

Epidem & Data Sci

Disease progress over time
Dispersal

10 July 2018

Activities today

- Review syllabus
- Discuss epidemiology portfolio
- Disease progress over time
- Dispersal
- Break approximately here (1:45-2:00)
- Examples in R

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Template for Epidemiology Portfolio Proposal

- You can use the following slides (filling them in) to design the proposal for your Epidemiology Portfolio, as part of a 20 minute presentation
- In the individual projects, each participant will select a disease (or disease complex) of particular interest, assemble a portfolio of epidemiological information related to that disease (or similar diseases, if data are sparse), write a short proposal including an experimental design to collect important new data related to the disease, analyze simulated results from that proposal, and evaluate regional management strategies for the disease in an epidemiological synthesis.

1 – Background on disease

- What disease are you addressing?
- What is known about the “disease triangle” as it relates to this disease? (succinctly)
 - Competent pathogen(s)
 - Susceptible host(s)
 - Conducive environment
 - (Competent vector(s), if relevant)

2 - Sampling

- What methods are appropriate for sampling the abundance of this pathogen?
- What methods are appropriate for sampling the incidence and/or severity of this disease?

3 – Disease progress over time

- What is known in general about how this disease increases at a location over time?
- What are the main results from one or two studies of disease progress over time for this disease (or for closely related pathogens, or this pathogen on other hosts)?
- What are the strengths and weakness of these studies – what are logical next steps?

4 – Dispersal

- What is known in general about how this pathogen disperses across space?
- What are the main results from one or two studies of dispersal for this pathogen (or for closely related pathogens, or this pathogen on other hosts)?
- What are the strengths and weakness of these studies – what are logical next steps?

5 – Weather effects and disease forecasting

- What is known in general about how this disease responds to weather?
- What are the main results from one or two studies of weather effects on this disease (or for closely related pathogens, or this pathogen on other hosts)?
- What are the strengths and weakness of these studies – what are logical next steps?

6 – Management decision making

- What management options are available for farmers for this disease?
 - Tactical, within season
 - Strategic, across seasons
- What factors help to determine whether farmers will use these options?

7 – Yield loss

- What is known in general about the relationship between disease severity/incidence and yield?
- What are the main results from one or two studies of the severity/incidence – yield relationship for this disease (or for closely related pathogens, or this pathogen on other hosts)?
- What are the strengths and weakness of these studies – what are logical next steps?

8 – Regional management

- What management options are available for for this disease, for groups such as phytosanitary organizations and policy makers?
 - Tactical, within season
 - Strategic, across seasons
- What factors help to determine whether these options are practical?

9 – Research frontier for this disease

- For this disease, what have been important research emphases?
- What is the frontier for research related to the disease, to build on this knowledge?

10 – Introduction to research proposal

- A summary of the general motivation (drawing on the earlier material)?
 - Overview of what is known and not known

11 - Objectives

- 3 or 4 objectives, building on the arguments above, addressing aspects such as disease progress over time, dispersal, weather effects, management effects, yield loss, regional analyses
- Objectives framed in terms of hypotheses (or questions), with basis in the literature

12 – Methods: Field components

- Locations, and rationale for their selection
- Experimental design, and rationale for it
- Sampling methods

13 – Methods: Analysis

- Statistical methods
- Graphics

14 – Planned interpretation of results

- What are potential results?
- How will they be interpreted?
- What are the implications?

15 – Intellectual merit and broader impacts

- Intellectual merit

- NSF:

- https://www.nsfgrfp.org/applicants/application_components/merit_review_criteria

- How will this project help to move a scientific community forward?

- How will this analysis inspire other studies?

- Broader impacts

- What societal goals will this analysis ultimately help to support?

- How does understanding the networks you evaluate help to reach these societal goals?

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Tools for studying disease progress



- Techniques for evaluating pathogen and disease 'levels'
- Methods for analyzing observations

