle leaves; lesions severe on top leaves and some defoliation of top leaves evident.	61- 80
9	Defoliation of almost all leaves leaving bare stems; some leaflets may be present, but with severe leaf spots.	81-100

Subrahmanyam et al. (1980) discussed the procedure for screening for the leaf spot diseases in the greenhouse. Plants are grown in plastic pots (15-cm diameter) containing a mixture of soil and farmyard manure (4:1 v/v). In each pot, two plants are grown.

Inoculum (conidia) of *Phaeoisariopsis personata* (late leaf spot) is collected from incubated, infected, and detached leaves of the susceptible cultivars. Conidia are suspended in sterile tap water

containing a few drops of wetting agent, 'Tween 80'. Before spraying the inoculum concentration is adjusted to approximately 50 000 conidia mL^{-1} of solution.

Inoculation is carried out, first when seedlings are 30-day old and the second time when plants are 50-day old.

Disease development is recorded at 28 and 42 days after inoculation to evaluate the following parameters:

a. **Defoliation.** The number of leaflets on the main stem and the number of abscised leaves are counted on each plant to calculate defoliation percentage.

b. **Leaf area damage.** It is estimated for each leaf on the main stem in comparison to the diagram depicting the known percentage of the area affected (Fig. 5).

c. **Infection frequency.** The number of lesions on each leaf of the main stem is counted 28 days after inoculation. The leaf area is estimated using a leaf area meter. Infection frequencies are reported as number of lesions cm^{-2} of leaf area.

d. **Lesion diameter.** The diameter of 10 randomly selected lesions are measured on the leaves of the main stem.

e. **Sporulation.** Five leaflets are taken from each main stem 42 days after inoculation and incubated on moist filter paper in petri dishes at 25°C under continuous illumination in a percival plant growth chamber for 5 days. On the 6th day, the lesions are examined under a stereoscopic-microscope (x20) to score the degree of sporulation on a 5-point scale.

f. Subject the percentage value of recorded data to arcsine/angular transformation for analysis.

g. **Rating description (5-point scale).**
   1 No sporulation
   2 Very few spores
   3 Moderate sporulation
   4 More sporulation than score 3
   5 Extensive sporulation

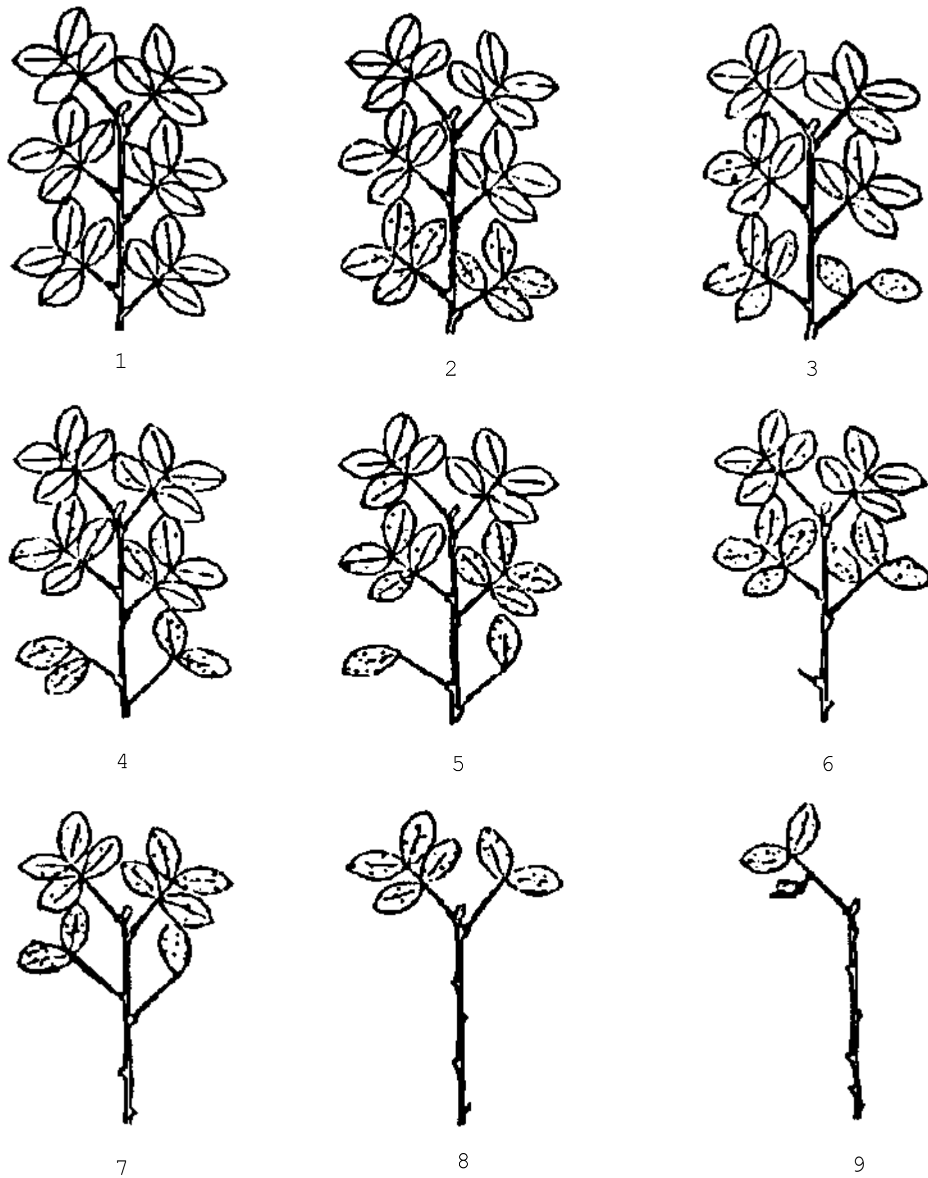

Figure 4. Leaf spots damage (1 to 9 scale) on groundnut plants.
(Source: P. Subrahmanyam, ICRISAT, personal communication 1990)

