

Graduate Level Diagnostics Course taught by NPDN Diagnosticians



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Course topics:

1. Introduction: Signs and symptoms, types of pathogens, important information needed for diagnostics, looking at samples with a compound and stereomicroscope, how to prepare slides for microscope viewing.



7. Quarantine: Discussion about pathogens of quarantine significance in California and what steps you need to take if you find a new pathogen/disease (Guest lecturer)

8. Phytophthora diseases: Using morphological, immunological and molecular detection methods.

9. Virus detection: virus symptoms, detection methods, challenges associated with viral diseases (Guest lecturer).

10. Wilts and Canker Diseases: Symptoms associated with vascular wilts, how to culture from vascular tissue, important vascular wilt diseases in California, class wrap up and course evaluations

2. Resources: Books, lists, websites. (Handout)



3. Root and crown diseases: Below and aboveground plant symptoms of root rot, culturing from roots, sampling for an ELISA based assay for *Phytophthora* spp.

4. Sequence Analysis data: benefits, limitations, genes used in fungal identification, how to interpret sequence data and perform BLAST searches.

5. Powdery and Downy mildews: how to distinguish between them, morphological characters of each group, how to make scotch tape mounts (Handout)

6. Rusts: Common genera, urediniospore and teliospore morphology (Handout).

	Powdery Mildews	Downy Mildews
Classification	True fungi (Kingdom Fungi, Phylum Ascomycota)	Water molds (Kingdom Stramenopila)
Nutrition	Obligate parasite	Obligate parasite
Symptoms and signs	White to grayish powdery growth on (young plant tissues). Both sides of the leaf, can affect the roots and fruit.	Yellowish blotches on the upper leaf surfaces; later white to gray to violet growth on the lower surfaces; also affects roots and fruit.
Hypae	Septate. Much external hyphae on the lowest surface	Coenocytic. Lack of external hyphae on tissue surface
Sporelation	Conidia formed in chains or singly on conidiophores on the leaf surfaces (pedicels). Lenticular spores penetrate through stomata	Conidia are produced on branched conidiophores emerging through the leaf stomata
Optimum Conditions	Cool to warm weather; medium to high humidity; does not require free water for germination	Cool, wet weather; requires free water for spore germination and infection
Overwintering structures	Chasmothecia, asexual	Oospores, mycelium

Rust Handout:
Five rust spore types:

- Spermatia (S):** Produced by minute spermatogonium, small, oval called hyaline spores with no diagnostic value; dikaryotizing elements
- Aeciospores (A):** Non-repeating spores produced as a result of dikaryotization from spermatia; Produced in cup-shaped aecia; Unicellular spores that germinate to form dikaryotic mycelium
- Urediniospores (urediniospores, urediniospores) (U):** Produced in aecidia from dikaryotic mycelium and they themselves produce dikaryotic mycelium (repeating stage in life cycle); Uredinia usually subepidermal, erumpent; Urediniospores typically globose, echnulate, often with germ pores
- Teliospores (T):** Typically the resting spore stage; Vary greatly in morphology (one to multi-celled); usually with thick walls, typically stalked; Produced in telia that can be found either in the mesophyll layer, within the epidermal cells, the subepidermal layer, erumpent cushions or as hair-like columns.
- Basidiospores (B):** Produced on basidia following germination of teliospores; Vary in size and shape but with no diagnostic value.