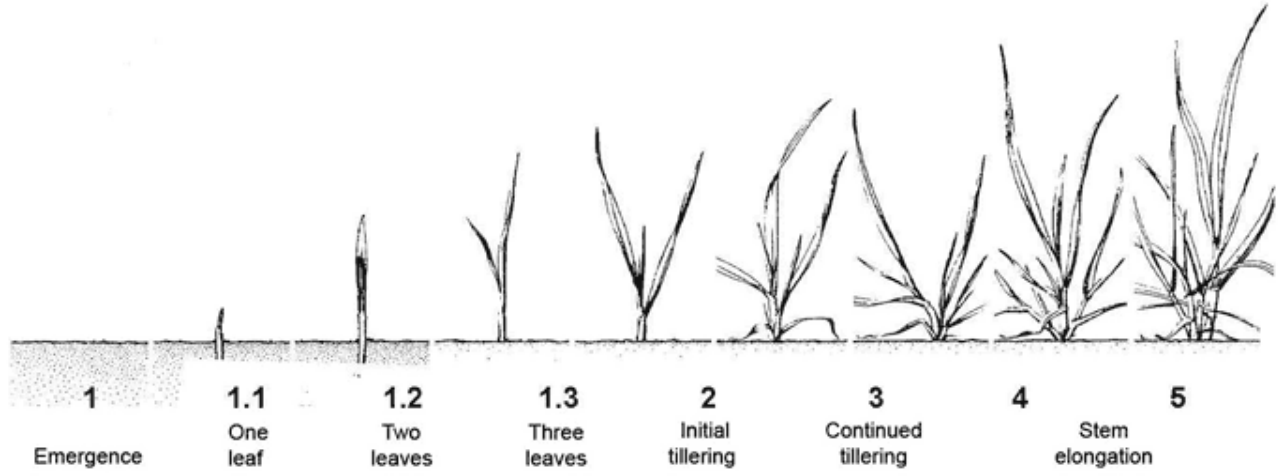
Monitoring the host

- Often not given enough attention
 - If the percentage diseased leaf area remains constant, but the host has doubled in size – then the amount of disease in absolute units has also doubled
- Some characteristics of host are routinely included
 - Cultivars, geographic location, planting and harvesting time, plant density,...
 - Also useful are host growth stage, leaf or root area,...
 - Other conditions such as soil type and fertility and density of competing plants may be useful

Host measures

- Host growth stages – key times in the growth and reproduction of plants that have meaning in relation to host physiology or yield
 - Phenology – discipline dealing with the relations between environment and periodic biological events such as growth stages
 - Ex – Feekes Scale for wheat and other small grains
 - Germination
 - (1) Seedling growth – one shoot = “braiding”
 - (2) Beginning of tillering
 - (3) Tiller formed, leaves often twisted spirally...
 - (4) Stem elongation – beginning of the erection of the pseudo-stem, leaf sheaths beginning to lengthen
 - ...

Germination Seedling growth Tillering Stem elongation



Stem extension Heading and flowering



6 One node (jointing)	7 Two nodes	8 Appearance of last leaf (flag leaf)	9 Early boot stage	10 Late boot stage (sheath split)	10.1 Ear emergence	10.5 Ear clear of leaf sheath	10.51 Beginning of flowering
10.54 Flowering completed	11 Formation of kernels (early milk)	11.1 Kernels full size, content milky (medium milk)	11.2 Kernel content starchy, still readily crushed leaves drying; glumes yellow (soft dough)	11.3 Kernels semi-hard, shiny, leaves dry, nodes yellow	11.4 Kernels hard, plants dry		

Host measures, cont.

- Measuring host growth
 - Less important for diseases where epidemics occur after canopy closure has occurred and a large portion of the leaf area has been produced (late blight is a potential example...)
 - More important when a host may be attacked at any growth stage – mildews, leaf spots, rust diseases

Host area

- Measuring leaf/root area
 - Electronic methods – leaf area meters, video image analysis systems, spectral radiometers
 - Related-measures methods – regression model of leaf area as a function of fresh or dry weights or height – need to be estimated for each new scenario
 - Visual estimates – leaves or roots placed over grids in which each small square equals a fixed area – counting of the number of squares covered
 - Root length – several methods for approximation

Time

- Choice of time scale
 - Many plant growth models relate growth to accumulation of degree-day units above a specific base temperature
 - Could also consider other environmental components – e.g., centibar-day, referring to the occurrence of certain levels of soil moisture over days, for some root diseases
- Plant growth curves (more later)

Monitoring the environment

- Environment varies over three dimensions
- Measurement systems should be matched to the objectives of the investigation in terms of accuracy, repeatability, ...

Meteorological variables and their measurement

- Temperature
 - Governs host growth, disease development, pathogen reproduction
- Rain - Timing, frequency, duration, intensity, splash characteristics, thermal effects, acidity level

Moisture

- Life cycle of most plant pathogens contains one or more phases that are affected by the states, forms, and energy of environmental water
- Relative humidity (the ratio of the amount of water vapor in the air at a given temperature to the amount of water vapor that could be contained in the air at that temperature, multiplied by 100) may be a determining factor in the success of leaf-infecting fungi
- Example: $RH > 95\%$ for spore germination and infection of peanut leaves by *Cercospora arachidicola*
- Root pathogens may exist in a soil environment where RH usually exceeds 95% and a film of water among soil particles may be nearly continuous
- Many fungi are dispersed by rain splash or in irrigation water

