[*]HIERARCHICAL POPULATION STRUCTURE OF *PHAEOCRYPTOPUS GAEUMANNII* IN THE PACIFIC NORTHWEST: *FROM NEEDLES TO LANDSCAPES*

Patrick Bennett[1] and Jeff Stone[1]

[1]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR

INTRODUCTION

Swiss needle cast (SNC), a foliage disease of Douglas-fir (*Pseudotsuga menziesii),* is caused by the physical blockage of the stomata by pseudothecial ascocarps of the fungus *Phaeocryptopus gaeumannii*. Occlusion of the stomata results in a decrease in the ability of the host to exchange gases with the environment leading to reduced carbon assimilation (Manter et al., 2000). This results in premature foliage loss and subsequent growth reduction due to decreased photosynthetic leaf area (Manter et al., 2003). Prior to the 1980's, it was known to cause severe disease only in exotic Douglas-fir plantings and was considered an innocuous needle endophyte throughout the native range of Douglas-fir, where it historically caused little impact (Hansen et al., 2000). It has since emerged as a significant forest health issue in the western Coast Ranges of Oregon and Washington for reasons that are not well understood. Climate has a significant influence on the abundance of *P. gaeumannii*, with factors such as mean-daily winter temperature and spring/summer precipitation being the best predictors of disease severity (Stone et al., 2008). Changes in the local climate on the Oregon coast, with winter temperatures increasing significantly in recent decades, may be a contributing factor to the intensification of SNC (Stone et al., 2008). There may also be variation in the virulence of two coexisting fungal lineages that may have some influence in the recent emergence (Winton, 2001). This study aims to determine the distributions of two previously identified cryptic lineages of *P. gaeumannii* in the Pacific Northwest, and assess spatial variation in genotypic diversity and population structure using DNA microsatellites known as simple sequence repeats (SSRs). This information will be important in determining whether the distribution of these lineages, or the genetic structure of their populations, is associated with SNC disease severity at the landscape level.

METHODS

The analyses presented here includes genotypes from isolates collected near Tillamook, Oregon and southwestern Washington in 2014 (Bennett and Stone, 2016) as well as 304 isolates collected from nine sites in western Washington in 2015 (Table 1, Figure 1). To determine whether geographic trends in the distributions of the two *P. gaeumannii* lineages similar to those observed for western Oregon also occurred in Washington, sites managed by the WA Department of Natural Resources (WA DNR) were selected along latitudinal transects from the coast to approximately 56 km inland (Figure 1). These transects consisted of five sites on the northern Olympic Peninsula and four sites along the northwestern edge of the Quinault Indian Reservation. Three to four shoots bearing ascomata of *P. gaeumannii* were collected from the mid-crowns of five haphazardly selected trees at each site. Foliage samples were stored in cooler boxes and returned to OSU where spores were isolated from this foliage within 72 h of collection for culturing and DNA extraction. Single-spore isolates of *P. gaeumannii* were obtained by suspending infected needles

[*]In: Goodrich, B. & P. Palacios (Comps). Proceedings of the 64th Annual Western International Forest Disease Work Conference, 2016 May 9-13, Sitka, AK

above the surface of water agar in Petri dishes, allowing ascospore discharge, and then isolating individual spores within 48 hours. Fungal isolations, culturing, genomic DNA extraction, PCR amplification, and microsatellite genotyping, and the population genetics analyses were performed using the methods described in Bennett and Stone (2016), with the microsatellite markers described in Winton et al. (2007).

Table 1. (A) Summary of sample sizes and diversity statistics for each of the WDNR sites sampled in 2015. **(B)** Summary statistics of the two Lineages from the 2015 Washington sites.

