

Guide to African Soybean Seedborne Diseases and Pests

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Soybean Production in Sub-Saharan Africa

Soybean is a valuable crop primarily grown for its seed which has a broad range of uses from nutritional to industrial purposes. Soybean seeds consist of more than 36% protein as well as substantial amounts of dietary fiber, vitamins and minerals. Soybean is also a valuable source of oil.

Smallholder farmers produce the majority of the 1.5 million hectares grown in Africa with Nigeria, South Africa, and Zambia as the top soybean-producing countries. Soybean production is increasing at a rate of 6.8% per year in Sub-Saharan Africa compared to a worldwide increase of 4.7%. This is in

part due to the efforts of the Feed the Future Innovation Lab for Soybean Value Chain Research (SIL) to increase soybean production. SIL's mission is to build a foundation for soybean production throughout Sub-Saharan Africa by developing the knowledge, innovation, and technologies to enable successful and sustainable soybean production.

This laboratory guide is part of the SIL mission to increase soybean production in Africa by providing researchers and technologists with tools to improve all aspects of soybean production including seed health.

The Importance of Seed Health

Seed health is important for all aspects of soybean production and utilization. Healthy seed results in high rates of seed germination and seedling vigor.

Proper storage is important to keep seeds protected against storage molds and pests which can result in poor seed quality and germination. Many factors, including biotic (bacteria, fungi, pests, and viruses) and abiotic (mechanical and environmental) stresses can deteriorate seed health both in the field and in storage. By identifying the causes of decreased seed health and utilizing best

management practices to improve seed health, growers can increase seed quality and yield and, therefore, profitability.

This guide provides an overview of protocols for seed testing, and a guide to seedborne biotic organisms associated with soybean production in Sub-Saharan Africa.



Unhealthy soybean seeds that are discolored, shriveled, and/or damaged by insects

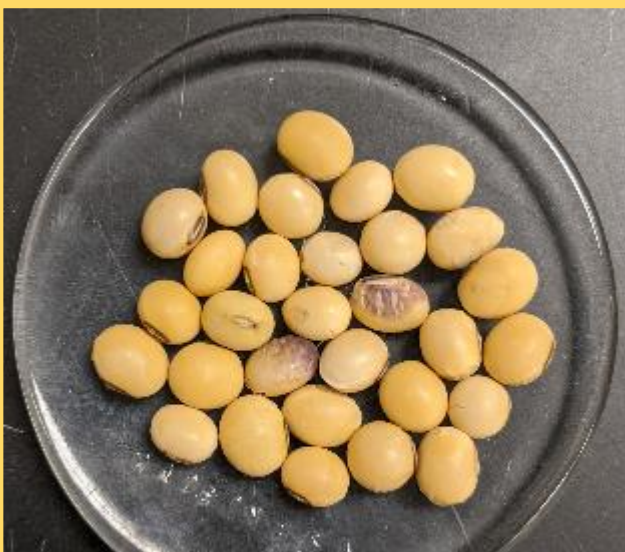
Visual Seed Quality Rating

Seed ratings based on visual evaluation are a quick way to assess seed lots. Fast evaluation methods are useful for high throughput systems, such as testing seed health in breeding programs. The seed rating scale described here (see next page) is determined by percent incidence of damaged and diseased seed within a seed lot. For instance, a rating of 0 indicates the lot has no visible symptoms like abrasions, wrinkles, or seed coat discoloration. A rating of 5 indicates 91-100% of the seed sampled has visible symptoms of pathogen infection, pest feeding, or mechanical damage.

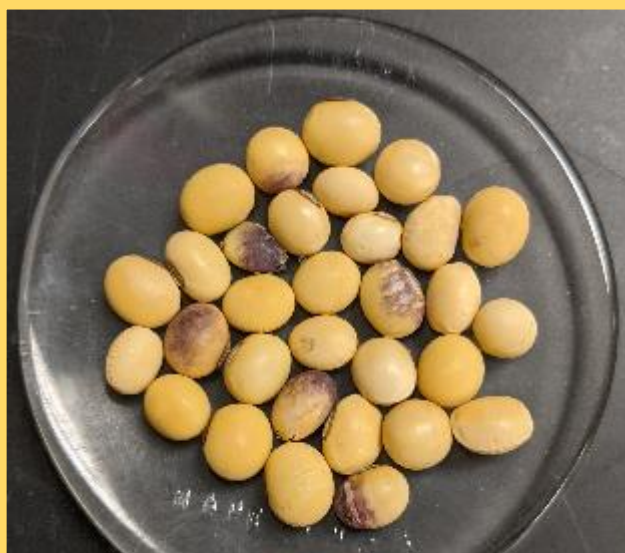
- A random sample, of approximately 20-50 seeds, is taken from a lot of interest.
- Ratings are based on the scale presented on the next page.
- To increase accuracy of ratings, each seed lot should be randomly sampled 2-4 times or more depending on the variability observed.



