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Research

Pollinator Ecology and Management

Examination of a Managed Pollinator Strategy for Almond Production Using *Apis mellifera* (Hymenoptera: Apidae) and *Osmia lignaria* (Hymenoptera: Megachilidae)

Theresa L. Pitts-Singer,[1,4] Derek R. Artz,[1] Stephen S. Peterson,[2] Natalie K. Boyle,[1] and Gordon I. Wardell[3]

[1]USDA-Agricultural Research Service, Pollinating Insects Research Unit, Logan, UT 84322, [2]AgPollen, LLC, 14540 Claribel Road, Waterford, CA 95386, [3]The Wonderful Company, 33141 E Lerdo Highway, Bakersfield, CA 93308, and [4]Corresponding author, e-mail: theresa.pitts-singer@ars.usda.gov

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Abstract

Pollination services provided by managed bees are essential for California almond (*Prunus dulcis* Mill.; Rosales: Rosaceae) production. Currently, pollination needs are met by rented or owned *Apis mellifera* L. (Hymenoptera: Apidae; honey bee) colonies. Excessive demand on a challenged *A. mellifera* industry to provide strong colonies in early spring has caused sharp increases in rental prices over the past decade, inviting the consideration of alternative pollinators in addition to, or in place of, *A. mellifera*. *Osmia lignaria* Say (Hymenoptera: Megachilidae; the blue orchard bee) is an excellent pollinator of fruit and nut trees, but its pollination impacts when used in tandem with *A. mellifera* have yet to be evaluated in commercial almond orchards. A 2-yr study was conducted in California orchards to compare almond pollination and production using *A. mellifera* as sole pollinator to an alternative practice of adding *O. lignaria* as a co-pollinator with *A. mellifera.* Almond orchard managerial decisions, such as for pesticide use and irrigation intensity, vary between almond growing regions because of local climates. Therefore, both north-central and southern sites of California's San Joaquin Valley are represented. We compared bee visitation, nut set, and nut yield between orchards and between tree rows within orchards. Also, *O. lignaria* reproductive success was recorded to assure that these bees remained in the orchards as pollinators and to assess the ability to sustain these bees under regional orchard conditions. We demonstrated that augmenting large commercial almond orchards with *O. lignaria* can significantly increase nut set and sometimes nut yield in both regions evaluated.

Key words: Blue orchard bee, honey bee, crop pollination, reproductive success

Pollination services performed by *Apis mellifera* L. (Hymenoptera: Apidae; the honey bee) fill a critical role in meeting global food, fuel and fiber demands (Klein et al. 2007). Unquestionably, the *A. mellifera* industry is vital for providing bees for production of many crops in modern agriculture, as illustrated in California's absolute reliance on *A. mellifera* for pollinating its 380,400 ha (940,000 ac) of almonds (USDA NASS 2017). To service this enormous mass-blooming monoculture, approximately 1.9 million commercial honey bee colonies are needed. Although many *A. mellifera* colonies are maintained in California (Souza 2011), many hives are transported to California from other states where honey bees spend the winter so that they arrive in time for February almond bloom. A loss of almond pollination services would devastate California's ability to produce 81% of the world's almonds (Almond Board of California 2016), which boasted a state economic value of $5.33 billion in 2014 (CDFA 2015). The steady annual increase in California almond acreage coincides with an on-going struggle to maintain robust and abundant *A. mellifera* colonies. A broad spectrum of stressors, including mite infestation, disease, pesticide exposure, and poor nutrition continue to inhibit the ability to maintain strong colonies (Oldroyd 2007, vanEngelsdorp et al. 2009, Le Conte et al. 2010). Over the past decade, beekeepers have experienced high annual losses (~30%) of overwintering colonies (Steinhauer et al. 2016). Understandably, this intense demand by the almond industry on beekeepers to provide healthy colonies has resulted in exceptionally high prices for rentals and a scarcity of available colonies (Aizen and Harder 2009, Ward et al. 2010, Seitz et al. 2016). More than ever, orchardists would benefit from the adoption of alternative pollination strategies to bridge the widening gap between *A. mellifera* colony supply and demand.

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Osmia lignaria Say (Hymenoptera: Megachilidae; the blue orchard bee), is native to North America and offers great potential as an alternative pollinator of commercial almond orchards (Artz et al. 2013, 2014; Peterson and Artz 2014). As a solitary, cavity-dwelling species, managed *O. lignaria* readily nest in drilled wooden holes, hollow reeds, or cardboard tubes that can be distributed throughout orchards at sheltered sites (Bosch and Kemp 2001). *O. lignaria* overwinter as adults in cocoons and emerge naturally in early spring. These bees reliably visit rosaceous orchard and berry flowers in western U.S. growing regions (Bosch and Kemp 2000), although when distributed in orchards in the eastern United States, *O. lignaria* show a strong preference for visiting Eastern redbud (*Cercis canadensis*, F.) over orchard blossoms (Kraemer and Favi 2005, 2010; Kraemer et al. 2014). Under managed conditions, adult emergence can be manipulated to occur when bees are needed by implementing temperature-controlled winter storage followed by spring incubation. This ability is particularly valuable as it provides flexibility for delivering this bee to flowering crops that typically have a narrow bloom window (Bosch and Kemp 2001). Considering the high *O. lignaria* fidelity to orchard crops and the ability to manipulate their activity, the potential to apply *O. lignaria* has already been realized in several agricultural systems, including almonds, apples, and cherries (Torchio 1985, Bosch and Kemp 2000, 2002; Bosch et al. 2000, 2006; Artz et al. 2013; Boyle and Pitts-Singer 2017). For example, a study in a large almond production orchard (~61 ha) focusing on nest site distribution for *O. lignaria* flown in concert with *A. mellifera* resulted in at least an average nut yield for that orchard, even though only half of the recommended stocking density of each species was used (Artz et al. 2013, 2014). Furthermore, a study that engaged bees with almond trees revealed a functional synergy between *O. lignaria* and *A. mellifera* in the form of an increase in 1) pollen tubes reaching the base of the styles (successful pollination event) and 2) proportion of flowers that produced a nut, compared to when either species was caged alone with trees (Brittain et al. 2013). However, in a conventional almond orchard, fruit set and nut yield can vary substantially due to many factors that are independent of pollination efficacy. Some such factors are growing region, local climate, almond variety and management practices (e.g., watering, pesticide applications, and fertilization) (Tufts and Philip 1922, Flint 2002).

The goal of this study was to quantify the direct impact of *O. lignaria* on almond production when used to supplement a full stocking rate of *A. mellifera* for open-field pollination of conventionally managed, commercial orchards. We sought to determine: 1) whether the improved cross-pollen deposition and nut production observed by Brittain et al. (2013) in cages is upheld in an open, conventional context and 2) if geographic location, climate, and orchard management influence the relative success of mixed species pollination. By comparing nut set (pollination efficacy) and yield (pollination plus orchard management and environment) resulting from orchards pollinated by one or both pollinators, we evaluated the direct contributions of *O. lignaria* visitation to almond production. Furthermore, *O. lignaria* activity in the orchards was confirmed and evaluated by recording floral visitation and assessing *O. lignaria* nesting success (i.e., reproduction). Measuring nesting success of *O. lignaria* is critical to evaluating the feasibility of the use of these bees, because the wetter northern and central regions of California almond production are more frequently treated with fungicides during bloom than are southern regions. Wetter climates promote fungal diseases (California Department of Pesticide Registration 2013; CDFA 2014, 2015), and some fungicides have been implicated in disrupting *O. lignaria* retention and nesting behavior (Ladurner et al. 2008, Artz and Pitts-Singer 2015).

Materials and Methods

Study Sites and Conditions

In 2013 and 2014, we concurrently performed pollination experiments in orchards located in the north-central and southern San Joaquin Valley (hereafter NV and SV orchards, respectively). We worked in eight NV commercial almond orchards in 2013 (mean [±SE] orchard size: 35.1 ± 5.5 ha, range: 15.6–54.0 ha). Due to orchard conditions and availability of cooperating orchardists, only four of the same orchards were used in 2014, and four were selected anew (2014 mean orchard size: 25.8 ± 2.1 ha, range: 15.6–31.7 ha). NV orchards were in Stanislaus (37°42′N–120°43′W) and Merced Counties (37°27′N–120°33′W). In the SV, we worked in 12 commercial orchards in Kern County (35°30′N–119°42′W) in 2013 and 2014 (mean [±SE] orchard size: 97.4 ± 25.7 ha, range: 59.7–367.6 ha). All of the SV orchards were located in one of two large ranches (A and B) belonging to Wonderful Orchards Co. These ranches are continuous acreages of orchards, uniformly sectioned as rectangular blocks separated by roads used as 'beeways' where pallets of *A. mellifera* hives are located; they are managed by different ranch managers (in the same company). Ranch A is located northwest of Ranch B and separated by at least 12 km.

