LiveSeeding

Training in organic breeding

Module 15: **Hot Water Treatments for Seed Health**

Unit 15.2: **Contamination thresholds and detection**

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UK Research and Innovation

Location of pathogens in seed

- **Biotrophs**
  - **Obligate parasite, do not kill the host, have narrow host range, can not be cultured; viruses some fungi; typically in embryo**

- **Necrotrophs**
  - **Kill host cells, feed on dead tissue, wide host range, can be cultures; fungi and some bacteria; typically seed coat and/or endosperm**

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Pathogens on the seed coat

- *Xanthomonas campestris* pv. *campestris* - black rot in crucifers
- *Pseudomonas syringae* pv. *tomato* - bacterial speck in tomatoes
- *Alternaria* spp.- causes blights
- *Cladosporium* spp. - causing leaf mold
- *Botrytis cinerea* - causes gray mold
- Tobacco mosaic virus (TMV)

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Pathogens on the seed coat

- *Fusarium spp.* – causing willt and root rot
- *Verticillium spp.* – Verticilium willt
- *Colletotrichum spp.* - causing anthracnose
- Tomato Brown Rugose Fruit Virus (ToBRFV)

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Pathogens in the edosperm

- *Fusarium spp.* – causing willt and root rot
- *Aspergillus* spp.
- *Penicillium* spp.

Pathogens in the embryo

- *Fusarium spp.*
- *Colletotrichum spp.*
- *Pseudomonas syringae pv. phaseolicola* - causes halo blight in beans
- *Xanthomonas campestris pv. vesicatoria* - causes bacterial spot in peppers and tomatoes
- Tomato Mosaic Virus (ToMV)
- Tobacco Mosaic Virus (TMV)

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Seedborne inoculum trasholds

- Lettuce mosaic virus (LMV)
  - zero tolerance in USA and Australia
  - 0,1% in Europe
- *Xanthomonas campestris* pv. *campestris* in brassicas
  - 1 in 30 000 seeds
- *Phoma lingam* in brassicas
  - 1 in 10 000 seeds
- *Ascochyta fabae* in broad beans
  - 1 in 600 seeds
- *Cylindrocladium parasiticum* in peanuts
  - 1 in 400 seeds

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Seed health testing

- **Sensitive** – able to detect pathogen at low concentration
- **Specific** – able to detect targeted pathogen in the presence of non-targets
- **Speed** – rapid turnaround
- **Reliable** – unaffected by slight variation in test conditions
- **Repeatable** – consistent performance
- **Reproducible** – transferable, consistent performance across different laboratories
- **Cost-effective**

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Methods of detection of seed borne pathogens

Test type	Fungi	Bacteria	Virus	Nematodes
Visual examination	√	x	x	x
Grow-out	x	√	√	x
Bioassay	x	x	√	x
Wash or Soak - microscope exam	√	x	x	√
Blotter test	√	x	x	x
Agar media test	√	√	x	x
ELISA	x	√	√	x
PCR	√	√	√	√

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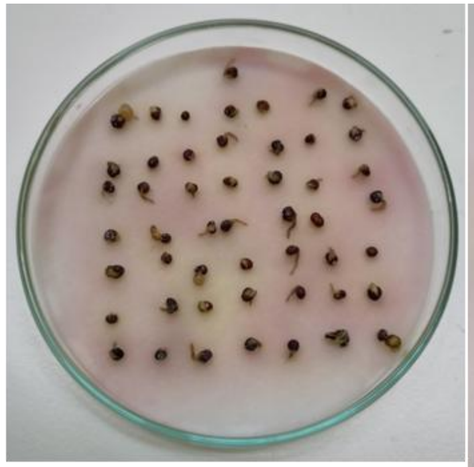
Source: The American Phytopathological Society (APS)

Blotter Tests

- For fungal pathogens
- **Advantages -** inexpensive, versatile, direct observation of viable pathogen
- **Limitations** – small numbers of seeds is tested, expertise needed

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ISTA

Seed Health Methods 2023 (Desktop Version)

Price	Free
Associate Member-Price	Free

7 001a Detection of Alternaria dauci in Daucus carota (carrot) seed by blotter method	Download
7 001b Detection of Alternaria dauci in Daucus carota (carrot) seed by malt agar method	Download
7 002a Detection of Alternaria radicina in Daucus carota (carrot) seed by blotter method	Download
7 002b Detection of Alternaria radicina in Daucus carota (carrot) seed by malt agar method	Download
7 003 Detection of Botrytis cinerea in Helianthus annuus (sunflower) seed	Download
7 004 Detection of Leptosphaeria maculans and Plenodomus biglobosus in Brassica spp seed	Download
7 005 Detection of Ascochyta pisi on Pisum sativum (pea) seed	Download
7 006 Detection of Colletotrichum lindemuthianum in Phaseolus vulgaris (bean) seed	Download
7 007 Detection of Alternaria linicola Botrytis cinerea and Colletotrichum lini in Linum usitatissimum (flax linseed) seed	Download
7 008 Detection of Caloscypha fulgens in Picea engelmannii and P glauca (spruce) seed	Download
7 009 Detection of Fusarium circinatum in Pinus spp (pine) and Pseudotsuga menziesii (Douglas fir) seed	Download

- International Seed Federation (ISF)
- National Seed Health System (NSHS) in the United States

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To summarize:

- Pathogens can be **located** in the seed coat, endosperm, or embryo, and this determines both risk and treatment options
- **International and national thresholds** for seedborne inoculum vary by pathogen and reflect their epidemiological importance
- **Effective seed health testing** must be sensitive, specific, reliable, and cost-effective.
- **Methods** such as blotter tests, agar plate methods, and molecular assays are commonly used, with ISTA protocols providing standardized procedures
- **Reliable detection and threshold-based decision**-making are essential prerequisites for deciding whether hot water treatment or other measures should be applied

In the next unit, we will turn to the process of hot water treatment itself, looking first at general considerations and workflow.

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