PLP 6404
Epidemiology of Plant Diseases
Spring 2015

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Lecture 6: Influence of pathogen on disease development: soil-borne pathogens

Pathogen characteristics that influence disease development:

- Ecological type (root inhabiting or soil inhabiting)
- Pathogen population size (number of propagules)
- Fitness (competitive saprophytic ability and inoculum potential)
- Level of virulence
- Reproduction (sexual, asexual, rate)
- Dispersal mechanisms
- Pesticide resistance (fungicides, antibiotics)

Ecological types of soilborne pathogens

- soil inhabitants
    - competitive saprotrophic ability (on dead organic matter)
    - wide host range
    - secondary pathogens or pathogens of young plants
- root inhabitants
    - no competitive saprotrophic ability
    - specialized parasites, primary pathogens
    - small or wide host range

Soil inhabitants may have a good or less well developed competitive saprophytic ability. Root inhabitants generally have very poor or no competitive saprophytic ability.

Examples of ecological types:

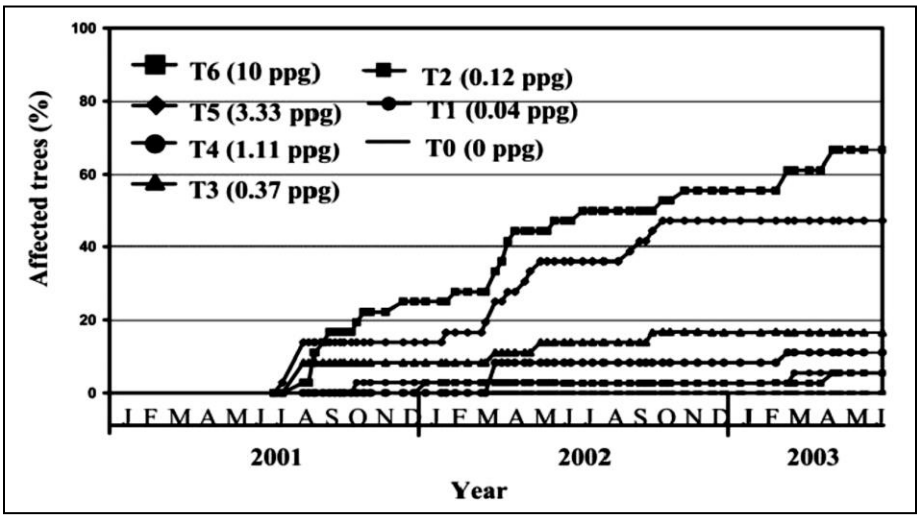
- Soil inhabitant, good CSA: *Pythium, Rhizoctonia, Fusarium* (fungi), *Rhizomonas* and *Ralstonia* (bacteria)
- Soil inhabitant, limited CSA: *Phytophthora, Verticillium* (fungi)
- Soil inhabitant, no CSA: *Pratylenchus penetrans* (nematode)
- Root inhabitant, no CSA: *Plasmodiophora* (protozoa), *Meloidogyne*(nematodes)

Some important terms:

- Rhizoplane- on the root surface
- Rhizosphere- near the root but within the influence of the root (from exudates, volatiles etc.)
- Spermoplane- on the surface of seed (in the soil)
- Spermosphere- near the seed in the soil

Pathogen population size

The greater the number of propagules near susceptible hosts, the earlier the epidemic starts and the greater the final intensity of the epidemic



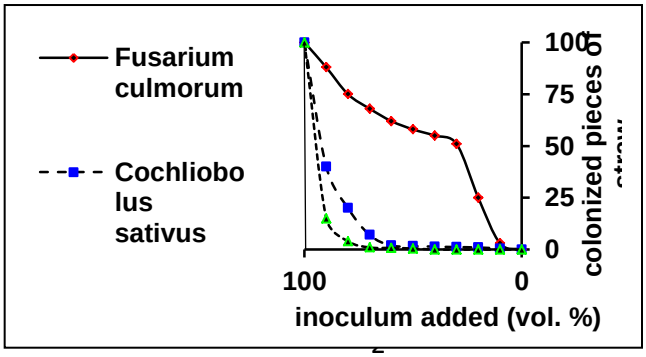
Progress of Verticillium wilt of olive in microplots infested with different levels of initial inoculum (ppg = # microsclerotia per gram of soil)