Although varying in size and shape, all of the experimental NV orchards in 2013 and 2014 were bordered by other almond orchards and either grassy rangelands, wetland habitat, or oak and willow riparian habitat (Fig. 1A). In most NV orchards, non-crop plants were kept at low densities by mowing and applying herbicides. All experimental SV orchards were bordered by almond, pistachio, or pomegranate orchards; therefore, none of the surrounding landscape offered non-almond floral resources for bees at this time of the year (Fig. 1B). According to company management standard practices, all of the SV orchard floors were nearly or completely devoid of vegetation. The distance between experimental orchards in each valley region was at least 1.6 km. Because the foraging range of female *O. lignaria* within a blooming orchard normally does not exceed 60 m from the nest site (Rust 1974, Biddinger et al. 2013 for *O. cornifrons*, Koh et al. 2017), this distance between orchards would prevent *O. lignaria* released for this study from dispersing to, or moving between, experimental orchards with nesting sites.

To obtain representative weather conditions of NV and SV, we searched 2013 and 2014 archived data from the National Environmental and Atmospheric Administration (NOAA; www.noaa.gov) stations for Modesto and Bakersfield, CA (located in Stanislaus and Kern Counties, respectively). We retrieved 2013 and 2014 maximum, mean, and minimum temperatures and precipitation data for February and March almond bloom season and for April–June early nut maturation season.

Northern Valley Orchard Set-up and *O. lignaria* Management

All eight NV orchards had a normal complement of *A. mellifera* colonies (two colonies per 0.4 ha), which were placed along orchard edges. Four of these orchards were selected for supplemental pollination with *O. lignaria*. Although orchard sizes were large, the areas in which we placed *O. lignaria* nesting sites and released bees were limited to 2 ha. Prior to almond bloom, *O. lignaria* artificial nesting blocks were affixed to trees (by plastic zip ties) at regular spacing (15 per 0.4 ha; 75 total per 2 ha plot) starting at 15 or 16 tree rows from the western orchard edge and extending to rows 44

Fig. 1. Aerial photographs of almond orchards (Google Maps) reveal (A) heterogeneous and nonuniform margins of one representative Northern San Joaquin Valley, CA orchard (NV) compared to (B) three homogeneous, uniformly rectangular orchards in the Southern Valley (SV). Trees rows are oriented north to south. Nesting sites were (C) laminated wooden layers stacked and bound to form blocks in NV orchards and (D) plastic corrugated boxes with suspended cardboard tubes in SV orchards. (E) Wooden sampling rectangle for collecting nuts with husks and debris from swept harvest rows between almond trees for gathering using a broom and bin.

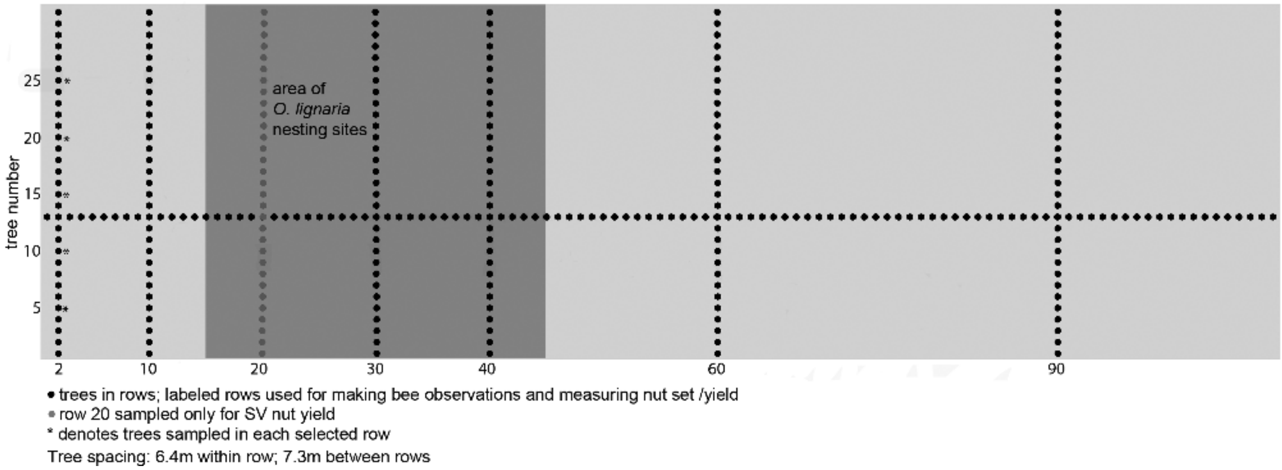

Fig. 2. Diagrammatic representation of almond orchard design demarking sampled tree rows and areas of *O. lignaria* nesting sites. The diagram represents one uniform, rectangle orchard (~16 ha; 40 ac). In sites in the Southern Joaquin Valley, CA, the adjacent orchard to the south of this orchard (e.g., Fig. 1) was also supplied with bees and bee nest boxes and was included in nut yield sampling. No bee observations were made nor was nut set assessed in the second, southern orchard.

or 45 (Figs. 1 and 2). Nesting blocks consisted of grooved wooden laminates with corrugated plastic roofs (Fig. 1C). Each of these blocks had 64 tunnels (7.5 mm diameter, 15.2 cm deep), and the face of each block was sprayed with a patented nest attractant (a version of an known attractive blend composed of three free fatty acids plus ethanol as solvent and dispensed with an aerosol can; Pitts-Singer et al. 2016).

In both years, all *O. lignaria propinqua* (the western U.S. subspecies of *O. lignaria*) used in NV orchards originated from wild-trapped Utah populations, but had been reared in California for several generations. After overwintering at 4°C, in 2013, a total of 8,000 *O. lignaria* females and approximately 16,000 males were incubated at 22°C beginning on 10 February for a planned stocking density of 1,000 females per ha (400 per ac). Bees were collected as they emerged and held in refrigeration until the orchards were nearing 10% of full bloom. Approximately 91% of the females emerged for an actual release rate of 910 *O. lignaria* females per ha (364 per ac). Fully-emerged *O. lignaria* males and females were mass-released on two dates in the center of each plot in each orchard (i.e., less than 60 m from any nesting site), with the first release (of mostly males) occurring on 23 February and the second on 24–25 February. A subsample of nesting sites was examined at night by peering into tunnels using a fine-focused light on 1 March and 11–12 March to estimate *O. lignaria* female retention in the orchard. Herbicides (glyphosphate, oxyflourfen), fungicides (pyraclostrobin/boscalid, iprodione, pyrimethanil), and/or both were applied in all eight orchards during bloom. No insecticides were sprayed during bloom. Nest blocks remained in the orchard until late April/early May when they were removed and then placed in storage under a fluctuating temperature regimen (14:27°C, 8:16 h). Once the bees had reached the cocooned adult (overwintering) stage in late July, storage temperature was dropped in steps to 4°C by 12 August 2013.

Using overwintered 2013 brood and purchased Utah bees, in 2014, a total of 7,963 *O. lignaria* females and approximately 16,000 males were incubated at 22°C beginning on 5 February for a planned stocking density of 995 females per ha (398 per ac). Bees were collected as they emerged and held in refrigeration until ready to release in the orchards. In this year, bloom was relatively early, and consequently, the bee release occurred when orchards were at about 55% of full bloom. Approximately 90% of the females emerged for an actual release rate of 358 *O. lignaria* females per 0.4 ha (895 per ha). Fully-emerged *O. lignaria* males and females were released as in 2013, with the first release occurring on 18–20 February and the second on 20–21 February. A subsample of nest blocks was examined at night on 25 February and 5 March to estimate *O. lignaria* female retention and nest establishment. All eight NV orchards were treated with fungicides twice during bloom from 17 to 26 February. No herbicides or insecticides were applied during bloom. Nest blocks remained in the orchard and managed as in 2013, and adults were placed in winter storage by 30 July 2014.

In both years, once orchard-produced progeny had reached adulthood in September while still in nest blocks, the blocks were opened to assess reproductive success. By carefully extracting cells from nests to preserve live bees, the number of nests and of all adult males and female cells were counted from all the nesting blocks. For a subsample of 10 nest blocks in 2013 and 20 nest blocks in 2014 from each orchard, we summed the number of cells (a 'cell' refers to an individual compartment within a nest tunnel) with a live cocoon, unconsumed provision, or parasite(s). Female and male cells were distinguished from each other using cell size and the position of the cell within the nest, with female cells being larger and in the first few positions in the back of the nest. Percent females per nest and unconsumed provisions per nest were estimated for NV orchards from these subsample counts. Eventually, all cells were removed from laminates, and only cells with live bees were retained for future use.

