# THE EFFECT OF POLLEN DIVERSITY AND ARTIFICIAL PROTEIN SUPPLEMENT ON HONEY BEE (*Apis mellifera* L.) HEALTH, PHYSIOLOGY, AND IMMUNOLOGY

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## Introduction

The pollination requirement is huge for commercial agriculture in the United States. Honey bee pollination is estimated to be worth more than $20 billion in the U.S and worth $2 billion in the Pacific Northwest. Our current agricultural production involves extensive monocultures and enclosed greenhouse production with routine pesticide use. These production systems are without the presence of cover crops, which had once provided supplemental pollen sources for honey bees (Dimitri, 2005). Within this current system, honey bees are left with few pollen species in their diet for a significant amount of time as they are transported from bloom to bloom. These landscapes may not provide pollen from enough sources in order to maintain a healthy nutritional diet. Poor nutrition is correlated with a weakened immune system and heightened susceptibility of pathogens (Ritz and Gardner 2006, Eischen and Graham 2008, Alaux et al., 2010). Without proper nutrition, colonies may generate enough stress to negatively influence other important health factors (Stanley and Linskens 1974). Stress resulting from mono-cropping and habitat loss has been speculated to be one of the contributing factors for current colony population decline (Oldroyd 2007, Naug 2009).

The goal of this project is to evaluate effects of pollen diversity and artificial protein supplements on honey bee physiology, colony growth and immunology. This project will enable us to distinguish differences in colony fitness of more pollen-diverse and protein-rich pollen diets.

## Materials and Methods

### Pollen collection

Pollen loads were collected from foragers by fitting standard pollen traps onto hives that visit each pollinated crop. Almond, cherry, meadowfoam, and blueberry were the targeted crops. Pollen traps were installed on the hives when the bloom was 70 to 100 percent on target crops. Pollen from traps underwent analysis light microscopy and acetolysis in order to identify the floral resources in each pollen collection. Pollen treatments were formulated to represent a wide range of pollen diversity. Almond is a monofloral source and was used as the least diverse treatment diet. The 3 other treatments held increasingly large amounts of pollen species.

Experimental Design

Colonies were established in 5-frame nucleus boxes with naturally-mated sister queens and equal numbers of adult bees and stores. The nucleus colonies were situated in flight cages placed within the same apiary. Top feeders with 50% sucrose solution and external water feeders were applied to all colonies. One frame in each colony was empty built-comb; pollen treatments were inserted into these frames by distributing the raw pollen into the cells followed by a spray of 50% sucrose solution. This simulated workers to pack pollen into the cells thus initiating the pollen processing procedure (Dreller and Tarpy, 2000). Bee-Pro®, MegaBee®, and Global Patties® are three commonly used artificial protein supplements and served as three treatments groups for artificial diets.

Measurements

Honey bee physiology will be assessed by measurements of total gut proteolytic enzyme activity and hypopharyngeal gland protein quantification. Total gut proteolytic enzyme activity is associated with digestibility rate in the gut and will follow the same procedure as Sagili et al. (2005) and Michaud et al. (1995). Hypopharyngeal gland protein quantification within 'nurse' bees conveys the total amount of protein fed to developing brood. Nurse bees will be cold euthanized, their hypopharyngeal glands will be dissected and analyzed for protein using Bradford assay described by Sagili et al. (2005).

Phenoloxidase (PO) and prophenoloxidase (ProPO) enzymes are part of humoral immune responses that are present in the hemolymph of honey bees. PO and ProPO are associated with wound healing response and immunity. PO and ProPO analysis will be prepared following the method of Laughton & Siva-Jothy (2010). Measurements will be reported as optical density per minute and normalized by total protein (mg) using a standard BCA assay. Glucose oxidase (GOX) is associated with social immunity of the colony. GOX is utilized in nurse bees' hypopharyngeal glands to sterilize food before administered to developing brood. GOX measurements will be performed following the methods of Ohashi et al. (1999).

The various measurements of colony growth will be used to assess whether or not treatment diets can translate to the colony-level. A standardized grid will be used to assess colony resources: open brood, capped brood, pollen stores, nectar/honey stores (Pankiw et al., 2004). Colony growth was evaluated every 7 days. Queen supersedure and laying production (i.e. spotty-brood syndrome) was monitored as well.

**Significance**

This study will help us better understand the role of protein nutrition in colony growth and survival. Results will lead to a better understanding of how the quality of foraging landscapes and supplemental protein feed influence the nutritional health of colonies.

This project is underway and no data has been statistically generated yet. However, if diets with less pollen diversity are shown to be harmful to honey bee health, this project provokes pesticide use on forage landscape effects. Heavy pesticide use may constitute less diversity of bee-collected pollen within agricultural landscapes.

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