Fitness of the pathogen:

- Saprophytic growth in soil, spermo- and rhizosphere
- Competitive saprophytic ability (CSA)
- Inoculum potential
- Ability to colonize the spermo- or rhizoplane
- Ability to infect the roots

Competitive saprotrophic ability = characteristics ('weapons') of the colonist or
all physiological characteristics that make an organism suited to colonize dead organic matter

There is a standard method to determine CSA:
Add different inoculum levels to pieces of straw and determine the % of pieces colonized by a particular pathogen in the presence of competition.



Factors affecting CSA:

- fast germination of survival structures
- fast growth of mycelium
- fast formation of enzymes needed to break down the substrate
- formation of antibiotics that inhibit competitors
- tolerance for antibiotics formed by other organisms
- able to survive unfavorable periods

Inoculum potential = the number of spores and their vitality or aggressiveness.

- Inoculum potential = inoculum density x aggressivity
- Inoculum density = # spores / g soil or meters of hyphae /g soil
- Aggressivity = number of infection units - can be determined only in bioassays!

→ so: inoculum potential can only be determined in bioassays

.	quantification	interpretation
inoculum density	easy	difficult
inoculum potential	difficult	easy .

Level of virulence

Pathogenic races
Modern definition of physiological race or race: a group of parasites (particularly fungi) characterized by specialization to different cultivars of one host species

Table 1. Differential hosts used to distinguish pathogenic races of Fusarium wilt of peas. (From: Maloy. 1993. Plant Disease Control)

	Pea Wilt Race			
Differential Variety	1	2	5	6
Little Marvel	S	S	S	S
Darkskin Perfection	R	S	S	S
New Era	R	R	S	S
WSU 23	R	R	R	S

Reproduction

Many soilborne pathogens have been considered monocyclic until recently. In monocyclic plant pathosystems, the disease increases usually **without** successive generations of inoculum and infection within a cropping season. Consider a field with microsclerotia of *Verticillium dahliae* in the soil. When transplants of tomatoes are set in the field, exudates from the roots of these plants stimulate the microsclerotia to germinate and infect the roots. The inoculum at the start of

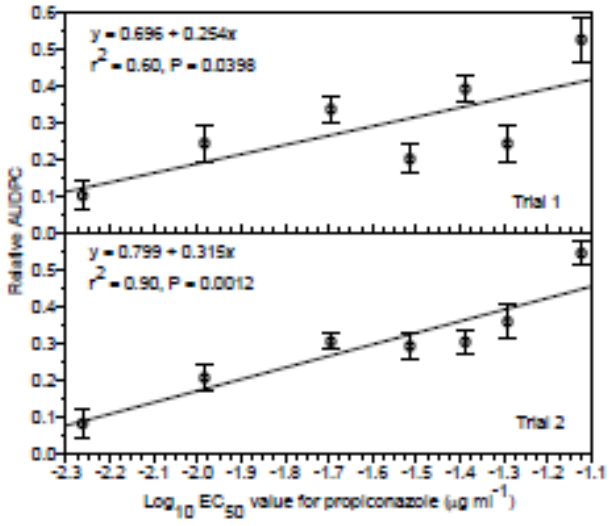
the season is the main (or sole source) of inoculum. The inoculum does not increase very much nor does it move about very much except by the assistance of man. The increase in the number of wilting tomato plants is NOT due to the fungus moving from plant to plant, but due to the increasing numbers of roots encountering inoculum. Most monocyclic epidemics are caused by soil-borne pathogens and the diseases are damping-off, root rots, stem rots, and wilts. However, more and more, plant pathologists discover that soil-borne pathogens can be polycyclic, for example *Fusarium oxysporum* f.sp. *radici lycopersici* (and other forma speciales) can form macro- and microconidia at the soil level in humid climates.

