

Research



# Apiculture & Social Insects

# Assessment of Pollen Diversity Available to Honey Bees (Hymenoptera: Apidae) in Major Cropping Systems During Pollination in the Western United States

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#### **Abstract**

Global western honey bee, *Apis mellifera* (L.) (Hymenoptera: Apidae), colony declines pose a significant threat to food production worldwide. Poor nutrition resulting from habitat loss, extensive monocultures, and agricultural intensification is among the several suggested drivers for colony declines. Pollen is the primary source of protein for honey bees; therefore, both pollen abundance and diversity are critical for colony growth and survival. Many cropping systems that employ honey bee colonies for pollination may lack sufficient pollen diversity and abundance to provide optimal bee nutrition. In this observational study, we documented the diversity and relative abundance of pollen collected by honey bees in five major pollinator-dependent crops in the western United States. We sampled pollen from pollen traps installed on honey bee colonies in the following cropping systems—almond, cherry, highbush blueberry, hybrid carrot, and meadowfoam. The pollen diversity was estimated by documenting the number of different pollen pellet colors and plant taxa found in each pollen sample. The lowest pollen diversity was found in almond crop. Relatively higher quantities of pollen collection were collected in almond, cherry, and meadowfoam cropping systems. The information gleaned from this study regarding pollen diversity and abundance may help growers, land managers, and beekeepers improve pollen forage available to bees in these cropping systems.

Key words: honey bees, pollen diversity, pollen abundance, crop pollination

The western honey bee, Apis mellifera (L.) (Hymenoptera: Apidae), is the major pollinator of fruit, nut, vegetable, and seed crops that depend on bee pollination for high quality and yield (Klein et al. 2007). During critical crop bloom periods, growers rent large numbers of honey bee colonies to pollinate their crops. Approximately 2.5 million commercially managed honey bee colonies are used for crop pollination in the United States every year (USDA-NASS 2018). Renting colonies to growers for pollination services is a significant source of income for commercial beekeepers, but it also requires them to repeatedly transport the colonies between crops throughout the growing season (Burgett 2010, Sagili and Caron 2017). Although migratory beekeeping contributes to the pollination needs of many cropping systems, intensive agricultural sites may affect the health of honey bees (Smart et al. 2016). Since 2007, commercial beekeepers in the United States have reported annual colony losses averaging over 30% (vanEngelsdorp and Meixner 2010). Previous studies have attributed these losses to a multifactorial combination of causes, which include lack of proper

nutrition, pesticide exposure, and prevalence of common pests and diseases (Higes et al. 2008, Naug 2009, Mullin et al. 2010).

Nectar and pollen provide essential nutrients for honey bees. Nectar serves as a source of carbohydrates (primarily monosaccharides and oligosaccharides) and trace amounts of vitamins, minerals, and amino acids (Ball 2007). A honey bee colony's protein source is pollen, which has varying amounts of amino acids, lipids, vitamins, and minerals (Stanley and Linskens 1974). These nutrients obtained from pollen are essential for honey bee larval development (Stanley and Linskens 1974). Pollen largely contributes to the growth of fat bodies in larvae and egg development in the queen (Pernal and Currie 2000, 2001). The nurse bees consume pollen so that hypopharyngeal glands can biosynthesize proteinaceous secretions that are progressively fed to the larvae (Winston 1987, Knecht and Kaatz 1990, Crailsheim et al. 1992).

Pollen is also critical for maintaining colony health. Honey bee colonies consuming adequate amounts of high quality pollen are less susceptible to the gut parasite *Nosema ceranae*, have lower

pathogen loads, and overwinter successfully compared with colonies receiving scanty or poor quality nutrition (Eischen and Graham 2008, Di Pasquale et al. 2013, DeGrandi-Hoffman et al. 2016, Jack et al. 2016, Glavinic et al. 2017). Well-nourished individuals in a honey bee colony are able to withstand the effects of other stressors such as parasites, insecticides, and the long-distance transport of colonies that is inherent in migratory management (Brodschneider and Crailsheim 2010, Mao et al. 2013, Schmehl et al. 2014, Simone-Finstrom et al. 2016). Hence, optimal nutrition is a colony's first line of defense, enabling it to successfully cope with both biotic and abiotic stressors.

