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# Mass Screening Techniques for Resistance to Maize Diseases

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**ICAR-INDIAN INSTITUTE OF MAIZE RESEARCH**

PAU Campus, Ludhiana 141 004, Punjab (India)

# Mass Screening Techniques for Resistance to Maize Diseases


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Front Cover : DMRH 1301: Medium maturing IIMR hybrid for NEPZ and CWZ
Back Cover : DMRH 1417: Suitable Early maturing IIMR hybrid for rainfed ecology
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# PREFACE

Diseases caused by pathogens are one among the major causes of yield loss in maize. These diseases not only affect its yield but can greatly impair the quality and year-wise stability of production, undermining the efforts to promote sustainable agriculture. Moreover, environmental and health hazards, resulting from the non-judicious application of numerous chemical fungicides are another cause of concern. The All India Coordinated Research Project on Maize (AICRPM) upgraded to Directorate of Maize Research in 1994 and lately to ICAR-Indian Institute of Maize Research in 2015; developed disease resistant and/or tolerant varieties of maize since 1957 through identification of sources of resistance and their utilization in the breeding program.

Maize is prone to several diseases incited by fungi, bacteria and viruses. The germplasm screening for disease resistance requires standard screening methods. Current publication is the third publication in this area since publication of *'Techniques of Scoring for Resistance to Important Diseases of Maize*" by All India Coordinated Maize Improvement Project in 1983. Towards the high precision and accuracy in scoring the germplasm, existing 1-5 rating scales for foliar diseases were long felt to be revised to 1-9 rating scale for scoring the disease severity particularly in view of global revision of these disease rating scales. Narrow scale of 1-5 is prone to bring errors in the process of scoring due to oversight and ambiguity of scores. Moreover, diseased leaf area (DLA)/ severity and per cent disease index (PDI) have been included for determination of disease reaction. The publication elaborates briefly existing names of the pathogens with their synonyms, their symptoms of infections on maize, host range, economic importance, morphological identification, epidemiology, disease cycle and disease screening techniques. Determination of disease reaction based on score, DLA (severity) and PDI have been incorporated in the methodology. We are sure that the bulletin will be of great help to researchers and students.

The contributions of collaborators from within the institute and from All India Coordinated Research Project (AICRP) on Maize is duly acknowledged. We sincerely acknowledge the support received from Indian Council of Agricultural Research (ICAR) in executing various activities.

Authors

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# INTRODUCTION

Maize (*Zea mays* L.) is the most versatile crop with the highest yield potential and wider adaptability among cereals. It is cultivated in all climates such as tropics, sub-tropics and temperate conditions. It has diverse types of uses like field corn, sweet corn, popcorn, amylose corn etc. Apart from maize used as food, feed and fodder, it is raw material for several food and non-food based industries including biofuel. Maize is being grown in more than 166 countries over an area of 184 million ha producing 1041 million tonnes with an average productivity of more than 5.5 t/ha. Three countries namely USA, China and Brazil together constitute more than 45 per cent of global maize area and produces more than 65 per cent of global maize. USA has the highest productivity of more than 10 t/ha followed by China (6.1 t/ha) and Brazil (5.2 t/ha). India ranks fourth in maize area with more than 9.5 million ha and sixth in the production with 27.14 million tonnes (2$^{nd}$ advance estimate of DAC 2017-18) with an average productivity of more than 2.8 t/ha. Though maize area, production and productivity have increased continuously since 1950s, the phenomenal increase was recorded in the last decade principally due to adoption of single cross hybrid (SCH) technology by the farmers. The benefit of this increased productivity from SCH has been realized by the farmers from whole country, particularly the endured agro-ecosystem.

The Indian maize improvement programme i.e. All India Coordinated Research Project on Maize (AICRPM) was the first of its kind among all AICRPs which was started in 1957. The project was upgraded to Directorate of Maize Research in 1994 and lately to ICAR-Indian Institute of Maize Research in 2015. The research efforts in the last 60 years have led to development and release of more than 400 cultivars. The increased maize production has become a driving force for maize and maize based industries like poultry feed, livestock, starch, beverages/alcohol etc. It is expected that maize demand would be 45 million tons by 2030 with the annual growth of 5-9 per cent. To achieve this target, there is need for development of low cost high yielding hybrids along with appropriate production and protection technology package.

Though maize is affected by more than 60 diseases, in India about a dozen are of serious concern. The four major diseases *viz*; turcicum leaf blight (TLB), maydis leaf

blight (MLB), post-flowering stalk rots (PFSR), and banded leaf and sheath blight (BLSB) are of national importance. Besides these, six diseases are of regional importance and these are polysora rust, brown stripe downy mildew (BSDM), sorghum downy mildew (SDM), Rajasthan downy mildew (RDM), late wilt and cyst nematode. The level of disease intensity and extent of damage due to various pathogens largely depend on edaphic factors, inoculum density of pathogens and host plant resistance. Host plant resistance (HPR) is considered to be most practical, feasible and effective way to control plant diseases. Though diseases can be managed through chemicals, these are neither farmer- nor environment-friendly. These are serious threat to soil and human health too. Further use of chemicals makes maize cultivation costly, reducing profit margin. Therefore, exploitation of HPR helps in minimising inoculum in soil and yield loss in an eco-friendly and economic manner.

Towards identification of sources of resistance, it is necessary to screen materials at

| S.No. | Zone | Hot Spot Location | Diseases[#] | |
|---|---|---|---|---|
| | | | ***Kharif* / Spring** | **Rabi** |
| 1. | North Hill Zone (NHZ) | Almora (Uttarakhand) | TLB | - |
| | | Larnoo (Kashmir) | TLB | |
| | | Bajaura (Himachal Pradesh) | TLB | - |
| | | Barapani (Meghalaya) | TLB | - |
| | | Dhaulakuan (Himachal Pradesh) | CLS, BSR | |
| | | Larnoo (Kashmir) | TLB | - |
| 2. | North West Plain Zone (NWPZ) | Delhi | MLB, BLSB | - |
| | | Karnal (Haryana) | MLB, BLSB | CR |
| | | Ludhiana (Punjab) | MLB, ChR | ChR |
| | | Pantnagar (Uttar Pradesh) | BLSB, BSR | - |
| 3. | North East Plain Zone (NEPZ) | Dholi (Bihar) | MLB | TLB |
| | | Kalyani (West Bengal) | MLB | MLB, TLB |
| 4. | Peninsular Zone (PZ) | Coimbatore (Tamil Nadu) | - | ChR |
| | | Dharwad (Karnataka) | TLB, CR | ChR |
| | | Hyderabad (Telangana State) | ChR | ChR |
| | | Mandya (Karnataka) | TLB, PR, SDM | TLB, SDM |
| | | Udaipur(Rajasthan) | CLS, RDM, MCN | FSR |

[#]BLSB- Banded leaf and sheath blight; BSDM- Brown stripe downy mildew; BSR- Bacterial stalk rot; ChR- Charcoal rot; CR- Common rust; CLS- Curvularia leaf spot; FSR- Fusarium stalk rot; MLB- Maydis leaf blight; PR- Polysora rust; PFSR- Post Flowering stalk rot; RDM- Rajasthan downy mildew; SDM- Sorghum downy mildew; TLB- Turcicum leaf blight; MCN- Maize cyst nematode

locations having high disease intensity (referred to as '*Hot Spots*') under natural conditions and artificially created sick plots. Artificial epiphytotics are created to confirm the resistance level at identified hot spots as detailed in table. Over years, germplasm screening at these hot spots helped in identification of stable sources of resistance, which have been deployed in the development of disease resistant cultivars of maize.

Worldwide 1-9 rating scales for foliar diseases are very commonly followed. This prompted us to consider making a uniform system of scoring for maize diseases in India as well. Accordingly, the existing 1-5 rating scales for foliar diseases were revised to more precise and accurate 1-9 rating scales. In earlier narrow scales (1; 1.5, 2.0; 2.5 etc.), always there were chances of errors in scoring the germplasm due to oversight or ambiguity. In the revised system, diseased leaf area (DLA) i.e. severity and per cent disease index (PDI) have also been included for determination of disease reaction of all major diseases of maize. This document aimed at providing a comprehensive details of screening techniques for disease resistance in maize germplasm. The document will be of immense practical use to maize researchers and students.

# 1. MASS SCREENING TECHNIQUES FOR RESISTANCE TO LEAF BLIGHTS

## 1.1 Maydis Leaf Blight (MLB)/ Southern Corn Leaf Blight (SCLB)

### 1.1.1 Causal organism:

**1.1.1.1 Teleomorph:** *Cochliobolus heterostrophus* (Drechsler) Drechsler, 1934

**1.1.1.2 Anamorph synonyms:**

1. *Bipolaris maydis* (Y. Nisik. & C. Miyake) Shoemaker, 1959
2. *Drechslera maydis* (Y. Nisik. & C. Miyake) Subram. & B.L. Jain, 1966
3. *Helminthosporium maydis* Y. Nisik. & C. Miyake, 1926

There are three physiological races of *B. maydis:* Race T, Race O and Race C. Race T and Race C are pathogenic to cytoplasm male-sterile T and cytoplasm male-sterile C germplasm of maize respectively. Confirmation of the physiological race can be achieved by assessing symptoms and pathogenecity on various host germplasm.

### 1.1.2 Host range:

*Zea mays* L., *Z. mays* var. Praecox bonaf. Popcorn, *Zea. mays* var. Rugosa bonaf. Sweet corn, *Eriochloa prooera, Paspalum baseianam, Zingiber officianale*

### 1.1.3 Economic importance:

Maydis leaf blight is most serious in warm and wet temperate and tropical areas, where yield losses close to 70 per cent have been reported due to the disease. Several races of *B. maydis* are pathogenic to maize. Symptoms and severity of *B. maydis* depends on the pathogen race and host germplasm.

### 1.1.4 Symptoms:

Symptoms vary according to the causal race and host germplasm.

- **Race O** produces lesions that are initially small and diamond-shaped. These lesions elongate as they mature, although growth of lesions is restricted by leaf veins. Final lesions are rectangular (2-6 × 3-22 mm), restricted by leaf veins, and tan in color. Lesions caused by isolates of Race O are restricted to leaves.
- **Race T** produces lesions that are oval and slightly larger (6-12 × 6-27 mm) than those caused by Race O. Lesion borders are usually characterized by dark, brown borders. Race T causes lesions on all above ground parts of the plant (including stems, sheaths and ears) and can also cause ear rots. Seedlings from seeds infected with Race T often wilt and die within 3 to 4 weeks.
- Under severe disease pressure, usually when infection occurs prior to silking, lesions may coalesce, blighting the entire leaf. In these circumstances, sugars

may be diverted from the stalk for grain filling, thus predisposing the plant to lodging.

(Courtesy: Anonymous, 2001)

### 1.1.5 Morphological identification of *Cochliobolus heterostrophus*:

**1.1.5.1 Conidia:** Olivaceous brown, spindle-shaped, and taper to round ends, size 15-20 × 70-160 µm in dimension and Conidia are 5 to 11 septate and are characterized by *bipolar germination*.

**1.1.5.2 Asci:** cylindrical, hyaline.

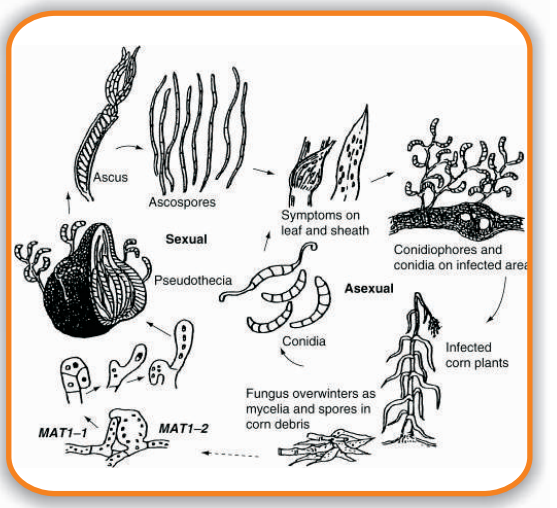
**1.1.5.3 Ascospores**: Size 6-7 × 130-340 µm, dark, and have 5 to 9 septa.

### 1.1.6 Epidemiology:

- Regions with a warm (20 to 32°C) and damp growing season are most at risk from maydis leaf blight.
- Long, dry, sunny periods during the growing season are unfavorable for the disease.
- The fungi overwinters in infected crop debris and therefore regions where infected crop debris overwinters and where maize is cultivated continuously are at risk.
- Race O pathotypes are widely distributed, although Race T pathotypes are only prevalent where hybrids have the Texas source of male sterility.

### 1.1.7 Disease cycle:

The fungus overwinters as mycelium in infected crop debris that remains on the soil surface between growing seasons. At the onset of the subsequent growing season, in response to favorable temperatures and humidity, mycelia within the crop debris begin to sporulate (producing condia). Conidia are then disseminated through wind and rain splash to freshly planted maize in




(Courtesy: Turgeon and Scott 2007)

the vicinity. Conidia germinate and infect plants through stomata, giving rise to characteristic lesions within which conidia are produced, which leads to secondary disease cycles. In favorable environmental conditions, the disease cycle can be completed in as little as 72 hours.

### 1.1.8 Disease screening of maize germplasm

There are three techniques of preparation of MLB inoculum (AICMIP, 1983) for creation of artificial MLB epiphytotics:

#### 1.1.8.1 Technique I

Original cultures of the organism are isolated by collecting leaf lesions and placing in a moist chamber. Newly-formed spores on the surface of the lesions (4-5 days later or even earlier) are picked up with a fine flattened needle under a dissecting microscope, placed in a droplet of sterile water and streaked across the surface of hardened, acidified water agar in petri plates. After allowing a few hours for the spores to germinate, they are cut out of the agar and transferred to hard, acidified potato-dextrose agar. After two weeks of incubation at 20-25°C, these cultures may be transferred to fresh plates of acidified potato dextrose agar for multiplication. When the fungus growth has covered the surface of the agar the cultures are ready for use. About 20 petri dishes of culture are macerated in water in a waring blender for 15-30 seconds, strained through a layer of cheese or muslin cloth and made up to four-five litres of suspension. This stock suspension is taken to the field and diluted in a compressed air sprayer (which should not have been used for fungicidal or insecticidal spray) at the rate of one litre in about 12 litres of water. Four litres of stock suspension diluted as indicated is enough to inoculate about 1,000 plants. Inoculum should be directed into whorls of the plants, where it will be retained long enough to permit spore germination. If inoculum is sprayed over the leaves, moisture evaporates before germination leading to dessication of spores. Inoculations should be made at least twice per week for three weeks beginning when plants are 30-45 cm high. On the basis of 1,000 plants this will require 120 petri dishes of pure uncontaminated cultures. This method has been uniformly successful over several years in establishing heavy infection so that resistant plants could be selected before silking.

#### 1.1.8.2 Technique II

Inoculum is most easily prepared by gathering, in the previous year, leaves heavily infected with maydis leaf blight. This should be done before leaves become fully mature, but not too green that spoilage would occur in storage. It is best to store leaves in large gunny bags in some dry room protected from moisture and rodents. Just before inoculation, the dry leaves are ground a meal of about the coarseness of wheat bran.

This may be done in a mill, or, if not available, the leaves may be placed in tightly woven sacks and beaten with sticks of stamped on to break up the leaves. By sifting through a screen, fragments of the right size can be obtained. About 30 kg of such leaf meal is sufficient to inoculate twice 20,000 to 22,000 plants. This requires the collection of 25 to 30 bags of infected leaves in the previous season. Inoculation is done by placing a pinch of leaf meal (a heaped thimbleful) into the whorl of each plant when the latter are about 30-45 cm high. A second inoculation may be made five to ten days later. This method of inoculation will be ineffective if dry weather prevails following application of the leaf meal. To avoid this and to facilitate infection during dry weather, water (10-12 ml) can be applied in the whorls by means of an 8-10 litre sprayer. Second inoculation can be followed if the symptoms do not appear even after a week of first inoculation. If there is some rain or heavy dew soon after application of inoculum, the addition of water is not necessary.

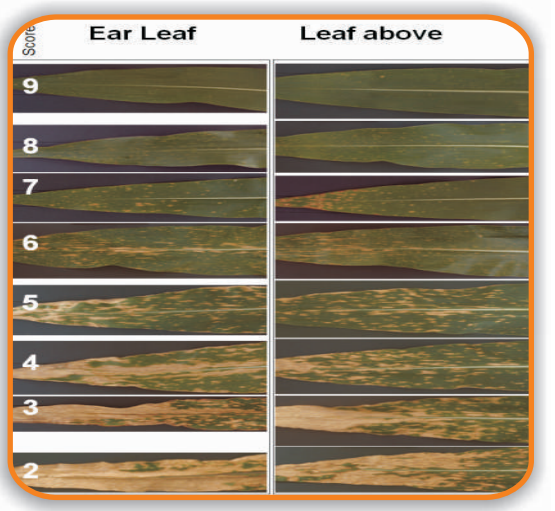
#### 1.1.8.3 Technique III

Grain (sorghum seed) culture of the blight pathogen (include as many virulent isolation as possible) are stored three weeks before planting the test material. When the pathogen has grown over the grain, grind it in a food chopper and store at 6-9°C. At the time of inoculation, this inoculum can be diluted to the desired level by adding to its ground sorghum seeds. Inoculation is done by placing a small quantity into the whorl on a cloudy day or towards evening to avoid mortality by direct exposure to sun sight.

### 1.1.9 Procedure of recording disease reaction based on disease ratings, diseased leaf area and PDI:

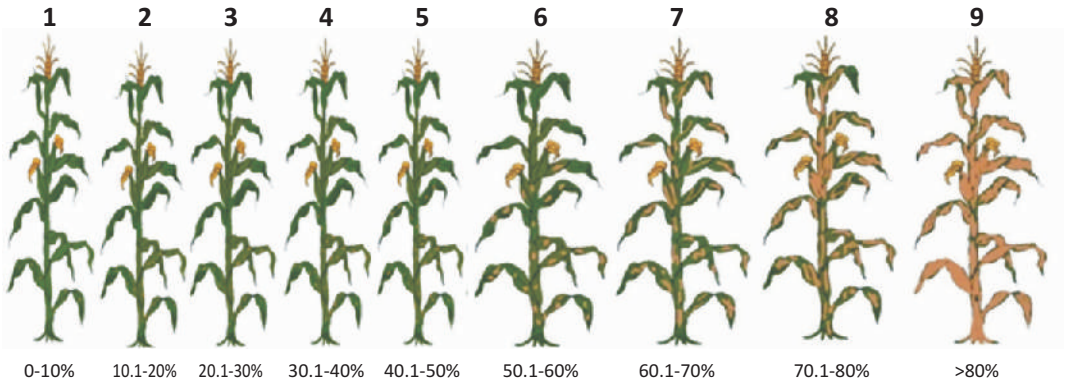
The rating scale for recording MLB reaction consists of 9 broad categories designated by numerals 1 to 9 (Balint-Kurti *et al.*, 2006; Chung *et al.,* 2010 and Mitiku *et al.,* 2014).

Disease reaction to MLB can be determined based upon disease ratings, disease leaf area (DLA) and PDI at 30-35 days after inoculation as mentioned below:




(Courtesy: Peter Balint-Kurti, NCSU, NC)

## Diagrammatic scale for assessment of MLB severity on maize plants




| Rating scale | Degree of infection (per cent DLA*) | PDI** | Disease reaction |
|---|---|---|---|
| 1.0 | Nil to very slight infection (≤10%). | ≤11.11 | Resistant (R) |
| 2.0 | Slight infection, a few lesions scattered on two lower leaves (10.1-20%). | 22.22 | (Score: ≤ 3.0) (DLA:≤ 30%) |
| 3.0 | Light infection, moderate number of lesions scattered on four lower leaves (20.1-30%). | 33.33 | (PDI: ≤ 33.33) |
| 4.0 | Light infection, moderate number of lesions scattered on lower leaves, a few lesions scattered on middle leaves below the cob (30.1-40%). | 44.44 | Moderately resistant (MR) (Score: 3.1–5.0) (DLA: 30.1-50%) |
| 5.0 | Moderate infection, abundant number of lesions scattered on lower leaves, moderate number of lesions scattered on middle leaves below the cob (40.1-50%). | 55.55 | (PDI: 33.34-55.55) |
| 6.0 | Heavy infection, abundant number of lesions scattered on lower leaves, moderate infection on middle leaves and a few lesions on two leaves above the cob (50.1-60%). | 66.66 | Moderately susceptible (MS) (Score: 5.1-7.0) (DLA: 50.1-70%) (PDI: 55.56-77.77) |
| 7.0 | Heavy infection, abundant number of lesions scattered on lower and middle leaves and moderate number of lesions on two to four leaves above the cob (60.1-70%). | 77.77 | |
| 8.0 | Very heavy infection, lesions abundant scattered on lower and middle leaves and spreading up to the flag leaf (70.1-80%). | 88.88 | Susceptible (S) (Score: >7.0) (DLA: >70%) |
| 9.0 | Very heavy infection, lesions abundant scattered on almost all the leaves, plant prematurely dried and killed (>80%). | 99.99 | (PDI: >77.77) |

***DLA- Diseased leaf area; **Per cent disease index (PDI)**

## 1.2 Turcicum Leaf Blight (TLB)/ Northern Corn Leaf Blight (NCLB)

### 1.2.1 Causal organism:

**1.2.1.1 Teleomorph**: *Setosphaeria turcica (Luttr.)* K.J. Leonard & Suggs, 1974

**1.2.1.2 Anamorph synonyms:**

1. *Exserohilum turcicum* (Pass.) Leonard and Suggs, 1974
2. *Helminthosporium turcicum* Pass., 1876
3. *Bipolaris turcica* (Pass.) Shoemaker, 1959
4. *Drechslera turcica* (Pass.) Subram. & B.L. Jain, 1966
5. *Luttrellia turcica* (Pass.) Khokhr., 1978
6. *Helminthosporium inconspicuum* Cooke & Ellis, 1878

### 1.2.2 Host range:

**1.2.2.1 Primary hosts**: *Zea mays* (maize), *Zea mays subsp. mays* (sweet corn), *Sorghum bicolor (*sorghum), *Pennisetum glaucum* (pearl millet).

**1.2.2.2 Wild hosts**: *Sorghum halepense* (Johnson grass), *Panicum miliaceum* (millet), *Pennisetum purpureum* (elephant grass), *Sorghum sudanense* (Sudan grass).

| Races of *E. turcicum* | Resistance genes | | | |
|---|---|---|---|---|
| | Ht1 | Ht2 | Ht3 | HtN |
| 0 | R | R | R | R |
| 1 | S | R | R | R |
| 2 | R | S | R | R |
| 12 | S | S | R | R |
| 23 | R | S | S | R |
| 23N | R | S | S | S |
| 123N | S | S | S | S |

The fungus attacks maize, Sudan grass and sorghum. The maize isolate does not go on to sorghum and *vice versa*. The presence of race II of *E. Turcicum* capable of attacking lines with monogenic resistance (gene ht) was recorded in early seventies (1974). Subsequently occurrence of strains capable of monogenic resistant source (ht.N), B57 was also reported.

### 1.2.3 Economic importance:

In India, the disease was for the first time reported by Butler during 1907 from Bihar. The disease is prevalent in almost all the maize growing areas in India. Severe losses in grain yield due to epiphytotics have been reported in several parts of India and these losses vary from 25 to 90 per cent depending upon the severity of the disease. The disease is responsible for premature death of blighted leaves and results in significant yield reductions. It is considered to be one of the most devastating diseases as it appears in sizeable form in Karnataka resulting in reduction of grain yield of maize by 28 to 91 per cent.

### 1.2.4 Symptoms:

- The disease starts at first as small elliptical spots on the leaves, greyish green in colour and water soaked lesions.
- The spots turn greenish with age and get bigger in size, finally attaining a spindle shape.
- Individual spots are usually 3/4" wide and 2" to 3" long.
- Spores of the fungus develop abundantly on both sides of the spot.

- Heavily infected field present a scorched appearance.
- The disease is recognised by long elliptical greyish or tan lesions. When fully expanded, the spots may be 1½" by 6" in size.
- These lesions appear first on the lower leaves and as the season progresses, the lesion number increases and all the leaves are covered.
- The plants look dead and grey.

