Agricultural Fungal Pathogen Identification and Preservation for an Evergreen Teaching Collection

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**Introduction**

The members of this group have a strong interest in sustainable agriculture, Although this concept may be defined in many ways, this proposal addresses the many impacts agricultural diseases in cultural, social, environmental, and economic domains. It is estimated that plant disease accounts for annual crop losses of $9.1 billion in the United States alone (Agrios, 2005). One out of every eight crops in the world will not be successful because of a fungal pathogen, even with current methods of plant protection (Moore, 2001). The Pacific Northwest, and Washington in particular, are key agricultural spots in the United States. Washington state is the number one producer of eleven major crops in the U.S., including apples and cherries (wa.gov). Therefore, pathogens that affect Pacific Northwest crops are not only detrimental locally, but also have the potential to impact food production on a broader scale. Identifying and studying the behavorial patterns of these pathogens is important for the better understanding of how to mitigate their negative impacts on agriculture.

Agricultural and silvicultural plant diseases are diagnosed to inform growers and aid in disease management decision making, as many control measures to treat symptoms do not address root causes or long term plant health prospects (Schurtleff & Averre III, 1999; Scheuerell 2012). Disease prevention and suppression requires growers to be familiar with pathogen history in a given place and pathogen life cycles, as most mechanisms of action regarding physical, biological and chemical controls are effective only during certain periods of a target organism's (i.e. fungal pathogen) life. This information is essential to low-input agricultural and silvicultural systems. Accurate identification of both the host plant and causal agent is the first step to confirm a field diagnosis based on plant symptoms in the field (Scheuerell, 2011). At a given place in time, a single biotic agent or an abiotic factor can cause multiple symptoms of a disease, while multiple biotic agents or abiotic factors can simultaneously cause symptoms similar to one another.

Fungal organisms include the True Fungi, or the Chytridiomycetes, Zygomycetes, Glomeromycetes, Ascomycetes and Basidiomycetes, while Fungal-like organisms include the Myxomycota, Plasmidiophoromycetes and the Oomycetes (Agrios, 2005). Other common plant disease causing organisms include bacteria, viruses, nematodes and mites (Agrios, 2005). Examples of plant diseases we expect to encounter, describe and identify include Apple Scab (causal agent *Venturia inaequalis*), Cereal Rust (causal agent *Piuccinia gramnis*) and Clubroot of Crucifers (causal agent *Plasmophoria brassicacae*).

Apple Scab

Apple (*Malus sp.*) Scab occurs worldwide, but is more severe in the Pacific Northwest because of the cool, moist springs and summers (Agrios, 2005). Scab reduces the quality, size and storage life of apples; it can cause premature fruit drop, defoliation and poor bud development; and, economic losses may amount to 70% of the total fruit value (Agrios, 2005). *V. inaequalis*, an ascomytic fungi, “overwinters in dead leaves on the ground as immature pseudothecia. Pseudothecia complete their growth in late winter and spring, and ascospores mature as the weather becomes favorable for growth and development of the host” (Agrios, 2005). Mycelium create intercellular cavities within dead leaves and dropped fruit to form pseudothecia, asci are formed within this cavity and sporulate to cause primary infections when temperature and moisture regimes negatively correlate between temperatures of 6 to 26 degrees Celsius and 12 to 28 hours of leaf wetness (Agrios, 2005; du Toit et al., 2012). After pseudothecia forcibly discharge ascospores and air currents move the spores to infect young developing fruit and foliage, the fungus reproduces asexually via conidia, which cause new infections whenever weather is favorable, continuing the disease cycle (du Toit et al., 2012). Thus, when an orchardist at any scale has the ability to recognize symptoms of scab and the knowledge of how the pathogen survives winter, that orchardist can make the decision to physically eradicate or reduce inoculum, i.e., by raking leaves in the fall. In regards to large scale mechanized orchards, Agrios writes, “[s]hredding of apple leaf litter or treating them with urea in the fall reduced the risk of scab by 65%. Apple scab, however, can be controlled thoroughly by timely sprays with the proper fungicides” (2005).

