

Identification, Assessment and Management of Soilborne Plant Pathogens in Vegetable Production Systems



Sampling and Assessing for Soilborne Pathogens

Beth K. Gugino

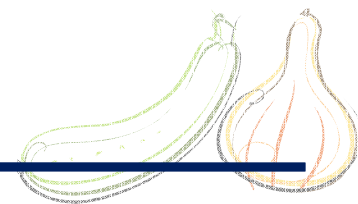
Department of Plant Pathology and
Environmental Microbiology

Penn State



Project LNE10-296

Outline



- ➔ Why sample and assess?
- ➔ Field scouting
- ➔ Plant and soil sample collection
- ➔ How to submit a sample to a plant disease clinic
- ➔ Baiting/isolation techniques
- ➔ Assessment tools



Why sample & assess for soilborne pathogens?



Because disease management begins with an accurate diagnosis!



Which is Fusarium wilt and Verticillium wilt?



How to scout fields for soilborne pathogens

- ➔ Know what a healthy crop looks like
- ➔ Know what soilborne pathogen are mostly likely to be a problem on your crops
- ➔ Learn to identify symptoms and signs of these diseases and what environmental conditions favor disease development



How to scout fields for soilborne pathogens

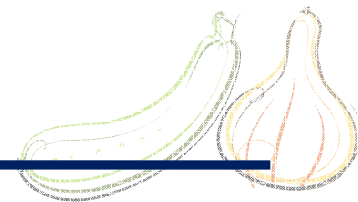
➔ Physically **scout plants regularly**



➔ Monitor temperature, dew periods and other environmental parameters favorable for pathogens.



Field scouting...



➔ **Observe patterns** to determine prevalence of problem



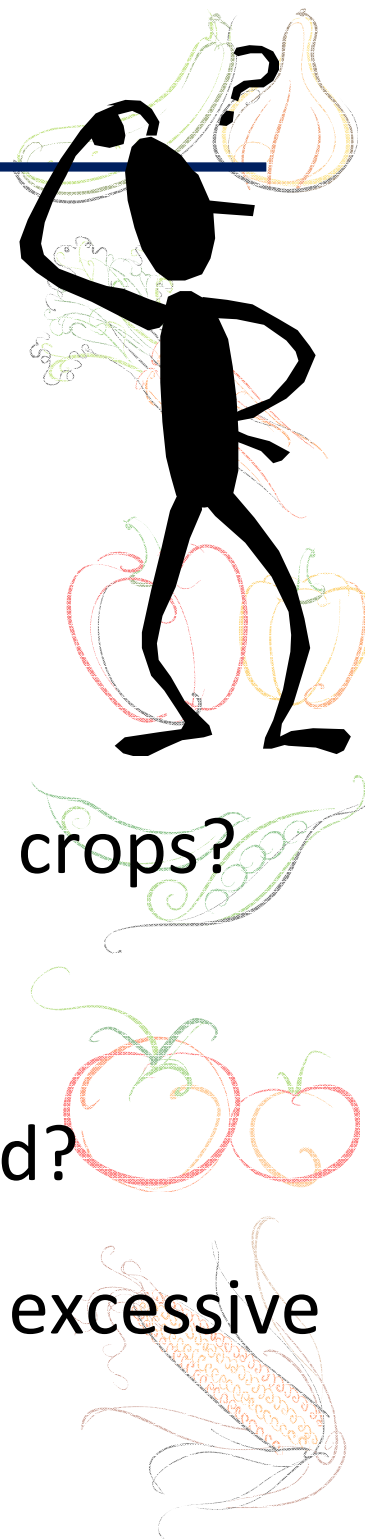
All plants in a large area or scattered and localized?



Field scouting....

➔ Ask questions

- ✓ When was the problem noticed?
- ✓ Was the damage sudden or gradual?
- ✓ How old are affected plants?
- ✓ Are the symptoms on one variety? Multiple crops?
- ✓ Percentage of plant affected?
- ✓ What cultural practices have been employed?
- ✓ Environmental conditions? Extreme temps, excessive rain?

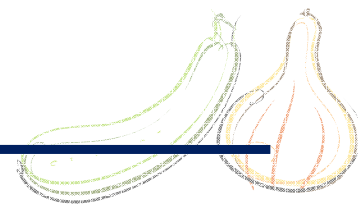


Field scouting...

➔ Herbicide drift damage



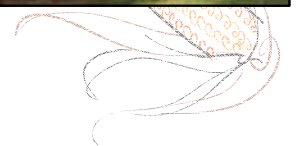
2,4-D – commonly used in lawn care



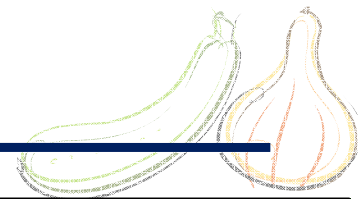
Field scouting...



Walnut Wilt
(juglone)



Field scouting...



➔ Check for symptoms and signs

- ✓ **Symptoms** – plant reactions or alternations of a plant's appearance due to a disease or disorder
- ✓ **Signs** – actual pathogen, parts or by-products seen on a diseased host plant



Collecting plant samples for diagnosis



➔ Representative sample – varying levels of severity of all plant parts

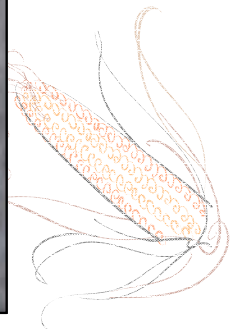
Dead plants tell no tales!



Clemson University - USDA Cooperative Extension
Slide Series, Bugwood.org

UGA1234077

Fusarium wilt of tomato



Speaking of sample condition...



Virginia Tech Plant Pathology Archive, VA Tech, Bugwood.org

5334004



Mary Ann Hansen, VA Tech, Bugwood.org



5334001



Speaking of sample condition...



Plant, soil, and sample form jumbled together in the bag!



**Dried and Dead
Sorry, we can't do an Autopsy!**



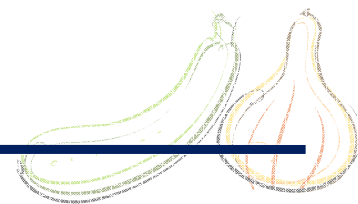
Small dried leaves



Soup/Stewed tomatoes?



Speaking of sample condition...



PSU Clinic has received samples as small as this and smaller!



Still too small! Not enough material to culture or test

Mary Ann Hansen, VA Tech, Bugwood.org

Submitting a sample to a Plant Disease Clinic



- ✓ Fresh sample in good condition
- ✓ Rapid delivery may be critical
- ✓ Keep soil on roots
- ✓ No extra water
- ✓ Wrap in dry paper then double bag in plastic
- ✓ Disinfect exterior of bags
- ✓ Strong crush-proof box, tape all seams



Photos: Tom Creswell, North Carolina State University,
www.forestryimages.org

Plant Disease Clinic Specimen Information Form



PENNSYLVANIA STATE UNIVERSITY



Lab Specimen No. _____

Specimen Information Form Plant Disease Clinic, Department of Plant Pathology

220 Buckhout Laboratory
University Park, PA 16802

Please Complete All Sections of this Form

Client Information

Name _____
Address _____
City, State, Zip _____ County _____
Phone _____ Fax _____ E-Mail _____

Submitter information

Cooperative Extension Office for Commercial Grower/Company Homeowner
 Cooperative Extension Office for Homeowner Commercial Grower/Company
 Certified Organic

Specimen Information

Plant _____ Variety _____ Date Collected _____

Describe the problem and explain what concerns you:

Plant Part Affected

Leaves
 Roots
 Stems, branches
 Flowers

Symptoms

Yellowing: Interveinal General Marginal
 Browning: Interveinal General Marginal
 Wilting Distortion Mottling Leaf Spots
 Other _____

Type of Planting

Garden Nursery
 Yard Orchard
 Indoor/house Plantation
 Field Vineyard
 Forest Golf course
 Greenhouse High Tunnel
 Other _____

Disease Distribution

General High areas
 Scattered plants Low areas
 Dry areas Foundation
 Wet areas Next to drive or road
 Shaded areas Near vents/fans
 Sunny areas End/Edge of planting
 Other _____

Soil Type

Sandy
 Clay
 Loam

Soil Moisture

Excessive
 Adequate
 Deficient

Drainage

Good
 Moderate
 Poor

Terrain

Sloped
 Level
 Low

When did the symptoms first appear? _____

Has the problem occurred before? _____ When? _____

please continue on page 2

page 2

Size of Planting _____ Acres _____ Extent of Problem _____
Number of plants _____ Percentage of plants affected or Number of plants affected _____