### 1.2.5 Morphological identification of *Setosphaeria tutrcica:*

**1.2.5.1 Ascomata:** Black, globose to elliptical, 350-725 x 345-500 µm, ostiolate.

**1.2.5.2 Setae:** Rigid, dark brown, thick-walled, septate which measure up to 150 µm long, 4-6 µm thick.

**1.2.5.3 Pseudoparaphyses:** Filiform, hyaline, septate, branched, anastomosing.

**1.2.5.4 Asci:** Cylindric-clavate, short pedicellate, bitunicat, thick-walled when young, 1-8 spored, 175-250 x 24-31 µm.

**1.2.5.5 Ascospores:** Hyaline, fusoid, 1-6 (mostly 3) - septate, constricted at the septa, straight to slightly curved, 40-78 x 12-18 µm, surrounded by a thin, hyaline mucilaginous sheath which may extend beyond the ends of the spore after discharge.

**1.2.5.6 Conidiophores:** Single or in small groups, simple, cylindrical, olivaceous brown, paler towards the apex, geniculate above, up to 300 µm long, 8-10 µm thick. They emerge in groups of two to six or more through stomata, or less frequently directly through the epidermis.

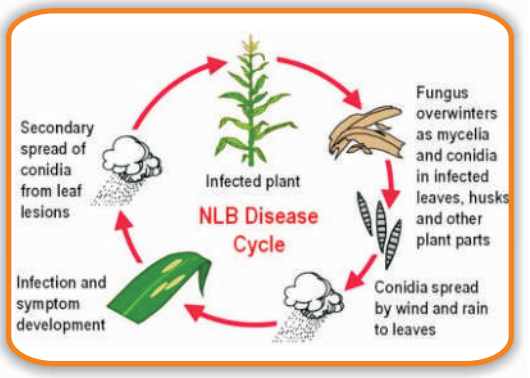
**1.2.5.7 Conidia:** Ellipsoidal to obcavate, pale to olivaceous brown, straight to slightly curved, smooth, 4-9 distoseptate, 50-144 x 18-33 µm with a distinct protuberant hilum. Conidia germinate commonly from one or both polar cells, rarely from intermediate cells (Description taken from Sivanesan, 1987).

## 1.2.6 Epidemiology:

- Maximum severity was noticed from tasselling and six to eight weeks after silking which resulted in heavy loss.
- Saphrophytic survival capacity of the fungus *E. turcicum* in maize field and found that the pathogen survival was 80 per cent up to three weeks of incubation but fell to 10 per cent after 12 weeks.
- The meteorological factors like temperature 22 to 38°C, relative humidity 72 to 98 per cent and rainfall 134 to 165 mm were correlated with increased disease intensity.

## 1.2.6 Life cycle:

Mycelia and conidia of this fungus from infected crop residue, in or on the soil, act as the primary inoculum for the next crop.




(Courtesy: httpwww.pestnet.orgfact)

Conidia can overseason by thickening of their walls to become chlamydospores. The secondary inoculum comes from lesions which produce conidia that are dispersed by wind and can be transported for long distances.

### 1.2.7 Disease screening of maize germplasm:

There are three techniques of preparation of TLB inoculum (AICMIP, 1983) for creation of artificial TLB epiphytotics:

#### 1.2.7.1 Technique I

The most convenient method is by using leaves heavily infected with leaf blight which should have been collected in the season previous to the one during which inoculations are proposed to be done. Care is taken to collect only mature leaves avoiding green leaves. These leaves are stored in loosely packed gunny bags in a cool and dry place. Prior to inoculations the leaves are ground coarse by milling or by putting, them in tightly woven sacks and beating them into coarse powder. By sieving through a suitable sieve powder of proper coarseness can be obtained. About 30 bags of leaves will yield about 10,000 plants four times. A pinch of the leaf powder is dropped into the whorl of each plant which is about 50 cm in height. This is followed by a spray with water using a knapsack sprayer, while directing the spray to the whorl. The inoculations are preferably done late in the afternoon. The inoculations are repeated 3-4 times at weekly intervals.

#### 1.2.7.2 Technique II

Portions of infected leaf with lesions are placed in a moist chamber. Three or four days later new spores develop on the surface of the lesions. These are picked up by means of flattened needle under a dissecting microscope and placed in a droplet of sterile distilled water which is streaked on acidified agar contained in petri plates. The spores after they are allowed to germinate (which they do in a few hours) are cut out with the agar and transferred to acidified PDA in petri plates. Transfer to fresh set of acidified PDA plates can be made after two weeks. When the plates are covered with the fungus the culture is ready for use. About 20 of these plates are put in blender which is run for about 20 seconds, after which it is strained through muslin cloth, to which is added about five litres of water. This is the stock suspension. This stock suspension is diluted by mixing about 12 litres of water to one litre stock suspension. The diluted suspension is sprayed by using a knapsack sprayer which has not been used for spraying any chemical before. Plants which are about 50cm high are sprayed with the suspension with the nozzle directed into whorl.

#### 1.2.7.3 Technique III

Inoculum is increased on whole sorghum grains. About an inch deep layer of sorghum

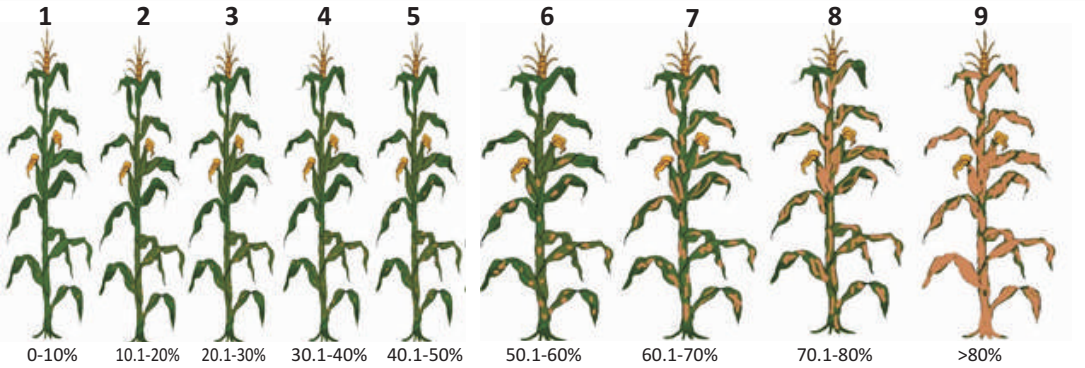
grain contained in a conical flask is soaked in water about 4-6 hours, after which the excess water is drained off. To this is added potato dextrose just enough to provide a thin coating on the grain. The flasks containing the sorghum grain are autoclaved and seeded with the fungus. The flask is shaken once in 2-3 days to facilitate growth on the grains. After incubation of about a fortnight material is ready for inoculation. Two teaspoonful of the grain are mixed with ½ litre of water and blended for two minutes in a blender. After straining the suspension is diluted by adding three parts of water. This diluted suspension is used for inoculating plants as described earlier.

Artificial creation of TLB Epiphytotics through leaf whorl inoculation technique

### 1.2.8 Procedure of recording disease reaction based on diseased leaf area and PDI:

The disease rating of TLB is done at dough stage following 1-9 scale (Chung *et al.,* 2010; Mitiku *et al.,* 2014).

## Diagrammatic scale for assessment of TLB severity on maize plants:




Disease reaction to TLB can be determined based upon disease ratings, disease leaf area (DLA) and PDI at 30-35 days after inoculation mentioned below:

| Rating scale | Degree of infection (per cent DLA*) | PDI** | Disease reaction |
|---|---|---|---|
| 1.0 | Nil to very slight infection (≤10%). | ≤11.11 | Resistant (R) |
| 2.0 | Slight infection, a few lesions scattered on two lower leaves (10.1-20%). | 22.22 | (Score: ≤ 3.0) (DLA:≤ 30%) |
| 3.0 | Light infection, moderate number of lesions scattered on four lower leaves (20.1-30%). | 33.33 | (PDI: ≤ 33.33) |
| 4.0 | Light infection, moderate number of lesions scattered on lower leaves, a few lesions scattered on middle leaves below the cob (30.1-40%). | 44.44 | Moderately resistant (MR) (Score: 3.1–5.0) (DLA: 30.1-50%) |
| 5.0 | Moderate infection, abundant number of lesions scattered on lower leaves, moderate number of lesions scattered on middle leaves below the cob (40.1-50%). | 55.55 | (PDI: 33.34-55.55) |
| 6.0 | Heavy infection, abundant number of lesions scattered on lower leaves, moderate infection on middle leaves and a few lesions on two leaves above the cob (50.1-60%). | 66.66 | Moderately susceptible (MS) (Score: 5.1-7.0) (DLA: 50.1-70%) (PDI:55.56-77.77) |
| 7.0 | Heavy infection, abundant number of lesions scattered on lower and middle leaves and moderate number of lesions on two to four leaves above the cob (60.1-70%). | 77.77 | |
| 8.0 | Very heavy infection, lesions abundant scattered on lower and middle leaves and spreading up to the flag leaf (70.1-80%). | 88.88 | Susceptible (S) (Score: >7.0) (DLA: >70%) |
| 9.0 | Very heavy infection, lesions abundant scattered on almost all the leaves, plant prematurely dried and killed (>80%). | 99.99 | (PDI: >77.77) |

***DLA- Diseased leaf area; **Per cent disease index (PDI)**

## 1.3 Banded Leaf and Sheath Blight (BLSB)

### 1.3.1 Causal organism:

**1.3.1.1 Teleomorph:** *Thanatephorus cucumeris* (Shirai) Tu and Kimbro., *1956*

**1.3.1.2 Anamorph synonyms:**

1. *Rhizoctonia solani* J.G. Kühn, 1858
2. *Pellicularia solani* (J.G. Kühn) Exner, 1953
3. *Moniliopsis solani* (J.G. Kühn) R.T. Moore, 1987
4. *Rhizoctonia ferrugena* Matz (?)
5. *Rhizoctonia napaeae* Westend. & Wallays, 1846
6. *Rhizoctonia napi* Westend., 1861
7. *Rhizoctonia napae* Westend., 1861
8. *Rhizoctonia betae* Eidam, 1887
9. *Rhizoctonia fusca* Rostr., 1893
10. *Moniliopsis aderholdii* Ruhland, 1908
11. *Rhizoctonia mucoroides* G.E. Bernard, 1909
12. *Rhizoctonia potomacensis* Wollenw., 1913
13. *Rhizoctonia microsclerotia* Matz, 1917
14. *Sclerotium griseum* J.A. Stev., 1919
15. *Rhizoctonia melongenae* Matz, 1921
16. *Rhizoctonia dimorpha* Matz, 1921
17. *Rhizoctonia ferruginea* Matz, 1921
18. *Rhizoctonia macrosclerotia* Matz, 1921
19. *Rhizoctonia gossypii* Forsten., 1931
20. *Rhizoctonia gossypii* var. *aegyptiaca* Forsten., 1931
21. *Rhizoctonia solani* var. *hortensis* Schultz, 1936
22. *Rhizoctonia solani* var. *lycopersici* Schultz, 1936
23. *Rhizoctonia solani* var. *ambigua* E. Bald. & Cabrini, 1937
24. *Corticium microsclerotia* (Matz) G.F. Weber, 1939
25. *Rhizoctonia borealis* J.T. Curtis, 1939
26. *Rhizoctonia choussii* Crand. & Arill., 1955
27. *Rhizoctonia dichotoma* H.K. Saksena & Vaartaja, 1960
28. *Rhizoctonia solani* var. *typica* Sneh, Burpee & Ogoshi, 1991
29. *Rhizoctonia solani* Kühn *f. sp. saskii Exnr., 1988*

### 1.3.2 Host range:

*R. solani* is also pathogenic to a wide range of cultivated crops. In addition to maize, anastomosis group AG1-IA is also pathogenic to rice, wheat, sorghum, bean (*Phaseolus* species) and soybean.

### 1.3.3 Economic importance:

The banded leaf and sheath blight (BLSB) is a very destructive disease of maize and considered to be the major constraint for maize production. This pathogen causes losses in grain yield ranging from 11.0 to 40.0 per cent (Singh and Sharma, 1976). Lal

*et al.* (1985) reported that the losses in grain yield to the extent of over 90.0 per cent. The disease is prevalent in hot humid foothill region in Himalayas and in plains.

### 1.3.4 Symptoms:

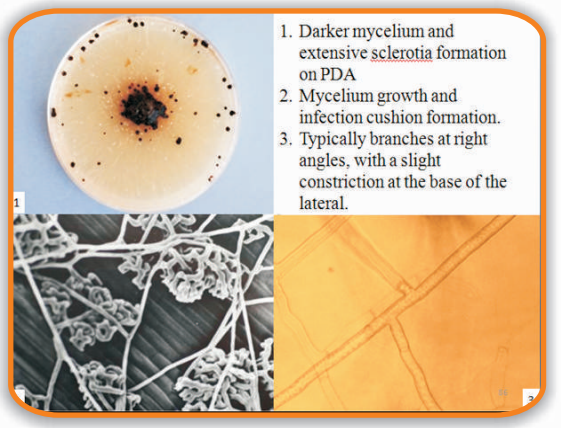
- The disease appears on leaves and sheaths on 40-50 days old plants and later on spread to the ears.
- The characteristic lesions are first seen on lower leaves and sheaths (first and second) in the form of concentric bands and rings.
- The affected plant produces large, gray, tan or brown discoloured areas alternating with dark brown bands.
- Sclerotia later on appear in these diseased areas.
- The developing ear is completely damaged and dried up prematurely with cracking of the husk leaves.
- Brown rotting of the ears may develop which show conspicuous light brown cottony mold with small, round black sclerotia.


(Courtesy: http://petikam.tripod.com/)

### 1.3.5 Morphological identification of *Rhizoctonia solani* Kühn *f. sp. saskii Exnr.*:

#### 1.3.5.1 Mycelium:

Brown pigmentation, typically branches at right angles, with a slight constriction at the base of the lateral and in size 5 to 14 µm diameter of hyphae. It has dolipore septa, and multinucleate cells. The fungus lacks asexual spores, and it commonly forms sclerotia in culture, and may form barrel-shaped cells in plant tissue.



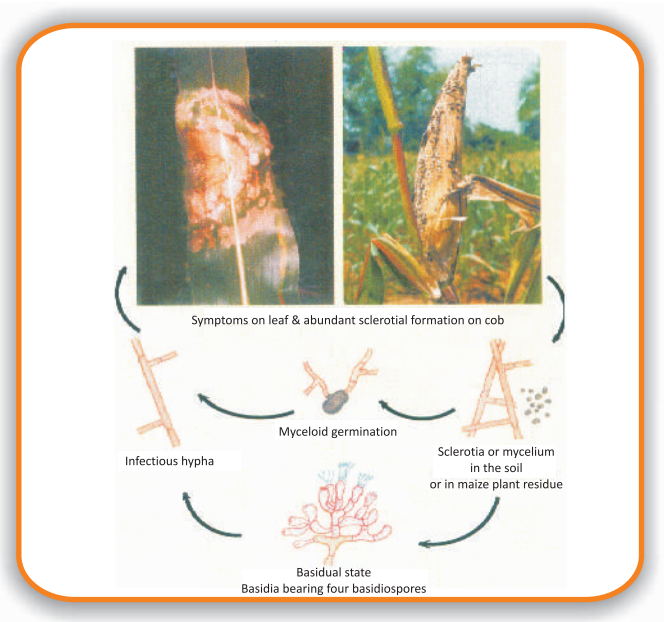

**1.3.5.2 Sclerotia:** Loose type, darken to reddish-brown in colour and central part is made up by pseudoparenchymatous tissue.

### 1.3.6 Epidemiology:

The pathogen is known to prefer warm wet weather, and outbreaks typically occur in the early summer months. Most symptoms of the pathogen do not occur until late summer and thus most farmers do not become aware of the diseased crop until harvest. A combination of environmental factors have been linked to the prevalence of the pathogen such as: presence of host plant, frequent rainfall/irrigation and increased temperatures in spring and summer. In addition, a reduction of drainage of the soil due to various techniques such as soil compaction are also known to create favorable environments for the pathogen. The pathogen is dispersed as sclerotia, and these sclerotia can travel by means of wind, water or soil movement between host plants.

### 1.3.7 Disease cycle:

Pathogen can survive in the soil for many years. Thick outer layer of sclerotia allow for their survival in overwinter. In rare cases (in teleomorph) the pathogen may also take on the form of mycelium. The fungus is attracted to the plant by chemical stimuli released by a growing plant. The process of penetration of a host can be accomplished in a number of ways. Entry can occur through direct penetration of the plant cuticle/epidermis/ natural openings in the plant. Pathogen produces an appressorium which penetrates the plant cell. The pathogen can also release enzymes that break down plant cell walls. Pathogen colonize and grow inside dead tissue and forms sclerotia. New inoculum is produced on or within the host tissue. A new cycle is repeated when new plants become available. The disease cycle begins as such-




(Courtesy: Dr. R.S. Rathore, Ex-Prof. & Head, MPUAT)

1. The sclerotia/mycelium overwinter in plant debris, soil or host plants
2. The young hyphae and fruiting basidia (rare) emerge and produce mycelium and rarely basidiospores.

## 1.3.8 Disease screening of maize germplasm:

### 1.3.8.1 Technique I

Germplasm screening in sick plot is most accepted approach for disease screening against soil borne diseases irrespective of any crop. Evaluation of breeding material can be accomplished easily and directly in the field with little expense by the use of naturally or artificially infested fields or plots ('sick plots', SP). The main advantage of SP is that they allow simultaneous screening of a large amount of genetic material under environmental conditions similar to those of cultivated plants. SPs should be established based on the presence of the disease as indicated by visual symptoms and reisolation of the causal fungus. In contrast to diseases caused by foliar fungi where natural epidemics are unpredictable, incidence of disease caused by soil-borne fungal pathogens in a SP is much more reliable.

Sick plots are being developed at hot spot locations of BLSB of maize in the country by fixing the an isolated plot and following maize monocropping since last few years, its incorporation in soils and amending the soils with grain culture of *Rhizoctonia solani Kuhn f. sp. saskii* Exnr over the years. Detailed procedure for development of BLSB sick plot is as follows:

1. Select an isolated plot of adequate size to avoid spread of the fungus inoculum from this plot to others.
2. The plot should have had maize crop in the previous year, and at least traces of BLSB incidence should have been observed.
3. Incorporate chopped small pieces of BLSB infected plants collected from this plot and other fields uniformly in the surface soil of the selected plot.
4. Plant a sole crop of a highly susceptible cultivar in this plot. Ensure a good plant population and carry out normal agronomic operations.
5. By the end of the season, at least 20 per cent of the plants should show BLSB symptoms. After harvesting and threshing; scatter the debris uniformly all over the plot and incorporate it by dicing.
6. Repeat step 3; this will help in increasing the level of the inoculum to make the soil "sick".
7. Repeat steps 3 and 4 in the next season. By the end of this season, 90 per cent BLSB incidence should be recorded. If the incidence is less than 70 per cent, repeat steps 3 and 4 one more time.
8. Initiate screening in the next season and plant a susceptible cultivar after every ten test rows in the whole field. These rows will serve as checks, and will help

in monitoring and maintaining the sickness of the plot. The susceptible check rows should show more than 90 per cent infection.

9. From the 4th or 5th year onwards, a susceptible check can be planted after every 20 test rows. Always include susceptible and resistant checks for comparison for estmating the disease pressure in the plot.
10. Record disease incidence/ diseased leaf area (DLA)/ disease score/ PDI at 45 days after inoculation.

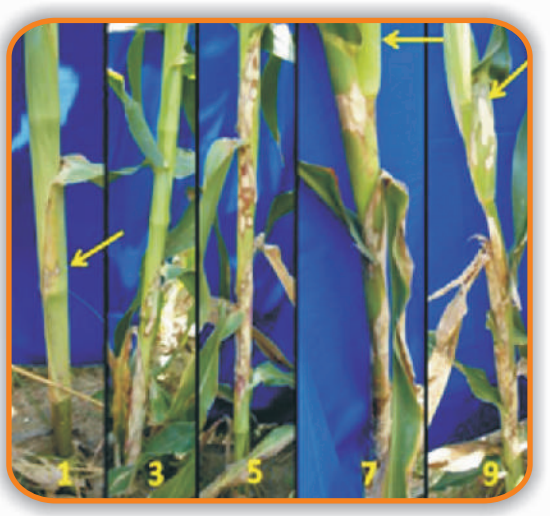
**1.3.8.2 Technique II**

In absence of sick plot, germplasm screening is undertaken in artificially created BLSB epiphytoics. Grain culture of *Rhizoctonia solani Kuhn f. sp. saskii* Exnr. is prepared in laboratory condition for inoculation in the field for creation of epiphytotics. The detailed procedure of inoculum multiplication is described hereafter.

Soak barley grains in water for 24 hours and dispense 40g in 250ml Erlenmeyer flask after removing excess water; autoclave at a pressure of 1.05kg/sq cm for 30 minutes. In case of 100ml flasks only 10g of material may be used. Homogenise 2-3 days old growth of pathogen taken from potato dextrose agar in sterile water and seed 5ml in each flask. Incubate at 27$^0$C for ten days. The barley grain culture can be immediately used for inoculation or can be air dried at room temperature and stored in paper bags (but not in plastic or butter paper) or in the flask themselves at 15$^0$C for subsequent use (Ahuja and Payak, 1978)

## 1.3.9 Inoculation techniques:

**1.3.9.1 Under field conditions:** Inoculations should be made during the rainy season (July and August) on 30 to 45 day old plants with grain culture (using four grains) inserted between stalk and sheath at second or third intermodal level from soil. Grains placed at junction of sheath and leaf can also create optimum disease level and do not fall away with strong wind or heavy rain (Ahuja and Pathak, 1978).



(Courtesy: Vimla et.al., 2018)

**1.3.9.2 Laboratory evaluation:** Single grain culture is placed on adaxial surface of leaf piece (8.0 x5.0cm), collected

from 30 to 45 day old plants and floated on distilled water in petri dishes. Inoculated leaf pieces are incubated at 27±1$^0$C for two days (Ahuja and Pathak, 1981a).

