USING A POPLAR HYBRID TO INVESTIGATE HOST PLANT GENETIC CONTROL OF ASSOCIATING FUNGAL AND INSECT COMMUNITIES

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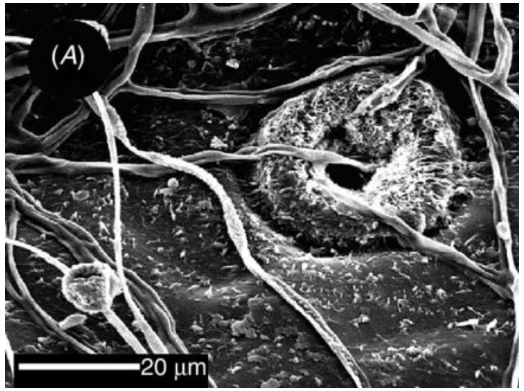
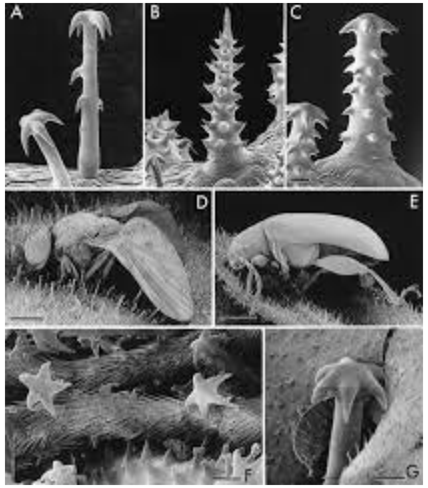
Genetically Based Host Plant Resistance

1. Antixenosis- morphological, physical, and structural barriers
   - Interferes with oviposition/tissue invasion/host-plant recognition
2. Antibiosis- biophysical and biochemical defenses
   - Interferes with pest or pathogen feeding/development
3. Tolerance- ability to withstand and survive attack
   - Support pest or pathogen load
   - Stable yields