There are several factors that influence the adequacy of a colony's pollen diet. Pollen stores in a honey bee colony are regulated around a homeostatic set point. Unlike nectar foraging behavior, which does not change in response to fluctuating honey stores, pollen foraging effort responds to changes in pollen stores (Fewell and Winston 1992, 1996). However, honey bees encounter significant temporal variations with nutritional resource availability in intensive agricultural systems (Di Pasquale et al. 2013). Even if pollen stores within the hive are dwindling, a colony's foragers may not always be able to gather enough pollen to return stores to the optimal level of 1 kg (Jeffrey and Allen 1957). Indeed, in such environments, periods of limited pollen forage may adversely influence colony development (Odoux et al. 2012). Another consideration is that the nutritional value, specifically protein content, varies greatly among different species of pollen (Standifer 1967, Roulston et al. 2000). Every plant species produces pollen with a unique composition, and any one pollen species may not meet the complete nutritional requirements for honey bees (Stanley and Linskens 1974). A diet low in pollen diversity negatively affects a colony's defense system, which consequently increases disease susceptibility and pesticide sensitivity (Wahl and Ulm 1983, Alaux et al. 2010, DeGrandi-Hoffman et al. 2010, Foley et al. 2012, Di Pasquale et al. 2013). Thus, some cropping systems may put bees at risk for temporary nutritional deficiency if the crop plant's pollen is deficient in certain nutrients and bees are unable to find an alternative source of these nutrients. Hence, it is imperative for beekeepers and crop producers to understand the pollen abundance and diversity that honey bees encounter during crop pollination to mitigate nutritional deficiencies by providing supplemental food or forage.

This observational study primarily documents the diversity of pollen available to honey bees in five major crops pollinated by managed honey bee colonies in the western United States (California and Oregon). We use descriptive statistics to report our findings. We identified the number of pollen pellet colors and plant taxa types found in pollen collections from pollen traps on honey bee colonies placed next to almond, cherry, highbush blueberry, hybrid carrot, and meadowfoam cropping systems.

#### **Materials and Methods**

We collaborated with 17 migratory commercial beekeepers from the Pacific Northwest region for pollen collection from honey bee colonies in five different cropping systems from late February to August of 2012 (Fig. 1).

#### Pollen Collection

We collected the corbicular pollen loads from returning honey bee foragers by installing Sundance bottom mount pollen traps (Brushy Mountain Bee Farm, NC) on experimental colonies in the following five crops: almond [Prunus dulcis (Mill.) D.A. Webb], cherry (Prunus avium L.), highbush blueberry (Vaccinium corymbosum L.), hybrid carrot seed [(Daucus carota L.), (Hoffm.)], and meadowfoam (Limnanthes alba Hartw. ex Benth.) (Fig. 1). These crops require the most honey bee colonies for pollination provided by Oregon commercial beekeepers (Caron et al. 2012). We sampled colonies at a total of 17 sites in Oregon and California (Table 1; Fig. 2). All colonies at a given sampling site were owned by a single beekeeper and all but one of the 17 collaborating beekeepers were sampled at only one site. We chose the sampling sites based on pollination contracts held by the collaborating beekeepers, ensuring each site was at least three miles away from any other sampling site to minimize any overlap of bee-foraging areas. The number of colonies used for pollen collection in each cropping system and site was dependent on specifications provided by beekeepers. We installed pollen traps on at least five colonies at each site (Table 1). We collected pollen from the colonies when each target crop bloom was about 80 to 100% (Fig. 1).

For pollen collection, we installed pollen traps on colonies with entrances facing south, as south-facing colonies receive abundant morning sunlight and generally exhibit good foraging activity. We also selected these colonies as defined by the frequency of pollen foragers entering the colony. To assess pollen foraging frequency, we counted foragers at the colony entrances for 2 min (Pettis et al. 2013). We engaged pollen traps on the colonies for 7 d. The pollen collected from all colonies at a given sampling site was pooled together and stored at -20°C until further analysis.

### Hand Collection of Pollen and Anther Samples

We surveyed the landscape surrounding each sampling site for blooming flowers. We collected mature anthers from blooming plant species at the time of sampling. The pollen from these anthers served as voucher specimens for morphological identification of our collected pollen. We stored anther samples collected from each location at -20°C within 6 h after collection. We likely were unable to sample every flowering species within the foraging radius of bees. Thus, we

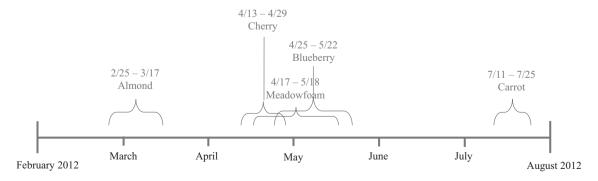


Fig. 1. Timeline of pollen sampling when colonies were placed in seven different cropping systems (software used: Microsoft Visio by Microsoft Office; v14.0). All pollen samples were collected in 2012.

Table 1. Colony sample size per site for the five cropping systems

| Crop       | Site ID | Number of colonies |
|------------|---------|--------------------|
| Almond     | 1       | 15                 |
|            | 2       | 5                  |
|            | 3       | 10                 |
|            | 4       | 5                  |
|            | 5       | 15                 |
| Blueberry  | 1       | 15                 |
|            | 2       | 15                 |
|            | 3       | 10                 |
|            | 4       | 15                 |
| Carrot     | 1       | 5                  |
|            | 2       | 5                  |
| Cherry     | 1       | 10                 |
|            | 2       | 10                 |
|            | 3       | 20                 |
| Meadowfoam | 1       | 15                 |
|            | 2       | 10                 |
|            | 3       | 10                 |

supplemented our identification process with curated pollen samples from previous reference collections held at the United States Department of Agriculture, Agriculture Research Service Unit, College Station, TX (Jones et al. 1995).