### 1.3.10 Procedure of recording disease reaction based on disease ratings, diseased leaf area and PDI:

**1.3.10.1 Under field conditions:** Disease is recorded after 45 days of inoculations on basis of following modified 1-9 rating scale of AICMIP (1983) and Muis and Quimio (2006).

| Rating scale | Degree of infection (per cent DLA) | PDI | Disease reaction |
|---|---|---|---|
| 1.0 | Disease on one leaf sheath only; few small, non-coalescent lesions present (≤10%). | ≤11.11 | Resistant (R)<br>(Score: ≤ 3.0)<br>(DLA:≤ 30%)<br>(PDI: ≤ 33.33) |
| 2.0 | Disease on two sheaths; lesions large and coalescent (10.1-20%). | 22.22 | |
| 3.0 | Disease up to four sheaths; lesions many and always coalescent (20.1-30%). | 33.33 | |
| 4.0 | As in disease rating symptoms of 3.0, + rind discolored with small lesions (30.1-40%). | 44.44 | Moderately resistant (MR)<br>(Score: 3.1–5.0)<br>(DLA: 30.1-50%)<br>(PDI: 33.34-55.55) |
| 5.0 | Disease on all sheaths except two internodes below the ear (40.1-50%). | 55.55 | |
| 6.0 | Disease up to one internode below ear shoot; rind discoloration on many internodes with large depressed lesions (50.1-60%). | 66.66 | Moderately susceptible (MS)<br>(Score: 5.1-7.0)<br>(DLA: 50.1-70%)<br>(PDI:55.56-77.77) |
| 7.0 | Disease up to the internodes bearing the ear shoot but shank not affected (60.1-70%). | 77.77 | |
| 8.0 | Disease on the ear; husk leaves show bleaching, bands and cracking among themselves as also silk fibers; abundant fungal growth between and on kernels; kernels formation normal except being lusterless; ear size less than normal; some plants prematurely dead (70.1-80%). | 88.88 | Susceptible (S)<br>(Score: >7.0)<br>(DLA: >70%)<br>(PDI: >77.77) |
| 9.0 | In addition to disease rating symptoms of 8.0, shrinkage of stalk; reduced ear dimension; wet rot and disorganization of ear; kernel formation absent or rudimentary; prematurely dead plants common; abundant sclerotia production on husk leaves, kernels ear tips and silk fibres (>80%). | 99.99 | |

**1.3.10.2 Laboratory conditions:** Disease is recorded on basis of extent of area affected following the modified method of Ahuja and Payak (1981a&b).

| Rating scale | Diseased leaf area (%) | Disease reaction |
|---|---|---|
| 1.0 | ≤10.00 | Resistant (R) (Score: ≤ 3.0) (DLA: ≤30%) |
| 2.0 | 10.1-20 | |
| 3.0 | 20.1-30 | |
| 4.0 | 30.1-40 | Moderately resistant (MR) (Score: 3.1–5.0) (DLA: 30.1-50%) |
| 5.0 | 40.1-50 | |
| 6.0 | 50.1-60 | Moderately susceptible (MS) (Score: 5.1-7.0) (DLA: 50.1-70%) |
| 7.0 | 60.1-70 | |
| 8.0 | 70.1-80 | Susceptible (S) (Score: >7.0) (DLA: >70%) |
| 9.0 | >80 | |

## 2. MASS SCREENING TECHNIQUES FOR RESISTANCE TO LEAF SPOTS

### 2.1 Brown Spot (BS)

**2.1.1 Causal organism:** *Physoderma maydis* (Miyabe) Miyabe, 1909

**Teleomorph synonym:** *Cladochytrium maydis* Miyabe, 1903

**2.1.2 Host range:**

*Zea mays* (maize), *Zea mays* subsp. *mexicana* (teosinte)

**2.1.3 Economic importance:**

Brown spot of maize caused by the fungus *Physoderma maydis* was first reported from India by Shaw in 1910. Initially it was named as *Physoderma zeae maydis* Shaw which was later changed to *P. maydis* (Shaw) Maybe. Subsequently the disease was recorded from USA, Brazil, Colombia, Mozambique, and Guatemala. Mexico, Panama, Kenya, Congo, Chamois and Cameroon. In India the disease has been found in different states *viz;* Maharashtra, West Bengal, Rajasthan, Himachal Pradesh and Kashmir. Tisdale (1919) reported a loss of 5-10 per cent in the yield of maize due to brown spot. Simmonds (1956) reported 30 per cent foliage loss by the disease. Broyles (1959) recorded losses of 67 per cent and 25 per cent in grain yield in artificial and natural

epidemic conditions, respectively, in a susceptible single cross WC7 x CI21; however, a large scale field estimate showed a reduction of only 1.9 per cent. Thakore (1974) found reduction in grain yield up to 27 per cent and 24 per cent in artificially inoculated plants of Ganga 5 and Malan, respectively, while in naturally infected plants the reduction was up to 21 and 16 per cent, respectively.

### 2.1.4 Symptoms:

The disease normally occurs in areas of high rainfall and high mean temperatures

- It attacks leaves, leaf sheaths, stalks, and sometimes outer husk
- The first noticeable symptoms develop on leaf blades and consist of small chlorotic spots, arranged as alternate bands of diseased and healthy tissue.
- Spots on the mid-ribs are circular and dark brown, while lesions on the laminae continue as chlorotic spots.
- Nodes and internodes also show brown lesions.
- In severe infections, these may coalesce and induce stalk rotting and lodging.

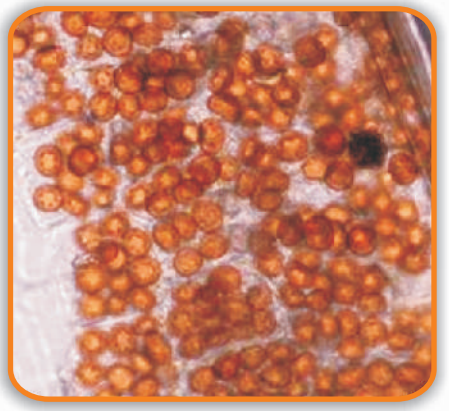
### 2.1.5 Epidemiology:

Favourable condition: Sporangia require water on leaf surfaces and relatively high temperature (23 to 30$^0$C).

### 2.1.6 Morphological identification of *Physoderma maydis*:

**2.1.6.1 Parasitic nature:** Obligate parasite

**2.1.6.2 Resting sporangia/ resting spore:** Yellow to brown, smooth, thick walled, oval or nearly circular in a median plane but markedly flattened on one surface, produced terminally or intercalarily on rhizomycelium



*(Courtesy: httpstwitter.comlaurajesseisu) Physoderma maydis sporangia in diseased leaf tissue.*

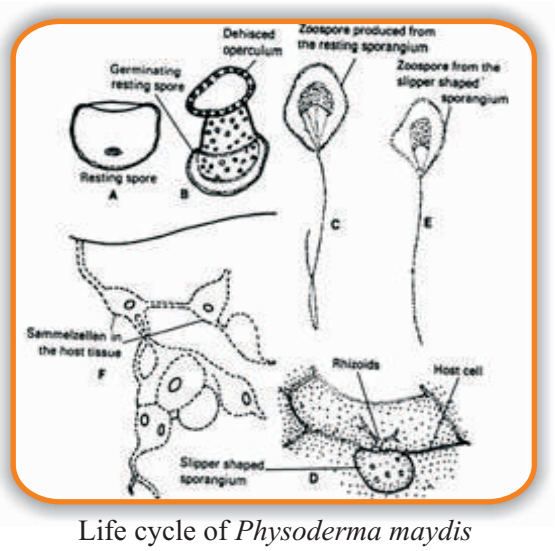
**2.1.6.3 Endosporangium:** Conical or pyriform, thin walled with saucer shaped lid with apical papilla.

**2.1.6.4 Zoospore:** Posterioraly uniflagellate and ellipsoidal, with a large eccentric hyaline refractive globule.

Infection hyphae expand within the host cells to form several enlarge storage cells called *Sammelzellen*.

### 2.1.7 Disease cycle:

The thick-walled, brown sporangia (resting spores) formed within infected cells enable *P. maydis* to overseason in corn debris or in the soil. The sporangia are released from infection pustules, disintegrating corn debris and soil are carried to susceptible plants by air currents, insects, splashing rain or flowing water, and humans. Free water is required for infection. When moisture is present in the whorl or behind the leaf sheaths and temperatures are relatively high (23 to 30° C), a sporangium "germinates" to release 20 to 50 swimming zoospores. The zoospores move about in water for 1 to 2 hours before settling down, becoming amoeba-like, and penetrating young meristematic tissue with fine infection hyphae. Infection commonly occurs in a diurnal cycle. Zoospores of *P. maydis* can infect corn tissue only during certain hours of the day and within a few hours after being released.




Life cycle of *Physoderma maydis*
(Anonymous, 1993)

The development of symptoms and the germination of new sporangia occur approximately 6 to 20 days after infection, completing the disease cycle.

### 2.1.8 Disease screening of maize germplasm:

The technique of preparation of brown spot inoculum (AICMIP, 1983) for creation of artificial epiphytotics is:

Whorl inoculation has been found to be most efficient. Five thousand sporangia per millilitre of water make optimum inoculum concentration. For preparation of inoculum the infected leaves (fresh or stored for 1-2 years) are taken and cut in small pieces. These are put in water for thorough moistening and then blended in a waring blender in tap water. The resultant is filtered through muslin cloth. The filtrate is then

diluted by pouring water to bring the concentration of sporangia up to 5000/ml of water. This inoculum is filled in small dropper bottles and the desired plants at susceptible stage (30±10 days) are inoculated by putting 2-3 drops of inoculum into the whorl. The disease appears after 10-20 days.

### 2.1.9 Procedure of recording disease reaction based on disease ratings, diseased leaf area and PDI:

Disease rating is done with following modified scale of AICMIP (1983*)*:

| Rating scale | Degree of infection (per cent DLA) | PDI | Disease reaction |
|---|---|---|---|
| 1.0 | Nil to very slight infection (≤10%). | ≤11.11 | Resistant (R)<br>(Score: ≤ 3.0)<br>(DLA:≤ 30%)<br>(PDI: ≤ 33.33) |
| 2.0 | Slight infection, a few lesions scattered on two lower leaves (10.1-20%). | 22.22 | |
| 3.0 | Light infection, moderate number of lesions scattered on four lower leaves (20.1-30%). | 33.33 | |
| 4.0 | Light infection, moderate number of lesions scattered on lower leaves, a few lesions scattered on middle leaves below the cob (30.1-40%). | 44.44 | Moderately resistant (MR)<br>(Score: 3.1–5.0)<br>(DLA: 30.1-50%)<br>(PDI: 33.34-55.55) |
| 5.0 | Moderate infection, abundant number of lesions scattered on lower leaves, moderate number of lesions scattered on middle leaves below the cob (40.1-50%). | 55.55 | |
| 6.0 | Heavy infection, abundant number of lesions scattered on lower leaves, moderate infection on middle leaves and a few lesions on two leaves above the cob (50.1-60%). | 66.66 | Moderately susceptible (MS)<br>(Score: 5.1-7.0)<br>(DLA: 50.1-70%)<br>(PDI: 55.56-77.77) |
| 7.0 | Heavy infection, abundant number of lesions scattered on lower and middle leaves and moderate number of lesions on two to four leaves above the cob (60.1-70%). | 77.77 | |
| 8.0 | Very heavy infection, lesions abundant scattered on lower and middle leaves and spreading up to the flag leaf (70.1-80%). | 88.88 | Susceptible (S)<br>(Score: >7.0)<br>(DLA: >70%)<br>(PDI: >77.77) |
| 9.0 | Very heavy infection, lesions abundant scattered on almost all the leaves, plant prematurely dried and killed (>80%). | 99.99 | |

## 2.2 Curvularia Leaf Spot (CLS)

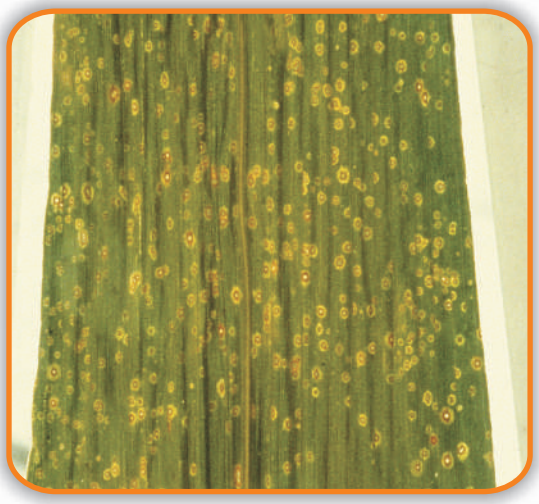
### 2.2.1 Causal organism: *Curvularia lunata* (Wakker) Boedijn, 1933

### 2.2.2 Host range:

*Vigna rediata, Glycene max, Arachis hypogea, Vigna mungo, Vigna sinensis, Crotolaria juncea, Trifolium alexandrianum, Oryza sativa, Zea mays, Pennisetum typhoides, Gossypium hirsutum, G. herbaceium and G. arboretum.*

### 2.2.3. Economic importance:

The leaf spot of maize is caused by *Curvularia lunata*. This disease is a very important seed and soil borne disease prevalent in the hot, humid maize areas. The disease produces small necrotic or chlorotic spot with a light colored halo and this cause significant damage to maize up to 60 per cent due to great loss of photosynthetic region of the crop.



Courtesy: httpswww.flickr.comphotoscimmyt

### 2.2.4 Symptoms:

- These fungi produce small necrotic or chlorotic spots with a light colored halo.
- Lesions are about 0.5 cm in diameter when fully developed
- The centre of each lesion is straw coloured to light brown, which is surrounded by a dark brown margin
- The disease is prevalent in hot, humid maize areas and can damage the crop significantly.

### 2.2.5 Morphological identification of *Curvularia* spp.:

**2.2.5.1 Culture:** Effuse, brown, grey or black, hairy, cottony or velvety.

**2.2.5.2 Stromata:** Often large, erect, black, cylindrical, somtimes branched.

Courtesy: https://atrium.lib.uoguelph.ca/xmlui

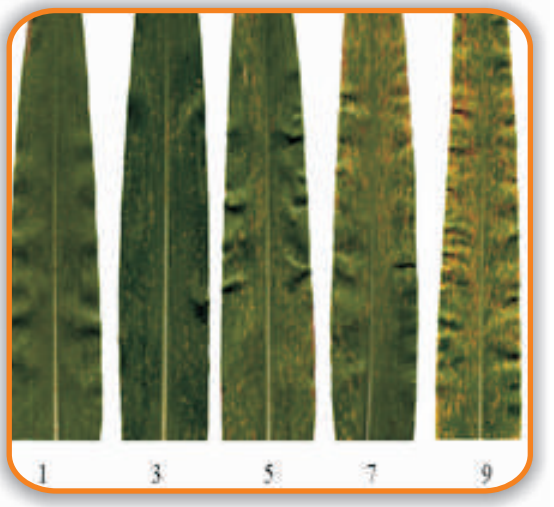
**2.2.5.3 Conidiophores:** Macronematous, mononematous, straight or flexuous, often geniculate, sometimes nodose, brown, usually smooth.

**2.2.5.4 Conidiogenous cells:** Polytretic, integrated, terminal, somtimes later becoming intercalary sympodial, cylindrical or occasionally swollen, cicatrised

**2.2.5.5 Conidia:** Solitary, acropleurogenous, simple, often curved, clavate, ellipsoidal, broadlly fusiform, obvoid or pyriform with three or more transverse septa. Pale or dark brown, Dark bands at the septa, smooth and verrucose, hilum (Paul, 1980).

### 2.2.6 Disease screening of maize germplasm:

The technique of preparation of CLS inoculum for creation of artificial epiphytotics:




Mass multiplication of culture is done on half cooked sorghum grains and after evaporating excess moisture from surface, the grains are filled in 500 ml conical flasks and plugged properly. These are autoclaved for two hours at 15 lbs pressure and inoculated when cooled down at room temperature with pure culture of *Curvularia lunata*. After completion of mycelial growth which may take 15-20 days at temperature around 25-27 degree C, these grains are washed in RO water to get conidial suspension of $5x10^4$ conidia per ml. A bucket full of suspension is enough for spray inoculation of two 480 meter strip. The washed grains are spread in a tray to get again mass of conidia. After two days gap, one more spray inoculation is done as per previous method, but this time conidial suspension should be half of the previous one.

### 2.2.7 Procedure of recording disease reaction based on disease ratings, diseased leaf area and PDI:

At least three observations are made and third observation at 80-85 DAS would be final based on leaf area covered by spots caused by pathogen. Observations are recorded using 1-9 rating scale (Hou *et al.,* 2013) as described below:

| Rating scale | Degree of infection (per cent DLA) | PDI | Disease reaction |
|---|---|---|---|
| 1.0 | ≤10% area of leaf infected | ≤11.11 | Resistant (R) (Score: ≤ 3.0) (DLA:≤ 30%) (PDI: ≤ 33.33) |
| 2.0 | 10.1-20% area of leaf infected | 22.22 | |
| 3.0 | 20.1-30% area of leaf infected | 33.33 | |
| 4.0 | 30.1-40% area of leaf infected | 44.44 | Moderately resistant (MR) (Score: 3.1–5.0) (DLA: 30.1-50%) (PDI: 33.34-55.55) |
| 5.0 | 40.1-50% area of leaf infected | 55.55 | |
| 6.0 | 50.1-60% area of leaf infected | 66.66 | Moderately susceptible (MS) (Score: 5.1-7.0) (DLA: 50.1-70%) (PDI:55.56-77.77) |
| 7.0 | 60.1-70% area of leaf infected | 77.77 | |
| 8.0 | 70.1-80% area of leaf infected | 88.88 | Susceptible (S) (Score: >7.0) (DLA: >70%) (PDI: >77.77) |
| 9.0 | >80% area of leaf infected | 99.99 | |

## 2.3 Zonate Leaf Spot (ZLS)

### 2.3.1 Causal organism: *Gloeocercospora sorghi* D.C. Bain & Edgerton, 1943

Synonyms: *Microdochium sorghi* (D.C. Bain & Edgerton ex Deighton) U. Braun, 1995

### 2.3.2 Host range:

*Agrostis capillaris* (common bent), *Agrostis stolonifera* var. *palustris* (bent grass), *Cynodon dactylon* (Bermuda grass), *Pennisetum glaucum* (pearl millet), *Pennisetum purpureum* (elephant grass), *Saccharum officinarum* (sugarcane), *Sorghum bicolor* (sorghum), *Sorghum halepense* (Johnson grass), *Sorghum sudanense* (Sudan grass), *Zea mays* (maize).

### 2.3.3 Economic importance:

*G. sorghi* produced typical zonate leaf spots on maize and sorghum in India, damaging up to 85 per cent of the photosynthetic leaf area under humid and cloudy weather conditions. Leaf weight decreased and leaf dry matter content increased with increasing severity of infection. This type of damage affects forage production.

### 2.3.4 Symptoms:

- The first visible symptoms are the appearance of small non-diagnostic lesions on the lower leaves.
- Lesions may occur anywhere on the leaf.
- As the lesions mature they become circular or target shaped on the interior of the leaf and semicircular on the leaf margins.


Courtesy: www.flickr.com/photos/cimmyt

- Lesion appearance and size is variable.
- Lesions elongate, run together, and whole leaves may be blighted.
- When the weather is favorable the disease progresses up the plant and lesions may occur on all leaves of the plant.

### 2.3.5 Morphological identification of *Gloeocercospora sorghi*:

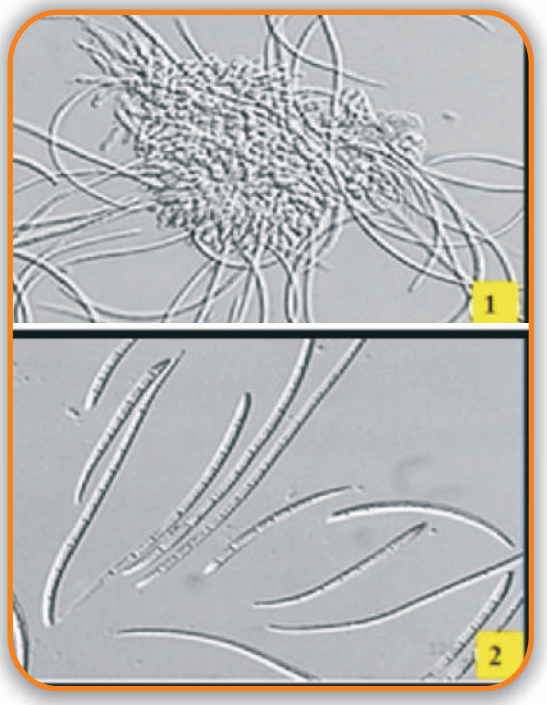
**2.3.5.1 Sporodochia:** Pink to salmon colored gelatinous spore masses

**2.3.5.2 Conidiophores:** Hyaline, septate, short, 5-10 µm in length, simple or branched

**2.3.5.3 Conidia:** Saptate, Hyaline, elongate to filiform, variable length 20-195x1.4-3.2 µm; generally born in a slimy matrix, salmon in mass

**2.3.5.4 Sclerotia:** 0.1-0.2 µm in diameter, black lenticular to spherical, in necrotic host tissue

**2.3.5.5 Mycelia:** Hyaline, septate, branching




### 2.3.6 Disease screening of maize germplasm:

The fungus is isolated from zonate leaf

spot infected maize plants on Potato Dextrose Agar (PDA) and incubated at 28 ± 1°C. The growing mycelium from the margin of distinct colonies is then sub-cultured on fresh petriplates containing (PDA) to obtain pure culture. Plants in the field are artificially inoculated by spraying the spore suspension of *Gloeocercospora sorghi* containing $5\times10^4$ spores/ml. The inoculum was sprayed between 6-7 pm as night temperature and humidity were conducive for infection.

### 2.3.7 Procedure of recording disease reaction based on disease ratings, diseased leaf area and PDI:

The observations on disease severity are recorded in 1-9 scale mentioned below:

| Rating scale | Degree of infection (per cent DLA) | PDI | Disease reaction |
|---|---|---|---|
| 1.0 | 0 to ≤1% leaf area covered/ no symptom | ≤11.11 | Resistant (R) (Score: ≤ 3.0) (DLA: ≤ 10%) (PDI: ≤ 33.33) |
| 2.0 | 1.1 to 5% leaf area covered | 22.22 | |
| 3.0 | 5.1 to 10% leaf area covered | 33.33 | |
| 4.0 | 10.1 to 20% leaf area covered | 44.44 | Moderately resistant (MR) (Score: 3.1–5.0) (DLA: 10.1-30%) (PDI: 33.34-55.55) |
| 5.0 | 20.1 to 30% leaf area covered | 55.55 | |
| 6.0 | 30.1 to 40% leaf area covered | 66.66 | Moderately susceptible (MS) (Score: 5.1-7.0) (DLA: 30.1-50%) (PDI:55.56-77.77) |
| 7.0 | 40.1 to 50% leaf area covered | 77.77 | |
| 8.0 | 50.1 to 75% leaf area covered | 88.88 | Susceptible (S) (Score: >7.0) (DLA: >50%) (PDI: >77.77) |
| 9.0 | >75% leaf area covered | 99.99 | |

## 3. MASS SCREENING TECHNIQUES FOR RESISTANCE TO MAIZE RUSTS

### 3.1 Polysora Rust (PR)/ Southern Rust (SR)

**3.1.1 Causal organism:** *Puccinia polysora* Underw., 1897

**3.1.1.1 Teliomorph**: *Dicaeoma polysorum* (Underw.) Arthur, 1906

**3.1.2 Host range:** *Poaceae* (grasses), *Themeda* (kangaroo grass), *Tripsacum dactyloides* (eastern gamagrass (USA)), *Zea mays* (maize), *Zea mays* subsp. *mays* (sweetcorn), *Zea mays* subsp. *mexicana* (teosinte)

### 3.1.3 Economic importance:

Polysora rust (Southern rust) is a major disease of maize in tropical and subtropical regions worldwide. Unlike common rust, polysora rust is most severe in warm growing conditions. In some seasons characterised by warm growing conditions, polysora rust can become a major constraint to maize production in temperate regions. Yield losses in excess of 45 per cent have been recorded due to polysora rust

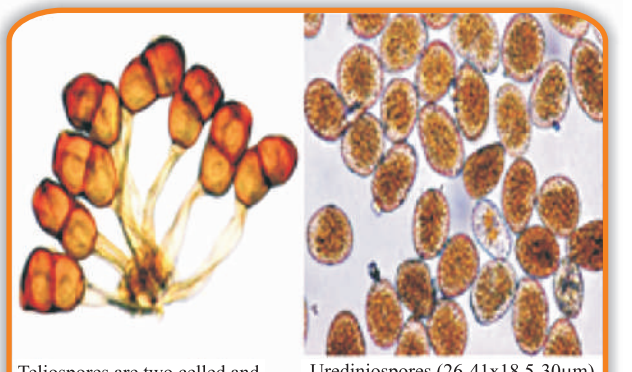
### 3.1.4 Symptoms:

- The pustules being lighter in color, smaller (0.2 to 2 mm long), and circular to oval.
- The orange-red to light cinnamon brown pustules are often much more numerous on the upper surfaces of leaves.
- But slower and less abundant on the lower leaf surface.
- The pustules are initially less powdery than common corn rust.
- The leaf epidermis remains intact covering the pustule for a long time, frequently opening by a longitudinal slit.
- Chocolate brown to black teliospores are produced in a circle around the uredial pustule later in the season

### 3.1.5 Morphological identification of *Puccinia polysori :*

Only uredial and telial stages have been observed.

