Using a poplar hybrid to investigate genetic control of associating insect and fungal communities

Sandra Simon[1] , Jared LeBoldus[2] , William MacDonald[3] , Wellington Muchero[4] ,
David Macaya-Sanz[1], and Stephen DiFazio[1]

[1]Department of Biology West Virginia University, [2]Department of Botany and Plant Pathology Oregon State University,
[3]Division of Plant and Soil Sciences West Virginia University, [4]Plant Systems Biology Oak Ridge National Laboratory

U.S. DEPARTMENT OF ENERGY
Office of Science

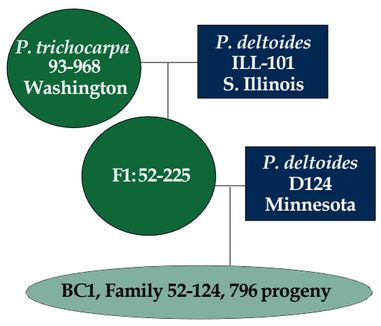
Introduction

In natural ecosystems, the dynamics of plant interactions with other living organisms are complex. To mitigate the effects of biotic attack, plants employ a diverse set of defense mechanisms including chemical, protein-derived molecules, and physical barriers. Insect and fungi must develop strategies in parallel to overcome these obstacles to survive. This relationship can leave lasting impacts on host plant genome structure for example, gene families, such as the Kunitz protease inhibitors (KPIs) and resistance genes (R-genes), have greatly expanded in response to insect and fungal attack through tandem duplication events. The analysis of these patterns is important in studying and understanding the complexities of plant/pest genetic interactions.

Given their rapid growth and vegetative reproduction, *Populus* species have become a focus for research into biofuel production making them a valuable commercial crop. *Populus* has also become an important genetic model for research of forest trees with the completion of the full genome sequencing of *Populus trichocarpa.* Using a pseudo-backcross hybrid family and parental genome comparisons we identify candidate genes important in the associations between *Populus* trees and several common *Populus* diseases and pests.

Experimental Approach

Pseudo-backcross pedigree Family 52-124



A pseudo-backcross family of hybrid poplars was established in two sites; Westport, Oregon and Morgantown, West Virginia.

These plantations were surveyed for a number of biotic pests including symptoms of the fungal pathogens *Melampsora* sp. and *Sphaerulina* sp. as well as the vagabond aphid (*Mordwilkoja vagabunda*), the poplar petiole galling aphid (*Pemphigus populitransversus*), the leaf folding sawfly (*Phyllocolpa* sp.), and the aspen serpentine miner (*Phyllocnistis populiella*).

3,700 single nucleotide polymorphisms (SNPs) were chosen to distinguish between the two species and these loci were genotyped for family 52-124 using an Illumina Infinium Bead Array assay in order to create a genetic map. DNA sequences from 93-968 and ILL-101 were aligned to the Phytozome *P. trichocarpa* and *P. deltoides* reference genomes for parental genome