Southern Valley Orchard Set-up and *O. lignaria* Management

In 2013, six of the experimental orchards were located in Ranch A and stocked solely with *A. mellifera* colonies, while the other six orchards were located in Ranch B and stocked with *A. mellifera* colonies in combination with *O. lignaria*. In 2014, to accommodate newly recognized differences in management across ranches A and B, three of each of the treatments (with or without *O. lignaria*) were located in orchards of each ranch. *A. mellifera* stocking rate was 1.9 colonies per 0.4 ha in both study years. Selected SV orchards consisted of 109 rows of trees by 30–31 trees per row. Tree rows ended with bee ways, which also separated experimental orchards from adjacent ones (see Artz et al. 2013 for a detailed orchard description) (Figs. 1 and 2).

For SV *O. lignaria*-supplemented orchards, we placed nest boxes and released bees in two adjacent orchards, resulting in two *O. lignaria* nesting plots (4-ha each), designated as northern and southern blocks (Figs. 1 and 2). 48 navy blue corrugated plastic nest boxes (Boxes, Bags, and Labels, Tehachapi, California), each filled with 200 cavities (cardboard tubes with paper straw inserts), were used as nesting sites in both northern and southern blocks (as in Artz et al. 2013, 2014) (Fig. 1). The front ends of the cardboard tubes were treated with the bee attractant. Starting with tree row 17 (from the western orchard edge) and ending in tree row 44, nest boxes were uniformly distributed within each 4-ha plot (12 per ha) by hanging them from tree branches with metal hooks (Fig. 2). Accordingly, distance between nest boxes was a minimum of 32 m (N–S) and 25 m (E–W).

O. lignaria propinqua used in SV orchards were acquired and managed by Pacific Pollination Co., LLC (El Dorado, AR; no longer an active business) and were from Utah and Idaho populations. At Wonderful Orchards Co. facilities near Lost Hills, CA, wintering bees were kept in cold storage in darkness until just prior to almond bloom in early February. A total of approximately 48,000 adult *O. lignaria* females and 96,000 males were then incubated at 27°C until adult emergence from cocoons after about 1 wk.

At about 10% full bloom, on 20 February 2013 and 13–14 February in 2014, approximately 4,000 *O. lignaria* females and 8,000 males were released in each of the northern and southern blocks of designated SV orchards, respectively. As in the NV orchards, bee releases occurred at the center of each of the areas containing nest boxes (Fig. 2). No herbicides, insecticides, or fungicides were applied in the orchards during bloom and while bee nests were present.

Brood in nesting tubes remained in nest boxes in the orchard until late April/early May, at which point they were removed and placed in warehouse storage at a constant 26°C. In early August, the storage temperature was stepped down gradually, then held at a constant 4°C over the winter. During late August, batches of nests were methodically and temporarily removed from storage for evaluating SV *O. lignaria* reproduction using x-radiography (8-s exposure at 20 kVp) (Faxitron 43804N; Faxitron Bioptics, Tucson, AZ) to diagnose nest contents. We conducted a census of all completed nests and partially-completed nests, and collected the total number of cells, number of male and female cells (and calculated sex ratio), percentages of brood cells with unconsumed provisions, and overall mortality. Partially completed nests included nesting cavities containing at least one provisioned cell, in which the distinctive 'mud plug' at the end of the nest (signifying that the nest is complete; Fig. 1D) had not yet been made.

NV and SV Bee Observations and Almond Production Sampling

For all NV orchards (both with and without *O. lignaria*), five rows of Nonpareil trees were selected for data collection: Rows 2, 10, 30, 40, and 60 (or Rows 1, 9, 29, 39, and 59, depending upon cultivar spacing); distance from the western edge of the orchard being 16, 73, 219, 292, 438 m, respectively. Rows were selected to include those within, and on either side of, the area of *O. lignaria* nesting blocks, if added (Fig. 2); Rows 30 and 40 represented rows with *O. lignaria* nesting sites. In 2013 for eight NV orchards and in 2014 for seven orchards, we observed and recorded bees visiting flowers in five evenly-spaced trees for each of the five tree rows.

In 2013 and 2014 in only the SV six orchards with *O. lignaria* nesting sites (and none of the orchards without them), we made bee observations in five designated trees in each of Nonpareil rows 2, 10, 30, 40, 60, and 90 (or Rows 1, 9, 29, 39, 59, and 89); distance from the orchard edge of 16, 73, 219, 292, 438, and 658 m, respectively (Fig. 2). As in NV orchards, Rows 30 and 40 represented tree rows within the *O. lignaria* nesting area.

In both NV and SV orchards for the trees described above, bee visitation to flowers was recorded weekly throughout bloom on days having sunny to lightly overcast skies and temperatures above 13°C, with wind speed less than 2.5 m/s, and between 1000 and 1600 hours PST. Each observer visually divided the trees into four quadrants: top interior, top exterior, bottom interior, and bottom exterior. Every week, each quadrant in a tree was observed for 20 s for a total of 80 s per tree, and the number of visits by any bee or other insect to flowers was recorded.

Nut set assessments (and fruit set in 2014 in SV) were performed in all orchard (for both treatments) on the same tree row numbers described for bee observations (Fig. 2). During February, one branch from each tree was randomly selected and flagged 1 m from the terminal end of the branch. All flowers, including all flower buds, open flowers, and flowers that had lost their petals were counted along the 1-m tagged area of the branch, and data were recorded. We returned to the same branches in April/May (and March 2014) to count the number of developing nuts to calculate nut set (and fruit set). Nut set was the proportion of blossoms counted during bloom that were developing into nuts. Fruit set was collected prior to nut set and is the proportion of flowers showing very early development along those same branches. Fruit and nut set measurements often exceeded nut yield, as almond trees naturally abort developing nuts that they would be not physiologically able to support to maturity after this stage (Tombesi et al. 2011).

At the time of commercial harvest in late August, nuts are mechanically shaken from trees and swept into rows between trees. We collected mature nuts similar to Artz et al. (2013), by uniformly bisecting the windrows of nuts using a wooden frame (61 × 15 × 15 cm) and bagging the contents (nuts and debris) within the frame (Fig. 1E). Nut samples for NV were only collected in 2014 (NV samples $n = 25$ trees per orchard), but were collected in both study years in the SV orchards. Samples were taken from windrows next to the same Nonpareil trees that were flagged previously to evaluate nut set. In the SV orchards, we sampled nuts not only next to the flagged trees, but also took samples from the trees rows of the adjacent orchard just south of the first orchard (thus doubling the number of trees for mature nut sampling) (Fig. 2). Furthermore, we included samples from one additional tree row (Row 20) so that a total of 70 nut samples were collected for the SV orchards. All nuts were sifted, sorted, and counted in California, and random subsamples of 50 nuts from each tree were shipped to the USDA-ARS Pollinating Insects Research Unit in Logan, UT for shelling and weighing (fresh weight). The number of nuts in each sample multiplied by the average weight of a subsample of the nuts gave the calculated relative almond yield (by weight).

Data Analysis

SAS version 9.4 (SAS Institute 2008) was used for all statistical analyses. Because treatment assignments and ranch location were confounded in 2013 for SV orchards, we first looked for any broad significant differences between SV orchards due to ranch management and inherent almond production capabilities of orchards in 2013 and 2014. Company-provided commercial yields (reported in lbs/ac) for Nonpareil trees were compared by ranch and year using a general linear model (PROC GLM) followed by Tukey-Kramer multiple comparison tests. We similarly compared the yields we collected (number nuts in sample × mean kernel weight in gm) from SV orchards by ranch and year. To ensure that our research sampling efforts were representative of commercially-collected orchard yields, we looked for correlation (using PROC CORR) between yields as recorded by the company and our calculated yield estimates. Similar commercial yield data was not provided for NV orchards.

Next, generalized linear mixed models (PROC GLIMMIX; Gbur et al. 2012) were used to look for effects on the proportion nut set in May (number of maturing nuts/ number of blossoms counted earlier), and nut yield (gm) in August for NV and SV orchards. For SV 2014 only, we also examined the proportion fruit set in March (number of developing fruits/ number of blossoms; described above). For the NV orchards in 2013 and 2014, a beta distribution with a logit function was used to look for effects on the proportion nut set according to treatment (*O. lignaria* added or not), the row in which a sampled tree occurred, and their interaction. Windrowed nuts were sampled only in 2014 in NV orchards, and a lognormal distribution with identity link function was employed for analysis. Replicate was treated as a random factor for all analyses. Tukey-Kramer was used for multiple comparisons, and significance assigned as adjusted P-values ≤ 0.05.

The 2013 treatment assignments for SV orchards were made such that orchards in which *O. lignaria* were added occurred only in Ranch B, while orchards with only *A. mellifera* present occurred in Ranch A; yields greatly differed by ranch (see results below) in 2013. Additionally in 2013, due to limited time and labor force, nut set data was obtained for only three of the six orchards with only *A. mellifera*, and nut yield was not obtained for one of the six orchards that received *O. lignaria*. Consequently, we could not look for a 2013 SV treatment effect because of the ranch associations and because too few nut set samples were collected for the *A. mellifera* only treatment. However, we did look for effects of tree row on nut set and nut yield among orchards within each ranch. A beta distribution with logit link function was employed for analyses of nut set, with replicate as a random factor. A normal distribution with an identity link function was used to evaluate nut yield, looking for effects of tree row; replicate and within-site orchard (northern and southern) were random factors. Tukey-Kramer was used for multiple comparisons, and significance was based on adjusted P-values ≤ 0.05.

