# GENOTYPIC DIVERSITY AND POPULATION STRUCTURE OF *PHAEOCRYPTOPUS GAEUMANNII* IN NEW ZEALAND

***Patrick Bennett*[1] and *Jeffrey Stone*[1]**

## BACKGROUND

Swiss needle cast (SNC) and its causal organism, *Phaeocryptopus gaeumannii,* were first detected in New Zealand in 1959 on the North Island near Taupo. Approximately 10 years later, the disease made its first appearance on the South Island (Kimberley *et al*. 2011). While the environmental factors influencing SNC severity in New Zealand have been well studied (Hood and Kimberley 2005, Stone *et al*. 2007, Watt *et al*. 2010, Watt *et al*. 2011), this study represents the first assessment of population genetic structure and diversity for introduced populations of *P. gaeumannii*.

## RESEARCH OBJECTIVES

This study aims to compare the genetic variation and diversity present in introduced populations of *P. gaeumannii* from throughout New Zealand, and determine the relative abundances and distributions of two previously described lineages of *P. gaeumannii* present in the country. Population genetic parameters estimated for these populations will be compared to those measured in native populations from Northwestern North America (PNW) to contribute to a more thorough understanding of reproduction, dispersal, and population dynamics in this foliar pathogen of Douglas-fir.

## MATERIALS AND METHODS

### *Field Sampling, Isolations, and Culturing*

The isolates included in this analysis were collected from 17 Douglas-fir plantation sites in New Zealand (Figure 1, Table 1) in 2005 and 2007. Eight of the sites were on North Island, and the remaining 9 were on South Island (Figure 1). Foliage samples were collected from secondary branches in the upper crowns of 10 Douglas-fir (*Pseudotsuga menziesii var. menziesii*) trees at each site. Foliage bearing pseudothecia was affixed to the lids of Petri dishes with double-sided tape to allow ascospores to discharge onto the agar surface below for approximately 24 h. Individual ascospores were then isolated onto 2% malt agar and incubated for 2-6 months to allow adequate growth for DNA extraction.

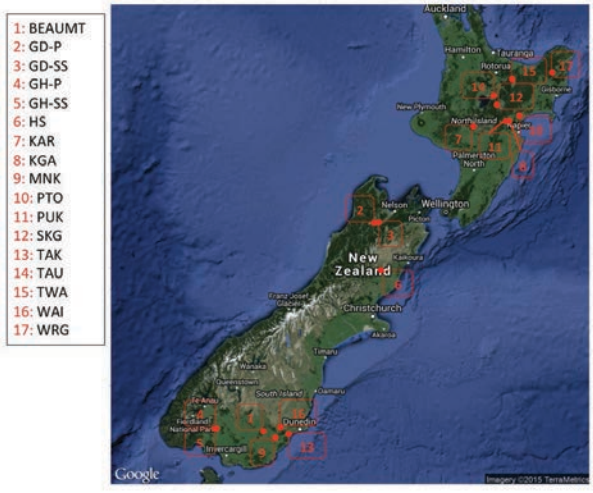



***Figure 1.*** *Map of New Zealand showing the distribution of sampling sites on the North and South Islands.*

### *Molecular Techniques*

Following thorough maceration of the *P. gaeumannii* mycelium, DNA extractions were performed with a CTAB extraction buffer followed by precipitation in 24:1 chloroform:isoamyl alcohol. The genomic DNA was then purified by passing through a QIAamp spin column. Ten microsatellite loci were amplified in 3 multiplexed PCR reactions using the primers described by Winton *et al*. (2007). Genotyping was performed via capillary electrophoresis at the Oregon State

In: Ramsey, A. & P. Palacios (Comps). Proceedings of the 63rd Annual Western International Forest Disease Work Conference; 2015 Sept. 21-15; Newport, OR. [1]Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon.

University Center for Genome Research and Biocomputing (CGRB). For more information on molecular techniques and data analysis see the companion publication in this proceedings: Bennett and Stone- Genotypic Diversity, Phylogeography, and Population Structure of Two Lineages of *Phaeocryptopus gaeumannii* in the Pacific Northwest.