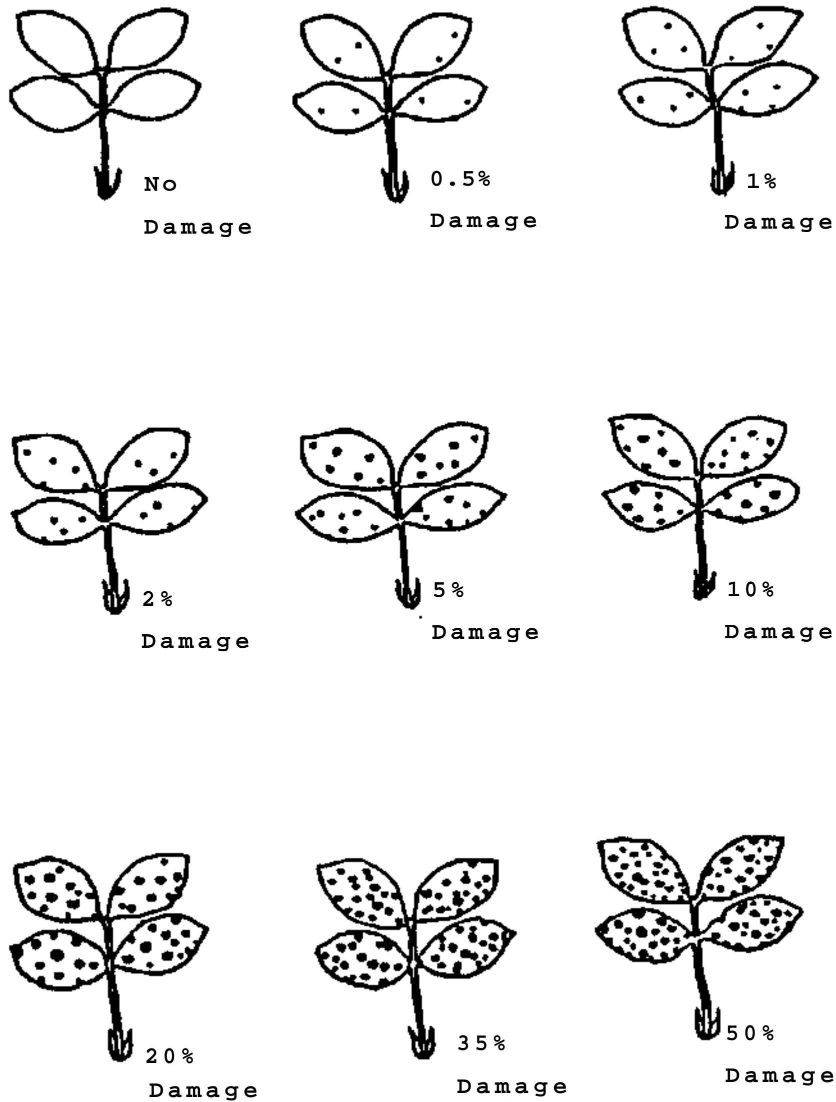

Figure 5. Leaf area damage (%) caused by leaf spots on groundnut. (Source: P. Subrahmanyam, ICRISAT, personal communication 1990)

MP 10. Use of Cob's Diagram for Percentage Leaf Area Damage by Rust and Early and Late Leaf Spots

A 1—9 scale is used for scoring foliar diseases of groundnut as it saves time. To study the disease development and to assess the efficacy of chemical applied, the Cob's diagram and percentage damage of leaves are considered. The diagram for scoring rust and leaf spots in field conditions can be used as follows:

1. The scoring for leaf area damage (%) is done 40-45 days after plant emergence at intervals of 10 or 15 days on separate data sheets (Annexures 1 and 2).
2. Five to ten plants are randomly selected and labeled plot"[1] for each genotype in each replication.
3. Observations of defoliation are taken on the main axis starting at the first leaf node and moving upward.
4. The damage caused by each disease (rust or leaf spot) is recorded using the diagram in Fig. 2 or Fig. 4. The data are converted to calculate percentage damage for each leaflet. In this way, complete data are recorded on 5 plants giving percentage damage on each leaflet at regular intervals of 15 days.
5. Mean disease damage (%) based on the individual leaf infection is calculated.

Example. The following data were taken from an experiment. There were eight treatments with three replications where rust (R), late leaf spot (LS) and percentage defoliation (DEF) data were recorded (Table 5).

Table 5. The observations recorded for treatment 2 of replication 1, plot 1.

	Leaf area damage (%)		
Leaf no.	Rust	Late leaf spot	Defoliation (%)
1	—	—	100
2	—	—	100
3	—	—	100
4	—	—	100
5	50	5	-
6	50	4	-
7	50	3	—
8	50	-	-
9	40	—	-
10	20	—	-
11	10	—	—
12	5	—	—
Mean	23%	1%	400/12 = 33%

Explanation. The data showed that the first four leaves were defoliated on most plants. The average rust infection on the remaining plants is 23%; while the mean for late leaf spot infection is only 1%. In the same way, the mean of each treatment can be calculated and the transformed value could be subjected to analysis and further interpretation.

MP 11. Bud Necrosis

Bud necrosis disease (BND) is caused by two serologically distinct viruses, bud necrosis virus (BNV) and tomato spotted wilt virus (TSWV). BND was first recorded in Brazil in 1941, and significant crop losses by this disease have been reported from Australia, India, and the USA (Reddy 1984a).

Symptoms

Initial symptoms are concentric rings or chlorotic spots on young leaflets. Subsequently terminal bud necrosis occurs especially when day temperatures exceed 30°C. Plants infected at early stages are severely stunted. Occasionally, necrosis may spread to the petioles and then to the stem leading to death of the plant. Later infected plants may only show bud necrosis on a few branches and axillary shoot proliferations may be restricted to the terminal portion (Reddy et al. 1991).

In early infection, pods are seldom produced. In late infections, pod size is reduced, shriveled, and mottled with discolored testa. The virus is not transmitted by seed; it is transmitted by thrips.

Control Measures

- Use resistant/tolerant cultivars: ICGS 11, ICGS 44, ICGV 87141, ICGV 87187, ICGV 87119, ICGV 87121, ICGV 87160, ICGV 87157, or ICGV 86590.
- Control of vector (thrips).
- Adjust date of sowing to avoid the peak disease incidence.
- Sow groundnut at a high plant density and maintain a good plant stand.
- Intercropping of groundnut with cereals, i.e., pearl millet will restrict spread of the virus.
- Avoid groundnut cultivation adjacent to the crops that are susceptible to BNV, such as green gram or black gram.

MP 12. Groundnut Rosette

Three rosette diseases have been recognized. They are "groundnut chlorotic rosette" (GCR), "groundnut green rosette" (GGR), and "groundnut mosaic rosette" (GMR). GCR and GMR are predominant in eastern and southern Africa, whereas GGR appears to be restricted to western Africa (Reddy 1984b).

Symptoms

Groundnut chlorotic rosette (GCR) is characterized by general chlorosis, with a few green islands on young leaflets. Early infected plants are stunted, progressively producing small chlorotic, curled, and puckered leaflets. Older leaflets are bright-yellow with dark-green patches. Plants infected late, show typical leaf symptoms without the marked stunting and bushy appearance (Reddy 1984b).

Groundnut green rosette (GGR) infected plants show mild and narrow chlorotic streaks on young leaflets. The older leaflets are dark-green and reduced in size with their margins rolled outward. Early infected plants are stunted and bushy, whereas on late infected plants a proliferation of axillary shoots may be observed (Reddy 1984b).

Control Measures

- Several long-duration cultivars with resistance to rosette are currently available. These include RG 1, RMP 12, RMP 91, KH 14-9 A, M 25-M 68, and M 69- M 101. Short duration rosette resistant cultivars are being developed.

- Aphis craccivora is mainly responsible for the spread of rosette disease. Spray of endosulfan 4% dust with 1 kg a.i. ha'^{1} or demeton-s-methyl 72-96 mL a.i. ha^{-1} provide effective control for aphids. It is essential to know the peak period of aphid migration before application of insecticides.