Physical Barriers

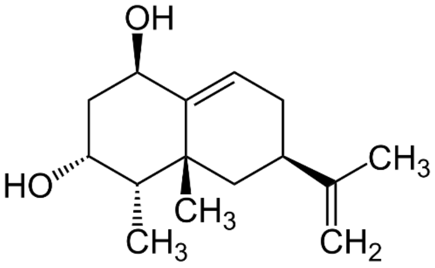


Mohammadian et al. 2009



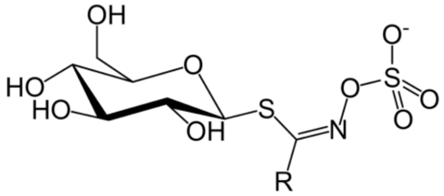
Eisner et al. 1998

Secondary Metabolites



Phytoalexin- Capsidol
Tobacco resistance to water-mold

Glucosinolates
Brassicaceae resistance to insect feeding

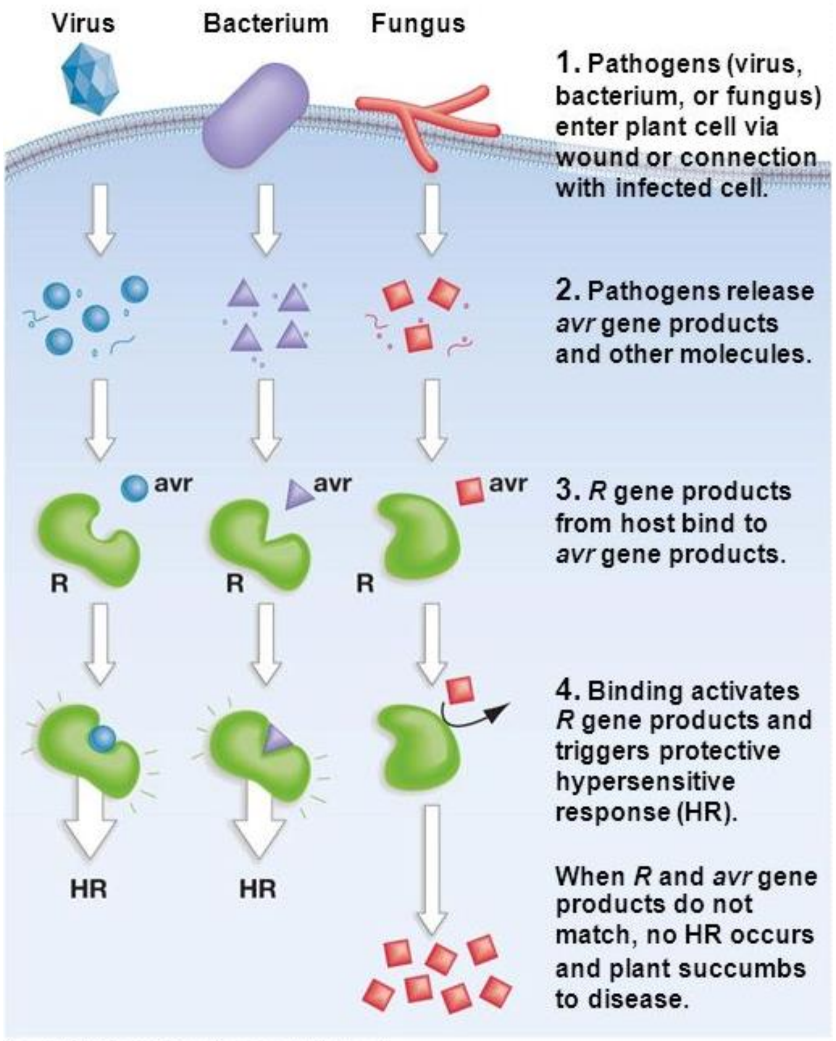


Pathogen/Pest-Host Plant Relationship

Abiotic vs Biotic Stress

Biotic interactions are complex

- Relationship between two genomes
- Both systems are evolving
- Gene-for-Gene Hypothesis
- Host-plant resistance/susceptibility – avirulent/virulent gene in corresponding locus of pest/pathogen
- Resistance is often polygenic



Potential Impacts on Host-Plant Genome Structure

- Diversifying selection pressure on host-plants
- Expansion of genes responsible for resistance
- Tandem-duplications
  - Duplication of exons within the same gene
  - Accumulate mutations leading to new function
  - Increasing genetic diversity for selection
- Examples:
  - Insect-plant relationships: Kunitz trypsin inhibitors (KTIs)
    - Disrupt insect digestion
  - Pathogen-plant relationships: Nucleotide-binding site leucine-rich repeat resistance genes (NBS-LRR R-genes)
    - Signal for hypersensitive response

Forest tent caterpillar *(Malacosoma disstria)*

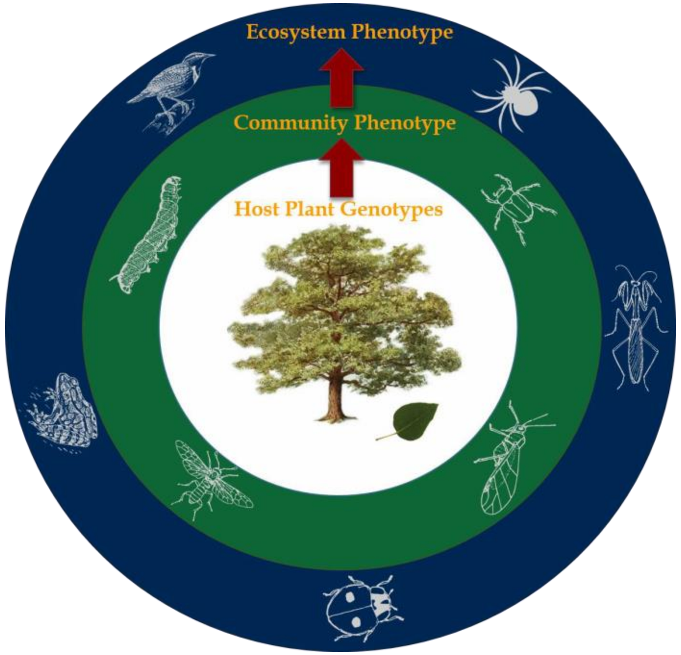
Venturia sp. shoot blight

Melampsora sp. leaf rust

Andrej Kunca, National Forest Centre - Slovakia, Bugwood.org

Significance

- Similarities among genome patterns for pathogen and insect resistance
- Understanding genomic mechanisms of resistance
  - Community genetics- host-plant genetic structure impacts on ecosystem function
  - Co-evolution pathogen/pest and host-plant systems
  - Breeding for resistance



