15.

Detection survey protocol for

Peronosclerospora sorghi (W. Weston & Uppal) C.G. Shaw

in Nepal

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Centre
Hariharbhawan, Lalitpur

March, 2025

Approved
Pandey
March, 2025

Detection survey protocol for *Peronosclerospora sorghi* (W. Weston & Uppal) C. G. Shaw
NPPO-Nepal, 2025

Endorsed by NPPO-Nepal on March 12, 2025

1. Background information

With entry into the WTO, Nepal has the opportunity to export its produce to the international markets. However, the exports from Nepal have not escalated to the same proportion as trade between developed nations. Developed countries have increased exports by using the rules of the SPS Agreement. At the moment, the Government of Nepal is obliged to use the SPS rules to exclude commodities that are posing a threat to the related industries within the country. Nepal should provide an adequate description of the health status of plant-based industries, while negotiating access to foreign trade. Prospective importers of Nepalese agriculture-related commodities assess the risk of introducing new pests based on the authentic pest information provided. Prospective importers also assess the phytosanitary measures being practiced in Nepal to reduce risk to an acceptable level. Extensive specimen-based records are the key for Nepal to negotiating with importing countries on a fair trading system. This document gives detailed guidelines for detection surveys of the pathogen *Peronosclerospora sorghi* in the field of agriculture. Besides, it will be applicable for monitoring, surveillance, import inspection and export certification and is the basis for specimen-based records to be developed by the NPPO-Nepal.

Under the Plant Quarantine and Protection Act, 2064, article 6(2), survey and surveillance functions and responsibilities are designated to NPPO-Nepal as per the sub-clause (i) "To perform such other functions as prescribed". This technical guideline to undertaking a pest detection survey of *Peronosclerospora sorghi* has been prepared with a view to guiding the survey activity. This protocol is prepared for researchers, plant protectionists, teachers, and other concerned professionals. This document will be a guide to submitting specimens to a laboratory for diagnosis and preservation.

1.1 About the target pest

Peronosclerospora sorghi, the causal agent of sorghum downy mildew (SDM), is a highly destructive pathogen that affects both sorghum and maize, leading to significant economic losses. Systemic infections result in severe symptoms, including chlorotic streaks, stunted growth, and, most critically, barren inflorescences, which directly impact grain production (Frederiksen et al., 1973). Yield losses can reach up to 20% (Jeger et al., 1998). In Israel, both forage sorghum and

maize have been severely affected, with disease incidence reaching up to 50% (Kenneth, 1976), while in the USA, some fields have recorded infection rates as high as 90% (Frederiksen et al., 1969).

1.2 Identity and taxonomy of the targeted pest (CABI, 2013)

1.2.1 Identity

Preferred scientific name: *Peronosclerospora sorghi* (W. Weston & Uppal) C.G. Shaw

Preferred common name: Sorghum downy mildew of maize

Other scientific names: *Sclerospora andropogonis-sorghi* (Kulk.) Mundk.

Sclerospora graminicola var. andropogonis-sorghi Kulk.

Sclerospora sorghi W. Weston & Uppal

Sorosporium andropogonis-sorghi S. Ito

Nepali name: सेते रोग

EPPO code: PRSCSO

1.2.2 Taxonomic tree of the pathogen is presented below:

Domain: Eukaryota

Kingdom: Chromista

Phylum: Oomycota

Class: Oomycetes

Order: Peronosporales

Family: Peronosporaceae

Genus: *Peronosclerospora*

Species: *Peronosclerospora sorghi*

1.3 Host range

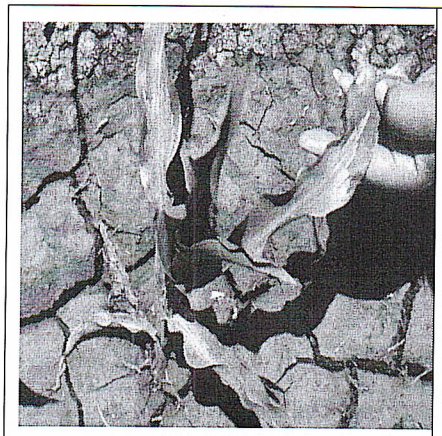
The host range of *P. sorghi* primarily includes maize (*Zea mays*), and sorghum (*Sorghum bicolor*) (Crouch et al., 2022). Some of the other wild hosts are *Andropogon sorghi, Sorghum almum, Sorghum arundinaceum* and *Zea diploperennis* (CABI, 2013).