- The eradication of volunteer groundnut plants is helpful to prevent perpetuation of virus inoculum during the off-season.

- Early sowing and maintenance of a good plant stand are helpful in reducing the disease incidence.

MP 13. Peanut Clump

Peanut clump is caused by peanut clump virus (PCV). Early-infected plants do not produce pods. Even in late-infected plants, yield losses of up to 60% have been observed.

Symptoms

Affected plants are severely stunted, and the new quadrifoliates exhibit mosaic mottling and chlorotic rings. Subsequently produced leaflets tur dark green with faint mottling. Infected plants become bushy and produc several flowers. Very few pods are produced on infected plants and the size of pod is reduced (Nolt and Reddy 1984).

Control Measures

- Avoid sowing virus-infected seed.
- Use soil solarization for at least 70 days during summer months.
- Use a soil biocide such as carbofuran.

MP 14. Detection and Inoculation of Plant Viruses

Virus detection requires a well-equipped laboratory and highly trained technicians. Techniques for the detection of plant viruses have been described in a manual prepared by the Virology Unit at ICRISAT Center. Steps include serology, electron microscopy, and transmission techniques (Reddy 1987).

Detection Techniques

Reaction on a set of hosts, and serological techniques are widely being used for detection and assay of mechanically transmitted plant viruses.

Mechanical inoculation of plant viruses

Mechanical or sap inoculation is the application of plant extracts or solutions containing viruses on the leaves of healthy plants. This is done in such a way that the virus can enter the plant cells. Viruses that are transmitted by aphids nonpersistently, those which multiply in epidermal or mesophyll cells, and those that reach very high concentration in plants are usually mechanically transmissible. The sap-transmissible viruses usually produce mosaic, mottle or ring spot symptoms. By contrast, viruses that are persistently transmitted by insects are restricted to the xylem or phloem and are not mechanically transmissible.

Preparation of phosphate buffer (PB) 0.05 M pH 7.0

a. Dissolve 7.08 g of dibasic potassium phosphate ($K_2HPO4.\ 3H_2O$) and 1.2 g of monobasic potassium phosphate anhydrous ($KH_2PO4.$) in 500 mL of distilled water. Make up the volume of the solution to 1000 mL with distilled water.

b. Add 1.56 mL of 2-mercaptoethanol or 0.75 mL of thioglycerol or 1.26 g of Na_2SO_3 to the above 1000 mL buffer. All of these are reducing agents that retard the inactivation of the virus by oxidizing enzymes, and thus preserve infectivity.

c. It is necessary to store the buffer at 4°C. If this is not possible, use freshly prepared and chilled buffer for each inoculation.

Preparation of plant extracts

a. Collect 1 g foliage showing typical virus symptoms (leaves showing initial symptoms are preferred).

b. Grind the tissue in 9 mL of cold phosphate buffer in a mortar and pestle. Keep the mortar and pestle on ice before use. Trituration is done till all the leaves are finely ground.

c. The extract is filtered through two layers of cheese cloth. The extract is ready for inoculation.

Procedure of inoculation

a. Expose plants to darkness before making the sap inoculation. It is better to do the inoculation either early in the morning or late in the afternoon (during summer months).

b. Dust 600 mesh carborundum on leaves using a spray bottle.

c. Hold the leaf on the palm of your left hand. Now gently rub the inoculum over the leaf surface. This is done either using a cotton swab or a piece of muslin cloth soaked with inoculum.

Precautions

Greater pressure and excessive carborundum dusted on the leaf surface may lead to scorching of the tissue. Soon after inoculation, the leaf surface is washed with tap water and plants are covered with moistened newspapers for one day. Wash hands after each inoculation either with soap or trisodium phosphate solution. Carborundum dust should not be inhaled as it can settle in the lungs. It takes at least 5 min after dusting to settle the carborundum on the leaf surface.

Procedure for local lesion assay

Extracts from either tomato spotted wilt virus or peanut mottle virus infected leaves are used. Several dilutions of the inoculum (1 : 10, 1:100, 1:1000) are applied using the half leaf technique. An edge of the leaf is punctured with a forceps to distinguish between the two primary leaves inoculated. All treatments are randomized and usually eight replications are used.

Procedures for grafting

The majority of plant viruses are transmitted by grafting. There are three types of graftings.

a. Approach grafting. In this system, the scion is not detached from the mother plant. A splice is made on the scion and the stock and both the surfaces are united.

b. Bud grafting. A small piece of stem tissue from the scion is grafted on to the stock after making a splice to fit the scion.

c. Wedge grafting. A wedge is made in the stock and the scion is cut to fit the wedge and inserted into the stock.

Enzyme-linked immunosorbent assay (ELISA)

This is by far one of the most widely used and sensitive serological test. It permits analysis of several samples under identical test conditions. The most simple form of ELISA is called the direct antigen coating (DAC) procedure. This is suitable for detecting viruses that reach high concentration in plant tissues, in seed, and in disease surveys.

Procedure (Fig.6)

Step 1. Add viral antigens present in purified preparations and adhered to the well surface of the ELISA plate by incubating the extracts for 1 h at 37°C. The plate is washed three times in PBS-tween allowing 3-min soaking for each wash. This will result in the attachment of virus to the walls.

Step 2. Add a high dilution of crude antiserum (usually over 1 : 1000) and incubate at 37°C for 1 h. Wash as in step 2, r-globulins are attached to virus particles that are attached to the walls of the well.

Step 3. Add anti-rabbit Fc-specific gamma globulins enzyme (prepared in goat's serum) conjugated with alkaline phosphatase. Incubate at 37°C for

1 h. Wash with distilled water and PBS-tween as in step 2. Anti-rabbit globulins are attached to the virus plate.

Step 4. Add the substrate for alkaline phosphatase (normally P-nitro phenyl phosphate is used) or the sodium penicillin in bromothymol blue.

In case of a positive reaction the r-globulins attached to the antigens attached to the well surface will react with gamma globulins. This will facilitate the attachment of conjugated anti-rabbit Fc-alkaline phosphatase anti-Fc reacts with the substrate and produces colored hydrolysates. The intensity of color is measured at 405 nm that is proportional to the concentration of viral antigens.

In case of a negative reaction, the specific gamma globulins are not retained, thus the anti-rabbit Fc-specific globulins are washed away during the washing. Since alkaline phosphatase is not retained, the substrate will now become yellow.

A special ELISA reader is used to measure the absorbance at 405 nm (for P-nitro phenyl phosphate that is converted into nitrophenol). In case of sodium penicillin, the positive reaction shows light-green initially which turns dark-yellow showing a strong positive reaction.

The composition of ELISA buffers are:

a. Composition of carbonate buffer.

Na_2CO_3 1.59 g (0.02 M)
Na_2HCO_3 2.93 g (0.02 M)

Add chemicals to distilled water and make up to 1 L. The pH of the buffer should be 9.60.

b. Composition of phosphate buffer saline (PBS-tween).

