Diagnosing Strawberry Root and Crown Diseases

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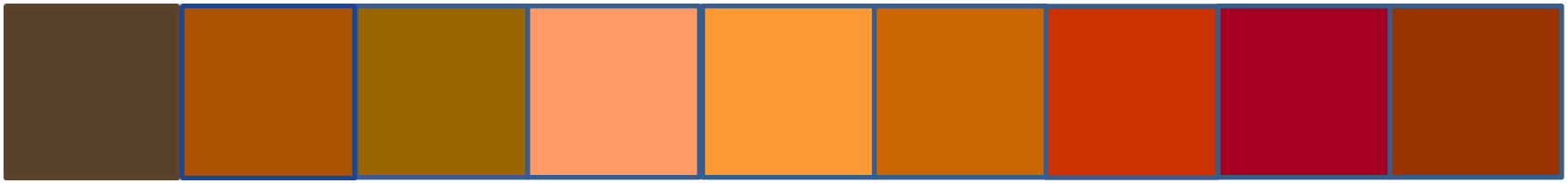
Soil Borne Pathogens

- Symptoms are not diagnostic – not even to separate biotic from abiotic
- There is no one single method for isolating or identifying all root or crown pathogens
- All cause “browning”, streaking and decay
- Field guides offer only clues: more red- than brown, more orange- than red, buff-colored, tan-colored, reddish- colored, rust-colored, cinnamon-red or chocolate- brown, brown- but not black ??

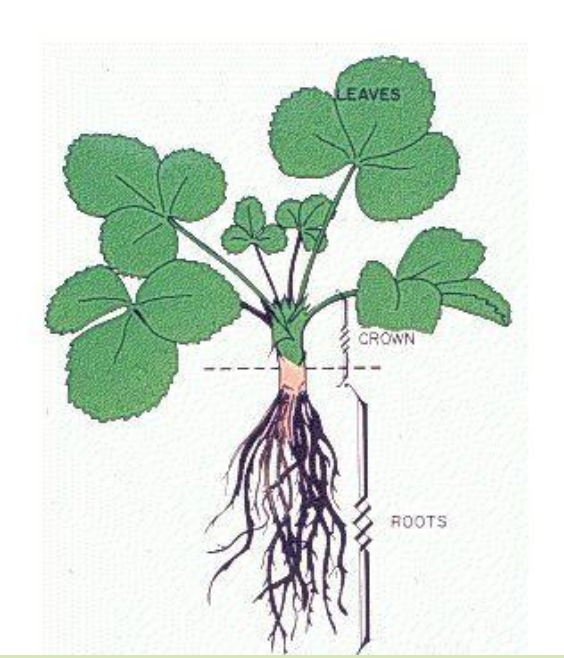


Soil Borne Pathogens

- Microscopy alone not usually an option
- For regulatory purposes, identification needs to be *at least* to species, often to a subspecies or variety level (highly accurate)
- Without selective isolation techniques, pathogens cannot be detected or confirmed
- Saprophytic competitors and secondary pathogens quickly invade diseased tissues making diagnosis impossible

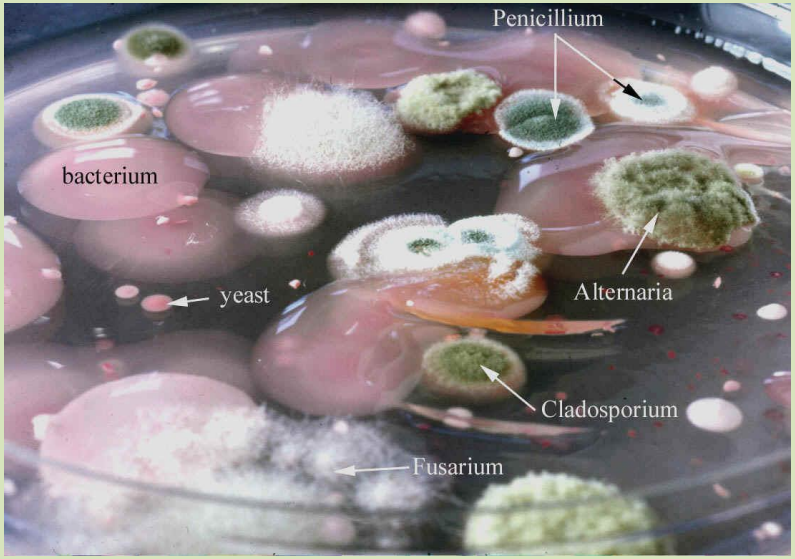
Soil Borne Pathogens- Culturing

- Diagnosis depends on the quality of the sample
- Always want the whole plant – not completely dead
- Include rhizosphere soil only for certain tests – sclerotial counts, nematodes



