Determining the ecological role of flowering plants as nutritional forage for pollinators: You are what you eat!

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Abstract

This project will develop a digital pollen library of the Mid-Atlantic flowering plants and different cultivars of indigenous species. The nutritional quality of pollen and nectar collected from flowering plants will be analyzed for total amino acid content, crude protein, fatty acids and sterols, total carbohydrates and vitamins and minerals. This project seeks to determine if there are differences in the nutritional value of pollen and nectar from different cultivars of plants, and to see if there are differences in the morphology of pollen among cultivars. This project also will track the attractiveness of foraging resources to determine which plants act as an ecological asset to the landscape. Finally this project will correlate nutritive value of foraging resources with pollinator abundance and diversity measures. From the data collected we hope to provide nursery managers, homeowners, growers and landscape artists with recommendations on planting assemblages that will add aesthetic value to their property while providing an ecological service; nutrition for pollinators.

Justification

The decline of honey bee populations has been of major concern since the mid-2000's soliciting lots of research attention and dollars. While research has indicated there is no one causal factor for these continual declines it has identified many stressors that play a role in diminishing honey bee health; the most notable being a combination of pests/pathogens, pesticide exposure, habitat fragmentation and nutritional health (Chauzat et al., 2006; Chen and Siede, 2007; Cox-Foster et al., 2007; Chen et al., 2008). The latter three stressors are not unique to honey bees but also have severe ramifications on other pollen collecting insect species, most notably native bees which also use pollen and nectar for growth and development and also provide crucial ecosystem services (Winfree et al. 2007; Breeze et al. 2011).

Bees are thought to have evolved from wasps during the late cretaceous period when angiosperms radiated across the landscape. During this radiation the bees switched from a mostly carnivorous diet to one dependent upon plant produced products namely pollen, nectar and in some species, plant produced oils (Michener, 1974). Both social and solitary bee species are specialized for collection of pollen and nectar. The co-evolved characteristics between different flowering plant species and their respective pollinators has been a topic of much study and these pollinator syndromes typically focus on floral traits, however, nectar characteristics and pollen composition are also considered as co-evolved traits that are intrinsically tied to the fitness of both the plants and pollinators.

Nutritional quality:

Bees like most animals require carbohydrates, proteins, fats, sterols, minerals, vitamins and water in order to live a long and healthy life. They obtain these nutrients through the assimilation of nectar and pollen. Nectar is a plant-made liquid primarily produced in the flowers, but other sources include extrafloral nectaries. Nectar is the main source of carbohydrates for the developing and adult bees and can be easily broken down to provide energy at the cellular level. It is known that carbohydrates are essential for fueling flight muscles, thermoregulatory functions inside the hive and proper wax gland function. Not all nectars are made equal and bees can discriminate between nectars with low and high sugar concentrations preferring the latter (Nicolson 2007, Nicolson 2011). The sugar content can vary greatly with reports of 4% to 60 % and higher (Waller, 1972). Nectars with higher sugar contents are preferred by foragers and studies by Waller (1972) found sugar solutions of 30- 50% to elicit a maximum collection response.

The amount of nectar and honey that is needed for optimal colony health and longevity depends on many variables. The size and the strength of a colony determines the amount of nectar and honey the developing brood and adults will need to sustain themselves. The weather directly affects the timing of flower bloom and the availability and amount of different types of plants and nectars. Other environmental variables also affect nectar production from soil to temperature and humidity, and finally sunlight. As we can see from observing bees forage on different plants not all nectar is as equally enticing and sugar concentration is one of the main factors that influences foraging decisions. Early studies by Von Frisch (1967) also show that bees prefer certain sugars over others. In fact honey bees recognize only a few sugars as being sweet: glucose, fructose, sucrose, melizitose and maltose. Early studies (Waller, 1972) also show that bees actually show a preference, when given a choice, of sucrose followed by glucose, maltose and fructose. This preference, not surprisingly transfers over to a higher nutritive value as well. Studies by Barker and Lehner, (1974; 1978) reveal that bees fed sucrose lived longer and developed better than bees fed diets composed of other sugar sources.

All organisms require adequate protein levels in order to ensure proper development. Protein consumption during early honey bee development is essential for the development of body tissue, muscles and glands and increases overall longevity (Haydak, 1970). One of the main sources of protein in a honey bee diet comes from pollen, specifically pollen that is processed via fermentation to a storable protein source called bee bread. Pollen contains protein, nitrogen, amino acids, starch, sterols and lipids (Roulston and Cane, 2002.)The nutritional quality of pollen also varies greatly among plant species but is also caused by differences in geographic origin, climatic conditions and micro-climatic factors such as soil attributes. Early studies (Maurizio, 1953; Standifer, 1967; Loper and Berdel, 1980) found that different types of pollen resulted in differences in honey bee development (specifically the fat bodies and hypopharyngeal gland)- basically how pollen converts to brood. This early work also demonstrated that bees seem to prefer mixed pollen sources.