# Sample Preparation

For taxonomic identification, we selected a 30-g composite pollen sample from each pooled collection. We sorted individual pollen pellets by color from this sample. For some color groups, there were as few as 4–5 pellets; other groups contained relatively more pellets. We acetolyzed three pollen pellets from each color group as well as the pollen from our frozen voucher anther samples in preparation for morphological analysis via light microscopy (LM; see below). We were able to identify some taxa to the species level and some only to the genus or family level.

For acetolysis and slide preparation of both pollen pellets and pollen from anthers, we used methods adapted from previous publications (Jones and Bryant 2004, Jones 2012). We dehydrated each pollen sample by agitating them in glacial acetic acid on a vortex

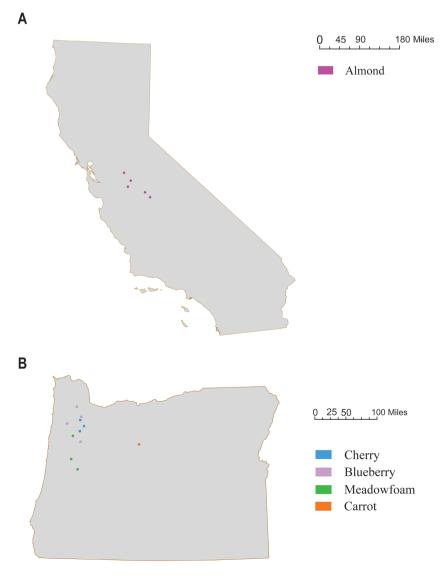


Fig. 2. Locations in (A) California and (B) Oregon for collection of different crop pollens.

mixer (1 min, 700 rpm); centrifuged the samples (3 min, 3,500 rpm, room temperature); discarded the supernatant and heated the resulting pellets in a 9:1 solution of acetic anhydride:sulfuric acid on a heat block (80°C, 5 min). We recentrifuged (as above) and then rinsed the pollen residues first in glacial acetic acid, then in water, and finally in 95% ethanol, centrifuging and decanting after each rinse. Next, we mixed the pollen residues with two to five drops of Safranin O stain (LC223408, LabChem Inc., Zelienople, PA); rinsed and centrifuged the samples in 95% ethanol; and resuspended the precipitate in three to five drops of glycerin (LC148501, LabChem Inc., Zelienople, PA). Finally, after evaporating any remaining ethanol from the samples (room temperature, overnight), we mounted one to two drops of the pollen-glycerin solution on a slide and sealed the cover slip with nail polish (Clear 450B, Wet and Wild Shine Nail Protector, Los Angeles, CA). We took micrographs of pollen grains in several diagnostic positions with an Aus Jena Jenaval compound light microscope (Carl Zeiss, Jena, Germany) at 400× magnification using bright field, phase contrast, and Nomarski phase.

#### Limitations of the Study

Pollen collections in our study took place at diverse locations and during different times of the year. Weather conditions at time of pollen sampling varied among the different crops, potentially altering the foraging preferences of bees and the distance foragers traveled for pollen collection. Each cropping system also differed in field size. This might have influenced the diversity of nontarget crop pollen types within our pollen sample collections.

#### **Results**

#### Pollen Collection

The quantity of pollen collected from pollen traps on honey bee colonies from the five different cropping systems varied widely. Honey bee colonies placed in meadowfoam, cherry, and almond cropping systems collected substantial amounts of pollen in each collection site:  $693 \pm 265$  g/colony/week in meadowfoam,  $533 \pm 284$  g/colony/week in cherry, and  $303 \pm 105$  g/colony/week in almond. Colonies placed in highbush blueberry and hybrid carrot cropping systems collected relatively smaller amounts of pollen from collection site(s):  $81 \pm 6$  g/colony/week in highbush blueberry and  $14 \pm 9$  g/colony/week in hybrid carrot.

