Insect Interactions in the Salicaceae

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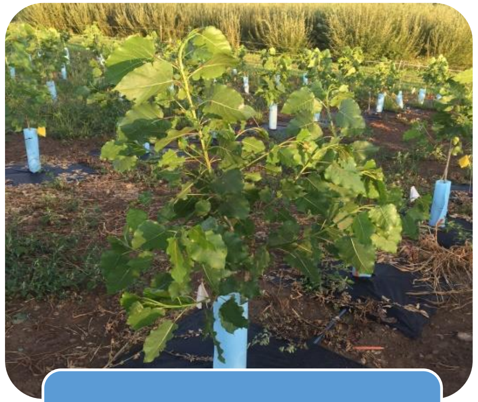
Doctoral Research Proposal

Why care about bugs

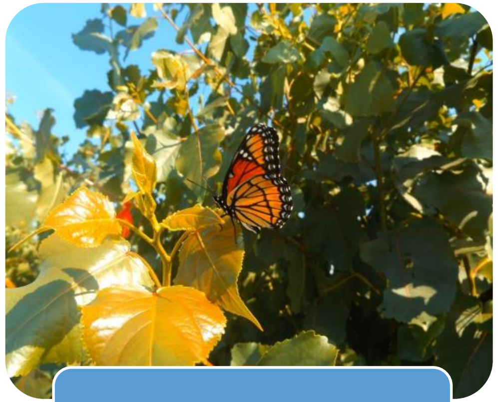
- Pollinating insects
  - 80% wild plants and 75% food crops rely on pollinators for sexual reproduction
  - $3 billion ecological services such as gardening, education, aesthetic enjoyment
- Insect herbivores
  - Destruction 6 million acres of forest trees in 2015 (USDA)
  - 16-18% all agricultural losses and $12.5 billion pesticide management in the US in 2006
- How do insects locate a potential host?

Host plant selection

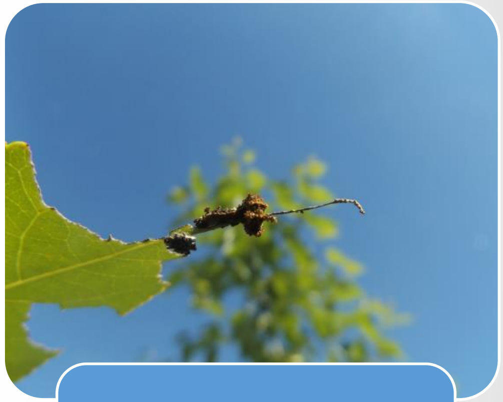
Habitat location



Host location



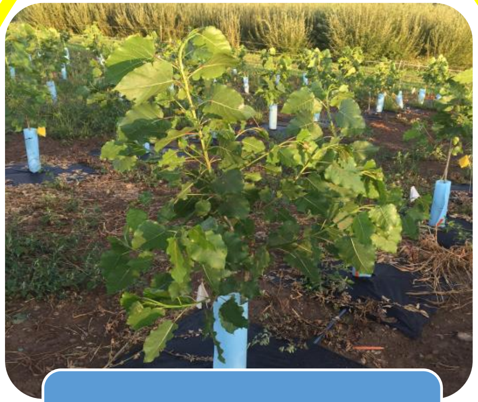
Host acceptance



Pollination/ Oviposition/ Feeding

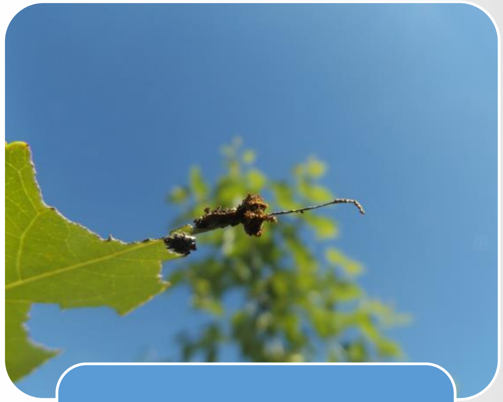
Host plant selection

Habitat location



Host location

Host acceptance



Pollination/ Oviposition/ Feeding

Host plant location

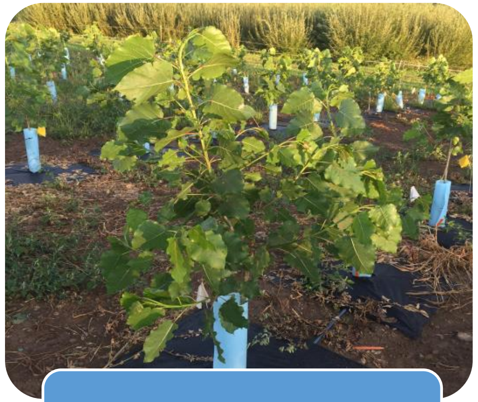
- How does an insect locate a host plant prior to visual stimuli?
  - More likely to "smell" plant before seeing it
- Plant volatiles
  - Chemical compounds that disperse in the air at ambient temperatures
  - Pollinating insects
    - Flower volatile compounds such as terpenoids, benzenoids, and fatty acid derivatives
  - Insects herbivores
    - Brassicaceous plants produce isothiocyanates, volatile catabolites of the glucosinolates

Cabbage aphid

Cabbage seed weevil

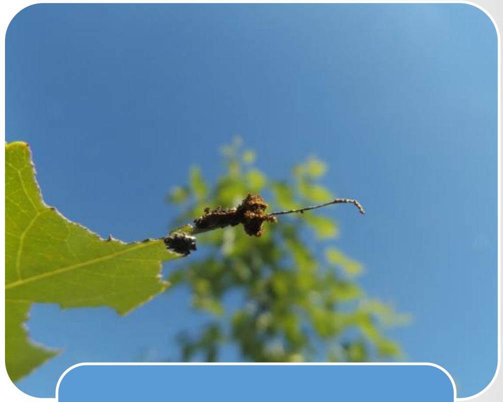
Host plant selection

Habitat location



Host location

Host acceptance



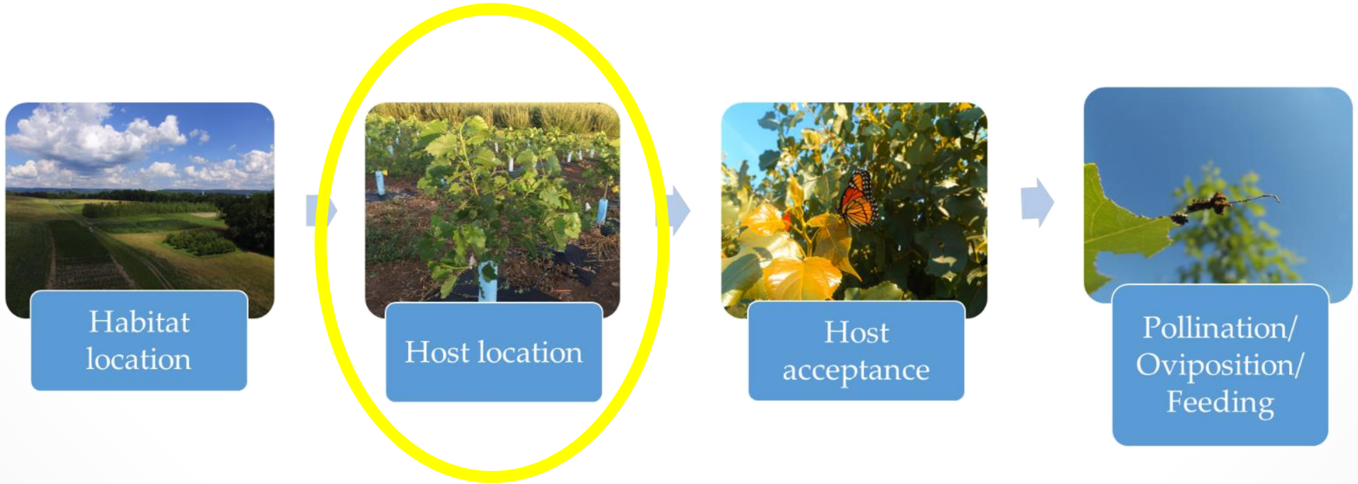
Pollination/ Oviposition/ Feeding

Host plant acceptance

- Testing plant suitability for offspring
  - Palatability, tissue toughness, nutritional quality, secondary metabolites, and rewards
- Require olfactory and visual stimuli
- Pollinating insects
  - Pollen and nectar quality
- Herbivore insects
  - Abdominal drumming
  - Probing with mouthparts
  - Chemoreceptors on legs
- Volatiles and tissue quality are genetically variable