1.4 Disease symptoms

In maize, local lesions are rare, but it is common in sorghum and are discreet, chlorotic areas, elongated with parallel edges (Kenneth, 1976). Systemic infection in sorghum and maize is characterized by leaf chlorosis (which invariably includes the leaf base), which usually appears ~2 weeks or more after sowing (symptom expression being dependent on the timing of infection). The intersection between the diseased and healthy tissue is sharply defined (resulting in the 'half-leaf symptom') (Figure 1). Progressively greater proportions of the lamina on subsequently emerging leaves show chlorosis, until most or all of the lamina are chlorotic.

In cool and humid weather, the asexual structures (conidiophores and conidia) of the pathogen appear on the surface of the diseased leaves, giving a white, down-like appearance. Infected plants can be stunted. On maize, whitish streaks develop from the base of the younger leaves, which turn brown as the oospores are produced in rows (Figure 2). In maize, leaf shredding is rare, but it is common in sorghum. Oospores are formed and scattered throughout the leaf mesophyll (Malaguti, 1978).

The leaves of infected maize plants tend to be narrower and more erect than those of healthy plants; the plants are often stunted and can die, and they are typically sterile, or have abnormal seed set. The pith of systemically-infected maize plants can show a mottled, brown discoloration, with the stem showing excessive brace root formation, abnormal tallness, as well as being barren (Warren et al., 1974). On maize in some regions, tassels affected by phyllody (crazy top) have been observed (Figure 3).



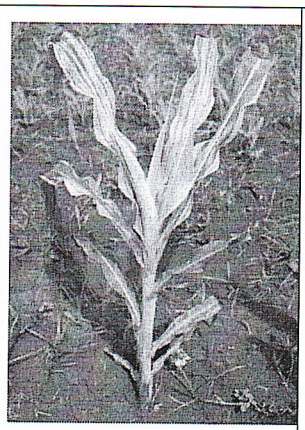


Figure 1. Maize leaf showing stunted plant growth and chlorotic striping and downy growth on leaves resulting from sorghum downy mildew (*P. sorghi*) (Source: EPPO, 2024)

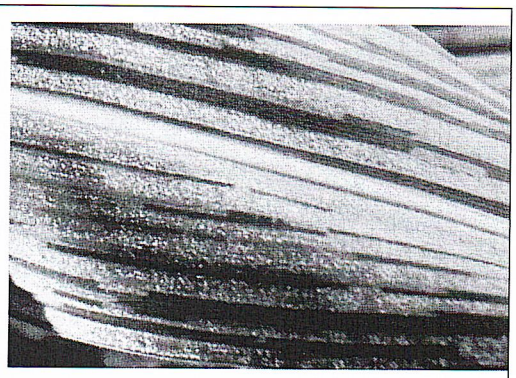


Figure 2. Leaf streaks on maize showing asexual spore production (Source: EPPO, 2024)

Figure 3. Crazy top of maize due to systemic infection of *P. sorghi* (Source: EPPO, 2024)

1.5 Epidemiology

The optimum temperature for sporulation is between 21 and 23°C (Safeeulla & Shetty, 1978). The optimum temperature for conidial germination is 23 °C. Germination does not occur at temperatures below 10 °C or above 32 °C (Safeeulla & Shetty, 1978). For infection to occur, a wet period and temperature between 10 and 33 °C for 4 h are necessary (Bonde et al., 1978). Conidia are typically produced between midnight and 05.00 A. M., with temperature of about 20°C and a minimum of 85% RH, and are dispersed by air currents (Kenneth, 1970; Shenoi &

Ramalingham, 1979; Bock et al., 1998). Oospore germination is optimal at 26 to 28°C (French & Schmitt, 1980). Maximum infection with oospores occurs between 24 and 29°C (Kenneth, 1970).