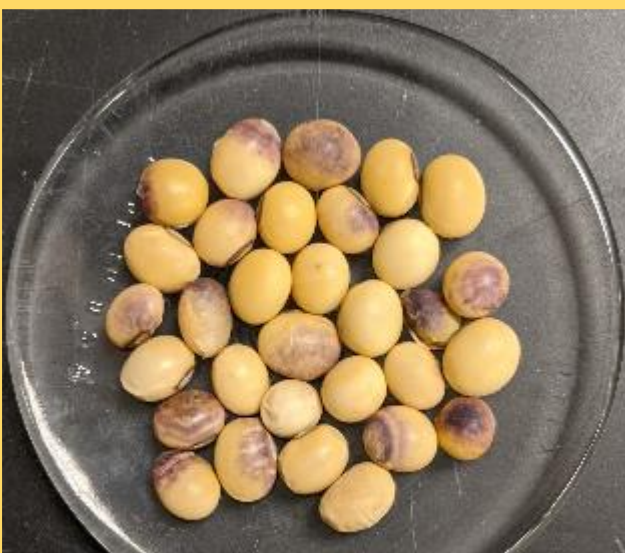
0: 0%



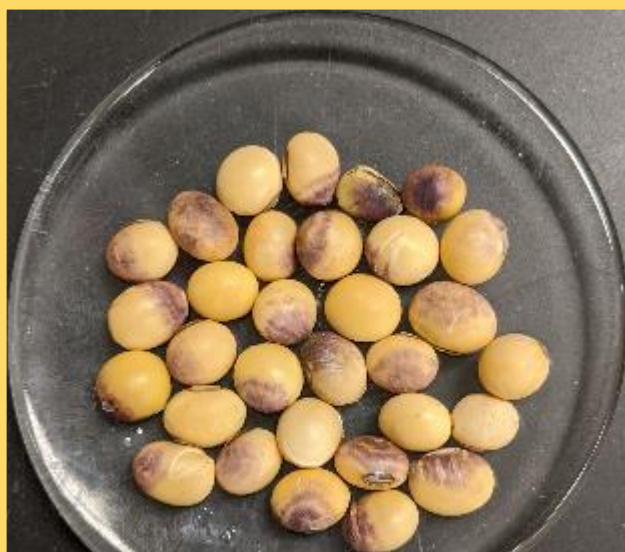
1: 1-10%



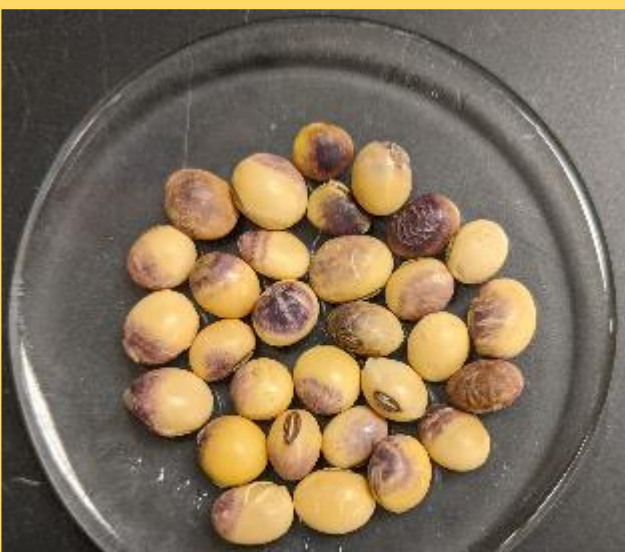
2: 11-32%



3: 33-66%



4: 67-90%



5: 91-100%

Seed Germination Testing

Seed germination tests determine seed viability and vigor depending upon the test. There are several methods that use different environmental conditions to evaluate seed viability, but all methods include selecting a random sample of seed. Most tests place seed in a moist environment to initiate germination. This can be done using a seed germinator, a home-made moist chamber (using a closed container and moist paper towels), or agar plates. Soybean seeds typically germinate within 3-6 days.



Agar Plate



Moist Chamber



Germinator

Homemade Moist Chamber

Moist chambers create a high humidity environment that is ideal for germinating seed and producing fungal structures, such as fruiting bodies that contain spores, to aid in microbe identification. Moist chambers can easily be made by using any closed container, paper product, and distilled water.

1. Obtain a clean glass or plastic storage container with a lid. Containers are ideally clear to allow for easy inspection of materials.
2. Cover the bottom of the container with clean paper towel or filter paper.
3. Moisten paper with distilled water by pouring water into container or using a spray bottle.
4. Drain out any excess water from container.
5. Place seed inside container on top of moistened paper.
6. Close lid and store at room temperature.

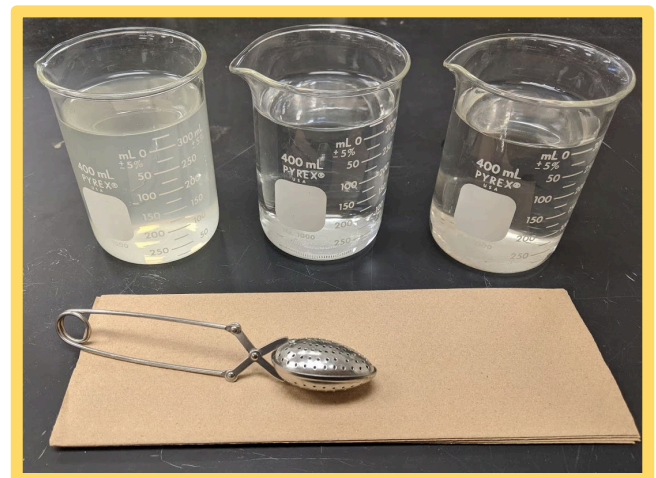
Seed Surface Disinfection for Seedborne Microbial Growth

Seed surface disinfection is a method to remove microbes and debris from the surface of the seed prior to plating. This ensures microbes that grow on the media are from within the seed and not a surface contaminant. This guide will show one method of surface disinfection. However, there are many ways to do this. You will need:

- A timer
- A tea strainer or container with drainage holes
- A container of bleach (0.6% sodium hypochlorite)
- Two containers of sterile water
- Paper towels

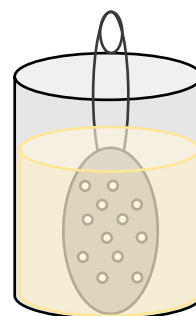
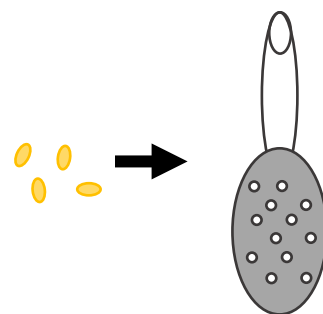


Large plastic containers with ice cube trays with holes carved into the bottom used for high-through put seed screening

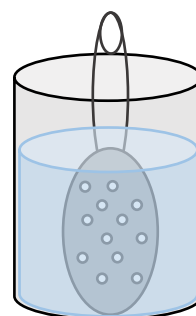


Tea strainer and 600 ml Pyrex beakers used for smaller quantities of seed

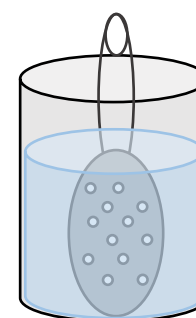
1. Place seed in container with drainage holes.
2. Submerge container with seeds into bleach solution (0.05% NaClO) for 5 minutes.
3. Remove container from bleach solution and allow excess to drain.
4. Submerge container in sterile water for 1 minute, then drain.
5. Submerge container in a second sterile water bath for 1 minute, then drain.
6. Shake off any excess water before placing on sterile paper towels to remove any excess liquid.



