# Assessing the Value of Supplemental Forage During Almond Pollination by Longitudinal Monitoring of Honey Bee Colonies for Nutritional Status, Colony Growth and Survival

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## **Project Cooperators and Personnel:**

Dr. Neal Williams, UC Davis Oregon Beekeepers Project Apis m.

## **Objectives:**

Project No.:

Evaluating effects of supplemental forage prior to and after almond bloom on honey bee nutrition, colony growth, immune system and survival.

#### Interpretive Summary:

Honey bee colonies employed for almond pollination face two challenges with respect to nutrition: a) lack of adequate foraging resources before and after almond bloom and b) lack of floral diversity during almond bloom. To address this nutritional stress, organizations such as Project Apis m are providing alternate forage for bees before and after almond bloom in California. To successfully implement and promote this strategy we need to understand the potential of these supplemental bee forages in promoting honey bee colony health. We measured honey bee colony health and nutrition in colonies adjacent and at a distance from supplemental forage. Data pertaining to bee health and nutrition for this study is currently being analyzed. Field observations show during peak almond bloom, honey bees focused their foraging efforts in the almonds. Alternative forage was clearly utilized as the almond bloom waned and concluded. The pollen collection data demonstrate the ideal pollination scenario: honey bees focus on pollinating almond blossoms when in bloom with minimal distraction to other forage, and upon bloom conclusion, honey bees have continuous resources available to sustain their growing population. We look forward to repeating this study again during 2015-16.

# Materials and Methods:

#### Field sites

We identified 4 field sites in Sacramento Valley almond orchards: two sites were adjacent to land planted with mustard, and two sites had no mustard within at least a 1-mile radius of the honey bee colonies. We marked 24 colonies near mustard plantings (among two sites) and we marked 24 colonies with no mustard nearby (among two sites)

In the South San Joaquin Valley, we identified two field sites: one site was adjacent to UC Davis' wildflower planting project, and one site was at least 2 miles away from the wildflower site. We marked 20 colonies at the site near the wildflower plantings and we marked 20 colonies at the site far from wildflower plantings.

### Colony measurements

On the following dates: February 12, February 21, and March 12, 2015, we measured each marked colony for comb area occupied by brood (eggs, larvae, and pupae), honey, pollen, and empty space. We also measured the area covered by adult bees on each frame to estimate bee population.

We collected a sample of 300 honey bees from the brood nest of each marked colony in a wide-mouth plastic jar. Samples were stored on dry ice and transported to the lab for further analysis.

We also installed pollen traps (front porch type) at the entrances of marked colonies. The traps force honey bees to squeeze through a small opening while scraping pollen loads from their corbiculae into a collection drawer below. Traps were activated for 24 hours during each of our three visits. Pollen was collected, sorted by color, and then sent to UC Davis / Dr. Neal Williams for identification.

## Lab analysis

Lab analysis of honey bee samples included Varroa mite counts, nosema spore counts, and hypopharyngeal gland protein content. To estimate Varroa mite infestation, we used a 70% ethanol wash to dislodge mites from honey bees. The number of mites was divided by the number of bees in the sample to yield a percent infestation of Varroa in the colony. The prevalence and intensity of *Nosema* infection was determined by light microscopy techniques followed by Cantwell (1970).

From each sample, we selected ten honey bees for hypopharyngeal gland dissection. Glands were pooled together and placed in 2ml microcentrifuge tubes containing saline buffer. We quantified protein content with the PierceTM Biotech BCA Assay Kit (Cat #23225, Thermo-Scientific, IL, USA) microplate procedure. Absorbance at 562 nm was measured using a microplate spectrophotometer (BioTek Synergy 2, Gen5 2.00 software) and protein content concentration in  $\mu$ I /ml was calculated from the resulting standard curve.

Immunocompetence analysis is underway. Haemolymph will be extracted with micro capillaries from the second abdominal tergite of the individual bee and number of haemocytes per microlitre of haemolymph will be counted with the help of haemocytometer (Alaux et al. 2010). Phenoloxidase and prophenoloxidase enzyme activity levels, indicators of immunocompetence in honey bees, will be measured following the method of Laughton & Siva-Jothy (2010).

## **Results and Discussion:**

Pollen identification data received from UC Davis is summarized in **Table 1** (courtesy of Kimiora Ward, UC Davis). During peak almond bloom, honey bees focused their foraging efforts in the almonds. Alternative forage was clearly utilized as the almond bloom waned and concluded.

Wildflower	Site	Pollen	23 Feb	27 Feb	6 Mar	10 Mar
Far	MA	Almond	94	76	6	
		Wildflowe	3	9	42	
		mustard	0	7	6	
		Unknown	3	5	20	
		Weed	0	3	31	
Near	MW	Almond	72	58	0	0
		Wildflowe	7	15	8	23
		mustard	16	42	23	57
		Unknown	4	6	6	2
		Weed	1	9	66	18
Mustard			12 Feb	21 Feb		12 Mar
Far	НА	Almond	97	96		76
		mustard	0	0		14
		Unknown	3	4		86
		Weed	0	0		0
	KA	Almond	91	50		0
		mustard	1	48		91
		Unknown	8	3		12
		Weed	0	0		1
Near	KM	Almond	91	31		0
		mustard	0	66		64
		Unknown	10	8		36
		Weed	0	0		0
	PM	Almond	93	31		1
		mustard	3	73		83
		Unknown	4	6		77
		Weed	0	0		0

**Table 1.** Percent pollen collected by honey bee hives positioned adjacent almond orchards and "Far" or "Near" to forage plantings.

Data analysis pertaining to colony parameters such as colony growth, pest/disease incidence and hypopharyngeal gland protein is currently in progress. Preliminary results reflect the challenges/difficulties we encountered in identifying control sites (far from mustard or wildflowers) as late winter rains in California yielded a flush of blooming weeds, mustard, and wildflowers in fallow fields and roadsides. Colonies in the "control" sites may have foraged on these volunteer blooming plants as honey bees can fly up to 3 miles for forage. Therefore, the variability we are experiencing in the data may be due to most honey bees in the experiment having access to alternative forage.

Furthermore, the duration of almond bloom in Southern California was among the shortest in recent history (Gordon Wardell, personal communication). Our sampling dates covered prebloom (February 10), petal fall (February 23), and leaf/nut formation stages (March 10). The Northern California bloom duration was slightly longer. This unique circumstance may affect our final results. We will repeat this study again during 2015-16.

The pollen collection data demonstrate the ideal pollination scenario: honey bees focus on pollinating almond blossoms when in bloom with minimal distraction to other forage, and upon bloom conclusion, honey bees have continuous resources available to sustain their growing population.

# **References Cited:**

- Alaux.C, Ducloz, F., Crauser, D. and Le Conte, Y. 2010. Diet effects on honeybee immunocompetence. Biology Letters doi:10.1098/rsbl.2009.0986.
- Cantwell, GE. (1970). Standard methods for counting Nosema spores. *American Bee Journal*. 110: 222-223.
- Laughton, A., Siva-Jothy, M. 2011. A standardized protocol for measuring phenoloxidase and prophenoloxidase in the honey bee, Apis mellifera. Apidologie, Springer Verlag (Germany), 42 (2), pp.140-149.