Previous Crop (name): _____

Trees/Shrubs: Approximate age: _____ Height: _____
How long has the plant been growing in the present site? _____

Treatments Applied This Season and Previous Year: (Fertilizer, Fungicide, Insecticide, Herbicide, Other)

Material	Rate	Date applied	Material	Rate	Date applied

Disturbances

High winds Excavation, _____ ft away Other _____
 Hail recently Construction nearby
 Frost Gas or sewer lines None

Turfgrass: If sample is turfgrass, please describe the infection center:

Grass killed No distinct pattern; irregular areas
 Grass thinned Definite pattern to affected areas:
 Circular areas Size _____
Size of affected area: _____ Rings Size _____

Greenhouse Specimens: If sample is a greenhouse specimen, please complete the following:

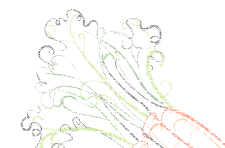
Raised beds Heating system: _____
 Ground beds Irrigation system: _____
 Pots or containers Soil mixture: _____
 Growth regulators applied (list materials and date): _____
 Fertilizer used (list type and analysis): _____
Fertilizer application technique:
 soil incorporation foliar spray dry on surface liquid on surface
Temperature in production area: Days: _____ to _____°F Nights: _____ to _____°F

Additional Comments:

Diagnostic Lab Use Only:
Sample Condition _____ Information Received _____ Photo or digital image _____

February 2011

Plant Disease Clinic Specimen Information Form

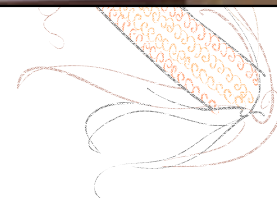
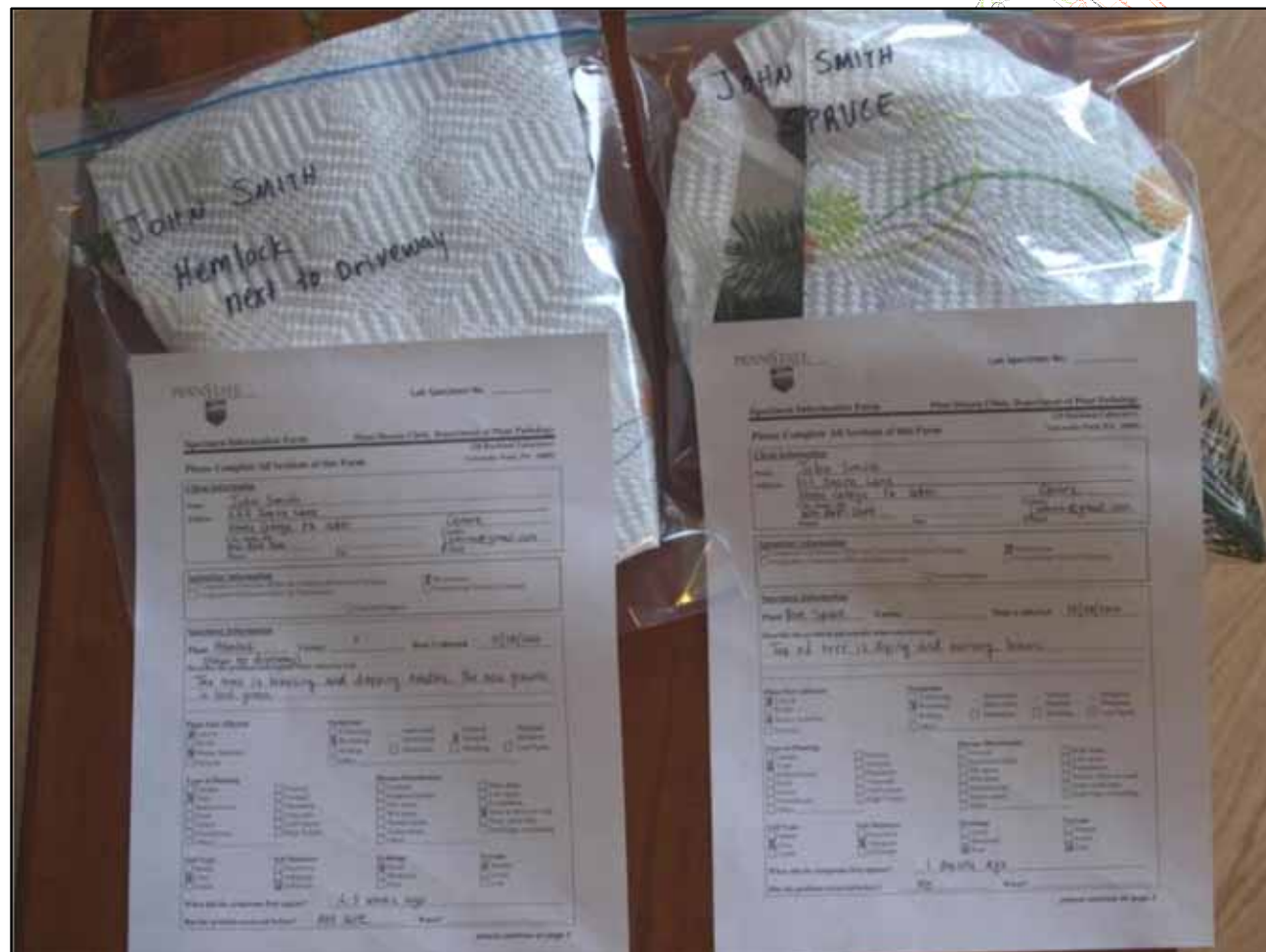


➔ Submit one form per sample

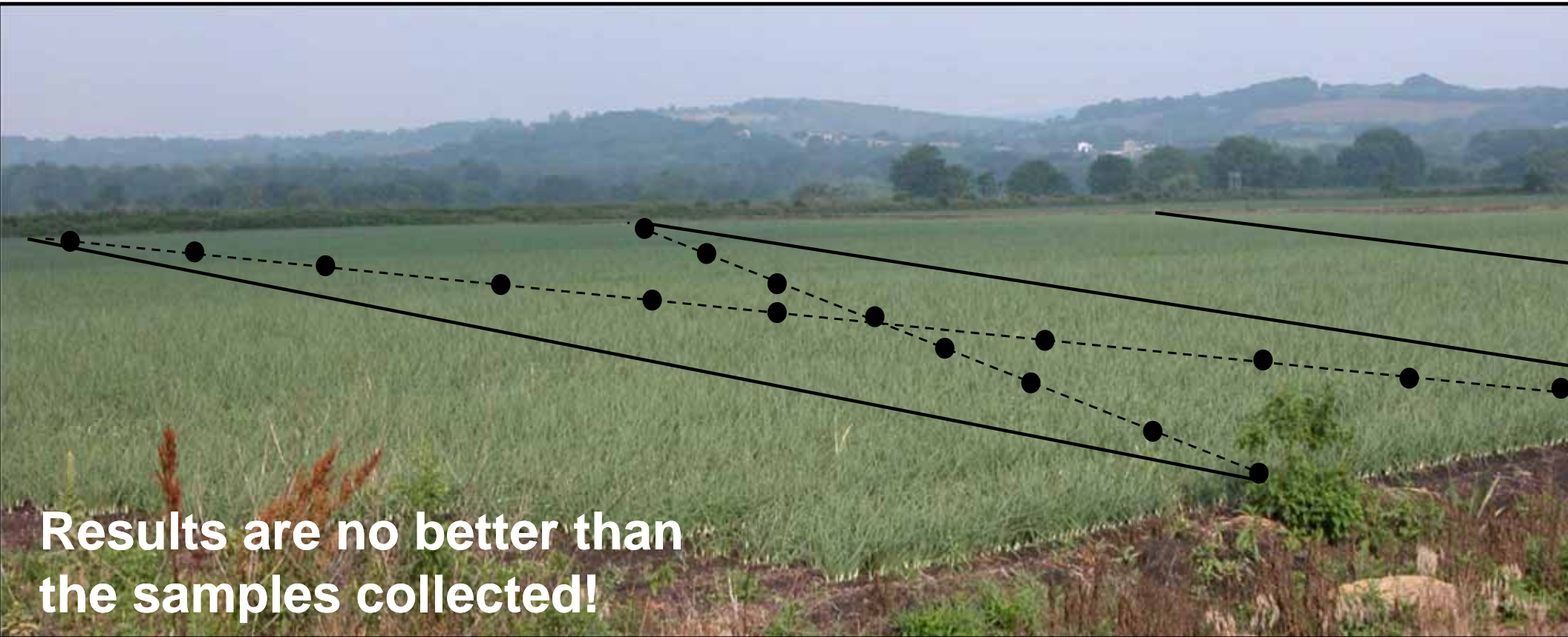
Many different diagnostics tests are used to determine the cause of symptoms

Easier to keep track of important information

Information on the form provides diagnostic clues

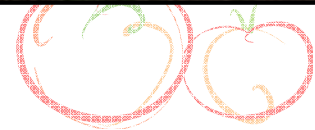


Collecting soil samples for assessment



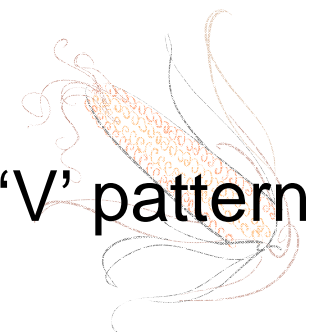
Results are no better than the samples collected!