Clubroot

*P. brassicae* is a fungal-like organism responsible for creating galls in the roots *Brassica* plant family members. Plaguing the United States since before 1952, *P. brassica* has devastated entire crops of cabbage, mustard, and other *Brassicas* (Hirai, 2006). Clubroot reaches hosts via floods, agricultural tools, and *Brassicaceae* weeds such as wild mustards, that can be found in full strength throughout the Pacific Northwest (Dixon 2009). Infection of a host initiates sporulation, and *P. brassica* transform the infected cell into a plasmodium, functionally a spore sack. The plasmodiums then create secondary infections throughout the plant roots, affecting the hosts’ vascular systems (Agrios, 2005). The protazoon’s motile zoospores can survive without a host for long duration, but Dixon believes this  
is the only vulnerable stage in clubroot’s life cycle (2009). Currently, there are many strategies being implemented to mitigate *P. brassicae*, even inoculation with endophytic fungi, but farmers and scientists continue to struggle with the disease (Dixon, 2009; Narisawa, 2000).

Project Significance

This project has the potential to serve as a catalyst for a permanent plant pathology resource in Evergreen’s Natural History and Teaching Collection, creating protocol for collecting, describing, identifying and preserving fungal plant pathogens. As Evergreen’s academic catalog includes interdisciplinary programs every year focused on sustainable and ecological agriculture with upwards of hundreds of students enrolling in said programs, this project is not only of academic significance, but relevant.

**Proposed Work**

Our group will conduct bio-blitz surveys of fungal pathogens from TESC organic farm, Kirsop farm, Calliope farm, Common Ground farm, Sunbreak farm, Maple Grove farm and the Kiwanis food bank garden, photographing and collecting tissues of infected plants. This means we will visit each farm and collect as many different pathogen specimens from each site as possible. Plant and pathogen specimens collected will be taken to the lab for description, identification and preservation. We will create an ongoing iNaturalist account of our data so field diagnosis and photographs are preserved for future study, and so disease management techniques can be shared with the community. We will start an herbarium collection of positively identified farm pathogens, and build a pathogen slide collection/ digital image library for future Evergreen program use. Materials needed include compound and dissecting microscopes, taxonomic keys, a plant pressing rack, a hot plate, and semi-permanent slide mounting medium.

**Methods**

Creating Plant Presses

Following methods provided by The Evergreen State College Natural History Museum, we will preserve herbarium quality sample via a dry plant press. We will store drying specimens will be stored at 45°C inside plant presses to retain plant tissue pigments for future display. Infected plant tissue will be pressed between newspaper, blotting paper, cardboard, and finally wooden frames in outward order (Smith 2012).

Creating Semi-Permanent Slides:We will prepare a semi-permanent medium and slide preparation will be following a six step process acquired from Zander (1997).

1) We will then create a gelatin mounting medium. The ingredients we will use are: 7g gelatin, 5cc water, 35cc glycerol, and a few grains on phenol or thymol). We will follow the steps below:

a) Add the gelatin to heated water

b) Add the glycerol and the phenol or thymol

c) Heat and occasionally stir until the liquid is clear

d) Pour the glycerin jelly into a pan to cool

e) After the glycerin jelly has cooled, cut and use the jelly as needed

2) After the medium is made we will heat, soak and dissect (into paper thin slices) the sample in a small drop of pure glycerol.

3) Then we will place a slice of glycerin jelly onto a slide.

4) The slide will then be placed onto a hotplate heated to 65° C.   
5) After the glycerin jelly has melted over our sample, we will slowly add a cover slip, using caution to not trap air bubbles beneath the cover slip.   
6) Lastly, our completed slides will be viewed under a compound microscope.

**Expected Results**

Our project seeks to identify which crop plant diseases are caused by fungal and fungallike organisms in Olympia, WA, and create protocol for preserving and organizing this information within the Evergreen Natural History and Teaching Collection. Because farms are pulling plants at this time of year to prepare for winter, our time to collect pathogens is limited. We hope to identify as many pathogens as possible, focusing on pathogens that infect economically important crops in the region. If we do not have enough to fall collections, we will bait pathogens of storage crops (Schurtleff & Averre III, 1999). We will also create a power point presentation of our work to be shared with our program and fungal expo at the end of the quarter.

Our timeline for this project is as follows:

* Nov. 2: meet with Whitney Smith to discuss use of the herbarium resources
* Nov. 5 through end of fall quarter: collect and preserve specimens (goal of one farm visit per week, depending on farm availability)
* Winter quarter weeks 1-8: identify specimens, creating permanent slides and iNat page that will continue through the project
* Winter quarter weeks 8-10: Organize information for fungal expo

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