#### Pollen Analysis (Identification)

Table 2 summarizes pollen diversity parameters of each cropping system. Table 3 provides information about pollen types collected in each cropping system. Each pollen pellet color contained either a single plant taxa or a mixture of several plant taxa (Table 3). On average, we found  $3.0 \pm 0.5$  pellet colors and  $3.2 \pm 1.2$  plant taxa per site in almonds, both relatively low numbers when compared

with all the other cropping systems (Table 2). Of the eight distinct pellet colors found in pollen collected in almonds, three (cream, tan, and yellow gold) contained pollen from Prunus species. Other (nontarget) pollen found in almond collection sites were Brassicaceae, Cornus stolonifera L. (red osier dogwood), Crataegus sp. (hawthorns), Pedicularis sp. (louseworts), Taraxacum officinale F.H. Wigg (common dandelion), Trifolium incarnatum L. (crimson clover), Trifolium repens L. (white clover), and Viburnum sp. (viburnums). Pollen collected from sites in the four remaining cropping systems contained more pellet colors and more plant taxa than the pollen collections from almond sites. We found an average of 6.0  $\pm$  2.0 pellet colors and 8.0  $\pm$  1.5 plant taxa per site in cherry,  $8.8 \pm 1.4$  pellet colors and  $13.5 \pm 2.0$  plant taxa per site in highbush blueberry,  $7.0 \pm 1.0$  pellet colors and  $11.0 \pm 0.0$ plant taxa per site in hybrid carrot, and 10.0 ± 0.0 pellet colors and 13.0 ± 1.5 plant taxa per site in meadowfoam. Irrespective of the crop, pollen collections from all collection sites contained pollen from Trifolium spp. (clovers), including Trifolium arvense L. (rabbitfoot clover)., Trifolium incarnatum L. (crimson clover), Trifolium pretense L. (red clover), and Trifolium repens L. (white clover). Additionally, in all cropping systems, the collected pollen contained Taraxacum officinale F.H. Wigg (common dandelion). Other pollen types commonly observed in this study were Cornus stolonifera L. (red osier dogwood), Viburnum sp. (viburnums), Crataegus sp. (hawthorns), Medicago sativa L. (alfalfa), Prunus sp., Pedicularis sp. (louseworts), and Limnanthes alba Hartw. ex Benth. (meadowfoam; Table 3). Notably, we found no highbush blueberry (Vaccinium corymbosum L.) pollen in any of the pollen samples collected in highbush blueberry crops (Table 3). Target crop species were found in collections from meadowfoam (Limnanthes alba Hartw. ex Benth.) and carrot (Daucus carota L.). *Prunus* spp. was found in almond and cherry cropping systems.

#### **Discussion**

Our study provides valuable insights into the pollen abundance and diversity available to honey bee colonies employed for pollination in five major crops in the western United States. Our findings suggest that almond, cherry, and meadowfoam crops are able to provide ample pollen to honey bees, whereas highbush blueberry and hybrid carrot seed crops may not supply sufficient pollen. Meadowfoam and almond are considered to produce significant amounts of pollen. Some studies have demonstrated that meadowfoam plants (Sagili et al. 2015) and almond trees (Hill et al. 1985) produce significant amounts of pollen with almond flowers yielding pollen from 29 to 126 mg per 100 flowers. The honey bee colonies employed for almond pollination gain strength relatively quickly and at the end of almond bloom these colonies are almost twice the size of colonies that are stationary (not transported for pollination) in temperate climates during that time of year (Sagili and Burgett 2011). Such strong colonies can thus more efficiently pollinate

Table 2. Mean number of pellet colors (±SE), mean number of plant taxa (±SE), and total taxa identified of pollen collected from pollen traps in five cropping systems

| Crop       | Mean number of pollen pellet colors/site (SE) | Mean number of plant taxa/site (SE) | Tot    | al taxa identi | ified   |
|------------|---|-------------------------------------|--------|----------------|---------|
|            |   |                                     | Family | Genus          | Species |
| Almond     | 3.0 (0.5)                                     | 3.2 (1.2)                           | 4      | 3              | 4       |
| Blueberry  | 8.8 (1.4)                                     | 13.5 (2.0)                          | 5      | 10             | 13      |
| Carrot     | 7.0 (1.0)                                     | 11.0 (0.0)                          | 3      | 5              | 6       |
| Cherry     | 6.0 (2.0)                                     | 8.0 (1.5)                           | 4      | 7              | 5       |
| Meadowfoam | 10.0 (0.0)                                    | 13.0 (1.5)                          | 5      | 4              | 14      |

Table 3. Plant taxa with associated pellet color and cropping systems found in pollen collection