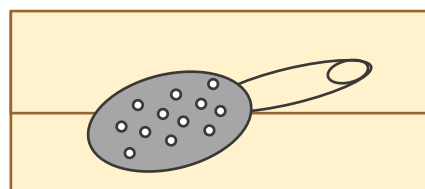
0.05% NaClO
5 min



dH₂O
1 min



dH₂O
1 min



Seed Plating for Seedborne Microbial Growth

After seed are disinfected and rinsed, they are plated on agar to evaluate germination and seed microflora

You will need:

- Petri dishes
 - Potato dextrose or other nutrient-rich media to encourage microbial growth with 10-15% agar
 - Container of 95% ethanol
 - Alcohol burner
 - Tweezers
1. Sterilize tweezers by submerging the end of the tweezer into ethanol and then holding in flame for 20 seconds. Let tweezers cool before use.
 2. Place 10 sterile seed using sterilized tweezers onto media, equally spaced from each other.
 3. Use tweezer to press seed into media securing seed in place.
 4. Close petri dish and incubate in the dark at 25°C for 6 days.
 5. After 6 days, record the number of seeds germinated and identify any seed-borne microbes.



Day 0

Seed is plated on agar.



Day 2

Plated seed begins to swell. Beginning stages of germination and radical formation are visible.



Day 4

Seed continues to germinate (as shown on right) and microbial growth starts to be visible on the plate.



Day 6

Microbes have grown and developed characteristics to identify to genus level. Viable seedlings have all germinated.

Examples of Plated Seeds Six Days After Plating



7 Germinated seed
2 *Cercospora* spp.
1 *Fusarium* sp.

5 Germinated seed
1 Bacteria
1 *Alternaria* sp.
1 *Diaporthe* sp.
3 *Fusarium* spp.



2 Germinated seed
1 *Aspergillus* sp.
1 *Alternaria* sp.
1 Bacteria
2 *Diaporthe* spp.
2 *Fusarium* spp.

Microbial Identification Tools

Bacteria

Although there are some morphological characteristics that differ between bacteria, such as color and cell wall composition (determined by gram staining), other physiological and molecular based assays are often used for identification and may include the use of polymerase chain reaction (PCR) assays and gel electrophoresis.

Fungi

There are many morphological characteristics that are visible that may help identify fungi to the genus level, including fruit body structures, spore formation, and pigments. There are many illustrated mycological glossaries and keys available to aid in fungal identification, such as the [Illustrated Genera of Imperfect Fungi](#). Serological tests are also available to aid in fungal diagnostics. Molecular based assays to speciate fungi are also common and sequencing specific genes are often used to compare and confirm species.

Viruses

Viruses are extremely small particles that require an electron microscope to visualize. Identifying viruses requires molecular techniques. Serological tests like the enzyme-linked immunosorbent assay (ELISA) are common methods to identify viruses by using virus-specific antibodies to adhere to viral particles and change color if the virus is present. There are also PCR-based assays for virus detection and identification.

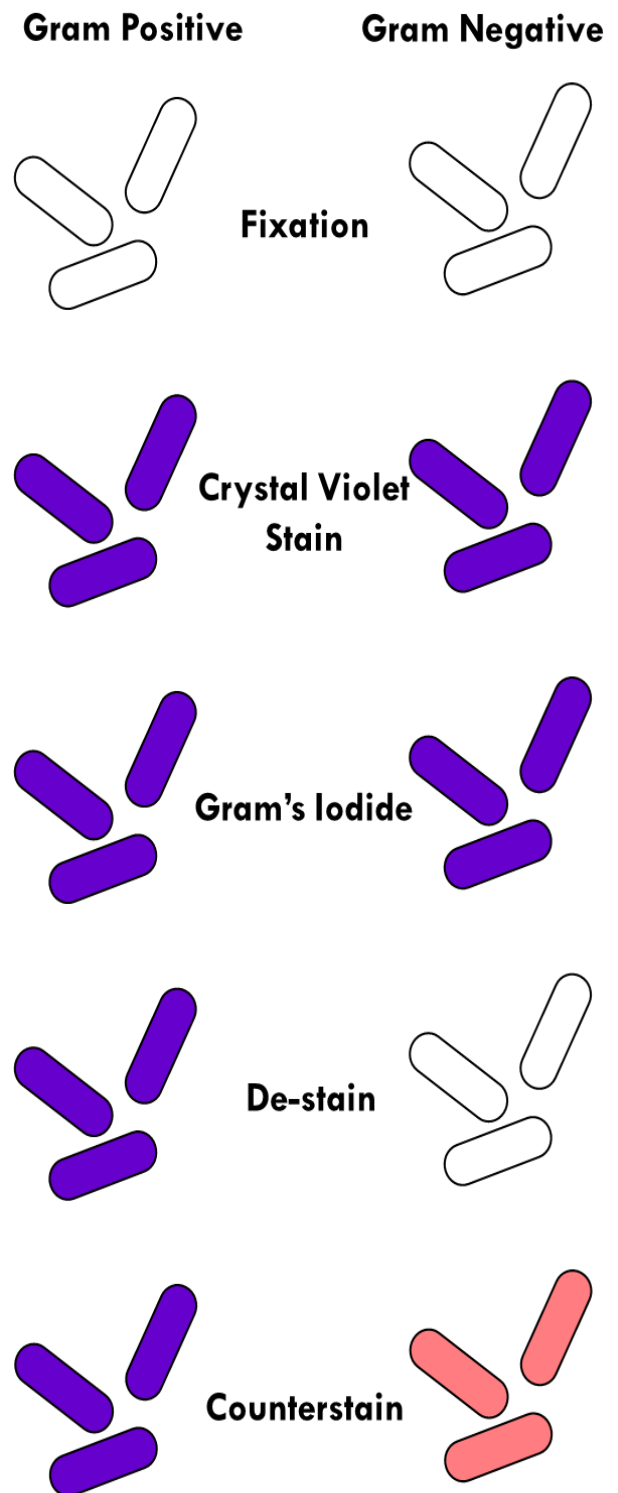
Gram Staining for Bacterial Identification

Gram staining is a multi-step staining technique used to help identify bacteria. Bacteria with thinner cell walls stain pink or red and are classified gram positive. Gram negative bacteria have thicker cell walls, and they stain violet. Most pathogenic bacteria are gram negative but some, such as the bacteria that cause bacterial tan spot and bacillus seed decay, are gram positive.