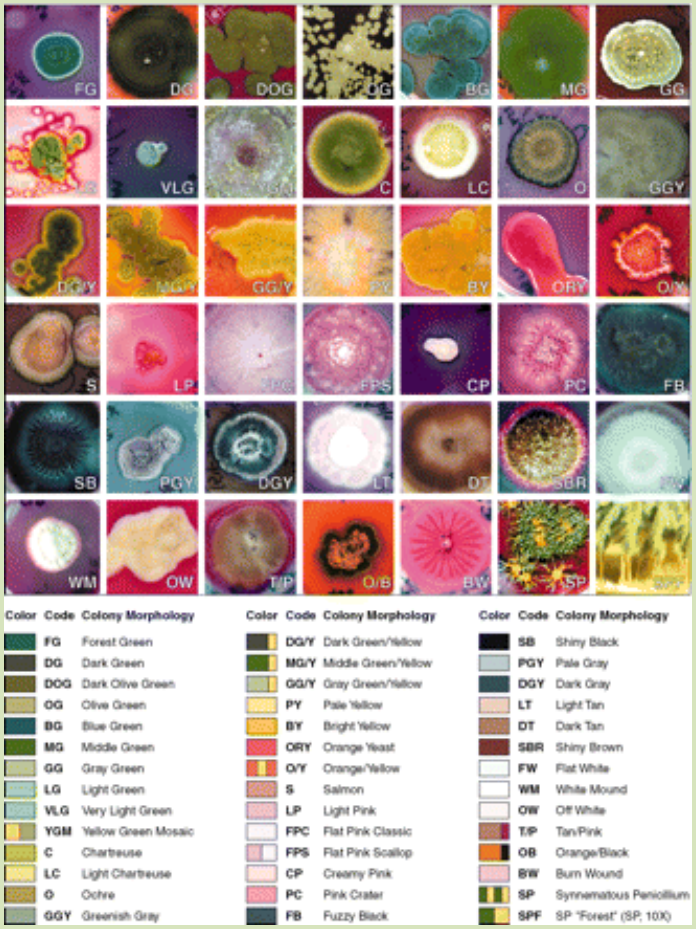
Soil Borne Pathogens- Culturing

- Non-selective media – generally supportive to bacterial and fungal growth, favors saprophytes (soil is dirty)
- Semi-selective or selective media adds anti-bacterials or anti-fungals, surfactants, amino acids, or sugars - available for some pathogens not all, value is variable