References for Plant Pathology Diagnostics
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For Fungi:
General:
• <http://www.ncbi.nlm.nih.gov/pubmed/12753020>
• *Systematic Botany and Mycology* (Wheeler, an update of Fay, C.P. ed. 1989). Fungus Species and Their Products in the United States APFS press, St. Paul, MN pp. 1-1252
List of larger plant pathogens by tribe, relevance, common name, scientific name, host.
<http://www.ces.ncsu.edu/>
• *CABI Datasheets* (Index Fungorum - current names of fungi, author's names and original references)
<http://www.ces.ncsu.edu/>
Plant viruses:
<http://www.ces.ncsu.edu/>
Bacterial names:
<http://www.ces.ncsu.edu/>
Books: many available in the department library:
• *200 California Insect APFS press*
• *Arthur, J.C. 1920. Manual of the Fungi in United States and Canada*. Horner Publishing Company, New York, New York
• *Barnett, L.L. and Hunter, Barry B. 1987. Illustrated General of Imperfect Fungi*. Macmillan Publishing Company, New York, New York
• *Chapin, Charles 1924. A Monograph of the Erythrales (formerly redwings) Nova Henaga 63-1-100*
• *Heavens, G.H., Greville, G.W., Nordlie, M.E., and Hansen, M.E.C. 2004. Phytochemical Manual*. CABI Publishing, Cambridge MA.
• *Chapin, Charles 1924. A Monograph of Cestomycetes (self published) Stock, New York*
• *Crous, Paulo V. & Braun, Uwe 2003. Microfungi and its sequencers. Names published in Centropore and Centropore Centropore names: Schimmelpilze, Schwämme, The Netherlands*
• *Ellis, M.A. 1971. Desmoussieux Mycophana (MS). Commonwealth Mycological Institute, Kew, Surrey, England*
• *Ellis, G.C. & Saccardo, P.A. 1951. Phytopathogenic Fungi. APFS press, St. Paul, MN*
• *Geisley, M.E. & Hsing, C. 2008. Phytophthora-Identifying Species by Morphology and DNA Fingerprint APFS press, St. Paul, MN*
• *Geisley, M.E. & Hsing, C. 2007. Phytophthora-Identifying Species by Morphology. Academic Press, San Diego, CA*
• *Leslie, J.F., Somers, D.A. 2006. The Fungus Laboratory Manual*. Blackwell Publishing, Ames, Iowa
• *More, H.C. (Thomas of Bristol 1928). Bull. British Agric. Fish. Com. for 1928 (in appendix 1928)*
• *Re, Neil 1993. Compendium of Plant Pathology with Agriculture-Related Canada. Mycology Publications, Ontario, Canada*
• *Geisley, M.E. 2007. Abstracts in Identification Manual, CDS Biosecurity Centre, Utrecht, Netherlands*
• *Snyder, Wayne A. & Lyman, Howard H. 2005. Diseases of Trees and Shrubs (2nd Ed.) Cornell University Press, Ithaca, New York*
• *Sutton, Brian 1980. The Coelomycetes. Commonwealth Mycological Institute, Kew, Surrey, England*



Unexpected surprises:
Instructors and students had a lot of fun. One hour limited what we could cover. Room was carpeted and had no sink. Electrical power strips were lacking. Dissecting microscopes had severe limitations.

Why design a new course? Diagnostic training is not always available in current Plant Pathology curricula and most graduate students are not able to add an additional full-time course to their schedules. In response to the requests of graduate students interested in disease diagnosis, we designed a 1 hour per week seminar class. The main objective of the course was to give students a practical hands-on experience in the diagnosis of diseases and other plant problems.

Instructors:

Name	Crop expertise
Suzanne Rooney-Latham	Grapevines, ornamentals, trees
Cheryl Blomquist	Ornamentals, trees
Doug Gubler	Fruits, nuts, vines
Mike Davis	Vegetables, field crops

Who were the students? Course participants included registered graduate students, graduate students on filing fee status, undergraduates, post-doctoral researchers, junior researchers and other staff members in the department and related departments



Course Breakdown: The class met for one hour each week during the spring 2009 quarter. Individuals were graded on a Pass/No Pass system, based solely on participation. Each week a different topic was presented to the students. An introduction and short lecture, including useful handouts, was typically presented during the first 10-20 minutes of each class. The remainder of the class time was spent on "unknown" disease samples that pertained to the weekly topic (or previous topics). Students were allowed to work independently or in small groups to try and diagnose each sample. Appropriate resources were made available to the students each week and included literature and reference manuals (i.e. compendia), compound and stereomicroscopes and necessary supplies for preparing specimens on slides. A group discussion for each plant sample was held toward the end of the class.