Insect interactions in the Salicaceae

Objective one: Classifying the pollinator community and investigating the attraction of floral visitors in the dioecious willow species *Salix nigra* (Black Willow)



Genus *Salix*

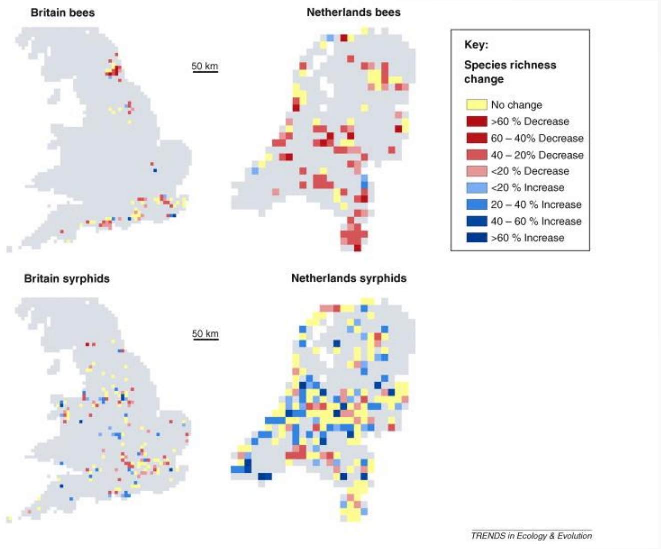
- Around 400 species of dioecious trees and shrubs
- Most species flower between April-June
- Non-showy catkin flowers
- Exhibits both wind and insect pollination
- Insect pollinated *Salix* provide early season resources for pollinators
  - Important in supporting communities that also pollinate agricultural systems

Salix nigra

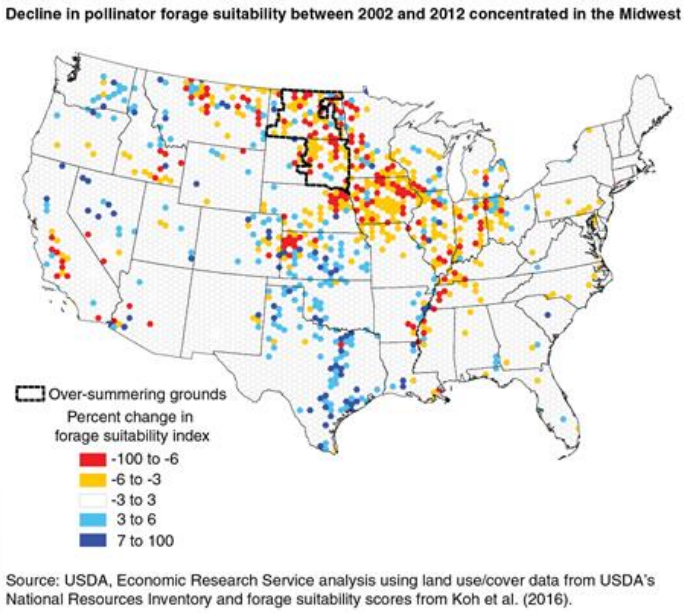
- Tree form willow
- Individuals bloom in early May
- Reproduces both sexually and asexually (delicate branchlets)
- Sexual productivity is completely determined by insect pollinators
- Volatile scents are different among male and female individuals (Collaborator Dr. Ken Keefover-Ring)

Global declines of pollinating insects

- Climate change, disease, and habitat loss
- *Apis melifera*, *Bombyx* sp., and Syrphid flies
- Surveys are needed to study distributions and evaluate declines
- Traditional methods of surveying insects
  - Visual surveys and sweep netting
  - Passive methods; sticky, pan, pitfall traps
  - Time consuming and often biased in species captured
  - Expert identification and keying also time consuming
- High-throughput methods required



Potts et al., 2010

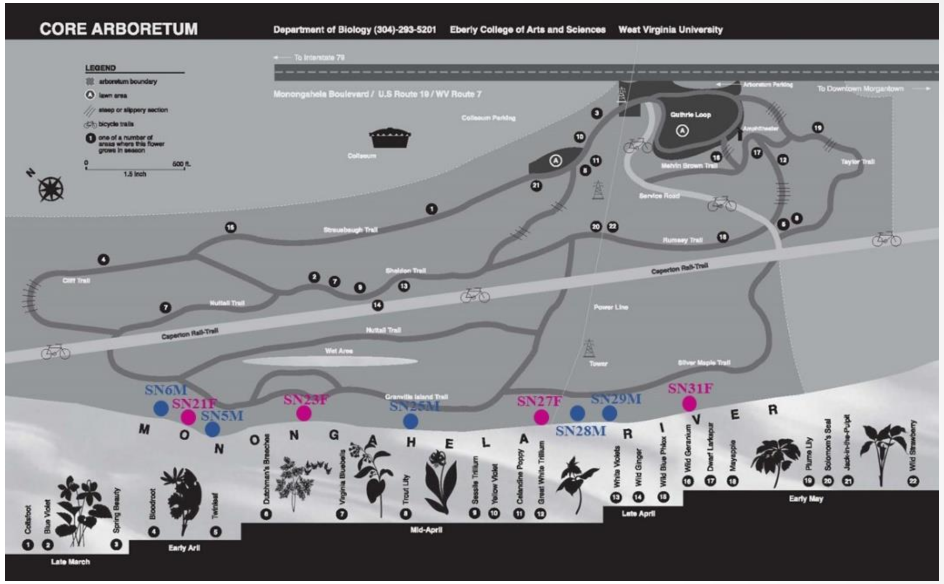


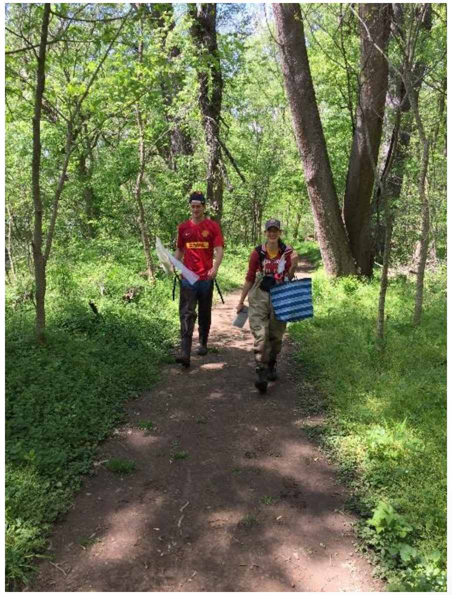
Question one

What is the community composition of floral visitors of *S. nigra*?