Protein levels in pollen can range from 2-60% (Rouslton et al. 2000) and studies looking at the relationship between pollen protein content and foraging preference have failed to find correlations between the two (Pernal and Currie, 2001; Roulston and Cane 2002). However, it is still believed that protein concentration may influence the foraging behavior of adult bees so as to insure they meet the protein quota for the developing larva (Neff and Simpson 1997). Further studies have shown that threshold protein levels are necessary for complete metamorphosis, an increase in adult body size and adult longevity (Greenberg, 1982; Schmidt et al. 1987; Regali and Rasmont, 1995).

Different pollens can have different mixtures and quantities of amino acids. A study by De Groot (1953) showed that most pollen contains all the essential amino acids: arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, isoleucine and valine. Amino acid composition is a major factor in the nutritive value of different pollens. However, the predominant amino acid in mature fermented pollen is proline (Stanely and Linskens, 1974). Proline is found in high levels in all insects and is an essential substrate for the citric acid cycle.

Proline is extremely important as a substrate for flight metabolism. More importantly it has been shown in honey bees that amino acid content is more important in determining the amount of pollen necessary for proper development than simple protein content, and that all ten essential amino acids are needed for maximum development (De Groot, 1953). In studies where honey bees were fed only one kind of pollen or pollen lacking in one or two of the essential amino acids larvae did not reach maturity, but upon the addition of these amino acids full brood rearing was restored (Herbert, 1992) The amino acid content of pollen has also been shown to increase foraging response in choice tests and is a benchmark for how much pollen is needed to sustain bee development (Nicolson, 2011).

Lipids and fatty acids are also found in pollen and are the building blocks of phospholipids which are a major component of cell membranes. Some solitary bee species have been shown to have the ability to completely digest pollen lipids. These complex lipids are the precursors to important hormones involved in the molting process during the insect's life cycle and serve as a primary energy store for insects in their fat bodies. These fatty acids also are used for the derivation of different defensive secretions in some insects. The loss and depletion of fatty acids is linked to the desiccation of many insects and they also aid in the lubrication of food.

With the detection of various chemistries associated with bee forage, i.e. nectar and pollen, what is the effect of these chemistries on the health of bees? First we need to understand the nutritional requirements of bees and what plants produce the optimal supply of these essential nutrients. So the first question should be- What do bees eat? The answer to this question may be quite different from what they actually forage on and collect. However the first step towards answering this question is to create a digital pollen library of regional plant species. The creation of this library will help us to identify the pollen collected and stored in bee nests so that a temporal pollen and nectar map can be developed for pollinators in the Mid-Atlantic region. The second step is to look at the nutritional profile of pollen and nectars produced by the various flowering plants and to create a nutritional index for plants visited by pollinators.

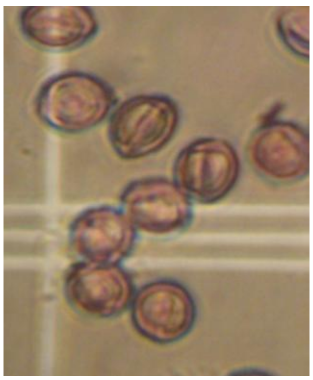
Objectives

1)Development of a regional digital pollen library to be used as a reference tool to identify pollen from inside bee nests.

-Using a Leica DM750 Brightfield Configurable microscope/ Leica EC3 Digital Camera and software kit with HD monitor we have begun preparing digital pollen library of various flowering plants throughout the Mid-Atlantic region. We have collected pollen from over 200 different flower species.

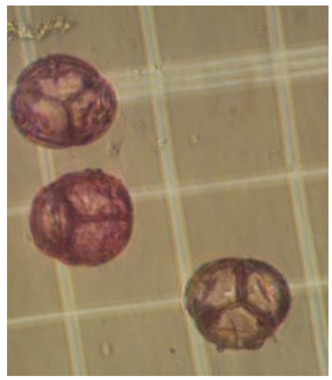
Figure 1. These pictures (A, B, and C) represent pollen grains collected by *Bombus impatiens* from their corbicula or pollen baskets.

A



Mock Orange

B



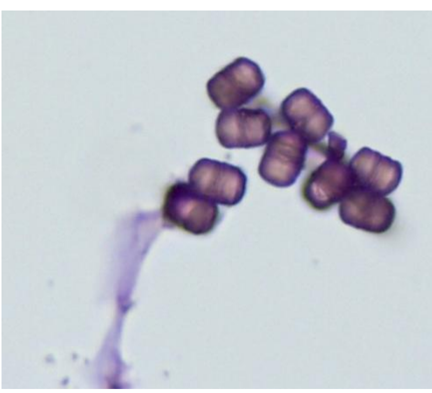
Blueberry

C

Czech Olive

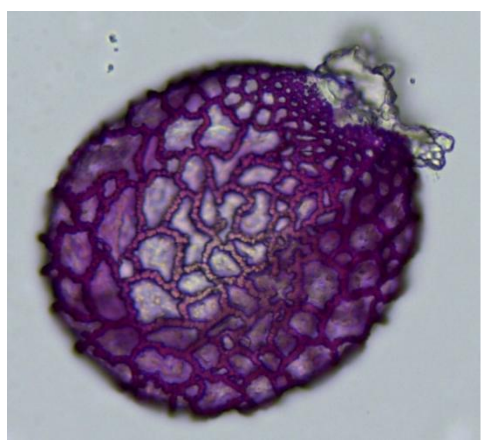
Figure 2. These pictures (A, B, and C) represent pollen collected straight from the anthers of flowers.