1.6 Mode of dispersion/Pathway

a) **Airborne conidia:** Sorghum downy mildew is commonly disseminated by air currents and rain splash. When the conidia are mature, they appear to be ejected from the conidiophore (Kenneth, 1970) and germinate shortly thereafter. This results in localized spread among neighboring hosts of the pathogen (Weston, 1923; Singh et al., 2020).

b) **Infected plant materials:** Plant materials, including crop residues from infested fields, can harbor the pathogen. When infected material is transported across regions or countries, the pathogen can be introduced into previously uninfected areas, especially if quarantine measures are not enforced (Irwin et al., 1999; Singh et al., 2020).

c) **Oospores:** The dissemination of oospores in the soil can be done by men or animals, attached to their feet or implements (Williams, 1984). Transmission by seeds is carried out by oospores immersed in the glumes or attached to the surface of the seeds, as well as by the mycelium located in the pericarp, endosperm or embryo (Chabrabarty et al., 1998; Pinto, 1999). Wind and water are also important in the dissemination of oospores (Jeger et al., 1998). Wind speeds greater than 2 m/s are capable of tearing infected leaves and dispersing these structures. Low wind speeds are sufficient to disseminate them over long distances (Bock et al., 1997).

1.7 Disease vector

Not known yet.

2. Detection survey

A detection survey is conducted in an area to determine if pests are present [FAO, 1990; revised FAO, 1995]. These surveys are more frequently carried out to determine pest status in an area, and they follow a definite survey plan, which is approved by NPPO-Nepal. These surveys are carried out either seasonally or annually and/ or following the eradication measures applied to a pest in a given area or production sites. These surveys are organized following a definite survey methodology based on statistical sampling, which is determined after taking into account the

biology of the pest and employing appropriate detection techniques such as field diagnostic kits, traps etc. The results of the survey are documented and communicated (PPD/NPPO-Nepal, 2071 BS).

2.1 Purpose and scope of detection survey

The purpose of the detection survey is to determine the presence or absence of *Peronosclerospora sorghi* in a given area or production sites. The scope will be limited to maize and other defined crops to be grown for haylage/silage production for export to China and other concerned countries.

2.2 Timing of survey

Detection surveys need to be done during the early seedling (5-15 days after emergence), vegetative stage (15-45 days after emergence), tasseling to silking stages (45–75 days after emergence) and reproductive stage (75 days onward) of the host crop (*i.e.*, maize) for detection of *Peronosclerospora sorghi* in the field.

2.3 Selection of survey area:

Field plots of maize and concerned crops in the target areas.

2.4 Materials required for survey

Paper bags (envelopes), blotter papers or stacks of newspaper, plant press, scissors, hand lens, loves, face masks, forceps, tags, permanent markers, GPS, Camera, and data sheets.

2.5 Identification method

To identify *Peronosclerospora sorghi,* a combination of diagnostic methods is typically used. Morphological identification of *P. sorghi* is based on known reference specimens, literature descriptions and taxonomic keys and descriptions.

2.5.1 Morphological features of *Peronosclerospora sorghi*

Conidiophores erect, spreading, comprising basal cell, main axis more or less complex, usually dichotomously branched, expanded top; 100–150 µm length to the septum (rarely by a partial, ring-like thickening); main axis 15–25 µm diameter; basal cell 7–9 µm wide, knobbed or bulbous at base (Figure 4). Branching comprises short, stout dichotomies usually with primary,

secondary, and tertiary branches terminating in tapering sterigmata; sterigmata 13 µm long. Conidia suborbicular, hyaline, 21–24.9 × 19–22.9 µm (range 15–28.9 × 15–26.9 µm) in diameter, thin-walled, germination direct by germ tubes. Oospores spherical, hyaline, 31–36.9 µm (mode 35–36.9 µm, range 25–42.9 µm) diameter; wall light Mars Yellow, 1.1–2.7 (range 0.3–4.3 µm) thick; contents finely granular with oil globules, positioned centrally or eccentric; germination direct by a branched, hyaline germ tube, 4.4 µm average width (range 2.5–8.3 µm) (Crouch et al., 2022).