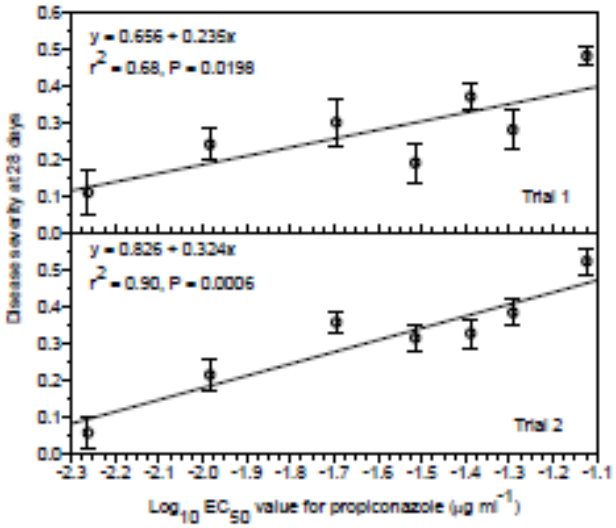
Dispersal Mechanisms

- Soilborne
  - localized (patchy) epidemics
  - pathogen movement and propagule dispersal physically restricted
  - epidemics can be significantly affected by pathogen-infested soil on field equipment (cultivation, etc.)
- Rain or irrigation water
  - soilborne pathogens may be dispersed throughout field in irrigation water

Pesticide Resistance

Multiple gene resistance (sterol inhibitors) - quantitative effect
Example: propiconazole resistance in *Sclerotinia homoeocarpa*
(From: Miller, et al. 2002. Plant Disease 86:1240-1246)





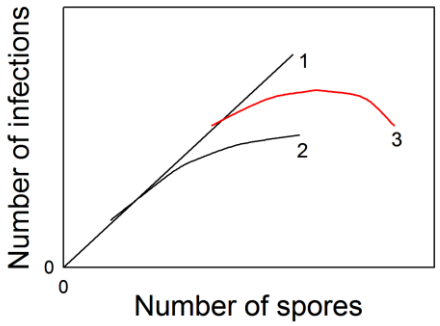
Quantification of soilborne inoculum

- Extraction and quantification of propagules from soil
    - Soil dilution plating for fungi and bacteria (caution! Only profusely sporulating fungi may be detected)
    - Enrichment with organic matter before dilution plating for pathogens with good competitive saprophytic ability
    - Warcup plate method (spread soil on plate cover with luke-warm agar or place soil on solidified agar)
    - Anderson sampler with appropriate sieves on layers of petriplates
    - Wet-sieving and flotation possibly in sucrose gradients (sclerotia, nematodes)
- Disease tests with naturally infested soil
    - Plant seeds or seedlings in soil, possibly in a dilution series wwith sterile soil (see most probable number technique)
- Baiting techniques
    - Place autoclaved leaves etc in soil, plate after incubation and count positive samples (not really quantitative; why not?)
- Dilution end point and most probable number methods
    - Mix field soil with sterilized soil or sand at different ratios
    - Place baits or plants in individual cups or wells with the mixes
    - Determine dilution at which no more infection occurs
    - Use several reps and dilutions for MPN (like 5 reps, 10 dilutions); use MPN table or computer program to calculate the estimated MPN per g dry soil
- Immunological techniques
    - Soil extracts in ELISA tests with monoclonal and/or polyclonal antibodies
    - Immunomagnetic capture (magnetic beads coated with monoclonal antibodies)
    - Test kits available, but quantification is still problematic
- DNA techniques
    - Extraction and lysis of DNA; separate DNA from tannins and humic acids (which interfere with PCR); amplify by PCR with specific primers (only qualitative)
    - Same but use quantitative or qPCR (also called real-time or rtPCR); this is semiquantitative, because we don't know how the cycle threshold value (Ct) relates to infective propagules (adding different inoculum levels to soil to prepare a calibration curve is not the same as different natural inoculum levels)

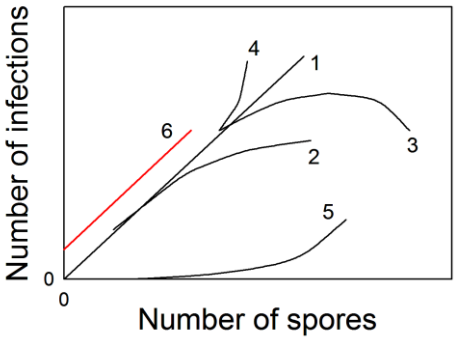
The relationship of disease intensity (DI) to inoculum density (ID) [DI:ID]