For 2014 SV orchards, treatments were reassigned to include orchards in each of the ranches used in 2013. Therefore, it was possible to examine the effects of ranch, treatment, tree row and their interactions on fruit and nut set using a beta distribution, with replicate as a random factor. Due to miscommunication over company harvest timing, nut yield was collected only for four orchard sites where only *A. mellifera* were present (two replicates each in Ranch A and Ranch B) and five sites where *O. lignaria* were added (two replicates in Ranch A and three in Ranch B). The model for nut yield analysis included a normal distribution with an identity link function. Ranch, treatment, and row were fixed factors; replicate and within-site orchard were random factors. As before, Tukey-Kramer was used for multiple comparisons.

Due to differences between NV and SV orchards in the assessments of reproduction of *O. lignaria*, no statistical comparisons for reproduction by location were conducted, but results were summarized and presented in relative terms.

Results

2013 and 2014 Weather Conditions

In the vicinity of NV orchards, the mean temperature during February bloom was 3.5°C cooler, and the area received slightly more (1 mm) precipitation, in 2013 than in 2014 (Table 1). However, for March 2013 compared to March 2014, mean temperatures were only less than 1°C cooler, but conditions were wetter with 7 mm more precipitation. The nearest national weather station to the SV orchards revealed that the 2013 mean temperatures were 3–4°C cooler in February and only about 1°C cooler in March than in 2014. Compared to 2014 levels, 2013 received 9 mm more precipitation in February and 12 mm more precipitation in March. Bloom was earlier in 2014 than in 2013, and therefore, bee releases were earlier in 2014 by 5 and 7 d for NV and SV orchards, respectively. During almond nut maturation (April–June), there was greater precipitation (13 mm) in 2014 than in 2013, but mean temperatures were nearly the same in both years, reaching between 35°C and 44°C in May–June in NV and SV locations.

To validate the claim that more fungicides are used in the northern and central areas of the San Joaquin Central Valley, queries were made into California pesticide databases to obtain reports on almond bearing acres and number of those acres sprayed with boscalid, a common fungicidal product. Records included applications for 1 February to 15 March in 2013 and 2014 for the counties of Butte (far northern region of almond production), Stanislaus (north-central region = NV orchards), and Kern (southern region = SV orchards). In Butte County, 40% and 56% of the bearing almond acres were sprayed in 2013 and 2014, respectively. For Stanislaus County almond acres, boscalid was sprayed on 20% in 2013 and 46% in 2014. For Kern County, 3 and 2% of the almond acres were sprayed in 2013 and 2014, respectively (California Department of Pesticide Registration 2013; CDFA 2014, 2015). Of the combined acres (of bearing and non-bearing trees) for these counties in the two study years, Butte County represented 11% of the total almond acres, Stanislaus County had 37% of the acreage, and Kern County hosted 52% (CDFA 2014, 2015).

Table 1. For 2013 and 2014, February and March temperature and precipitation from weather stations in Modesto and Bakersfield, CA representing North-central and Southern San Joaquin Valley, respectively

Modesto	2013	2014
Feb. mean maximum (°C)	16.7	19.9
Feb. mean (°C)	9.6	13.1
Feb. mean minimum (°C)	2.5	6.2
Feb. total precipitation (mm)	*8.1*	*7.1*
Mar. mean maximum (°C)	22.4	23.4
Mar. mean (°C)	15.3	15.9
Mar. mean minimum (°C)	8.2	8.3
Mar. total precipitation (mm)	*27.2*	*20.8*
Bee release in orchards	23–25 Feb.	18–21 Feb.
Bakersfield	2013	2014
Feb. mean maximum (°C)	17.7	19.9
Feb. mean (°C)	10.7	13.8
Feb. mean minimum (°C)	3.8	7.7
Feb. total precipitation (mm)	*15.2*	*8.1*
Mar. mean maximum (°C)	22.9	23.7
Mar. mean (°C)	15.8	16.8
Mar. mean minimum (°C)	8.8	9.8
Mar. total precipitation (mm)	*21.1*	*9.1*
Bee release in orchards	20 Feb.	13–14 Feb.

NV and SV Bee Visitation

In 2013 and 2014 in all NV orchards, *A. mellifera* and *O. lignaria* were the only bee species recorded on almond flowers during weekly observations. In 2013, only one *O. lignaria* was recorded (in Row 40 where nest sites were located) in any of the NV orchards (Fig. 1); all other recorded bee visits to almond flowers were by *A. mellifera* ($n = 315$). Also, one syrphid and one muscid fly were observed. Similarly, in 2014, *A. mellifera* was the only bee species recorded visiting almond flowers ($n = 284$) in any of the NV orchards; no *O. lignaria* adults were seen. Two syrphid and five muscid flies also were seen on almond flowers in this year.

In 2013 in SV orchards, mostly *A. mellifera* ($n = 487$) visited almond flowers during observations. Only 11 *O. lignaria* were observed on flowers, and they were seen in Rows 10, 30, 40, and 60; however, 71% of those visits were from Rows 30 and 40 where *O. lignaria* nest sites were located (Figs. 1 and 2). In 2014, 100% of the observed visits ($n = 7$) by *O. lignaria* females were on flowers in tree Rows 30 and 40. Again, the majority of visits recorded in 2014 were by *A. mellifera* ($n = 236$).

NV Orchards: Nut Set

Nut set in 2013 did not differ between treatments in NV orchards, i.e., when *A. mellifera* were present alone versus when *O. lignaria* were added to *A. mellifera* (Fig. 3A), although it was significantly affected by tree row (Table 2; Fig. 3B). The percentage of nut set in Row 2 was significantly lower than in Row 10 ($t = -5.13$, df = 180, $P < 0.0001$) and Row 60 ($t = -4.40$, df = 180, $P = 0.0002$); Row 10 nut set also was significantly higher than Row 30 ($t = 3.07$, df = 180, $P = 0.02$), where *O. lignaria* nests were located. In 2014, treatment affected nut set, with orchards containing *O. lignaria* achieving significantly higher nut set than when only *A. mellifera* were present (Fig. 4A). No significant differences were found by tree row or treatment × tree row (Fig. 4B).

Nuts were not collected from any NV orchards in 2013. In 2014, there were significant treatment and tree row effects, but no effect of treatment × row (Fig. 4A, C). Contrary to the percentage nut set recorded in May, orchards with only *A. mellifera* had higher nut yield than those with *O. lignaria* added. Across all orchards regardless of treatment, Row 10 yield was significantly greater than Row 60 yield ($t = -2.97$, df = 183, $P = 0.03$) (Fig. 4C).

SV Orchards: Fruit/Nut Set and Nut Yield

In 2013, May nut set (and no March fruit set) (Fig. 5A) was obtained for only three orchards stocked solely with *A. mellifera* colonies, and no statistically significant differences were found among the sampled tree rows of those three orchards (Table 2). For the six *Osmia*-supplemented orchards that were sampled, there was a significant difference in nut set among tree rows. Within these orchards, Row 2 had a significantly lower proportion of flowers that were maturing into nuts compared to Row 40, where *O. lignaria* nests were located ($t = -3.09$, df = 163, $P = 0.03$) (Fig. 5B). No significant differences in 2013 nut yield were observed between tree rows within each ranch (Fig. 6B).

Data obtained in 2014 included March fruit set for SV orchards (Fig. 6A). Fruit set was significantly affected by treatment; orchards supplemented with *O. lignaria* (Fig. 6A) showed higher fruit set

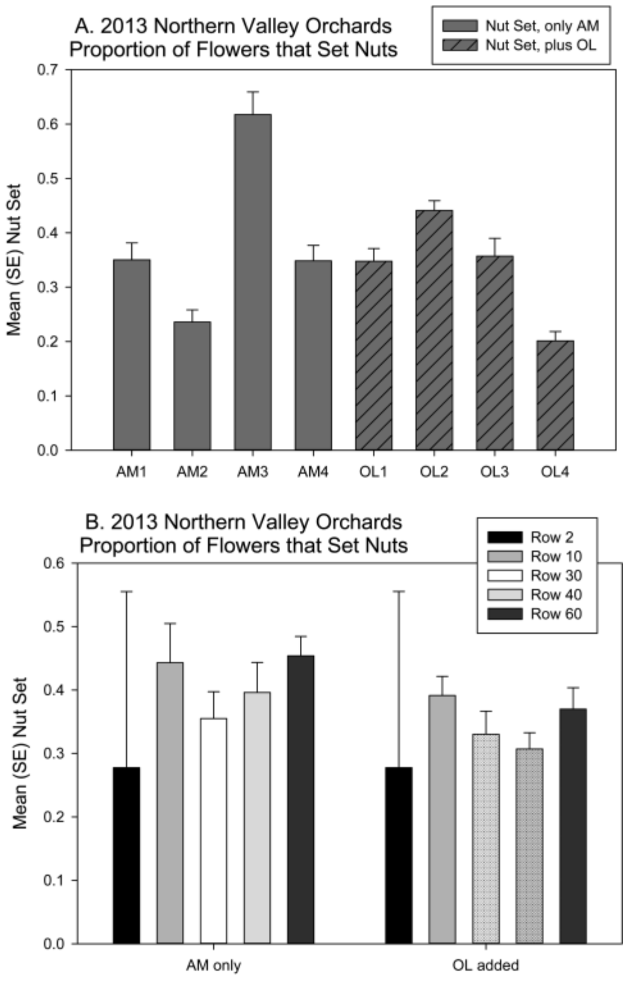

Fig. 3. 2013 mean (SE) nut set for eight commercial Northern San Joaquin Valley, CA almond orchards. (A) Comparison of orchards with only *A. mellifera* (AM) and with *O. lignaria* (OL) added. (B) Comparison of Nonpareil tree rows within orchards with only *A. mellifera* and with *O. lignaria* added. Dotted bars are where *O. lignaria* nest sites were located.