|  |   |                   |         |       |       | -      | :                  |   | -        | -1. Dod | n - ddie          | ;                 |   | = 2, |                   | 1 Puom     |                  |         |          |            |
|--|---|-------------------|---------|-------|-------|--------|--------------------|---|----------|---------|-------------------|-------------------|---|------|-------------------|------------|------------------|---------|----------|------------|
|  | Amber Army Ash Black Brown Cream Gold Gray Light<br>Green | Army Ash<br>Green | n Black | Brown | Cream | Gold G | ray Light<br>Brown |   | nge rurj | ole Keu | Keddish<br>Orange | h Tan Violet<br>e | Orange Purple Red Reddish Tan Violet White Yellow Yellow Yellow Almond Blue- Carrot Cherry Meadowfoam<br>Gold Green berry | Gold | Yellow A<br>Green | Holla<br>1 | Blue- C<br>berry | arrot C | herry M  | Ieadowfoam |
| Aceraceae, Acer macrophyllum                 | ×   |                   |         |       |       | ×      | ×                  |   |          |         |                   |                   |   | ×    |                   |            | ×                | ×       | ×        |            |
| rutsh, bigjear inapie<br>Aceraceae, Acer sp. |   |                   |         |       |       | ×      |                    |   |          |         |                   |                   |   |      |                   |            | ×                | ×       |          |            |
| Anacardiaceae (type 1)                       |   | ×                 |         |       |       |        |                    |   |          |         |                   |                   |   |      |                   |            | ×                |         |          |            |
| Anacardiaceae (type 2)                       |   |                   |         |       |       |        |                    |   |          |         |                   | ×                 |   |      |                   |            |                  |         | ×        |            |
| Anacardiaceae, Rhus glabra L.,               | ×   |                   | ×       |       |       |        |                    |   |          |         |                   |                   |   | ×    |                   |            |                  |         | ×        |            |
| smooth sumac                                 |   |                   |         | >     |       |        |                    |   |          |         |                   |                   |   |      |                   |            |                  |         | >        |            |
| Anacardiaceae, Toxicodendron                 |   |                   |         | ×     |       |        |                    |   |          |         |                   |                   |   |      |                   |            |                  |         | ×        |            |
| diversilobum (Torr. & A. Gray)               |   |                   |         |       |       |        |                    |   |          |         |                   |                   |   |      |                   |            |                  |         |          |            |
| Angestiscese Toxicoloudron                   |   |                   |         | >     | >     |        | >                  | > |          | >       |                   |                   |   | ×    |                   |            |                  | >       | <b>×</b> |            |
| rydbergii (Small ex Rydb.)                   |   |                   |         | <     | <     |        | ς                  |   |          | <       |                   |                   |   | <    |                   |            |                  | <       | <        |            |
| Greene, western poison ivy                   |   |                   |         |       |       |        |                    |   |          |         |                   |                   |   |      |                   |            |                  |         |          |            |
| Apiaceae, Anethum graveolens                 |   |                   |         |       |       |        |                    | × |          |         | ×                 | ×                 |   | ×    |                   |            | ×                |         |          | ×          |
| L., dill                                     |   |                   |         |       |       |        |                    |   |          |         |                   |                   |   |      |                   |            |                  |         |          |            |
| Apiaceae, Daucus carota L., carrot           |   |                   |         |       |       | . 1    | ×                  |   | ×        |         |                   |                   |   |      |                   |            |                  |         |          | ×          |
| Asteraceae, Carduus sp.                      |   |                   |         |       |       |        |                    | ; |          |         |                   |                   |   | ×    |                   | ;          | ;                | ;       | ;        | ×          |
| Asteraceae, Taraxacum officinale             |   |                   |         |       |       |        |                    | × |          |         |                   |                   |   | ×    |                   | ×          | ×                | ×       | ×        | ×          |
| F.H. Wigg, common dandelion                  |   |                   |         |       |       |        |                    | ; |          |         |                   |                   |   |      |                   |            |                  | ;       | ;        |            |
| Asteraceae, Cichorea                         |   |                   | ;       |       |       |        |                    | × |          |         | ;                 |                   |   |      |                   |            |                  | ×       | ×        | ;          |