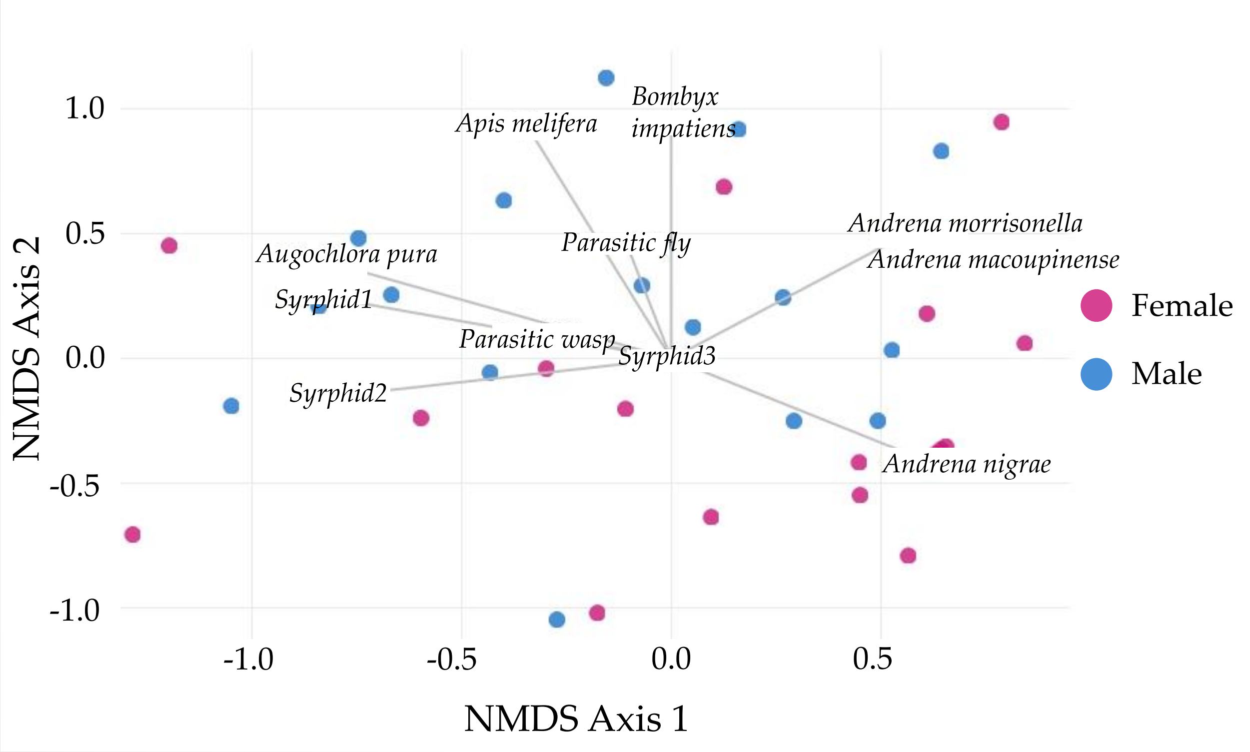
Site description and surveys

- *S. nigra* population Core Arboretum
- Branches were flagged on trees containing 300 catkins
  - Timed observation with number of different insects recorded (16 minutes)
  - Visual surveys were performed on sunny days throughout 2 week bloom (2017-2018)
  - Avoided days with heavy wind and rain to maximize observation of small floral visitors
  - Order of surveyed trees randomized for each day
  - Specimen collection was done using gentle sweep netting





Community data analysis



Grouping	ANOSIM R-Value	p-value
Tree	0.321	0.001
Sex	0.114	0.015
Survey Day	-0.005	0.482

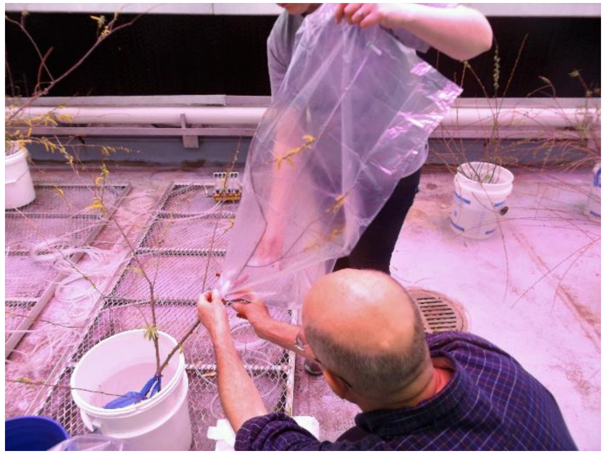
- 2017 data: high number of pollen predators attracted to male trees
- *Andrena* spp. cross pollinating
  - *Andrena morrisonella*
  - *Andrena nigrae* (state record)
  - *Andrena macoupinense* (state record)
- Floral visitor communities were unique among male and female trees as well as among individuals

Question two

How are volatile profiles influencing the community of floral visitors and individual pollinators?

Flower volatile collection

- Dormant branches collected from the field
- Flowered in buckets in the greenhouse
- Volatiles collected using the dynamic headspace method (Keefover-Ring, 2013)
  - Oven bags placed over branch and connected to flow meter and pump
  - Collected over three hours, chemical traps collected and rinsed with hexane
  - Flowers collected, freeze dried
  - All samples sent for analysis at the University of Wisconsin-Madison by the lab of Dr. Ken Keefover-Ring



Hand pollination

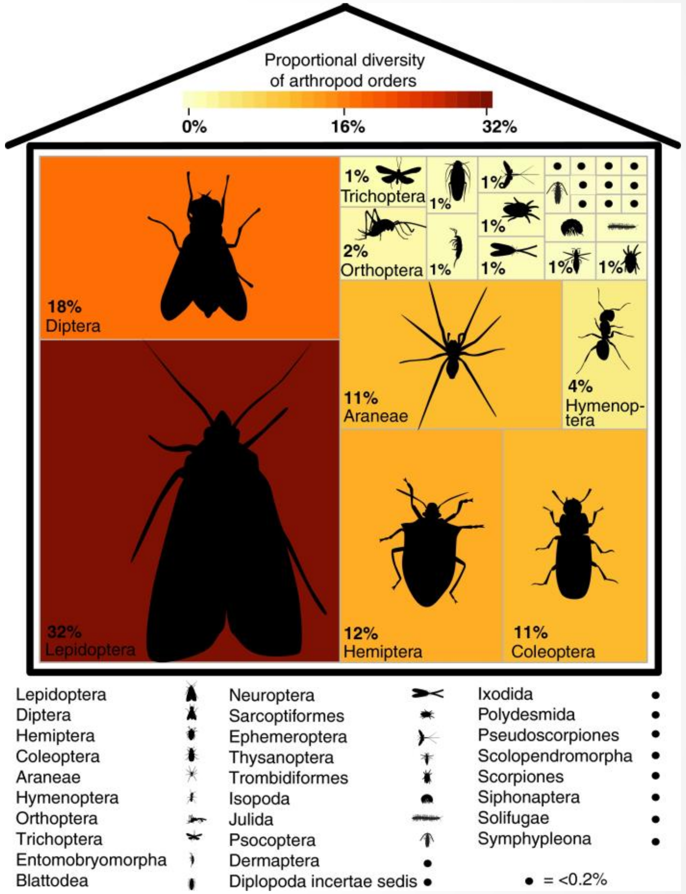
No hand pollination

Question three

How do molecular surveys compare to visual and net specimen collections?

Surveys with DNA barcoding

- Isolate insect DNA traces from floral tissue
- DNA barcoding
  - Target conserved genes with barcoded primers
  - Cytochrome c oxidase subunit 1 gene (Cox1/Co1/COI) in the mitochondrial genome
  - Sufficiently variable to identify taxonomic genus and species level
  - GenBank database contains 70,000 mitochondria sequences for hexapods
- Technique
  - Identify insects in animal diets
  - Insect communities in homes
  - Characterize soil arthropod communities



Madden et al., 2016

DNA barcoding

- Catkins from surveyed branches were gathered and frozen for 2017 and 2018 surveys
- DNA isolation and barcoding
  - Modified CTAB protocol to isolate insect DNA traces from flowers.
  - PCR amplify cytochrome c oxidase subunit 1 gene.
  - Sequence barcoded samples on Illumina MiSeq
- Analyze sequences using GenBank and comparisons to DNA sequences from collected specimens

Insect interactions in the Salicaceae

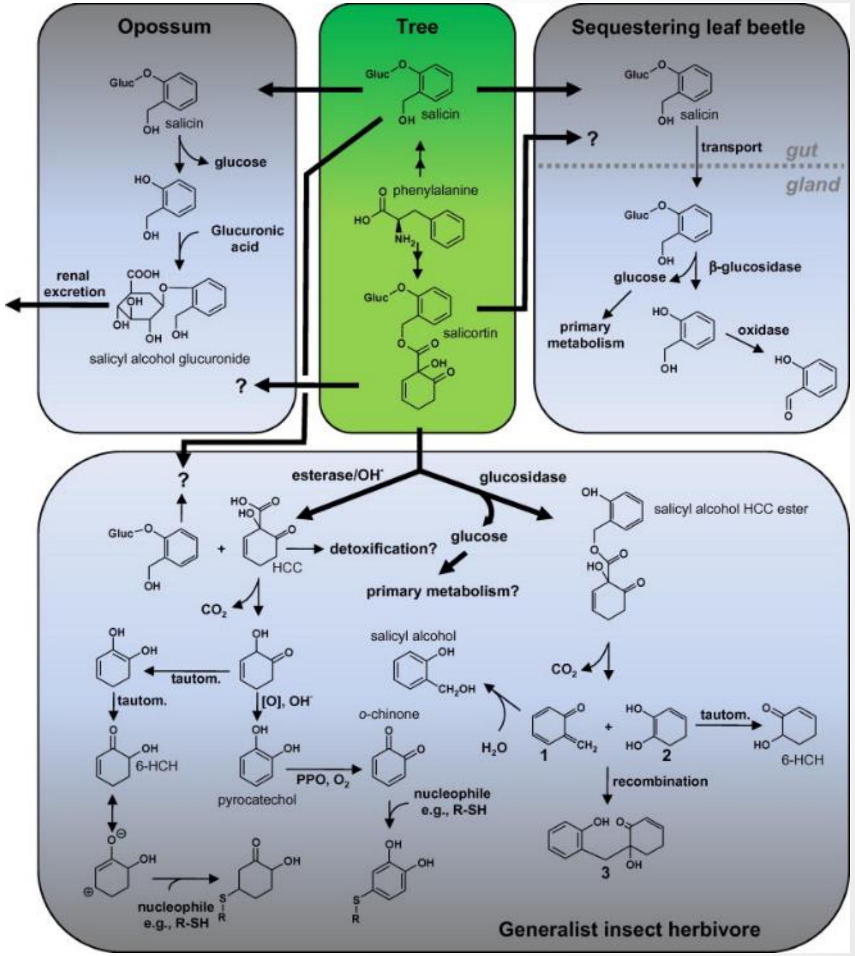
Objective two: Investigating the genetic mechanisms associated with *Phyllocolpa bozemani* oviposition preference in a hybrid *Populus* and a diverse collection of *Populus trichocarpa* (Black Cottonwood)

Populus as a model

- Forest tree genetic model
  - Resistance is often polygenic
  - Relationship between two evolving genomes
  - Full genome sequences available for *P. trichocarpa* and *P. deltoides*
  - Help find candidate genes mediating host-plant and insect interactions
- Commercial value
  - Research interest in production of biofuels
  - Breeding for biomass characteristics
  - Potential tradeoffs with production of secondary metabolites
  - High biomass may leave trees less defended

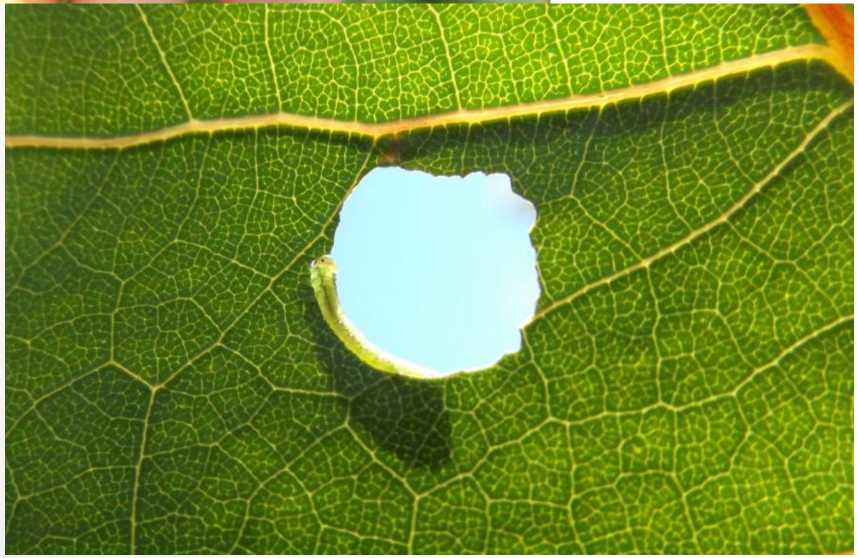
Secondary metabolites (SMs) in *Populus*

- Phenolics- plentiful secondary metabolites
  - Phenolic glycosides (PGs) and condensed tannins (CTs)
  - Highly variable among species
  - Within species levels differ by tissue type, sex, and expression
- Deterrent to generalist insects and mammals
  - Often inhibit insect development and reproduction
- Attractant of specialist insects.
  - Some species, such as *Chrysomela scripta,* transform SMs for their own protection
  - Possible ovipositional/feeding stimulant



Boeckler et al., 2011

Phyllocolpa bozemani

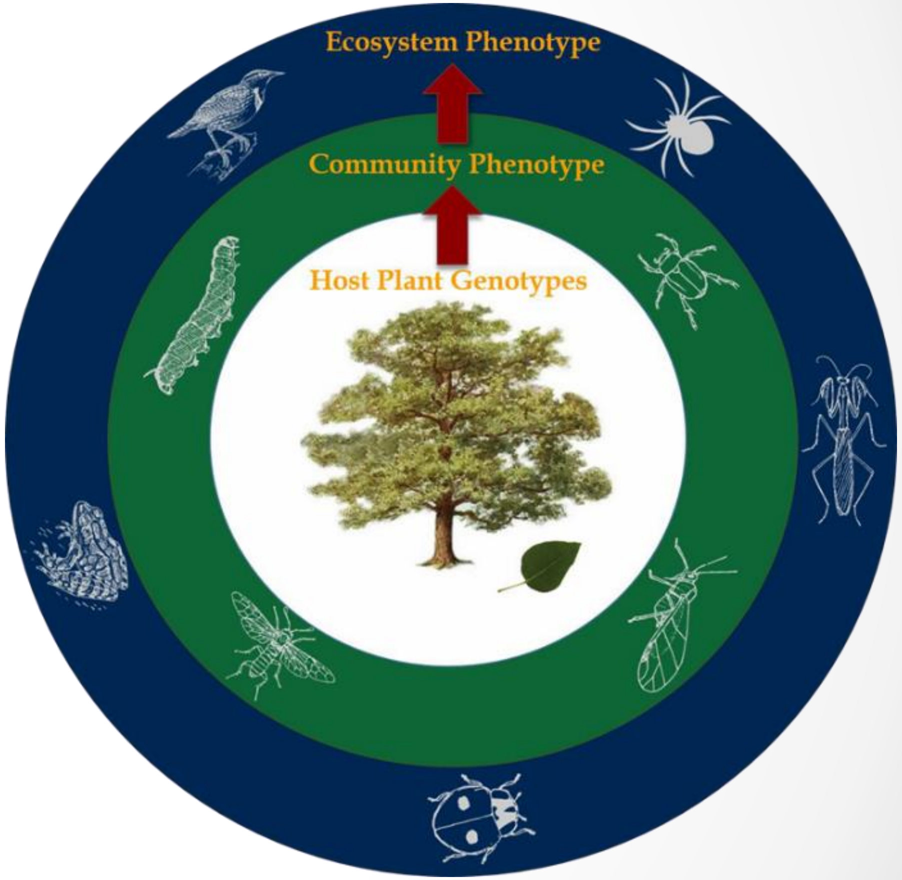


- *Phyllocolpa* spp.
  - Specialist of *Populus* and *Salix*
  - Female sawflies inject leaf edge with small amounts of fluid
  - Leaf edge swells and creates a fold where a single egg is laid
  - Larvae feed on tissue in fold
- Leaf folds create habitat for a variety of insect species
  - Earwigs, caterpillars, ant tended aphids
  - Arthropod species richness and abundance increase with presence of *Phyllocolpa* sp. leaf-folds in Aspen forests
  - Potential impacts on community structure

Bailey, J. K., & Whitham, T. G. (2006). Biodiversity is related to indirect interactions among species of large effect. Indirect Interactions Webs: Nontrophic Linkages through Induced Plant Traits. https://doi.org/10.1017/CBO9780511542701.014

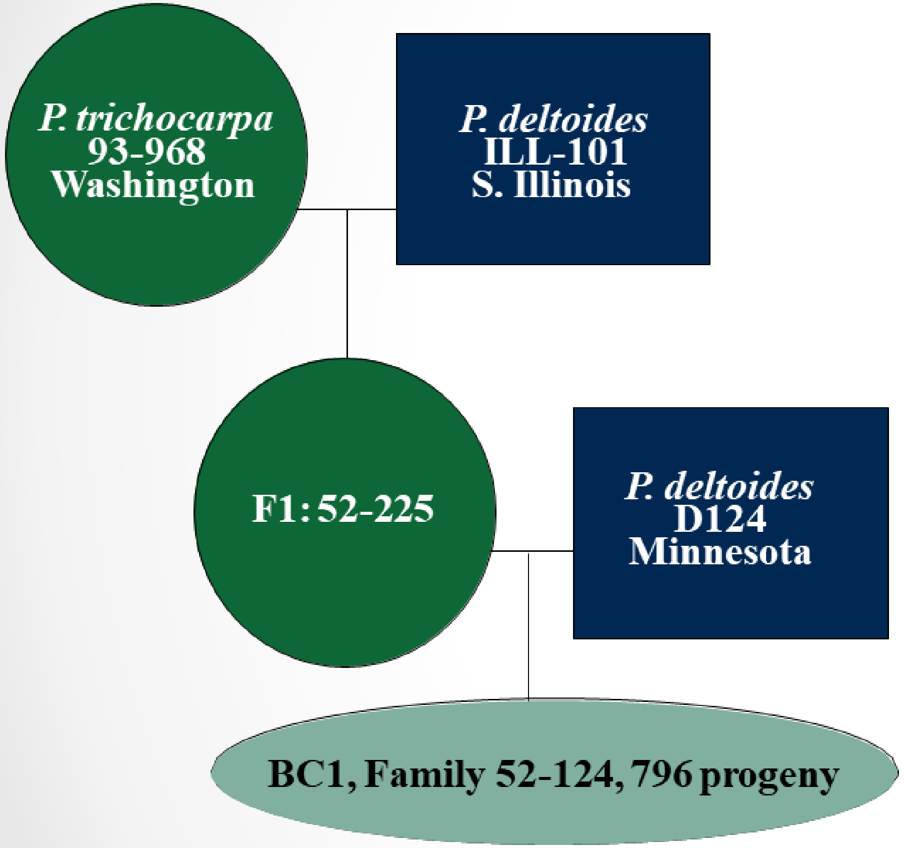
Parental species and hybrids

- Many community studies have focused on natural hybridizing ranges in *Populus*
  - Hybrids tend to show more significant responses to biotic community interactions
  - Hybrids are rarely more resistant to insects than their parents
  - Hybrids may differently express secondary metabolites
- Not necessarily reflective of the parental species populations
  - Hybrids can be used as a tool to identify compounds of interest
  - Variation of SMs in parental populations may have a similar impact on insect distributions

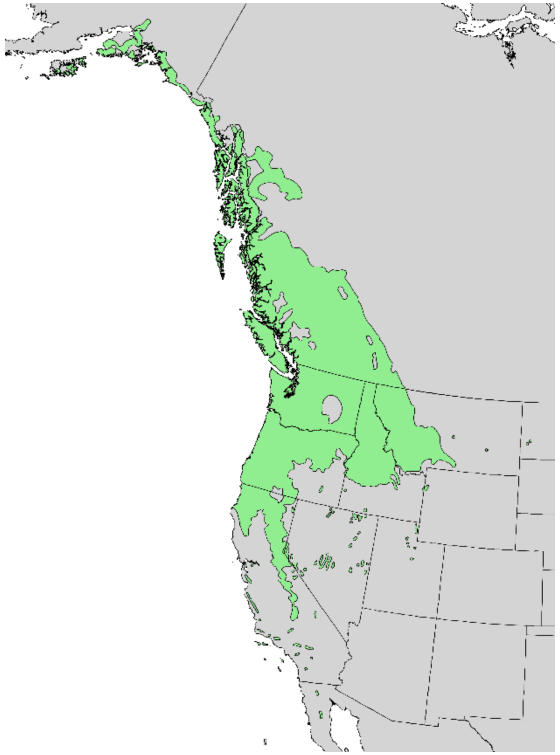


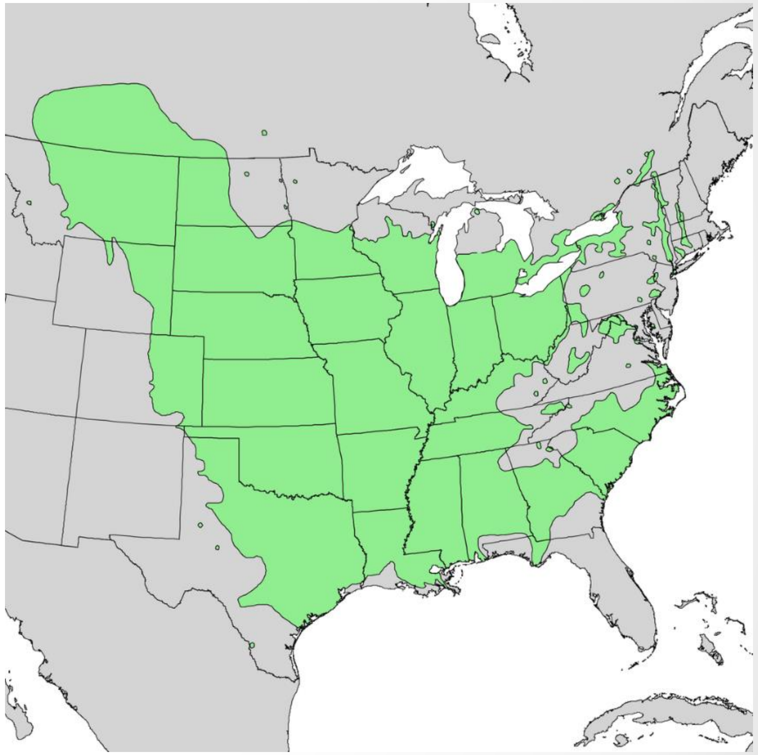
Cheng, D., Vrieling, K., & Klinkhamer, P. G. L. (2011). The effect of hybridization on secondary metabolites and herbivore resistance: implications for the evolution of chemical diversity in plants. Phytochemistry Reviews, 10(1), 107–117.

52-124 pseudo-backcross pedigree



P. trichocarpa range



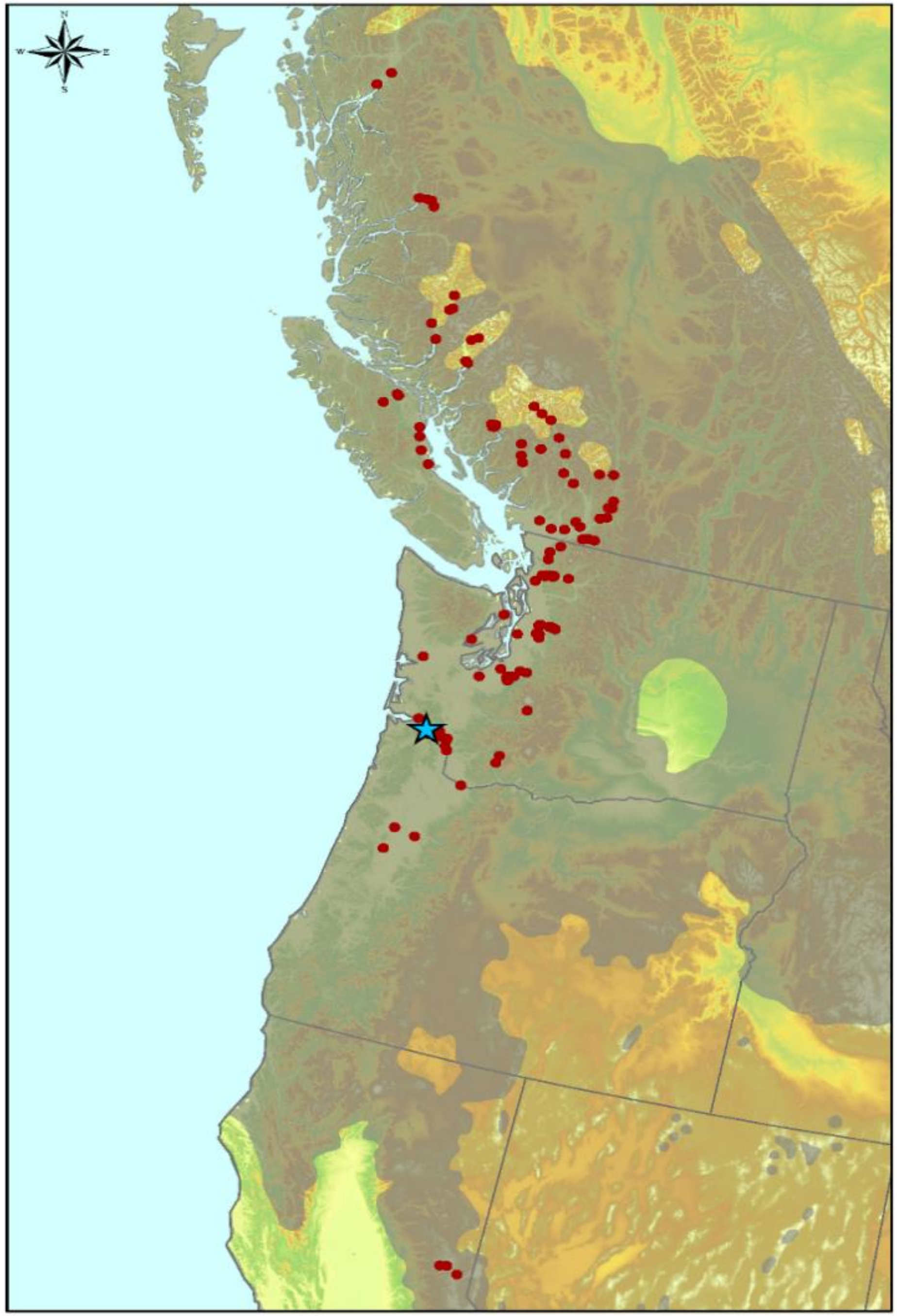


P. deltoides range

Hybrid family and diverse collection of *P. trichocarpa*

- 52124 pseudo-backcross hybrid plantation
  - Westport, Oregon
  - 339 progeny replicated in randomized three block design

- *P. trichocarpa* plantation
  - Clatskanie, Oregon
  - 1,100 *P. trichocarpa* genotypes collected from across the species range
  - Replicated in randomized three block design



Question one

Is *P. bozemani* oviposition choice heritable in the hybrid *Populus* family and the diverse collection of *P. trichocarpa*?

P. bozemani surveys

- 52124 pseudo-backcross hybrid site
  - July 2017; tree canopies were counted for number of *P. bozemani* leaf folds
- *P. trichocarpa* plantation
  - July 2012; five branches of equal biomass were surveyed for total number of *P. bozemani* leaf folds

Heritability

Site	H^2	p-value
Westport (n=333) Hybrid *Populus*	0.391	<0.0001
Clatskanie (n=39) *P. trichocarpa*	0.150	0.0819

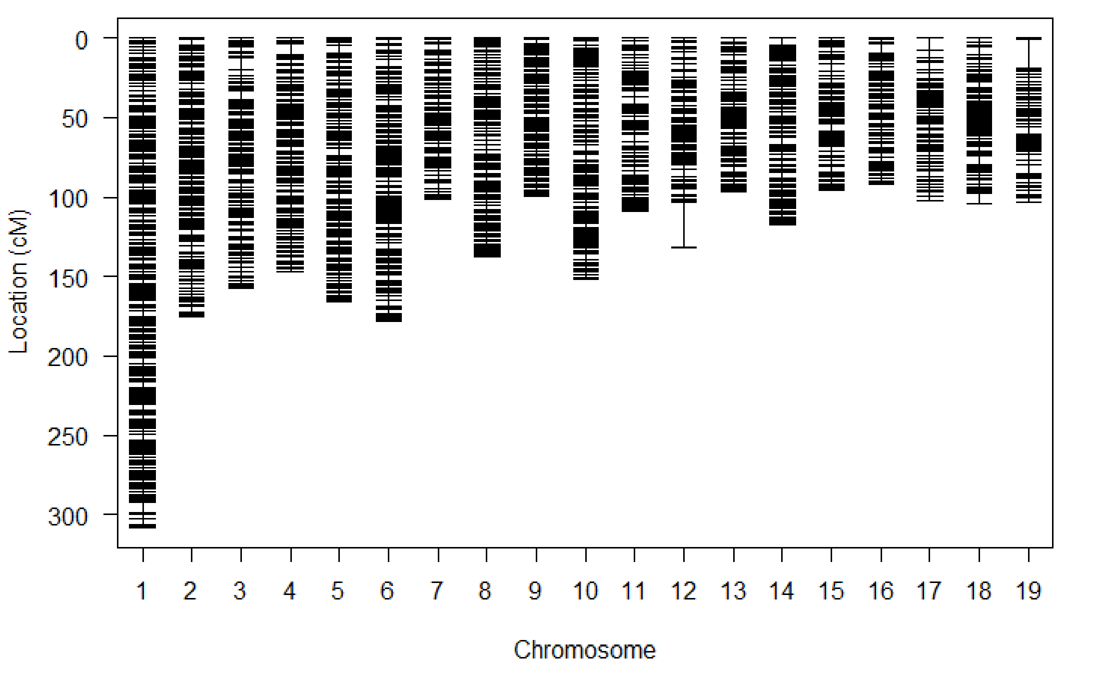
- H^2; contribution of tree genetics to variation in number of leaf folds
- Strong heritability in hybrids and weak heritability in *P. trichocarpa*
    - Hybrid site showed high segregation of sawfly oviposition

Question two

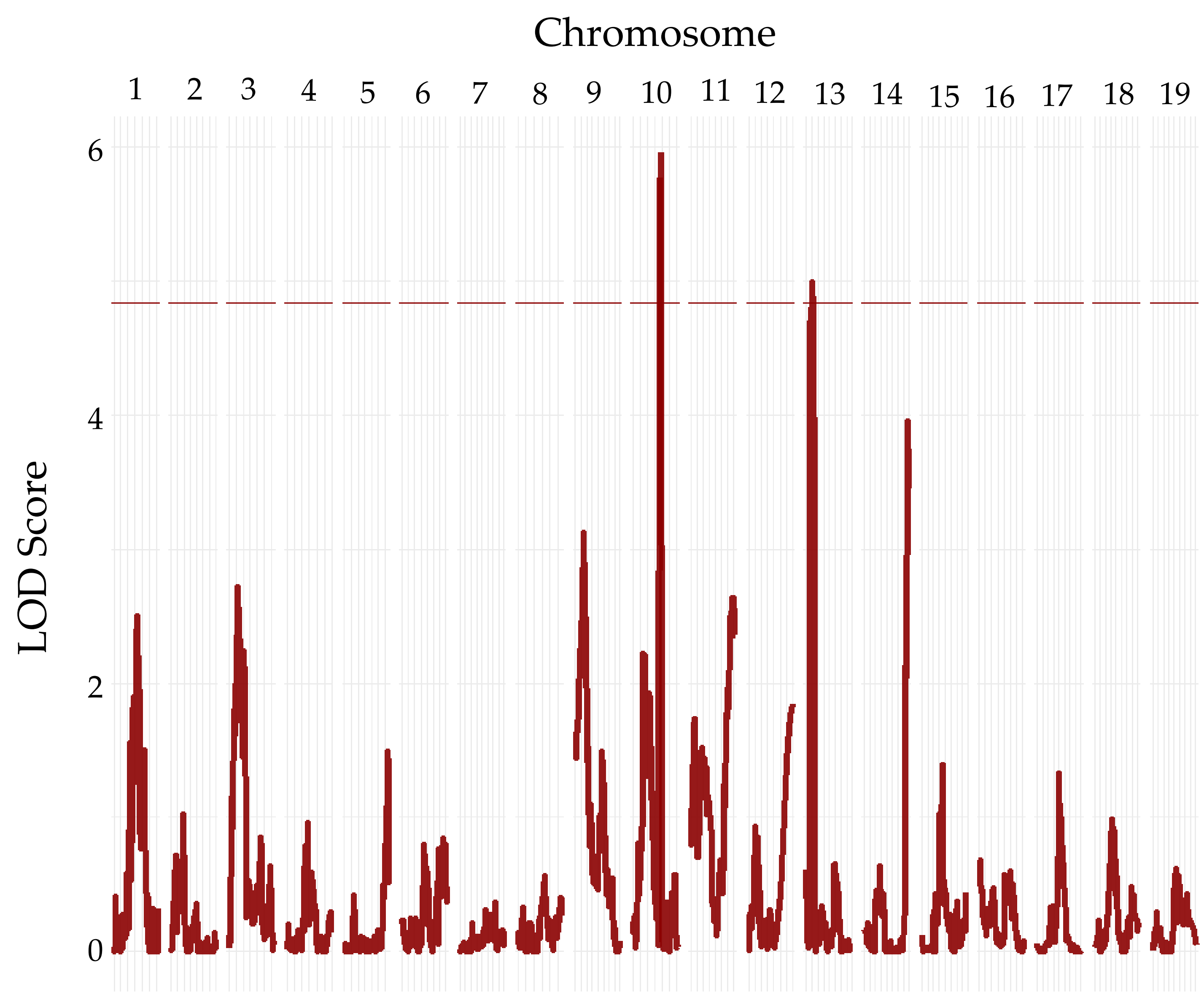
What regions of the genome associate with the leaf folding activity of *P. bozemani*?

Hybrid genetic map

- 52124 Hybrid Family
  - Species specific markers
  - Illumina Infinium Bead Array with 3,568 segregating loci
  - Identify which regions of the genome were inherited from *P. trichocarpa* parent and *P. deltoides* parent
  - Quantitative trait loci analysis (QTL)



P. bozemani QTL analysis



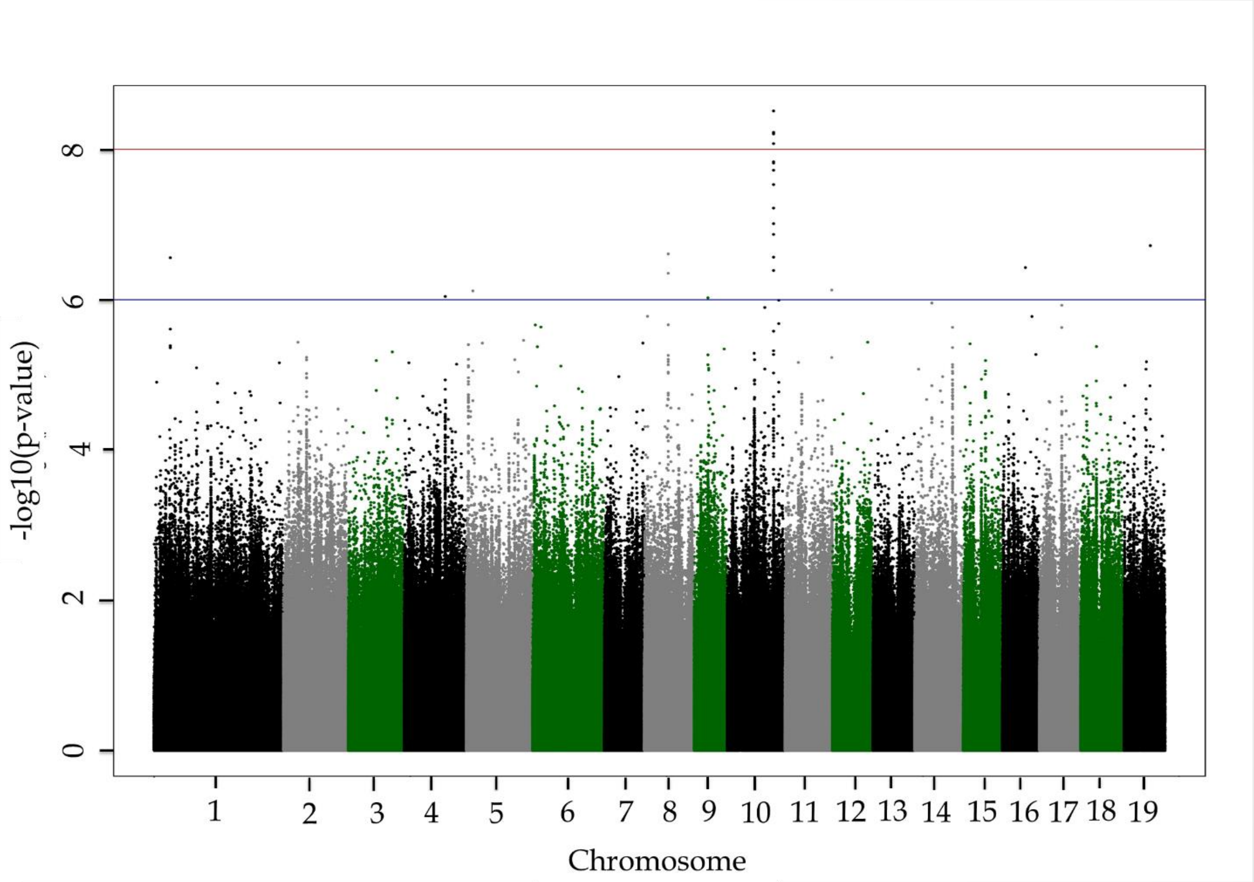
Marker	Resistance	Variance explained	p-value
Chromosome 10	*P. deltoides*	9.65%	0.015
Chromosome 13	*P. deltoides*	8.82%	0.045

- Female sawflies appear to avoid progeny with *P. deltoides* alleles in both significant regions of the genome
- *P. deltoides* parent D124- no signs of leaf folding in the field
- Chemical recognition?

Future Directions

P. trichocarpa genetic map

- Look for similar location associations in *P. trichocarpa* genome
- Sequenced to depth 15X and containing millions of segregating single nucleotide polymorphisms (SNPs)
- Genome wide association analysis (GWAS)



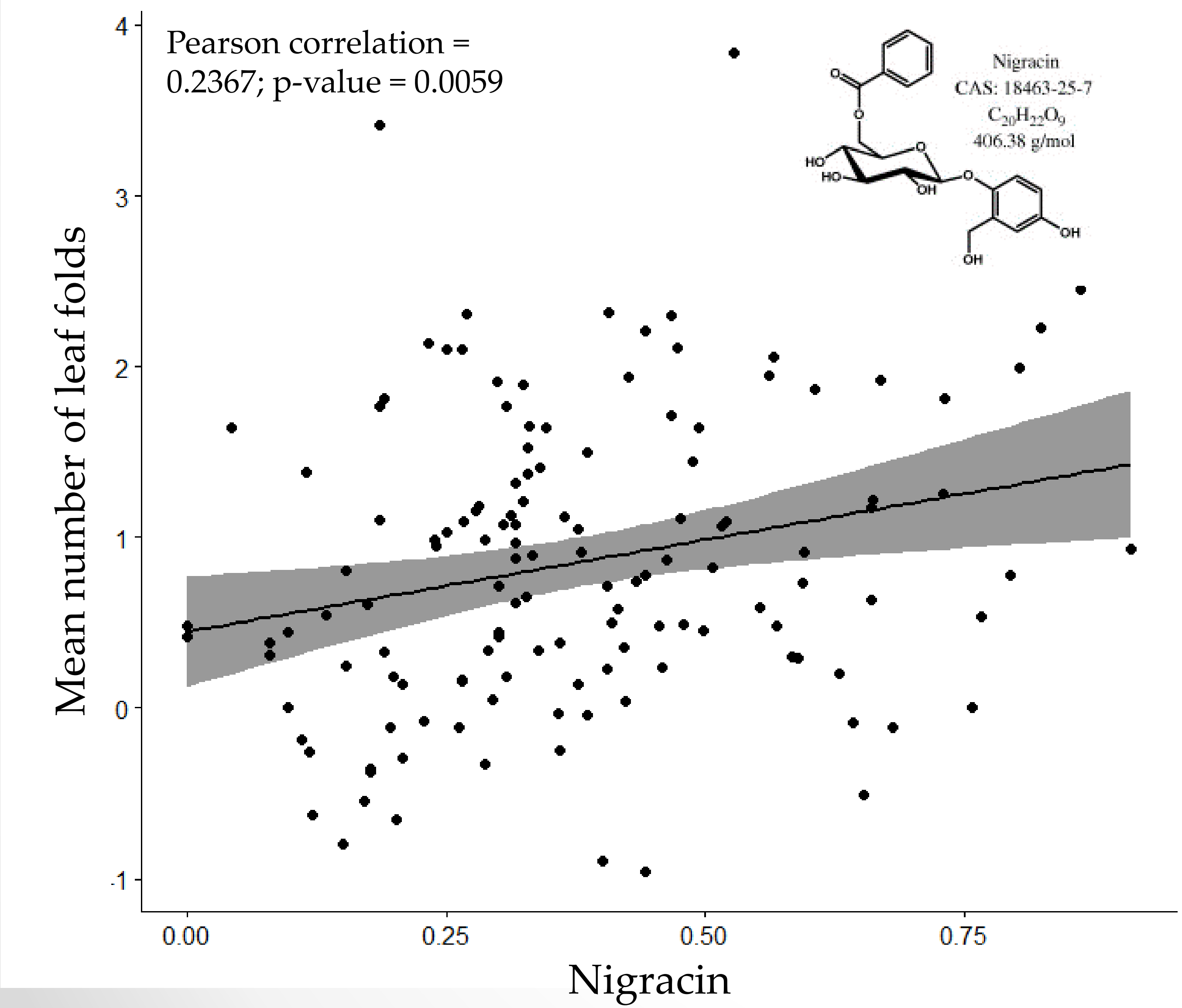
Question three

How do secondary metabolite profiles influence *P. bozemani* oviposition choice?

Hybrid secondary metabolite data

- 52124 Hybrid Family
  - Leaf tissue was collected from 40 individuals (20 high abundance; 20 low abundance)
  - Sent to University of Wisconsin-Madison to be analyzed for phenolic chemistry and nutritional characteristics by Dr. Ken Keefover-Ring
  - Seven higher order salicylates previously classified for 52124 progeny by Dr. Timothy Tschaplinski (Oak Ridge National Laboratory)

Hybrid nigracin correlation



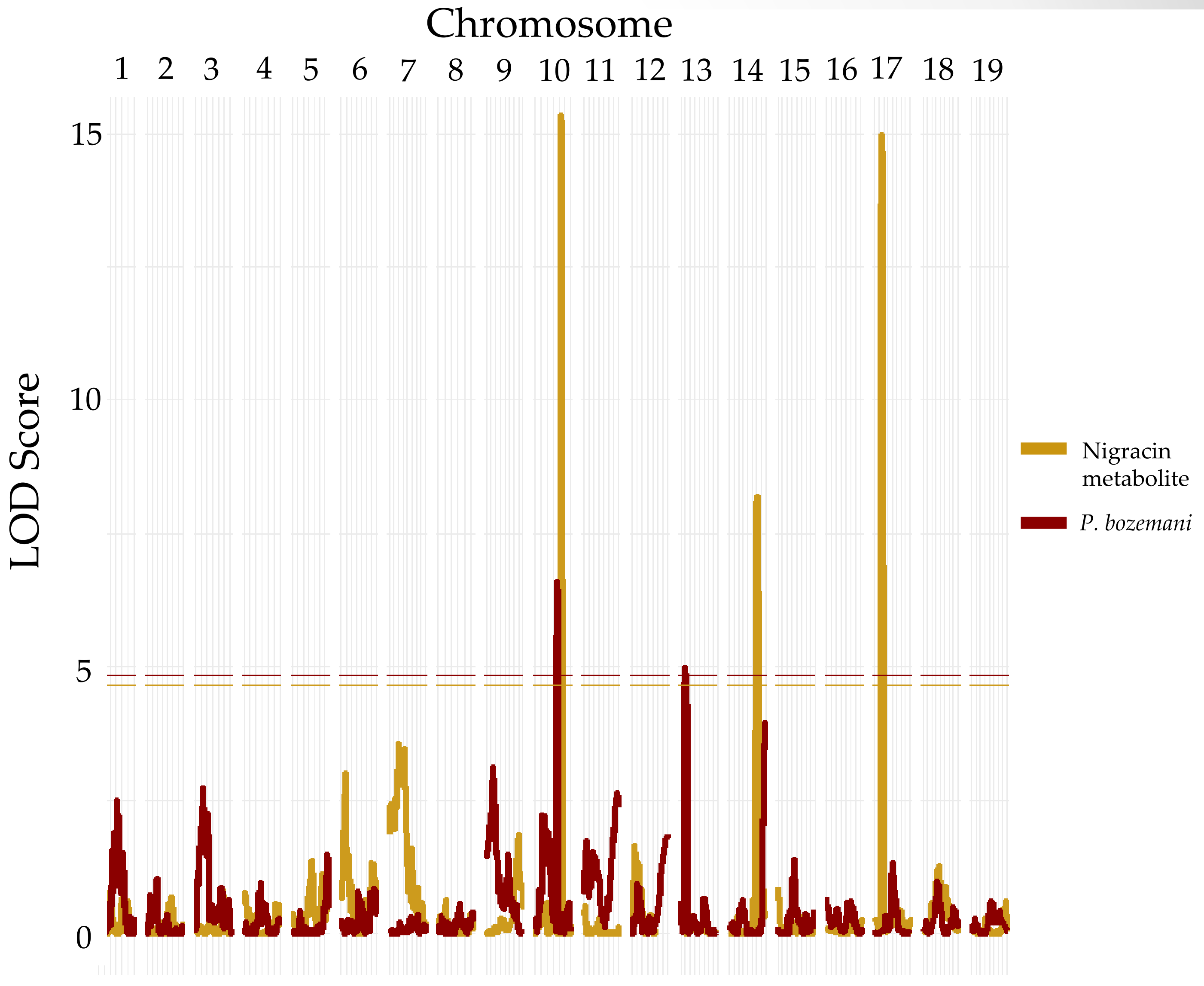
- Correlation between tissue levels of nigracin and abundance of *P. bozemani* leaf folds in hybrid family
- Ovipositional cue?

Parent	Mean number of *P. bozemani* leaf folds	Nigracin levels
Delt "ILL 101"	NA	0.53
Tricho"93-968"	NA	5.51
TxD "52-225"	17.0 ± 6.84	3.49
Delt "D124"	0.00 ± 0.00	0.35
Progeny 52124	1.05 ± 0.063	10.34

Hybrid nigracin QTL analysis

Marker	Positive allele	Variance explained	p-value
Chromosome 10	*P. trichocarpa*	27.0%	<0.0001
Chromosome 17	*P. trichocarpa*	22.8%	<0.0001
Chromosome 14	*P. trichocarpa*	10.6%	<0.0001

- Similar regions of the genome account for large variation of nigracin levels was also important in segregation of *P. bozemani* oviposition



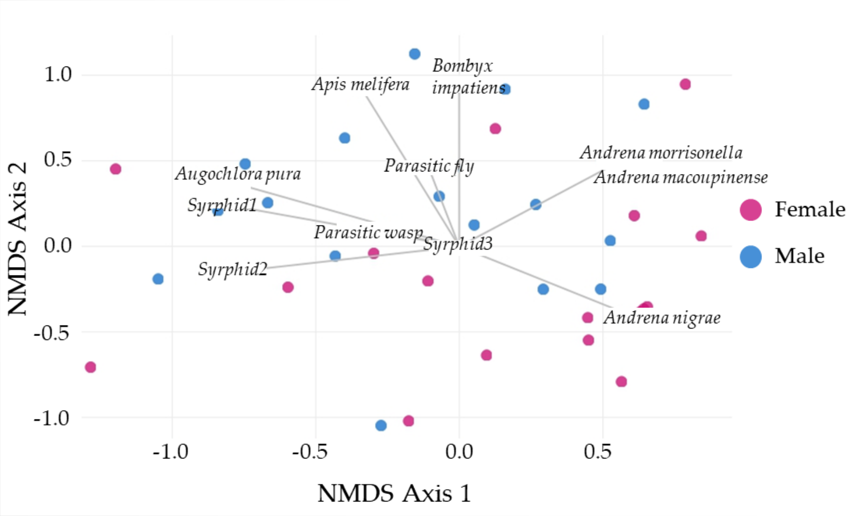
Future Directions

- *P. trichocarpa* population
  - Does nigracin also explain some of the variation in leaf folding activity in *P. trichocarpa*?
  - Phenolic and nutritional characteristics by Dr. Tschaplinski (Oak Ridge National Laboratory)

Objective one: host plant location

Classifying the pollinator community and investigating the attraction of floral visitors in the dioecious willow species *Salix nigra* (Black Willow)

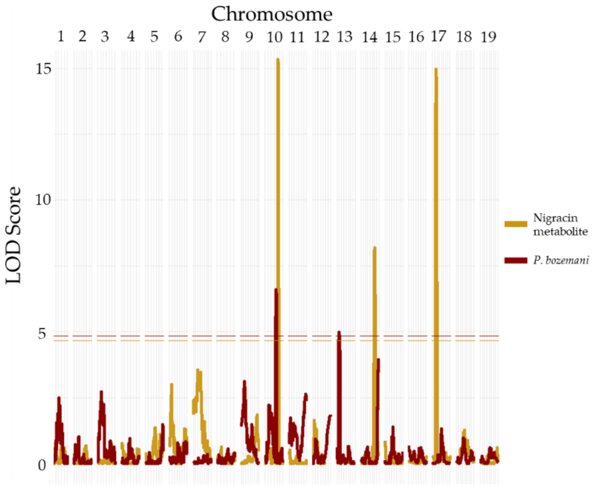
- Preliminary data analysis conclusions
  - Individual trees have a unique community of insects visiting flowers
  - Male and female trees have unique assemblages of floral visitors
  - The main pollinator in our site belongs to the *Andrena* bee genus
- Future directions
  - How are volatiles impacting insect attraction?
  - How does DNA barcoding compare to visual surveys and specimen collections?



Objective two: host plant acceptance

Investigating the genetic mechanisms associated with *Phyllocolpa bozemani* oviposition preference in a hybrid *Populus* and a diverse collection of *Populus trichocarpa* (Black Cottonwood)

- Preliminary data analysis conclusions
  - Host genetics accounted for variation in *P. bozemani* leaf fold counts in both sites
  - Chromosome 10 and 13 are important in mediating the interaction between *P. bozemani* and the hybrids
  - In hybrids similar genetic positions important in nigracin production are also responsible for insect preference
- Future directions
  - Do similar regions of the genome and secondary metabolite levels impact ovipositional activity in *P. trichocarpa?*



Acknowledgements

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Questions?