- Leaf wetness (from dew, fog, guttation, irrigation, or rain) – duration as well as amount and form – a problematic measurement since artificial collecting or sensing surfaces may not have the same physical and chemical characteristics as the plant organs of interest
 - deWit leaf wetness meter – hemp string expands when wet and contracts as it dries
 - electronic sensors – wires, wire clips, and metal strips as electrodes mounted in parallel to provide an artificial leaf surface – water completes the circuit and current flows only when moisture is present
 - estimates may be produced by considering the amount of time during which $RH \geq 90\%$ - easier but inaccurate for dense canopies (alternative formulations based on percent cloud cover, wind speed, and timing of precipitation) – calibration needed



- Soil moisture – often neglected

- Comparison of dry and wet weights of soil samples
- Continuous measurement – resistance block - when placed in soil, a porous block with embedded electrodes equilibrates with the soil matric potential – electrical conductivity of the block is a function of absorbed water
- Continuous measurement – thermocouple psychrometers or hygrometers – soil water potential at equilibrium is equal to the potential of water vapor in the soil air

Other weather measures

- Radiation – not often measured directly (more often indirectly through temperature)
- Wind – transport of inoculum and vectors, modification of temperature and available moisture on leaf surfaces and soil, abrasion of plant parts to allow entry of pathogens
 - Anemometers measure average wind speeds (cup anemometers disrupted by turbulence more than are thermoelectric and sonic anemometers)
- Interactions between these variables...

Monitoring pathogen populations



- Types of pathogen propagules to monitor
 - Fungal spores are discrete propagules that are often easily recognizable
 - Some soilborne fungi, such as *Rhizoctonia solani*, lack a discrete propagule
 - Other fungi and relatives may have several types of propagules – zoospores, sporangia, chlamydospores
 - Other fungi may have true sclerotia or microsclerotia
 - Plant parasitic nematodes also produce several types of quantifiable propagative units: cysts, eggs, egg masses, juveniles, male or female adults
 - Virus “propagule levels” often are measured by quantifying pathogen titer in host plants or the proportion of the vector population that carries the plant pathogen
 - Bacteria generally not measured as individuals – often colony-forming units on a growth medium
 - Propagules of parasitic plants are most often true seeds, though plant segments may also serve as propagative units (ex, dodder, *Cuscuta* spp.)

Measurement options

- Means of dispersal, survival, and infection must be known for development of an effective monitoring technique – ex, ascospores (from sclerotia) of *Sclerotinia sclerotiorum* would be more useful to enumerate than sclerotia, since these actually initiate new infections – importance of frequency, timing, and location of sampling

- Quantifying inoculum of airborne pathogens
 - Methods similar to those for sampling airborne dust and pollen
 - Simplest method: settling from still air or wind – expose horizontal surface onto which particles can settle under the influence of gravity – ex, glass microscope slides coated with an adhesive, open petri plate – problems with turbulence and wind speed
 - Inertial methods – vertical or inclined microscope slides, vertical cylinder
 - Impaction using movement through air – ex, Rotorod sampler
- Forces airflow impactors – ex, suction samplers giving a volumetric reading under field conditions – decrease effect of changing wind speed

Viruses

- Quantifying viruses in vectors
 - Range of insect traps analogous to those for airborne pathogens
 - Ex, water pan trap for aphids, sticky traps, vertical nets for catching live insects, suction traps
 - Then the fraction of vector individuals that have acquired the pathogen and are capable of transmission is determined
 - Live insects can be placed on plants to check for transmission
 - ELISA (enzyme-linked immunosorbent assay) can be used to test for presence of virus in vectors
 - Viral DNA or RNA sequencing

Soilborne pathogens

- Quantifying inoculum of soilborne pathogens
 - Direct enumeration
 - Physical extraction and counting of the propagules obtained
 - Appropriate when macroscopic propagules, such as sclerotia, or nematodes are being counted
 - It may also be possible to count spores

- **Selective culture media**
 - Selectivity provided by changes in nutrients, pH, antibiotics, or pesticide amendments
 - Soil dilutions may be used to enhance identification of organisms by reducing the total number of organisms – or as part of a dilution end point or most-probably-number technique
 - MPN techniques – testing aliquot samples of soil to determine whether the organism is present or not – the proportion of aliquot samples that contain the organism can be related to the number of infective propagules in a given volume of the original soil
 - Generally soil is sieved or diluted before being placed on medium

- **Substrate (or bait) colonization**

- Provides estimate of number of propagules and ability of these propagules to colonize a substrate effectively
- Often used for pathogens such as *Rhizoctonia solani* and *Phytophthora* spp.
- Testing for colonization of plant parts such as mature buckwheat stems, beet seed, bean and cotton stem segments, avocados, apples,...
- Plant parts are placed in soil for a period of time and then tested for the presence of the pathogen

- **Bioassay**

- Other methods may be problematic if pathogen is present at extremely low but epidemiologically significant levels – or if formae speciales, races, or varieties of the pathogen exist
- Bioassays most reliable method for determining the relative populations of some nematodes when populations are low
- For root-know nematode, susceptible tomato seedlings are grown in the greenhouse in the soil to be assayed