A

Site	N	MLG	Lineage 1	Lineage 2
WDNR70	27	21	18	9
WDNR71	8	8	6	2
WDNR49	47	40	33	14
WDNR68	31	27	29	2
WDNR66	54	39	53	1
WDNRQ	31	25	7	24
WDNR64	38	28	12	26
WDNR63	21	17	9	12
WDNR32	47	36	47	0
Total	**304**	**227**	**214**	**90**

B

Lineage	N	MLG
1	214	143
2	90	84
Total	**304**	**227**

N= sample size, MLG = number of multilocus genotypes

RESULTS AND DISCUSSION

Phylogeography

Both *P. gaeumannii* lineages previously identified in western Oregon were present and abundant in samples from the Olympic Peninsula and southwestern Washington. Lineage 1 was most abundant overall as it accounted for approximately 70%, or 214 of the 304 total isolates from Washington (Table 1B). Lineage 2 was detected at eight of the nine sites and accounted for 30% of the total isolates recovered. Lineage 2 had a greater diversity of multilocus genotypes (MLGs) than Lineage 1, with 85% and 69% of isolates exhibiting distinct MLGs in each lineage, respectively (Table 1B). The distributions of the two lineages in Washington closely resembled the geographic trends identified in our preliminary studies of their distributions in western Oregon (Bennett and Stone 2016). Sites within 40 km of the coast comprised mixtures of both lineages, with the greatest proportions of Lineage 2 occurring in stands nearest to the coast, while sites 40 km or more from the coast were predominantly Lineage 1. While isolates of Lineage 2 were detected at all of the sites sampled within 40 km of the coast, the coastal site near Queets (WDNRQ), in the Quinault Indian Nation, had particularly high proportions of this lineage (Figure 1). Approximately 40 km to the east, at the site near Quinault (WDNR32), Lineage 2 was totally absent. Although the relationship between the presence of either lineage and disease severity is not understood, in Oregon, the regions where these lineages co-occur correspond with the regions where the greatest SNC disease severity and growth impacts have been observed. It has also been observed that populations consisting of only one of the two

lineages, such as the southern Oregon coast where Lineage 2 predominates (Winton 2001) and inland sites where populations consist exclusively of Lineage 1, generally have less severe defoliation due to SNC.