Non-selective medium

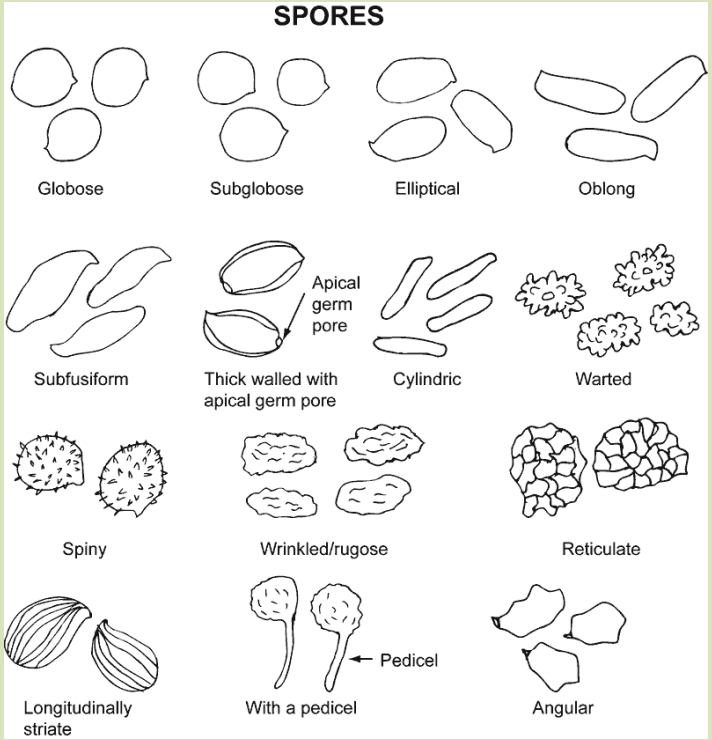
Soil Borne Pathogens- Culturing

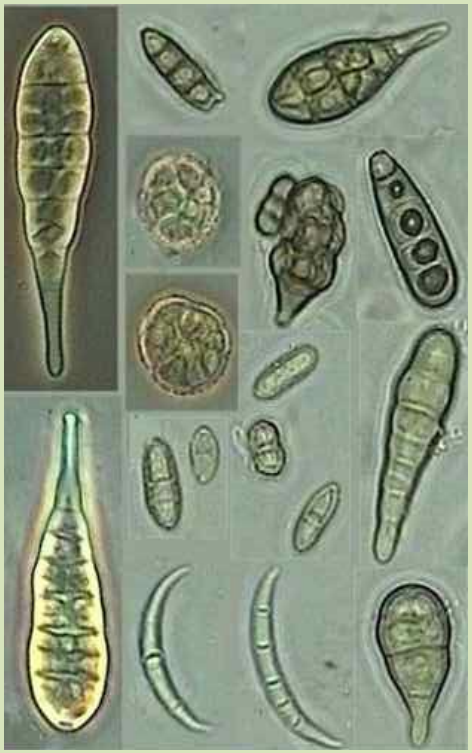


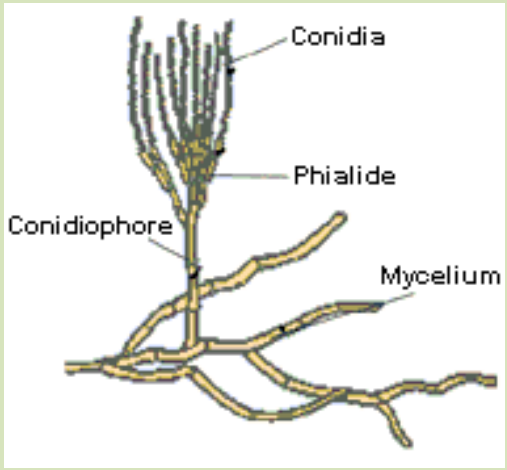
- Look at colony shape, size, color and texture
- Variable depending on the growth medium used, age of colony, growth conditions (light spectrum and day length, temperature)

Soil Borne Pathogens- Culturing

- use microscopy to look at spore sizes, shapes, colors, and the structures that produce spores







Soil Borne Pathogens – other methods

- **ELISA**: Enzyme-linked Immunosorbent Assay
- No need to culture and gives very rapid results
- Very sensitive to low pathogen density
- Doesn't require the pathogen to be alive (+/-)

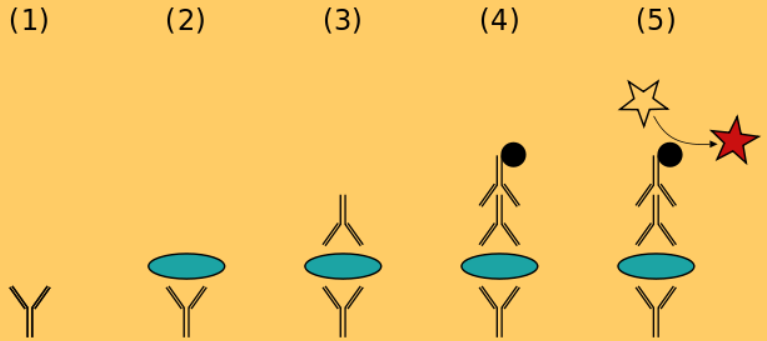
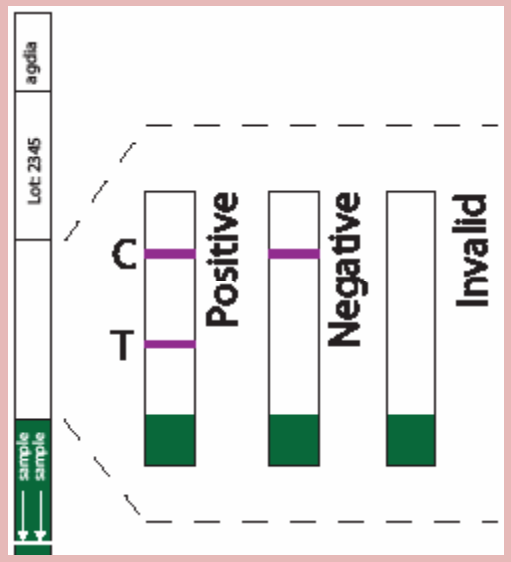


Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form (color change)