Sequencing

- Direct assessment of DNA and/or RNA
- Metagenomics
- Making good use of this data is a new frontier for epidemiology

Attributes of disease to monitor

- Disease intensity – quantity of disease
 - Disease **incidence** – number of plant units that are visibly diseased
 - Disease **severity** – area or volume of plant tissue that is diseased
 - For viral diseases, sometimes expressed on an arbitrary scale indicating the degree of whole-plant symptom development
 - For some diseases, calibrations to calculate from one measure to another are available



- **Timing and frequency of assessment**

- Calendar scale
- Physiological-environmental scale
- Growth stage scale



- **Procedures for disease assessment**

- Try to optimize
 - Accuracy (closeness to true value)
 - Precision (degree of minimization of variability)
 - Reproducibility (from one evaluator to another)
- Designating disease classes may be quick, but give less information
- Keys are available for clarifying assessment (giving examples of different percentages leaf are covered with symptoms)
- Remote sensing
 - Technique for measuring the characteristic manner in which a substance emits, absorbs, transmits, or reflects electromagnetic radiation at some distance from the surface of that substance
 - Healthy and unhealthy plants absorb and reflect radiation differently
 - Challenges for calibration when trying to determine disease levels
 - Aerial infrared photography used to estimate disease levels since 1933
 - Explosion in data availability from satellite imaging in the past decade, but calibration always a challenge



- **Relationship between disease incidence and severity**
 - Motivation: incidence may be easier to measure but severity may be more useful for analyses such as predicting yield loss
 - Somewhat specific to particular host-pathogen systems and even particular seasons
 - Relationship explored using correlation and regression analyses
 - Diseases with discrete lesions may be easiest to work with
 - Poisson distribution may be used to estimate relationship under assumption that lesions are independently distributed

Disease rating

- Evaluating accuracy, precision, and reliability of disease estimates
 - Can formally consider how variance is partitioned between leaves, between stems, between quadrats for a particular disease
- Can formally consider the inter-rater reliability for a particular method (perhaps using computer programs that simulate infected leaves)

AUDPC

- Area under the disease progress curve
- The AUDPC is the sum of the weighted observations (generally measures such as percent disease severity).
- Mean observations are often weighted by the length of time between the two observations that go into the estimate of the mean

AUDPC, cont.

- The AUDPC has the advantage of being simple to calculate and understand
- It has the disadvantage of being difficult to compare across experiments. Also, it doesn't reveal **when** high and low disease levels occurred. The effects of disease on yield may vary with time of infection and this effect is not easily analyzed using the AUDPC
- The AUDPC can be standardized by dividing by the length of time during which the epidemic was studied. It can also be truncated to study the earlier stages of epidemics.

Days after inoculation	Disease severity (%)	Mean disease severity over interval	Number of days in interval	AUDPC up to this point	Standardized AUDPC up to this point (AUDPC divided by # days)
0	0	-	-	-	-
5	2	1	5	$1 * 5 = 5$	$5 / 5 = 1$
10	10	6	5	$5 + 6 * 5 = 35$	$35 / 10 = 3.5$
20	50	30	10	$35 + 30 * 10 = 335$	$335 / 20 = 16.75$
30	83	66.5	10	$335 + 66.5 * 10 = 1000$	$1000 / 30 = 33.33$

Participants' examples of disease progress

- Part 1: disease progress over time
 - Describe how pathogen populations increase over time within a location
 - Ideally, reference a study about this pathogen (or related species) that evaluates pathogen population increase over time and relates the rate of increase to other factors (such as environmental factors, pathogen genotypes, host genotypes, etc.)

Annu. Rev. Phytopathol. 2004. 42:61–82
doi: 10.1146/annurev.phyto.42.040803.140427
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First published online as a Review in Advance on March 5, 2004

ANALYSIS OF DISEASE PROGRESS AS A BASIS FOR EVALUATING DISEASE MANAGEMENT PRACTICES

M.J. Jeger

Models and epidemiology

- **‘COMPONENTS OF THE DISEASE CYCLE** Examples of models developed include the following:
- the relationship between lesions of *Phytophthora infestans* and oospore production;
- a biophysical model of splash dispersal applied to *Colletotrichum acutatum* ;
- the effect of weather on the development, reproduction, movement, and survival of the aphid vectors of *Barley yellow dwarf virus*’

Models and disease management

- ‘Much empirical modeling ...has been concerned with the effects of weather and other locational variables on infection-related variables, as a basis for disease management’
- **POPULATION SCALING-UP OF CONTROL PRACTICES**
- **PHYSIOLOGY OF DISEASE-DAMAGE RELATIONSHIPS** A mechanistic model of how downy mildew affects the rates of photosynthesis in grapevine was used to define economic thresholds for control interventions