proportions compared to orchards without them. There were no effects of ranch, row, treatment × row, ranch × row, and ranch × treatment × row (Table 2). 2014 May nut set also was significantly affected by treatment and by treatment × row (Table 2). Ranch, row, ranch × row, and ranch × row × treatment were nonsignificant factors. Orchards supplemented with *O. lignaria* had significantly higher nut set (Fig. 6A) compared to orchards with only *A. mellifera*. Within the supplemented orchards, Row 2 had significantly lower nut set than Row 30 (where *O. lignaria* nest boxes were located) ($t = -3.37$, df = 292, $P = 0.04$), Row 60 ($t = -3.89$, df = 292, $P = 0.007$), and Row 90 ($t = -3.47$, df = 292, $P = 0.03$) (Fig. 6B). Other significant differences for any treatment × row effect were found only between rows of different ranches, so no further discussion is offered.

Nut yield was collected for nine SV orchard locations (north and south blocks of two adjacent orchards in most locations) in 2014, and significant effects on nut yield were found by treatment, tree row, ranch × treatment, and treatment × ranch × row. Contrary to the result for nut set, the yields where *O. lignaria* were added were less than where only *A. mellifera* was present. This was likely due to the very poor performance of the Ranch B orchard B2 (Fig. 6A), which is supported by the Tukey-Kramer comparison that found a significant result for only the effect of treatment within Ranch B, and not within Ranch A. No effects of ranch, ranch × row, or treatment × row were revealed. Table 3 reports the many significant pairwise results for tree rows, ranch × treatment, and treatment × ranch × row.

SV Orchards: Ranch Yields According to Company Records and Experimental Sampling

Commercial yields provided for the SV orchards were found to be significantly different according to ranch ($F = 16.85$, df = 1, $P = 0.0009$) and ranch × year ($F = 5.34$, df = 1, $P = 0.04$). Ranch A nut yield in 2013 exceeded nut yield in Ranch B in both 2013 ($P = 0.002$) and 2014 ($P = 0.02$) (Figs. 5A and 6A). Nut yield for Ranch A in 2013 was similar to Ranch A yield in 2014 ($P = 0.22$), and 2014 Ranch A yield was no different from 2013 or 2014 Ranch B yield (2013 $P = 0.15$; 2014 $P = 0.67$).

For nut samples taken from windrows in our study, a significant difference was found between ranches ($F = 10.22$, df = 1, $P = 0.006$), but there was no year ($F = 0.47$, df = 1, $P = 0.51$) or ranch × year ($F = 0.33$, df = 1, $P = 0.57$) effect. As with commercially obtained yields, Ranch A orchards had greater nut yields than Ranch B orchards (Figs. 5 and 6).

There was a strong positive correlation between commercial yield data provided by company cooperators and the experimental windrow sample yields that we collected (Pearson Correlation = 0.86, $n = 19$, $P < 0.0001$).

NV Orchards: *O. lignaria* Reproduction

O. lignaria nest site use and reproductive success greatly differed between years. Using the counts of females roosting in nest blocks during bloom, female establishment in the orchard ranged from 8 to 36% in 2013, and 6 to 23% in 2014. More than six times as many total nests and cells were made in NV nest blocks in 2013 (nests = 3,716; cells = 20,184) than in 2014 (nests = 620; cells = 3,186) (Table 4). In 2013, the production of female progeny matched or nearly matched the number of females released in two orchards (OL1 = 87% and OL2 > 100% female recovery, respectively), and there was a recovery range of 32–100% from in-orchard reproduction in this year. In 2014, the recovery of females for all orchards was only 7–22% across all orchards in the study. The poor recovery of bees in some 2013 orchards and all 2014 orchards was not due to mortality as failed cells (unconsumed provisions), which comprised less than 15% of cells made in each year. Fungicides were sprayed at least once in all orchards during nesting in both years, and, therefore, we cannot conclude that fungicide effects on *O. lignaria* females explain the severe lack of retention and reproduction in 2014. There was simply much less establishment and nesting in the provided nest blocks in 2014 compared to 2013 (Table 4). However, bee manager (author S.S.P.) records of bee release indicate that almond bloom in 2014 began earlier (13 February) than in 2013 (22 February) (Table 1). Anticipation of bloom-timing in 2014 was partly based on 2013 timing, and, therefore, the bees were incubated somewhat late for matching the early onset of 2014 bloom. Thus, the release of emerged adults occurred in orchards at about 55% of full bloom in 2014, compared to a better-timed bee release at 10% bloom in 2013. Bee nesting began in early March 2013 when bloom was reaching about 85%, but in 2014 nesting began when bloom was down to the last 15%. Mean March temperatures and precipitation also could have affected bee nesting, although they were similar in 2013 and 2014 (Table 1).

The proportion of female cells was similar across orchards at around 30% in 2013, but was generally higher in 2014. There were

Table 2. By region and year, the statistical output from a generalized linear mixed model for fruit set, nut set, and nut yield in Northern and Southern San Joaquin Valley, CA almond orchards

Northern Valley		2013			2014		
		F-value	df	*P*-value	*F*-value	df	*P*-value
Nut set	Treatment	1.61	1, 180	0.21	9.70	1, 187	0.002
	Tree Row	8.07	4, 180	<0.0001	0.20	4, 187	0.94
	Trtmt × Row	1.28	4, 180	0.28	1.39	4, 187	0.24
Nut yield	Treatment	-	-	-	3.95	1, 183	0.05
	Tree Row	-	-	-	2.74	4, 183	0.03
	Trtmt × Row	-	-	-	1.86	4, 183	0.12

Southern Valley		2013			2014		
		F-value	df	*P*-value	*F*-value	df	*P*-value
Fruit set	Treatment	-	-	-	491.59	1, 285	<0.0001
	Ranch	-	-	-	3.66	1, 285	0.06
	Ranch × Trtmt	-	-	-	1.20	1, 285	0.27
	Tree Row	-	-	-	1.85	5, 285	0.10
	Trtmt × Row	-	-	-	1.15	5, 285	0.33
	Ranch × Row	-	-	-	1.49	5, 285	0.19
	Ranch × Trtmt × Row	-	-	-	1.16	5, 285	0.33
Nut set	Treatment	-	-	-	82.89	1, 292	<0.0001
	Ranch	-	-	-	0.38	1, 292	0.54
	Ranch × Trtmt	-	-	-	0.00	1, 292	0.95
	Tree Row	2.47	5, 163	0.03	1.86	5, 292	0.10
	Trtmt × Row	-	-	-	2.30	5, 292	0.05
	Ranch × Row	-	-	-	1.47	5, 292	0.20
	Ranch × Trtmt × Row	-	-	-	2.04	5, 292	0.07
Nut yield	Treatment	-	-	-	9.38	1, 678	0.002
	Ranch	-	-	-	0.64	1, 678	0.43
	Ranch × Trtmt	-	-	-	11.47	1, 678	0.0007
	Tree Row	0.92	7, 807	0.49	5.86	7, 678	<0.0001
	Trtmt × Row	-	-	-	1.48	7, 678	0.17
	Ranch × Row	-	-	-	1.48	7, 678	0.17
	Ranch × Trtmt × Row	-	-	-	3.24	7, 678	0.002

The two treatments were 1) adding only *Apis mellifera* as pollinators and 2) adding both *A. mellifera* and *Osmia lignaria* as pollinators.

nearly two females per nest in all orchards, except two orchards in 2014 that had only about 1.5 females per nest.