**3.1.5.1 Uredinia:** Small, circular to oval cinnamon brown, uniformly distributed



Teliospores are two celled and measure 26-50x16-27 µm

Urediniospores (26-41x18.5-30µm) of *P. polysora*

(Courtesy: (http://www.plantwise.org)

**3.1.5.2 Urediniospores:** Yellowish to golden brown, ellipsoid, echinulate, 4-5 equatorial pores (26-41x 18.5-30µm)

**3.1.5.3 Urediniospore release**– Up to 220 spores/day from early June to mid-August

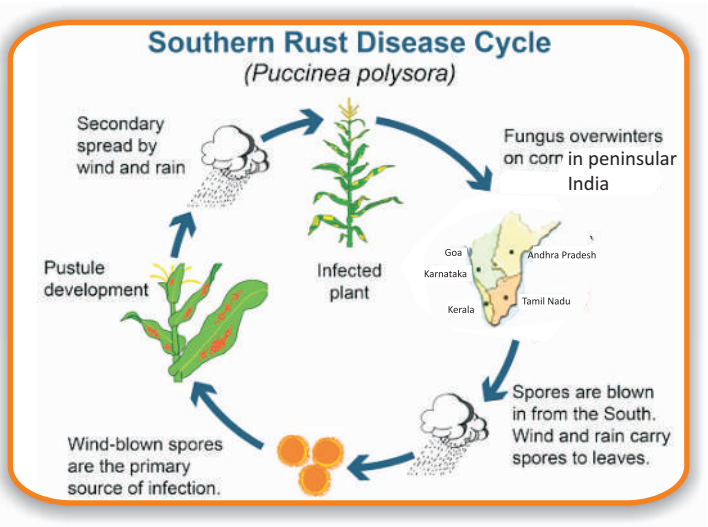
**3.1.5.4 Teliospore:** Bi-celled, dark red in colour and measure 26-50X 16-27µm

### 3.1.6 Epidemiology:

Factors favouring development are as follows:

- Periods of extended dew or high humidity.
- In temperate regions, polysora rust is more prevalent towards the end of the growing season when temperatures rise.
- Late planted and late maturing maize.
- Polysora rust is favored by warm and humid conditions.
- Optimal temperature for urediospore germination and infection from 24 to 28°C.
- Periods of extended dew or high humidity favor polysora rust.

### 3.1.7 Disease cycle:




(Courtesy: www.pioneer.com)

Urediniospores of this fungus are windblown from previously infected corn leaves, and are blown progressively northward during the growing season. Although teliospores are produced, they do not rupture the epidermis and have not been shown to germinate, and thus are unimportant in the disease cycle. Disease progress is most rapid when favorable temperatures of 77° to 80°F (25° to 28°C) and high humidity occur in fields of susceptible varieties. As with the common rust pathogen, free water as dew is needed on plant surfaces for urediniospore germination and initial penetration of the corn leaf. Symptoms appear about 3 to 6 days after infection and by 7 to 10 days, the pustules may rupture to expose

mature urediniospores. Southern rust is associated with high temperatures, high relative humidity, and heavy rainfall.

### 3.1.8 Disease screening of maize germplasm:

The technique of preparation of rust inoculum (AICMIP, 1983) for creation of artificial epiphytotics is:

The rust is an obligate parasite and thus, it is very difficult to grow it on artificial media under laboratory condition. Though, for some specific purposes small amount of inoculum can be grown under laboratory condition on detached leaf culture. But, this meagre amount of culture obtained by such method is not sufficient to be utilized for large scale screening trials under field conditions. Therefore, naturally infected leaves showing large number of uredopustules may be collected from different places so that all the prevalent races in the areas may be utilized for screening the materials against the prevalent rust fungus. The infected leaves thus collected should be macerated thoroughly in between two palms of the hands dipped under a bucket of water until the water gets sufficiently coloured. The uredospores can also be collected on a butter paper by tapping the severely infected leaves with fingers and then stored in glass vial or glass tube which can be sealed easily under a flame. The uredospores, thus obtained may be kept for longer period in the freezer at lower temperature i.e. 5-7°C and can also be easily carried to some distant places for inoculation purposes. For inoculating the plants in a field use of a knapsack sprayer is very useful. The spore suspension should be sprayed over the plants during the second half of the day when the sun becomes mild. While spraying inoculum, the nozzle of the sprayer should be kept over whorl of the plant and all the leaves may be sprayed thoroughly. The spore suspension must be stirred continuously during spraying as the light spores aggregate together on the upper surface of the water. Repeating the inoculation two to three times gives a good result. In addition 2-4 lines of susceptible varieties grown as border rows around the screening plots also help to spread the disease.

### 3.1.9 Procedure of recording disease reaction based on disease rating, diseased leaf area and PDI:

Disease rating is done as per scale devised by Lubberstedt *et al.* (1998) and Paterniani *et al.* (2000).

| Rating scale | Degree of infection (per cent DLA) | PDI | Disease reaction |
|---|---|---|---|
| 1.0 | No uredia or hypersensitive flecks | 0.00 | Immune/HR (Score: ≤ 1.0) (DLA: 0%) (PDI: 0.00) |
| 2.0 | Very slight infection, one or two pustules on lower leaves only (0.1-1%). | 22.22 | Resistant (R) (Score: 1.1-2.0) (DLA: 0.1-1.0%) (PDI: ≤ 22.22) |
| 3.0 | Very slight to slight infection, few scattered pustules on lower leaves only (1.1-10%). | 33.33 | Moderately resistant (MR) (Score: 2.1–4.0) (DLA: 1.1-20%) (PDI: 22.23-44.44) |
| 4.0 | Light infection, few scattered pustules on lower leaves only (10.1-20.0%) | 44.44 | |
| 5.0 | Moderate infection, moderate number of pustules on lower leaves only (20.1-30%) | 55.55 | Moderately susceptible (MS) (Score: 4.1-6.0) (DLA: 20.1%-40%) (PDI: 44.45-66.66) |
| 6.0 | Moderate infection, abundant pustules on lower leaves; few on middle leaves (30.1-40%) | 66.66 | |
| 7.0 | Severe infection, abundant pustules on lower and middle leaves (40.1-60%) | 77.77 | Susceptible (S) (Score: 6.1-7.0) (DLA: 40.1-60%) (PDI: 66.67-77.77) |
| 8.0 | Severe infection, abundant pustules on lower and middle leaves; extending to upper leaves (heavy infection) (60.1-80%) | 88.88 | Highly susceptible (Score: >7.0) (DLA: >60%) (PDI: >77.77) |
| 9.0 | Severe infection, abundant pustules on all leaves, plant may dry prematurely or killed by the disease (very heavy infection) (>80%) | 99.99 | |

## 3.2 Common Rust (CR)

### 3.2.1 Causal organism: *Puccinia sorghi* Schw., 1832

#### 3.2.1.1 Teliomarph synonym:

1. *Dicaeoma sorghi* (Schwein) Kuntz., 1851
2. *Puccinia maydis* Berenger, 1844
3. *Puccinia zeae* Berenger, 1851

### 3.2.2 Host range: *Zea mays* (maize), *Oxalis* spp.

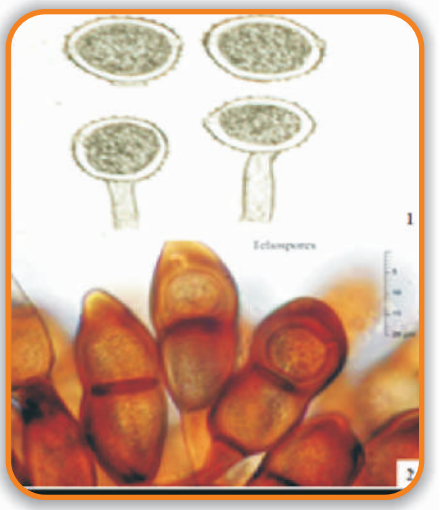
### 3.2.3 Economic importance:

Common rust on corn is caused by the fungus *Puccinia sorghi*. Epidemics of this disease can cause serious losses in yield and quality of corn. High rust susceptibility of many popular sweet corn hybrids is a major factor contributing to rust epidemics. Another factor is that sweet corn is usually planted over an extended period from May

through June for fresh and processing uses. The staggered planting schedules result in high concentrations of fungal spores in the air, originating from early planted fields, at the time when late-planted fields contain young actively growing susceptible plants.

### 3.2.4 Symptoms:

- Symptoms are oval to elongate cinnamon brown pustules scattered over upper and lower surfaces of the leaves .
- The pustules rupture and expose dusty red urediniospores, which are spread by wind and have the ability to infect other corn leaves directly.
- As the pustules mature, they turn brownish black and release the dark-brown overwintering teliospores.
- In severe epidemics, pustules may also appear on the ears and tassels, and the leaves may yellow and become easily tattered in strong winds.
- Partial resistance is expressed as chlorotic or necrotic hypersensitive flecks with little or no sporulation.



### 3.2.5 Morphological identification of *Puccinia sorghi*:

**3.2.5.1 Uredia:** Without paraphysis, circular to elongate, cinnamon brown, and produced on both surfaces of leaf

**3.2.5.2 Urediniospores:** Echinulate, cinnamon brown, globoid to ellipsoid, (23-29 x 26-32µm) and have 3-4 equatorial pores

**3.2.5.3 Telia:** Circular to elongate and brownish-black toward plant maturity

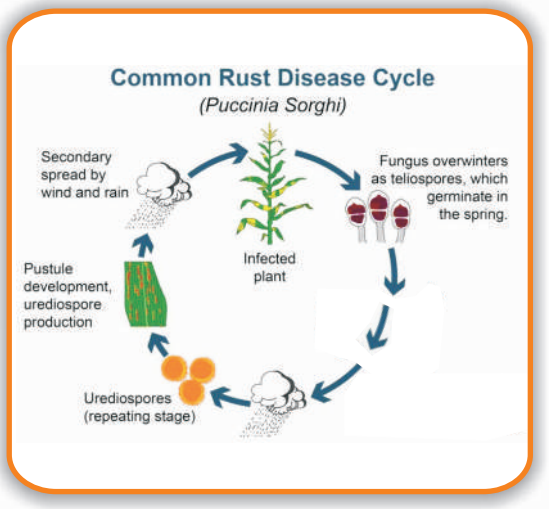
**3.2.5.4 Teliospore:** Bi-celled, chestnut brown, slightly constricted at the septum with a thickened apex and 16-23X 29-54µm. The side wall and pedicel measure 1-2, 5-7 and 50-80 µm respectively.

### 3.2.6 Epidemiology:

- Teliospores dormancy that must be broken by low temperatures and weathering before germination occurs.
- Dry urediospores remain viable for several days at moderate temperatures and storage for long periods at 10° to –26°C.
- Urediospores have a self inhibitor, and spore dispersal is required for germination.
- The optimum temperature for urediospore germination and germ tube growth is in the range of 15°–25°C. The minimum is near 4°C and the maximum is under 31°C.
- Germination is maximum at 100 per cent relative humidity and most spores do not germinate at 98 per cent or lower.
- Light favors urediospore germination. A minimum of about 4 hr is required for infection.

### 3.2.7 Disease cycle:

The complete disease cycle of *P. sorghi* includes five different spore types and two hosts, corn and species of wood sorrel (*Oxalis* spp.). The spore types and the hosts they infect are teliospores (o), basidiospores (o), pycniospores (o), aeciospores (c), and urediniospores (c). The aecial stage (called "cluster-cups") appears on the underneath surface of *Oxalis* leaves, producing aeciospores, which are windborne and infect corn leaves. Urediniospores occur on corn leaves throughout the growing season and continue cyclic infections. Three major factors interact to influence the




(Courtesy: www.pioneer.com)

outbreak of rust epidemics on sweet corn: (1) the quantity of urediniospores available to initiate rust epidemics, (2) environmental factors, and (3) the level of rust susceptibility in the sweet corn varieties in use. Urediniospores are unable to overwinter successfully in northern climates. Temperatures of 60 to 75°F (16-24°C) and heavy dews or high relative humidity (close to 100 per cent) favor rust development. Moisture is required for spore germination. Infection will occur when leaves are wet for a minimum of 3 to 6 hours.

| Southern vs. Common Rust: | Southern Rust | Common Rust |
|---|---|---|
| **Ideal Environment** | Warm to hot and moist $77^0$ F | Cool to warm and moist $60\text{-}77^0$F |
| **Appearance of Pustules** | Small circular, pinhead appearance | Large, circular to elongated |
| **Color of Pustules (spores)** | Reddish orange | Brown to cinnamon-brown |
| **Location of Pustules** | Upper leaf surface<br>May also infect husks | Both upper and lower leaf surfaces<br>Infects leaves only |

### 3.2.8 Disease screening of maize germplasm:

The technique of preparation of inoculum (AICMIP, 1983) for creation of artificial epiphytotics is:

The technique of preparation of inoculum of common rust is similar to that of polysora rust.

### 3.2.9 Procedure of recording disease reaction based on disease ratings, diseased leaf area and PDI:

Disease reaction is determined similar to that of polysora rust.

## 4. MASS SCREENING TECHNIQUES FOR RESISTANCE TO MAIZE DOWNY MILDEWS

Downy mildews are caused by up to ten different species of oomycete fungi in the genera *Peronosclerospora, Sclerophthora* and *Sclerospora*. Downy mildews originated in the Old World although they have since been introduced to many regions of the New World. The important seven downy mildews are mentioned below:

1. *Peronosclerospora sorghi* (Sorghum downy mildew)
2. *P. maydis* (Java downy mildew)
3. *P. philippinensis* (Philippine downy mildew)
4. *P. sacchari* (Sugarcane downy mildew)

5. *Sclerophthora rayssiae* var. *zeae* (Brown stripe downy mildew)
6. *Sclerospora graminicola* (Graminicola downy mildew or green ear)
7. *Sclerophthora macrospora* (crazy top)

### a) Economic importance:

Downy mildews are important maize diseases in many tropical regions of the world. They are particularly destructive in many regions of tropical Asia where losses in excess of 70 per cent have been documented.

**Morphological characteristics of various downy mildew pathogens of maize**

| Pathogen (Disease name) | Morphological characteristics | | |
|---|---|---|---|
| | Conidiophores/ Sporangiophores | Conidia/Sporangia | Oospores |
| *Peronosclerospora sorghi* (Sorghum downy mildew) | Erect, dichotomously branched, 180 to 300µm in length. Emerge singly or in groups from stomata. | Oval (14.4-27.3 × 15-28.9µm), borne on sterigmata (about 13µm long. | Spherical (36µm diameter average), light yellow or brown in color. |
| *P. maydis* (Java downy mildew) | Clustered conidiophores (150 to 550µm in length) emerge from stomata. Dichotomously branched two to four times. | Spherical to subspherical in shape (17-23µm x 27-39µm). | Not reported. |
| *P. philippinensis* (Philippine downy mildew) | Erect and dichotomously branched two to four times. 150 to 400µm in length and emerge from stomata. | Ovoid to cyclindrincal (17-21µm x 27-38µm), slightly rounded at apex. | Rare, spherical (25 to 27µm in diameter and smooth walled. |
| *P. sacchari* (Sugarcane downy mildew) | 160 to 170µm in length erect and arise singly or in pairs from stomata. | Elliptical, oblong (15-23µm x 25-41µm) with round apex. | 40 to 50µm in diameter, globular, yellow. |
| *Sclerospora graminicola* (Graminicola downy mildew or green ear) | Average length of 268µm. | Borne on short sterigmata, elliptical (12-21 x 14-31µm) with distinctive papillate operculum at apex. | Pale brown and 22 to 35µm in diameter. |
| *Sclerophthora macrospora* (crazy top) | Very short (14µm on average). | Lemon shaped (30-65 x 60-100µm), operculate. | Pale yellow, circular (45-75µm). |
| *Sclerophthora rayssiae* var. *zeae* (Brown stripe downy mildew) | – | Oval to cyclindrical (18-26 x 29-67µm). | Spherical (29-37µm in diameter), brown in color. |

**(Courtesy: http://maizedoctor.org/downy-mildew-extended-information)**

## Geographic distribution, alternate hosts, and yield losses of various downy mildews of maize

| Pathogen (Disease name) | Geographic distribution | Host range | Yield loss |
|---|---|---|---|
| *Peronosclerospora sorghi* (Sorghum downy mildew) | Americas (North, Central, and South), Asia, Africa, Europe, Australia. | Cultivated and wild sorghum, Johnson grass, teosinthe, wild grasses (*Panicum, Penniselum, Andropogon* species). | Severe outbreaks have occured in India, Israel, Mexico, Nigeria, Texas, Thailand, and Venezuela. In Nigeria, yield loss as high as 90% has been reported. |
| *P. maydis* (Java downy mildew) | Indonesia and Australia. | Teosinthe, wild grasses (*Penniselum, Tripsacum* species). | Very serious in Indonesia. Up to 40% crop loss. |
| *P. philippinensis* (Philippine downy mildew) | The Philippines, China, India, Indonesia, Nepal, Pakistan, and Thailand. | Oats, teosinthe, cultivated and wild sugarcane, cultivated and wild sorghum. | Very serious in the Philippines where yield losses range between 15 and 40%. Yield losses in excess of 70% have been recorded. |
| *P. sacchari* (Sugarcane downy mildew) | Australia, Fiji, Taiwan, Japan, Nepal, New Guinea, India, Philippines, and Thailand. | Sugarcane, teosinthe, sorghum and wild grasses. | Important disease of maize in Australia and Asia. Yield losses range from 30 to 60%. |
| *Sclerospora graminicola* (Graminicola downy mildew or green ear) | USA and Israel. | Wild grasses, millet. | Only known to infect maize in USA and Israel. Minor disease of maize in both regions. |
| *Sclerophthora macrospora* (crazy top) | Americas, eastern and southern Europe, parts of Africa and Asia. | Oats, wheat, sorghum, rice, finger millet, various grasses. | Rare in tropical areas and causes extensive loss only in localized area. |
| *Scleropthora rayssiae* var. *zeae* (Brown stripe downy mildew) | India, Nepal, Pakistan and Thailand. | *Digitaria* species. | Very serious in India where yield losses in excess of 60% have been recorded. |

**(Courtesy: http://maizedoctor.org/downy-mildew-extended-information)**

**Infection characteristics of various causal pathogens of downy mildew in maize**

| Pathogen (Disease name) | Initial source of inoculum | Seed-borne | Means of sporangia germination | Optimum temp. for sporangia production | Optimum temp. for sporangia germination |
|---|---|---|---|---|---|
| *Peronosclerospora sorghi* (Sorghum downy mildew) | Oospores and sporangia | Yes | Germ tubes | 17-29°C | 21-25°C |
| *P. maydis* (Java downy mildew) | Sporangia | Yes | Germ tubes | Below 24°C | Below 24°C |
| *P. philippinensis* (Philippine downy mildew) | Sporangia | Yes | Germ tubes | 21-26°C | 19-20°C |
| *P. sacchari* (Sugarcane downy mildew) | Sporangia | Yes | Germ tubes | 20-25°C | 20-25°C |
| *Sclerospora graminicola* (Graminicola downy mildew or green ear) | Oospores and sporangia | No | Zoospores | 17°C | 17°C |
| *Sclerophthora macrospora* (crazy top) | Oospores and sporangia | No | Zoospores | 24-28°C | 12-16°C |
| *Scleropthora rayssiae* var. *zeae* (Brown stripe downy mildew) | Oospores and sporangia | Yes | Zoospores | 22-25°C | 20-22°C |

**(Courtesy: http://maizedoctor.org/downy-mildew-extended-information)**

### b) Disease screening procedures:

The five main methods of screening maize germplasm against downy mildew (AICMIP, 1983) are as follows:

**I. Use of soil-borne oospores, usually in a 'sick plot'**: Oospores 'sick plots' are used effectively at the University of Mysore. The limitation on their use in screening at most other locations is that:

- Oospores are not formed,
- When formed, germination and infection is erratic,

- Infection in a field is irregular or patchy and
- Oospores are generally unreliable as a source of inoculum for dependable, uniform tests.

**II. Natural infection from conidia:** Natural infection from conidia is a satisfactory method in areas of heavy infection. But, again is too often subject to disappointment from lack of uniformity.

**III. Conidia produced from a collateral host grown on the sides and often in rows of fields:** Use of a collateral host is quite effective and energy saving. The problem in most cases is identification of a collateral species in use.

**IV. Direct inoculation with conidia:** Most laboratories use conidia by direct inoculation, use of donor plants, or a combination of the two methods:

- Prior to the sowing season, a downy mildew susceptible (DMS) variety is grown on about 1/8 ha.
- Plants are inoculated at first whorl stage 2-4 times. This provides the inoculum source.
- About two weeks before planting the test materials, DMS donor plants are established in both front (3 hills) and back (1 hill) alley-ways and inoculated up to four times; the first inoculation is made at about whorl formation.
- Inoculation of the test plant is done twice, but it is not necessary.
- Inoculation is ordinarily done by knap-sack sprayer. However, we can use a tractor mounted spray rig (12 rows at a pass) which requires much more inoculum, but uses less labour; and, plastic squeeze bottles where inoculum is directed at whorl, is labour intensive, but conserves inoculum. A surfactant (1 drop/ 50-100 mls of Tween 80) is incorporated in the aqueous based conidial inoculum. Inoculum density should be at least 40,000 conidia/ml.

**V. Conidia supplied from infector (donor) plants within the field:** Formerly sporulating leaves are collected from the field at 0200-0300 hrs or harvested diseased leaves in the evening and incubated them in dark, moist conditions at 20-23°C. From these the conidia were harvested, the dilutions made and inoculations were completed from 0430 hrs to-day-break. It was found that a photosynthetic period was required by the maize plant. The length of period depended upon light intensity and degree of sporulation the previous night. On clear, bright days diseased leaves are collected at 1100 hrs, on overcast days at 1300 hrs and washed (rubbed) off the old conidiophores, place the leaves in plastic buckets containing 4-5 cm water in the bottom and incubate them at 20-23°C for 8 hrs. Spores are harvested by washing leaves in water.

## 4.1 Sorghum Downy Mildew (SDM)

### 4.1.1 Causal organism: *Peronosclerospora sorghi* (W. Weston & Uppal) C.G. Shaw, 1978

**4.1.1.1 *Synonym:*** *Sclerospora sorghi* W. Weston & Uppal, 1932

### 4.1.2 Economic importance:

Maize crop is uniquely attacked by ten different downy mildew pathogens world over. It was first mentioned by Butler (1907) who considered it to be *Scleropora graminicola.* Sorghum downy mildew (*Peronosclerospora sorghi*) is found in Gujarat, Maharashtra, A.P., Karnataka, Tamil Nadu. Plant less than one month old is highly susceptible to this disease.

### 4.1.3 Symptoms:

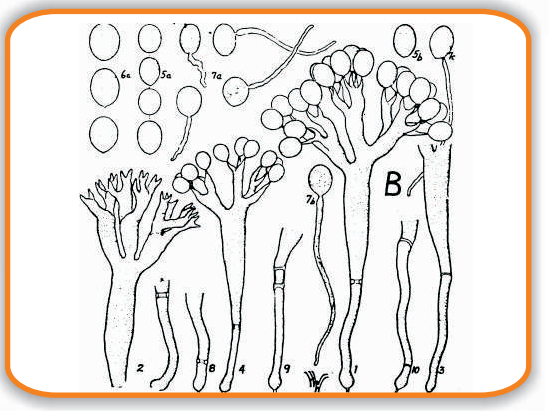
- In systemic infection, the disease appears in the seedlings soon after their emergence.
- The affected seedlings have pale yellow, narrow leaves covered with a fine downy growth consisting of the conidial stage of the fungus.
- Infected leaves have a downy growth more common on lower surface toward the basal part.
- Due to the formation of oospores, infected plants are chlorotic, stunted and sterile and have striped leaves.

- High populations of spores are produced on the leaf surface; they are short-lived and require extended periods of high humidity for infection.
- Overwintering spores produced between leaf veins exist in the soil for long periods.

### 4.1.4 Morphological identification of *Peronosclerospora sorghi*:

- It is an obligate parasite.
- The fungus produces coenocytic mycelium .

- The sterigmata are longer than those of *S. graminicola,* being upto 16 µm.
- Major difference lies with the sporangia which always germinate by germ tube (Conidia-like), not produce zoospores.
- The sporangium is commonly spherical instead of oval and lack the apical papilla.
- The size of conidia is 15-29 µm in diameter.




(Courtesy: www.eagri.org)

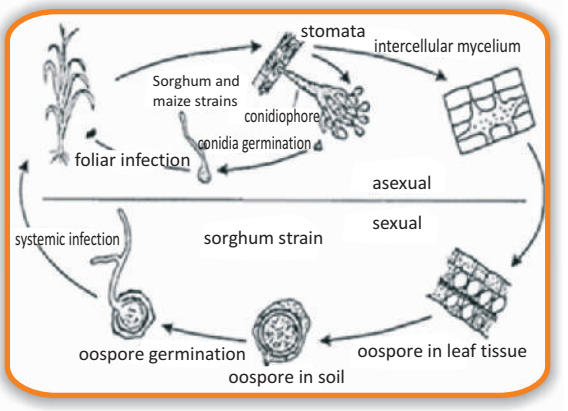
- Oospores is spherical and three walls, the exosporium, mesosporium and endosporium. The endosporium is smooth, yellow in colour and of even thickness.

