Lab Tuesday: Electron Microscopy and Nematode Extraction

- Quiz for Viruses (pp 75-83) and Observation of Viral Movement in Plants (p. 119-120), and intro sections for Electron Microscopy (pp.21) and Nematode Extraction from Soils (p. 84 week #1).
- Finish Koch's postulates (re-examine symptoms)
- Demonstration of electron microscopy
- Extract nematodes lab
- Observe movement of GFP-expressing virus under black light
- Record data on from virus inoculations

NOVA scienceNOW

Review of RNA silencing

- A wayward petunia leads to the discovery of modest little molecules with enormous medical and agricultural promise*.

http://www.pbs.org/wgbh/nova/sciencenow/3210/02.html

*Potential to eliminate virus diseases from agriculture

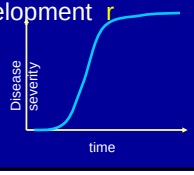
*Potential to cure many human afflictions

Principles of Plant Disease Control

Review

Disease management strategies revolve around three manageable factors:

- reduce the amount or efficacy of initial inoculum x_o or amount of initial disease y_o
- reduce the rate of disease development r
- change the time frame t



Principles of Disease Control

Pathogen

Exclusion: preventing the entrance of inoculum or establishment of a pathogen within an uninvaded area

Eradication: reducing, eliminating or destroying inoculum at the source.

Therapy: curing diseased plants by use of chemicals (drugs), surgery or an altered environment (temperature, drainage).

Host

Host resistance: reducing efficiency of inoculum via host genetics

Environment

Protection: reducing the efficiency of inoculum by interposing a barrier between host and the pathogen

Avoidance: Avoiding disease by avoiding the pathogen or by altering the environment

Principles:	Methods:
• **Exclusion:**	certification, quarantine
• **Eradication:**	rotation, pasteurization, fumigation
• **Therapy:**	surgery, drug treatment, heat therapy
• **Host resistance:**	R-genes, polygenic, pathogen-derived
• **Protection:**	chemical paints, biological agents
• **Avoidance:**	planting site, date, depth and maturity irrigation method, drainage

Exclusion of pathogens

Purpose: to prevent the introduction of a pathogen(s) into an area where susceptible plants will be grown (this area can be as small as a green-house or as large as a country)

Goal is to produce susceptible plants in a pathogen-free environment

Pathogens distributed with propagative parts are vulnerable to control by exclusionary procedures

Methods to achieve pathogen exclusion

- legally enforced **quarantines**
- seed **certification**
- Distribution of **pathogen-free** propagation stock

Quarantine

'Governmental action to prevent spread of disease'

Quarantine

- legislative control of the propagation, culture or transport of plants or plant parts to prevent the spread of pests or pathogens

Areas where alternate hosts susceptible to wheat stem rust are restricted

Other examples: Sudden Oak Death in PNW, Plum Pox in Pennsylvania, Citrus canker in Florida

Plant Quarantine Act of 1912 regulates importation of plants from foreign countries as well as trade of commodities (and consequently, trade disputes can result).

Inter-state transport can be regulated similarly.

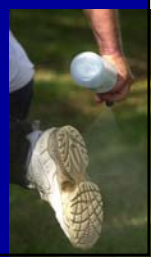
Citrus canker quarantine and eradication effort in southern Florida - 2005

Quarantine area within county

Citrus canker on grapefruit

These areas are under quarantine. No citrus trees, fruits or plant parts may be moved from the quarantine areas. No citrus trees may be planted in the quarantine area. No potted citrus may be kept in a quarantine area unless in a registered nursery.

Citrus Canker: Taking action within a quarantined, residential area



Questions about Quarantine?

Disease Certification Seed and Propagation Stocks

'Governmental assurance of quality'

- Seed and Propagation Stocks
  Certification 'tag' on the stock (states)
- Exported produce
  Phytosanitary certificate with shipment
  (feds: USDA APHIS)

Disease Certification

purpose is to ensure *low disease* in planting stocks

Certification programs exist for nursery stock, ornamentals, seed potatoes, vegetable seeds, grass and cereal seeds, fruit trees, berries, mint, etc.

Inspectors **visually certify** propagation fields and shipped plant parts. They do this by looking for **symptoms** of target **diseases.**

For every crop that is certified, there is a list of diseases (also weed seeds) and the amount of each tolerated. The inspection reports are public records and are available to the buyer.

Maximum tolerances for diseases in seed potatoes

Disease	Tolerance (Gen III)
mosaic	0.1%
potato leaf roll	0.05%
ring rot	**0.0%**
blackleg	0.1%

Questions about Certification?