A



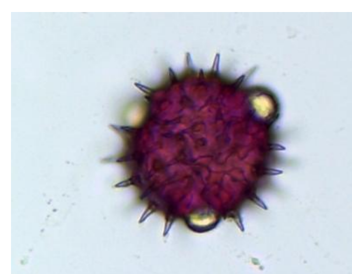
Forget-me-not

B



Madonna Lily

C



Sunflower

2)The development of a nutritional index of forage plants

-Measure the nutritional quality of plants, via pollen and nectar analysis from various indigenous flowering plants and specifically different cultivars.

-Identify pollen using honey bee and bumble bee colonies as model systems. Pollen will be collected from honey bee colonies using pollen traps fitted onto the front entrance of the honey bee colonies.

3)To determine nectar production and recharge ratse of nectar for different cultivars
-Multiple flowers from each cultivar will be bagged prior to bloom and nectar will be collected using a calibrated microcapillary pipette. Within each cultivar, flowers will be resampled at different time intervals to assess nectar recharge rates and amounts.

4)To calculate pollinator species diversity and abundance estimates for different cultivars
-Pollinators will be collected from various cultivars three times a week during bloom time in the morning and afternoon. A pinned reference collection will be established with specimens identified to species.

5)To understand the ecological role of different cultivars on pollinator species
-Colony productivity measurements, species diversity and abundance measurements and pollen and nectar nutritional value will be correlated to understand how nutritional quality and plant phenotype affect pollinator health and productivity.

Methods:

Regional Pollen Library:
Pollen and nectar will be sampled and collected from over 200 species of flowering plants sampled from forest fragments from March to October (typical foraging time for many bee species). Pollen and nectar will be collected from plots of cultivars established at Mt. Cuba. Flowers will be bagged in glassine envelopes and marked with date, time of collection and *Genus species* name. Nectar collection will follow an established protocol whereby flowers will be collected in the field in glass jars and stored in a cooler and taken back to the lab. Nectar samples will be collected through the use of a calibrated microcapillary tube (Rusterholz and Erhardt, 1998; Pozo et al. 2011; Álvarez-Pérez and Herrera, 2013). Filter papers with absorbed nectar will be placed into a tube labeled with date, time of collection and *Genus* and *species* name. Nectar samples will be stored in a freezer till analysis.

Nutritional Assays
Pollen and nectar will be sampled shortly after flower bloom from various cultivars as well as flowering plants found in the Mid-Atlantic region. Stored pollen and nectar will be prepared and sent off for nutritional analysis to the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories http://www.aescl.missouri.edu/index.html. Nutritional screens

will consist of a full amino acid screen, crude protein, carbohydrate analysis, fatty acid and sterol breakdown, and vitamin and mineral screen.

Pollen traps will be placed on colonies for one day every other week from March to October. Bumble bee foragers will be collected at the colony entrance once a week in the morning and afternoon from March to October. Pollen pellets from bumble bee and honey bee curbiculae will be diluted to a concentration of 1.4×10^{-4} μL/g with DI water and 5 μL of fuschin stain solution. Then, 20 μL of the pollen mixture will be inserted into a hemocytometer and all pollen grains within the middle square will be counted and identified. Pollen will be identified to species using the digital pollen library as a reference. Honey bee and bumble bee colonies will be assessed monthly to track colony productivity and performance over the spring and summer of 2014. Colony growth and productivity will be assessed throughout the season by measuring adult and brood population, honey and pollen stores, and presence/absence of various pests and pathogens. Pollen type, diversity and nutritional value will be tested for any possible correlations.

Forage Attraction

Various cultivars will be monitored three times a week in the morning and afternoon from early March to late October for pollinator visitation. Pollinators will be collected and identified to species. Pollinator diversity and abundance will be measured between cultivars, and diversity and abundance measures for each cultivar will be correlated with nutritional information for each cultivar.

Nectar Load and Recharge Rates and Amounts

Flowers from various cultivars will be bagged and sampled at bloom to quantify nectar loads to assess if there are differences in nectar production. A series of flowers will be resampled at different time intervals to assess nectar recharge rates.

Plant Assemblage Recommendation

Cultivar and plant species lists with nutritional value for each will be provided to nursery managers, landowners, growers and landscape artists. These lists will specify the nutritional value of the forage, time of year forage is present, and what pollinators are most abundant on the flowers from each plant. A colorful picture/color plate section will provide users with a picture during bloom, a picture of the pollen and pictures of common pollinator visitors.

Specifics related to number of cultivars/plots and honey bee and bumble bee colony numbers and placement will be based on previous literature and discussion with other members of the research team. At least 5 honey bee and bumble bee colonies will be established on Mt. Cuba grounds, and the pollinator observation will take place in the trail garden at Mt. Cuba.

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