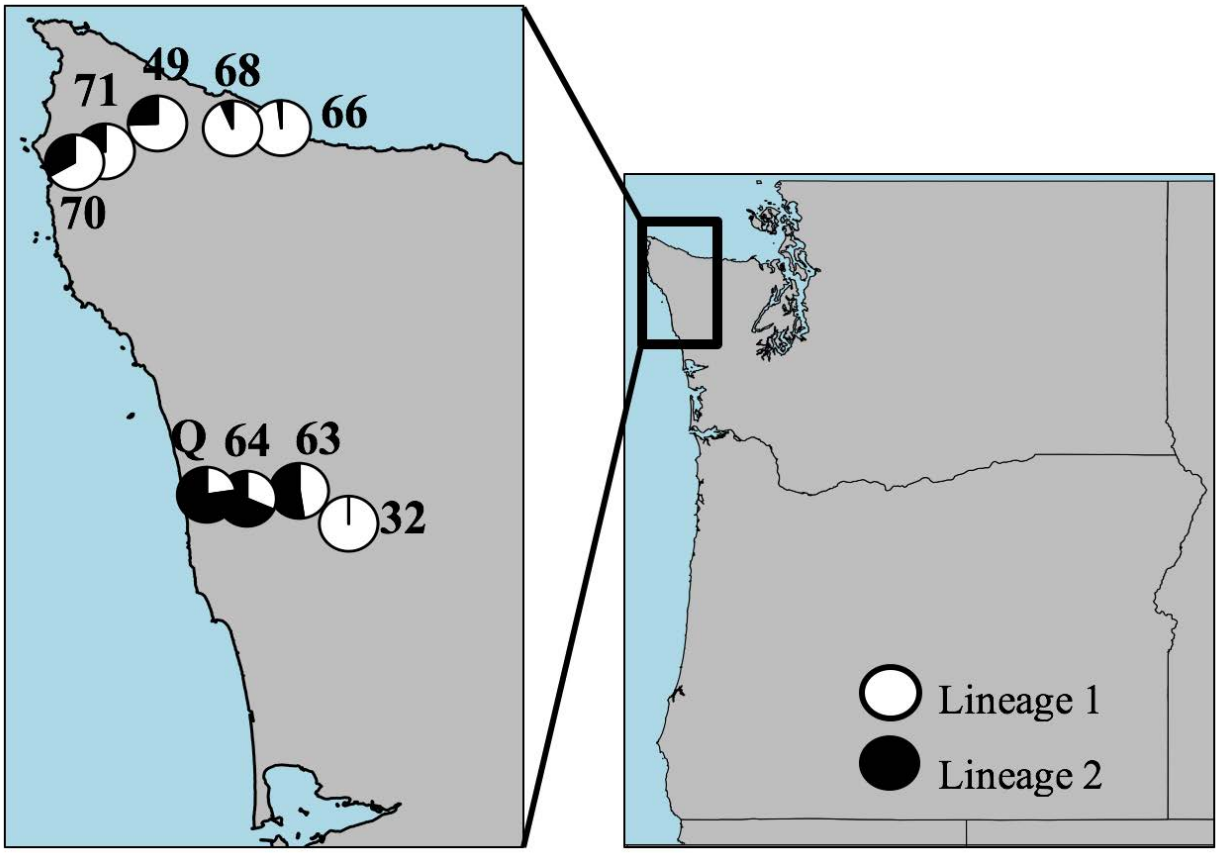

Figure 1. Map showing the distribution of the two lineages across the nine sampling sites. The labels next to each pie chart represent the site name as listed in Table 1A.

Population Structure

We aimed to assess population structure, or the occurrence of genetic subdivision within populations, at several spatial scales ranging from sites to needles. The discriminant analysis of principal components (DAPC) revealed that the sites were genetically differentiated with coastal (0-16 km inland), intermediate (24-40 km inland), and inland sites (40-56 km inland) separating into distinct genetic clusters in both the northwestern Oregon sites (near Tillamook, OR) (Figure 2) as well as the transects sampled in Washington (Figure 3). These results suggest that genetically distinct populations of *P. gaeumannii* occur within the SNC epidemic areas and that these populations may exhibit some level of local adaptation. This level of genetic differentiation could also occur due to limitations on spore dispersal distance. These observations of the distributions of the two lineages in Washington have also reaffirmed our previous observations that the areas with the greatest disease severity, as assessed by aerial surveys, occur where the ranges of the two lineages overlap near the coast. This trend of greater disease severity where the lineages coexist may be related to competition between isolates of the two lineages, but more likely reflects climatic preferences of the two lineages. These lineages might coexist in these areas simply because the coastal climate in the Sitka Spruce vegetative zone is particularly conducive to dispersal, infection, and reproduction of these fungi.

With these data, we have also made the first observation of Lineages 1 and 2 co-occurring within a single needle (Figure 4). A total of six isolates were genotyped from a single needle from the WDNRQ site (near Queets, WA), and eleven isolates were genotyped from a single needle from WDNR66 (near Pysht, WA). Of the six isolates collected from the tree at WDNRQ, four were identified as Lineage 2, and two were identified as Lineage 1. All of the isolates from the second needle were identified as Lineage 1 (Figure 4). It is not known whether co-occurrence of both lineages within needles is significant to disease, but if both cryptic species are in very close spatial proximity to one another it is likely that they are competing for space and resources.