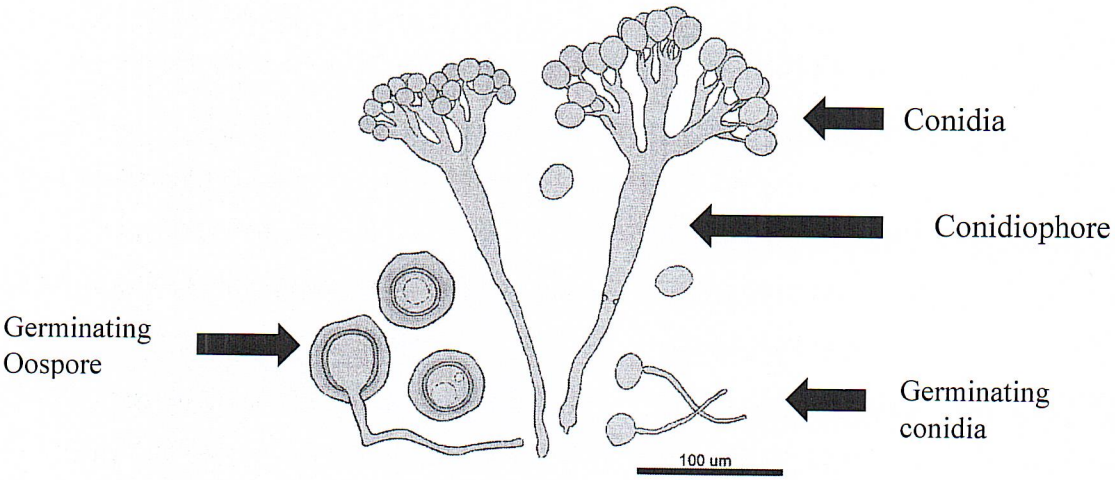


Figure 4. *Peronosclerospora sorghi*, conidiophore, conidia (including germinating conidia), and oospores (Crouch et al., 2022)

Table 1. Key differences between various *Peronosclerospora* species

Pathogen	Host range	Optimum temp. for sporangia production	Conidiophores/ Sporangiophores	Conidia/ Sporangia	Oospores
P. philippinensis (Philippine downy mildew)	Oats, teosinte, cultivated and wild	21-26°C	Erect and dichotomously branched two to four times. 150 to	Ovoid to cyclindrincal (17-21µm x 27-38µm),	Rare, spherical (25 to 27µm in diameter and

	sugarcane, cultivated and wild sorghum.		400µm in length and emerge from stomata.	slightly rounded at apex.	smooth-walled.
P. sacchari (Sugarcane downy mildew)	Sugarcane, teosinte, sorghum and wild grasses.	20-25°C	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
Peronosclerospora sorghi (Sorghum downy mildew)	Cultivated and wild sorghum, Johnson grass, teosinte, wild grasses (*Panicum, Pennisetum, Andropogon* species)	17-29°C	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.
Sclerophthora macrospora (crazy top)	Oats, wheat, sorghum, rice, finger millet, various grasses	24-28°C	Very short (14µm on average).	Lemon-shaped (30-65 x 60-100µm), operculate.	Pale yellow, circular (45-75µm).
Scleropthora rayssiae var. zeae (Brown	*Digitaria* species	22-25°C	Short determinate and produced from hyphae in	Oval to cyclindrical (18-26 x 29-67µm).	Spherical (29-37µm in diameter), brown.

stripe downy mildew)			the substomatal cavity		

2.6 Number of plants to be sampled

The number of maize plants to sample for the survey and surveillance of *Peronosclerospora sorghi* depends on the purpose of the survey, such as detection, delimitation, or monitoring; the field size; and the required confidence level for disease detection (Fletcher et al., 2010).

a) Based on the area of the field (Aggarwal et al., 2022)

- Small fields (up to 1 hectare): Sample at least 100 plants randomly across the field.
- Large fields (>1 hectare): Use a systematic approach such as a transect or grid pattern to sample 1-2% of the total plants in the field.

b) Initial detection

- For initial disease detection, sample 10 plants for 100 m^2 for inspection (FAO, 2023).

2.7 Sampling method

Several sampling methods are available to assess crops for plant disease. A common technique is to sample plants at random or uniform intervals along a path of a predetermined design. This technique is used to obtain samples from a field within a reasonable time. Conventional sampling includes the diagonal, W, V and X (Basu et al., 1977; Lin et al., 1979), which cover an entire field or are restricted to subdivisions of a field. These are referred to as whole-field and partial-field designs, respectively. The entire-field X and W designs are equivalent to one another and are the most precise; the diagonal design is intermediate, and the partial field designs are the least precise (Lin et al., 1979).