### 4.1.5 Epidemiology:

Production of conidia requires temperature of 17-29°C (optimum 24-26°C). Germination of conidia requires a saturated atmosphere or free water and moderate temperatures of 21-25°C. High levels of systemic infection occur at 11-32°C with a wet period of 4 hours or longer.

### 4.1.6 Disease cycle:

The major inoculum source for *P. sorghi* is oospores which can survive for several seasons in soil. Susceptible seedlings are attacked through underground parts, which become systemically infected. Airborne conidia, which are produced on the leaf surfaces, serve as secondary inoculum that can induce systemic infection in plants up to four weeks old. The pathogen is also seed borne, however, transmission of the pathogen to seedlings occurs most readily from freshly harvested or immature seeds, although systemically infected plants are usually sterile and




(Courtesy: Fredriksen 1986)

produce no seed for harvesting. Seeds dried to below 13 per cent are unlikely to carry viable fungal material.

### 4.1.7 Disease screening of maize germplasm:

The technique of collection and maintenance of inoculum (Lal and Singh, 1984) for creation of artificial epiphytotics is mentioned below:

**A. Screening through direct inoculation with conidia:**

i. *Collection and maintenance of inoculum*: Sorghum plants showing systemic infection of downy mildew from the farmer's fields are collected during morning hours, preserved in polythene bags and brought to the laboratory. Conidiophores and conidia from the white bloom found on the lower surface of the leaves are washed with a fine jet of distilled water and conidial suspension is collected from the sorghum leaves. The seedlings of susceptible cultivar are spray inoculated at 2 leaf stage (6-7 days old) with the conidial suspension collected from the sorghum leaves. The inoculation of the seedlings is continued till the plants reached 15 days and systemic symptoms are seen. The inoculum from these plants is multiplied by spray inoculating to the fortnightly sowings of maize. The infected plants are maintained in the plot throughout the experimental period. Artificial inoculation technique developed by Lal and Singh (1984) is followed to induce the disease infection by spraying conidial suspension between 2.30 a.m. and 4.00 a.m.

ii. *Evaluation of maize genotypes under artificial inoculation:* Maize genotypes are evaluated against sorghum downy mildew by artificial inoculation. Artificial inoculation is done when the plants are at two leaves stage as described by Lal and Singh (1984). Diseased plants from which inoculum required to be drawn is sprayed with water at 6.00 PM so that leaves would have a thin film of water for good sporulation. By 2.00 AM, the inoculation crew assembles in the field with cleaned sprayers, torches and buckets. By 2.30 AM the diseased leaves with good sporulation are searched and washed in the water at the rate of 15 leaves per litre of water collected in the buckets. This operation is completed by 3.00 AM. Then the collected spore suspension in different buckets is thoroughly mixed and made up to 25 litres. The 25 litres of conidial inoculum is collected from 375 diseased leaves. The inoculation is completed by 4.00 AM with hand compression sprayer. Between 6.00 AM and at 6.00 PM water spray is given to the inoculated plot to create the required humidity artificially. With this method 100 per cent disease incidence can be created.

**B. Spreader row technique:**

Spreader rows are sown 15-20 days prior to the sowing of the entries in 2.5 meter bands

with a row spacing of 60 cm and plant to plant spacing of 30 cm. each band consisting of four rows surrounding on all the four directions. For this, highly susceptible variety is used. Inoculation of these spreader rows is done by following the above artificial inoculation procedure. Test entries were sown as mentioned above.

### 4.1.8 Procedure of recording disease reaction based on disease incidence:

Per cent disease incidence is recorded 35 days after sowing and the entries are classified according to their disease reaction as described by Lal and Singh (1984).

| Disease incidence (%) | Disease reaction |
|---|---|
| ≤ 10 | Resistant |
| 10.1 –25.0 | Moderately resistant |
| 25.1 –50.0 | Moderately susceptible |
| ≥ 50.0 | Susceptible |

## 4.2 Rajasthan Downy Mildew (RDM)

### 4.2.1 Causal organism: *Peronosclespora heteropogoni* Siradhana *et. al.*, 1980

### 4.2.2 Host range: *Heteropogon melanocarpus* and *H. contortus*

### 4.2.3 Economic Importance:

Rajasthan downy mildew was first reported from northern India in 1968 at Regional Research Station, Vallabh Nagar, Udaipur in Rajasthan. RDM causes heavy yield losses under conditions favouring infection. Infection may occur at early stage (2-3 leaf) of crop growth. Following infection, the disease spreads quickly and infects large number of plants. On some systemically infected plants rudimentary cobs may be formed, but these cannot be harvested for grain purpose at all and therefore results in the complete yield loss.

### 4.2.4 Symptoms

- The typical symptoms are "*half diseased leaf*" which are characterised by the pale appearance of bases of second & third diseased leaves of the seedling.
- On infected leaves, yellow stripes at the base also extends up to upper green portion.
- Severely infected plants give yellowish appearance even from a distance.

- Most of the infected plants die at about knee-high stage.
- Under humid conditions whitish fluffy growth due to abundant fructification of the fungus can be observed on the lower and upper leaf surfaces.
- In some cases leaves tend to become yellow in colour, erect and closer in position on the stem.
- No oospore formation by *P. heteropogoni* has been observed on maize.
- Systemically infected maize plants generally do not form cob.
- In some cases when cobs are formed, these are small, poorly filled and even if tassel appear, there is no pollen formation in anthers.
- Infected plants have weak and thin stems and poor root growth, but no excessive tillering or malformation of any part has been observed.

### 4.2.5 Morphological identification of *Peronosclerospora heteropogoni*:

**4.2.5.1 Conidiophores:** Determinate, macronemous with swollen base, dichotomously branched, measuring, 81.6 – 142.8 x 14.3- 25.5 µm with an average of 101.8 x 20.1 µm.

**4.2.5.2 Conidia:** Globose, hyaline, thin walled and are 14.3 – 22.4 x 14.3 – 20.4 (17.7 x 16.2) µm. Conidia are nocturnally (during night) formed on both *H. contortus* and maize (Dange et al., 1973) and germinate instantly through germ tube.

**4.2.5.3 Oospore:** Spherical with persistent oogonial wall, and measure 24.5 – 26.7 (29.0) µm.

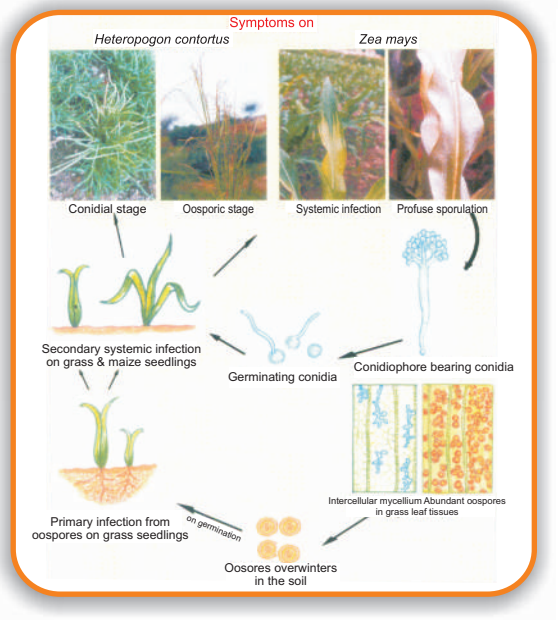
### 4.2.6 Epidemiology:

Number of conidia in a given inoculum determine the extent of disease likely to occur. Maize plants were inoculated with one drop of different concentrations of conidia *viz*; 1,00,000, 50,000, 25,000, 12,500, 6,260, 3125, 1562 and 781 $ml^{-1}$. Direct correlation between inoculum density and per cent infection was observed. The data suggested that the inoculum density of 6000 conidia $ml^{-1}$ may induce about 80 per cent disease incidence (Rathore 1983; Rathore and Siradhana 1987b). Siradhana *et al.* (1978) also found highest *P. heteropogoni* infection with temperature range 23.5 – 0.1ºC, rainfall being 154.4 mm and RH of 87.3 per cent. Conidial germination occurred between 10ºC to 35ºC, maximum at 25ºC, and no germination occurred at 40ºC.

### 4.2.7 Disease cycle:

*P. heteropogoni* produces abundant conidia and oospores on leaves of *H. contortus* which acts as a collateral host, but produces only condia on maize leaves. *H. contortus*

is an annual grass, it grows during rainy season and forms seeds in late rainy season. The roots of *H. contortus* harbour abundant mycelium and oospores of the pathogen and give rise to diseased seedling. The most susceptible growth stage was 3rd day after germination. Oospores formed on this grass are thus the primary source of inoculum which first infect the grass and then maize at 2-3 leaves stage. Systemically infected leaves of maize produce abundant conidia in between 2.30-3.0 AM. on both the surfaces. The conidia are the main source of secondary spread of the disease within and among fields. Under favourable environment infected leaves continue to produce conidia until they become necrotic or senesce. Young plants, particularly 3-4 days after germination, are most susceptible. Oospores are produced in infected leaves of *Heteropogon* only late in *kharif* season. Oospores remain in the soil along with infected leaf and root residues and cause primary infection in the following years.




(Courtesy: Dr. R.S. Rathore, Ex-Prof. & Head, MPUAT)

### 4.2.8 Disease screening of maize germplasm:

The technique of preparation of inoculum for creation of artificial epiphytotics is mentioned below:

Downy mildew nursery is required for artificial inoculation purposes. Susceptible maize cultivar is grown in cage house and the plants are inoculated at seedling stage by placing bits of downy mildew infected grasses *Heteropogon contortus* and *H. melanocarpus.* Humidity around 90 per cent is maintained in the cage house. Chlorotic symptoms along with light green color extends up to upper green portion are typical symptoms. During midnight hours a layer of conidia can be seen. These plants serve as source of inoculum for artificial inoculation.

Since the pathogen is of nocturnal nature and produces conidia during 12:00 to 6 AM, hence the freshly harvested conidia are collected in distilled water or RO water. Before collecting conidia the leaves can be washed before an hour so as to get fresh viable conidia. For screening the test entries, susceptible entries should be planted before 15

days and should be inoculated first. Since this pathogen does not form oospores on maize, hence sick plot technique does not work. The conidial suspension of harvested conidia is filled in dropping bottle to put drops of inoculum at seedling stage (6-7 days old) in the whorl (a cup like structure of upper leaf) during 3-5 AM. This should be done for 4-5 days regularly to avoid any escape. After 15-20 days symptoms become visible.

Artificial creation of RDM Epiphytotics

### 4.2.9 Procedure of recording disease reaction based on diseased leaf area and PDI:

The observation is recorded as per cent infected plants in a row out of total plants. At least three observations are taken at 30, 50 and 80 DAS. The last observation is considered as final, but number of plants is considered as of first observation. This is because some plants die and disappear due to infection. The entries are classified according to their disease reaction as described by Lal and Singh (1984) for SDM.

## 4.3 Brown Stripe Downy Mildew (BSDM)

### 4.3.1 Causal organism: *Sclerophthora rayssiae* var. *zeae* Payak and Renfro, 1967

### 4.3.2. Symptoms:

- *Sclerophthora rayssiae* var. *zeae* causes leaf lesions only.
- In early stages of infection leaves will show narrow chlorotic or yellowish stripes, 3 to 7 mm wide.

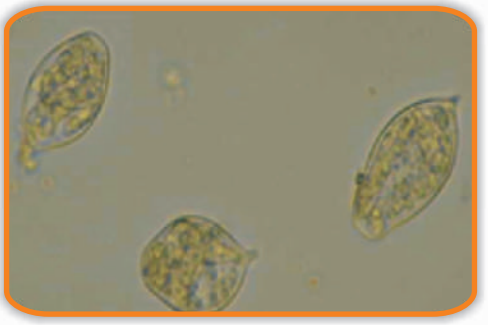
Courtesy: www.plantmanagementnetwork.org

- Some maize genotypes, these stripes will be reddish to purple.
- The lesions have well defined margins and extend parallel with and are delimited by the leaf veins.
- Advanced striping and blotching occurs with confluence of adjacent lesions.
- The disease may first be noticed on the lower leaves, which will show the greatest degree of striping.
- The pathogen apparently does not systemically infect the plant

### 4.3.3 Morphological identification of *S. rayssiae* var. *zeae*:

**4.3.3.1 Sporangiophore:** Sporangiophore are short, determinate, and produced from hyphae in the substomatal cavities.

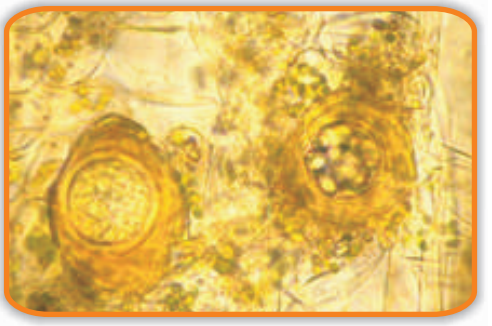
**4.3.3.2 Sporangia**: Sporangia are formed sympodially in groups of two to six, arising in basipetal succession. Sporangia are hyaline; ovate, obclavate, elliptic or cylindrical; smooth-walled; and are papillate, possessing a projecting truncate, rounded or tapering poroid apex. The sporangia are caducous, with a persistent, straight or cuneate peduncle. Sporangia range in size from 18.5-26.0 µm × 29.0-66.5 µm; there may be lens shaped pores through which zoospores or cytoplasm may escape. Four to eight zoospores are formed in the sporangia and may encyst within or outside of it.



Zoosporangia

**4.3.3.3 Zoospores**: Zoospores are hyaline, spherical, and vary from 7.5 to 11.0 µm in diameter.

**4.3.3.4 Oogonia**: Oogonia are hyaline to light and 33.0 to 44.5 µm in diameter. Thin-walled, they may have one or two paragynous antheridia.



Oospores

**4.3.3.5 Oospores**: Oospores are pleurotic, spherical or subspherical; and are hyaline, with one prominent oil globule. Cell walls are smooth, glistening, and uniformly about 4 µm thick, confluent with the oogonial wall. They range from 29.5 to 37.0 µm in size.

### 4.3.4 Epidemiology:

Downy mildew can develop when RH>80 per cent for >10 hours at night or when RH is 100 per cent for > 4 hours in the night, when temperature is 10–33°C in the night.

| Process | Temperature | Humidity | Time |
|---|---|---|---|
| Sporulation | 15–23°C | RH > 80% | 5–6 hours in the dark |
| Germination | 12–20°C (12–32°C) | Dew period | 2 hours |
| Infection | 10–33°C | Dew period | 4 hours (2 hours) |

### 4.3.5 Disease cycle:

Primary inoculum comes from oospores overseasoning in soil or plant debris or from mycelium in infected seed. The seed surface may carry plant debris containing viable oospores and the seed may carry oospores or mycelium within the embryo. Oospores in air-dried leaf tissue can remain viable for 3 to 5 years although infected seed dried to 14 per cent moisture or less and stored for 4 or more weeks will not be capable of transmitting the disease. Oospores generally undergo indirect germination, producing sporangiophores that bear sporangia which may contain four to eight zoospores. Less frequently, the sporangium may germinate directly and produce a germ tube capable of penetrating maize leaves. Rapid spread of the pathogen in the field occurs with the production of sporangia (secondary inoculum), which are dispersed in wind and water splash, or from physical contact with an infected plant. Sporangia have been trapped 1.65 m from an infected field, but the greatest numbers of sporangia were found to move less than 1 m, suggesting long distance transport via wind is unlikely. Sporangia production, germination, and infection require a film of water. Twelve hours of leaf wetness were required for infection via zoospores, with longer periods producing greater numbers of infected plants. Sporangial release occurs in the afternoon of sunny days when high moisture is present, rather than on cloudy or rainy days. Generation time of secondary inoculum (sporangia) from primary inoculum (oospores) can be rapid. Infected leaves placed in a moist environment at 22 to 25°C can produce sporangia in as little as 3 h, with a second generation of sporangia arising 9 h later. Young plants are most susceptible to inoculation, with susceptibility decreasing as the plants age. Warm soil (28 to 32.5°C) is required for disease development when seeds are inoculated with infected plant debris.

### 4.3.6 Disease screening of maize germplasm:

The techniques of creation of artificial BSDM epiphytotics (AICMIP, 1983) are mentioned below:

#### 4.3.6.1 Technique I

Artificial epiphytotic conditions can be created by placing the powdered infected maize leaves containing spores collected during the last season containing oospores in furrows just before planting. This inoculum could also be prepared by collecting infected leaves supposed to be full of oospores from early plantings of maize of the same season, drying leaves and making powder out of the debris. Inoculum should be placed in furrows in such a manner that seeds were in proximity of inoculum.

#### 4.3.6.2 Technique II

Artificial epiphytotic condition could also be created by putting 2-3 cm pieces of freshly infected leaves containing sporangia of the fungus in the whorls of seedlings. This should be done during cloudy weather in the evening between 5 and 7 P.M. at 17, 24 and 30 days after planting. In experimental plots, where disease occurs year after year, only this method is adequate for creating epidemics. In areas of low disease incidence, both the methods of inoculation can be combined to obtain better results.

### 4.3.7 Procedure of recording disease reaction based on diseased leaf area and PDI:

Disease rating of individual maize varieties can be done by evaluation all plants of the row (s) using modified rating scale of AICMIP (1983*)* as described below:

| Rating scale | Degree of infection (per cent DLA) | PDI | Disease reaction |
|---|---|---|---|
| 1.0 | Nil to very slight infection (≤10%). | ≤11.11 | Resistant (R) (Score: ≤ 3.0) (DLA:≤ 30%) (PDI: ≤ 33.33) |
| 2.0 | Slight infection, a few lesions scattered on two lower leaves (10.1-20%). | 22.22 | |
| 3.0 | Light infection, moderate number of lesions scattered on four lower leaves (20.1-30%). | 33.33 | |
| 4.0 | Light infection, moderate number of lesions scattered on lower leaves, a few lesions scattered on middle leaves below the cob (30.1-40%). | 44.44 | Moderately resistant (MR) (Score: 3.1–5.0) (DLA: 30.1-50%) (PDI: 33.34-55.55) |
| 5.0 | Moderate infection, abundant number of stripes scattered on lower leaves, moderate number of stripes scattered on middle leaves below the cob (40.1-50%). | 55.55 | |

| Rating scale | Degree of infection (per cent DLA) | PDI | Disease reaction |
|---|---|---|---|
| 6.0 | Heavy infection, abundant stripes on lower leaves, moderate infection on middle leaves and a few stripes on two leaves above the cob (50.1-60%). | 66.66 | Moderately susceptible (MS) (Score: 5.1-7.0) (DLA: 50.1-70%) (PDI:55.56-77.77) |
| 7.0 | Heavy infection, abundant stripes on lower and middle leaves and moderate number of stripes on two to four leaves above the cob (60.1-70%). | 77.77 | |
| 8.0 | Very heavy infection, stripes abundant on lower and middle leaves and spreading up to the flag leaf (70.1-80%). | 88.88 | Susceptible (S) (Score: >7.0) (DLA: >70%) (PDI: >77.77) |
| 9.0 | Very heavy infection, stripes abundant all leaves. No cob formation. Plant may be killed prematurely (>80%). | 99.99 | |

## 5. MASS SCREENING TECHNIQUES FOR RESISTANCE TO MAIZE STALK ROTS

### 5.1 Pre-Flowering Stalk Rots (PrFSR)

#### 5.1.1 Pythium Stalk Rot (PSR)

**5.1.1.1. Causal organism :** *Pythium aphanidermatum (*Edson) Fitzp., 1923

**5.1.1.2 Synonyms**:

1. *Rheosporangium aphanidermatum* Edson, 1915
2. *Nematosporangium aphanidermatum* (Edson) Fitzp., 1923
3. *Pythium butleri* Subraman., 1919
4. *Nematosporangium aphanidermatum* var. *hawaiiensis* Sideris, 1931

**5.1.1.3 Host range:**

*Pythium aphanidermatum* is known to infect a wide range of cultivate crops, including cereals and grassess, cucurbits, horticultural crops, and cotton.

**5.1.1.4 Economic importance:**

Pythium stalk rot is generally confined to hot and humid regions where maize is cultivated in poorly drained soils. Valley areas and river bottom fields are particularly susceptible to infection. Damage is usually localized in a field. Occasionally entire parts of a field may lodge prematurely, although extensive damage in the field is rare as the pathogen dispersal by natural means is often limited.

### 5.1.1.5 Symptoms

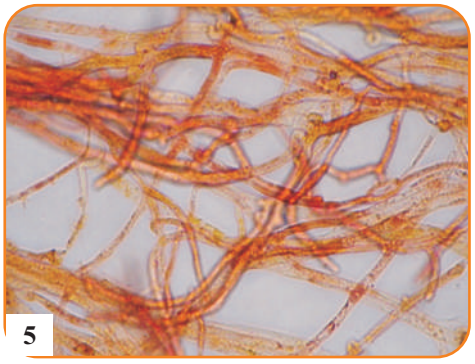
- Pythium stalk rot is usually confined to the first internode above the soil. Stalks become soft, water soaked, darkened, and collapsed.
- The stalk at the site of infection becomes twisted as the stalk tissue rots, eventually leading to stalk lodging.
- Stalks do not usually break off completely.
- Infected plants may appear healthy for extended periods as vascular bundles often remain intact for some time.
- Pythium stalk rot can occur prior to flowering.


1


2


3


4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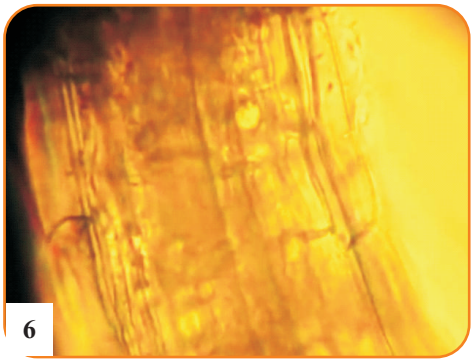
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1- External first internode infection of maize

2- Internal first internode infection of maize

3- Mycelium growth on infected internode of maize (5X-ZSM)

4- Culture of *P. aphanidermatum.*

5- Mycelium (ceonocytic) stain with safrenin (40X-BM).

6- Oospore present within infected rootlet of maize(100X-BM).

**5.1.1.5 Morphological identification of *Pythium aphanidermatum*:**

**5.1.1.5.1 Hyphae:** Non-septate and hyaline

**5.1.1.5.2 Sporangium:** Inflated (balloon like), branched or unbranched and measure 4-20 × 50-1,000µm.

**5.1.1.5.3 Zoospore:** Kidney shaped, laterally biciliate, and measure about 7.5 × 12µm.

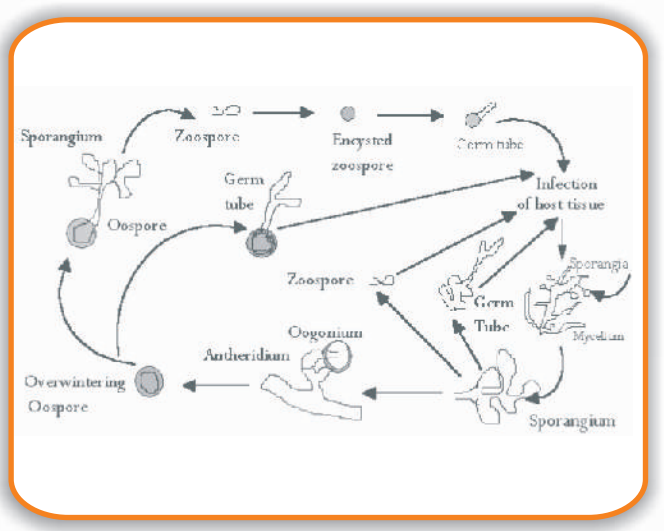
**5.1.1.5.4 Oogonium:** Spherical and 22-27µm in diameter with straight stalks.

**5.1.1.5.5 Antheridium:** Intercallary.

**5.1.1.5.6 Oospores:** Thick walled and 17 to 19µm in diameter. Typically oospores will be observed from diseased stalk tissue.