NaCl = 8.0 g
KH_2PO_4 = 0.2 g
Na_2HPO_4 $2H_2O$ = 2.9 g or Na_2HPO_4 = 1.15 g
KCl = 0.2 g
Tween-20 = 0.5 Ml

Dissolve the above chemicals in distilled water and make the volume up to 1 L.

c. Composition of conjugate buffer.

PBS (T)	=	500 mL	
Polyvinyl pyrollidone	=	10 g	(2%)
Ovalbumin	=	1 g	(0.2%)

d. Substrate buffer (pH 9.8)

97.0 mL diethanolmine
800 mL distilled water
0.2 g $NaNO_3$

Add HCl to obtain a pH 9.8 and make the volume up to 1 L.

Precautions

Never rinse new ELISA plates before coating with the antigen, use them as they are supplied by the manufacturer. Thorough washing between each step is required to remove the excess reagents, thus avoiding a nonspecific reaction. Carbonate buffer is used for coating the antigens. The phosphates buffer saline containing Tween-20 (PBS-tween) is used for washing ELISA plates between the steps. Antibodies and conjugated gamma-globulins are diluted in a conjugate buffer and the substrate is prepared in a substrate buffer.

> **Note.** The details of PAC and DAS discussed in the 'Identification and detection of legume viruses' are available in the Virology Unit at ICRISAT.

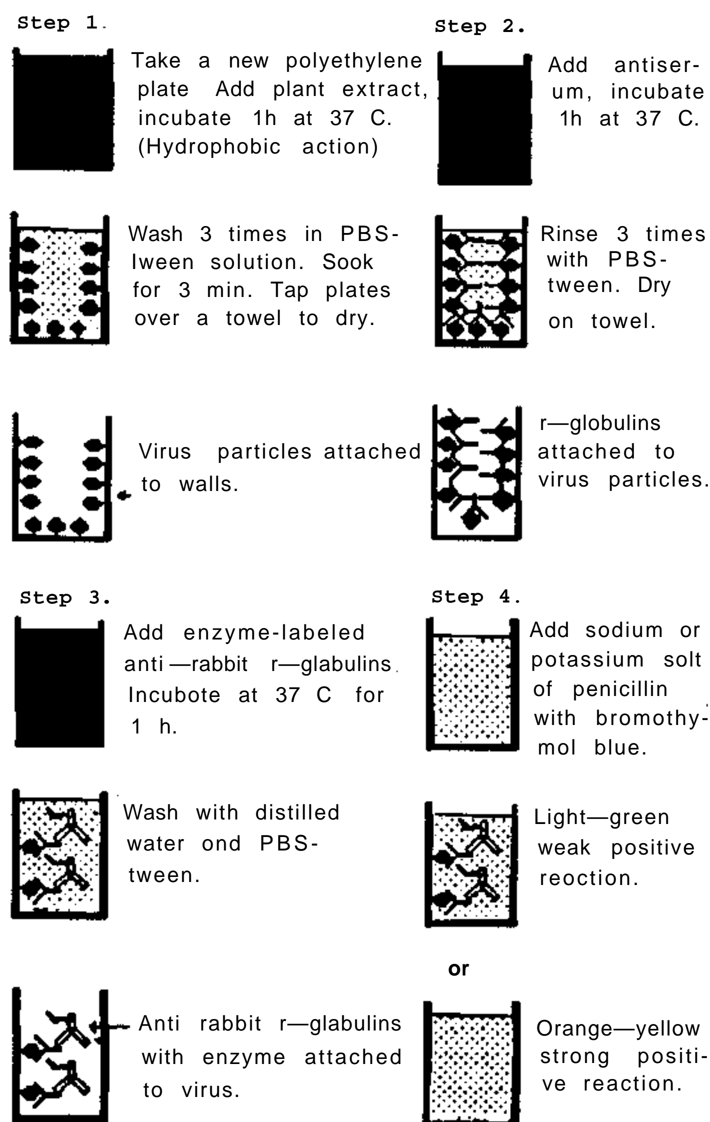

Figure 6. Direct antigen coating ELISA.

Technical terns used in ELISA

PBS	Phosphate buffer saline
Virus	Transmissible agent possessing definite shape. They are intracellular, and cannot be cultured on artificial media. They can be seen only under an electron microscope.
Antiserum	Blood serum containing antibodies.
r-globulins (IgG)	Commonest type of antibodies in serum.
Anti-rabbit (IgG)	Antibodies raised animals in other than rabbits.
Antigen	A substance that stimulates the production of an antibody when introduced into animal tissues.
Serum	A watery liquid that separates from coagulating blood.

(Source: Personal communication with Dr D.V.R. Reddy and Mr Sudarshan Reddy, ICRISAT 1991).

MP 15. Bacterial Wilt of Groundnut

Symptoms

Young infected plants show sudden wilting of stem and foliage with leaves on dead plants remaining green. In mature infected plants a gradual decline causes the foliage to turn yellow. Infected plant roots become discolored and are dead. Dying branches often curl to form a "shepherd's crook". The disease can be identified by dark-brown spots in the xylem and pith. The streaming masses of bacteria from cut ends of stems placed in water can be seen (Gitaitis and Hammons 1984).

Control Measures

- A crop rotation with cereal may reduce the incidence of a bacterial wilt.
- Use the seed produced in unaffected areas.
- The groundnut variety, Schwarz 21, has a high level of bacterial wilt resistance.
- Follow plant quarantine regulations to prevent the disease being introduced into new areas.

MP 16. Methods to Identify Plant Parasitic Nematodes

The identification of nematodes requires collection, preservation, and preparation of slides of nematodes for microscopic examination followed by their identification.

A. Extraction from soil

Nematode infested soil samples are collected with a spade or sampling tube from the vicinity of plant roots and stored in plastic bags in the laboratory at a low temperature (15-25°C).

1. Recovery by decantation of a soil suspension

Add about 500 cm^3 of soil to 3-4 L of water in a bucket and stir vigorously to break up the soil particles so that nematodes are released. Leave the solution for 1 min to let the soil particles settle to the bottom of the bucket, leaving the nematodes in suspension. Transfer the suspension into another bucket through a nest of sieves with opening of 850 µm, 250 µm, and 38 µm (=20, 250, and 400 mesh $inch^{-1}$). Return the third to the first bucket. Repeat the process, again by stirring, leaving the soil to settle, and by decanting through sieves. Finally, pass the water through sieve in the second bucket again. Collect the nematodes from the sieve by washing them with a fine stream of water from behind the sieve into a beaker.

2. Use of differential centrifugation with a solution of high specific gravity

Prepare a suspension of 100 cm^3 soil in 600 mL of water and stir well. Pour through a course sieve (850 µm pore size) into a container to remove stones and plant particles. Now transfer the soil suspension to centrifuge tubes and centrifuge the mixture at 1000 x gravity for 5 min. Add a small amount of kaolin to the soil before centrifugation, if clay content in soil is low. Pour off the supernatant, whip the upper part of the centrifuge tubes to remove debris. Add a sucrose solution to the tubes (490 g sucrose dissolved in 1 L of water). Resuspend soil in the tubes and centrifuge as above. Let it settle in water.