Questions

Objective 1: Identification of loci responsible for genetic control of biotic organisms (hybrid family analysis).

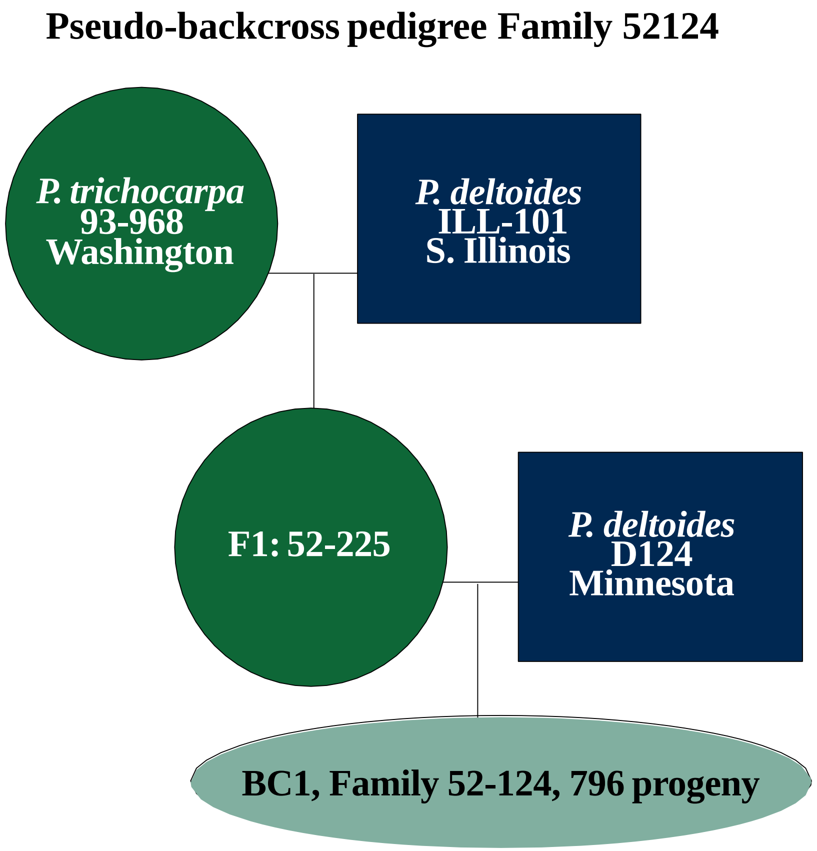
1. Is there heritable, host genetic control of insect and fungal species?

2. What genetic intervals associate with each insect and fungal species?

Objective 2: Identification of candidate genes for resistance through genome comparisons of *P. trichocarpa* and *P. deltoides* parents.

3. What genes and recent tandem duplications are unique in genetic intervals upon comparison of the *P. trichocarpa* and *P. deltoides* genomes?

Plantation Surveys



Hybrid *Populus* Pedigree in Common Gardens

WVU Agronomy Farm

- 2008- *Melampsora* sp. leaf rust and *Sphaerulina* sp. leaf spot
  - 1353 trees (851 unique clones)
- 2016- *Mordwilkoja vagabunda* (Vagabond aphid) and *Pemphigus populitransversus* (Petiole galling aphid)
  - 218 trees (201 unique clones)

Westport, Oregon

- 2017- *Phyllocolpa bozemani* (Leaf-folding sawfly) and *Phyllocnistis populiella* (Aspen serpentine miner
  - 1,020 trees (534 unique clones)

Quantitative Trait Loci (QTL) Mapping

- ~3,500 segregating single nucleotide polymorphic (SNP) markers

Host Plant Genetic Control

Fungus/Insect	H^2	Clone Variance	Error Variance	RLRatio	p-value
Melampsora sp. leaf rust	0.622	0.631	0.382	294	< 0.0001
Sphaerulina sp. leaf spot	0.227	0.099	0.337	32.0	< 0.0001
Phyllocolpa bozemani	0.391	11.9	18.5	129	< 0.0001

Mordwilkoja vagabunda were binary trait; only used in the QTL analysis.