**5.1.1.6 Epidemiology:**

Pythium stalk rot is more prevalent in areas where wet condition and air drainage is poor and humidity is high. The disease occurs in depressed areas of the field or in river bottom fields that are characterized by wet soils. High temperatures (25-35°C), high crop density, and high levels of nitrogen fertilizer all favour disease severity.




(courtesy: www.projects.ncsu.edu)

**5.1.1.7 Disease cycle**

*P. aphanidermatum* survives in the soil as mycelium or oospores. Oospores can survive in the soil for several years. They produce sporangia which release motile zoospores. Zoopores swim towards plant or root tissue in response to exudates which

serve as nutrient or chemotrophic stimulants. Zoospores infect the plant directly at or just below the soil surface where free water is present, leading to the development of characteristic lesions. Mycelium within the infected host tissue gives rise to oogonium and antheridium, the sexual stage of the life cycle. The antheridium fertilizes the oognium producing oospore, which is able to overwinter in crop debris or in the soil. The pathogen is dispersed with either the movement of infected crop debris or with flooding or excess wetness, which transports oospores and enables zoospores to swim freely.

### 5.1.1.8 Disease screening of maize germplasm:

#### 5.1.1.8.1 Technique I

Germplasm screening in sick plot is most accepted approach for disease screening against soil borne diseases irrespective of any crop. Evaluation of breeding material can be accomplished easily and directly in the field with little expense by the use of naturally or artificially infested fields or plots ('sick plots', SP). The main advantage of SP is that they allow simultaneous screening of a large amount of genetic material under environmental conditions similar to those of cultivated plants. SPs should be established based on the presence of the disease as indicated by visual symptoms and reisolation of the causal fungus. In contrast to diseases caused by foliar fungi where natural epidemics are unpredictable, incidence of disease caused by soil-borne fungal pathogens in a SP is much more reliable.

Sick plots can be developed in the country by fixing the an isolated plot and following maize monocropping since for at least five years, its incorporation in soils and amending the soils with grain culture of *Pythium aphanidermatum* over the years. Detailed procedure for development of pythium sick plot is as follows:

1. Select an isolated plot of adequate size to avoid spread of the fungus inoculum from this plot to others.
2. The plot should have had maize crop in the previous year, and at least traces of PSR incidence should have been observed.
3. Incorporate chopped small pieces of infected plants collected from this plot and other fields uniformly in the surface soil of the selected plot.
4. Plant a sole crop of a highly susceptible cultivar in this plot. Ensure a good plant population and carry out normal agronomic operations.
5. By the end of the season, at least 20 per cent of the plants should show PSR symptoms. After harvesting and threshing; scatter the debris uniformly all over the plot and incorporate it by dicing.

6. Repeat step 3; this will help in increasing the level of the inoculum to make the soil "sick".
7. Repeat steps 3 and 4 in the next season. By the end of this season, 90 per cent PSR incidence should be recorded. If the incidence is less than 70 per cent, repeat steps 3 and 4 once more.
8. Initiate screening in the next season and plant a susceptible cultivar after every ten test rows in the whole field. These rows will serve as checks, and will help in monitoring and maintaining the sickness of the plot. The susceptible check rows should show more than 90 per cent infection.
9. From the 4th or 5th year onwards, a susceptible check can be planted after every 20 test rows. Always include susceptible and resistant checks for comparison for estimating the disease pressure in the plot.

**5.1.1.8.2 Technique II**

The technique of preparation of pythium inoculum (AICMIP, 1983) for creation of artificial epiphytotics is mentioned below:

The inoculum for field studies is increased on round sterilized bamboo toothpicks, partially submerged in potato dextrose broth supplemented with 0.1 per cent yeast extract. Round hard bamboo toothpicks of 5.5cm length with tapering ends are boiled several times thoroughly in water to remove resin, gum or any other toxic substance that might inhibit the growth of the fungus. After washing, they are dried in sun. Keeping the tapering ends upwards the dried toothpicks are stacked loosely in screw capped or cotton plug jars. Prior to autoclaving, potato dextrose broth is added. The level of broth is so adjusted that it covers one-third length of toothpicks after autoclaving. Subsequently, the sterilized jars are seeded with the fungus and incubated at 30°C for one week. Abundant cottony white mycelial growth covers the toothpicks and these are used for inoculating the plants in the field.

The plants are inoculated within five to seven weeks after planting (but the prior to flowering). About 2cm deep hole is made in an oblique manner in the centre of a basal internode by means of a jabber. The toothpicks are then inserted into the hole. The round toothpicks thus effectively seal the orifice in the stalk and prevent drying of the stalk tissue.

**5.1.1.9 Procedure for recording disease reaction based on incidence of pythium stalk rot:**

Disease assessment (AICMIP, 1983) is made on the basis of percentage of plants lodged/ toppled in each test entry and disease reaction interpreted as below:

| Disease incidence (%) | Disease reaction |
|---|---|
| ≤ 10 | Resistant |
| 10.1 -25.0 | Moderately resistant |
| 25.1 -50.0 | Moderately susceptible |
| ≥ 50.0 | Susceptible |

### 5.1.2 Bacterial Stalk Rot (BSR)

**5.1.2.1 Causal organism**: *Dickeya zeae* Samson *et al.* 2005

**Synonyms**:

1. *Bacterium carotovorum* f.sp. *zeae* Sabet, 1954
2. *Erwinia carotovora* (Jones, 1901) Bergey et al., 1923
3. *Erwinia carotovora* f.sp. *zeae* Sabet, 1954
4. *Erwinia carotovora* var. *chrysanthemi* (Burkholder et al., 1953) Dye, 1969
5. *Erwinia chrysanthemi* (Burkholder et al., 1953) Young et al., 1978
6. *Erwinia chrysanthemi* corn pathotype Dye, 1969
7. *Erwinia chrysanthemi* pv. *zeae* (Sabet, 1954) Victoria et al., 1975
8. *Erwinia maydis* Kelman et al., 1957
9. *Pectobacterium carotovorum* f.sp. *zeae* (Sabet) Dowson, 1957
10. *Pectobacterium carotovorum* var. *graminarum* Dowson & Hayward, 1960
11. *Pectobacterium chrysanthemi* (Burkholder et al., 1953) Brenner et al., 1973 emend. Hauben et al., 1999
12. *Pectobacterium chrysanthemi* pv. *zeae* Kelman 1974

**5.1.2.2 Host range:**

1. *Aechmea fasciata*
2. *Allium fistulosum* (Welsh onion)
3. *Ananas comosus* (pineapple)
4. Araceae
5. *Chrysanthemum morifolium* (chrysanthemum (florists'))
6. Cyclamen
7. *Daucus carota* (carrot)
8. *Dracaena marginata* (Madagascar dragon tree)
9. *Euphorbia pulcherrima* (poinsettia)
10. *Imperata cylindrica* (cogon grass)
11. *Ipomoea batatas* (sweet potato)
12. *Megathyrsus maximus* (Guinea grass)
13. *Musa* (banana)
14. *Oryza sativa* (rice)

15. Paspalum
16. *Pennisetum purpureum* (elephant grass)
17. *Petunia hybrida*
18. *Phalaenopsis*
19. *Philodendron*
20. *Saccharum officinarum* (sugarcane)
21. *Saintpaulia ionantha* (African violet)
22. *Solanum lycopersicum* (tomato)
23. *Solanum tuberosum* (potato)
24. *Sorghum bicolor* (sorghum)
25. *Sorghum sudanense* (Sudan grass)
26. *Urochloa mutica* (para grass)
27. *Zea mays* (maize)
28. *Zea mays* subsp. *mays* (sweet corn)

**5.1.2.3 Economic importance:**

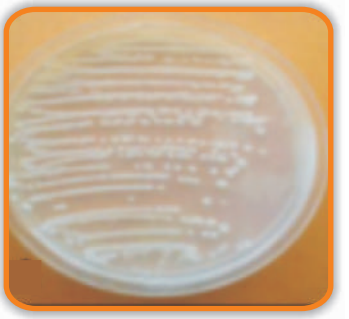
*D. zeae* is a major disease of maize in tropical and subtropical countries. It is particularly severe under conditions of high temperature and humidity (Saxena and Lal, 1984; Sah, 1991). It is one of the four major stalk diseases of maize in India. Incidence up to 80-85 per cent has been observed in nature and yield losses of 98.8 per cent in artificial epiphytotics. A sweet corn population grown during the 1979/80 rainy season in Brasilia (Brazil) was heavily attacked by *D. zeae.* In temperate regions such as the USA, the disease is only a problem with overhead irrigation.

**5.1.2.4 Symptoms:**

- Symptoms appear late on maize plants when they are 40-60 days old.
- The first sign is the premature withering and drying up of the tips of the uppermost leaves, soon followed by the lower leaves.
- *Basal Rot*- Leaves become yellow and the infected tissue becomes brown, soft, and water-soaked. Internally, the stalk turns into a soft mass of disintegrated tissue. At this stage, the plants usually topple over.
- A foul odour and the presence of dipterous larvae on and in decaying tissues are characteristic symptoms of this disease. The rot may involve only one or two internodes, or the entire length of the stalk, which finally dries up and its interior turns into a shredded mass of fibrous tissue.

- *Top Rot*- The first visible symptoms are wilting and drying up of the tips of the middle leaves of the whorl. At the same time, a soft rot develops in the stalk at the base of the whorl. A rapid decay downwards through the stalk is observed and the tops of affected plants droop. The cluster of leaves can easily be pulled out, showing a soft, rotted condition at the breaking point near the base of the whorl.
- When the ears along with the husks are infected, they first become water-soaked, turning slimy and, later, drying up. The plants may bear undeveloped ears with many rotting grains and a covering of slime, or the infected ear completely rots (cob rot) and does not bear any seed.

**5.1.2.5 Morphological identification of *Dickeya zeae:***



**5.1.2.5.1 *Bacterium***: *D. zeae*is motile, gram-negative, rod shaped bacterium with size varying from 0.8-3.2 x 0.5-0.8 µm (average 1.8 x 0.6 µm). There are 3-14, but more usually 8-11, peritrichous flagellae.

**5.1.2.5.2 Colony characters**: The bacterium produce off white, slimy and shiny colonies on King's B Medium (Kumar *et al*. 2015).

**5.1.2.6 Epidemiology**:

- This disease is prevalent in heavy soil under high temperature and humidity conditions.
- Warm temperature and frequent rains in the month of August and September aggravate this problem in farmer's field.
- Its attack on maize crop is often witnessed in *Kharif* sown crop as it has the most susceptible stage coinciding with the annual monsoon rainfall particularly in Punjab (NW India) which aggravates the disease development.

**5.1.2.7 Disease cycle:**

The pathogen is soil borne and may survive in crop residues in the soil. It attacks the plant through its natural openings like the stomata, hydathodes, wounds in the leaf whorl, stalks, and roots caused by insects; and injuries brought about by strong winds or mechanical means. The plant debris have important role for long survival of *D. zeae*. The bacteria can survive almost nine months in the soil containing naturally infected maize stalks. High temperature and soil moisture promote build-up of bacterial population and its continuous survival. Bacterial cells could be preserved in silica gel for approximately 3 years without losing their viability while in the field its virulence exists only for single crop season. It can also be spread from plant to plant and field to

field through rainwater and its runoff. Certain insects like maize borer play a vital role in the initial infection and subsequent spread of the disease.

**5.1.2.8 Disease screening of maize germplasm:**

The technique of preparation of inoculum (AICMIP, 1983) for creation of artificial epiphytotics is mentioned below:

For quick and reliable scoring of breeding material for resistance to bacterial stalk rot, creation of epidemics by artificial inoculation is essential. Available data supports that hypodermic syringe method of inoculation is the best for bacterial stalk rot pathogen. The stalk rot becomes progressively more severe in the internodes from bottom as compared to the top of the plant. Difference in the susceptibility among internodes may be due to the difference in carbohydrate content. Sugar and carbohydrate in the lower parts of the stalk are comparatively lesser than upper parts of the plants. It is now accepted that tasseling or silking stage is the most appropriate for artificial inoculations. For creating good epidemics, planting should be done early in the season, i.e. May-June planting, so that the flowering coincides with the period of frequent rains. This results in good disease development.

A virulent isolate of *Dickeya zeae* maize pathotype should be selected for inoculation. To maintain the virulence of the bacterium, it should be inoculated on healthy plants and then reisolated every year before mass inoculation. In order to isolate a virulent strain, the inoculated plants showing characteristic symptoms of the disease were selected. A small piece of rotten internodes is removed aseptically from the selected diseased plant. After rinsing the piece in spirit, it is immediately dipped into mercuric chloride solution (1:1000) for 15 seconds and passed through three changes of sterile water. The piece is then cut into the halves with sterilized blade, put into little sterile water and then teased apart with sterile needle. The small quantities of resulting suspension are then removed with a flamed wire loop and streaked out on well dried crystal violet pectate agar plates, the aim being to separate the cells so that they produce individual colonies. The characteristic colonies were identified after 2 days of incubation at $27^0$ C and used for sub-culturing on King's B medium. The culture is used for testing the pathogenicity. The culture which induced the typical symptoms of the disease within 48 hours of inoculation is used for mass inoculation. The inoculum is increased for mass inoculation on nutrient broth for 48 hours at $27^0$ C. The inoculum is diluted 10 times with sterile water. The concentration of the bacterium is maintained approximately $1x10^{7-9}$cells/millilitres.

The inoculation is done when the crop is at pre-silking stage until 75 per cent flowering. To inoculate the plants, a diagonal hole, deep up to the pith, is made in the

middle of second internodes from the ground with the help of a jaber. One millilitre of bacterial suspension is injected in the plant through that hole by a hypodermic syringe. After inoculation, the plants are frequently irrigated to maintain high humidity and soil moisture which is important for disease development. If necessary, one week after, the second inoculation may be done in the third internodes from the ground.

**5.1.2.9 Procedure for recording disease reaction:**

The inoculated plants in a plot are rated individually for their disease reaction using 1-9 rating scale (for precise information) or wilted and healthy plants in each plot are counted after 15 days of inoculation. The recording may be done near dry silk stage of the crop. During recording, the plants are cut from the ground in such a way that the first basal internode is intact. These plants are split open longitudinally from the first internode upward to clearly observe the spread of disease in the internal tissue.

**a. Procedure for recording disease reaction based on disease ratings and PDI:**

Disease reaction to BSR can be determined based upon disease ratings, disease leaf area (DLA) and PDI as mentioned below:

| Rating scale | Degree of infection (per cent severity) | PDI | Disease reaction |
|---|---|---|---|
| 1.0 | The infection is limited to a very small spot in the pith at the site of inoculation (≤10 %). | ≤11.11 | Resistant (R)<br>(Score: ≤ 3.0)<br>(Severity: ≤ 30%)<br>(PDI: ≤ 33.33) |
| 2.0 | Disease infection spreads in one fourth of the length of the inoculated internodes in the pith and cortical tissue Rind not infected (10.1-20%). | 22.22 | |
| 3.0 | Disease infection spreads in half of the length of the inoculated internodes in the pith and cortical tissue. Rind not infected (20.1-30%). | 33.33 | |
| 4.0 | Disease infection spreads in three fourth of the length of the inoculated internodes in the pith and cortical tissue (30.1-40%). | 44.44 | Moderately resistant (MR)<br>(Score: 3.1–5.0)<br>(Severity: 30.1-50.0%)<br>(PDI: 33.34-55.55) |
| 5.0 | Disease infection covers the entire length of the inoculated internodes but does not cross the nodal plates. The rind is green and the symptoms are not visible externally, but plant shows sign of wilting (40.1-50%). | 55.55 | |

| Rating scale | Degree of infection (per cent DLA) | PDI | Disease reaction |
|---|---|---|---|
| 6.0 | The nodal plates are crossed and the increasing infection also covers adjacent internode of the inoculated plants. (50.1-60%). | 66.66 | Moderately susceptible (MS) (Score: 5.1-7.0) (Severity: 50.1-70.0%) (PDI:55.56-77.77) |
| 7.0 | The disease spreads in two internodes. The pith and cortical tissues are degenerated. The rind of the inoculated internodes is affected and the plant wilts. (60.1-70%) | 77.77 | |
| 8.0 | The disease spreads in three internodes. The pith and cortical tissues are degenerated. The rind of the inoculated internodes is affected and the plant wilts. Ear length and size is considerably reduced as compared to healthy plants. (70.1-80%) | 88.88 | Susceptible (S) (Score: >7.0) (Severity: >70%) (PDI: >77.77) |
| 9.0 | The disease spreads in more than three internodes. The pith, cortical tissues and vascular bundles rot and disorganised. Rind discoloured, plants wilt and may topple down (>80%) | 99.99 | |

**b. Procedure for recording disease reaction based on incidence of bacterial stalk rot:**

Disease assessment is made on the basis of percentage of plants toppled/ rotten in each test entry and disease reaction is interpreted as below:

| Disease incidence (%) | Disease reaction |
|---|---|
| ≤ 10 | Resistant |
| 10.1 -25.0 | Moderately resistant |
| 25.1 -50.0 | Moderately susceptible |
| ≥ 50.0 | Susceptible |

## 5.2 Post-Flowering Stalk Rots (PFSR)

### 5.2.1. Charcoal Rot (ChR)/ Dry Weather Wilt (DWW)/ Summer Wilt (SW)/ Ashy Stem Blight (ASB)

**5.2.1.1 Causal organism**: *Macrophomina phaseolina* (Tassi) Goid., 1947

**Synonyms:**

1. *Macrophoma phaseolina* Tassi, 1901
2. *Tiarosporella phaseolina* (Tassi) Aa, 1977
3. *Macrophoma faseoli* Maubl. (?)
4. *Macrophoma phaseoli* Maubl, 1905
5. *Rhizoctonia lamellifera* W. Small, 1924

**5.2.1.2 Host range:**

1. *Arachis hypogaea* (peanut)
2. *Beta vulgarius*
3. *Brassica oleracea* (Cabbage)
4. *Capsicum annuum* (pepper)
5. *Cicer arietinum* (chick pea)
6. *Citrus* spp.
7. *Corchorus* sp.
8. *Cucumis* spp.
9. *Fargaria* sp.
10. *Glycine Max* (soybean)
11. *Gossypium* sp.
12. *Helianthus annuus* (sunflower)
13. *Ipomoea batatas* (sweet potato)
14. *Medicago sativa*(alfalfa)
15. *Phaseolus* spp., *Pinus* spp.
16. *Prunus* spp.
17. *Sesamum indicum* (sesame)
18. *Solanum tuberosum* (potato)
19. *Sorghum bicolor* (sorghum)
20. *Vigna unguiculata* (bean)
21. *Zea mays* (corn)

**5.2.1.3 Economic importance:**

*Macrophomina phaseolina* causes charcoal rot disease on more than 500 plant species throughout the world. This disease is economically important throughout the world, particularly in arid maize growing regions where extensive yield losses occur when the crop is infected early. Yield losses as high as 70 per cent have been documented in Africa. The disease is particularly prevalent in drought years and in arid regions where maize is regularly cultivated in rotation with other host crops. The disease is heat and

stress (drought) driven and is therefore rare in cooler climates and irrigated fields. Increased losses may be experienced where maize is mechanically harvested due to lodging. This has serious implications on the selection of follow-up crops since crops like sunflower and soybeans are also seriously affected by the disease.

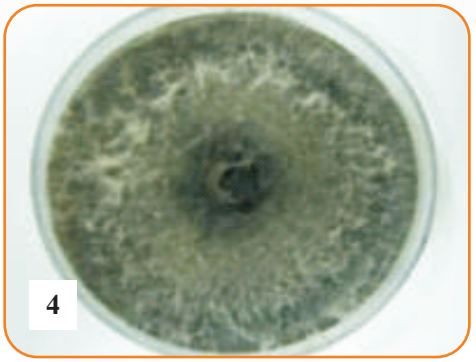
**5.2.1.4 Symptoms:**

- After flowering, initial symptoms are the abnormal drying of upper leaf tissue, stem lodging and premature death.
- At maturity, the lower stem internodes (usually limited to the first 5 nodes) show a typical charcoal, grey-black discolouration.
- Stem is cut open numerous minute black specks (microsclerotia) are visible on the shredded vascular bundles and on the inside of the stem, giving the interior parts of the stem a charred appearance.
- Survival bodies or microsclerotia can best be seen through a magnifying glass or hand lens.
- Brown, water- soaked lesions, which later turn black, are present on the roots.
- Infected kernels showing symptoms are pale yellow with black streaking below the pericarp, and the ear is loose and chaffy.
- Kernels are easily removed from the cob, and they show small, round, black, pinhead-like sclerotia on the surface.



1. Lodging of the plant in the field
2. Kernels are pale yellow with black streaking below the pericarp.


3


4

(Courtesy: http://padil.gov.au/thai-bio/Pest/Main/140461/30923)

3. Many tiny black sclerotia form on the stalk interior wall, it looks like charcoal dust
4. Growth of *M. phaseolina* on PDA

**5.2.1.5 Morphological identification of *M. phaseolina*:**

**5.2.1.5.1 Culture:** Black and homogeneous on agar

**5.2.1.5.2 Hyphae:** Dark grey-green mycelium, in size 2.5-7.5 µm wide.

**5.2.1.5.3 Sclerotia:** In size 60-120 µm in diameter.

**5.2.1.5.4 Microsclerotia:** Black and homogeneous in size

**5.2.1.5.5 Pycnidium:** Black and globose with ostiolate apically. In size 130-230 µm in diameter; ostioles 15-35 µm in diameter.

**5.2.1.5.6 Conidiophores:** Hyaline, simple, cylindrical, narrowing apically and in size 13-23 x 3-6 µm

**5.2.1.5.7 Conidia:** hyaline, cylindrical, 1-celled and in size 14-35 x 6-11.5 µm

**5.2.1.6 Epidemiology:**

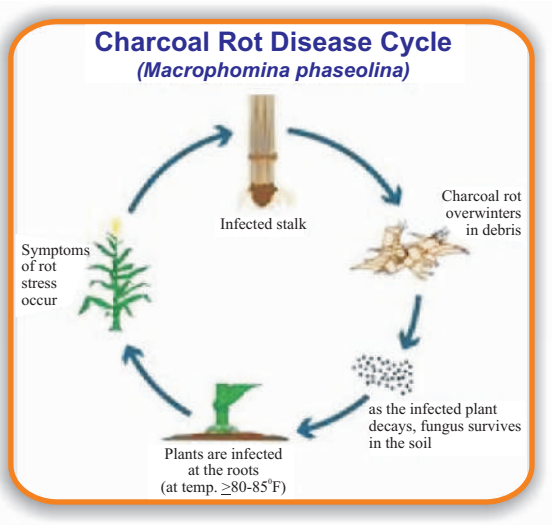
The severity of disease caused by *M. phaseolina* in various hosts is associated with high soil temperatures (30-42°C) and low moisture or when unfavourable environmental conditions stress the plant. Maximum infection occurs in plants subjected to moisture stress during the post-flowering period. Post-flowering stresses due to high plant population or drought coupled with heavy applications of nitrogen fertilizer, hail or insect damage promote disease development. The disease is especially widespread during extremely hot and dry seasons.

Charcoal rot overwinters or survives as resting structures (microsclerotia) on lower stem residues that remained in the field after harvesting. The main risk of dispersal is by the movement of soil, contaminated with microsclerotia, on tractors, ploughs and other farm machinery and packing material.

The alternate hosts are also a major source of inoculum causing infection in the following seasons. The incidence of seed borne infection is generally low, with no strong evidence to suggest transmission by seeds.

**5.2.1.7 Disease cycle:**

*M. phaseolina* overwinters as sclerotia in the soil and can remain viable for several years. In dry and hot conditions fungi infect the roots of maize plants and colonize the lower stalk, eventually giving rise to characteristic symptoms (abundant, minute, black sclerotia and charring and shredding of the pith tissue). Isolates that are pathogenic to maize are not known to produce conidia. Charcoal rot is a soil borne disease.




(Courtesy: www.pioneer.com)

**5.2.1.8 Disease screening of maize germplasm:**

**5.2.1.8.1 Technique I**

Since germplasm screening in sick plot is most accepted approach for disease screening against soil borne diseases irrespective of any crop and therefore, screening for resistance against charcoal rot can easily be done in sick plots (SP) (AICMIP, 1983). The main advantage of SP is that they allow simultaneous screening of a large amount of genetic material under environmental conditions similar to those of cultivated plants. SPs should be established based on the presence of the disease as indicated by visual symptoms and reisolation of the causal fungus. In contrast to diseases caused by foliar fungi where natural epidemics are unpredictable, incidence of disease caused by soil-borne fungal pathogens in a SP is much more reliable.