SV Orchards: *O. lignaria* Reproduction

O. lignaria reproduction was less successful overall in SV orchards than in NV orchards, and SV bee reproduction in 2014 was less than in 2013 (Table 5). The number of nests made in nest boxes in 2013 (*n* = 16,234) was nearly 1.5 time more than in 2014 (*n* = 10,905). The 2013 cell production (*n* = 79,994) was 1.7 times that of 2014 production (*n* = 47,613). Across orchards in 2014, female progeny per nest was ≤1.5, and was <1.0 in most orchards. As such, the recovery of the female pollinators ranged from 3 to 77% in 2013 and 6 to 68% in 2014. The proportion of unconsumed provisions in one orchard in 2013 was quite high (33%) compared to all other orchards (range in both years = 2–18%), which helps to explain the very poor return on bees in that orchard. Another orchard had extremely poor return in 2014 (6%) that cannot be explained by the proportion of unconsumed provisions found in those nests, which was quite low (2%).

Similar to NV, the weather was somewhat cooler and wetter in 2013 than in 2014 for February and March, which may contribute to the differences in nesting success between years. The release of bees was similarly matched with almond bloom timing in each year (Table 1).

Discussion

Our study in large commercial almond orchards in north-central and southern growing regions of California's San Joaquin Valley demonstrates that nut set can be improved by the activity of *O. lignaria*, even when fungicides have been applied to orchards during bloom. However, the increase in nut set was not a certain outcome. Furthermore, May nut set (while nuts were still maturing) did not always match nut yield at the time of commercial harvest, and March fruit set that was measured only in 2014 was well above both nut set and yield. As such, nut yield often appeared unaffected by adding *O. lignaria*, even though nut set (i.e., potential nut production) often revealed high pollination efficiency when the two-pollinator species were present.

From an experimental design point of view, it was unfortunate that the treatment assignments in 2013 for the work done in SV orchards positioned all the orchards containing the addition of *O. lignaria* (in only small sections) in one ranch that had overall poorer nut yield compared to the ranch with *A. mellifera*-only orchards. Consequently, we were compelled to obtain and analyze recorded commercial Nonpareil yields from SV study orchards study according to ranch. In 2013, it is notable that when Ranch B commercial nut yields were comparatively low, the mean nut yield of research samples (dots in Fig. 5A) almost always were higher values than were their matching average commercial

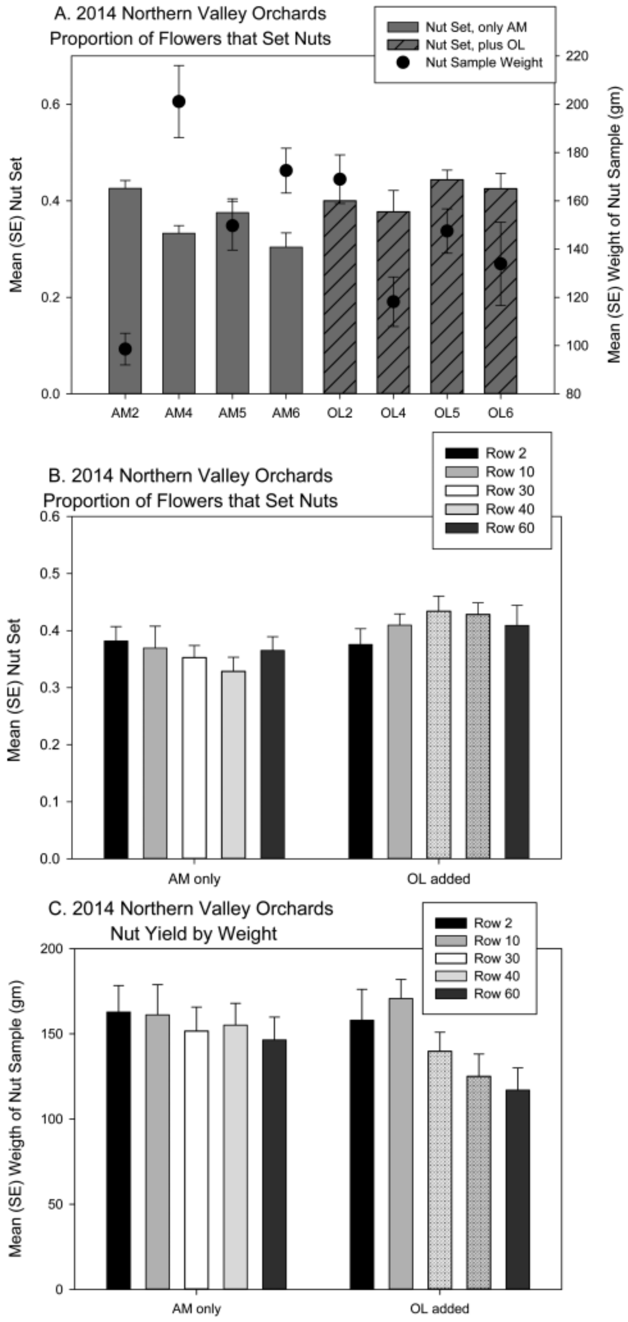

Fig. 4. For 2014, (A) mean (SE) nut set and nut sample weight for eight commercial Northern San Joaquin Valley, CA almond orchards, comparing orchards stocked only with *A. mellifera* (AM) to those with *O. lignaria* (OL) added. (B) Mean (SE) nut set comparing Nonpareil tree rows within orchards with only *A. mellifera* and with *O. lignaria* added. (C) Mean (SE) nut sample weight comparing rows within orchards with only *A. mellifera* and with *O. lignaria* added. Dotted bars are where *O. lignaria* nest sites were located.

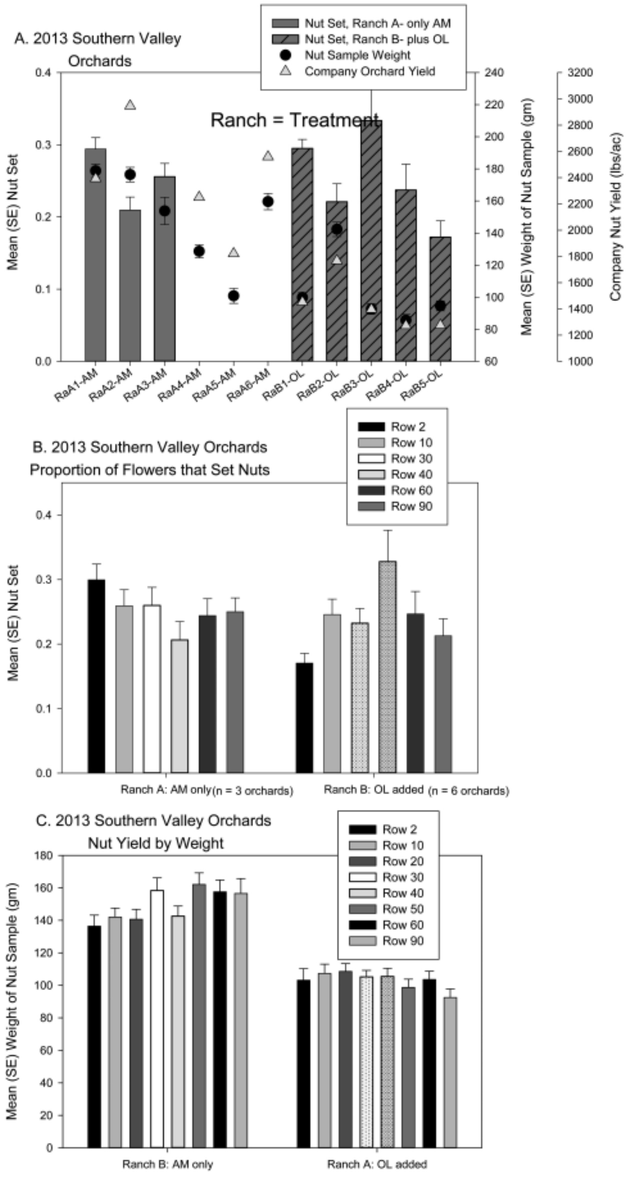

Fig. 5. For 2013, (A) mean (SE) nut set, nut sample weight, and company-reported yield for commercial almond orchards comparing orchards stocked only with *A. mellifera* (AM) to those with *O. lignaria* (OL) added. (B) Mean (SE) nut set comparing Nonpareil tree rows within orchards with only *A. mellifera* and with *O. lignaria* added. (C) Mean (SE) nut sample weight comparing rows within orchards with only *A. mellifera* and with *O. lignaria* added. Dotted bars are where *O. lignaria* nest sites were located.

yields. Our research samples were taken from tree rows largely within the foraging range of *O. lignaria*, and, thus, may be showing a local pollinator impact relative to the whole orchard performance. The same result was not seen in the better performing Ranch A that had only *A. mellifera*. We also did not see this kind of provocative result in 2014, when both ranches had similarly good commercial nut yield. We suggest the possibility that *O. lignaria* activity caused an increase in the nut set and yield of areas of the orchard (especially interior areas) where *A. mellifera* may be less efficient, perhaps due to environmental circumstances and forager preferences.