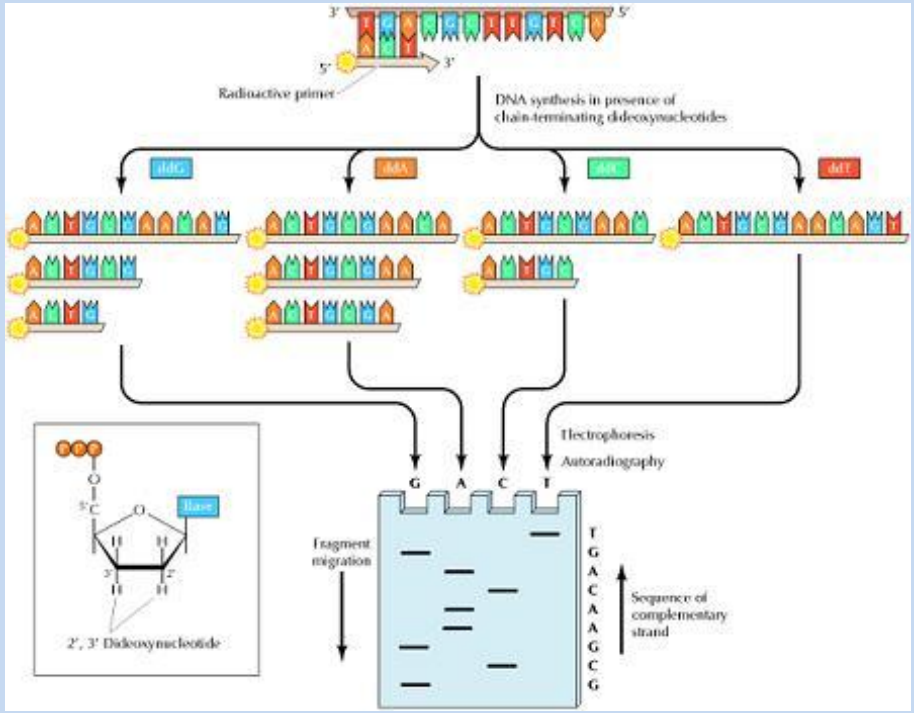
Soil Borne Pathogens – other methods

- **ELISA**: Enzyme-linked Immunosorbent Assay
- Quick tests for some select pathogens (mostly viruses)
- Can be used in the field – give rapid results but maybe only to genus (*Phytophthora*), sometimes to species (*X. fragariae)*



Soil Borne Pathogens- Molecular methods

- Nucleotide sequencing – most accurate method
- **ITS** -internal transcribed spacer- non-functional RNA sequence. Widely used because it is easy to amplify even from small quantities and has a high degree of variation even between closely related species.



Most Common Strawberry Root and Crown Pathogens

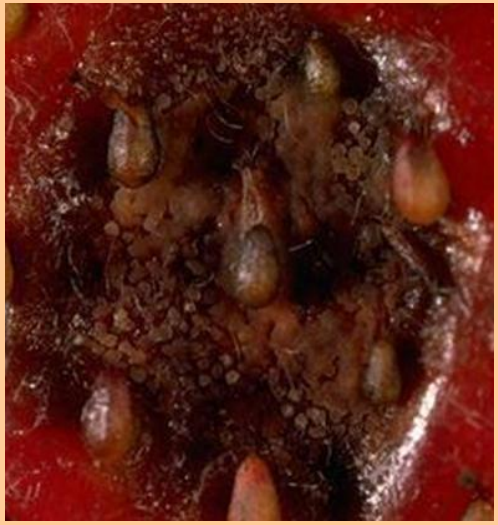
- Anthracnose – *Colletotricum acutatum*
- Black Root Rot –*Cylindrocarpon spp.*
- Fusarium Wilt – *Fusarium oxysporum*
- Charcoal Rot –*Macrophomina phaseolina*
- *Phytophthora* –several species
- Verticillium Wilt – *Verticillium dahliae*
- Nematodes – several species

Anthracnose Crown and Root Rot

- Little is known about how it survives in the soil
- Grows also on decaying tissue and plants could be exposed during normal practices of digging, trimming and packing
- Cinnamon – to – red discoloration of the crown

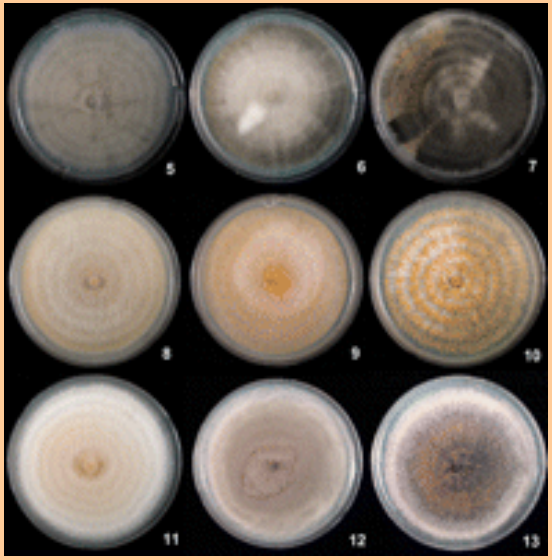
Anthracnose Crown and Root Rot

- Ascomycte fungus *Colletotrichum acutatum*
- When above ground structures infected, you see the signs of the pathogen as spore masses (rarely seen below ground)
- Produces spores in an acervulus
- Primarily a fruit rotter, also infects stolons and petioles