Comparative epidemiology

- **'RELATIONSHIP WITH CLIMATE** Some epidemiological studies have analyzed the influence of climate on entire disease progress curves, for example African cassava mosaic disease
- **SOURCES OF INOCULUM** The effect of inoculum density on disease progress has been studied for many soil-borne diseases. Incidence, severity, and AUDPC of wirestem in cabbage caused by *Rhizoctonia solani* AG-4 increased nonlinearly with increases in inoculum density'

Disease and yield loss

- 'In the extensive literature on the relationships between yield losses and disease, contrasts have been made between critical point and multiple point relationships.
- Today the main emphasis is on AUDPC assessment over defined periods of crop growth, or with approaches using healthy area alternatives to AUDPC.'

Plant breeding and genetic analysis

- **'GENETIC ANALYSIS AND MAPPING** Increasingly, analysis of disease progress curves has been linked with molecular markers in characterizing quantitative resistance.
- **IDENTIFICATION OF RESISTANCE IN BREEDING PROGRAMS**
Determination of genotype x environment interaction based on AUDPC can identify genotypes to serve as sources of resistance in regional breeding programs, as with sorghum anthracnose'

Pathogen isolates and strains

- ‘A few studies have analyzed disease progress curves to characterize pathogen variability.’
- ‘Fifteen isolates of *Fusarium graminearum* were compared for degree of virulence, ergosterol production, and mycotoxin (trichothecenes) accumulation on two winter wheat genotypes.
 - A significant correlation was found between AUDPC (based on head blight ratings) of the isolates and deoxynivalenol (DON) contents of the infected grain.
 - As DON and total trichothecene values were highly correlated, DON-producing capacity was considered a decisive virulence factor in the isolates.’

Disease progress and disease management: Chemical control

- **‘RELATING CHEMICAL TREATMENTS TO DISEASE DEVELOPMENT AND CROP YIELD**
- The EIPRE advisory system is based on a simple exponential model for cereal diseases, enables calculation of expected damage at harvest, and provides guidance over whether chemical control benefits exceed their costs.
- More typically, disease progress curves have been used retrospectively to quantify the effectiveness of chemical control.’

Field performance of resistant cultivars

- '**SOILBORNE DISEASE** Disease incidence of carnation cultivars to *Fusarium oxysporum* f. sp. *dianthi* was highly correlated with disease severity.
- A disease incidence of 75% recorded 180 days after planting gave a reliable separation into resistant and susceptible groups.'

- **'BACTERIAL DISEASE** Development of rice bacterial blight varies with environment, even within similar irrigated lowland production systems.
- Partial resistance of cultivars possessing the Xa4 gene was evaluated by comparison with the standard susceptible control IR24 .
- Disease severity, AUDPC, and rate of disease increase were reduced in cvs Laxmi and IR54 and were sufficient to characterize partial resistance.'

Biological control

- 'Disease progress curves can be used in at least two ways to investigate the effects of a biological control agent (BCA).
- In the first direct approach, especially relevant for mycoparasitism, the plant pathogen is considered the host, with the BCA the pathogen.
- In the second indirect approach, the effects of the BCA can be represented in terms of effects on disease progress on the plant host.'

Cultural control

- Tillage and residue management
- Irrigation
- Fertilization
- Sowing and transplanting dates
- Host growth habit, maturity, and type
- Intercropping
- Variety mixtures and multilines
- Integrated management