Our evidence of improved pollination efficacy due to *O. lignaria* in most of our evaluations agrees with another study that revealed an increase in fruit set due to interspecific interactions of *O. lignaria* and *A. mellifera* when they co-occurred in cages with almond trees compared to when either species were contained in separate cages (Brittain et al. 2013). Interactions between been species have not been observed directly, but Brittain et al. (2013) suggested an increase in bee foraging distance or a shift in honey bee foraging from within row flower visits to between row visits due to resource competition, or possibly the effect of scent-mark deposition on visited flowers that deters other bees from future visits to these flowers. Some of

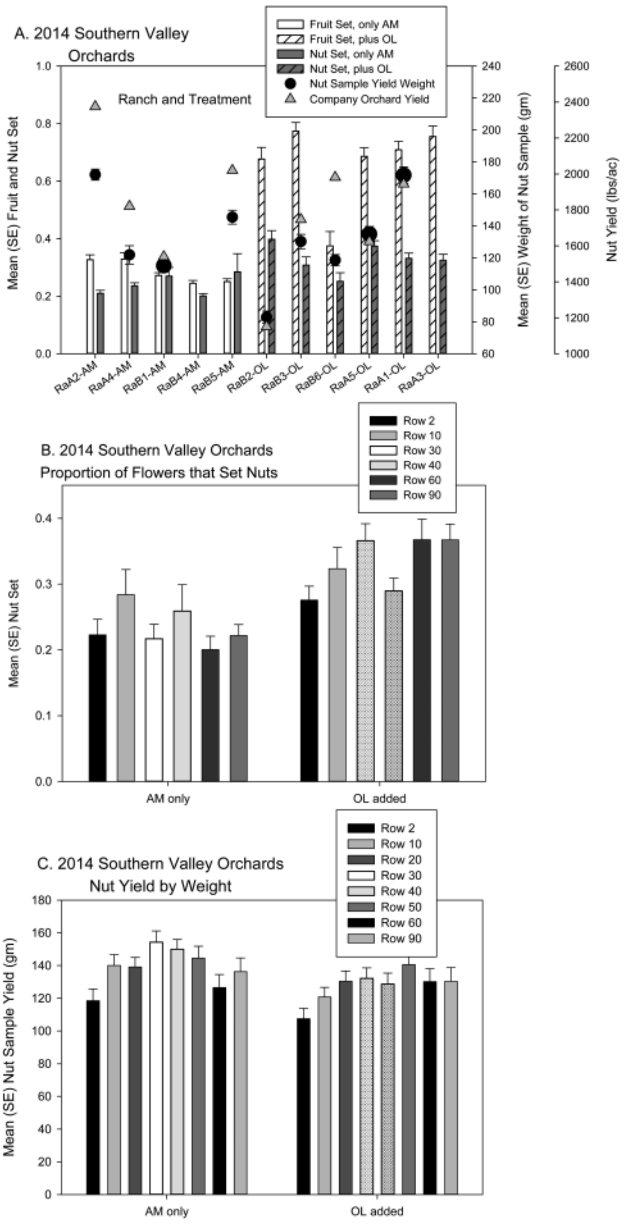

Fig. 6. For 2014, (A) mean (SE) fruit set, nut set, nut sample weight (yield), and company-reported yield for commercial almond orchards comparing orchards stocked only with *A. mellifera* (AM) to those with *O. lignaria* (OL) added. (B) Mean (SE) nut set comparing Nonpareil tree rows within orchards with only *A. mellifera* and with *O. lignaria* added. (C) Mean (SE) nut sample weight (yield) comparing rows within orchards with only *A. mellifera* and with *O. lignaria* added. Dotted bars are where *O. lignaria* nest sites were located.

our results also support a previous study where *O. lignaria* was used with half the recommended number of *A. mellifera* colonies, which resulted in improved nut yield near sites of high *O. lignaria* nesting activity (Artz et al. 2013, Koh et al. 2017). Other empirical studies have shown that synergisms emerging from the activity of different interspecific co-pollinators can alter bee foraging behavior or can increase the per-visit pollinator performance in other agriculturally-important crops (Greenleaf and Kremen 2006, Fulton et al. 2015, Park et al. 2016, Pisanty et al. 2016).

Table 3. Significant results for Tukey-Kramer multiple comparisons for Southern Joaquin Valley, CA orchards in 2014 for factors affecting nut yield (gm): tree row, treatment × ranch, and treatment × ranch × row

Comparison	*t*	*P*-value
Ol B, *Am* B	−5.56	<0.0001
r2, r10	−3.04	0.051
r2, r20	−3.81	0.004
r2, r30	−5.29	<0.0001
r2, r40	−4.66	0.0001
r2, r50	−5.16	<0.0001
r2, r90	−3.91	0.003
Ol A r2, *Am* A r30	−4.22	0.01
Ol A r90, *Am* A r60	3.91	0.03
Am A r2, *Am* A r30	−4.95	0.0004
Am A r2, *Am* A r40	−3.95	0.03
Am A r30, *Am* A r60	5.30	<0.0001
Am A r30, *Ol* B r1	4.93	0.0005
Am A r40, *Am* A r60	4.30	0.008
Am A r40, *Ol* B r1	3.91	0.04
Am A r60, *Am* B r20	−3.93	0.03
Am A r60, *Am* B r60	−4.05	0.03
Am A r60, *Am* B r90	−3.99	0.03
Ol B r2, *Am* B r10	−4.00	0.02
Ol B r2, *Am* B r20	−4.30	0.008
Ol B r2, *Am* B r40	−4.07	0.02
Ol B r2, *Am* B r60	−4.43	0.004
Ol B r2, *Am* B r90	−4.36	0.006

Degrees of freedom = 678. Negative *t*-values indicate that the least square (LS) means for the first component of the comparison is significantly less than the LS mean of the second component; positive *t*-values indicate that the LS means for the first component is significantly greater than that of the second component. A and B = Ranches; r = row; *Ol* = *Osmia lignaria* added; *Am* = only *Apis mellifera* present.

Most within-orchard comparisons between tree rows revealed only significant differences in nut set or nut yield between the orchard edge (Row 2) and the interior (Rows 10–90). Although we predicted that samples taken from tree rows within the areas bearing *O. lignaria* nesting sites (Rows 30 and 40) would have higher nut set values than those for other tree rows or from any row in orchards with only *A. mellifera* present, this prediction was not upheld. In most cases (NV 2014, SV 2013 and 2014), it appears that nut set was higher where *O. lignaria* nest sites were located in addition to rows further into the interior of the orchards (Rows 60 and 90). For the same rows in *A. mellifera*-only orchards, values for the far interior rows were lower than Rows 30 and 40. Again, the addition of *O. lignaria* may be boosting pollination activity and/or efficacy in areas of the orchard less efficiently visited by *A. mellifera*, even in areas beyond the vicinity of *O. lignaria* nest sites but still within foraging range.

In this study, we added *O. lignaria* in small portions of almond orchards that also contained full stocks of *A. mellifera* colonies. Retaining the full *A. mellifera* stocking rate mitigated the risk of yield loss while increasing pollination services by providing *O. lignaria*. We suspect that, in some instances, the addition of *O. lignaria* on top of the standard colony stocking rate oversaturated some of the orchards with pollinators and created additional competition (or synergistic interactions) between species for resources. A lack of significant increase in nut set or yield in orchards with *O. lignaria* (compared to those without) may be due to very high flower visitation and pollination in orchards with environmental and tree physiological conditions that did not permit nuts to develop. For instance, there are poorly understood variations in the annual

Table 4. Measures of *Osmia lignaria* reproductive success in commercial North-central Valley (NV) almond orchards in Stanislaus and Merced Counties, CA in 2013 (A) and 2014 (B)

A.

Orchard (% female establishment)	Total no. completed nests[b]	Total no. adult bee cells[b]	Total no. female cells[b]	Total no. male cells[b]	% Female cells	Females per nest[a]	% Unconsumed provisions (*n*)[a]	% Female recovery
OL1 (33%)	949	5,276	1,737	3,539	33	1.82	7% (338)	87%
OL2 (36%)	1,210	6,466	2,074	4,392	32	1.95	10% (742)	>100%
OL3 (31%)	1,180	5,871	1,804	4,067	31	1.84	8% (567)	90%
OL4 (8%)	377	2,571	648	1,923	25	1.97	8% (152)	32%

B.

Orchard (% female establishment)	Total no. completed nests[b]	Total no. adult bee cells[b]	Total no. female cells[b]	Total no. male cells[b]	% Female cells	Females per nest[a]	% Unconsumed provisions (*n*)[a]	% Female recovery
OL2 (10%)	127	586	186	400	32	1.21	14% (64)	9%
OL4 (6%)	134	893	402	491	45	1.96	3% (17)	20%
OL5 (23%)	91	348	135	213	39	1.57	13% (54)	7%
OL6 (21%)	268	1,359	432	927	32	1.92	10% (134)	22%

A total of ~400 *O. lignaria* females per 0.4 ha (actual females per 2 ha = 1,820 in 2013, and 1,990 in 2014) were released in each orchard along with males. Percent establishment of released females was obtained from highest count of females on 1 of 2 d when females were observed in nest cavities at night. Data in other columns refer to nests and offspring in them. Not all cells were counted for completed nests plus partial nests.