You will need:

- **Stain Solution A:**
 - 10g crystal violet in 100ml 95% ethanol
- **Stain Solution B:**
 - 1g ammonium oxalate in 100ml distilled water
- **Gram's Iodide**
 - 0.33g iodine + 0.67g potassium iodide in 100ml distilled water
- **De-staining Solution**
 - 95% ethanol
- **Counterstain Solution**
 - 2.5g Safranin O in 100ml 95% ethanol

1. Prepare Stain Solutions A and B and store for 24 hours and filter prior to use.
2. A thin smear of bacteria from a fresh culture are transferred to a microscope slide and 'fixed' by passing over a flame until dried.
3. Flood slide with Stain Solution for 1 minute.
4. Gently wash slide with distilled water for 2 seconds to remove excess stain.
5. Flood slide with Gram's Iodide Solution for 1 minutes.
6. Gently wash slide with distilled water for 2 seconds.
7. Flood slide with De-staining Solution until solution runs clear off slide.
8. Flood slide with Counterstain Solution for 1 minute.
9. Gently wash slide with distilled water until runoff is clear and then blot dry slide.
10. Observe under light microscope using oil immersion solution.



Bacteria

Bacterial pathogens cause important foliar diseases, including bacterial blight, tan spot, and pustule.

Bacteria can not directly infect and rely on natural openings, such as hydathodes, or wounds from hail or harsh winds to get into the plant. Once inside, bacteria may travel through the vascular system into the seed.

Effective management of bacterial diseases relies on multiple methods, especially preventative measures. This includes removal of infested debris from diseased plants, planting resistant cultivars, and using pathogen-free seed.

However, bacteria cannot be differentiated solely on morphological characteristics so molecular sequencing or pathogenicity testing will be needed for confirmation.



Bacterial blight

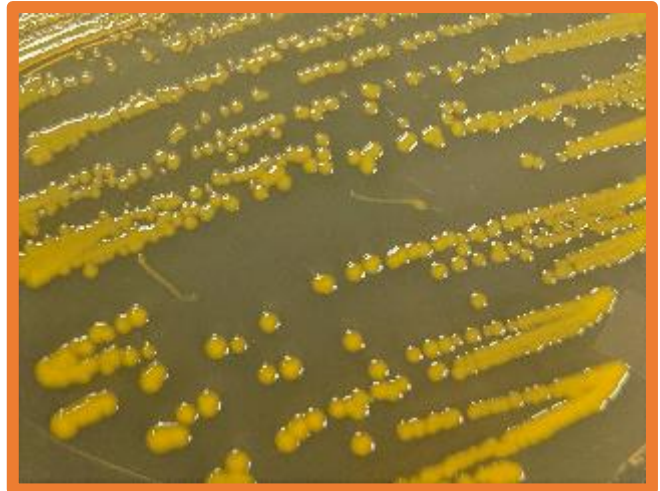
Bacterial tan spot

Bacterial pustule

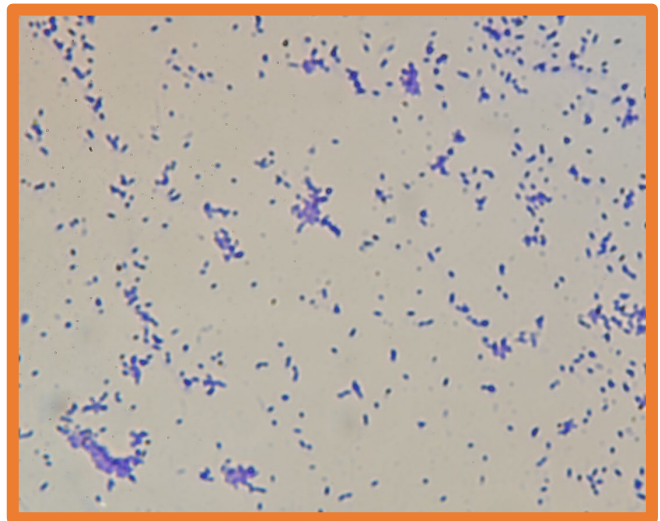
Bacteria

Curtobacterium flaccumefaciens* pv. *flaccumefaciens

Curtobacterium flaccumefaciens pv. *flaccumefaciens* (Cff) is the causal pathogen of bacterial tan spot. The bacterium is gram positive, rod-shaped, and 3 µm long. Colonies are yellow, orange, or pink in color. Seeds infested with Cff may have yellow discoloration.



Culture of Cff



Bacterial cells of Cff stained with crystal violet under a light microscope at 200x

Bacteria

Pseudomonas spp.

Pseudomonas is another genus of bacteria commonly found in soybean seed. Species within this genus are epiphytic, beneficial, or pathogenic. One pathogenic species, *P. syringae* pv. *glycinea* is the causal pathogen of bacterial blight on soybean. Colonies of *Pseudomonas* are gram negative and rod-shaped with polar flagella. Colonies are white in color.



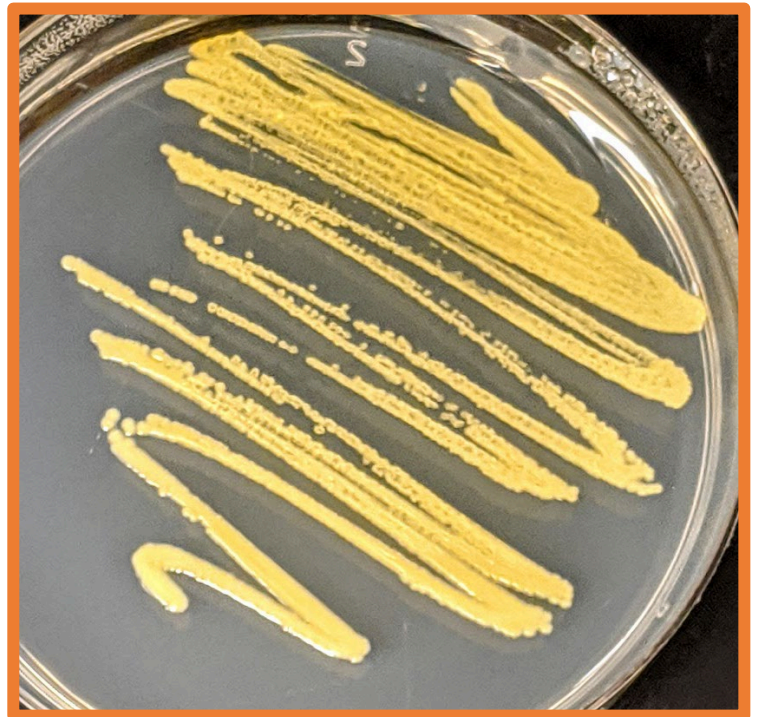
Culture of *Pseudomonas* sp.