Activities today

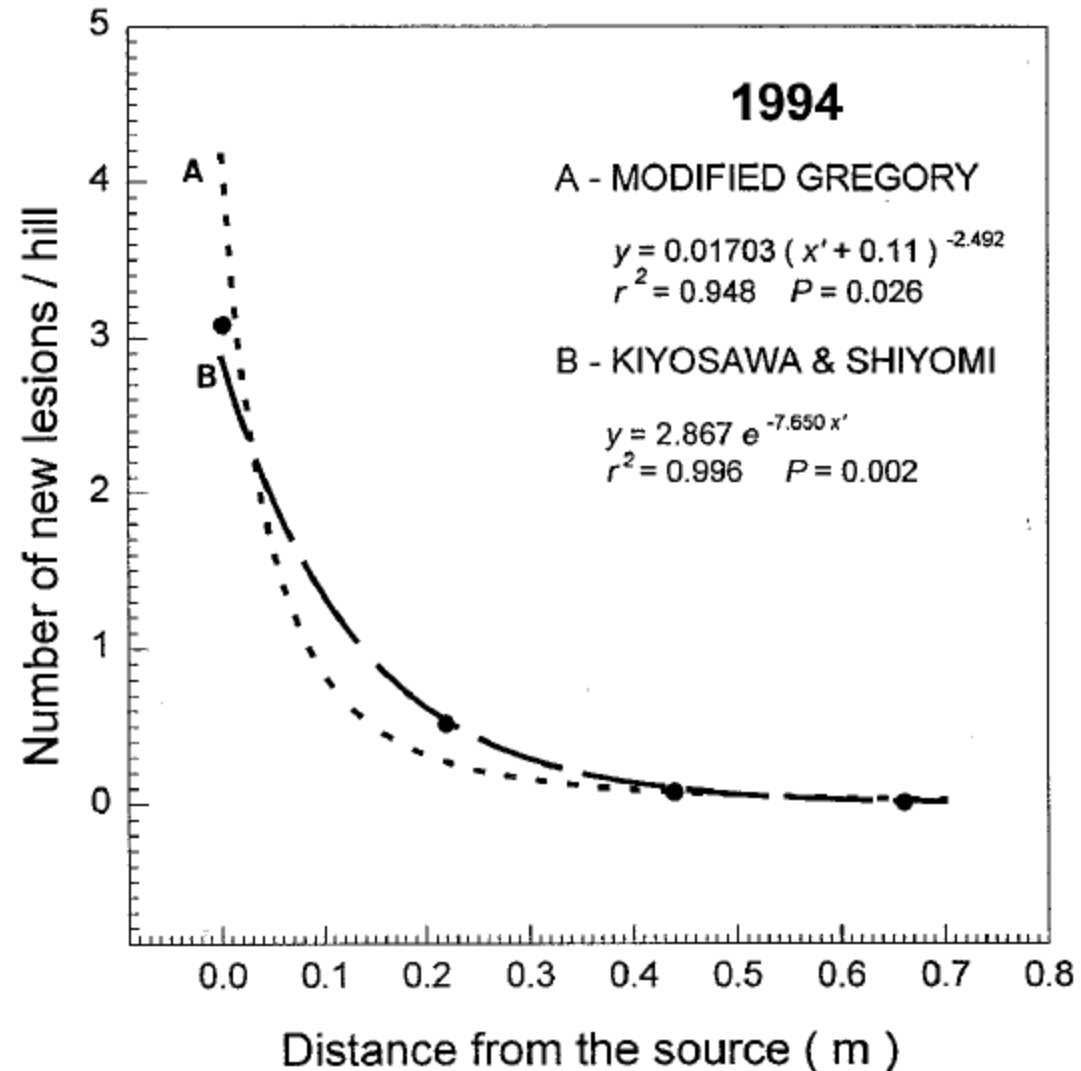
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Primary Disease Gradients of Bacterial Blight of Rice

Christopher C. Mundt, Hafiz U. Ahmed, Maria R. Finckh, Lorna P. Nieva, and Rizal F. Alfonso

1999 Phytopathology 89:64-67

Xanthomonas oryzae pv. *oryzae*



Principles of the Atmospheric Pathway for Invasive Species Applied to Soybean Rust