B. Preparation of slides

Prepare slides to examine the nematodes at high magnifications. This can be done by making temporary slides or permanent slides.

1. Temporary slides

Concentrate the nematodes in a small volume of water suspension in a small petri dish. Under a dissecting microscope, remove individual specimens by using a long-pointed pick. Lift a nematode from the bottom of the dish with the pick. When it is at the surface of water, pass the pick under the specimen and lift it through the air-water interface. It

will be held on the pick by surface tension. Then submerge the tip of the pick in a drop of water on a slide or small vial to release the nematodes from the pick. The drop should contain just enough fluid to spread completely to the edges of a cover slip. Cover the drop with a cover glass and seal the edges with melted paraffin, vaseline, or with nail polish to prevent rapid evaporation. Rapidly moving specimens can be temporarily immobilized by heating the slide briefly to about 50°C or use of anaesthetics like 0.25% propylene phenoxital in water or in 1% aqueous carboxymethyl cellulose.

2. Permanent slides

Fixation of nematodes. This requires fixation of specimen in aqueous solution of 11% formalin plus 6% glycerin. Concentrate the specimens in few milliliters of water in a vial. Bring the fixative to boil in a test tube and rapidly add a small quantity of fixative to the vial, equal to the amount of water in the vial. Cap the vial. The specimens are well fixed after a few hours and are usually straight.

Specimens are placed in pure glycerin for permanent slides. Lactophenol with a dilute concentration of cotton blue (0.0025%) is used for semipermanent preparation. Add a drop of the tinted lactophenol to a slide and warm it on a hot plate to 65-70°C. Transfer the specimens directly from the fixative to the hot lactophenol and maintain the temperature for 2-3 min. Then transfer them to nontreated tinted lactophenol, cover and seal.

Infiltration with glycerin. Water contents of the fixed specimens are gradually replaced to fix nematodes with pure glycerin. This could be accomplished in few hours or over a long period. The procedure involves the following:

a. Transfer the nematodes from the fixative to a small dish containing 0.5 mL solution of 96% ethanol 20 parts; glycerin 1 part; distilled water 79 parts.
b. Place the container with the nematodes in a desiccator containing 96% ethanol for 12 h at 35-40°C.
c. Remove the container with the nematodes, and fill with a solution of 5 parts glycerin in 95 parts of 96% ethanol, place in a partly closed petri dish and maintain at 40°C for 3 h or until the alcohol evaporates. Now the nematodes should be in pure glycerin. There are several other procedures (Dropkin 1980).

Mounting. Pickup the nematodes individually and transfer them to a drop of glycerin on a slide. Place a few glass fibers in the drop as spacers. Add a cover glass and seal to prevent gradual loss of the fluid and gradual absorption of moisture from the atmosphere.

C. Extraction from plants

The simplest way is to cut samples of infected plant parts (roots or other plant tissues) into 1-cm pieces and place them in an electric blender together with water. After 20-30 sec of maceration, the tissues will be separated into small pieces, but the nematodes remain intact and

can be recovered from the suspension.

Another way is to use a mist chamber where the water is sprayed intermittently from nozzles with small openings. The flow of water is controlled by a solenoid and a timer to operate the spray about 10% of the time. Funnels are placed under the spray, each draining into a test tube. The flow of water is very slow so that nematodes are washed into the tube and remain at the bottom while aerated water slowly circulates through the tube and flows out of the top. Both plant tissues and soil may be extracted in a mist chamber. In cool conditions, the water must be heated to avoid low temperatures during evaporation of the spray. The extraction usually proceeds for up to 1 week for complete recovery of nematodes from roots or soil (Dropkin 1980).

Staining nematodes within root tissues. This requires preparation of two lots of lactophenol solution (phenol 20 g; lactic acid 20 g; glycerin 40 g; distilled water 20 mL). To one solution add 5 mL of a 1% aqueous solution acid fuchsin (red) or cotton blue. Leave the other without dye.

Wash the root thoroughly in tap water. Bring tinted lactophenol to boil in a well ventilated place, and boil roots in the staining solution for 1 min. Wash out excess stain with tap water and blot off the excess water. Place in clear lactophenol solution until the roots are translucent in 1-2 days. Examine the roots in lactophenol or glycerin under the dissecting microscope at 20X. The nematodes retain the dye while the plant tissues are destained. Meristematic regions will also be stained (Dropkin 1980).

MP 17. Root-knot Nematode

The root-knot nematodes (*Meloidogyne* spp.) are the most important nematode species causing damage ranging from 20% to 90% in infested fields of groundnut (Rodriguez-Kabana 1984a).

Root galls contain white swollen adult females. The body tapers anteriorly to a narrow neck and mobile head with stylet, massive median bulb and large esophageal glands. An egg sac often protrudes posteriorly from the female to the exterior of the gall. It contains several hundred eggs. Often one or more elongate males are present in an egg sac. The females are 0.5 mm to 0.8 mm long. At the center of its posterior region, the female cuticle has a pattern of cuticular markings surrounding the anus and vulva. The second stage of juveniles invade roots at or close to the tip and migrate to the site of differentiating vascular tissues. Consequently several giant cells form around the nematodes head. The complete life cycle takes 3 weeks or more, depending on host and temperature. Males average about 1.1 mm in length. The posterior is characteristically twisted through 90 degrees or more. Larvae are about 400 µm long and have a delicate stylet (Dropkin 1980).

Disease Symptoms

The symptoms of damage caused by *Meloidogyne hapla* are similar to those caused by *M. arenaria.* Root-knot nematodes enter and damage groundnut roots, pegs, and pods. Infected plants develop enlarged roots and pegs. Galls develop into various sizes resulting from an internal swelling from the root tissue. Infected pods develop knobs, protuberances, or small warts. Infected plants with root-knot nematodes may show various degrees of stunting and chlorosis. Root development is reduced, and vascular systems of infected tissues are disrupted, resulting in the poor flow of water and nutrients from the roots (or pegs) to the shoot. Infected plants tend to wilt under drought conditions.

Control Measures

- A crop rotation of cereal-cereal-groundnut can significantly decrease the level of root-knot nematode infestation in soils.

- Nematicides used in groundnut are fumigant and nonfumigant types with contact or systemic properties. Application of a fumigant nematicide like ethylene dibromide (EDB) is made 18 cm deep at a soil temperature between 15—21°C @ 18 or 19 L ha^{-1}. Nonfumigant nematicides are aldicarb, carbofuran, and phenamiphos. These nematicides are effective when applied at sowing @ 2-3 kg a.i. ha^{-1}. The best results are obtained when applications of nematicides are made in a band 17-25 cm wide and incorporated 2-4 cm into the soil (Rodriguez-Kabana 1984a).

- Soil solarization during the hot dry season, also helps to control nematodes.