Anthracnose Crown and Root Rot

- Isolate from the margin of healthy and discolored tissue
- Grows on semi-selective media for fungi amended with antibacterial and antifungal compounds
- Identification based on colony size and shape plus on size and shape of conidia



Cylindrocarpon Black Root Rot

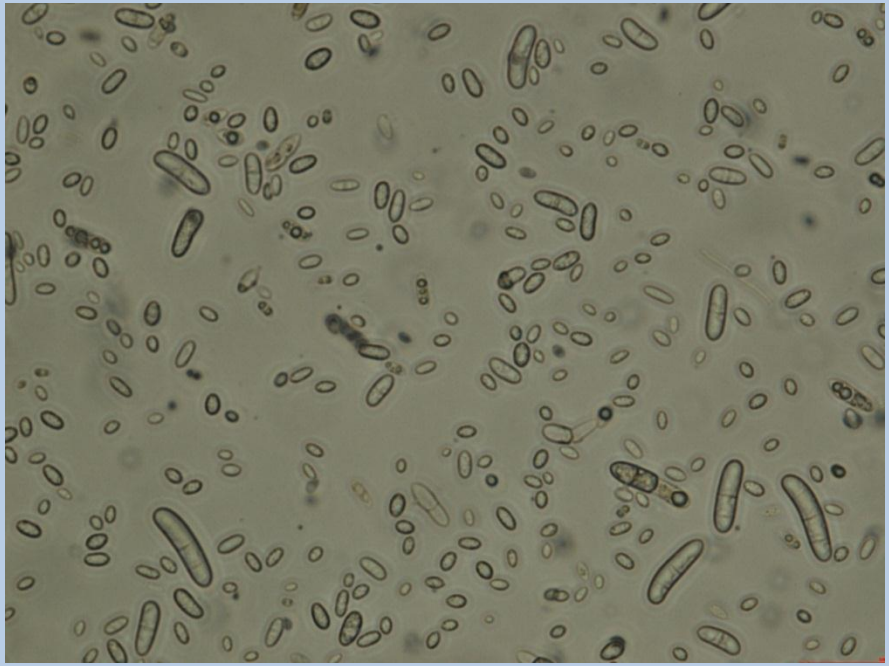
- Cosmopolitan pathogen with a large host range
- Isolated on semi-selective media
- Species difficult to separate on characters – and under taxonomic review