***Table 1.*** *Diversity statistics for each of the sampling sites.. N= sample size, MLG= number of multilocus genotypes, H= Shannon-Weiner diversity index. L1= Abundance of Lineage 1, L2= Abundance of Lineage 2.*

| Site | N | L1 | L2 | MLG | H |
|---|---|---|---|---|---|
| BEAUMT | 144 | 144 | 0 | 42 | 3.15 |
| GD-P | 109 | 107 | 2 | 20 | 1.73 |
| GD-SS | 47 | 46 | 1 | 18 | 2.40 |
| GH-SS | 91 | 91 | 0 | 21 | 2.29 |
| GH-P | 112 | 112 | 0 | 29 | 2.56 |
| HS | 132 | 132 | 0 | 37 | 2.84 |
| KAR | 87 | 87 | 0 | 31 | 2.88 |
| KGA | 119 | 119 | 0 | 36 | 2.46 |
| MNK | 48 | 48 | 0 | 12 | 1.74 |
| PTO | 18 | 18 | 0 | 10 | 1.98 |
| PUK | 20 | 20 | 0 | 12 | 2.35 |
| SKG | 15 | 15 | 0 | 8 | 1.86 |
| TAK | 17 | 17 | 0 | 11 | 2.17 |
| TAU | 129 | 129 | 0 | 73 | 3.95 |
| TWA | 16 | 16 | 0 | 6 | 1.12 |
| WAI | 123 | 123 | 0 | 30 | 2.37 |
| WRG | 14 | 14 | 0 | 8 | 1.77 |
| **17** | **1241** | **1238** | **3** | **242** | **3.87** |

## RESULTS AND DISCUSSION

Of the 1241 isolates analyzed for this study, 242 unique multilocus genotypes (MLGs) were detected (19.5%) (Table 1). A total of 1238 of the isolates were identified as Lineage 1, while only 3 isolates corresponded with Lineage 2. The Lineage 2 isolates were all isolated from foliage collected at two adjacent plantations in the northern South Island, GD-SS and GD-P (Table 1, Figure 1). Tauhara (TAU), a site in the central North Island, had the highest diversity of genotypes among all sites (H= 3.95) while the lowest genotypic diversity was found in the Hawkes Bay region of the North Island at TeWaka (TWA) (H= 1.12) (Table 1, Figure 2). Total genotypic diversity was significantly lower for the collection of isolates from New Zealand ($H_{total}$ = 3.87) compared to those from the PNW ($H_{total}$ = 6.09) (Table 1, Figure 2).

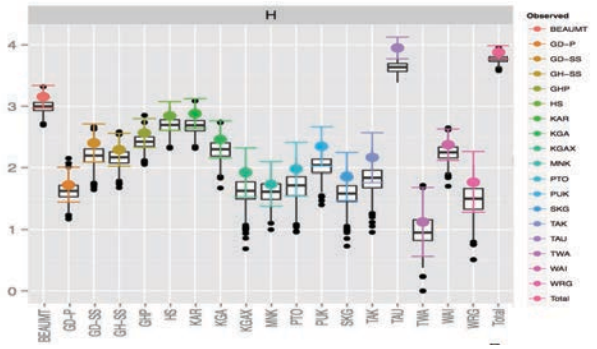



***Figure 2.*** *Shannon-Weiner diversity index calculated for a rarefied sample of 14 isolates from each site. Error bars represent 95% Confidence Intervals. Boxplots represent values obtained from bootstrap replicates.*

A partitioning of molecular variance (AMOVA) within and among sampling sites indicated that most of the variation is found within sites, but the degree to which this estimate reflects the true underlying genotypic variance depended upon whether we chose to maintain the "clones", or repeated MLGs, in the data. When these repeated MLGs were included in the analysis, approximately 78% of the variance occured within sites, and the remaining 22% of the variance was due to among-site differentiation (PhiPT = 0.220, P = 0.001) (Figure 4A). However, when the data was "clone-corrected", the within-site variance was estimated as. 91.4%, and 8.6% of the variance was due to differentiation between sites (PhiPT = 0.086, P = 0.001) (Figure 4B). This result indicated that the apparent strong subpopulation structure was due to the presence of repeated MLGs that arose due to homothallic reproduction and did not reflect the true subpopulation differentiation, which was relatively weak.