- Grow resistant cultivars: NC 343, NC 3033, NCAC 17090, or ICGS 2.

MP 18. Root-lesion Nematode

Root-lesion nematodes (*Pratylenchus brachyurus)* are small vermiform nematodes. The adults are generally less than 0.5 mm in length. Females have a long slender stylet with rounded knobs, whereas males usually have less developed or no stylets. Body annulations are fine. The vulva is in the posterior portion of the body. There is a single ovary. The procorpus and metacarpus are fused and the crescentic plates are large.

Disease Symptoms

Lesion nematodes are migratory endoparasites that attack groundnut roots, pegs, and pods. They feed within parenchymatous tissues. Both mechanical and chemical damage result from the nematodes feeding within the tissues. Root lesions develop and with large nematode populations these lesions coalesce, causing extensive discoloration and damage that results in reduced growth and pod production.

The pod lesions begin as tiny, tan to brown, pin-point areas on the surface. As the nematodes feed and reproduce the affected area becomes larger and darker. Old lesions are characterized by their blotchy appearance and indistinct margins. This is caused by the darker necrotic parenchyma, the outer cells of the pod and the necrotic areas become diffused. Sometime nematodes are established in the roots without visual symptoms above the ground, and cause yield reduction (Boswell 1984).

Control Measures

- o Apply carbofuran @ a.i. 4-8 kg ha^{-1} in the infected rows.
- o Grow resistant genotypes such as PI 390606, PI 395233, or PI 365553.
- o Solarize the soil during the hot dry season to control root lesion nematodes. The soil temperature during solarization should rise above 60°C to kill the nematodes as well as soilborne fungi.

MP 19. Ring Nematode

Ring nematodes (*Macroposthonia xenoplax*) are short and thick bodied nematodes. They move slowly and cannot be collected from the soil in Baermann funnels, but may be recovered after centrifugal flotation or by migration from soil in shallow layers.

The females are fusiform and have less than 200 annuluses. These are broad, often overlapping, and have smooth, irregular posterior margins of the annulations. They have a single outstretched ovary. The vulva is located posteriorly, close to the anus. Males have longitudinal incisures in lateral fields and candal alae. These nematodes are ectoparasites, partly embedded in root tissues with a long stylet, reaching well into the root (Dropkin 1980).

Disease Symptoms

Usually only a large population of nematodes produce symptoms of a chlorotic appearance that have been called "peanut yellows." In micropots inoculated with about 10 000 nematodes $plants^{-1}$, the roots, pods, and pegs of Argentine and Starr cultivars were found severely discolored with brown necrotic lesions. Many root primordia and young roots were killed, resulting in a few lateral roots. Pod yields from nematode-infested plants were only 50% of the healthy plant yields (Minton 1984).

Control Measures

- o Follow a crop rotation such as tobacco-maize-groundnut to reduce the population of nematodes,
- o Fumigants and organophosphate nematicides are effective against ring nematodes,
- o Solarize the soil during the hot dry season.

MP 20. Testa Nematode

Testa nematodes (*Aphelenchoides arachidis)* feed on fungi and parasitize buds and leaves of the plants. They are long, slender nematodes with a large metacarpus and blunt or pointed tails. The intestine joins directly to the metacarpus and the esophageal glands overlap the intestine in a long lobe. Males lack caudal alae and have a characteristic thorn shaped spicule. Many species possess a sharp tip on the tail called the mucron. The lateral fields have a few incisures (Dropkin 1980).

Symptoms

Testa nematode is an endoparasite of groundnut. This causes discoloration of seed tissues, reduces seed size, and causes seed shriveling. This nematode occurs within the tissues of pods, testa, roots, and hypocotyl. Infected groundnut seed coats are discolored when more than 2 000 nematodes testa^{-1} are present, often more than 25 000 nematodes are present. Heavily infected seeds, immediately removed from fresh mature pods have a translucent testa. Infected testa of dry seed are often wrinkled and dark brown. Infection also reduces the seed mass and seedling emergence (Rodriguez-Kabana 1984c).

Control Measures

- Avoid sowing the infected seed.
- Soak the infected seed in cold water for 15 min, followed by a hot water (60°C) treatment for 5 min.
- Dry the harvested pods in the hot sun or drier to a low moisture content (8%).

MP 21. Sting Nematode

Sting nematodes (*Belonolaimus longicaudatus)* are large slender nematodes with an off-set head separated by grooves into four lobes. A stylet is about 100 µm long. The procorpus has a long coiled lumen when the stylet is retracted. There is a single incisure in the lateral field. Two ovaries are present. The female tail is cylindrical and equal in length to three times the body width at the anus, the terminus is round. The caudal alae of the male are large. The esophageal glands overlap the intestine (Dropkin 1980). Males and females of this nematodes are morphologically similar. They range in length from 2 mm to 3 mm and have a strongly striated cuticle (Rodriguez-Kabana 1984b).

Disease Symptoms

Affected plant roots become gnarled and stubby, with the tap root frequently being the only root left over. Feeding results tiny lesions along the roots, affected plants become chlorotic with stubby, sparse root. Affected roots and pods have small, dark necrotic spots. They are ectoparasitic and rarely found internally in roots and pods (Rodriguez-Kabana 1984b).

Control Measures

Use of the nematicide, fensulfothion, provides effective control for sting nematodes.

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Annexure 1

Rust Scoring Data Sheet

Entry not
Replication :
Field not

Date:
Treatment :
Observation not

Line no.	Plot no.[1] LAD (%)	Plot no. LAD (%)	Plot no. LAD (%)
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			
13.			
14.			
15.			
Total			
Mean			

1. LAD (%) = Leaf Area Damage (%)

Annexure 2

Leaf Spot Scoring Data Sheet

Entry no:
Replication:
Field no:

Date:
Treatment:
Observation no:

Line no: no.	Plot no.[1] LAD (%)	Plot no. LAD (%)	Plot LAD (%)
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			
13.			
14.			
15.			
Total			
Mean			

1. LAD (%) = Leaf Area Damage (%)

Evaluation

Select the most appropriate answer and check the correct answer at the end of the booklet.

1. Any disturbance in the physiological processes of a plant is a
   a) causal organism. b) disease,
   c) pest. d) stress.

2. Nonchlorophyllous, nucleated, unicellular or multicellular filamentous bodies that are reproduced by sexual or asexual spores are
   a) bacteria. b) virus.
   c) fungi. d) microbes.

3. Biotrophs are active only in
   a) nonliving host. b) living host.
   c) vectors. d) soil.

4. The fungi that are mostly propagated by motile zoospore formed in sporangia, without production of hyphae are
   a) Ascomycetes. b) Basidiomycetes.
   c) Deuteromycetes. d) Phycomycetes.

5. The unicellular organisms of very small cells that lack organized nucleus are
   a) fungi. b) viruses.
   c) bacteria. d) mycoplasma.

6. Generally bacteria causing plant diseases are
   a) gram positive. b) gram negative.
   c) gram neutral. d) none of the above.