Charcoal rot sick plots are being developed at hot spot locations in the country by fixing an isolated plot and following maize monocropping since last few years, its incorporation in soils and amending the soils with grain culture of *M. phaseolina* over the years. Detailed procedure for development of *M. phaseolina* sick plot is as follows:

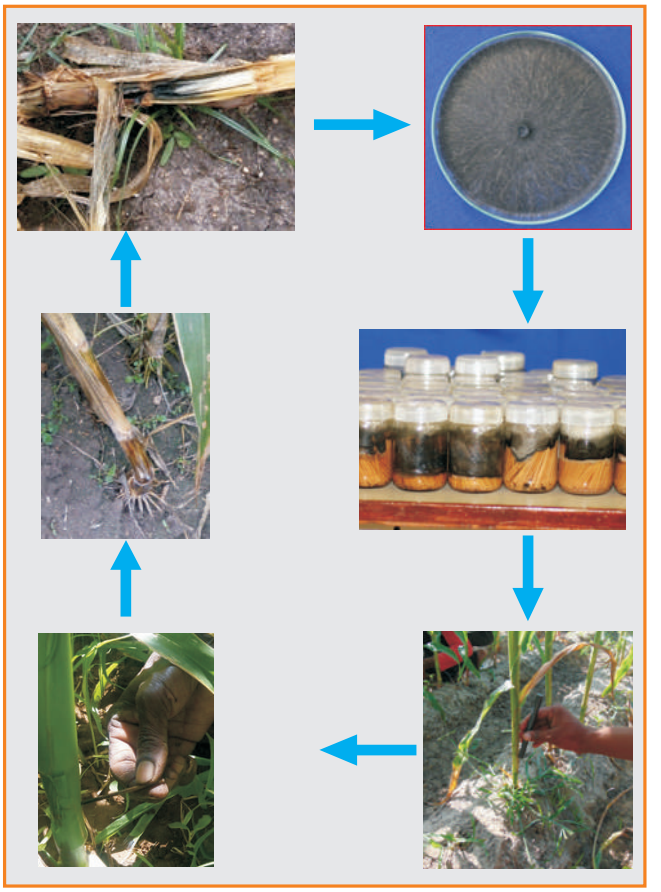
1. Select an isolated plot of adequate size to avoid spread of the fungus inoculum from this plot to others.
2. The plot should have had maize crop in the previous year, and at least traces of *M. phaseolina* incidence should have been observed.
3. Incorporate chopped small pieces of *M. phaseolina* infected plants collected from this plot and other fields uniformly in the surface soil of the selected plot.
4. Plant a sole crop of a highly susceptible cultivar in this plot. Ensure a good plant population and carry out normal agronomic operations.
5. By the end of the season, at least 20 per cent of the plants should show *M. phaseolina* symptoms. After harvesting and threshing; scatter the debris uniformly all over the plot and incorporate it by dicing.
6. Repeat step 3; this will help in increasing the level of the inoculum to make the soil "sick".
7. Repeat steps 3 and 4 in the next season. By the end of this season, 90 per cent charcoal rot incidence should be recorded. If the incidence is less than 70 per cent, repeat steps 3 and 4 one more time.
8. Initiate screening in the next season and plant a susceptible cultivar after every ten test rows in the whole field. These rows will serve as checks, and will help in monitoring and maintaining the sickness of the plot. The susceptible check rows should show more than 90 per cent infection.
9. From the 4th or 5th year onwards, a susceptible check can be planted after every 20 test rows. Always include susceptible and resistant checks for comparison for estimating the disease pressure in the plot.
10. Record disease incidence at 65-75 days after sowing.

**5.2.1.8.2 Technique II:**

Artificial inoculation is necessary where sick plots are not available. For this purpose the fungal material should be isolated from the infected stalks, cultured and multiplied in the laboratory as described below.

Small bits cut from the infected stalks should be surface sterilized with 0.1 per cent mercuric chloride solution for one minute followed by washing in sterile distilled water. Finally a single bit is to be aseptically transferred to sterilized potato dextrose agar at 26±2°C for getting the fungal hyphae to come out from the infected bits. Finally, the fungal hyphae is to be aseptically transferred to culture tubes containing the sterile PDA medium and to be incubated for about 10 days to get the stock culture of the pathogen to be used for increase of the inoculum in the laboratory for field inoculation.

Among various methods of field inoculation, the toothpick inoculation is followed for these diseases under the coordinated programmes. Round bamboo toothpicks about 6.5 cm long are boiled three times (about 1 hour each time) in tap water to remove toxic substances. After each boiling these are thoroughly washed in fresh water and dried in the sun. When these are thoroughly dry, they are loosely packed in bundles and put into the glass jars/ bottles and enough potato dextrose broth (one- third length of toothpicks) is added to thoroughly moisten the toothpicks plus some quantity in the bottom of the jars. The jars with the toothpicks are autoclaved immediately after the broth is added. Later the sterilized toothpicks are inoculated with the culture of the pathogen aseptically. The growth of the fungus covers the toothpicks and inoculum is ready for use in about 10 days.



Inoculations should be made just after flowering stage of plants (45-50 days old). For inoculating plants, the lower internode (second/third) above soil level is opened with a jabber and the toothpick is inserted into the hole. The jabber is made by driving a nail of the diameter of the toothpick into a wooden handle. The head of the nail is ground off to a point and to the desired length (2cm). The round toothpicks effectively seal the hole in the stalk and prevent drying. Symptoms may appear in inoculated plants 15-20 days after inoculation.

**5.2.1.9 Procedure for recording disease reaction based on disease ratings and PDI of charcoal rot:**

The measurement is based on the proportion of disease present in the inoculated internodes and its subsequent spread. For scoring disease severity of charcoal rot, 1-9 rating scale (AICMIP, 1983*)* is followed:

| Rating scale | Intensity and extent of severity (%) | PDI | Disease reaction |
|---|---|---|---|
| 1.0 | 25 per cent of the inoculated internode discoloured | ≤11.11 | Resistant (R) (Score: ≤ 3.0) (PDI: ≤ 33.33) |
| 2.0 | 26-50 per cent of the inoculated internode discoloured | 22.22 | |
| 3.0 | 51-75 per cent of the inoculated internode discoloured | 33.33 | |
| 4.0 | 76-100 per cent of the inoculated internode discoloured | 44.44 | Moderately resistant (MR) (Score: 3.1–5.0) (PDI: 33.34-55.55) |
| 5.0 | Discolouration of less than 50 per cent of adjacent internode | 55.55 | |
| 6.0 | Discolouration of more than 50 per cent of adjacent internode | 66.66 | Moderately resistant (MS) (Score: 5.1–7.0) (PDI: 55.56-77.77) |
| 7.0 | Discolouration of three internodes | 77.77 | |
| 8.0 | Discolouration of four internodes | 88.88 | Susceptible (S) (Score: ≥ 7.0) (PDI: ≥77.77) |
| 9.0 | Discolouration of five or more internodes and premature death of plant | 99.99 | |

### 5.2.2 Fusarium Stalk Rot (FSR)

**5.2.2.1. Causal organism:** *Fusarium verticillioides* (SAcc.) Nirenberg., 1976

**Anamorph synonyms:**

1. *Oospora verticillioides* Sacc., 1881
2. *Alysidium verticillioides* (Sacc.) Kuntze, 1898
3. *Fusarium moniliforme* J. Sheld., 1904
4. *Fusarium celosiae* Abe, 1928
5. *Oospora cephalosporioides* Luchetti & Favilli, 1938

**5.2.2.2 Host range:**

*Fusarium verticillioides* infects a range of cultivated crops, including sorghum, sugarcane, wheat, cotton, banana, pineapple, and tomato.

**5.2.2.3 Economic importance:**

The pathogens are seed borne with up to 75 per cent of all seed planted infected with *F.*

*verticillioides*. The stalk rots are present worldwide where maize or cereals are cultivated. These pathogens are home nearly in every soil. Infection pressure during the growing season is continuously hard. Stressed (by other pathogens, draught or other factors) plants are more susceptible. Infected plants fall down, this may cause high yield losses. Early infection is rare, but may kill out the plants. Late infection in the summer is common; the hazard of yield losses depends on the cultivated variety.

**5.2.2.4 Symptoms:**

- Infected plants typically wilt, leaves turn dull grayish-green and the lower stalk turns from dark green to straw-colored.
- Rotting at roots, crown and lower internodes.
- When split opened, inner stalk shows a light pink to tan discoloration.
- Pith disintegrates, vascular bundles remain intact.

**5.2.2.5 Morphological identification of *Fusarium verticillioides*:**

**5.2.2.5.1 Macroconidia:** Hyaline, slightly curved, and tapered at both ends. They are 3 to 5 septate and measure 2-5 × 15-60µm.

**5.2.2.5.2 Microconidia:** Single-celled and measure 2-3 × 5-12µm. They are produced prolifically and are borne in chains.

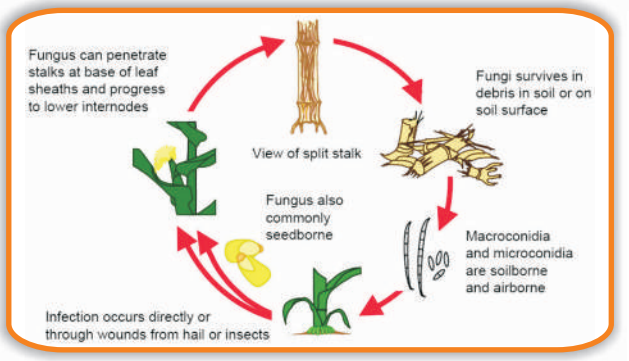
**5.2.2.6 Epidemiology:**

- Overwinters as mycelia in infected crop debris, spread by wind and rain splash.
- Can infect the plant directly through the roots, causing root and lower stalk rot.
- Can also infect at the nodes when dispersed to leaves and washed down into the sheath.
- Favoured by warm, relatively dry weather; plant stress following pollination; and other diseases.
- Disease generally progresses during reproductive stages of corn development.
- Typically occurs in a complex with other root/ stalk rots including *M. phaseolina*.
- Corn borer adults have been shown to vector the disease from plant to plant. Corn borer larvae create wounds that allow the fungus to enter the plant.

**5.2.2.7 Disease cycle:**

The fungi overwinters in the soil and in the plant residues as mycelia, hard-wall developed chlamydospora or sexual body in some species. Therefore, it is important to avoid cultivation without crop rotation. The fungus attacks the stalk as mycelia by

spores developed in a sexual or asexual way. Loose tissues and injuries are the best gates to this infection. The pathogens feed the internal parts of the internodes, the stem will be empty. In the plant tissue, the mycelia develop chlamydospores when the feeding material is at its end. Asexual spores develop outside, at the nodes in rings. Sexual bodies and spores develop at some species on the basal part of the stalk. The genetic material of these fungi varies a lot by sexual multiplication and anastomosis between mycelia and asexual spores. Disease-inflicting species have several strains, isolates, which are difficult to distinguish. Their multiplication rate, toxin and pigment developing disposition is also varied.




(Courtesy: www.pioneer.com)

**5.2.2.8 Disease screening of maize germplasm:**

The screening techniques are same as described under disease screening against charcoal rot.

**5.2.2.9 Procedure for recording disease reaction based on disease ratings and PDI of fusarium stalk rot:**

Measurement of disease on standard rating scale and determination of disease reaction are same as in case of charcoal rot.

## 5.2.3 Late Wilt (LW)

**5.2.3.1 Causal organism:**

**5.2.3.1.1 Teliomorph:** *Magnaporthiopsis maydis* (Samra, Sabet & Hing.) Klaubauf, Lebrun & Crous, 2014

**5.2.3.1.2 Anamorph synonyms:**

1. *Harpophora maydis* (Samra, Sabet & Hing.) W. Gams, 2000
2. *Cephalosporium maydis* Samra, Sabet & Hing., 1963

**5.2.3.2 Host range:**

*Zea mays* is the major crop so far known to be damaged, but the fungus may have other hosts, particularly if it originates from Egypt or India rather than with *Z. mays* from the western hemisphere.

**5.2.3.3 Economic importance:**

The late wilt disease has been reported only in Egypt and India. Both diseases kill the plants near flowering time. They are most common in humid, heavy soils in hot areas. The pathogens are soil and seed borne.

**5.2.3.4 Symptoms:**

- The first symptoms observed as moderately rapid wilting of the leaves beginning at tasseling time.

- The leaves turn dull green and then dry.
- Vascular bundles in the stalk are discoloured.
- Later, lower portion of the stalk become dry, shrunken and hollow with or without wrinkling turn purple to dark brown which is more prominent on lower 1-3 internodes.

(Courtesy: www.flickr.com/ photos/cimmyt)

- Some secondary organisms also develop on stalk rots that cause wet rot with some typical sweetish smell.

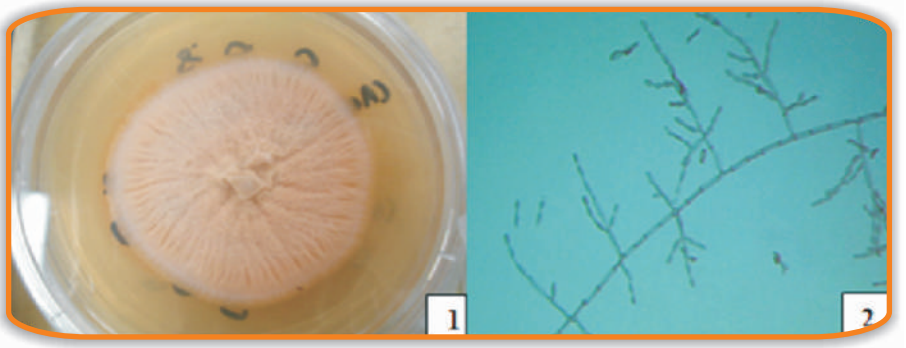
**5.2.3.5 Morphological identification of *Magnaporthiopsis maydis*:**

**5.2.3.5.1 Culture:**

- White, pale grey or pale pink compact colony, flat or folded, and occasionally raised in the center. It is glabrous, velvety, and membrane-like at the beginning. Powdery texture may also be observed.
- The color of the colony is on the surface.
- The reverse side is either uncolored or a pink to rose colored pigment production is observed

**5.2.3.5.2 Vegetative hyphae:**

- Unbranched, hyaline, septate hyphae, solitary, erect phialides are formed directly on the hyphal tips, the hyphal ropes, or both.
- The phialides are separated from hyphae by a septum and taper towards their apices. At the apices of the phialides are the hyaline conidia 2-3x4-8µm in size.
- They usually appear in clusters, in balls or rarely as fragile chains. The conidia are bound by a gelatinous material.




**5.2.3.6 Disease screening of maize germplasm:**

The screening techniques are same as described under disease screening against charcoal rot.

**5.2.3.7 Procedure for recording disease reaction of late wilt:**

Measurement of disease on standard rating scale and determination of disease reaction are same as in case of charcoal rot.

# 6. MASS SCREENING TECHNIQUES FOR RESISTANCE TO MAIZE EAR ROTS

## 6.1 Stenocarpella Ear Rot (SER)

### 6.1.1 Causal organisms:

*Stenocarpella maydis* (syn. *Diplodia maydis*), *Stenocarpella macrospora* (syn. *Diplodia macrospora*)

### 6.1.2 Host range:

Both *S. maydis* and *S. macrospora* infect maize as the primary host. However, *S. maydis* has also been reported to infect bamboo.

### 6.1.3 Economic importance:

Stenocarpella (Diplodia) ear rot is caused by *Stenocarpella* species and commonly found in hot, humid maize growing regions. The causal agents of Stenocarpella ear rot also cause Stenocarpella stalk rot and Macrospora leaf stripe. *Stenocarpella* species can produce various mycotoxins that are harmful to birds

### 6.1.4 Symptoms:

- Early infection of ears (just after flowering) results in development of husks that are bleached (straw colored) and dry although the maize plant remains green and healthy.
- Ears are also shrunken, bleached, and chaffed, with gray-white cottony mycelium growth between and over the kernels.
- Infected kernels are typically dull grey to brown.
- Symptoms of Stenocarpella ear rot usually first appear at the base of the ear, before the entire ear is colonized by the fungi. Infected ears may be totally rotted and light in weight.
- Late in the season, abundant, black, miniature pycnidia form on kernel and ear tissues. Some pathogen isolates are known to cause premature germination of kernels.

### 6.1.5 Morphological identification of stenocarpella ear rot:

**6.1.5.1 Pycnidia:** Stenocarpella ear rot is characterized by the formation of abundant, miniature, black pycnidia on kernels and ear tissue late in the season. Pycnidia of both *S. maydis* and *S. macrospora* are flask-shaped (spherical with circular, protruding papillate ostiole) and multicellular. Pycnidia are 150-450µm in diameter, while the ostiole is typically 30-40µm in diameter. Pycnidia contain pale brown, straight to slightly curved conidia with rounded ends.

**6.1.5.2 Conidia:** Conidia of *S. macrospora* are 0-3 septate measuring 7-12 × 44-82µm. Conidia of *S. maydis* are 1-2 septate and measure 5-8 × 15-34µm. Occasionally, pycnidia contain colorless, narrow spores that measure 1-2 × 25-35µm. A sexual stage for these fungi has not been recorded.

### 6.1.6 Epidemiology:

Wet weather immediately following silking increases disease severity. The disease is also more prevalent where maize follows maize and crop rotation is not used. Additionally, the disease is more prevalent where ears are damaged due to insect injury (e.g. stalk borer feeding). Generally Stenocarpella ear rot is predominantly a problem in the field, although it can emerge as a problem post-harvest if grain is stored at high moisture content (above 20 per cent).

### 6.1.7 Disease cycle:

The causal fungi overwinter as pycnidia in infected maize stalk debris. During wet weather, conidia are produced and are rain splash disseminated to silks. The fungi then grow down the silks and infect the ear. Generally, ears are most vulnerable to infection in the three week period following silking. Infection can also occur through the base of the ear or through ear injuries caused by birds and insects. If infection occurs early (following flowering), the fungi colonizes the ear, giving rise to pycnidia late in the season.

## 6.2 Fusarium and Gibberella Ear Rot (FGER)

### 6.2.1 Causal organisms:

**6.2.1.1 Gibberella ear rot:**

- **Teleomorph:** *Gibberella zeae* (Schwein.) Petch, 1936**;** **Anamorph:** *Fusarium graminearum* Schwein, 1839.

**6.2.1.2 Fusarium ear rot:**

- **Teleomorph**: *Gibberella fujikuroi* (Schwein.) Wollenw., 1931; **Anamorph**: *Fusarium verticillioides* (SAcc.) Nirenberg., 1976 (predominant)

### 6.2.2 Host range:

*i.* ***Gibberella zeae*:** It infects a range of other cereals including wheat, barley, oat and rye. It is also known to infect species of *Lycopersicon, Pisum, Trifolium* and *Solanum* in addition to carnations and other ornamentals.

*ii.* ***Fusarium verticillioides:*** It infects a range of cultivated crops including sorghum, sugarcane, wheat, cotton, banana, pineapple and tomato.

### 6.2.3 Economic importance:

Gibberella and Fusarium ear rot occur widely throughout maize growing regions of the world. They are of particular concern as the causal pathogens produce mycotoxins that are harmful to humans and livestock. The causal pathogens of Gibberella and Fusarium ear rot also cause stalk and seedling blights of maize.

### 6.2.4 Symptoms:

**Gibberella ear rot:**

- Ear infection is initially characterized by the presence of white mycelium on the ear tips that gradually moves towards the base of the ear.
- The mycelium turns a distinctive reddish-pink color in infected kernels.
- Early infection can result in entire ears being colonized by reddish-pink mycelium, extensive kernel rotting, and husks adhering tightly to ears.
- Bluish-black perithecia may also form on the husks.

**Fusarium ear rot:**

- Fusarium ear rot is characterized by cottony mycelium growth that typically occurs on a few kernels or is limited to certain parts of the ear, unlike Gibberella ear rot.
- Mycelium is generally white, pale pink or pale lavender.
- Infected kernels typically display white streaking (also known as 'starburst' symptoms) on the pericarp and often germinate on the cob.
- Typically, infection occurs close to ear tips and is commonly associated with damage and injury caused by ear borers.
- Under severe infestation, the entire ear appears withered and is characterized by mycelium growth between kernels.

### 6.2.5 Morphological identification of fusarium and gibberella ear rot:

#### 6.2.5.1 *Gibberella zeae:*

- **Conidia:** Conidia of the asexual stage of *Gibberella zeae (Fusarium graminearum*) are hyaline, slightly curved, tapering at both ends, and 3 to 5 septate. Condia measure 4-6 × 10-30µm.
- **Perithecia:** Perithecia can form within infected husks and stalks (Gibberella stalk rot) late in the season. Perithecia of *G. zeae* are bluish-black and contain 8 ascospores.

- **Ascospores:** Ascospores are hyaline, up to 3 septate, slightly curved, and taper at both ends. Ascospores measure 3-5 × 10-30µm. The fungus frequently overwinters as perithecia and ascospores only mature at the onset of the subsequent season when they are the primary source of disease inoculum.

**6.2.5.2 *Fusarium verticillioides:***

The perfect stage of *F. verticillioides* is rarely seen in contrast to that of *G. zeae*. During the imperfect (asexual) stage both macroconidia and microconidia are produced.

- **Macroconidia:** Macroconidia are hyaline, slightly curved and taper at both ends. They are 3 to 5 septate and measure 2-5 × 15-60µm.
- **Microconidia:** Microconidia are produced prolifically and are borne in chains. They are single-celled and measure 2-3 × 5-12µm.

## 6.2.6 Epidemiology:

**6.2.6.1 Gibberella ear rot:**

- Gibberella ear rot is favored by cool, wet weather immediately following silking.
- Gibberella ear rot can be particularly serious when water collects between husks and kernels at the base of the plant following heavy rainfall.
- Gibberella ear rot is typically more prevalent where infected crop debris is allowed to overwinter.

**6.2.6.2 Fusarium ear rot:**

- Fusarium ear rot is more prevalent where dry and hot weather occurs during flowering. The disease is commonly associated with injury to ears caused by borers.

## 6.2.7 Disease cycle:

**6.2.7.1 *Gibberella zeae*:** Ascospores and conidia are wind and rain splash disseminated from overwintering perithecia and stalk lesions and infect the ear through silks. The fungus advances towards the ear base during grain filling, giving rise to characteristic symptoms. The fungus overwinters as perithecia, which are produced on husks.

**6.2.7.2 *Fusarium verticillioides*:** *Fusarium verticillioides* and other *Fusarium* species that cause Fusarium ear rot overwinter in infected crop debris. Mycelium in infected crop debris produce macroconidia and microconidia that are wind and rain splash disseminated, infecting ears through silks and colonizing kernels. *F. verticillioides* can also infect maize plants systemically in which case ears may be infected through the ear shank. Insects such as the European corn borer have also been reported to act as

vector and transfer *F. verticillioides* spores between plants or cause plant injury that enable the fungi to infect the plant.

## 6.3 Aspergillus Ear Rot (AER)

### 6.3.1 Causal organisms:

*Aspergillus flavus* and *A. parasiticus* (predominant), *Aspergillus niger, A. glaucus, A. ochraceus, A. toxicarius, A. oryzae*

### 6.3.2 Host range:

*A. flavus* is known to infect a variety of stored grains, nuts and seeds including groundnut, Brazil nut, beans, rice, sorghum, wheat and cucurbits. *A. flavus* occurs worldwide as a saprophyte in soil and decaying organic matter.

### 6.3.3 Economic importance:

Aflatoxin is a liver toxin and a potent carcinogen. Livestock that consume aflatoxin can experience a variety of health issues, including suppressed immune systems, reduced weight gain, cancer, and death. Toxicity varies among animal species, and young animals are most sensitive to the toxin. Furthermore, when lactating animals consume contaminated grain, the aflatoxin is present in the animal's milk.