Fungal Pathogens

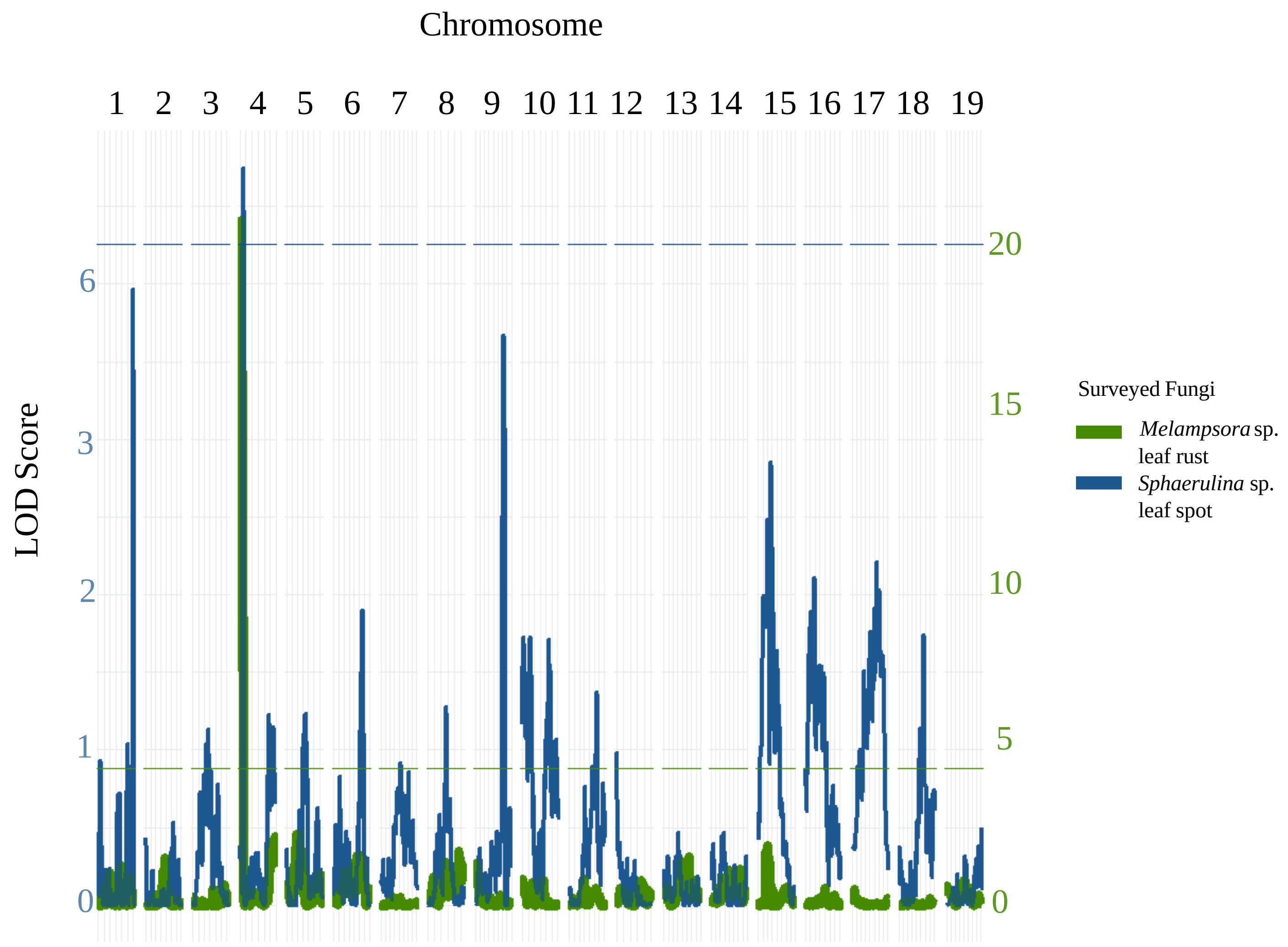
Melampsora sp. leaf rust

- Biotrophic fungi
- Uses poplar and willow tissue to perpetuate spores, intensifying the infection.
- Requires poplar and conifers to complete life-cycle.

Sphaerulina sp. leaf spot

- Necrotrophic fungi
- Dead lesions on leaves
- After infection obtains nutrients from and reproduces in dead host tissue.

QTL Analysis: *Melampsora* sp. leaf rust and *Sphaerulina* sp. leaf spot



Resistance- *P. trichocarpa*