➔ Sample following harvest or in early spring

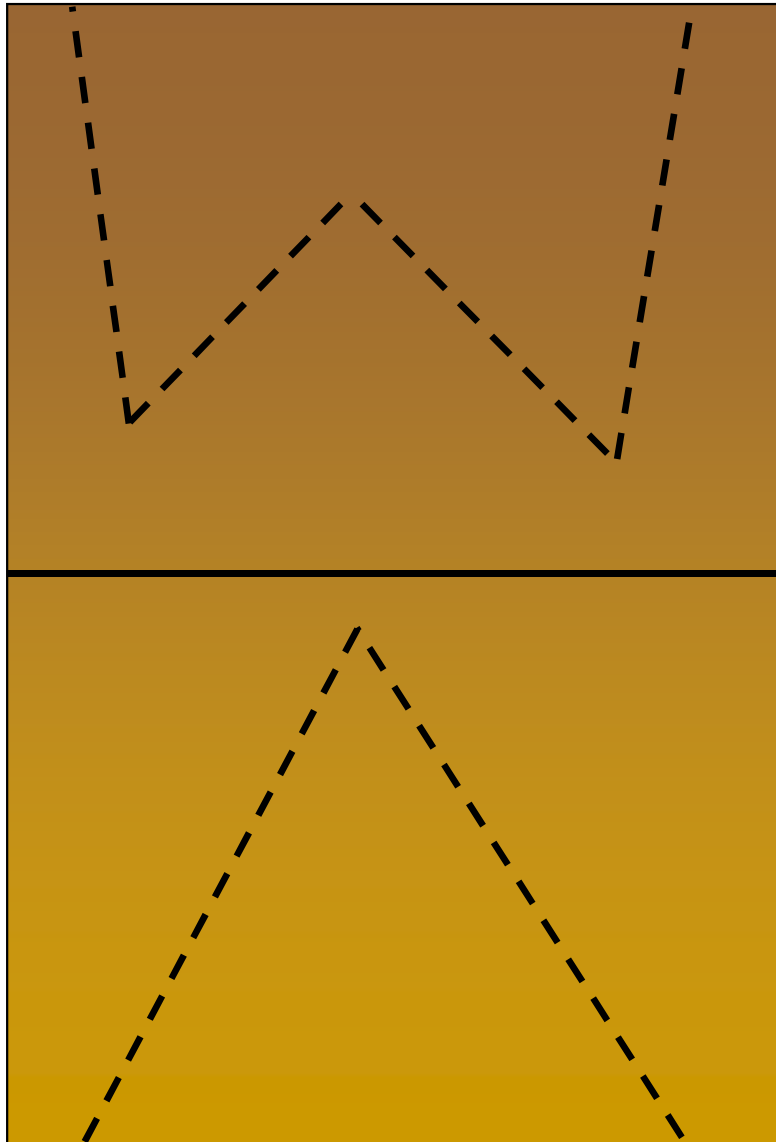


➔ Divide field into at least 4 quadrants - variability

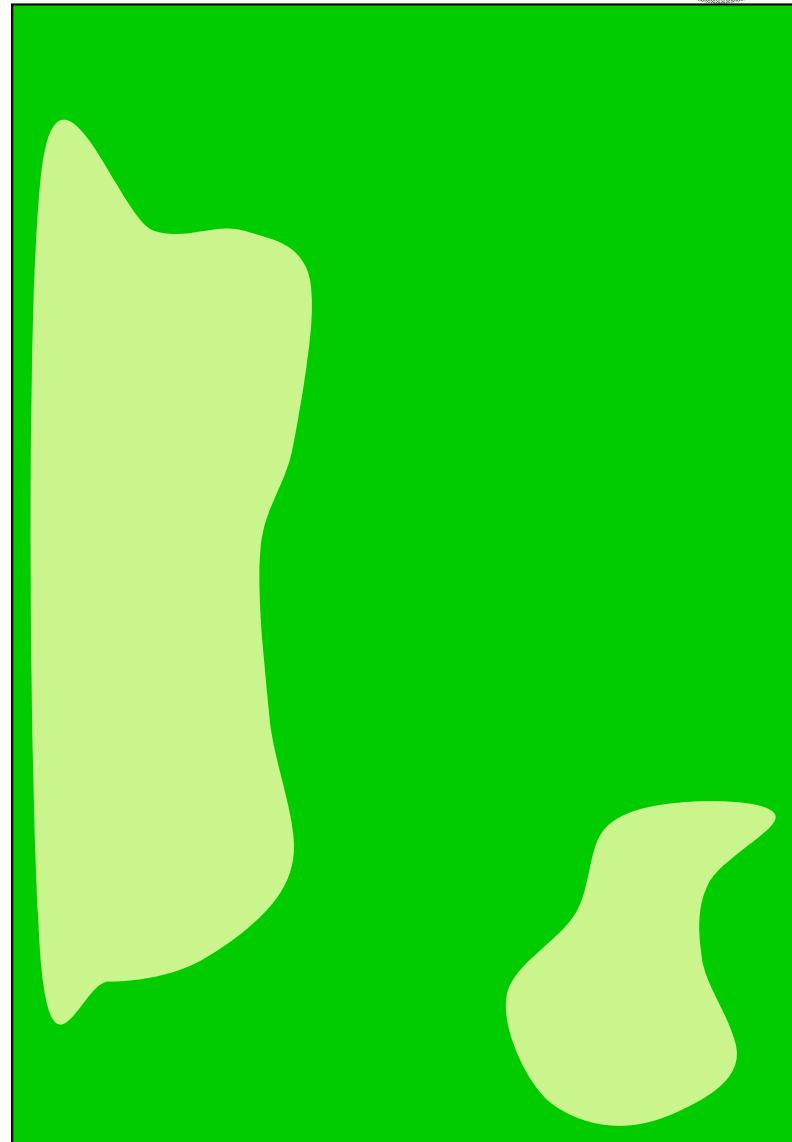
➔ Collect 15 to 20 sub-samples following an 'X' or 'V' pattern