October 2005 / Vol. 55 No. 10 • BioScience 851

SCOTT A. ISARD, STUART H. GAGE, PAUL COMTOIS, AND JOSEPH M. RUSSO

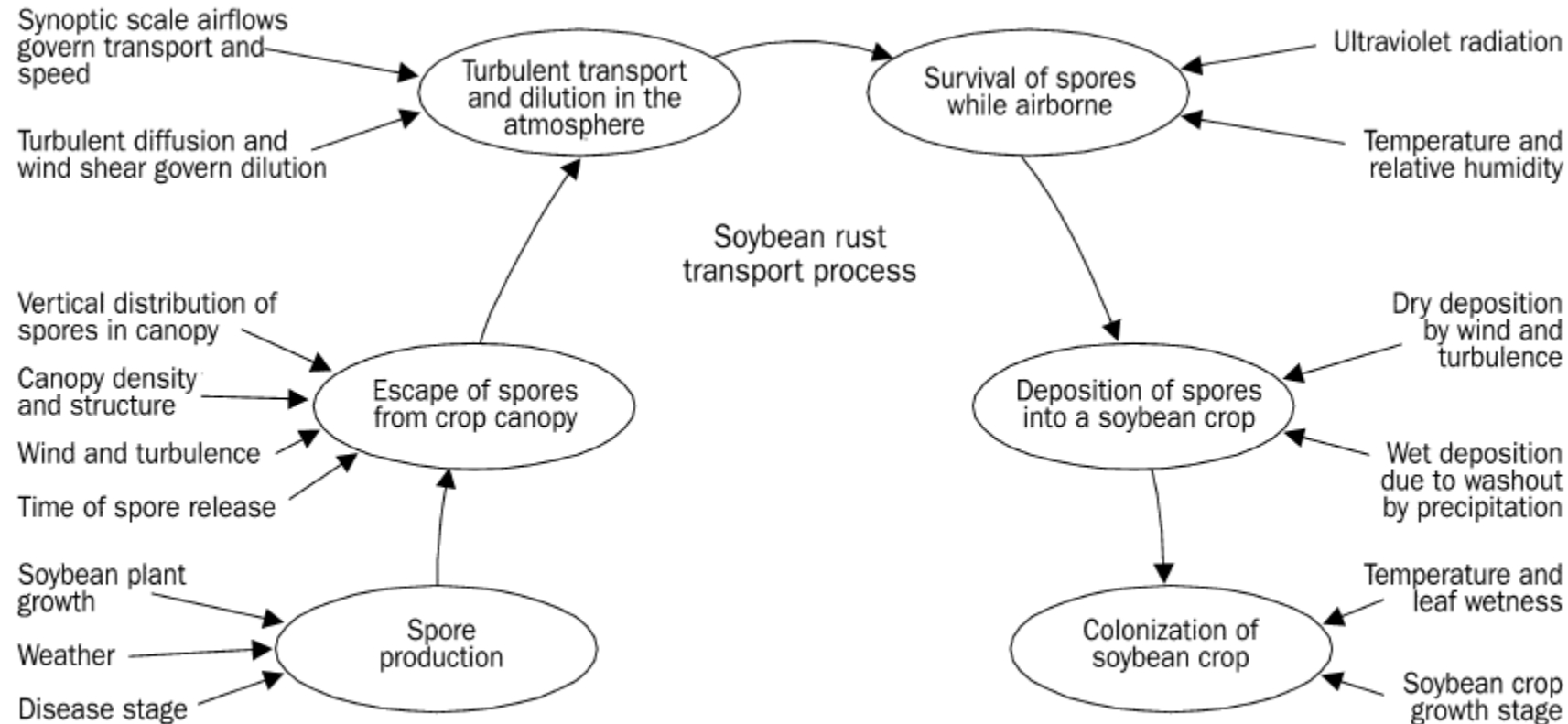


Figure 3. Conceptual model of the soybean rust transport process, depicting the processes that affect the movement of fungal spores as influenced by environmental and ecological controls.

Participants' examples of disease progress

- Part 2: dispersal
 - Describe how the pathogen disperses
 - Ideally, reference a study about this pathogen (or related species) that evaluates pathogen dispersal with estimates of dispersal gradients (i.e., how likely the pathogen is to disperse across a range of distances)

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An introduction to the R programming environment – using base R

K. A. Garrett, P. D. Esker, and A. H. Sparks. 2007. The Plant Health Instructor. DOI:10.1094/PHI-A-2007-1226-02. Available at <http://www.apsnet.org/edcenter/advanced/topics/EcologyAndEpidemiologyInR/IntroductionToR/Pages/default.aspx>

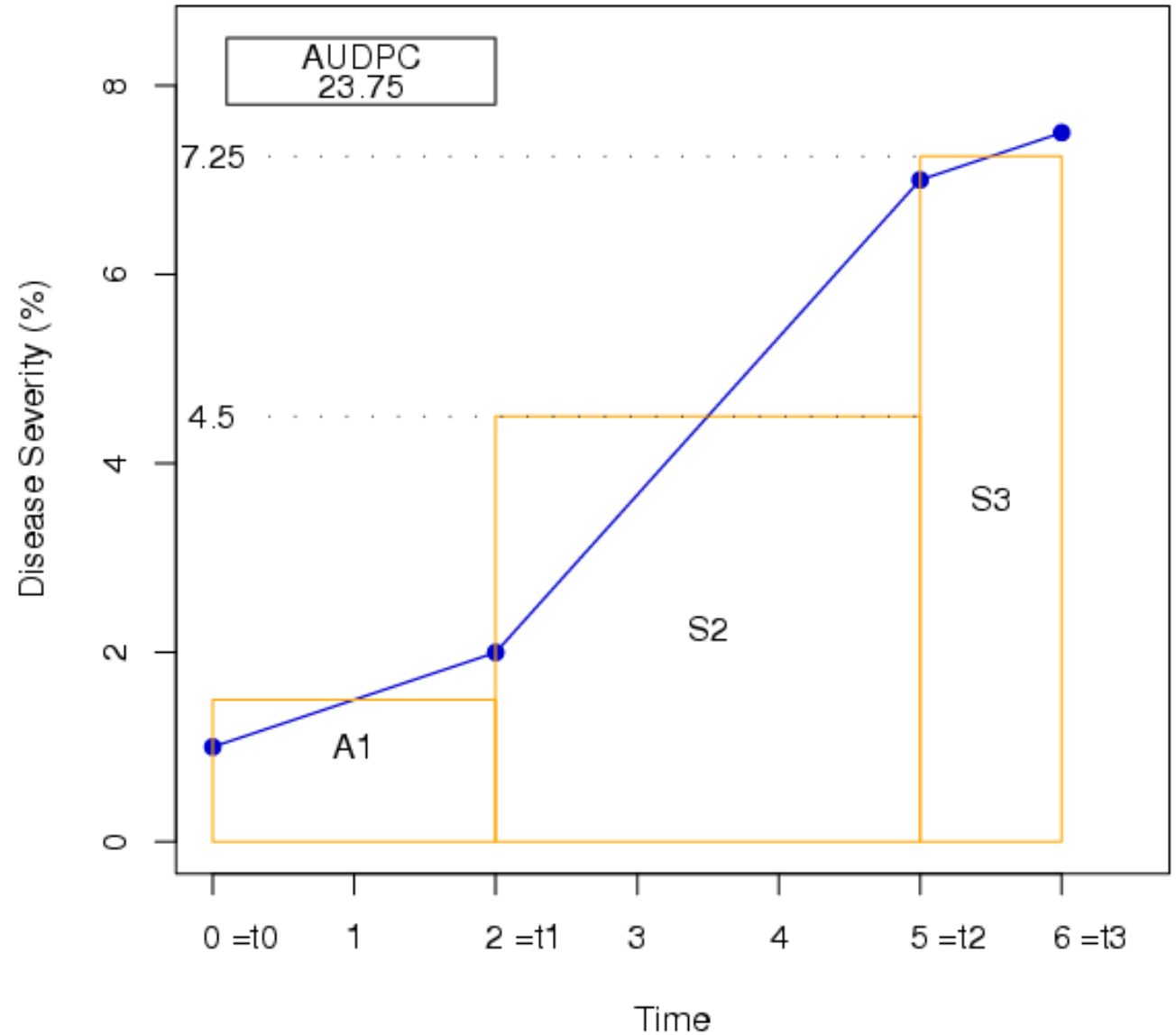
Ecology and epidemiology in R: Disease progress over time

