Characterization of *Salix nigra* floral insect community and activity of three native *Andrena* bees

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Introduction

Oligolectic solitary bees, which only collect pollen from either related plant species or a single species, rely heavily on predictable timing of available native floral resources. Upon emergence from nests oligolectic bees must locate flowers, feed, breed, build new nests, lay eggs, and collect resources to provide their larvae with food for development throughout the remainder of the year, all within the bloom time of their specific host. In addition to being a valuable resource for early emerging generalist floral insects, willow species belonging to the genus *Salix* are the primary hosts of many oligolectic bees, especially those belonging to one of the largest bee genera, *Andrena*.

Salix species have a non-showy fluorescence arranged in a catkin form where male catkins are typically yellow and female flowers tend to be green. Additionally, *Salix* species emit a complex mixture of volatile organic compounds that are important as olfactory signals to insects, and both male and female plants offer nectar rewards. *Salix nigra*, known by its common name black willow, is a tree-form, entomophilous willow that grows throughout the Eastern United States. The extensive range and productivity of *S. nigra* as well as its early bloom, February in its southern range through late June in more northern states, makes it an ideal resource for early emerging insects. Studying the mechanisms that *S. nigra* employs to attract insects as well as the influence of sex of tree on floral insect community through time is important in determining the competition for and potential stability of catkin resources, native oligolectic bee activity, and *S. nigra* reproductive success.

Objectives

1) Characterize the community of insects that visit *S. nigra* catkins in the WVU Core Arboretum using visual observation and examine the impact that survey year (2017-2019) and sex of tree had on the floral visitors and activity of native bees *A. morrisonella*, *A. macoupinense* and *A. nigrae*.

2) Evaluate the community of floral insects captured using visual survey techniques and bee-bowl traps placed in tree canopies in 2019.

3) Examine the total floral community captured using two techniques in 2019 to determine the impact of location, sex of tree, volatiles and secondary metabolites.

Floral community characterization and analysis

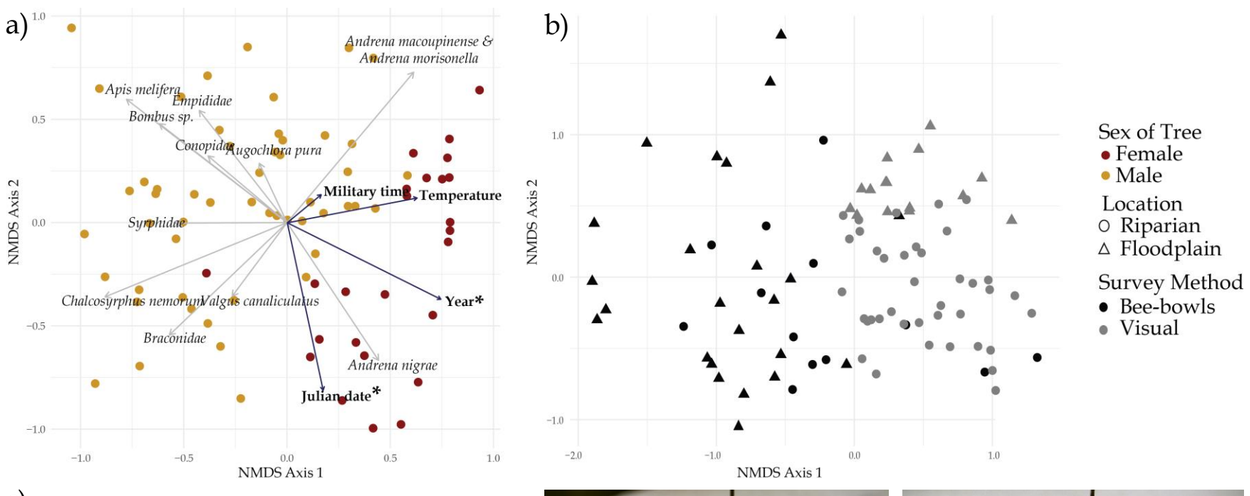

Figure 1. Non-metric multidimensional scaling (NMDS) analysis of floral insect communities for a) most common insects visiting overlapping trees through time (2017-2019), b) visual survey method vs bee-bowl trap collected in 2019 and c) dataset merging visual and bee-bowl traps in 2019. Points that cluster in multidimensional space are more similar in their insect communities (grey vectors). An additional fit was performed for 2017-2019 and 2019 NMDS analyses to look for correlation of survey conditions (blue vectors) with the three NMDS dimensions (* indicates significant correlation).

Analysis of community similarity

Table 1. Analysis of similarity results testing grouping variables. A significant p-value (<0.05) indicates that insect community is more similar within groups rather than between groups.

Community analysis	Variable	ANOSIM R	p-value
2017-2019 (Figure 1a)	Year	0.1669	0.001
	Sex of tree	0.1018	0.032
Survey comparison (Figure 1b)	Survey type	0.6656	0.001
	Sex of tree	0.07105	0.032
2019 (Figure 1c)	Location	0.165	0.003
	Sex	0.3077	0.001

Community metric and individual species activity over time

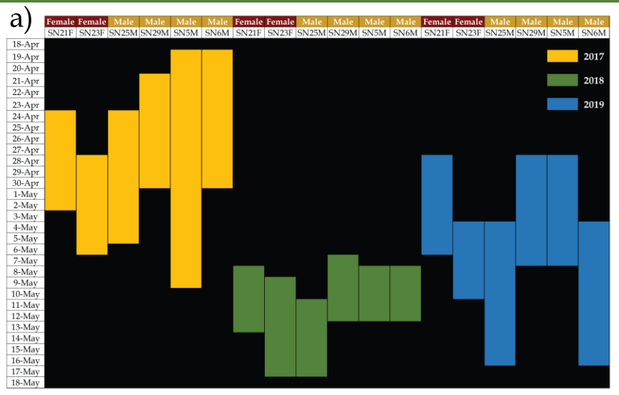

Figure 2. a) Bloom time of individually surveyed trees in 2017 (yellow), 2018 (green), and 2019 (blue). Analysis of covariance revealed that b) the activity of *A. nigrae* was dependent upon year and c) the diversity of visiting insects and number of species (richness) that visited flowers was dependent on the sex of the tree. Letters above error bars indicate significantly different means as determined by Tukey's HSD test (p-value < 0.05).

Bee-bowl capture and 2019 survey evaluation

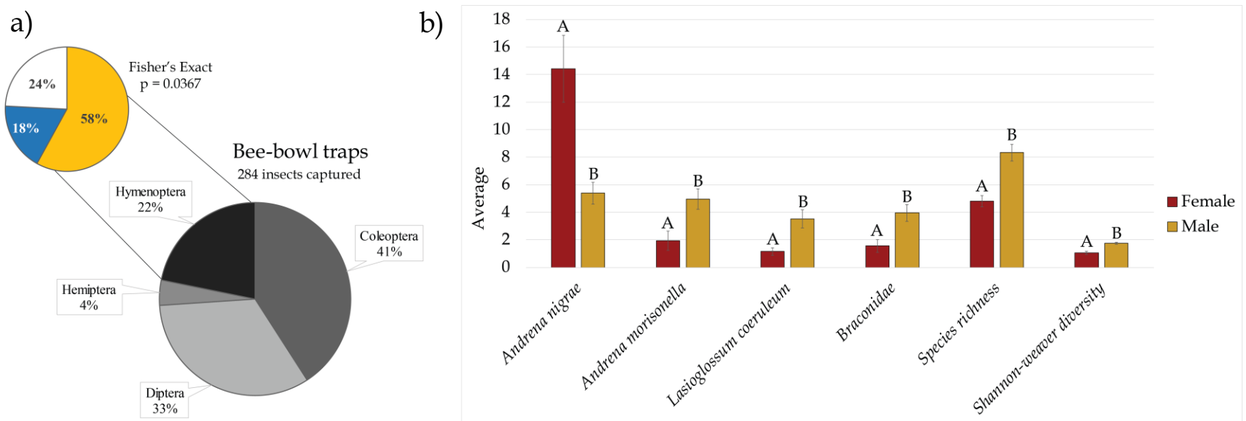

Figure 3. a) Percentage breakdown of insect orders captured in bee-bowls. A Fisher's exact test indicated hymenopterans occurred in yellow traps over other colors more than expected by chance. Analysis of covariance revealed that b) the average count of individual native bees as well as average species richness and diversity were all dependent on the sex of the tree rather than location, individual tree, or Julian date. Letters above error bars indicate significantly different means as determined by Tukey's HSD test (p-value < 0.05).

Main findings and future directions

Male trees attracted more diverse and unique insects when compared to female trees through time. This effect of sex is even more pronounced in the 2019 dataset as the combination of two survey methods allowed us to capture a fuller community sampling. For generalist bees this may be due to the additional visual cue of pollen on males as seen in a trend of yellow bee-bowl traps to preferentially attract hymenopterans. In 2019, there is also an effect of sex on distributions of *A. nigrae* (higher activity on females) and *A. morrisonella* (higher activity on males). However, through time there was no effect of tree sex on number of *Andrena* visits indicating all three species are contributing to successful pollination of *S. nigra* catkins.

Finally, year had a large effect on *A. nigrae* activity with far fewer catkin visits in 2017. The bloom time of the trees in 2017 occurred earlier than subsequent years (Figure 2a) due to uncharacteristically warm weather followed by much cooler temperatures. The abnormality of temperature may have decoupled the availability of floral resources from local *A. nigrae* population emergence. Alternatively, cooler temperatures may have had a negative effect on volatilization of catkin scent leading to the inability of native bees to effectively locate their host. Future work will be focused on how individual tree volatile profiles contribute to floral community distributions and activity.