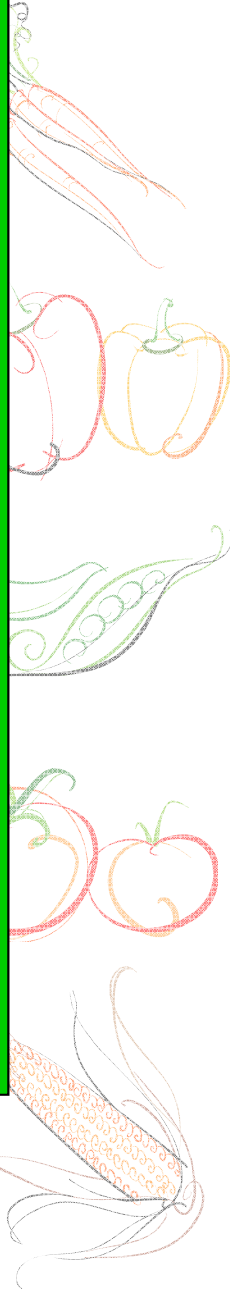
Collecting soil samples for assessment



10 acre field with two soil types



5 acre field with patches of poor crop growth



Collecting soil samples for assessment



- ➔ Highly dependent on production practices and cropping history

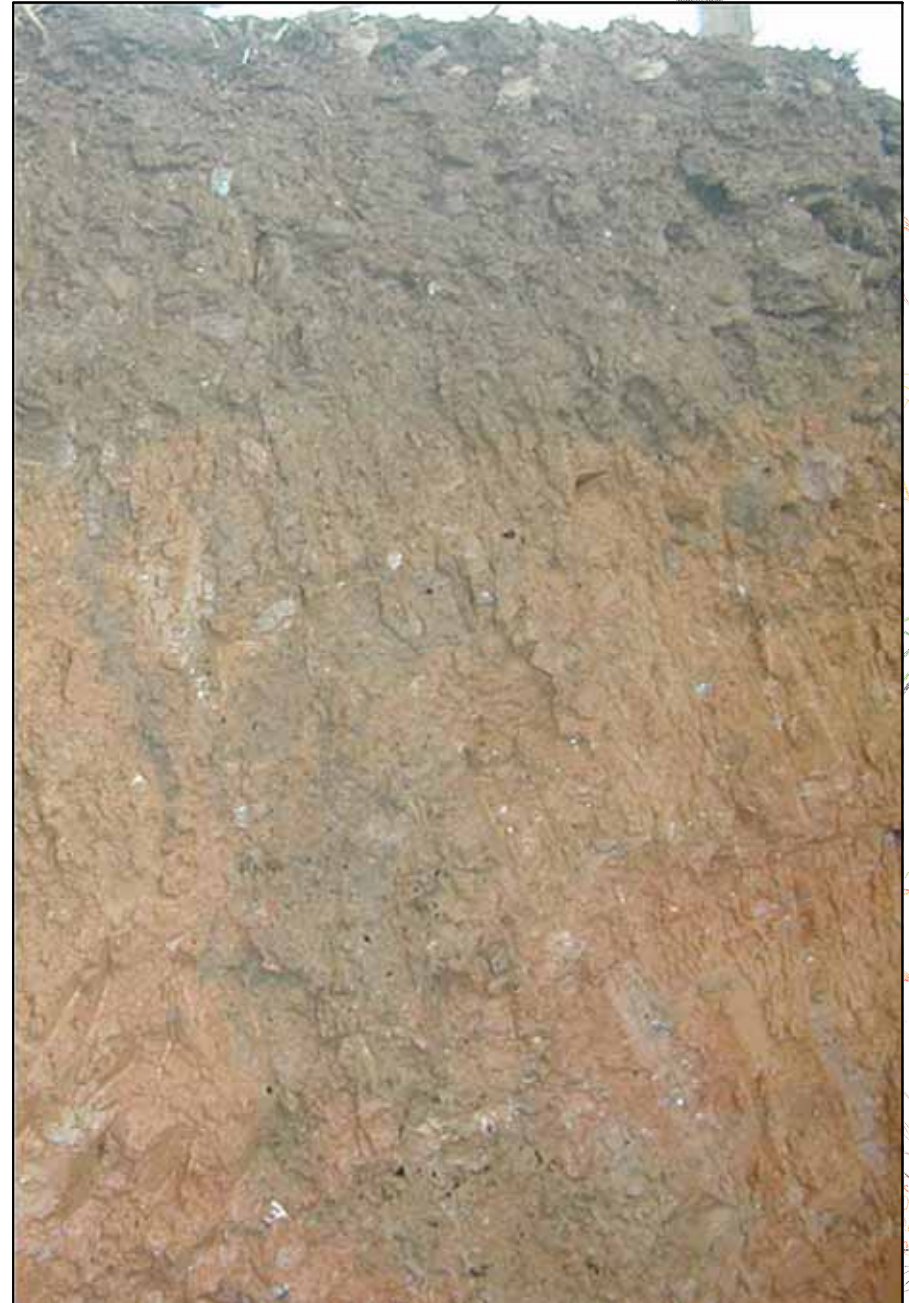


Impacts scouting and sampling

Collecting soil samples for assessment



- ➔ Uneven and patchy distribution within and between fields
- ➔ Variability at depth
- ➔ Seasonal fluctuations in nematode populations (in roots vs in soil)



Collecting soil samples for assessment



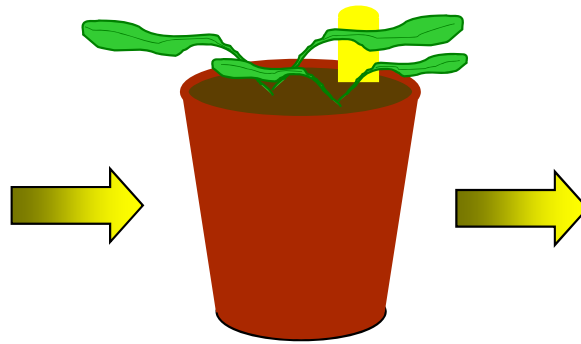
Strip-tilled beans 2007 then strip-tilled carrots 2008



Bioassay techniques...root-knot nematode



Soil collection



2 lettuce seedlings
planted in field soil



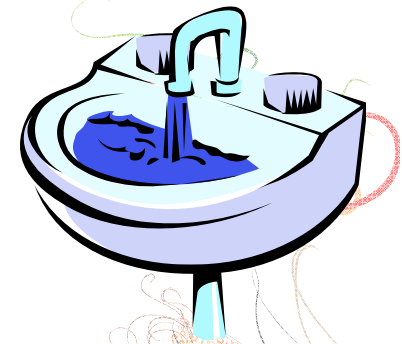
Lettuce grown in greenhouse
5 to 6 weeks