Phenotypes Scored

Quantitative Trait Loci (QTL) Analysis

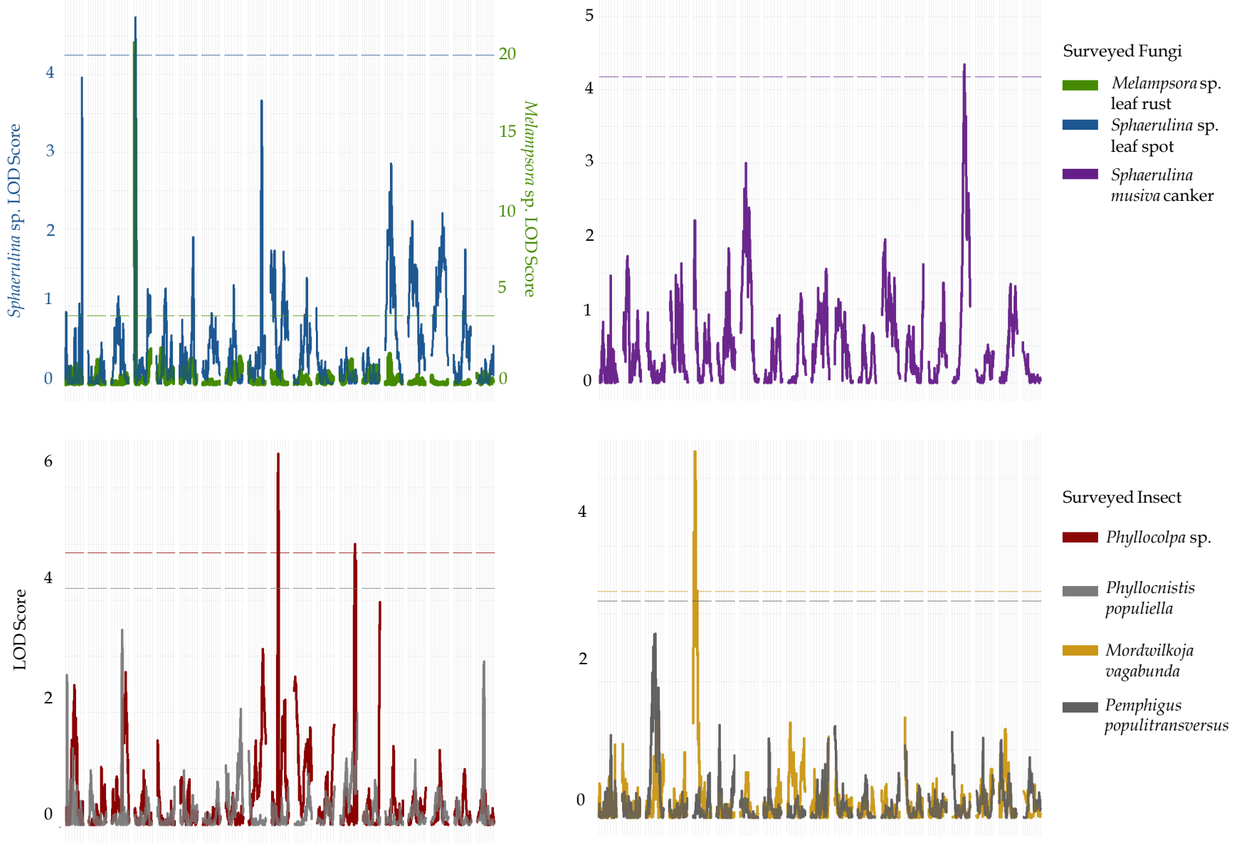
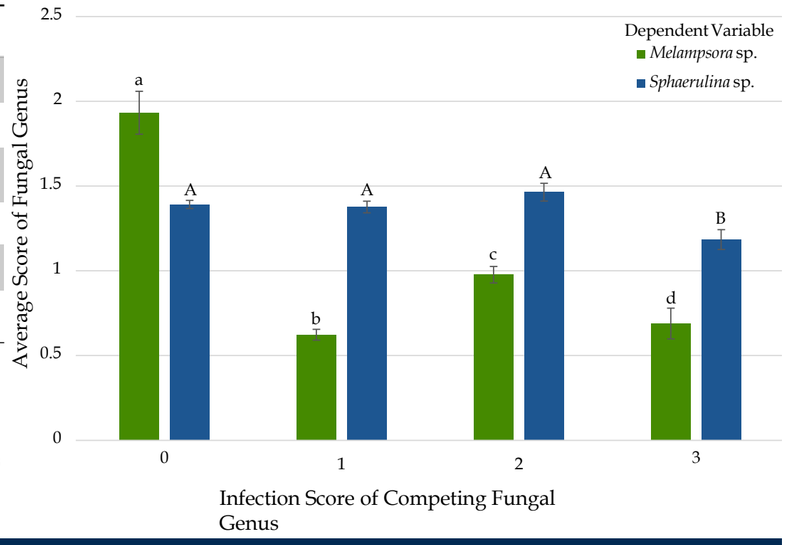


Figure 1. QTL interval plots showing peaks across the genome that associate with biotic surveys. Lines on the plots indicate p-value thresholds as determined by running 1000 permutations of mapping model

Factor	DF	Sum of Squares	Mean Square	F-ratio	p-value
Test #1: Dependent Variable- *Melampsora* sp. leaf rust					
Clone	732	1120	1.53	4.69	<0.001
Sphaerulina sp. score	3	34.4	11.5	35.2	<0.001
Test #2: Dependent Variable- *Sphaerulina* sp. leaf spot					
Clone	732	379	0.518	1.77	<0.001
Melampsora sp. score	3	27.6	9.19	31.5	<0.001

Figure 2. Mean and standard error of fungal infection for individuals with varying category levels of competing fungus infection. Letters indicate significantly different means as determined by Tukey's Honest Significant Difference test. Lower case letters indicate Test #1, upper case letters indicate Test #2.