C. destructans

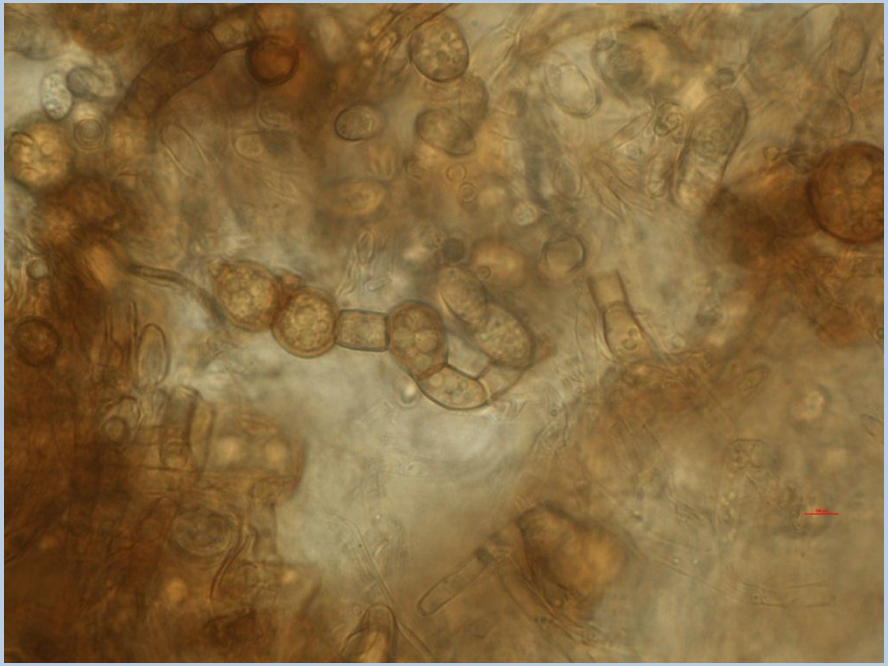
C. liriodendri

C. macrodidymum

Cylindrocarpon Black Root Rot



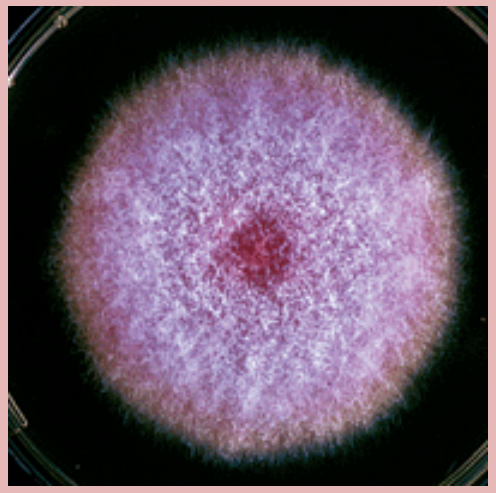
Most have predominately microconidia (and a few 2-3 celled macroconidia)

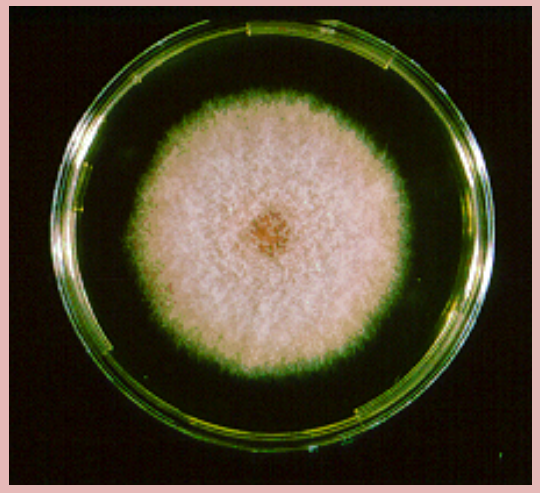


Most make large numbers of thick-walled chlamydospores in chains or clusters

Fusarium oxysporum

- Easy to isolate on semi-selective media
- Easy to speciate to *Fusarium oxysporum*
- Many non-pathogenic strains commonly found in field soils – need Pathogenicity test or known DNA sequences from Strawberry to confirm

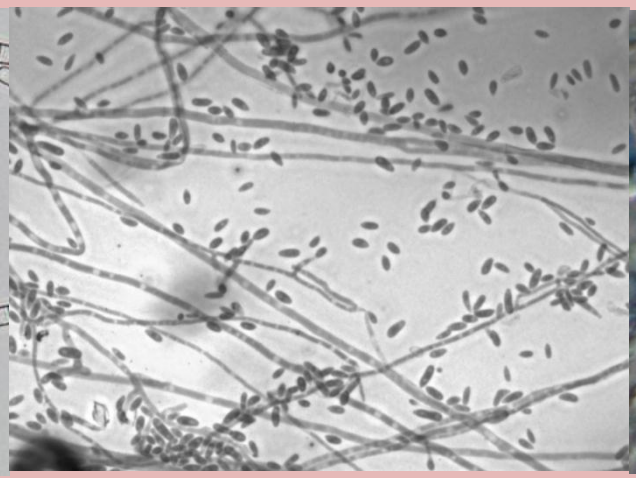




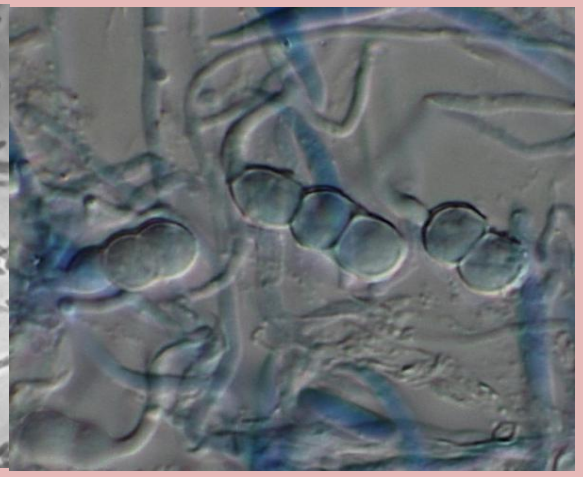
Fusarium oxysporum

- Identification can be based on the size and shape of multiple types of spores, but microscopy cannot give proof of pathogenicity – diagnose with that disclaimer
- Needs ITS test protocol or greenhouse tests

Macroconidia



Microconidia



Chlamydospores

Charcoal Rot - *Macrophomina phaseolina*

- Cutting the crowns of affected plants reveals reddish-brown necrotic areas on the margins
- May find sclerotia, but could be many other 2^{o} fungi