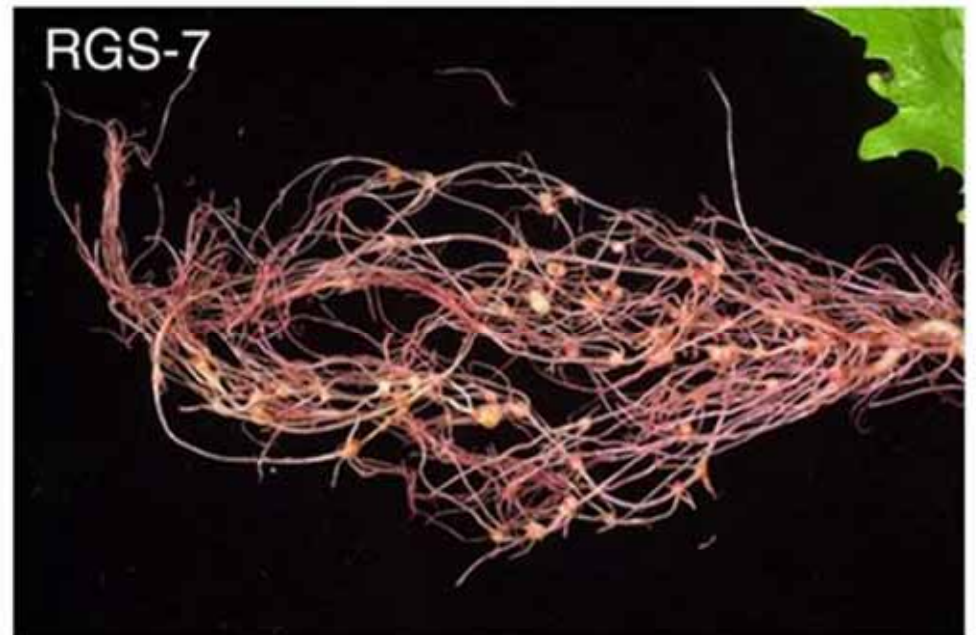
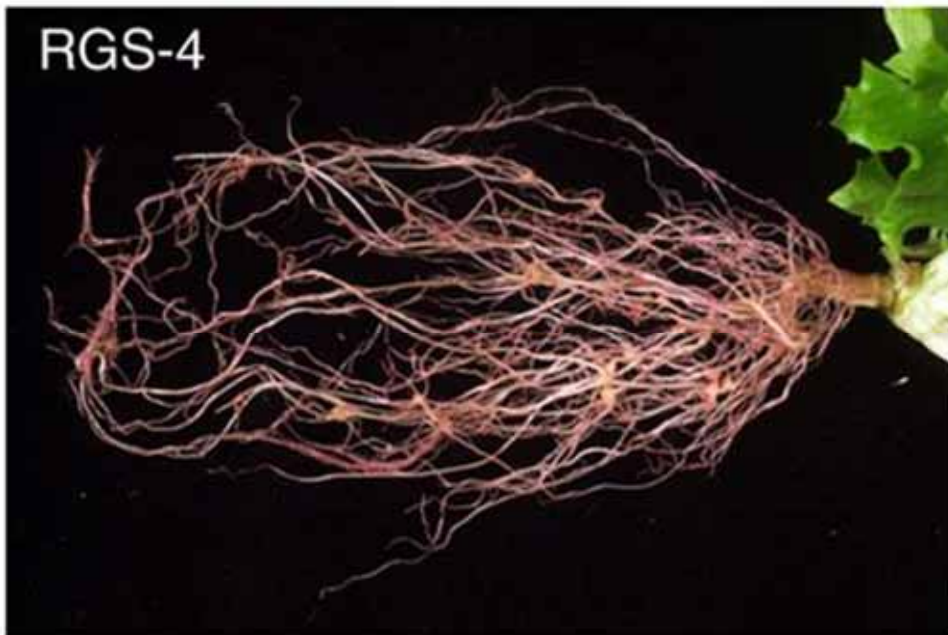
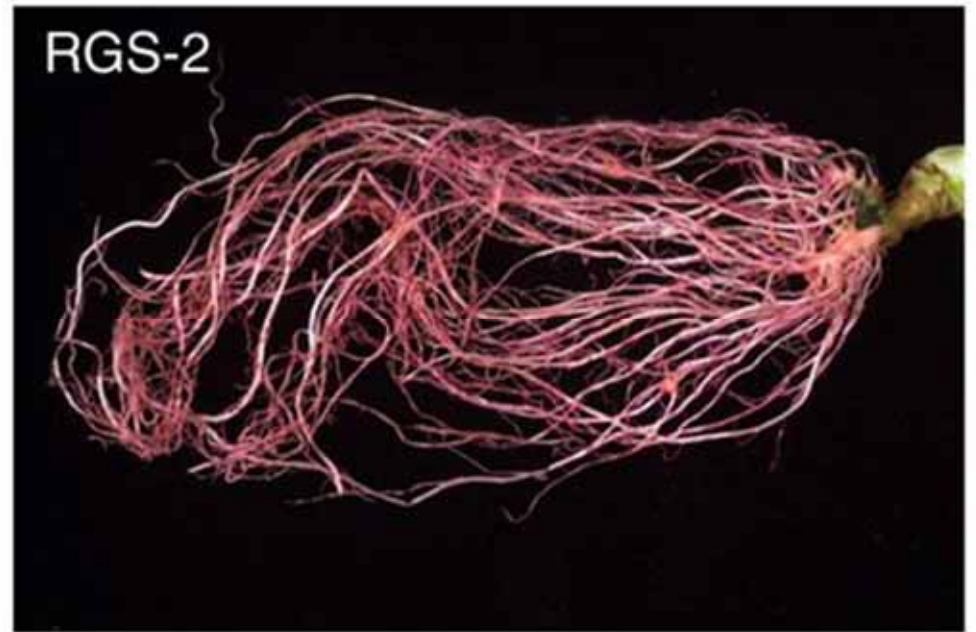
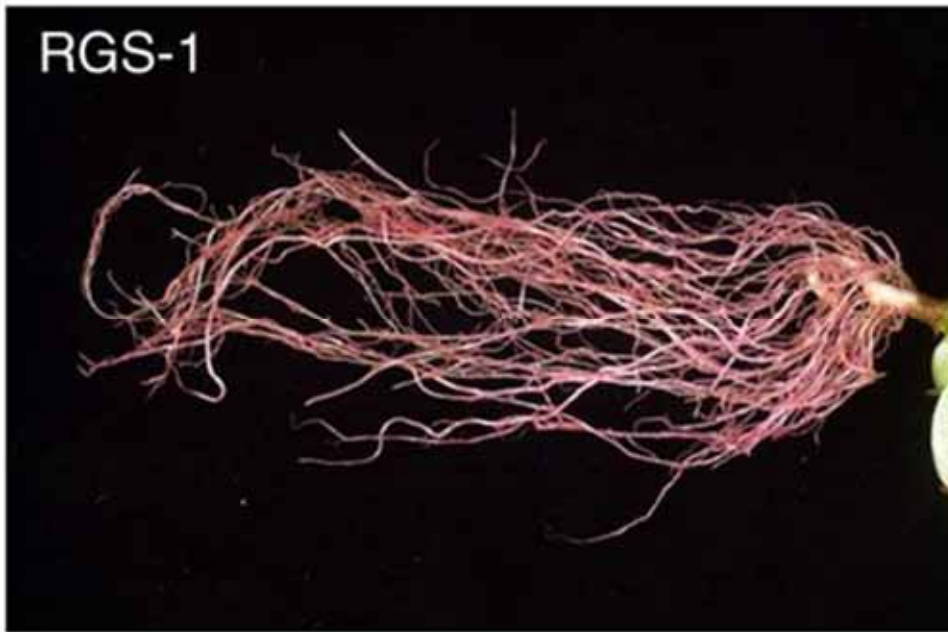


Root galling severity rating
(1 = healthy to 9 = severely galled)



Lettuce roots are washed





Lettuce roots illustrating a portion of the root-galling severity scale of 1 - 9. The scale values shown are 1 = no observable galls (healthy), 2 = 1-3% roots galled (one gall is shown in the photo), 3 = 4-10% roots galled, and 7 = 50-65% roots galled.

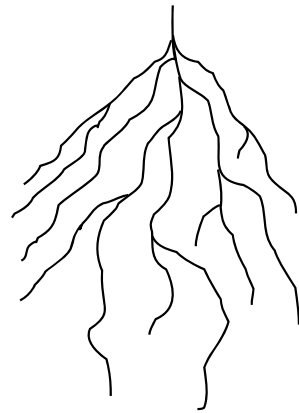
Visual Assessment of Root-Knot Nematode Soil Infestation Levels using a Lettuce Bioassay



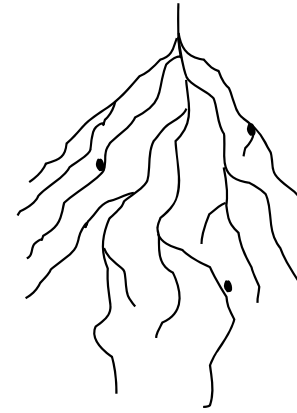
Cornell University
New York State Agricultural Experiment Station



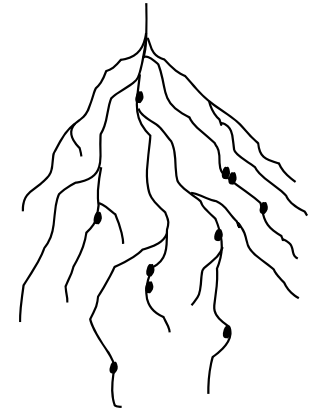
Modified root-galling severity scale for on farm