| Asteraceae, Cirsium sp.                      |   |                   | ×       |       |       |        |                    | ; | ×        |         | ×                 |                   |   | ;    |                   |            |                  |         |          | ×          |
| Asteraceae                                   |   |                   |         |       |       |        |                    | × |          |         |                   |                   |   | ×    |                   |            |                  |         |          | ×          |
| Asteraceae, Ratibida columnifera             |   |                   |         |       |       |        |                    | × |          |         | ×                 |                   |   |      |                   |            |                  |         |          | ×          |
| (Nutt.) Wooton & Standl.,                    |   |                   |         |       |       |        |                    |   |          |         |                   |                   |   |      |                   |            |                  |         |          |            |
| Mexican hat                                  |   |                   |         |       |       |        |                    |   |          |         |                   |                   |   |      |                   |            |                  |         |          |            |
| Brassicaceae (type 1)                        |   | ×                 |         |       |       | ×      | ×                  |   |          |         | ;                 | ×                 | × ;   | ×;   |                   | ×          | × ;              | × ;     | × ;      |            |
| Brassicaceae (type 2)                        |   |                   |         |       |       |        | ;                  |   |          |         | ×                 |                   | ×   | ×    |                   |            | ×                | ×       | ×        |            |
| Brassicaceae (type 3)                        |   | ;                 |         |       |       |        | ×                  |   |          |         |                   |                   | ×   | ×    |                   |            |                  | × ;     | × ;      |            |
| Calycanthaceae, Calycanthus                  |   | ×                 | ×       |       |       |        |                    |   |          |         |                   |                   |   |      |                   |            |                  | ×       | ×        |            |
| floridus L., eastern sweetshrub              |   |                   |         | ;     |       |        | ;                  |   |          |         | ;                 |                   |   | ;    | ;                 | ;          | ;                | ;       |          |            |
| Capritoliaceae, Viburnum sp.                 | ×   | ×                 | ;       | ×     |       |        | ×                  |   |          |         | ×                 |                   |   | ×    | ×                 | ×          | × ;              | ×       |          |            |
| Caryophyllaceae                              |   |                   | ×       |       |       |        |                    |   |          |         |                   |                   |   |      |                   |            | ×                |         |          |            |
| Cheno-Am (type 1) <sup>a</sup>               | . 1   | ×                 | ×       |       |       |        | ×                  |   |          |         |                   |                   |   | ×    |                   |            |                  |         |          | ×          |
| Cheno-Am (type 2) <sup>a</sup>               |   |                   | ×       |       |       |        |                    |   |          |         | ×                 |                   |   |      |                   |            |                  |         |          | ×          |
| Cheno-Am (type 3) <sup>a</sup>               | ۲,  | ×                 |         |       |       |        | ×                  |   |          |         | ×                 |                   |   |      |                   |            |                  | ×       |          | ×          |
| Cornaceae, Cornus stolonifera L.,            |   | ×                 | ×       |       |       |        |                    |   |          |         |                   |                   | ×   | ×    |                   | ×          |                  | ×       |          |            |
| red osier dogwood                            |   |                   |         |       |       |        |                    |   |          |         |                   |                   |   |      |                   |            |                  |         |          |            |
| Fabaceae, Medicago minima L.,                |   |                   |         | ×     |       |        |                    |   |          |         |                   |                   |   |      |                   | ×          |                  | ×       |          |            |
| little bur-clover                            |   |                   |         |       |       |        |                    |   |          |         |                   |                   |   |      |                   |            |                  |         |          |            |
| Fabaceae, Medicago sativa L., alfalfa        | r ¥   | ×                 | ×       |       |       |        |                    | ř |          |         |                   |                   |   |      |                   |            |                  | ×       | ×;       | Ì          |
| Fabaceae, Kobinia pseudoacacia               |   |                   |         |       |       |        |                    | < |          |         |                   |                   |   |      |                   |            |                  |         | <        | <          |
| L., Diack locust                             |   |                   |         |       |       |        |                    |   |          |         |                   |                   |   |      |                   |            |                  |         |          |            |