Discolored crowns

Sclerotia in tissues

Charcoal Rot - *Macrophomina phaseolina*

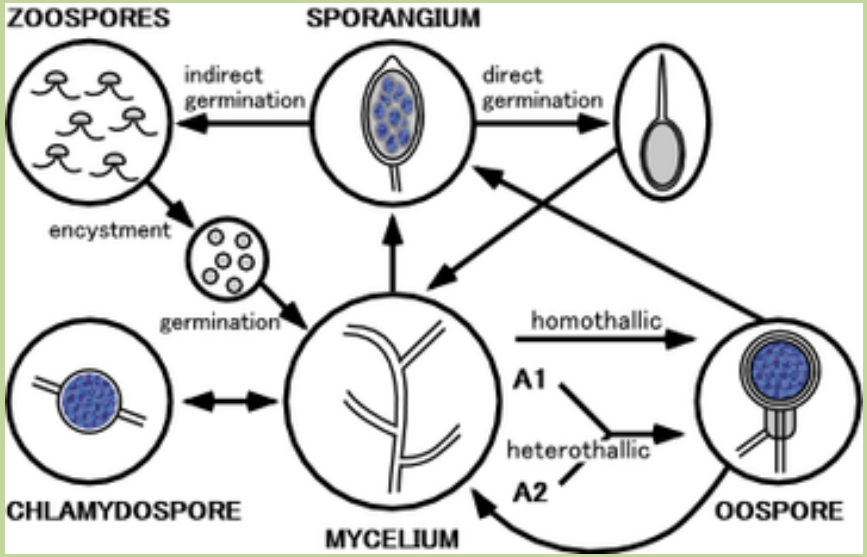
- Symptoms are similar to those caused by other crown-rot pathogens such as *Colletotrichum* and *Phytophthora* species.
- Plants initially show signs of water stress and subsequently collapse
- To confirm a diagnosis, the pathogen must be isolated in culture from the diseased crowns

Phytophthora Crown and Root Rot

- *Phytophthora cactorum, P. citricola, P. parasitica, P. megasperma* and *P. fragariae**
- brown discoloration in the crown, with or without a brown-to-black root rot.

Phytophthora Crown and Root Rot

- ELISA gets you easily to *Phytophthora spp*.
- CDFA regulates *P. fragariae* in Nursery Code – nurseries must be free-from Red Stele
- Very difficult to isolate *Phytophthora* from mushy rotted tissues
- Working on PCR test for *P. fragariae* but needs to be reviewed and accepted by stakeholders

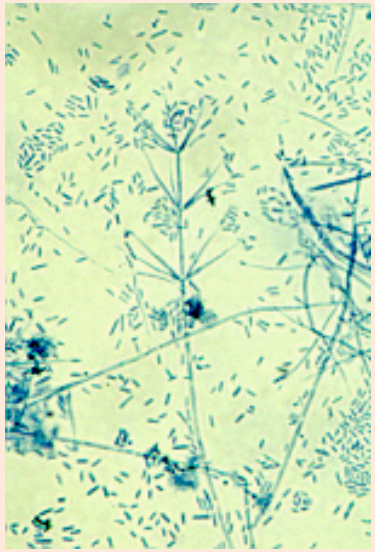


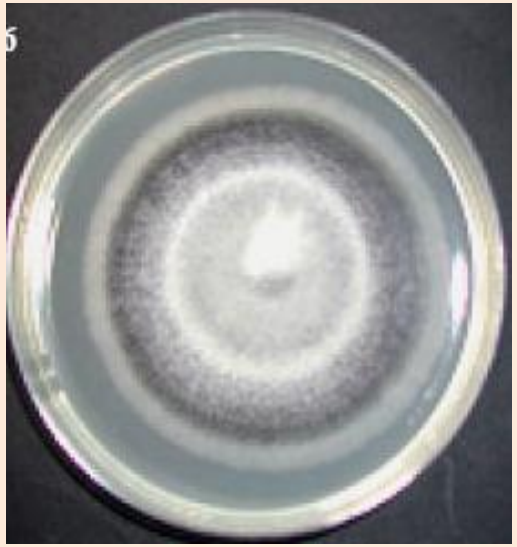
Verticillium Wilt

- *Verticillium dahliae* non host-specific and infects many weed species and crops
- Symptoms also nonspecific with stunting, wilting and browning of leaves and crowns