**Action levels for aflatoxin contaminated corn (USFDA)**

| Action Level (ppb) | End Use of Grain |
|---|---|
| 20 ppb | Animal feed and feed ingredients intended for dairy animals |
| 20 ppb | Human consumption |
| 100 ppb | Grain intended for breeding cattle, breeding swine, and mature poultry |
| 200 ppb | Grain intended for finishing swine of 100 pounds or greater |
| 300 ppb | Grain intended for finishing beef cattle |

(**Source:** FDA Regulatory Guidance for Toxins and Contaminants)

### 6.3.4 Symptoms:

- Gray-green, olive, yellow-green or yellow-brown powdery mold growth on and between kernels.
- Surface mold can develop anywhere on the ear.
- Symptoms are often found at damaged areas of ear.

(Courtesy: www.pioneer.com)

### 6.3.5 Morphological identification of *Aspergillus* spp:

**6.3.5.1 Conidiophores:** Colorless, smooth, biseriate and thick-walled, 400-600 × 3.9-4.8 µm. Conidial heads radiated and were, mostly, 38.7-48.4 µm (rarely 70 µm) in diameter.

**6.3.5.2 Primary phialide:** 4.8-5.8 × 2.9-3.1 µm,

**6.3.5.3 Secondary phialide:** 7.8-8.7 × 1.4-1.9 µm.

**6.3.5.4 Vesicles:** Ellipsoid, 17-21 × 12-16 µm in diameter.

**6.3.5.5 Conidia:** Globose, pea green, roughened, conspicuously echinulate, 3.0-4.0 µm in diameter.

**Colony colour in various *Aspergillus* species:**

| Species | Surface | Reverse |
|---|---|---|
| *A. clavatus* | Blue-green | White, brownish with age |
| *A. flavus* | Yellow-green | Goldish to red brown |
| *A. fumigatus* | Blue-green to gray | White to tan |
| *A. glaucus* group | Green with yellow areas | Yellowish to brown |
| *A. nidulans* | Green, buff to yellow | Purplish red to olive |
| *A. niger* | Black | White to yellow |
| *A. terreus* | Cinnamon to brown | White to brown |
| *A. versicolor* | White at the beginning, turns to yellow, tan, pale green or pink | White to yellow or purplish red |

**Microscopic features of various *Aspergillus* species:**

| Species | Conidiophore | Phialides | Vesicle | S | C | Hc | A |
|---|---|---|---|---|---|---|---|
| *A. clavatus* | Long, smooth | Uniseriate | Huge, clavate-shaped | - | - | - | - |
| *A. flavus* | Colorless, rough | Uni-/biseriate | Round, radiate | + (In some strains, brown) | - | - | - |
| *A. fumigatus* | Short (<300 µm), smooth, colorless or greenish | Uniseriate | Round, olumnar head | - | - | - | - |
| *A.glaucus* group | Variable length, smooth, colorless | Uniseriate | Round, radiate to very loosely columnar head | - | + (yellow -orange) | - | - |
| *A. nidulans* | Short (<250 µm), smooth, brown | Biseriate, short | Round, columnar head | - | + (red) | + | - |
| *A. niger* | Long, smooth, colorless or brown | Biseriate | Round, radiate head | - | - | - | - |
| *A. terreus* | Short (<250 µm), smooth, colorless | Biseriate | Round, compactly columnar head | - | - | - | + (solitary, round, produced directly on hyphae) |
| *A. versicolor* | Long, smooth, colorless | Biseriate | Round, loosely radiate head | - | - | + (in some strains) | - |

**S: Sclerotia, C: Cleistothecia, HC: Hulle cells, A: Aleuriconidia**

### 6.3.6 Epidemiology:

- Aspergillus ear rot is more prevalent in hot and dry years.
- Drought and high temperatures increase disease severity.
- Unlike many other pathogenic fungi and bacteria, *A. flavus* is thermotolerant and is able to survive high temperatures.
- Stressed plants (drought or nitrogen deficiency) and cobs damaged by insect feeding are more susceptible to Aspergillus ear rot.
- *A. flavus* can be a serious storage rot, particularly when grain is stored at high moisture levels.

### 6.3.7 Disease Cycle:

- Aspergillus fungal spores are produced on crop residue on the soil surface and on discarded kernels and fines around grain bins.
- Fungal spores become airborne and can infect kernels by growing down the silk channel when silks are yellow-brown and still moist.
- Infection is most common through kernel wounds caused by several types of insects.
- Aspergillus can occur on many types of organic material, including forages, cereal grains, food and feed products and decaying vegetation.
- Partially or completely burying infected residue reduces disease inoculum and incidence.
- Fungal spores overwinter on plant residue.
- Aspergillus can also produce specialized survival structures that enable it to survive in the soil for extended periods.

## 6.4 Penicillium Ear Rot (PER)

### 6.4.1 Causal organisms:

*Penicillium oxalicum* (most common). Other *Penicillium* species associated are *P. chrysogenum, P. glaucum, P. cyclopium, P. funiculosum.*

### 6.4.2 Host range:

*Penicillium* species are widely distributed and occur in soils and decaying vegetation. *P. oxalicum* is recognized as an important pathogen to cucurbits in greenhouses in addition to maize. *P. oxalicum* has also been reported to cause genital infection of water buffalo.

### 6.4.3 Economic importance:

Penicillium ear rot is most commonly caused by *Penicillium oxalicum,* although other *Penicillium* species are also involved in the disease complex. The disease is common worldwide wherever maize is grown though incidence is higher in warmer climates. *Penicillium oxalicum* is known to infect greenhouse crops in addition to water buffalo. Certain *Penicillium* species involved in *Penicillium* ear rot produce mycotoxins (ochratoxins) that are carcinogenic and acutely toxic to mammals.

### 6.4.4 Symptoms:

- Penicillium ear rot is characterized by a distinct light blue-green powdery mold that grows between kernels and on the ear surface.

- Infected kernels are typically bleached and streaked.
- Infection of stored grain results in blue-green discoloration of the embryo.
- Penicillium ear rot can be distinguished from Diplodia (Stenocarpella) and Gibberella ear rot which form a whitish-grey and reddish-pink mold respectively.

### 6.4.5 Morphological identification of penicillium ear rot:

**6.4.5.1 Growth**: Growth of *Penicillium* species on culture is filamentous, rapid, flat, and cottony in texture. Colonies are initially cottony-white but become blue-green as colonies mature and sporulate.

**6.4.5.2 Hyphae:** Microscopic examination of *Penicillium* species reveals septate, hyaline hyphae up to 5µm in diameter.

**6.4.5.3 Conidia**: Conidia are borne on long conidiophores that typically branch in a 'broomlike' manner. Conidia are single-celled, round (resembling glass beads), up to 5µm in diameter, usually green in color, and form in chains at the tips of conidiophores.

### 6.4.6 Epidemiology:

Penicillium ear rot predominantly occurs on ears that have been damaged mechanically or by insect injury. Penicillium ear rot is particularly common in fields that are infested with insect borers that attack the stem. The disease can also arise where maize is stored at high moisture levels, particularly if ear rot was observed prior to harvest. Humidity above 80 per cent after grain fill leads to increased disease severity.

### 6.4.7 Disease cycle:

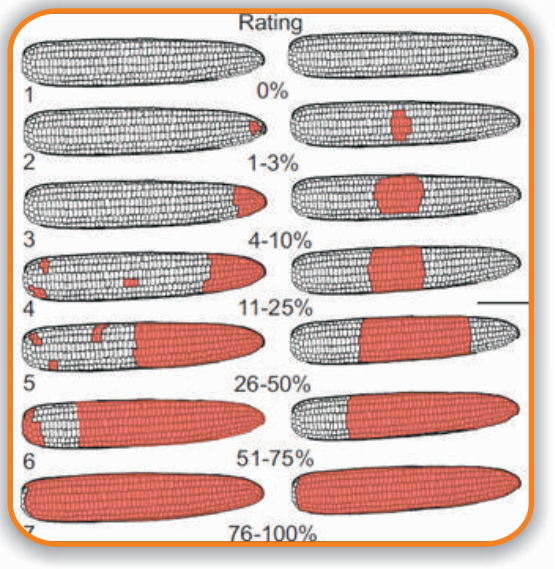
Conidia are predominantly wind and rain splash disseminated and infect ears through wounds. The ear is colonized and secondary sporulation occurs, giving rise to the characteristic green-blue color of the mold. The fungi overwinter in the soil and on alternate hosts.

### 6.4.8 Disease screening of maize germplasm against ear rots:

The screening techniques for creation of epiphytotics are:

**A. Inoculum production of *Fusarium verticilloides* and *Aspergillus flavus***: The pathogens are isolated and identified from infected kernels. Infected kernels are surface sterilized with in 50 ml of a 1:10 dilution of commercial sodium hypochloride and water (0.3 to 0.6 per cent final concentrations) for 2 minutes, rinsed in sterile water and blot dried on sterile paper. Three seeds are placed at equidistance in a Petri dish containing potato dextrose agar (PDA). After three to four days of incubation, the growth of the fungus would be sufficient for obtaining pure cultures of the pathogens.

Pure cultures of the suspected ear rot pathogen are prepared by transferring small sections (0.2 $mm^2$) of the growing tip of the mycelium that show no mixture of different types of mycelium or bacterial growth. After 2-3 weeks when the fungus has covered the surface of the agar, one of the representative cultures should be observed in the microscope to ensure that the correct fungus was isolated based on morphological structures. The cultures at this time should be stored in a sealed plastic bag in the refrigerator (5-$10^0$C) to maintain good quality cultures for preparing the inoculum.




For production of *Fusarium verticilloides* and *Aspergillus flavus* inocula for field inoculations, 10 to 20 ml of sterile distilled water is added to a Petri dish containing a pure culture of the fungus using sterile technique and the spores and mycelia are scraped from the agar using a small laboratory spatula and added to a jar containing 1 liter of sterile water. Protective rubber gloves should be used in the preparation of the inoculum since this fungus produces mycotoxins that are water soluble. The contents of the container are mixed and the solution is poured through two layers of gauze placed in a funnel to collect the concentrated spore solution. The spore concentration obtained from a one liter jar is in the order of $2x10^5$ spores/ml and this solution needs to be diluted with water to arrive at the concentration for field inoculations. The stock solution should be stored immediately in the refrigerator and can be used over a period of one week. A spore concentration of $2x10^5$ spores/ ml is prepared immediately before use (normally 5-10 ml of the stock solution added to one liter of water).

**Inoculation technique:** Inoculations for *Fusarium verticilloides* and *Aspergillus flavus* ear rots are done 7-10 days after pollination using a spore suspension with $2x10^5$ spores/ml. The period of 0-14 post-female flowering is the window where the ear is most susceptible to *Fusarium verticilloides* ear rot. For *Fusarium graminearum*, 1 ml of the spore suspension is injected in the silk channel using a repeater syringe used for vaccinating swine at 7-10 days after silking.

**B. Inoculum preparation of *Stenocarpella maydis***: Four hundred grains collected from ears with typical symptoms were placed in a moist chamber (seven days at 25ºC

and 95 per cent relative humidity) to stimulate the formation of pycnidia. Later, with the aid of a stereoscopical microscope and a histological needle, a pycnidium was removed from the grain, placed on a water drop and covered with a cover slip. The conidia were examined at 50x magnification for species identification. Once the species was identified, the conidia were transferred to a drop (approx. 1.0 mL) of sterile distilled water (SDW) placed on a plastic Petri dish containing potato-dextrose-agar (PDA), and spread on the surface of the substrate. The plates were incubated for three days at 23 to 27ºC, the colonies were transferred to new Petri dishes with PDA and incubated for an additional three to four days at 25ºC.

The substrate for inoculum production was prepared as follows. One hundred grams of sorghum grains in 1L Erlenmeyer flasks was washed in tap water, shaken by hand. The grains were absorbed in 125 ml of distilled water for approximately 12 hours; afterwards, the water not absorbed by the grains was discarded. After that, the substrate was autoclaved at 125ºC for 20 minutes, and this operation was repeated twice. Five discs of *S. maydis* colony, 5.0 mm in diameter, were transferred with the help of a histological needle, to an Erlenmeyer flask with the grain sorghum substrate. Flasks were then shaken to distribute the mycelial discs evenly in the substrate. The flasks were incubated at 25ºC until a black mass of spores was formed around the grains, and were then maintained in a shaker for five days until uniform distribution was achieved. This procedure was carried out with a mixture of six isolates, one from each corn hybrid mentioned in the previous section.

The inoculum from one flask was suspended in 250 mL of SDW, shaken for 30 minutes, and transferred to another flask by filtration through five layers of cheese-cloth, supported by a plastic funnel. Conidial concentrations were counted in a Neubauer chamber and the suspension was adjusted to 4x10 conidia $mL^{-1}$.

**Inoculation technique:** It consisted in pouring the spore suspension through the floral bracts on the peduncle with the help of an automatic dosing syringe. Five mL were poured on each ear, ten days after all the plants had flowered. The pouring technique was the easiest to carry out.

### 6.4.9 Procedure for recording disease reaction

Measurement of disease and disease reaction on standard rating scale of 1-9 (Henry *et al.,* 2009) are done as mentioned below:

| Rating scale | Severity (rotting of ear) (%) | PDI | Disease reaction |
|---|---|---|---|
| 1.0 | 0% rot on the cob | 0.00 | Resistant (R) (Score: ≤ 3.0) (Severity: ≤ 10%) (PDI: ≤ 33.33) |
| 2.0 | 0.1–5% rot on the cob | 22.22 | |
| 3.0 | 5.1–10% rot on the cob | 33.33 | |
| 4.0 | 10.1–25% rot on the cob | 44.44 | Moderately resistant (MR) (Score: 3.1–5.0) (Severity: 10.1-40%) (PDI: 33.34-55.55) |
| 5.0 | 25.1–40% rot on the cob | 55.55 | |
| 6.0 | 40.1–55% rot on the cob | 66.66 | Moderately susceptible (MS) (Score: 5.1–7.0) (Severity: 40.1-70%) (PDI: 55.56-77.77) |
| 7.0 | 55.1–70% rot on the cob | 77.77 | |
| 8.0 | 70.1–85% rot on the cob | 88.88 | Susceptible (S) (Score: ≥ 7.0) (Severity: >70%) (PDI: ≥77.77) |
| 9.0 | >85% rot on the cob | 99.99 | |

## 7. MASS SCREENING TECHNIQUES FOR RESISTANCE TO MAIZE CYST NEMATODE (MCN)

### 7.1 Causal organism: *Heterodera zeae* Koshy, Swarup & Sethi, 1971

### 7.2 Host range:

1. *Avena sativa* (oats)
2. *Hordeum vulgare* (barley)
3. *Oryza sativa* (rice)
4. *Poaceae* (grasses)
5. *Setaria italica* (foxtail millet)
6. *Sorghum bicolor* (sorghum)
7. *Sorghum sudanense* (Sudan grass)
8. *Triticum aestivum* (wheat)
9. *Zea mays* (maize)
10. *Zea mays* subsp. *mexicana* (teosinte)

### 7.3 Economic importance:

The MCN has been found in India, Pakistan, Egypt, and the United States. In India and Egypt it is considered to be of economic importance. In the United States, it is not considered to be of economic importance due to its limited distribution and high soil

temperature requirements for reproduction. Losses to the tune of 11-26 per cent by *H. zeae* have been reported in maize under sandy loam soils. The severity of losses caused by *H. zeae* on maize is higher in Rajasthan, due to favourable soil conditions, mono-cropping of maize and ignorance of management practices.

### 7.4 Symptoms:

- Yellowing of leaves
- Stunted and Patchy growth of plants
- Bushy root system *i.e.* excessive feeder roots
- Browning of roots
- Lesion and small cavities formation on roots
- Diseased plants easily pulled out
- Pearly white pin head like structures (females) seen on the roots which is the characteristic symptom of this nematode and later turns yellow to dark brown in colour.
- Diseased plants tasseled early and bear small cobs
- Qualitative and quantitative losses on yield.

### 7.5 Morphological identification of *Heterodera zeae*:

Maize cyst nematode, *Heterodera zeae* is identified by smaller sized yellow to brown colour of the cyst. Cyst is generally lemon shaped having well developed neck and vulva with thin walled cuticle. A thin crystalline layer is visible in young cysts. The eggs are retained in body of the white pin head like females and visible from the outside body of cysts. The *Heterodera zeae* can be identified by the following features:

#### 1. Second stage juveniles:

Body length: 36-440 µm a: 19.0-25.4 b: 4.0-6.5 b': 2.9-4.2 c: 8.0-13.0

Stylet length : 20-25 µm Tail length : 32-50 µm Hyaline tail terminal : 16-30 µm

lateral lines : 4

#### 2. Female :

Body length : 423-716 µm Body width : 325-684 µm

Length /width ratio : 1.0-1.5 Stylet length : 24-25 µm

#### 3. Cyst :

Body length : 342-684 µm Body width : 260-537 µm

Length /width ratio : 1.0-1.5 Fenestral length : 37-53 µm

Fenestral width : 20-32 µm Vulval slit length : 36-58 µm

Vulval bridge : 4.5 – 9.0 µm

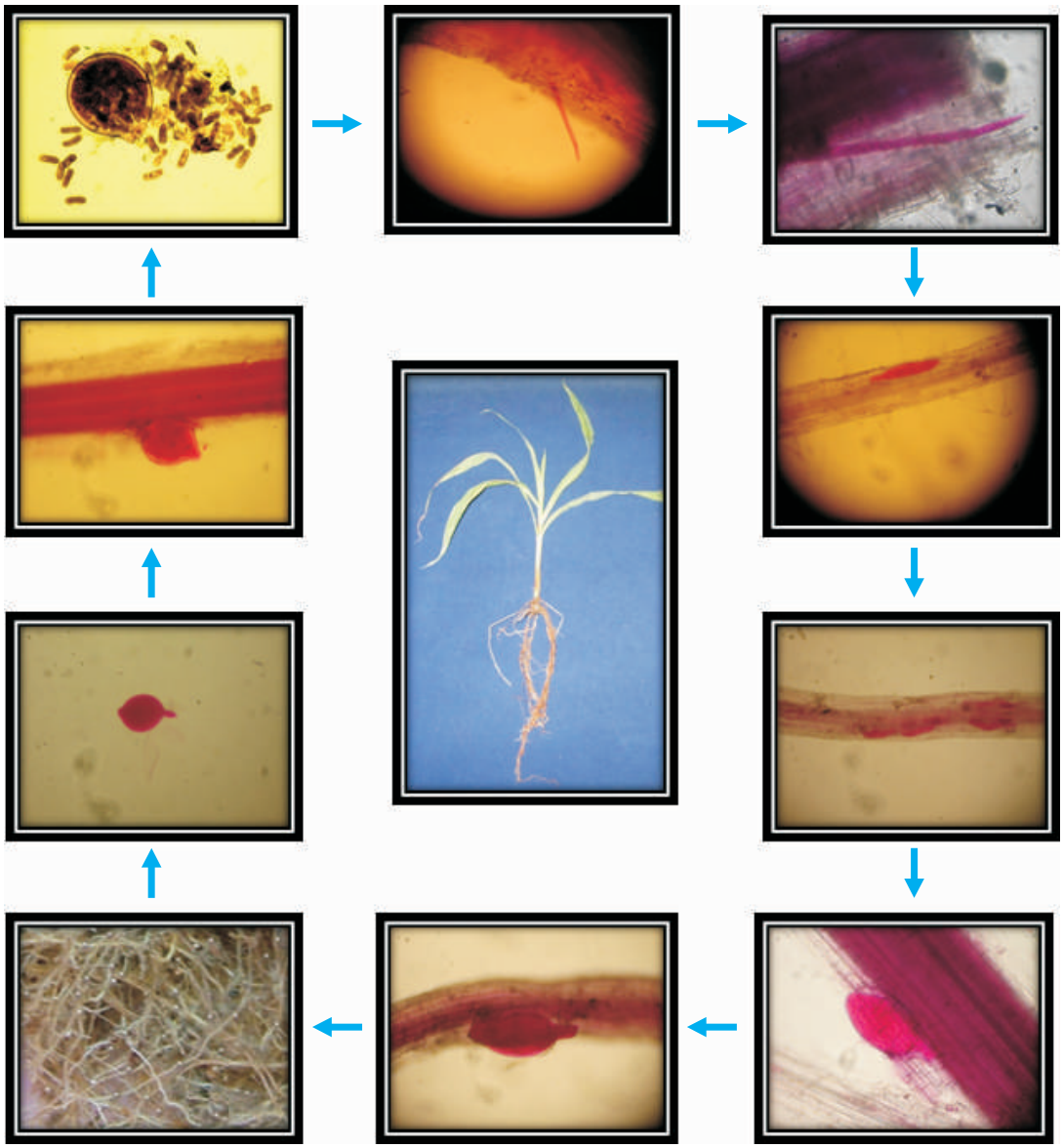
Vulval cone of *Heterodera zeae* is very prominent. Fenestrae ambifenestrate with two semifenestrae seperated by a fairly wide vulval bridge and surrounded by wide basin. There are four prominent finger like bullae arranged in a distinct fashion located immediately below the underbridge. This is a diagnostic and most reliable character for identification of this species.

### 7.6 Epidemiology:

The infective juveniles after penetrating the roots, move towards the stellar tissue to find a suitable site for feeding. Thereafter, they establish their head near the vascular tissues and become sedentary. For a successful parasitism, a juvenile induces the formation of syncytia in endodermal, pericycle, xylem or phloem cells surrounding the nematode head. The syncytia are formed due to partial dissolution of cell wall and protoplasm fusion of the neighbouring cells. As a result multinucleated cells with thickened wall are formed. The syncytium function's like transfer cell and is essential for growth and development of the nematode. The breakdown of conducting vessels leads to poor plant growth as the nutrient and water supply to the shoots is partially blocked. Ten days after penetration the syncytium continued to enlarge with a tendency towards rectal expansion. After 15-20 days the root section showed the stellar region was occupied by syncytium. A clear cell less zone was observed due to dissolution of cell wall around nematode body.

### 7.7 Disease cycle:

The life cycle of the MCN is similar to other cyst nematodes in the genus *Heterodera*. Individuals begin as eggs contained within a cyst, the hardened body of a dead female. Juveniles complete the molt from first stage to second stage within the egg, and it is the second stage juvenile or J2, which hatches from the egg. The J2 will seek out the roots of a suitable host plant and penetrate the root in search of a feeding site. The J2 will establish a permanent feeding site known as a syncytium and progress to adulthood. Males have not been described, but are assumed to exist. Female development is highly temperature dependent with development being minimal at 75°F and steadily increasing in rate from 80°F to 97°F. The optimal developmental temperature is $\geq$ 97 °F, which is considered high for cyst nematodes. In the greenhouse females will produce around 150 eggs when developing on corn. Upon completion of their life adult females will darken in color and become hardened forming protective cysts, which contain their eggs.



Life Cycle of Maize Cyst Nematode, *Heterodera zeae* on maize

## 7.8 Disease screening technique:

Plant parasitic nematodes are responsible to causes 10.2 per cent losses to maize. Though, large number of plant parasitic nematodes attacks on maize but maize cyst nematode (*Heterodera zeae*) is considered as most important and therefore, screening trials are carried out under artificially inoculated conditions in permanent plots to find out source of resistance against maize cyst nematode (*Heterodera zeae*). The observations on nematode infestation are recorded after 45 days of germination. The varieties/hybrids/ lines are categorized on the basis of cyst/plant as mentioned below:

| S. No. | Number of cyst/plant | Category |
|---|---|---|
| 1 | 0 -4 cyst/plant | Resistant |
| 2 | Above 4 -9 cyst/plant | Moderately Resistant |
| 3 | Above 9 cyst/plant | Susceptible |

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## Appendix 1

### Formula for calculation of Per cent Disease Index (PDI) of Foliar Diseases of Maize

Per cent disease index (PDI) is calculated using the following formula of Mckinney (1923).

$$\text{Per cent disease index (PDI)} = \frac{\textit{Sum of individual ratings}}{\textit{No. of leaves examined}} \times \frac{100}{\textit{Maximum disease rating}}$$

On the basis of PDI, the inbred lines/ varieties/ hybrids can be classified as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S).

The test inbred lines/ varieties/ hybrids with resistant reaction are considered acceptable for a breeding programme whereas test inbred lines/ varieties/ hybrids with moderately resistant are acceptable when lines with resistant reaction are not available.

# Notes

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