7. The submicroscopic organisms composed of protein and nucleic acid, that multiply only in the host cell and do not possess an enzyme system are
   a) fungi. b) bacteria.
   c) mycoplasma. d) viruses.

8. A vector is an agent which transmits
   a) bacteria. b) fungi.
   c) virus. d) mycoplasma.

9. The seed and seedling diseases caused by fungi in groundnut are
   a) bud necrosis, mottling, and stripe.
   b) root rot, wilt, and aflaroot.
   c) rust and leaf spots.
   d) root knot and stunting.

10. Groundnut rust is caused by
    a) *Cercosporidium personatum.* b) *Aspergillus niger.*
    c) *Puccinia arachidis.* d) *Cercospora arachidicola.*

11. Late leaf spot of groundnut is caused by
a) *Cercospora arachidicola.* b) *Puccinia arachidia.*
c) Cercosporidium personaturn, d) *Aspergillus flavus.*

12. Early leaf spot of groundnut is caused by
a) *Aspergillus niger.* b) *Puccinia arachidis.*
c) *Phaeoisariopsis personata.* d) *Cercospora arachidicola.*

13. The vector of peanut clump virus is
a) *Aspergillus niger.* b) *Aspergillus flavus.*
c) *Polymyxa graminis.* d) *Puccinia arachidis.*

14. Bacterial wilt of groundnut is caused by
a) *Pseudomonas solanacearum.* b) *Corynebacterium insidiosum.*
c) *Xanthomonas tumefaciens.* d) *Agrobacterium tumefaciena.*

15. Aflaroot or yellow mold in groundnut is caused by
a) *Aspergillus niger.* b) *Aspergillus flavua.*
c) *Botrytis cineria.* d) *Fusarium solani.*

16. Crown rot or collar rot of groundnut is caused by
a) *Aspergillus niger.* b) *Aspergillus flavua.*
c) *Botrytis cineria.* d) *Fusarium aolani.*

17. Stem rot of groundnut is caused by
a) *Cylindrocladium crotalariae.* b) *Pythium myriotylum.*
c) *Sclerotinia minor.* d) *Sclerotium rolfsii.*

18. In groundnut *Rhizoctonia aolani* and Pythium *myriotylum* cause
a) seed and seedling rot.
b) crown or collar rot.
c) damping off, root rot, and pod break down.
d) vascular wilt.

19. Groundnut pod rots are caused by
a) Fusarium *solani* and *F. oxyaporum.*
b) *Macrophomina phaaeolina* and *Rhizoctonia aolani.*
c) Sclerotium *rolfaii* and *Aspergillua niger.*
d) the combined attack of all the above.

20. In groundnut, the sudden wilting of lateral branches at an early stage with the affected branches becoming chlorotic and turning brown are symptoms of
a) blight. b) black rot.
c) stem rot. d) vascular wilt.

21. When germinating seeds are covered with masses of black conidia, or there is rapid drying of infected plants with a shaded dark brown color in the collar region, these are symptoms of
a) wilt. b) collar rot.
c) blight. d) stem rot.

22. Preemergence death of seedlings with shrunken elongated dark-brown areas on the hypocotyl are symptoms of
a) *Rhizoctonia solani.* b) *Fusarium solani.*
c) *Aspergillus flavus.* d) *Verticillium alboatrum.*

23. Wilting, curling, and chlorotic symptoms on leaflets and petioles are due to an infection caused by
a) Fusarium *oxysporum.* b) *Aspergillus flavus.*
c) *Rhizopus arrhizus.* d) *Verticillium alboatrum.*

24. Following a cereal-cereal-groundnut rotation, seed dressings, and clean groundnut cultivation are helpful to control
a) foliar diseases. b) seed and seedling diseases.
c) viruses. d) all the diseases.

25. When infected plants become stunted, with vein cleaning chlorosis on the leaflets, and where seedlings lack secondary root symptoms, the disease may
a) aflatoxin. b) toxin.
c) aflaroot. d) wilt.

26. The lines are identified as resistance to invasion and colonization by *Aspergillus flavus* when sporulating growth of fungus is up to
a) 11-20% of seeds. b) 1–16% of seeds,
c) 15–25% of seeds. d) 30–50% of seeds.

27. The appearance of orange colored pustules (uredinia) in groundnut plants and reddish brown uredospores on the abaxial surface of leaves are symptoms of
a) early leaf spot. b) late leaf spot.
c) rust. d) virus.

28. The main symptoms of groundnut rusts are confined to the
a) roots. b) stems,
c) leaflet and aerial parts. d) flowers.

29. Chlorothalonil is useful to control groundnut
a) early leaf spot. b) late leaf spot.
c) rust. d) all the above.

30. The nonreplicated test genotypes are grown with plants 60-cm apart in one-row plots of 4-m length with the infector rows for
a) advanced screening of rust.
b) preliminary screening of rust and leaf spots.
c) screening for virus.
d) screening for nematodes.

31. Test genotypes are replicated 2 or 3 times and each test plot is separated by an infector row for
a) advanced screening of rust only.
b) advanced screening of rust and late leaf spots.
c) screening for virus.
d) screening for nematodes.

32. For rust, infector rows are inoculated with urediniospore suspensions of
    a) volunteer plants. b) potted spreader plants,
    c) infector row plants. d) natural infection.

33. For rust inoculation in the laboratory, spores are collected in a glass tube with the help of
    a) a sucking pump. b) the hand.
    c) a cyclone spore collector. d) none of the above.

34. For inoculation purposes the rust inoculum is adjusted to ______ spores mL^{-1} of solution.
    a) 20 000 b) 30 000
    c) 40 000 d) 50 000

35. The spores in the inoculum are counted using a
    a) cyclone spore collector, b) microscope,
    c) hemocytometer. d) hygrometer.

36. After rust inoculation the disease symptoms start developing in
    a) 3 days. b) 5 days,
    c) 7 days. d) 9 days.

37. Leaf diagrams with a known percentage of disease damage are useful for estimation of
    a) leaf area damage. b) percentage of infection.
    c) disease severity. d) disease intensity.

38. Hogland solution is useful for
    a) inducing disease infection. b) reducing disease infection.
    c) enhancing plant growth. d) inducing root development.

39. Small necrotic flecks with a light to dark-brown center and a yellow halo are the symptoms of infection caused by
    a) early leaf spot, b) late leaf spot,
    c) rust. d) virus.

40. Small necrotic flecks that enlarge and become light to dark brown without a yellow halo and sporulations on the abaxial surface of leaves are symptoms of infection caused by
    a) rust. b) early leaf spot,
    c) late leaf spot. d) virus.

41. Excessive use of chlorothalonil for the control of foliar diseases increases the severity of
    a) Rhizoctonia blight. b) wilt.
    c) viruses. d) Sclerotinia blight.