'Pathogen-free' or 'pathogen-tested' seed and propagation materials

purpose is to ensure little to no *pathogen* resides in propagation stock

Note: this is a more stringent goal than is usually the case with a disease certification program

typically, 'pathogen-testing' requires lab testing by government agency or increasingly, by a private company

‘Pathogen-free’ or ‘pathogen-tested’ seed and propagation materials

Often goes hand-in-hand with *disease certification* but may involve testing planting stock for the presence of pathogen(s)

Obtaining pathogen-free seed:

Grow seed crop in area isolated from the pathogen or area where the climate is not suitable for the disease (dry climate)

Obtaining pathogen-free clonal plant material:

Use micro-propagation to cure infected plants and to (at least initially) mass produce pathogen-free plants

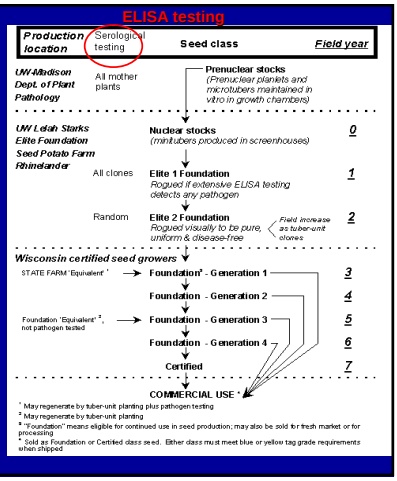
European Plant Protection Organization

Programs in pathogen tested planting stock

PM 4/1(1) Virus-free or virus-tested fruit trees and rootstocks
PM 4/2(1) Pathogen-tested material of carnation
PM 4/3(2) Pathogen-tested material of pelargonium
PM 4/4(1) Pathogen-tested material of lily
PM 4/5(1) Pathogen-tested material of narcissus
PM 4/6(1) Pathogen-tested material of chrysanthemum
PM 4/7(1) Nursery requirements - recommended requirements for participation in certification of fruit or ornamental crops
PM 4/8(1) Pathogen-tested material of grapevine varieties and rootstocks
PM 4/9(1) Pathogen-tested material of *Ribes*
PM 4/10(1) Pathogen-tested material of *Rubus*
PM 4/11(1) Pathogen-tested material of strawberry
PM 4/12(1) Pathogen-tested citrus trees and rootstocks
PM 4/16(1) Pathogen-tested material of hopPM
4/17(1) Pathogen-tested olive trees and rootstocks
PM 4/18(1) Pathogen-tested material of *Vaccinium* spp.
PM 4/19(1) Pathogen-tested material of begonia
PM 4/20(1) Pathogen-tested material of New Guinea hybrids of impatiens
PM 4/21(1) Pathogen-tested material of rose
PM 4/26(1)Pathogen-tested material of petunia

Example of a pathogen tested potato seed program

Once propagated from a pathogen-tested tissue culture, clonal plant material can exist in the program for up to 8 years.



Questions about Pathogen Tested?

Micropropagation

Obtain clean, 'mother' plants

Typically, systemic, obligate pathogens are targeted – e.g. viruses

Micropropagation

Goals:

Cure infected plants
Obtain pathogen-free plants
Maintain pathogen-free condition as number of plants is increased

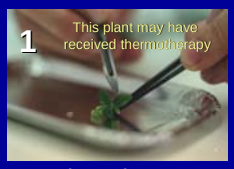
Procedures

1. meristematic tissue is isolated in tissue culture
(sometimes, prior to excision of meristem, the plant is held at high temperature 32-36 C for 2-6 wk to slow viral spread – this is called 'thermotherapy' (i.e., give the plant a fever)

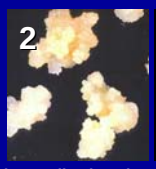
2. "index" (i.e., test) for pathogens to determine if the new plant is pathogen free

3. increase indexed plants in pathogen-free environment, then retest

Example of micropropagation program



Excise meristem



Produce callus in culture

Regenerate plantlet

Index (test) for pathogens

Grow plantlets

Micropropagation Program continued

Divide indexed plantlets

Root increased plantlets

Plant in 'soil-based' medium

Micropropagation Program continued

Increased indexed plantlets in pathogen-free and insect-free greenhouse

Harvest propagation unit that will go to the field – 'mini-tubers'

Micropropagation Program continued

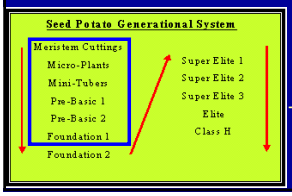
Start indexed plants for field production as transplants

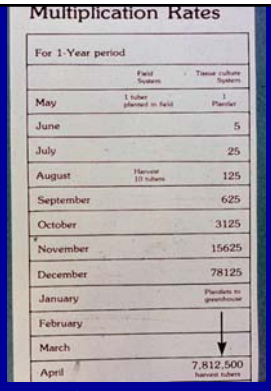
For potatoes, at this point, the indexed 'generation' could be grown as 'certified-seed' for several years.

Annually inspected then winter- tested in Florida

Produce pathogen-tested stock in isolated, intensely managed fields

Potential number of pathogen-free plants produced in potato micropropagation program compared to field-based program where pathogen status is uncertain





Multiplication Rates

For 1-Year period

	Field System	Tissue culture System
May	1 tuber planted in field	1 Plantlet
June		5
July		25
August	Harvest 10 tubers	125
September		625
October		3125
November		15625
December		78125
January		Plantlets to greenhouse
February		
March		
April		7,812,500 harvest tubers

Total no. of tubers: 10 Field 7,812,500 Tissue culture