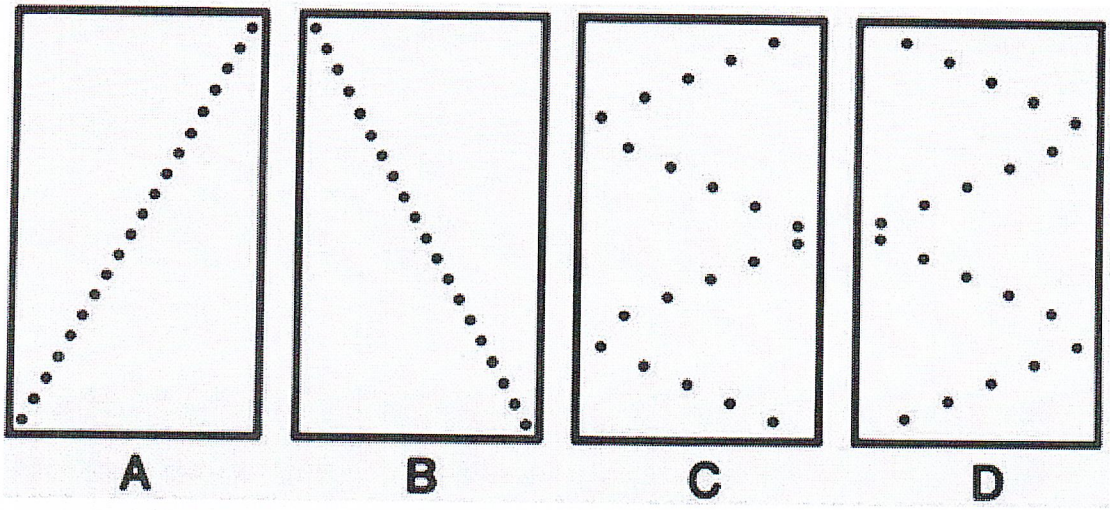


Figure 5. Sampling designs. Points represent sample sites. A. right diagonal, B. left diagonal, C. right W, D. left W (Source: Delp et al., 1986)

2.8 Plant parts to be observed

When surveying or monitoring maize for *P. sorghi*, the following plant parts should be carefully observed for symptoms and pathogen structures:

a) Leaves: Look for chlorotic streaks or patches on the upper leaf surface and white, downy fungal growth on the lower leaf surface (especially during early mornings).

b) Tassels: Look for malformation or phyllody (crazy top) in tassels.

c) Stem: Look for stunted growth and deformities in the stem.

d) Young seedlings: Look for chlorotic streaks and uneven or stunted growth

2.9 Specimen collection and preparation

For the proper collection and preparation of samples:

- Identify plants showing typical symptoms of the disease.
- Collect samples from various parts of the fields, especially from high-risk zones like field edges, low-lying areas, and regions near water sources.
- Collect leaves with visible symptoms and, if available, sample both early-stage and advanced-stage symptoms.
- Collect malformed or sterile tassels if symptoms are present.

- If seedlings are affected, uproot the entire plant carefully, ensuring the roots and above-ground parts remain intact.
- Place each sample in a separate paper envelope (do not use plastic bags) to avoid cross-contamination.
- Clearly label each sample with information related to field location, date of collection, crop variety, symptoms observed, and collector's name.
- Assign a voucher number for future reference.
- Transport samples to the diagnostic lab as soon as possible.

2.10 Herbarium preparation

For the proper herbarium preparation of samples:

- Select maize leaves with clear downy mildew symptoms.
- Collect multiple leaves from different plants for variation.
- Preferably collect in the early morning, when sporulation is visible.
- If whole leaves are too large, cut representative sections (~15–20 cm).
- Place the diseased leaves between sheets of newspaper or blotting paper.
- Arrange them flat and spread out to avoid overlap.
- Stack the papered samples in a plant press and apply even pressure.
- Change the blotting paper every 2-3 days to prevent fungal growth.
- Use herbarium sheets for mounting.
- Attach the dried leaves using glue or adhesive tape.
- Arrange to show key symptoms (both upper and lower leaf surfaces).
- Label the herbarium sheet with:
  - ✓ Scientific name: *Zea mays* (Maize)
  - ✓ Disease name: Downy mildew (*Peronosclerospora* sp.)
  - ✓ Collection details (date, location, collector's name, and specimen number)
  - ✓ Symptoms observed