A. H. Sparks, P. D. Esker, M. Bates, W. Dall'Acqua, Z. Guo, V. Segovia, S. D. Silwal, S. Tolos, and K. A. Garrett. 2008. The Plant Health Instructor. DOI:10.1094/PHI-A-2008-0129-02. Available at <http://www.apsnet.org/edcenter/advanced/topics/EcologyAndEpidemiologyInR/DiseaseProgress/Pages/default.aspx>

AUDPC

- <http://www.apsnet.org/edcenter/advanced/topics/EcologyAndEpidemiologyInR/DiseaseProgress/Pages/AUDPC.aspx>

Illustration of AUDPC Calculation



Growth models

- <http://www.apsnet.org/edcenter/advanced/topics/EcologyAndEpidemiologyInR/DiseaseProgress/Pages/GrowthModels.aspx>
- Exponential
- Monomolecular
- Logistic
- Gompertz
- Weibull

Ecology and epidemiology in R: Modeling dispersal gradients

P. D. Esker, A. H. Sparks, G. Antony, M. Bates, W. Dall'Acqua, E. E. Frank, L. Huebel, V. Segovia, and K. A. Garrett. 2007. The Plant Health Instructor. DOI:10.1094/PHI-A-2007-1226-03. Available at <http://www.apsnet.org/edcenter/advanced/topics/EcologyAndEpidemiologyInR/ModelingDispersalGradients/Pages/default.aspx>

Exponential and power law models of dispersal

- <http://www.apsnet.org/edcenter/advanced/topics/EcologyAndEpidemiologyInR/ModelingDispersalGradients/Pages/default.aspx>

Primary and secondary disease gradients

- <http://www.apsnet.org/edcenter/advanced/topics/EcologyAndEpidemiologyInR/ModelingDispersalGradients/Pages/PrimaryandSecondaryGradients.aspx>

Simulating an epidemic

- <http://www.apsnet.org/edcenter/advanced/topics/EcologyAndEpidemiologyInR/ModelingDispersalGradients/Pages/SimulatingEpidemic.aspx>
- You could consider including a simulation study in the epidemic portfolio
 - This isn't an expectation for this course, but we can help you develop this component if you would like to try this out

For discussion on Thursday, July 12

- Everyone should prepare a 1-2 minute contribution for the discussion, about the disease they are focusing on for the class
- Depending on the disease you have selected, you may find a lot or a little information about this point – “data-poor inference” is still valuable
- Sampling
 - What are effective ways to sample pathogen populations?
 - What are effective ways to sample disease levels?
 - Ideally, reference a study about this pathogen (or related species) that evaluates (or at least uses) sampling methods

Class Thursday

- Sampling and epidemic networks
- Decide about timing for leaders for journal article discussions
- **Now: schedule timing for individual meetings for feedback on drafts of epidemiology portfolios**