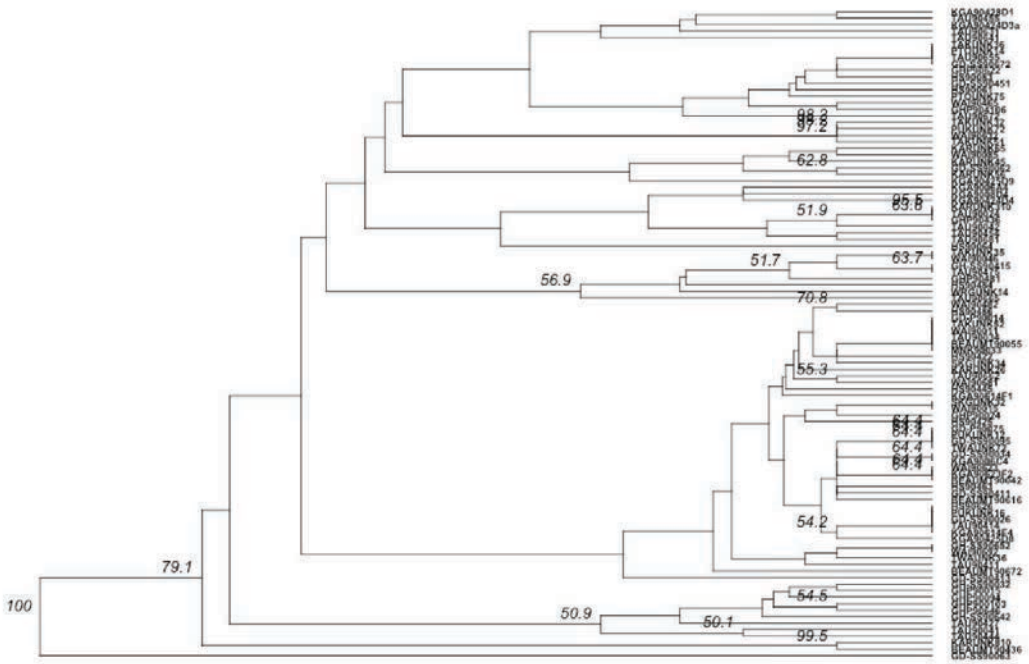
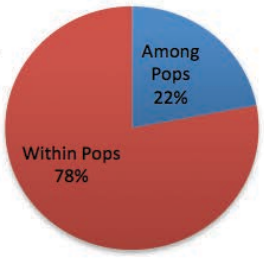



***Figure 3.*** *UPGMA dendrogram constructed with 100 randomly selected isolates from the clone-corrected data set. Node labels represent bootstrap values for 999 replicates. The basal node with bootstrap value of 100 represents the divergence of the two lineages.*

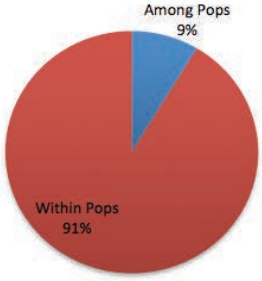
**A**




| Source | df | SS | MS | Est. Var. | % |
|---|---|---|---|---|---|
| Among Pops | 16 | 1255616.082 | 78476.005 | 1052.232 | 22% |
| Within Pops | 1225 | 4560463.170 | 3722.827 | 3722.827 | 78% |
| Total | 1241 | 5816079.253 | | 4775.059 | 100% |

| Stat | Value | P(rand >= data) |
|---|---|---|
| PhiPT | 0.220 | 0.001 |

**B**




| Source | df | SS | MS | Est. Var. | % |
|---|---|---|---|---|---|
| Among Pops | 16 | 135.629 | 8.477 | 0.252 | 8.6% |
| Within Pops | 387 | 1032.864 | 2.669 | 2.669 | 91.4% |
| Total | 403 | 1168.493 | 2.889 | 2.921 | 100% |

| Stat | Value | P(rand >= data) |
|---|---|---|
| PhiPT | 0.086 | 0.001 |

***Figure 4.*** *Analysis of Molecular Variance (AMOVA) comparing genetic variation within and among sampling sites. The P-value is from a randomization test with 999 permutations.* ***A.*** *Repeated multilocus genotypes were included in the AMOVA analysis.* ***B.*** *AMOVA with a "clone-corrected" dataset. (repeated multilocus genotypes were removed).*

A UPGMA analysis resulted in the placement of isolates from diverse sampling sites into groups on the branch tips, indicating the dispersal and admixture of isolates from across the region. There was insufficient variation among genotypes to assign them to genetic clusters by using a K-means algorithm, further supporting the genotypic homogeneity of *P. gaeumannii* populations in New Zealand. Much like the UPGMA dendrograms constructed with isolates from the PNW (Bennett and Stone, this proceedings), the isolates representing two lineages were separated at a basal node with high statistical support (Figure 3).

The DAPC analysis performed using a clone-corrected data set supported the AMOVA results, and confirmed that there is not significant differentiation among the isolates from various sites in New Zealand. The populations of *P. gaeumannii* sampled for this study appear to form a relatively coherent grouping on the DAPC scatterplot (Figure 5) due to shared allelic states.

The abundance of shared multilocus genotypes in populations of *P. gaeumannii* in New Zealand resulted in a highly clonal structure. This likely reflects the predominance of homothallism (self-fertilization) in these populations, as this fungus is not known to reproduce asexually. The homothallic mode of reproduction indicates a diminished capacity for adaptation, as recombination does not occur between dissimilar individuals. Genotypes comprised of alleles indicative of Lineage 2 were recovered at a very low frequency (3 isolates, or approximately 0.2% of the total). These isolates occurred at two adjacent sites on the South Island (GD-P and GD-SS, Table 1). Whether this is a result of random sampling, or indicative of a separate introduction of *P. gaeumannii* from the Pacific Northwest is not clear. There did not seem to be a relationship between genotypic diversity and phylogeography as observed for the native PNW populations.