Bacteria

Xanthomonas spp.

Xanthomonas spp. are almost exclusively found in plant tissues and infect a wide host range. Some species, like *X. axonopodis*, have what are known as pathovars (pv.). Pathovars are strains of the species that infect a specific host or group of hosts.

Xanthomonas axonopodis pv. *glycines* is the causal pathogen of bacterial pustule of soybean. The bacterium is gram negative and rod-shaped, measuring 0.7 to 2.0 μm long. Colonies are yellow and mucoid.



Culture of *Xanthomonas* sp.

Fungi and Fungal-like Organisms

Fungal and fungal-like pathogens are a diverse group of pathogens that cause most plant diseases. Important soybean disease caused by these pathogens include charcoal rot, frog eye leaf spot, and Fusarium root rot. Fungal-like pathogens, also known as oomycetes or water molds, differ from fungi based on cell wall composition and other characteristics but infect and cause disease like fungi. These pathogens may infect several parts of the plant, including seed. They enter indirectly, like bacteria, or directly by using a specialized structure called a penetration peg or by producing toxins or enzymes to kill and break down plant tissues.

Effective management methods for fungal diseases are dependent on the disease, but include planting disease resistant cultivars, in-season pesticide application, crop rotation, sanitation, and using pathogen-free seed.



Charcoal rot on soybean



Frog eye leaf spot on soybean

Fungi and Fungal-like Organisms

***Cercospora* spp.**

Several important soybean diseases, including frog eye leaf spot (FELS), *Cercospora* stem and leaf blight (CLB), and purple seed stain (PSS), are caused by species within the *Cercospora* genus. All of them can be seed-borne. Seed symptoms range from asymptomatic to brownish to pink to purple discoloration of the seed coat. In culture, species grow slowly, often pigment the agar, and produced translucent, multi-cellular conidia ranging in length from 38 to 445 μm for *C. kikuchii* (CLB and PSS) and from 40 to 60 μm for *C. sojina* (FELS).



Purple seed stain



***Cercospora* spp. growing out of seed
plated on potato dextrose agar**

Fungi and Fungal-like Organisms

***Colletotrichum* spp.**

Anthracnose is a disease caused by several species within the *Colletotrichum* genus, including *C. chlorophyite*, *C. incanum*, and *C. truncatum*. Cultures are slow growing and consist mainly of a stomata with a dense layer of small fruiting bodies called acervuli. Mature acervuli produce masses of conidia. Conidia are translucent, unicellular, and curved ranging from 15 to 20 μm long.



***Colletotrichum* sp. growing out of seed (top), setae on an acervulus oozing conidia (middle), and conidia (bottom)**

Photo of conidia taken by Hui-Ching Yang

Fungi and Fungal-like Organisms

***Diaporthe* spp.**

Species within the *Diaporthe* genus can cause stem canker, pod and stem blight, and Phomopsis seed decay on soybean.

The fungi generally grow fast in culture and are white to yellow in color. Colonies are appressed with irregularly shaped stromata forming throughout the culture and may produce fruiting bodies call pycnidia. Spores are translucent, unicellular, and fusiform to cylindrical in shape ranging from 2-5 μm .



Pycnidia oozing spores of *Diaporthe* sp. growing on soybean seed



Culture of *Diaporthe* sp.

Fungi and Fungal-like Organisms

Fusarium spp.

Fusarium is a widespread genus of pathogenic fungi that cause disease in all soybean production areas. *Fusarium* root rot is caused by a complex of species within the *Fusarium* genus.

In general, the species grow fast in culture often producing aerial mycelia that may be white or off white, pink, red or other colors. Some produce light pink to dark magenta pigments. Macroconidia are multi-cellular, fusiform, and 28-42 μm long. Microconidia are single-celled, kidney shaped, and 5-12 μm long.

Several species in this genus produce trichothecenes, which are toxic to humans and animals.



Fusarium spp. growing out of soybean seed plated on potato dextrose agar



Underside of *Fusarium* spp. cultures

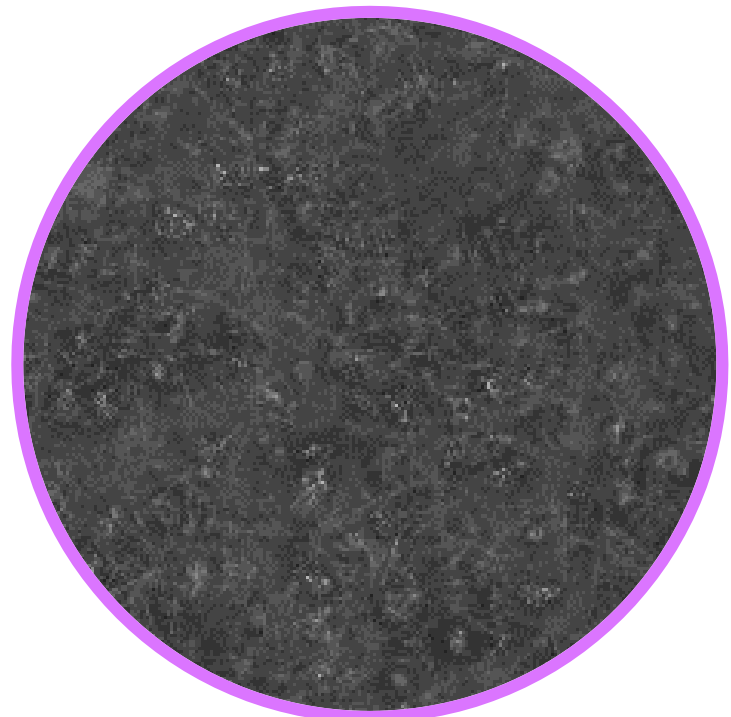
Fungi and Fungal-like Organisms

Macrophomina phaseolina

Macrophomina phaseolina is the only species within the genus *Macrophomina* and causes the disease charcoal rot. The fungus grows fast in culture. Juvenile mycelia are translucent and darken to black as they mature. Infected seed may turn a reddish color when plated out.

The most distinct sign of *M. phaseolina* is the presence of microsclerotia.