Parental Genome QTL Interval Structure

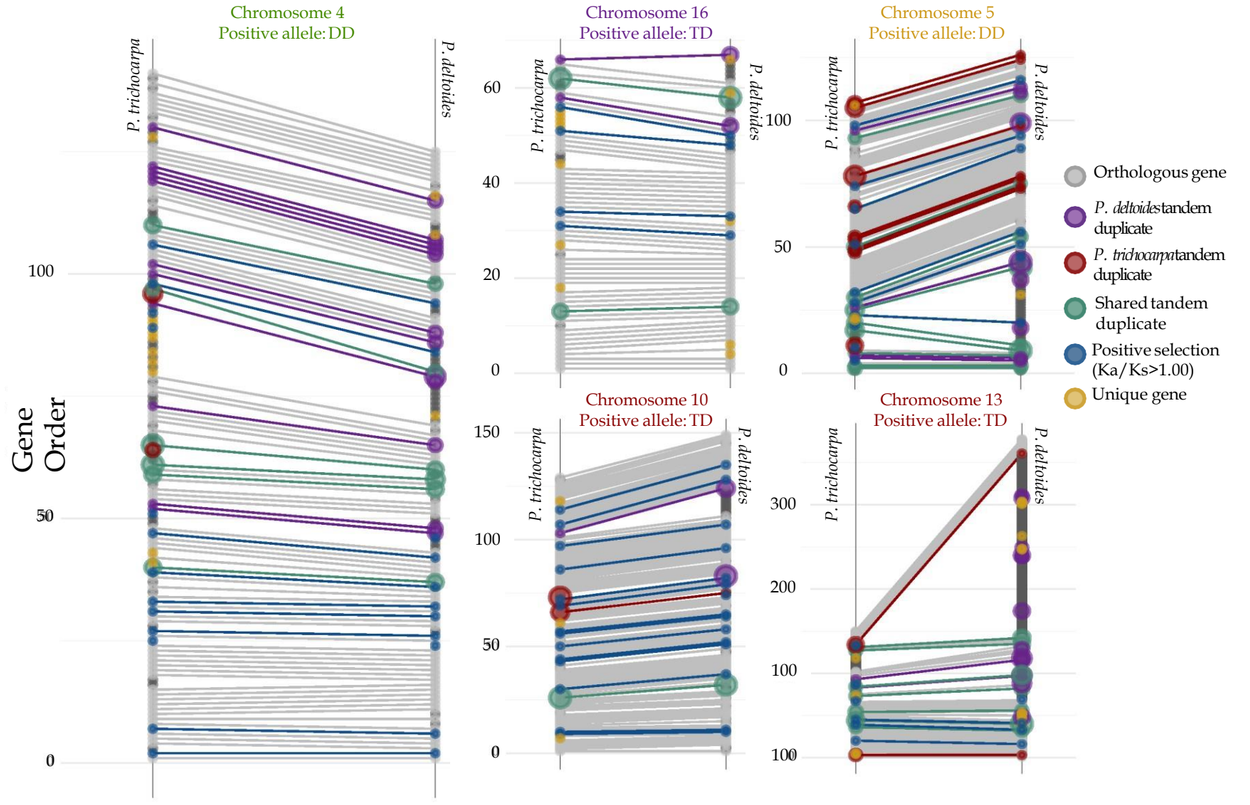


Figure 3. Comparison of gene content in *P. trichocarpa* and *P. deltoides* grandparents for significant genetic intervals. Size of gene point is relative to the number of genes in the tandem duplication expansion. The positive allele indicates genotype at interval that results in an increase in phenotype in the F2 progeny (DD indicates homozygous *P. deltoides* alleles and TD indicates heterozygous *P. deltoides* and *P. trichocarpa* alleles. To see an interactive version of these figures please visit my research webpage (sandraJsimon.weebly.com).

Candidate Genes and Tandem

Antixenosis: Cell wall loosening

Tolerance: Cell death signaling

R-genes: Novel pathways for resistance

Antibiosis: Secondary metabolite production

Susceptibility: Sink strength

Description	Tandem Duplicate Gene Copies *P. trichocarpa; P. deltoides*	QTL Interval Chromosome
Stigma-specific protein, Stig1*	Protein family enrichment (D)	4
Peroxidase superfamily protein	1;2	4
Early-responsive to dehydration stress protein	3; 3	4
Disease resistance protein (TIR-NBS-LRR class)	7;12	5
Cytochrome P450, family 76, subfamily G	5;4	5
Cytochrome P450, family 93, subfamily D	2;0	5
O-methyltransferase	Chromosome 13: 4; 4	13
Sugar Transporter	Protein family enrichment (T)	5, 13
Lipoxygenase; PLAT/LH2 domain	Chromosome 5: 4; 3	5, 10
Copper amine oxidase, enzyme domain*	Chromosome 10: 3; 0	10

* Indicates genes encoding protein type undergoing positive selection (Ka/Ks>1.00). (T)- protein family enriched in *P. trichocarpa* genome interval. (D)- protein family enriched in *P. deltoides* genome interval.

Results and

An overlapping interval on chromosome 4 was found to be associated with the activity of *Melampsora* sp. and *Sphaerulina* sp. fungi (Fig. 1). Both fungal species and their symptoms were surveyed in the same year and an interaction between the two influenced their infection severity on alternate clones (Fig. 2). QTL intervals contained a high abundance of disease resistance genes, secondary metabolite genes, and carbohydrate modifying enzymes with many in tandem duplicate arrays of different sizes in each species (Table 1, Fig. 3). Interestingly both intervals for galling insects contained a high number of genes encoding sugar transporters and the interval for the hemipteran *M. vagabunda* had tandem duplication of resistance genes (R-genes) which have been implicated in fungal pathogen and plant interactions.