(continued)

Table 3. Continued

| Plant taxa  |       |                 |       |       |       |       |      |             | Ь                | Pellet color |           |                   |          |        |          |                  |          |        | 0     | ropping    | Cropping system |  |
|---|-------|-----------------|-------|-------|-------|-------|------|-------------|------------------|--------------|-----------|-------------------|----------|--------|----------|------------------|----------|--------|-------|------------|-----------------|--|
|   | Ambeı | r Army<br>Green | 7 Ash | Black | Brown | Cream | Gold | Gray 1<br>B | Light C<br>Brown | Orange Pu    | ırple Red | Reddish<br>Orange | Tan Viol | et Whi | e Yellov | v Yellow<br>Gold | Yellow A | Almond | Blue- | Carrot     | Cherry M        | Amber Army Ash Black Brown Cream Gold Gray Light Orange Purple Red Reddish Tan Violet White Yellow Yellow Yellow Almond Blue- Carrot Cherry Meadowfoam  Green  Gold Green  Brown  Orange |
| Fabaceae, Trifolium arvense L., rahhirfoot clover             | ×     | ×               |       |       |       |       | ×    |             |                  | ×            |           | ×                 |          | ×      |          |                  |          |        | ×     |            | ×               |  |
| Fabaceae, Trifolium incarnatum                                | ×     |                 |       | ×     |       |       |      | ×           |                  |              |           | ×                 |          |        |          |                  |          | ×      |       | ×          | ×               | ×  |
| L., crimson clover<br>Fabaceae, <i>Trifolium pratense</i> L., |       |                 |       |       |       |       |      |             | ×                | ×            |           |                   |          |        |          |                  |          |        |       | ×          | ×               |  |
| red clover  |       | ;               | ;     |       | ;     |       |      |             |                  |              |           |                   |          |        | ;        |                  |          | ;      |       | ;          |                 |  |
| Fabaceae, <i>Trifolium repens</i> L.,<br>white clover         |       | ×               | ×     |       | ×     |       |      |             |                  |              |           |                   |          |        | ×        |                  |          | ×      |       | ×          |                 |  |
| Fagaceae, Quercus sp.   |       |                 |       |       |       |       |      |             | ×                |              |           |                   |          |        |          |                  |          |        | ×     | ×          |                 |  |
| Hippocastanaceae, Aesculus                                    |       |                 |       |       |       |       |      |             | ×                |              |           | ×                 | ×        |        |          |                  |          |        |       | ×          | ×               |  |
| hippocastanum L., horse                                       |       |                 |       |       |       |       |      |             |                  |              |           |                   |          |        |          |                  |          |        |       |            |                 |  |
| chestnut  |       |                 |       |       |       |       |      |             |                  |              |           |                   |          |        | >        | >                |          |        |       | >          | >               |  |
| Liliaceae   |       |                 |       |       |       |       | >    | >           |                  |              |           |                   |          |        | <        | <                |          |        | >     | <          | <               | >  |
| Linaceae, Annam sp.   |       |                 | >     | >     | >     |       | < >  | <           | >                | >            | >         |                   |          |        | >        |                  | >        |        | < >   | >          | >               | <  |
| Hartwex Benth meadowfoam                                      |       |                 | <     | <     | <     |       | <    |             | <                | <            | <         |                   |          |        | <        |                  | <        |        | <     | <          | <               |  |
| Oleaceae, Fraxinus sp.  | ×     |                 |       |       |       |       |      |             |                  |              |           |                   |          |        |          | ×                |          |        | ×     | ×          |                 |  |
| Pinaceae, Pinus sp.   |       |                 |       |       | ×     |       |      |             |                  |              |           |                   |          |        |          |                  |          |        |       | ×          |                 |  |
| Poaceae (type 1)  |       |                 |       |       |       |       |      |             |                  |              |           | ×                 |          | ×      |          |                  |          | ×      |       |            |                 | ×  |
| Poaceae (type 2)  |       |                 |       | ×     |       |       |      |             |                  |              |           |                   |          |        |          |                  |          |        |       | ×          |                 |  |
| Polygonaceae, Plantago sp.                                    |       |                 |       |       |       |       |      |             |                  |              |           | ×                 |          |        |          |                  |          |        |       |            |                 | ×  |
| Rosaceae, Fragaria sp.  |       | ×               |       | ×     |       |       |      |             |                  |              |           |                   |          |        |          |                  |          |        |       | ×          |                 | ×  |
| Rosaceae, Crataegus sp.                                       |       | ×               |       |       | ×     | ×     |      |             |                  |              |           | ×                 |          |        |          |                  | ×        | ×      | ×     |            | ×               |  |
| Rosaceae, Dryas drummondii                                    | ×     |                 |       |       | ×     |       |      |             |                  |              |           |                   |          |        | ×        |                  |          |        |       | ×          |                 |  |
| Richardson ex Hook.,  |       |                 |       |       |       |       |      |             |                  |              |           |                   |          |        |          |                  |          |        |       |            |                 |  |
| Decrees P.                |       | >               |       | >     |       | >     | >    |             | >                | >            | >         |                   | >        |        | >        | >                |          | >      | >     | >          | >               |  |
| Desaccae, France sp.  |       | <               | >     | <     | >     | <     | <    |             | <                | <            | <         |                   | <        |        | <        | <                |          | <      | <     | < >        | < >             |  |
| Nosaceae, 11010uiscus sp.                                     |       |                 | < >   |       | <     |       |      |             | >                |              |           |                   |          |        |          |                  |          | >      |       | < >        | <               |  |
| Rosaceae (type 1)   |       |                 | <     |       |       |       |      |             | <                |              |           |                   |          |        | >        |                  |          | < >    |       | < >        | >               |  |
| Kosaceae (type 2)   |       |                 |       | ì     |       |       |      |             | ,                |              |           |                   |          |        | < ;      |                  |          | <      | ļ     | <          | < ;             |  |
| Rosaceae (type 3)   |       | ;               | ;     | ×     |       |       |      |             | ×                |              |           |                   |          |        | ×        |                  |          | ;      | ×     | ;          | ×               |  |
| Scrophulariaceae, Fedicularis sp.                             |       | ×               | ×     |       |       |       |      |             |                  | >            |           |                   |          |        |          | >                |          | ×      |       | ×          | >               |  |
| Scrophulariaceae, Verbascum                                   |       |                 |       |       |       |       |      |             |                  | <            |           |                   |          |        |          | <                |          |        |       |            | <               |  |
| thapsus L., common mullein                                    | ;     | ;               |       |       |       |       |      |             |                  |              |           |                   |          |        |          |                  |          |        |       | ;          | ;               |  |
| Scrophulariaceae  | ×     | ×               |       |       |       |       |      |             |                  |              |           |                   |          |        |          | ,                |          |        |       | <b>×</b> ; | ×               |  |
| Stryacaceae, Halesia sp.                                      |       |                 |       |       |       |       |      |             |                  |              |           |                   |          |        |          | ×                |          |        |       | ×          |                 |  |
|   |       |                 |       |       |       |       |      |             |                  |              |           |                   |          |        |          |                  |          |        |       |            |                 |  |

<sup>a</sup>Cheno-Am refers to pollen samples within Chenopodiaceae or *Amaranthus* of Amaranthaceae.

subsequent crops such as blueberry, cherry, and pear when the colonies return to the Pacific Northwest.