Microsclerotia are small harden bits of mycelia 50-70 μm in diameter that serve as an overwintering structure. Microsclerotia can sometimes appear on the seed as well.

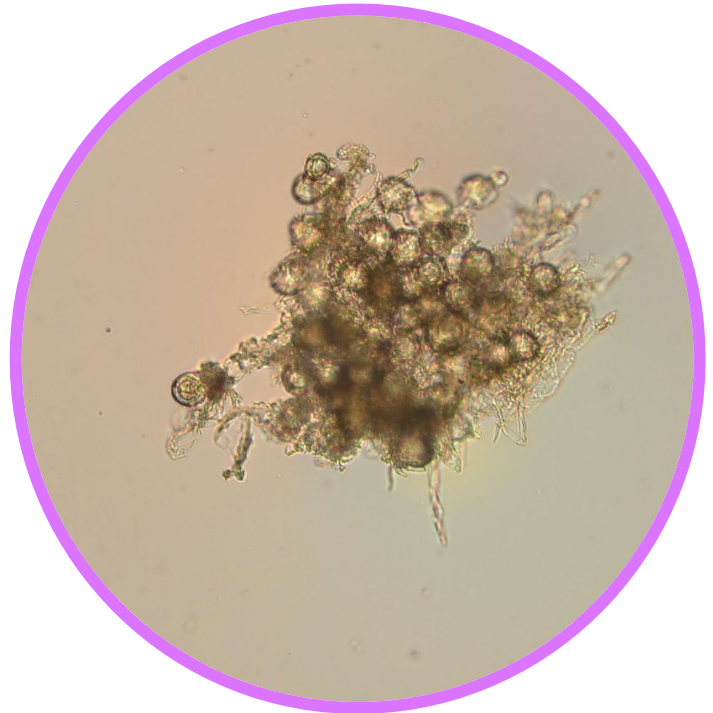


Culture of *Macrophomina phaseolina* (top)
and close up of microsclerotia (bottom)

Fungi and Fungal-like Organisms

Peronospora manshurica

Peronospora manshurica is a fungal-like organism and the causal pathogen of the foliar disease downy mildew. This pathogen is an obligate biotroph meaning it requires a live host to grow so it will not grow in culture. The pathogen will create a 'crust' on the outside of the seed and is chalky in appearance. This crust is the mycelia and thick-walled spores called oospores. Oospores are light brown or yellow and 20-45 μm in diameter.



P. manshurica on the surface of a soybean seed (top) and oospores under a light microscope at 200x (bottom)

Viruses

There are 46 known viruses that can infect soybean and many of these infect the seed. Symptoms on plants are not diagnostic to differentiate the viruses. Viruses are tiny particles only visible by electron microscope or detected by molecular diagnostic techniques.

There are some symptoms that are common with virus infection. This includes seed coat mottling, discoloration, or a bleeding hilum. Viruses commonly found on soybean include those that cause bean pod mottle, soybean mosaic, soybean vein necrosis, and tobacco streak.

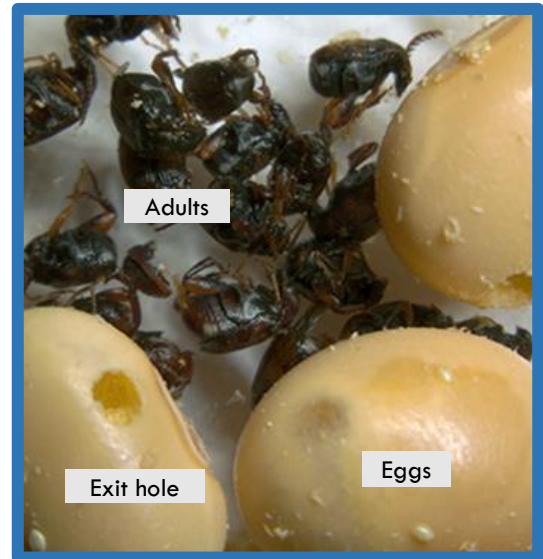


Soybean seeds with bleeding hila, a common symptom of virus infection

Pests

Bruchids

Bruchids, or weevils, are common storage pests that can cause up to 100% damage. There are two common types in Sub-Saharan Africa: the Chinese bruchid and the cowpea bruchid. Female bruchids will lay eggs on mature soybean seeds in the field. In storage, the eggs will hatch, and larvae will infiltrate the embryo of the seed and feed until adulthood. The most distinct characteristic of bruchids is the circular hole the adults make while eating their way out of the soybean seed.



Chinese bruchid eggs, adults, and exit holes from feeding

Photo taken by Doris Lagos-Kutz



Damage inside soybean from Chinese bruchid feeding

Photo taken by Doris Lagos-Kutz

Pests

Stink bugs

There are four species of stink bugs that can cause damage on soybean by feeding on the seeds in developing pods. One species is brown while the other three are green, but all have a distinct shield-shaped abdomen.

Stink bugs have a piercing-sucking mouth part called a proboscis. They use their proboscis to penetrate pods and suck nutrients out of the seed. Stink bug damaged seed will be severely shriveled and/or have yeast spots.



Adult stink bug

Photo taken by Leslie Ingram/bugwood.org



Damage on seeds from stinkbug feeding

Photo taken by Mississippi State University Extension, Mississippi-crops.com

Storage Pathogens

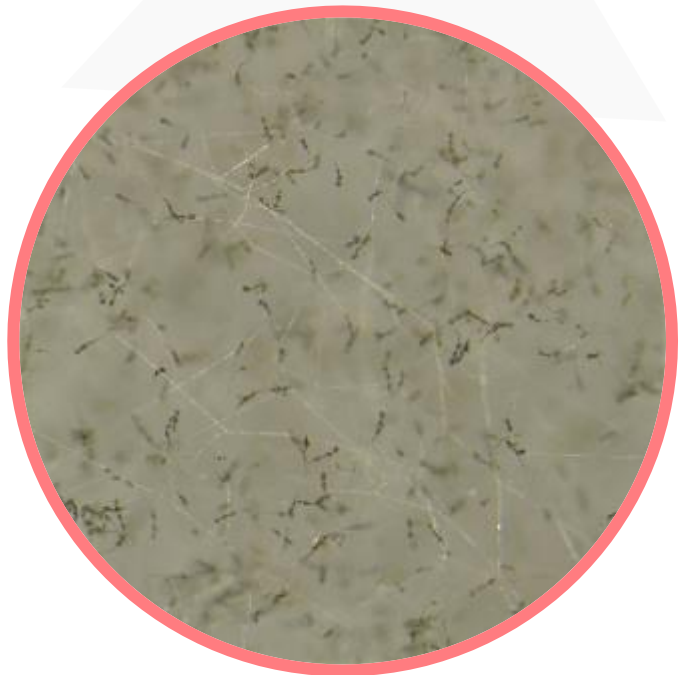
Over 150 microbes have been identified to be associated with stored soybean seeds. Some of these microbes are pathogenic and, in conducive conditions, can infect and colonize stored seed. This can cause substantial loss by deteriorating the seed and seed viability. These pathogens can also reduce the quality of seed by reducing protein and oil content. Some pathogens produce toxins which pose a health risk to animals and humans making infected seed unfit for consumption. Ultimately, these all will reduce the price of sale for the producer. Storage loss results in around \$100 million lost in price reductions in the United States.