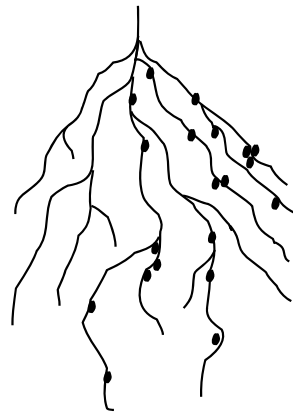
RGS 1 = no galls



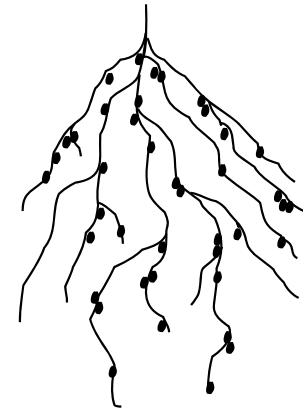
RGS 2 = 1 - 4 galls



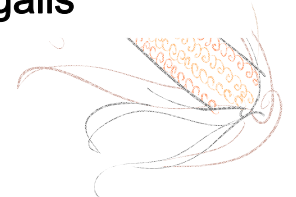
RGS 3 = 5 - 12 galls



RGS 4 = 13 - 40 galls



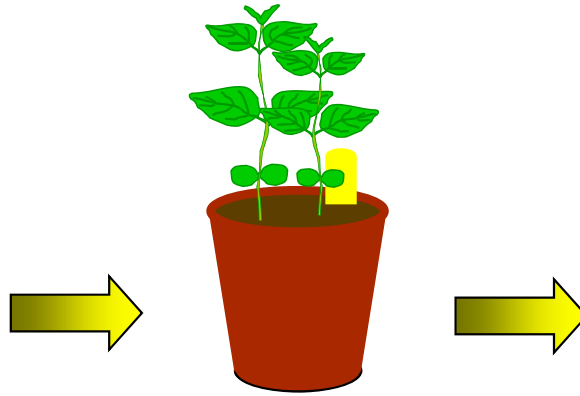
RGS 5 = >40 galls



Bioassay techniques...lesion nematode



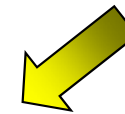
Soil collection



Field soil is planted with soybean seed



Plants grown in a growth chamber for 2 to 3 weeks



Roots are washed under running tap water



Lesion severity is assessed based on the number of lesions on the main taproot



Bioassay techniques...lesion nematode



Soybean bioassay roots

Bioassay techniques...lesion nematode



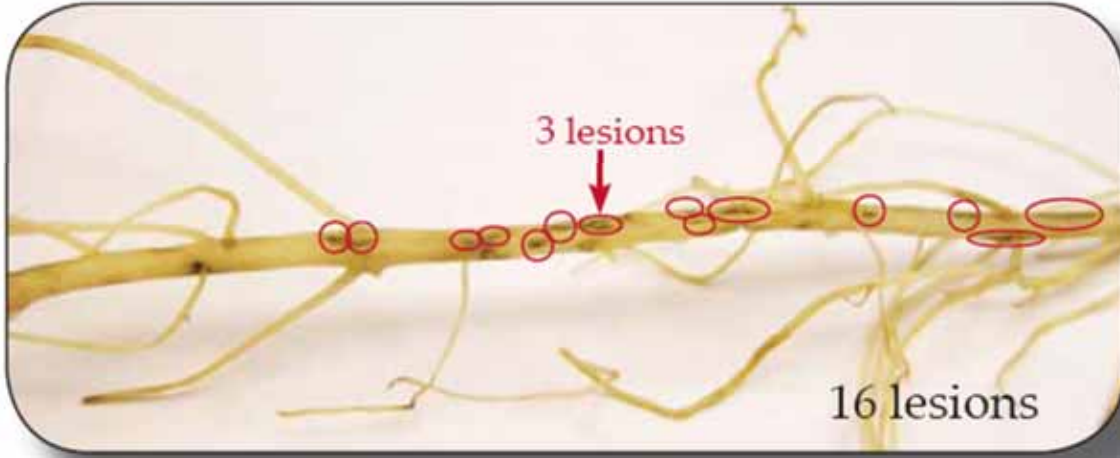
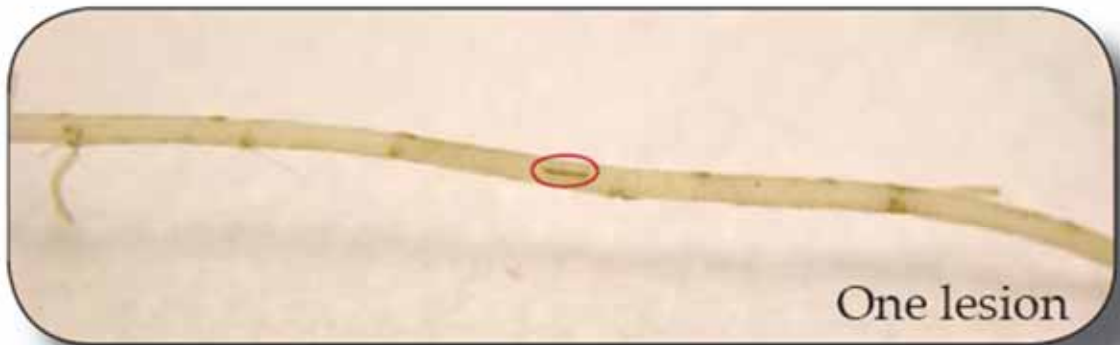
A Soil Bioassay for the Visual Assessment of Soil Infestations of Lesion Nematode



B.K. Gugino, J.W. Ludwig and G.S. Abawi
Department of Plant Pathology



Cornell University
New York State Agricultural Experiment Station



Bioassay techniques...fungal root pathogens



Soil collection



Snap bean seed 'Hystyle' planted in field soil (7 reps)

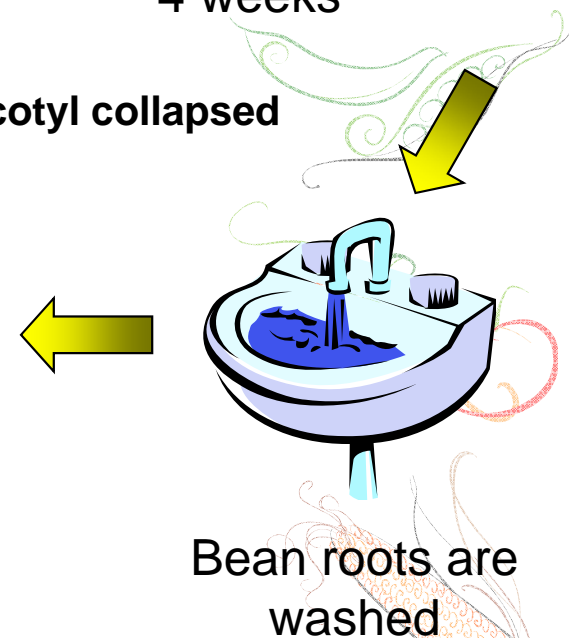


Beans grown in greenhouse 4 weeks

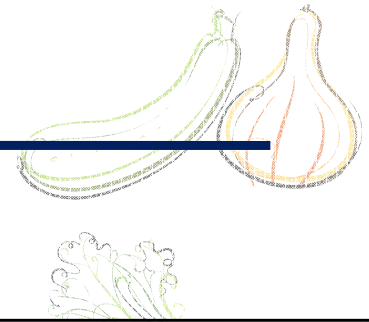


Overall root health rating

(1 = healthy to 9 = hypocotyl collapsed & primary roots rotted)



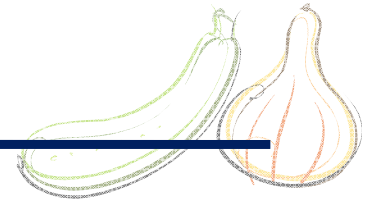
Isolation techniques...



➔ Thieviopsis isolation on carrot disks



Baiting techniques...



➔ **Phytophthora baiting from irrigation water**



Phytophthora “trap” baited with immature pears and a cucumber



Trap deployed in irrigation pond for 5-7 days to attract *Phytophthora* species



Baiting techniques...

➔ Phytophthora baiting from irrigation water



Baits with lesions



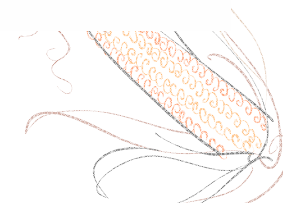
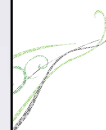
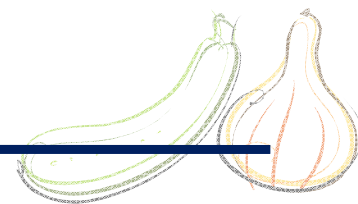
Phytophthora isolated from
bait tissue

Baiting techniques...

➔ Phytophthora baiting from soil



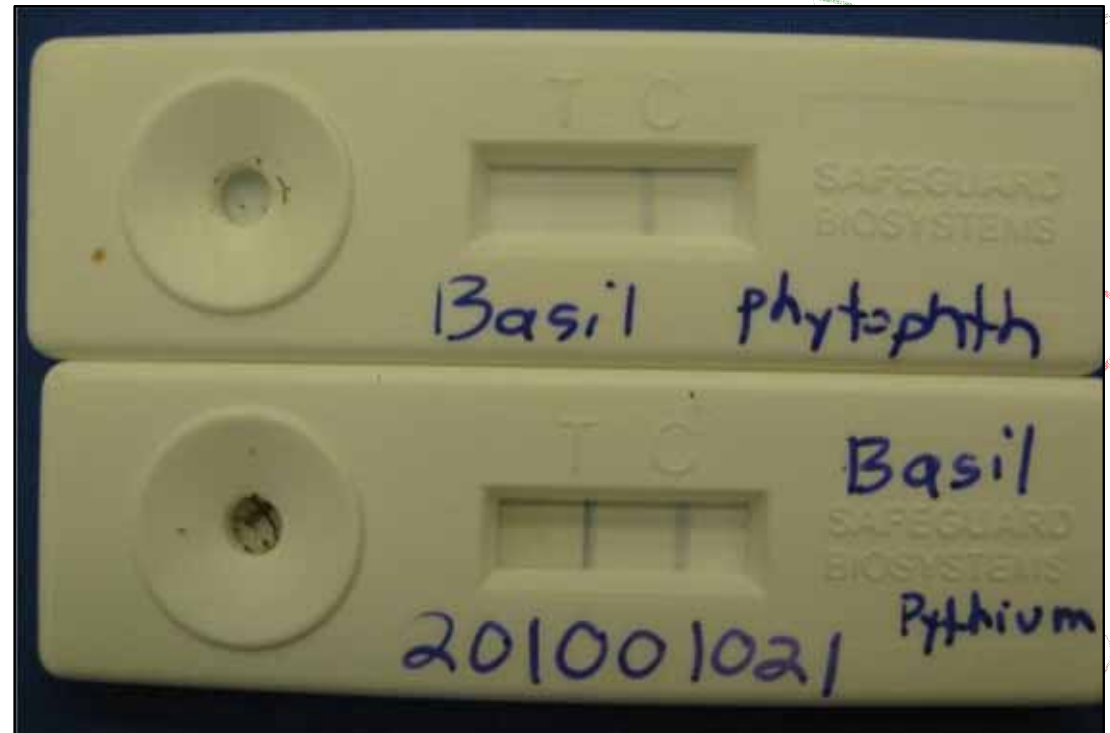
Fig. 26. A green tomato used to recover *Phytophthora* from soil. If the bait is successful, lesions like those on the tomato on the right will develop in 4 to 6 days.