There was a lot of discussion about this relationship in the 1970's, 80's and 90's (heated debates!). Van der Plank (1975 "Principles of Plant Infection") distinguished six basic types of responses for the DI:ID relationship:

1. Disease is directly proportional to inoculum dose (straight line passing through the origin (0,0); slope is infection efficiency; IE=constant); in this case, the spores neither interact nor compete for "sites".
2. DI:ID decreases as inoculum increases; something limits IE (availability of susceptible host sites or inhibitory interactions among propagules)
3. Antagonistic response: the DI decreases with increases in ID (accumulation of inhibitory products in the presence of "crowded" spores)



4. Synergistic response: DI:ID **increases** with increases in ID
5. ID has to exceed a certain threshold before the first infection (a large number of spores is needed to attain the probability that one spore will reach a susceptible site
6. Impossible response where disease seemingly occurs without inoculum! (curves like #6 in the literature; be suspicious!)



Basic assumptions under Van der Plank's curves:

- All propagules act independently, "independent-action model"
- Each organism has a certain probability of causing an infection
- Total probability of infection from a given dose is the statistical combination of the probabilities for all the individual organisms

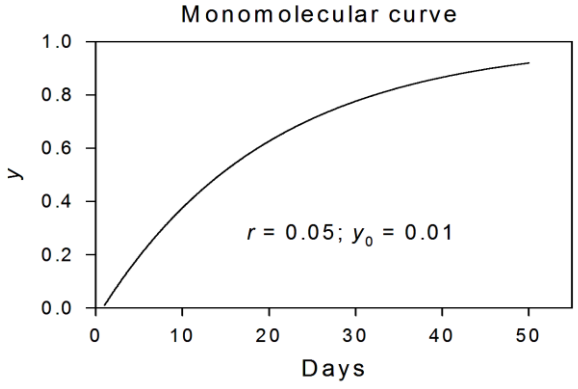
The relationship between DI and ID can be described by the model of independent action (especially when the relation is a saturation curve):

$$y = N\ [1 - \exp(-ax)]$$

- **y** = number of infections, ***x*** = number of spores, ***N*** = total number of susceptible sites, and ***a*** = the rate parameter that determines the proportion of effective units of inoculum
- High values of "***a***" mean very efficient inoculum
- Low values of "**N**" mean low numbers of susceptible sites
- With a high "***a***" and low "**N**", the monomolecular DI:ID curve has a strong curvature

Interlude: monomolecular disease progress

The shape of the curve for the increase of monocyclic diseases over time is a similar saturation curve: an asymptotic growth curve, shaped like an inverted "J". The preferred term for this shaped curve is **"monomolecular".** It is a variant type of an exponential curve.



If the rate of disease increase is a relative rate and the carrying capacity is 1, then the model for curves of this shape is the monomolecular equation:

$$Y_t = 1 - \exp(-r\,t)$$

In this equation, the rate, *r*, is is called *the monomolecular rate*, Yt is disease incidence (or severity) at time t.

The transformation equation for values along this curve is:

$$Y_t = \ln[1/(1-y)]$$

The rate (derivative) equation for the monomolecular curve is:

$$dy_t/dt = r\,(1- y_t)$$

In natural epidemics of "simple interest diseases", the epidemic rates are usually rather slow;

i.e., $r_m < 0.05$, and commonly about $r_m \approx 0.01$.

Where is y_0 in the monomolecular equation ? It isn't there, because all curves begin at 0.0.

The monomolecular curve is the "idealistic" shape for progress curves of simple-interest diseases. It is, by no means, the only shape. Some simple-interest diseases progress nearly linearly, other curves may be slightly ascending as a weak exponential, similar to the DI-ID relationships.

The DI:ID relationship debated

Ralph Baker (Colorado State Univ.) published extensively on the DI:ID relationship in the 1970s and 1980s, mainly based on work with *Rhizoctonia solani* on radish. He had interesting ideas about the effect of inoculum density in 3D soil space compared to 2D surfaces. Baker's concepts were severely criticized and a series of letters to the editor in Phytopathology resulted.