Since these pathogens thrive in moist, warm environments, controlling the temperature and humidity is the most effective management against storage pathogens. Seed should be dried to at least 12% moisture prior to storage. Seed should be stored at 10°C or less with 13-14% moisture. Any damaged or diseased seed should be removed prior to storage.

Storage Pathogens

***Alternaria* spp.**

Species of *Alternaria* can cause leaf spot, pod necrosis, and seed decay on soybean. Cultures have fluffy, aerial mycelia that darken as they mature and have a black underside. The most distinct characteristic of *Alternaria* is their conidia. They are darker in color, club-shaped, and multicellular measuring 23-34 μm long.



Alternaria sp. growing out of seed on potato dextrose agar (top) and conidia under a light microscope at 20x (bottom)

Storage Pathogens

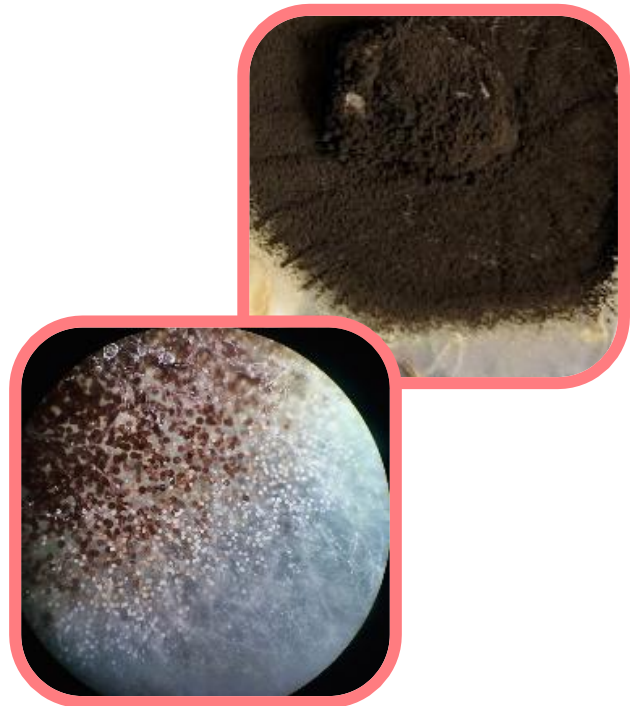
Aspergillus spp.

Aspergillus is a genus of saprophytic fungi that are known to produce mycotoxins that are carcinogenic to animals and humans.

Aspergillus species can be a broad range of colors including black, green, and orange on the top side of the colonies with a white to light yellow underside. A distinct characteristic of *Aspergillus* is their circular conidiophores that appear white when young and produce ample pigmented conidia as they mature. Conidia are globose and 3-6 μm in diameter.



A. flavus growing out of seed (top) and conidiophores (bottom)



A. niger growing out of seed (top) and conidia (bottom)

Storage Pathogens

***Bacillus* spp.**

Species within *Bacillus* genus are common storage bacteria that will cause a seed decay and can lead to 100% yield loss under moist, warm conditions. *Bacillus subtilis* is the most common species found on soybean.

Bacillus subtilis is a gram-positive, rod-shaped bacterium measuring 2-3 μm long. Colonies appear white to cream colored with wrinkled folds.



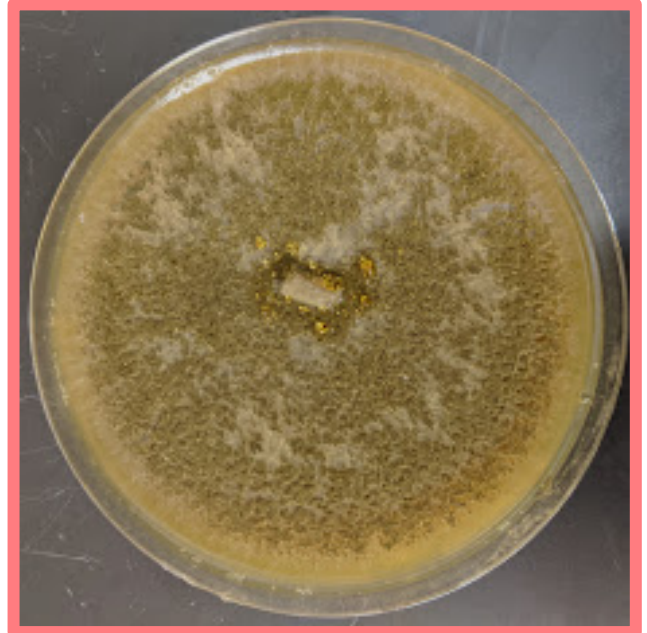
Bacillus sp. growing out of seed plated on potato dextrose agar

Storage Pathogens

***Chaetomium* spp.**

Species within the *Chaetomium* genus are common storage molds. These fungi do not rot the seed but may delay soybean germination. *Chaetomium* spp. also produce chaetoglobosins, which are mycotoxins harmful to humans.

The fungus is fast growing in culture with little mycelia. As the colony matures it will turn the agar a tan or yellow color. The most distinct sign of *Chaetomium* is the perithecium, a type of fruiting body, it produces. Perithecia are large and black in color with long setae around the surface.



Culture of *Chaetomium* sp.