✓ Host and pathogen identification details

2.11 Preparation for diagnosis

a) **Surface sterilization:** Surface sterilize small leaf sections showing symptoms with 1% sodium hypochlorite for 30 seconds, followed by rinsing with sterile water.

b) **Preparation of moist chamber:** Place two layers of filter paper at the bottom of the 9 cm Petri dish. Moisten the material with sterile distilled water. Ensure it is damp but not waterlogged to prevent leaf tissues from rotting.

c) **Incubation:** Place surface sterilized leaf sections onto the moistened blotter with the underside of the leaf facing upward. Place the Petri dish containing leaf sections in an incubator at 24°C for 24 hours. If sporulation is not observed, extend incubation up to 48-72 hours.

d) **Microscopic examination:** Examine the leaf tissue under a stereomicroscope to confirm the presence of conidiophores and conidia. For confirmation, slide preparation should be carried out (Cardwell et al., 1997; Janruang & Unartngam, 2018). To prepare slides, the following steps should be taken:

- Gently scrape the sporangia from the leaf surface using a fine brush or needle.
- Place the spores into a small drop of lactophenol on a clean glass slide.
- Carefully place a cover slip over the drop to avoid air bubbles.
- Gently press the cover slip to spread the sample evenly.
- Examine the slide under a compound microscope.
- Identify the sporangia
- Capture photographs for record-keeping and comparison.
- If needed, preserve slides by sealing the edges with nail polish for long-term storage.

Since *P. sorghi* is an obligate biotroph, culturing cannot be done in an artificial medium.

2.12 Data recording and mapping

- Data should be recorded in several respects like

- ✓ Date of collection
- ✓ Collection number
- ✓ Locality
- ✓ GPS coordinates
- ✓ Elevation
- ✓ Host plant type and infestation severity
- ✓ Plant growth stage
- ✓ Local name(s)
- ✓ Habit and habitat

- Use mapping tools like GIS to create infestation distribution maps

2.13 Diagnostic laboratories

- National Plant Pathology Research Centre, Nepal Agricultural Research Council, Khumaltar, Lalitpur
- Central Agricultural Laboratory, Department of Agriculture, Hariharbhawan, Lalitpur
- National Herbarium and Plant Laboratories, Department of Plant Resources, Godawari, Lalitpur
- Natural History Museum, Swayambhu, Kathmandu
- Private laboratories – Center for Molecular Dynamics Nepal (CMDN), Thapathali, Kathmandu Nepal Plant Disease and Agro Associates (NPDA), Balaju, Kathmandu, and others, identified if any.

2.14 Reporting

The responsible or concerned organizations (diagnostic laboratory) or an independent surveyor, after analysis and identification, should submit a report to the NPPO-Nepal for the reporting/declaration of the pathogen. The reports should include infestation maps, photographs and specimen vouchers.

2.15 Record keeping

NPPO-Nepal, in collaboration with responsible laboratories, will preserve the specimens and keep all the records safely. The documentation system should be well maintained by the NPPO-Nepal and the collaborating institutions will have access to it.

2.16 Molecular identification of *Peronosclerospora sorghi*

2.16.1 DNA extraction

Infected corn should be incubated as indicated above to get the fresh conidiophores and conidia produced on the upper and lower corn leaves. Conidiophores and conidia should be isolated using sterilized small syringes under a stereo-microscope and transferred to 50 ml of 5% (w/v) Chelex 100 buffer using sterilized distilled water in 1.5 ml Eppendorf tubes. Then, the solution should be boiled in a water bath for 8 min and mixed by vortexing three times and internal transcribed spacer 1 using PCR.