As a result of predominately homothallic reproduction in these populations, genotypic diversity was very low compared to native *P. gaeumannii* populations in the Pacific Northwest of the United States where outcrossing likely occurs at higher frequencies. This could be the result of a founder event in which a small number of genotypes was initially introduced to a site on the North Island of New Zealand that subsequently spread to populate the North and South Islands. Low genetic diversity may also reduce the capacity for evolutionary adaptation in these populations.

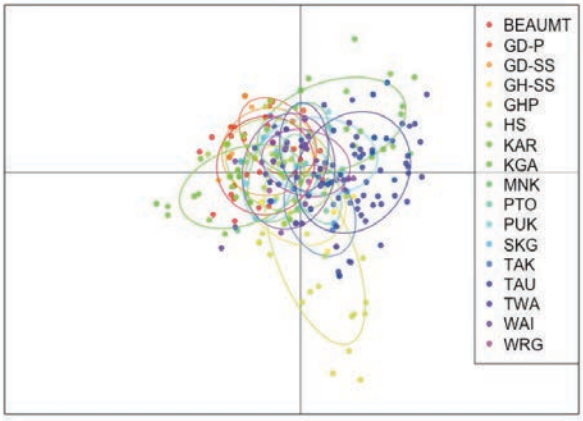



***Figure 5.*** *Discriminant Analysis of Principal Components (DAPC) showing relationships among sampling sites. Colors correspond to the 17 sampling sites, and points correspond to isolates.*

One site, Tauhara (TAU), had much higher genotypic diversity than the others, and is near the site of initial introduction on the North Island, Taupo (Kimberley *et al.* 2011) (Figure 2). Centers of diversity such as this are generally indicative of sites of origin or regional introduction, and could serve as sources of genetic variation on which natural selection may act. There was apparently no correlation between disease severity and genotypic diversity in New Zealand, as the site with the highest genotypic diversity, Tauhara, and that with the least diversity, TeWaka (Figure 4), both grouped among sites which had the greatest abundance of *P. gaeumannii* in Douglas-fir foliage (Stone *et al.* 2007). Furthermore, the sites where isolates of Lineage 2 were recovered, GD-SS and GD-P, had only moderate amounts of *P. gaeumannii* in foliage and little reduction in foliage retention (Stone *et al.* 2007).

The AMOVA result suggests that the distribution of *P. gaeumannii* in New Zealand is not limited by dispersal, and spores move freely among the sites sampled for this study. The apparent differentiation between the sites is reflective of the mode of reproduction, as clone-correction resulted in the negation of this subpopulation structure. The close grouping of isolates from all of the 17 sites on the DAPC biplot further supports the conclusion that these populations, exhibit low diversity, share allelic states, and have a clonal structure.

The continued intensification of SNC in Douglas-fir plantations in this region of the world is likely a complex combination of factors including climate, fungal population dynamics, and host tree seed source genetics. Strategies for SNC disease management in New Zealand may be better informed with knowledge of population structure and genotypic diversity.

## REFERENCES


Hood, I.A. and Kimberley, M.O. 2005. Douglas fir provenance susceptibility to Swiss needle cast in New Zealand. Australasian Plant Pathology 34:57.

Jombart, T. 2008. Adegenet: an R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403-1405.

Kamvar, Z.N., Tabima, J.F., and Grünwald, N.J. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281.

Kimberley, M.O., Hood, I.A., and Knowles, R.L. 2011. Impact of Swiss needle-cast on growth of Douglas-fir. Phytopathology 101:583–593.

Peakall, R. and Smouse P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

Peakall, R. and Smouse P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537-2539.

Stone, J.K., Hood, I.A., Watt, M.S., and Kerrigan, J.L. 2007. Distribution of Swiss needle cast in New Zealand in relation to winter temperature. Australasian Plant Pathology 36:445.

Watt, M.S., Stone, J.K., Hood, I.A., and Palmer, D.J. 2010. Predicting the severity of Swiss needle cast on Douglas-fir under current and future climate in New Zealand. Forest Ecology and Management 260:2232–2240.

Watt, M.S., Stone, J.K., Hood, I.A., and Manning, L.K. 2011. Using a climatic niche model to predict the direct and indirect impacts of climate change on the distribution of Douglas-fir in New Zealand. Global Change Biology 17:3608–3619.