Variance explained by marker- 54.1%
p-value = 0.001

Resistance- *P. trichocarpa*
Variance explained by marker- 3.31%
p-value = 0.026

Insect Pests




Leaf-folding sawfly (*Phyllocolpa bozemani*)

- Early in spring female sawflies sting leaf edge to create a fold.
- One egg is laid and larvae feed on tissue in the fold.

Aspen serpentine miner (*Phyllocnistis populiella*)

- Larvae feed on mesophyll between epidermal tissues of the leaf.

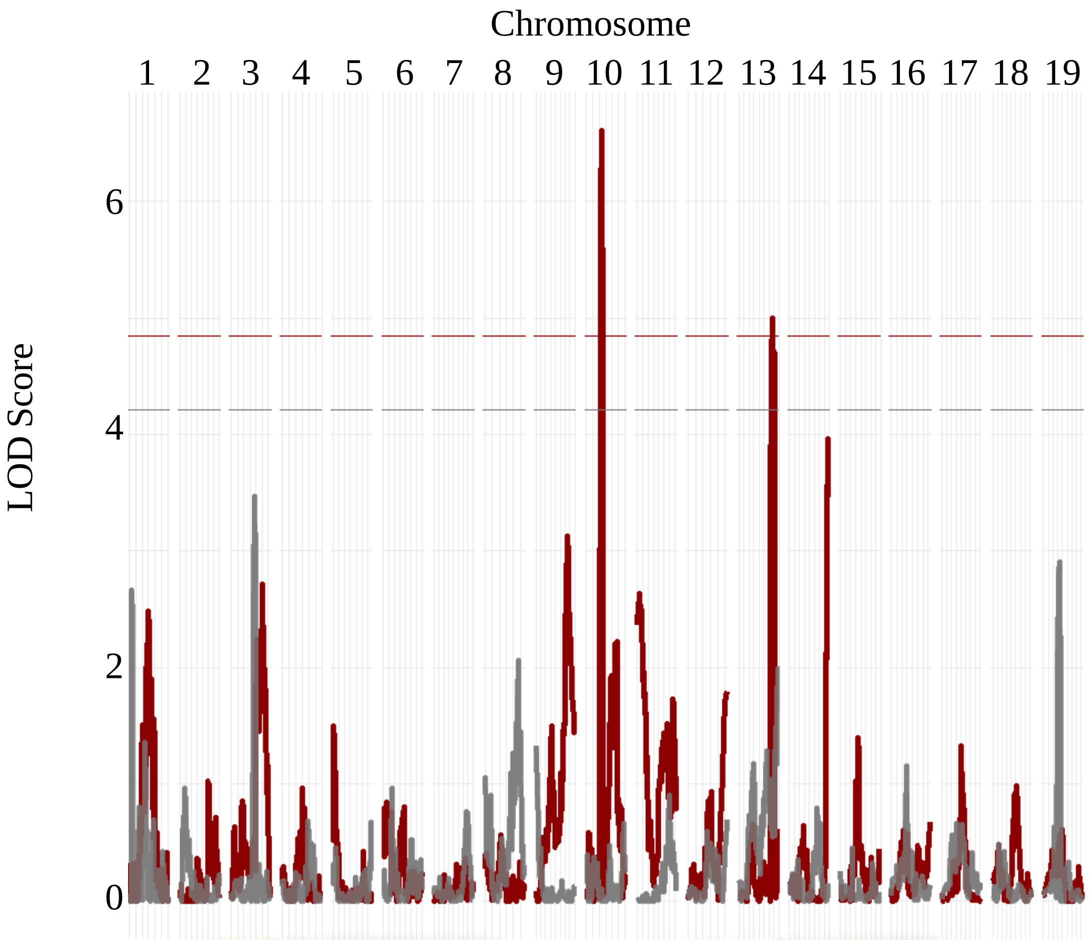
Vagabond aphid (*Mordwilkoja vagabunda*)