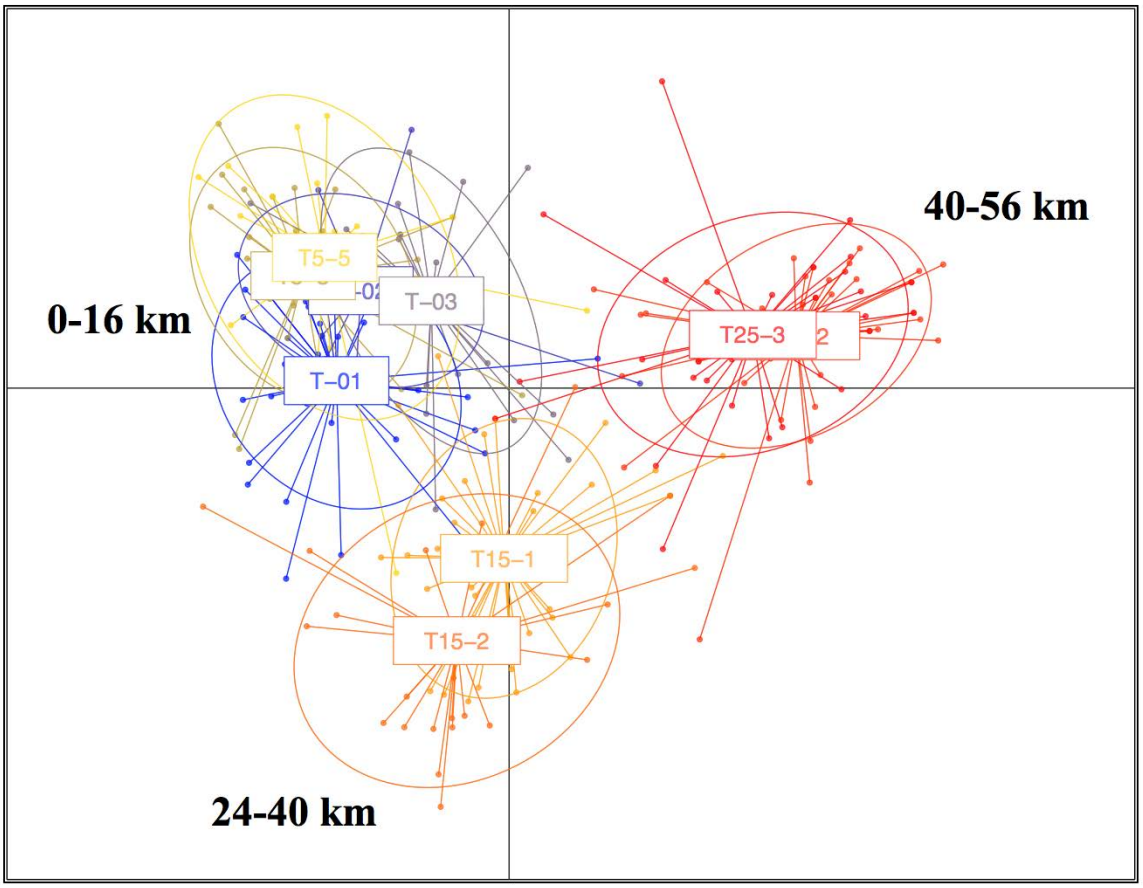

Figure 2. DAPC scatterplot showing genetic differentiation between sampling sites at various distances from the coast near Tillamook, Oregon (sampled in 2014).

Implications for Management

The relationship between presence, or relative abundance, of the two lineages and disease severity is not clear. It is known that both lineages of *P. gaeumannii* are able to cause disease and there is currently no strong evidence to suggest that either lineage is more virulent. Disease severity (i.e. defoliation) is related to the abundance of *P. gaeumannii* in needles, which in turn is related to environmental factors favorable to growth and reproduction of the fungus--abundant precipitation during May-June, and mild winter (Dec-Feb) temperatures. Both lineages are present in areas with the most severe disease but whether this is somehow due to competition or synergy, or simply to conditions that are very favorable for *P. gaeumannii*, is not clear. Recent trends in climate warming as well as expansion of Douglas-fir forestry to sites at higher

risk of SNC both appear to be involved in increasing in SNC severity in the region. There is no evidence of specific resistance to infection by either lineage in Douglas-fir. Because the physiological mechanism of disease (the inhibition of gas exchange due to stomatal occlusion) does not involve direct cell penetration by *P. gaeumannii*, it is unlikely that typical wound or pathogen-specific resistance reactions function in this host-parasite system. Considerable variation in foliage retention and growth among Douglas-fir provenances affected by SNC has been observed, at least where disease severity is moderate, though the specific genetic and morphological factors mediating this tolerance have yet to be investigated.