2.16.2 PCR reaction and sequencing

The DNA should be amplified in the regions of Domain 1- Domain 2 (D1/D2) of 28S rDNA using NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL4 primers (5'-GGTCCGTGTTTCAAGACGG-3'). PCR reactions should be carried out in 50 µl containing 5 µl of genomic DNA, 1x PCR buffer (10 mM Tris-HCl, 50 mM KCl), 2.5 mM MgCl2, 0.2 mM dNTP mixed, 20 pmol for forward and reverse primers and 1U Taq polymerase. Perform PCR Thermocycler with following steps: initial denaturation at 96°C for 1 min, followed 30 cycles of denaturation at 95°C for 30 sec, 848 annealing at 60°C for 30 sec and 72°C for 30 sec with a final extension of 4 min at 72°C. Conduct the amplification of the internal transcribed spacer1 (ITS1) region coupled with the 28S rDNA region using ITS1 (5'- TCCGTAGGTGAACCTGCGG-3') and ITS2 primers (5'- GCTGCGTTCTTCATCGATGC-3'). Perform PCR reactions in 40 µl containing 5 µl of genomic DNA, 1x PCR buffer (10 mM Tris-HCl, 50 mM KCl), 2.5 mM MgCl2, 0.2 mM dNTP mixed, 10 pmol for forward and reverse primers and 1U Taq polymerase. Perform PCR in a Thermocycler (Biometra) as follows: initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 1 min and 72°C for 1 min with a final extension of 10 min at 72°C. After amplification, 5 µl of all PCR products should be electrophoresed in a 1% (W/V) agarose gel and purified using Gene JET PCR purification kit (Thermo Scientific, Lithuania) and sent for sequencing (Janruang & Unartngam, 2018).

References

Aggarwal, S.K., Hooda, K.S., Bagaria, P.K., Debnath, S., Mallikarjuna, N., Gogoi, R., ... & Kaur, H. J. (2022). Surveillance of major maize diseases and their distribution scenario in different agroclimatic zones of India. *Maize Journal,* 11(2), 88–94.

Basu, P.K., Lin, C.S., & Binns, M.R. (1977). A comparison of sampling methods for surveying alfalfa foliage diseases. *Canadian Journal of Plant Science,* 57, 1091-1097.

Bock, C.H., Jeger, M.J., Fitt, B.D.L., & Sherington, J. (1997). Effect of wind on the dispersal of oospores of *Peronosclerospora sorghi* from sorghum. *Plant Pathology,* 46(3), 439–449.

Bock, C.H., Jeger, M.J., Mughogho, L.K., Mtisi, E., & Cardwell, K.F. (1998). Production of conidia by *Peronosclerospora sorghi* on sorghum crops in Zimbabwe. *Plant Pathology,* 47(3), 243–251.

Bonde, M.R., Schmitt, C.G., & Dapper, R.W. (1978). Effects of dew-period temperature on germination of conidia and systemic infection of maize by *Sclerospora sorghi*. *Phytopathology,* 68(2), 219–222.

CABI. 2013. *Peronosclerospora sorghi*. CABI Compendium, Wallingford, UK.

Cardwell, K.F., Kling, J.G., and Bock, C. 1997. Methods for screening maize against downy mildew *Peronosclerospora sorghi*. Plant Breeding, 116, 221-226.

Chabrabarty, S.K., Prasada Rao, R.D.V.J., Varaprasad, K.S., Singh, S.D., & Girish, G.A. (1998). The quarantine procedures for sorghum downy mildew—A note on the past experience. *Indian Journal of Plant Protection,* 26, 167–169.

Crouch, J.A., Davis, W.J., Shishkoff, N., Castroagudín, V.L., Martin, F., Michelmore, R., & Thines, M. (2022). *Peronosporaceae* species causing downy mildew diseases of *Poaceae*, including nomenclature revisions and diagnostic resources. *Fungal Systematics and Evolution,* 9(1), 43–86.

Delp, B.R., Stowell, L.J., & Marois, J.J. (1986). Evaluation of field sampling techniques for estimation of disease incidence. *Phytopathology*, 76, 1299-1305.

FAO. (2023). *Sampling and monitoring pests and diseases: Technical fact sheet*. Food and Agriculture Organization of the United Nations.

Fletcher, J., Luster, D., Bostock, R., Burans, J., Cardwell, K., Gottwald, T., ... & Smith, K. (2010). Emerging infectious plant diseases. *Emerging Infections,* 9, 337–366.

Frederiksen, R.A., Amador, J., Jones, B.L., & Reyes, L. (1969). Distribution, symptoms, and economic loss from downy mildew caused by *Sclerospora sorghi* in grain sorghum in Texas. *Plant Disease Reporter,* 53, 995–998.

Frederiksen, R.A., Bockholt, A.J., Clark, L.E., Cosper, J.W., Craig, J., Johnson, J.W., Jones, B.L., Matocha, P., Miller, F.R., Reyes, L., Rosenow, D.T., Tuleen, D., & Walker, H.J. (1973). Sorghum downy mildew—A disease of maize and sorghum. *Research Monograph No. 2.* Texas Agricultural Experiment Station.