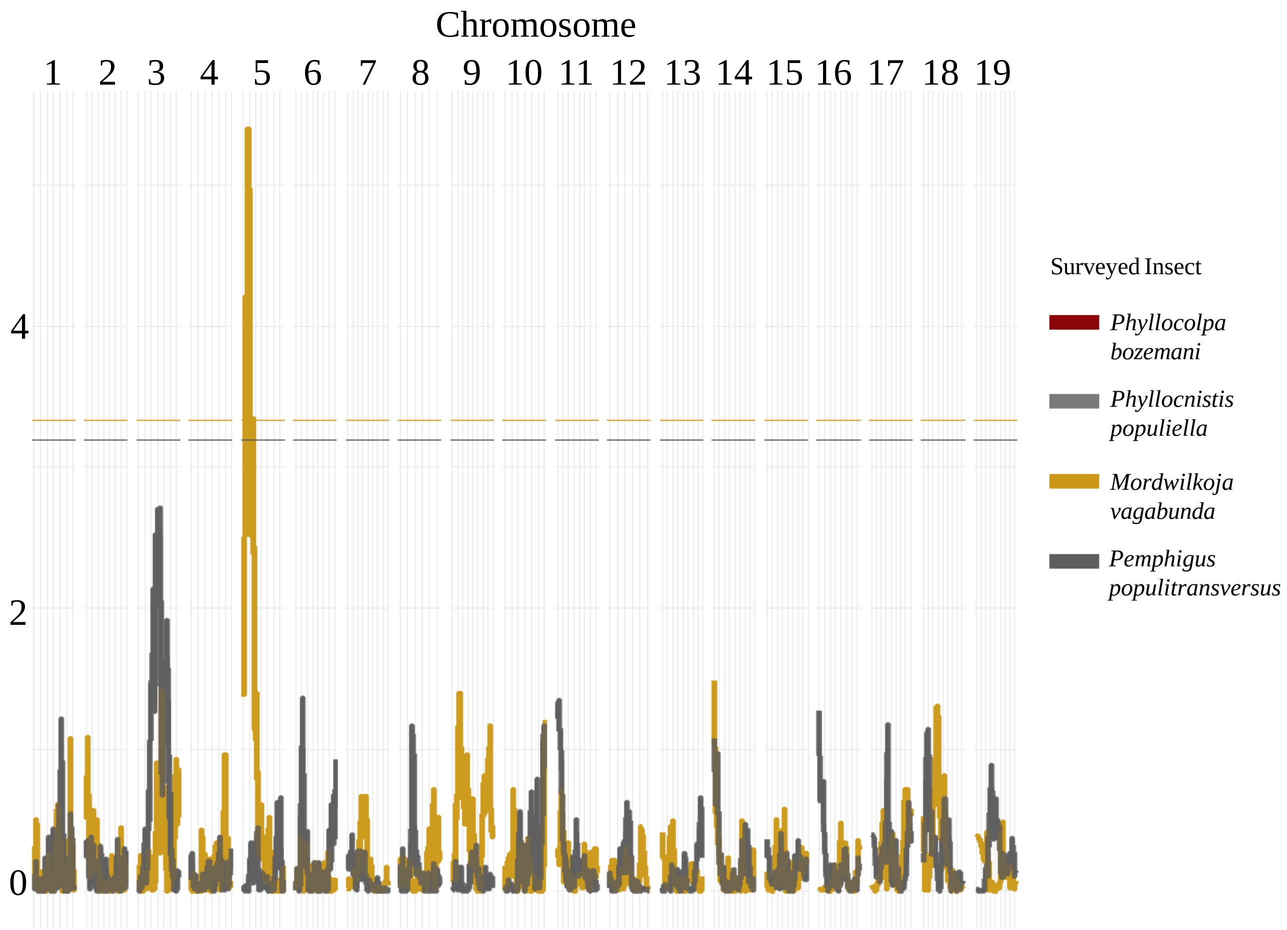
- Aphids feed on terminal buds forming bladder-like galls.