The colonies placed in hybrid carrot seed crop collected the least amount of pollen. Previous studies have reported low visitation of honey bees to hybrid carrot seed flowers, apparently due to low availability of pollen and nectar in the flowers (Sagili et al. 2015). With flowers producing low amounts of pollen, honey bees may not visit flowers frequently enough for properly cross-pollination. The hybrid carrot seed sites contained male-sterile carrot varieties. Malesterile varieties do not produce pollen and are unattractive to honey bees foraging for pollen (Erickson et al. 1979). In addition, in the intensely farmed high desert area in which the hybrid carrot seed crop is grown, very few other flowering plant species are present to provide additional forage during carrot bloom. These factors likely contributed to the poor pollen foraging of bees in the hybrid carrot seed crop.

Colonies in blueberries also collected relatively low amounts of pollen. Previous studies support this finding (Girard et al. 2012, Pettis et al. 2013, Colwell et al. 2017). Blueberry flowers require buzz pollination, to dislodge pollen from the anthers by vibrational force (Buchmann 1983). Unlike bumble bees, honey bees do not vibrate blueberry flowers and hence are unable to trigger pollen release (Javorek et al. 2002). Further, the bell shape of blueberry flowers appears to be a limitation in pollen collection (Colwell et al. 2017). However, the absence of blueberry pollen in pollen trap collections may not indicate a total lack of pollen collection or pollen transfer by honey bees. There is evidence that honey bees will collect smaller pollen pellets from blueberry flowers (Hodges 1974). Smaller pollen pellets on honey bee corbiculae could fail to dislodge in pollen traps. Furthermore, another recent study documented the presence of blueberry pollen on various parts of the honey bee bodies that were visiting blueberry flowers (Hoffman 2018).

Low amounts of pollen availability to honey bee colonies can dramatically affect brood rearing (Al-Tikrity et al. 1972). Beekeepers that employ their colonies for pollination of crops like hybrid carrot seed and highbush blueberry should frequently assess the amount of pollen stores in their colonies and provide protein supplements if pollen stores are low. Although protein supplements are not equivalent to pollen in nutrient composition, past studies have reported increased brood rearing and decreased disease susceptibility associated with the use of protein supplements (Waller et al. 1981, Nabors 2000, van der Steen 2007, Sagili and Breece 2012).

The species diversity observed in pollen collected by colonies in almond crops was low when compared with pollen collected in other crops. We speculate that as almonds bloom early in the year when there are so few plant species in bloom, bees have paltry other forage options and hence primarily rely on almond pollen. Furthermore, the expansive and largely unbroken acreage of almond monoculture in parts of the northern and southern ends of California's San Joaquin Valley may further contribute to poor availability of diverse pollen. Hence, almond pollen is the predominant source of protein for honey bees that are employed for almond pollination.

Honey bees employed for cherry, highbush blueberry, hybrid carrot, and meadowfoam pollination collected pollen with high diversity relative to almond in this study. In April and May, when most cherries, blueberries, and meadowfoam crops bloom in the Pacific Northwest, many other blooming plant species are also available and serve as pollen source to honey bees. Similarily, when hybrid carrot blooms in June and July, bees also have access to several other

blooming plant species in the surrounding areas. The high pollen diversity observed in these cropping systems reflects an abundance of forage resources other than the target crop.

The presence of *Trifolium* spp. (clover) pollen in samples from all seven crops indicates that *Trifolium* is quite prevalent in most agricultural landscapes in the western United States and is likely an important nutritional resource for honey bees. Furthermore, *Taraxacum officinale* F.H. Wigg (common dandelion) pollen was also present in samples collected from all cropping systems. Common dandelion is considered to be a consistent (and sometimes emergency) source of food for bees in most of the cropping systems (Donkersley et al. 2017) and our findings further support this assertion.

Pollen diversity is important for the growth and development of bees (Wohl et al. 2004, Finke and Snyder 2008, Drescher et al. 2014, Donkersley et al. 2017). Additionally, it enhances honey bee immunohealth (Alaux 2010, Di Pasquale et al. 2013, Filipiak et al. 2017). The nutritional value of pollen includes crude protein content and amino acid composition (Crailsheim 1990). Pollen with crude protein of 21% or greater is considered a high-quality protein source (Radev 2018). Honey bees also need 10 essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) in their diet for normal growth and development (de Groot 1953). The probability of satisfying these specific amino acid proportions is high in the case of a polyfloral, or diverse, pollen diet, whereas low pollen diversity might be a limitation (Alaux et al. 2010). Future studies should examine the nutritional value of pollen available to bees during pollination events in these five cropping systems.

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