From Rob Wick, UMass

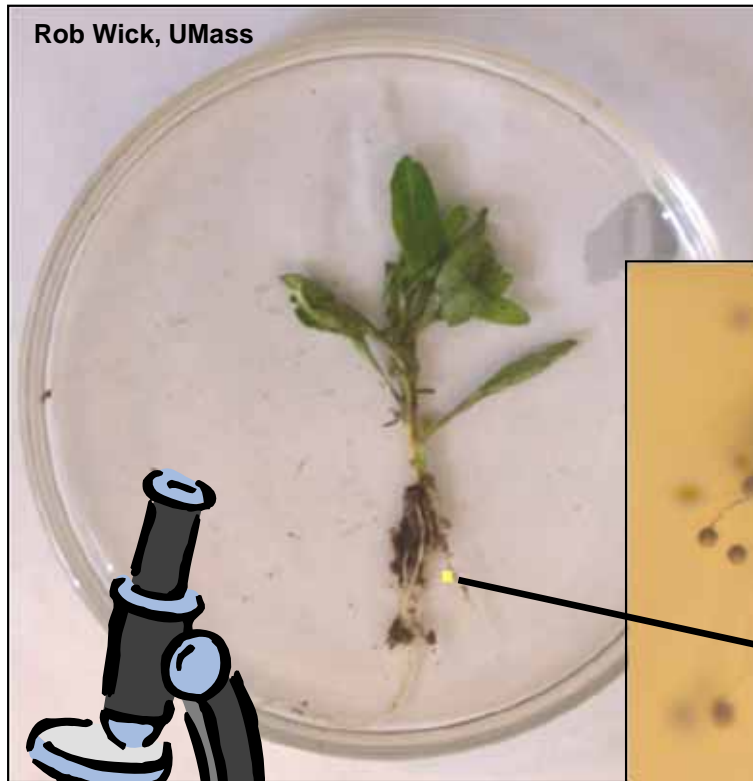
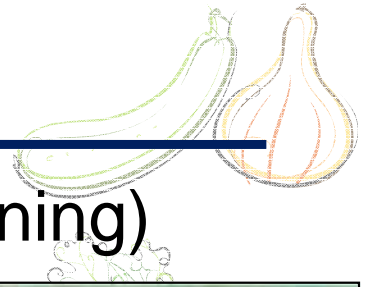
Serological techniques...

➔ Field test kits for Pythium and Phytophthora



Microscopy techniques...

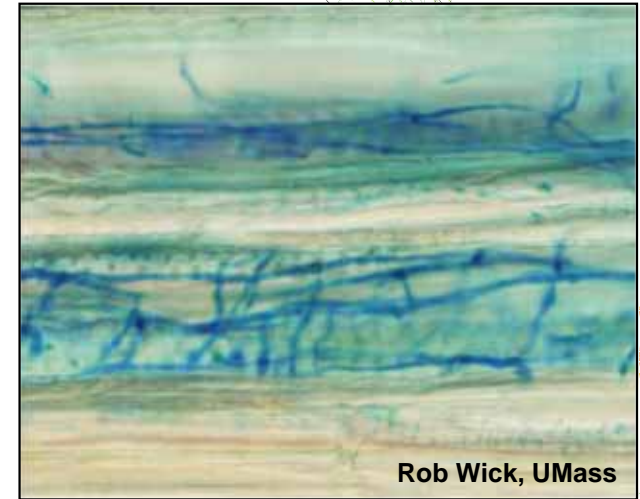
- ➔ Direct observation for signs of the pathogen (staining)
- ➔ Incubation in a moist chamber then observation



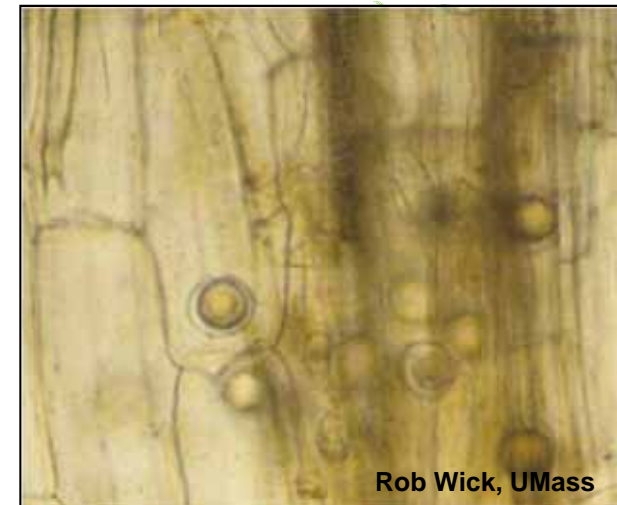
Sporangia of *Phytophthora* after incubation for 24hr



Pythium oospores in root tissue

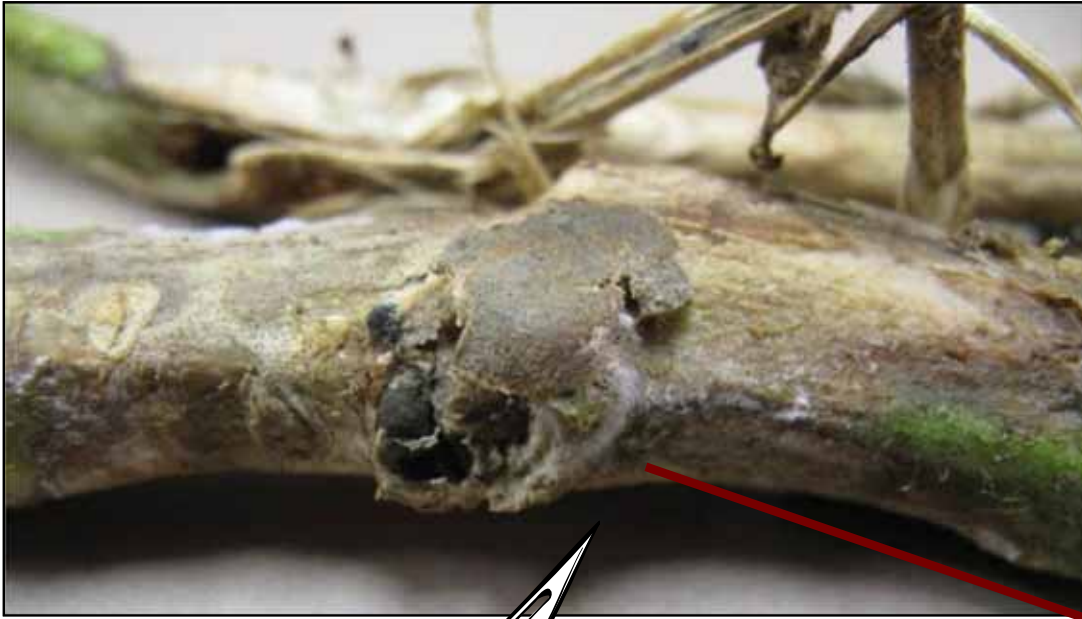
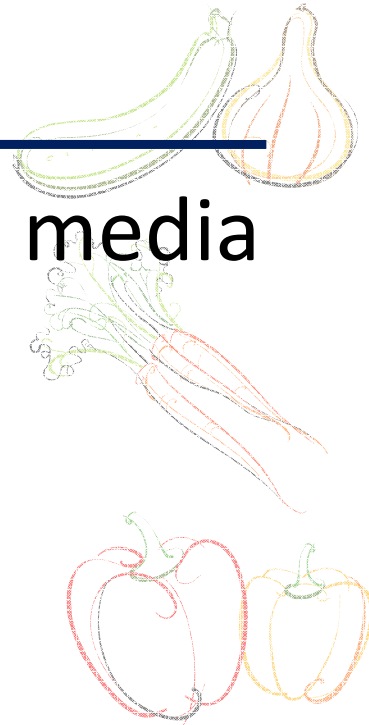


F. oxysporum in vascular tissue stained with aniline blue



Microbiological techniques...