Poplar petiole galling aphid (*Pemphigus populitransversus*)

- Gall formation on petiole to feed internally.
- Reproduce clonally on poplars and sexually on Brassica plants.

QTL Analysis: Insect Surveys





QTL Analysis: Insect Surveys

Leaf-folding sawfly
Chromosome 10 marker:

- Resistance- *P. deltoides*
- Variance explained – 9.65%
- p-value = 0.015

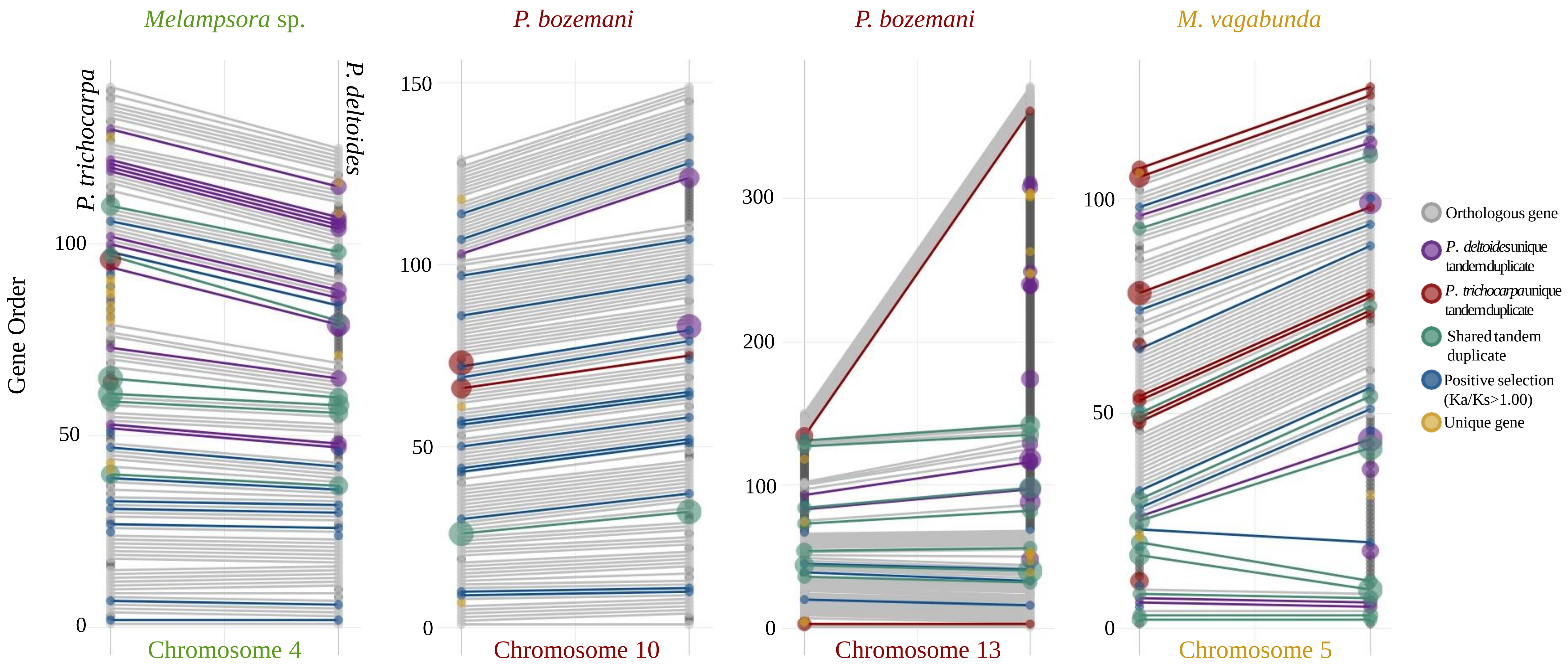
Chromosome 13 marker:

- Resistance- *P. deltoides*
- Variance explained- 8.82%
- p-value = 0.045

Vagabond aphid
Chromosome 5

- Resistance- *P. trichocarpa*
- Presence-absence score
- p-value = 0.001

P. trichocarpa and *P. deltoides* Genome Comparisons



Recent tandem duplications were enriched in biotic intervals relative to the rest of the genome in *P. deltoides* ($p = 0.0118$) but not in *P. trichocarpa* ($p = 0.1191$).

Candidate Gene List

Antixenosis: Lipid transfer protein; cell wall loosening

Tolerance: Cell death signaling; heat-shock; sugar transport

Resistance genes: Novel pathways for resistance

Antibiosis: Secondary metabolite production

Susceptibility: Sink strength

Description	Tandem Duplicate Gene Copies *P. trichocarpa; P. deltoides*	Protein Family	Enrichment p-value	QTL Interval Chromosome
Stigma-specific protein, Stig1*	NA	PF04885 (D)	1.31E-06	4
Peroxidase superfamily protein	1;2	NA	NA	4
Early-responsive to dehydration stress protein	3; 3	NA	NA	4
Disease resistance protein (TIR-NBS-LRR class)	7;12	NA	NA	5
Cytochrome P450, family 76, subfamily G	5;4	NA	NA	5
Cytochrome P450, family 93, subfamily D	2;0	NA	NA	5
O-methyltransferase	Chromosome 13: 4; 4	PF00891 (T)	1.79E-05	13
Sugar Transporter	NA	PF00083 (T)	1.39E-04	5, 13
Lipoxygenase; PLAT/LH2 domain	Chromosome 5: 4; 3	PF00305; PF01477 (T)	1.09E-05	5, 10
Copper amine oxidase, enzyme domain*	Chromosome 10: 3; 0	PF01179 (T)	3.73E-05	10

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

Conclusions

- The hybrid family cross segregated for pathogen and pest symptoms.
  - Identified multiple genetic associations with pathogens and pests.
- Recent tandem duplications appeared to be characteristic of genetic intervals that associate with biotic stressors.
- Identified multiple mechanisms of host plant resistance both within and among genetic intervals.
- Identified similar mechanisms of resistance and susceptibility in genetic intervals that associate with different insect pests.

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University of Wisconsin- Keefover-Ring Lab

Greenwood Resources

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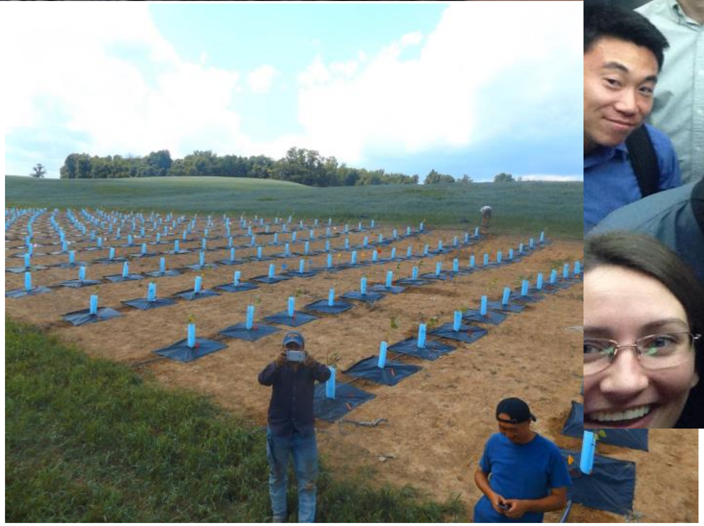

Questions?