42. A score of 1 on a 1—9 scale for leaf spots or rust in groundnut leaf disease severity is equal to ______ damage,
    a) 0% b) 1-2%
    c) 2-3% d) 4-5%

43. While scoring on 1 to 9 scale for leaf spot or rust disease severity (%) in groundnut a score of 3 indicates ________ damage.
a) 1-5% b) 6-10%
c) 11-20% d) 21-30%

44. Estimation of each leaf damage in comparison to the diagram depicting the known percentage of the area of late leaf spot damage is used for calculating
a) defoliation. b) leaf area damage.
c) infection frequency. d) sporulation.

45. The number of late leaf spot lesions cm^{-2} of leaf area is
a) leaf area damage. b) defoliation.
c) infection frequency. d) lesion diameter.

46. Concentric rings or chlorotic spots on young leaflets and subsequently terminal bud necrosis is caused by
a) wilt. b) peanut stripe virus,
c) tomato spotted wilt virus. d) iron chlorosis.

47. The vectors of tomato spotted wilt virus are
a) aphids. b) jassids.
c) thrips. d) spodopteras.

48. General chlorosis with a few green islands on young leaflets, stunting of plants, and small chlorotic, curled, and puckered leaflets are caused by
a) groundnut green rosette. b) groundnut mosaic rosette,
c) groundnut chlorotic rosette. d) peanut stripe virus.

49. The symptoms of narrow chlorotic streaks on dark green young leaflets, a reduced leaf size with upward rolled margins of older leaves are caused by
a) groundnut chlorotic rosette. b) groundnut mosaic rosette,
c) peanut stripe virus. d) groundnut green virus.

50. Groundnut rosette resistant cultivars are
a) ICGS 11 and ICGS 44. b) RG 1 and 69-M 101.
c) TMV 2 and JL 24. d) ICG(FDRS) 4 and ICG(FDRS) 10.

51. The insect vectors of groundnut rosette virus are
a) jassids. b) thrips.
c) aphids. d) spodopteras.

52. Symptoms of severe stunting of the plant with mosaic mottling, chlorotic rings, and a bushy appearance of infected plants are caused by
a) groundnut rosette virus. b) peanut stripe virus,
c) tomato spotted wilt virus. d) peanut clump virus.

53. Peanut clump virus is transmitted by
a) *Aphis craccivora* b) *Scirtothrips dorsalis.*
c) *Polymyxa graminis.* d) none of these.

54. The best way to detect plant virus is
a) mechanical inoculation. b) local lesion assay.
c) grafting. d) ELISA assay.

55. The application of virus extracts on to the leaves of healthy plant to facilitate virus infection is
a) grafting. b) ELISA.
c) mechanical inoculation. d) local lesions assay.

56. The viruses transmitted by insects are restricted to
a) leaves. b) stem.
c) roots. d) xylem and phloem.

57. When a substance introduced into animal tissue stimulates the production of an antibody it produces
a) a virus. b) an antiserum.
c) an antigen. d) a fungi.

58. A watery liquid that separates from coagulating blood is
a) an antigen. b) an antibody,
c) a virus. d) a serum.

59. The sudden wilting of the groundnut stem and foliage with gradual yellowing and curling of drying branches to form a shepherd's crook, with dark spots in the xylem and pith is caused by
a) fusarium wilt. b) tomato spotted wilt virus,
c) root rot. d) bacterial wilt.

60. A bacterial wilt resistant variety of groundnut is
a) RG 1. b) ICGS 11.
c) ICG(FDRS) 4. d) Schwarz 21.

61. An elongated tubular unsegmented organism that looks like thread is
a) fungi. b) bacteria,
c) nematode. d) virus.

62. The root-knot nematodes can cause damage ranging from _______
a) 1-5%. b) 5-10%.
c) 10-15% d) 20-90%

63. The nematode affected roots, pegs, and pods develop into galls caused by
a) root-lesion nematode. b) sting nematode.
c) root-knot nematode. d) ring nematode.

64. The most appropriate time for application of nematicide in nematode-infested field is
a) seed treatment. b) after sowing.
c) at the time of sowing. d) after infection is noticed.

65. The best result of nematicide application in affected areas is obtained by
a) broadcasting. b) mixing with seed,
c) band application 2 cm to 4 cm deep. d) foliar spraying.

66. One of the management practice useful for the control of nematodes is
a) deep plowing. b) shallow plowing.
c) deep irrigation. d) soil solarization.

67. The nematode which is a migratory endoparasite and can attack on groundnut roots, pegs, pods, and feed within parenchymatous tissue is
a) root-knot nematode. b) root-lesion nematode.
c) ring nematode. d) testa nematode.

68. The nematode that can develop in side root without visual symptoms above the ground and cause yield reduction is
a) root-knot. b) root-lesion,
c) ring. d) testa.

69. Root lesion nematode resistant genotypes are
a) ICGS 11 and ICGV 76. b) TMV 2 and JL 24.
c) PI 390606 and PI 395233. d) CS 9 and ICG(FDRS) 10.

70. The soil temperature during solarization to kill nematodes should rise above
a) 30°C. b) 40°C.
c) 50°C. d) 60°C.

71. The short and thick bodied nematodes that move slowly and can not be collected from the soil in Baermann funnels, but can be recovered after centrifugal flotation is
a) root-knot nematode. b) root-lesion nematode.
c) ring nematode. d) testa nematode.

72. The chlorotic symptoms called peanut yellows are caused by the infection due to
a) root-lesion nematode. b) root-knot nematode.
c) testa nematode. d) ring nematode.

73. The nematode that feeds on soil fungi and parasitize on buds and leaves of the plant is
a) root-lesion nematode. b) testa nematode.
c) root-knot nematode. d) ring nematode.

74. Discoloration of groundnut seed tissues, reduction in seed size, and shriveling of seeds are caused by
a) root-lesion nematode. b) root-knot nematode.
c) ring nematode. d) testa nematode.

75. Cold and hot water seed treatment can kill
a) root lesion nematode. b) testa nematode.
c) root-knot nematode. d) ring nematode.

76. Gnarled and stubby appearance of affected plants with barely left over tap roots showing necrotic spots are the symptoms caused by
a) sting nematode. b) testa nematode,
c) ring nematode. d) root- knot nematode.

Correct responses to the questions.

1. b); 2. c); 3. b); 4. d); 5. c); 6. b); 7. d); 8. c); 9. b);

10. c); 11. c); 12. d); 13. c); 14. a); 15. b); 16. a); 17. d);

18. c); 19. d); 20. c); 21. b); 22. a); 23. d); 24. d); 25. c);

26. b); 27. c); 28. c); 29. d); 30. b); 31. b); 32. b); 33. c);

34. d>? 35. c); 36. c); 37. b); 38. d); 39. a); 40. c); 41. d);

42. a); 43. b); 44. b); 45. c); 46. c); 47. c); 48. c); 49. d);

50. b); 51. c); 52. d); 53. c); 54. d); 55. c); 56. d); 57. c);

58. d); 59. d); 60. d); 61. c); 62. d); 63. c); 64. c); 65. c);

66. d); 67. b); 68. b); 69. c); 70. d); 71. c); 72. d); 73. b);

74. d); 75. b); 76. a).