French, R.C., & Schmitt, C.G. (1980). Effect of furfural on the *in vitro* germination of *Peronosclerospora sorghi* oospores. *Phytopathology,* 70(9), 877–880.

Janruang, P., & Unartngam, J. (2018). Morphological and molecular-based identification of corn downy mildew distributed in Thailand. *International Journal of Agricultural Technology*, 14(6), 845-860.

Jeger, M.J., Gilijamse, E., Bock, C.H., & Frinking, H.D. (1998). The epidemiology, variability, and control of the downy mildews of pearl millet and sorghum, with particular reference to Africa. *Plant Pathology,* 47(5), 544–569.

Kenneth, R.G. (1970). Downy mildew of *Graminae* in Israel. *Indian Phytopathology,* 23, 371–377.

Kenneth, R.G. (1976). The downy mildews of corn and other *Gramineae* in Africa and Israel and the present state of knowledge and research. *The Kasetsart Journal,* 10, 148–159.

Lin, C.S., Poushinsky, G., & Mauer, M. (1979). An examination of five sampling methods under random and clustered disease distributions using simulation. *Canadian Journal of Plant Science,* 59, 121-130.

Magill, C., Frederiksen, R., Malvick, D., White, D., Gruden, E., & Huber, D. (2006). *Philippine downy mildew and brown stripe downy mildew of corn.* The American Phytopathological Society.

Magill, C., Frederiksen, R., Malvick, D., White, D., Gruden, E., & Huber, D. (2013). *Recovery plan for Philippine downy mildew and brown stripe downy mildew of corn.* American Phytopathological Society. 23 p.

Malaguti, G. (1978). Oospore production in corn plants by *Peronosclerospora sorghi*. In *Proceedings of the 3rd International Congress of Plant Pathology*. Paul Parey. 114 p.

Pinto, N.F.J. de A. (1999). *Pathology of sorghum seeds*. Embrapa Corn and Sorghum. (Technical Circular, 32).

Safeeulla, K.M., & Shetty, H.S. (1979). Sorghum downy mildew in Asia: Assessment of present knowledge and future research needs. In *Sorghum Diseases—A World Review: Proceedings of the International Workshop at ICRISAT*.173 p.

Shenoi, M.M., & Ramalingam, A. (1979). Epidemiology of sorghum downy mildew II. Circadian and seasonal periodicities in conidia and oospores. *Proceedings of the Indian Academy of Sciences, B,* 88(II, 2), 95–102.

Singh, A.K., Singh, V.B., Srivastava, J.N., Singh, S.K., & Gupta, A. (2020). Diseases of maize crops and their integrated management. In: *Diseases of Field Crops: Diagnosis and Management*. Apple Academic Press. pp. 105–140.

Warren, H.L., Scott, D.H., & Nicholson, R.L. (1974). Occurrence of sorghum downy mildew on maize in Indiana. *Plant Disease Reporter,* 58(5), 430–432.

Weston Jr, W.H. (1923). Production and dispersal of conidia in the Philippine *Sclerosporas* of maize. *Journal of Agricultural Research,* 23(4), 239-278.

Williams, R.J. (1984). Downy mildews of tropical cereals. In: D.S. Ingram & P.H. Williams (Eds.), *Advances in Plant Pathology,* Vol. 2.. Academic Press. pp. 1–103.

ANNEXES

Annex- 1: Field datasheet

1. Name of field/Site visited:
2. Date/Time of visit:
3. GPS reference point Latitude:
   Longitude: Altitude:
4. Province: District:
   Municipality: Ward no./Place:
5. Climate data of locality: Average min. temp (in °C):
Average max. temp (in °C): Rainfall (in mm)
6. Survey/Field plot no.
7. Host plant species inspected: Variety:
8. Phenological stage of the plant:
7.1 Description of habitat (such as aspect, slope, vegetation type, soil type)

7.2 Alternate host plant species found infected, if any:
9. Sampling method:
10 Contact details of the local informant involved in the survey:
11. Details of pest recorded

SN	Scientific name	Common name	Plant parts affected	Symptom & Sign	Disease incidence	Severity % / Score

10. Any additional information (including collection of specimens for investigation):

11. Name/Signature of surveyor with date: