

## **The Effects of Increased Anthropogenic Nitrogen on Plant Characteristics and Pollinators**

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### **Introduction**

Most plants in temperate ecosystems are limited in their growth and reproduction by nitrogen (N) supply, and N fertilization often results in increased plant growth and reproduction (1). Human activities such as increased agricultural fertilizer inputs, increased growth of nitrogen fixing crops, and increased fossil fuel consumption have already doubled nitrogen inputs and are expected to continue to rise (2, 3). While it has been shown that greater N will lead to higher plant productivity (4), almost nothing is known about how this increase in N availability will affect plants indirectly through altering their mutualistic and antagonistic interactions with other species in natural populations. I will examine how changes in N availability influences mutualistic interactions between plants and their pollinators.

Variation in plant traits can influence pollinator behavior and subsequent plant reproductive success. For example, flower size, flower number, nectar quality and concentration, pollen color and number are plant traits that have been found to affect pollinator behavior (5, 6). Pollinator behavior can be described as pollinator preference (the degree to which plants prefer particular species, or particular morphs of a single species) and pollinator constancy (the degree to which an individual pollinator prefers one flower species to another that offers equal rewards); both affect plant fitness by altering the quality and quantity of pollinator visits (7, 8, 9). Previous studies on plants have also shown that increased soil N can alter plant traits that are associated with pollinator behavior. For example, N fertilization has been shown to lead to increased flower production (10) and pollen production (Hersch-Green Lab, unpublished data).

### **Significance**

The Hersch-Green lab is currently studying how nitrogen affects interactions with herbivores and plant growth traits; I will be adding data on how nitrogen affects interactions with pollinators. Since plants evolve as part of communities, we are working towards the big picture of how soil N affects pollinators, herbivores, and competitive interactions. This project will give me experience in measuring plant traits as well as providing data towards a larger goal in the Hersch-Green lab.

### **Objective**

The objectives of my experiment are (1) to determine how nitrogen fertilization alters plant physical characteristics (such as flower size and number, plant height, nectar quantity and sugar concentration, and number and color of pollen grains) that have been shown to be related to pollinator behavior, (2) to test whether increasing soil nitrogen concentrations affects pollinator behavior, and (3) to see if changes in plant traits influence associated changes in pollinator behavior.

### **Hypotheses**

My hypothesis is that increased soil N will lead to plants that are able to allocate more resources to reproduction and traits to attract pollinators- they will have more flowers, higher quantities of nectar and pollen grains, and more concentrated nectar. This, in turn, I believe will lead to increased pollinator visits to plants and higher plant fitness. I also predict that plants will show different responses depending upon their predominant pollinator guild, as different

pollinators are more attracted to plants with different traits (e.g. bee-pollinated flowers typically have more pollen than bird-pollinated flowers).

## Methods

I will conduct a potted experiment on land owned by Stacy Cotey (Biological Sciences). We will select four plant species native to Michigan's Upper Peninsula that occur in nearby populations and for which seeds have already been collected. Chosen plants will require pollinators for reproduction and will vary in terms of their dominant pollinator (e.g. bee vs. bird) and blooming period (June or July). These plants will be started at the MTU greenhouse and transported to the site of experimentation (which contains a greenhouse for storage). I will grow plants under three different N conditions- low, medium, and high N containing soil. Nitrogen levels will be determined by soil testing in the spring and adjusting accordingly. I will grow 20 individual plants (replicates) per plant species per treatment for a total of 240 plants. I will mark each pot with a unique number and will measure plant traits on each plant (number of pollen grains and nectar quantity and quality) to determine how N affects plant traits.

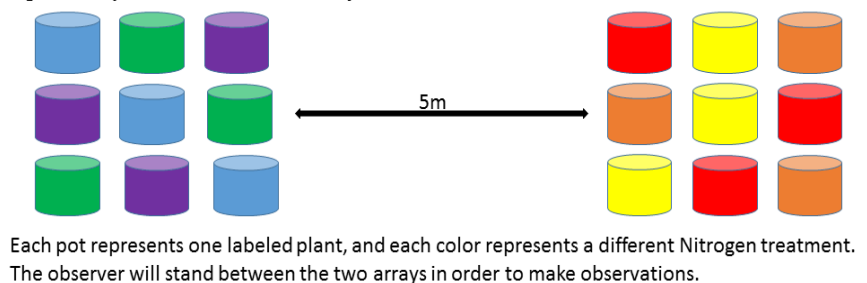
To observe pollinator behavior, I will set up experimental pollinator arrays (Fig. 1, see 7 for additional methodology); each array will consist of a single species, but I will watch 2 arrays simultaneously. Each array will have a different primary pollinator. I will record visits by different pollinator groups (bumblebees, flies, moths, birds) in 20-minute intervals (7). The plant pot visited, identity of pollinator, length of visit, number of flowers per plant and plant sequence will be noted for each pollinator entering my arrays. To avoid pollinator depletion of nectar or

pollen influencing results, plants in arrays will be rotated between observation periods and each pot will only be used once per day. Observations will occur over a six-week period. I will grow additional plants of each species and treatment in order to measure traits like pollen quantity

(which would leave the plant unable to fertilize others) and I will alter plants (whether in experimental arrays or measured for plant traits) daily. From plants used in my experimental arrays I will measure plant traits that are non-destructive such as plant height and flower number. Lastly, I will make attempts to collect insect pollinators for identification and to contribute to knowledge of pollinators of the UP.

At the conclusion of the project, statistical analyses will be performed with the assistance of Dr. Hersch-Green. We will examine correlations among traits measured. We will also examine for differences in plant traits among N treatments with analysis of variance models corrected for any trait correlations. We will then calculate measures of pollinator preference and constancy and test how plant traits influence pollinator behavior with multiple novels. My goals are (1) to learn new Ecology field and statistical methods that will prepare me for graduate school, (2) to present my findings at local and international meetings, such as the MTU Ecosystem Science Center undergraduate research forum and Ecological Society of America, and (3) to publish my findings as a scientific paper.

Figure 1: Experimental Pollinator Arrays



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