➔ Isolation and culturing on selective agar media



Howard F. Schwartz, CSU, Bugwood.org

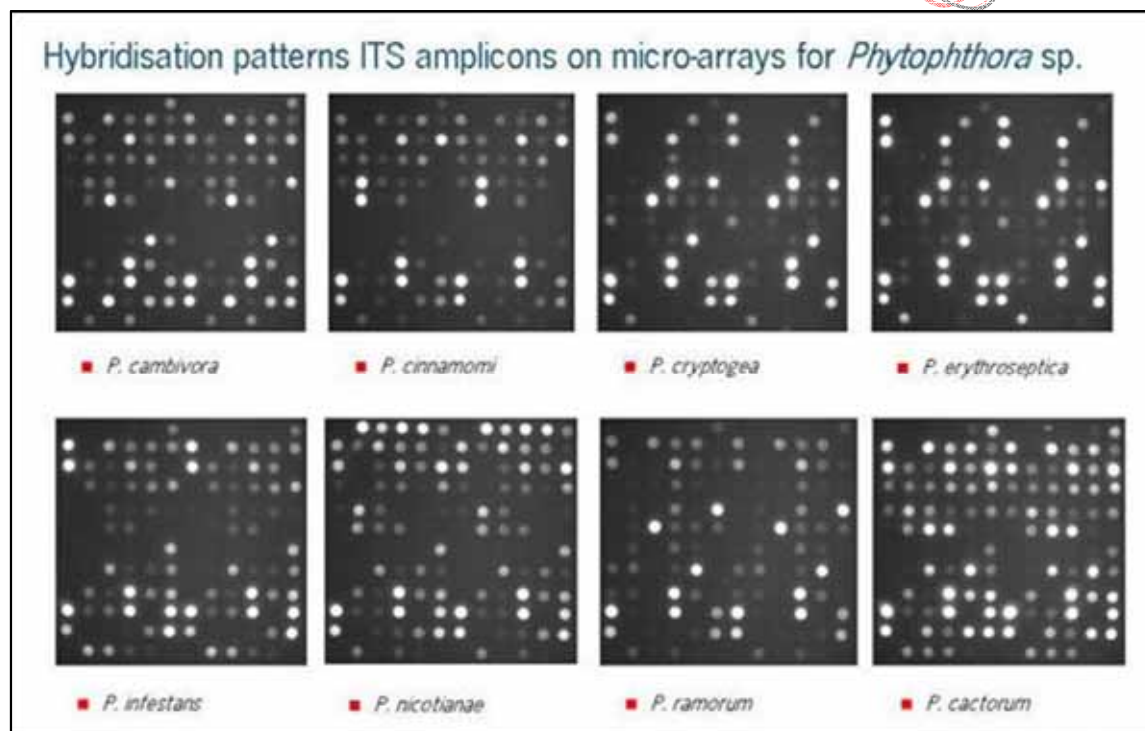
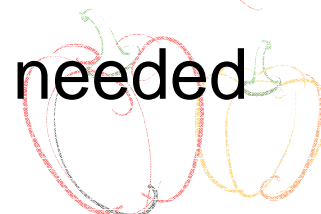
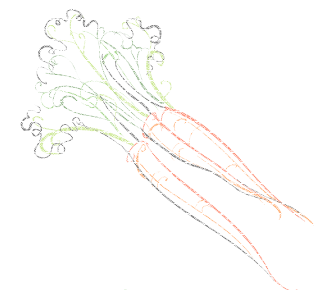
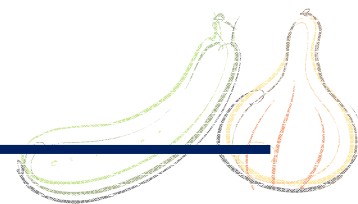
Molecular techniques...

➔ Benefits

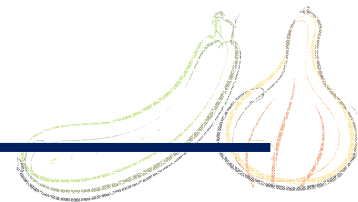
- ✓ Don't rely on ability of organism to be cultured
- ✓ Less time consuming and labor intensive
- ✓ Less taxonomic/morphological identification skills needed

➔ Drawbacks

- ✓ Target specific pathogens so difficult to ID complexes or screen for numerous pathogens

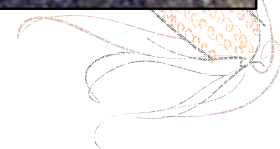
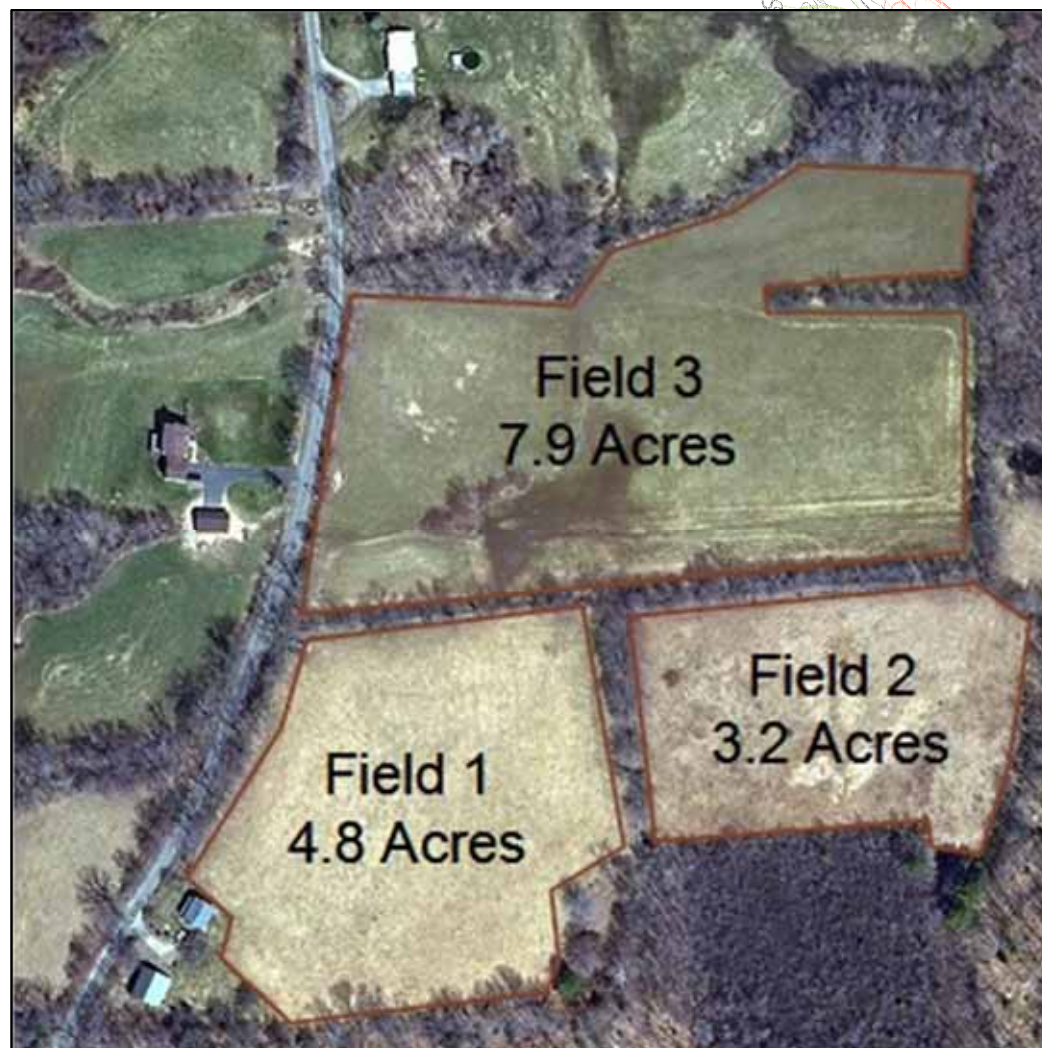


Field history disease maps...



➔ Map locations of areas most affected by disease outbreaks

- ✓ Monitor these areas more closely – target scouting efforts
- ✓ Use to inform disease management decisions





Questions about sampling and assessing for soilborne pathogens?

Beth K. Gugino

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Environmental Microbiology

Penn State