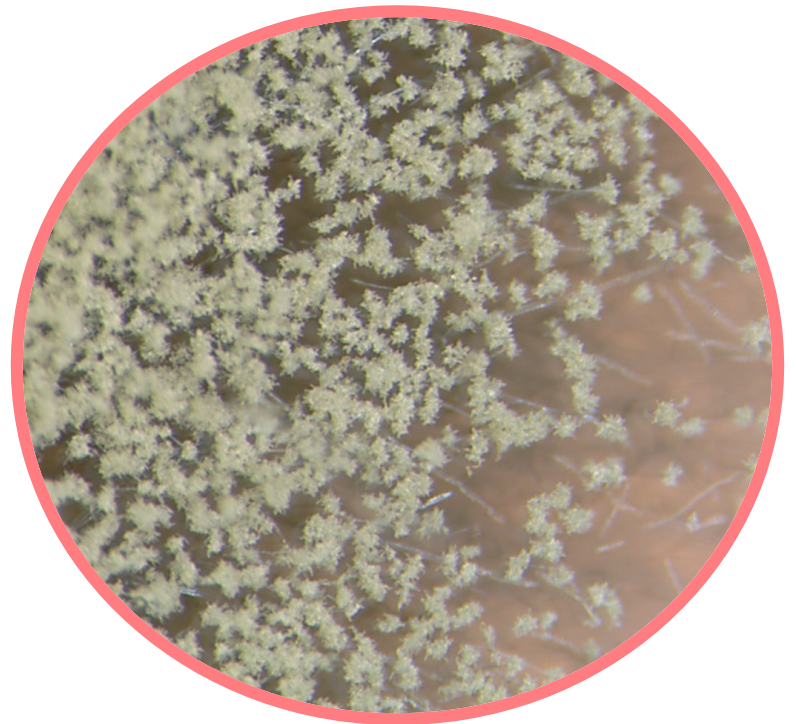


Perithecia of *Chaetomium* sp. on soybean

Storage Pathogens

Cladosporium spp.

Some species within the *Cladosporium* genus are common storage pathogens that reduce seed viability. In culture, *Cladosporium* spp. have a velvet-like texture and are olive green to black in color with a black underside. Mycelia are brown and segmented. Conidia are produced in chains that are pigmented and elliptical to cylindrical in shape. Conidia vary widely in size and are one to four celled depending on the species.

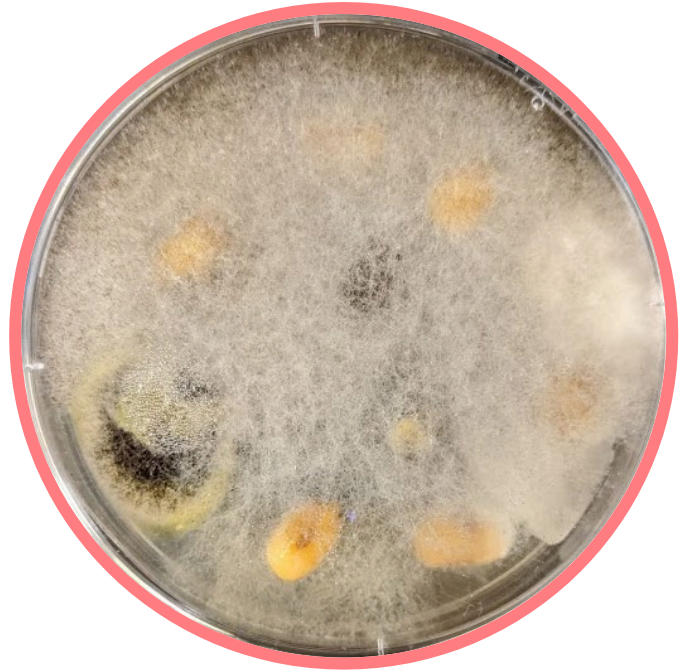


Cladosporium sp. growing out on potato dextrose agar (top) and conidia under a light microscope at 50x (bottom)

Storage Pathogens

***Mucor* spp. / *Rhizopus* spp.**

Mucor and *Rhizopus* are both genera within the Mucoraceae family and common storage contaminants. Both species produce fast growing, white, fluffy, aseptate mycelia. Mycelia can reach several centimeters in height in culture. Mature cultures will turn greyish brown and produce sporangia, which are spherical structures filled with spores. Spores are round and 4-8 μm in diameter.



Rhizopus sp. Growing out of seed plated on potato dextrose agar (top) and sporangia under a light microscope at 50x (bottom)

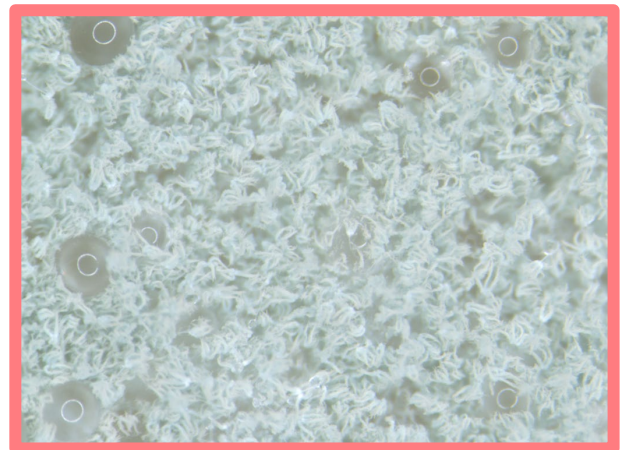
Storage Pathogens

***Penicillium* spp.**

Penicillium is another common fungal contaminant and some species produce mycotoxins. In culture, *Penicillium* is fast growing with a velvety texture. Colonies are initially white but turn blueish green or greyish green as they mature and produce spores. Spores are produced on round conidiophores as long chains creating a 'broom-like' structure. Conidia are unicellular, round, and range from 2.5 to 5 μm in diameter.



Culture of *Penicillium* sp.



Conidia of *Penicillium* sp. at 25x under light microscope

Additional Resources

- [American Microbiological Society's Gram Stain Protocols](#)
- [American Phytopathological Society](#)
- [Certified Integrated Pest Management for Africa Online Course](#)
- [Compendium of Soybean Diseases and Pests. 5th ed.](#)
- [Field Guide to African Soybean Diseases and Pests](#)
- [Plantwise Knowledge Bank](#)
- [Principles of Seed Pathology, 2nd ed.](#)
- [Soybean Innovation Lab](#)
- [Scouting for Common Soybean Seed Diseases](#)
- [The Identification of Fungi: An Illustrated Introduction with Keys, Glossary, and Guide to Literature](#)

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