Verticillium Wilt

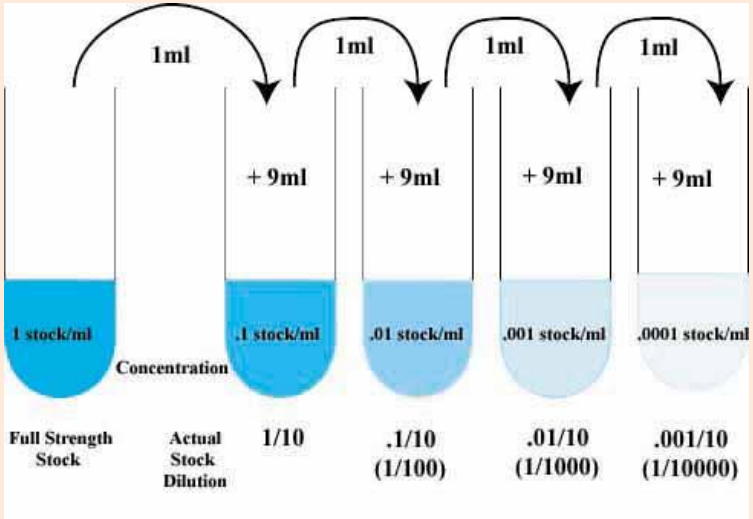
- Pathogen produces conidia and grows inside the vascular system
- Produces microsclerotia that go back into the soil and are long lasting



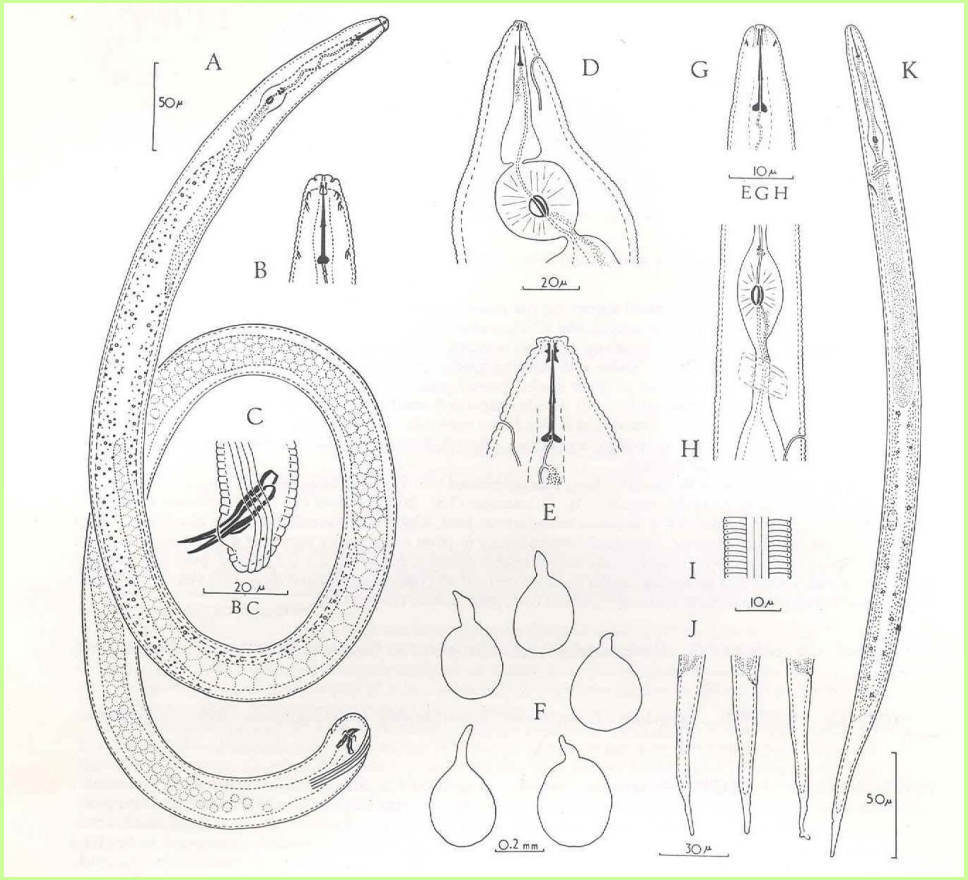


Testing soil pre-plant for Verticillium

- dilution plating method on a selective media (Sorenson's NPX) finds levels of Verticillium propagules per gram of soil (VPPG).
- Strawberry can only tolerate low numbers of VPPG



Strawberry Nematodes: Soil-borne endo- and ecto- parasites



Root Lesion
(Pratylenchus penetrans)

Stem
(Ditylenchus dipsaci)

Dagger
(Xiphinema americanum)

Needle
(Longidorus elongatus)

Root knot
*(Meloidogyne incognita, M. javanica, **M. hapla***)*

Strawberry Nematodes: Soil-borne endo- and ecto- parasites

- Field symptoms not diagnostic – stunting, poor growth, low yield
- Detection relatively easy
- Quantifying affect much more difficult

Summary- Strawberry Crown and Root Diseases

- Symptoms are not diagnostic – no accurate field ID for any of them
- Diagnosis by different methods depending on pathogen – no one test
- Improvements always needed, especially for regulatory work
- OK to ask questions about how the diagnosis was made