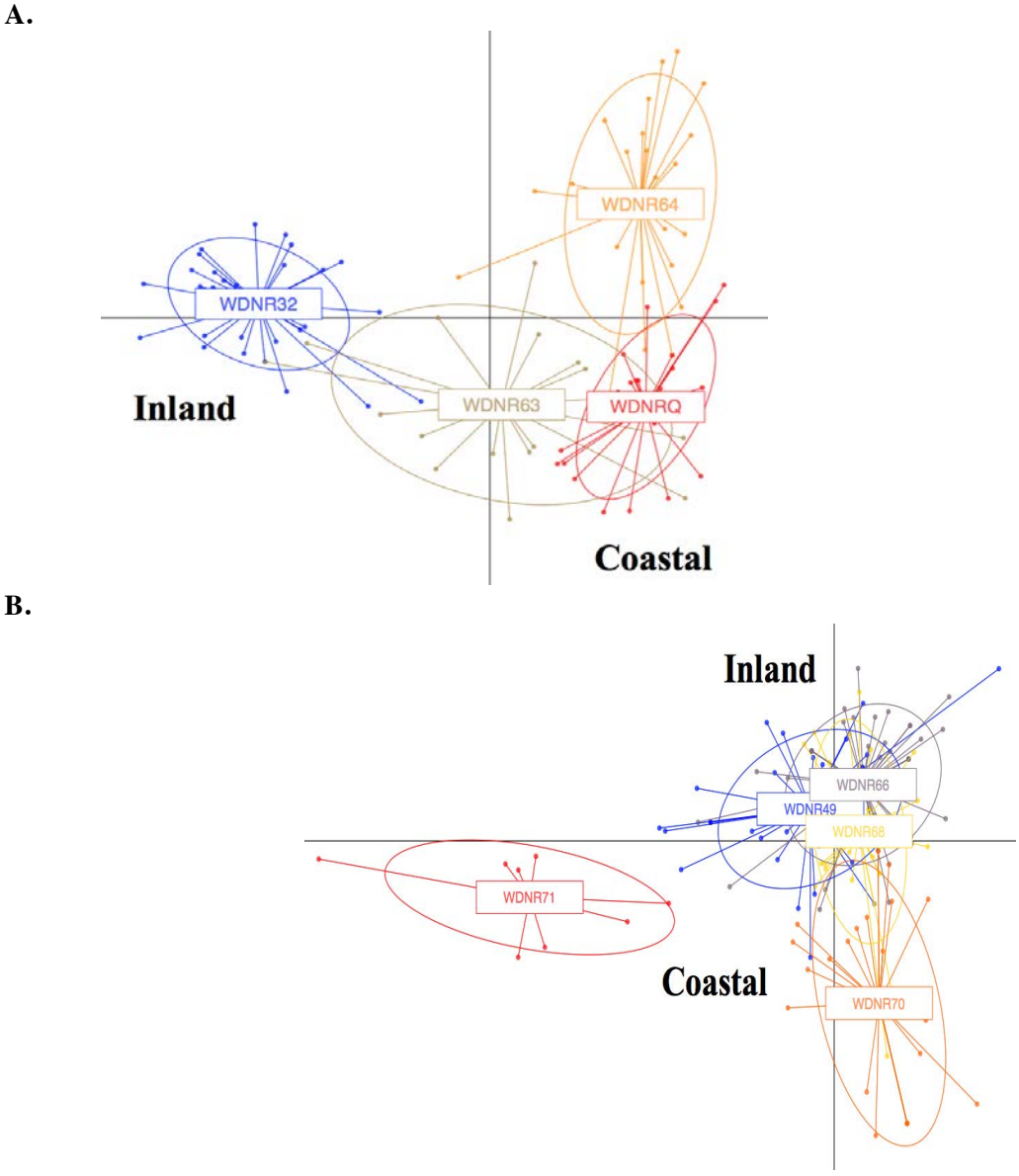

Figure 3. Discriminant analysis of principal components (DAPC) scatter plots showing genetic differentiation between isolates from each of the sampling sites. Each point represents an isolate, and the colors and labels correspond to sample sites listed in Table 1. **(A)** Differentiation between inland and coastal sites from the southern transect (Quinault) shown in Figure 1. **(B)** Differentiation between inland and coastal sites in the northern transect (Olympic Peninsula) shown in Figure 1.

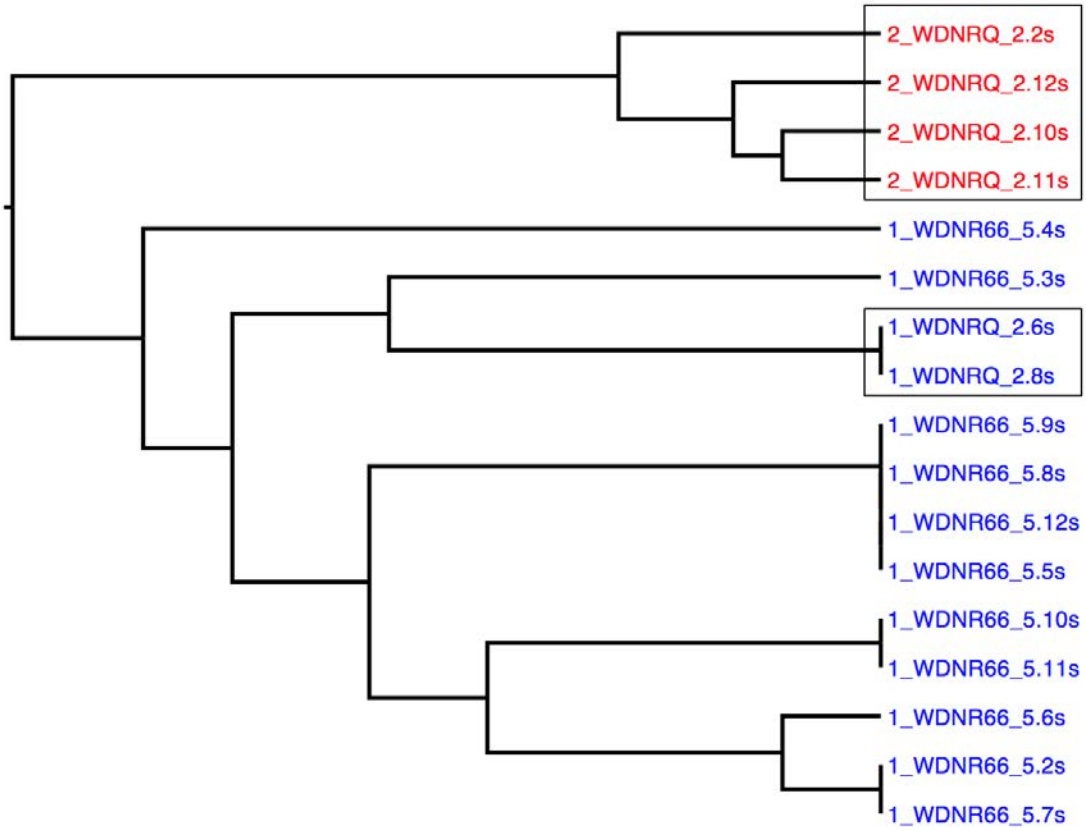

Figure 4. UPGMA dendrogram showing relationships between isolates collected from individual needles. The 6 isolates collected from a single needle at the WDNRQ (Queets) site include both Lineages 1 and 2, as indicated by the boxes. The 11 isolates from a single needle from WDNR66 are all Lineage 1. The labels for the taxa represent Lineage…Site…Tree…Isolate.

ACKNOWLEDGEMENTS

Special thanks to Dan Omdal and Amy Ramsey (WA DNR) for their guidance in selecting sites and collecting foliage samples. The Washington Department of Natural Resources and Jim Hargrove (Quinault Indian Nation) provided funding for this research. Their contributions to this research effort are greatly appreciated. The kind folks at WIFDWC provided travel funds for this research to be presented at the 2016 meeting in Sitka, Alaska.

REFERENCES

Bennett, P., and Stone, J. (2016). Assessments of population structure, diversity, and phylogeography of the Swiss needle cast fungus (*Phaeocryptopus gaeumannii*) in the U.S. Pacific Northwest. Forests 7(1):14. DOI: 10.3390/f7010014.

Hansen, E.M. et. al. (2000). Incidence and impact of Swiss needle cast in forest plantations of Douglas-fir in coastal Oregon. Plant Disease 84(7): 773–778. DOI: 10.1094/PDIS.2000.84.7.773.

Manter, D.K. et al. (2000). Pseudothecia of Swiss needle cast fungus, *Phaeocryptopus gaeumannii*, physically block stomata of Douglas fir, reducing CO_2 assimilation. New Phytologist 148(3):481–491. DOI: 10.1046/j.1469-8137.2000.00779.x.

Manter, D.K. et al. (2003). Modelling the impacts of the foliar pathogen, *Phaeocryptopus gaeumannii*, on Douglas-fir physiology: net canopy carbon assimilation, needle abscission and growth. Ecological Modeling 164(2-3):211–226. DOI: 10.1016/S0304-3800(03)00026-7.

Stone, J.K., Coop, L.B., and Manter, D.K. (2008). Predicting effects of climate change on Swiss needle cast disease severity in Pacific Northwest forests. Canadian Journal of Plant Pathology 30:169–176.

Winton, L.M. (2001). Phylogenetics, Population Genetics, Molecular Epidemiology, and Pathogenicity of the Douglas-fir Swiss Needle Cast Pathogen *Phaeocryptopus gaeumannii*. Oregon State University, Corvallis, OR.