Differences in actual versus estimated values are noted: [a]Estimated from subsample of completed plus partials nests; [b]Actual counts.

Table 5. Measures of *Osmia lignaria* reproductive success in commercial Southern Valley (SV) almond orchards in Lost Hills, CA in 2013 (A) and 2014 (B)

A.

Orchard	Total no. nests (complete & partial)	Total no. cells	Total no. female cells	Total no. male cells	% Female cells	Females per nest	% Unconsumed provisions (*n*)	% Female recovery
RaB OL1	4,395	23,714	6,202	14,587	26	1.41	5% (1,129)	77%
RaB OL2	3,053	15,275	3,942	8,203	26	1.29	14% (2,178)	49%
RaB OL3	2,051	9,056	1,815	4,924	20	0.88	14% (1,306)	23%
RaB OL4	640	1,408	264	298	19	0.41	33% (459)	3%
RaB OL5	3,499	19,047	5,560	11,256	29	1.59	7% (1,255)	70%
RaB OL6	2,596	11,494	3,057	5,631	27	1.18	18% (2,020)	38%

B.

Orchard	Total no. nests (complete & partial)	Total no. cells	Total no. female cells	Total no. male cells	% Female cells	Females per nest	% Unconsumed provisions (*n*)	% Female recovery
RaB OL2	2,226	9,149	2,023	4,945	22	0.92	12% (1,053)	25%
RaB OL3	2,231	9,635	2,107	5,050	22	0.96	11% (1,047)	26%
RaB OL6	3,890	19,247	5,428	10,198	28	1.40	14% (1,376)	68%
RaA OL7	673	2,334	496	1,040	21	0.70	2% (366)	6%
RaA OL8	1,142	4,872	967	2,293	20	0.88	12% (592)	12%
RaA OL9	743	2,376	596	924	25	0.76	16% (382)	7%

A total of 48,000 fully-emerged adult *O. lignaria* females and approximately 96,000 males were released (4,000 *O. lignaria* females and 8,000 males were released in each of two 4-ha plots in each orchard). Values reported are pooled data from the two 4-ha blocks within each orchard. Data refer to offspring of released females and are based on actual counts.

performance of orchards associated with the ability of the almond spurs (shoots that develop from dormant buds) to thrive and to bear fruit each year (Tombesi et al. 2011). We also have no data on the *A. mellifera* colony strength in each of the orchards evaluated, which also could have affected yield results. Differences between orchards in retention of *O. lignaria* females and in nesting success will also have been reflected in nut production.

We learned that there could be large variability between almond orchards and by year. In some years and locations, our results imply that the addition of *O. lignaria* to orchards may provide an almond grower the chance to achieve a greater than expected yield in some areas of orchards and/or under certain weather conditions for some orchards. Adding more *A. mellifera* colonies may not necessarily translate into higher nut set or yield (Traynor 1993), and the ability of an almond tree to approach 100% nut set from its flowers and bear all the nuts to maturity is currently limited and poorly understood (Tombesi et al. 2011). Adding a synergizing bee species may actually be a mechanism by which pollination efficacy can be enhanced at the level of the individual tree only as long as other physiological needs are met. Orchard-level yields may also be increased if the behavior of another pollinator promotes pollination service in less preferred areas of orchards, or if the co-pollinator is more amenable to harsh weather conditions (as is *O. lignaria*) and are pollinating flowers while *A. mellifera* remain in the hive.

A cost-savings opportunity for equaling or increasing nut yield by using only half the regular stocking rate of *A. mellifera* along with a complementary stocking rate of *O. lignaria* is a pollination strategy exemplified in previous studies (Artz et al. 2013, 2014). Economic viability would only be feasible if the cost of using *O. lignaria* was less than, or equal to, that of *A. mellifera* on a per hectare basis. It would further be prudent to investigate whether interspecific competition between these managed pollinators impedes *O. lignaria* reproduction and population sustainability or its increase. A negative economic impact on availability and health of a currently limited supply of this native solitary bee may negate any rewards of increased nut set and yield.

We evaluated *O. lignaria* nesting success to assure that there were bees actively foraging on orchard flowers and to assess the feasibility of in-orchard reproduction at a sustainable level. Bee establishment of nests in the provided artificial tunnels varied between orchards and years in both NV and SV regions. Good establishment requires a well-timed release of bees with orchard bloom, favorable weather conditions for bee flight, and presence of wet soil for nest-building. However, even if all conditions are favorable, the brief almond bloom period offers floral resources for nesting for only about 2 wk of an *O. lignaria* female's potential lifespan of 6 wk. In 2013, three of the four NV orchards had good establishment and production of *O. lignaria* progeny. The one NV orchard that had poor *O. lignaria* establishment (8%) in 2013 may have been a result of the pre-bloom application of esfenvalerate, which is a pyrethroid insecticide that is highly toxic to both *O. lignaria* and *A. mellifera* (Hooven et al. 2014), whose active ingredient has a half-life twice as long as some other pyrethroids (>14 d) (Dupont 2006) and was not applied to any of the other three NV orchards in this study. This same orchard with poor nesting establishment and reproduction observed also had the lowest nut set of the four *O. lignaria*-supplemented NV orchards. For all NV orchards, the benefit of the better nesting in 2013 did not result in significant increases in nut set or yield, as it did in 2014. For SV orchards in 2014, fruit set was dramatically higher in all *O. lignaria*-supplemented orchards compared to the *A. mellifera*-only orchards, and was higher than both the later nut set and yield within treatment. Contrary to expectation, nut yield in SV orchards where *O. lignaria* were added was lower than in orchards where they were absent. We cannot explain this counter-intuitive outcome without further investigation.

Another objective of this study was to explore differences between two growing regions, NV and SV. Based on anecdotes from *O. lignaria* researchers and users and on follow-up experimental evidence (Artz and Pitts-Singer 2015), we expected that the eminent and consistent use of fungicides in the NV orchards would disrupt *O. lignaria* nesting and possibly foraging behavior, which would be evidenced by poor bee retention (if not death) and low reproductive success. Such evidence was not gained by this study. Retention and nesting success of enough bees to provide a 100% return on released females only occurred in 2013 in one of the NV orchards (and never met 100% return in any SV orchards). Mean recovery of females was about 44% for both NV and SV orchards in 2013, and was much less in both regions in 2014, with 14.5% recovery in NV and 24% in SV. Field studies specifically designed for testing the effects of fungicides on bee retention and reproduction in commercial orchards are needed to fully understand interactions of these bees when stressed with pesticides or other various environmental conditions.

The higher rates of successful nesting in NV orchards may have been a consequence of the availability of supplementary floral resources within the surrounding, heterogeneous landscape, compared to the lack of any such resources near the SV orchards. Floral resources found within the grassy rangelands, wetland habitat, or oak and willow riparian habitats near the NV orchards could have extended the foraging season or enhanced the nutritional quality of *O. lignaria* larval food. Although *O. lignaria* generally inhabit a foraging range of around 60 m (Rust 1974), they are capable of foraging distances of at least 800 m for alternative floral resources (N.K.B., unpublished data). Additionally, riparian and wetland areas, by definition, would have likely provided reliable access to wet soil or mud—a critical component for *O. lignaria* nest-building and a resource that may have been more limited in the drier SV orchards.

In both NV and SV orchard locations, we rarely observed *O. lignaria* visiting almond blossoms despite the high rate of overall nesting observed in some orchards and the significant impact they often had on nut set. Besides the vast difference in the number of bees from *A. mellifera* colonies (tens of thousands per ha) versus the solitary *O. lignaria* populations (hundreds per ha), flight behavior and flower visitation differ between the two pollinator species. Compared to *A. mellifera*, *O. lignaria* fly higher in the canopy (D.R.A., N.K.B., and S.S.P., personal observation) and move more frequently between tree rows (Bosch and Blas 1994 for *O. cornuta*, Bosch et al. 2006 for *O. lignaria*). Therefore, researchers standing on the ground observed *A. mellifera* more frequently than they observed *O. lignaria*. In this case, observations of *O. lignaria* visitation to blossoms fail to provide an effective measure of their contribution to commercial pollination services.

Using many orchards in varying local and distant locations presented many challenges for this empirical study. First, orchards performed differently from each other and between years, and we were unable to sample all the same orchards in the two study years. Second, despite high nut set, June nut drop dramatically reduced potential mature nut yield. This natural thinning process is a period when developing fruits are aborted due to the physiological and photosynthetic constraints of the trees (e.g., water stress through poor irrigation strategies, high canopy temperatures and/or high irradiance substantially increasing water loss through crop evapotranspiration) (Brittain et al. 2014, Klein et al. 2015) as well as pre- and post-harvest orchard irrigation regimens (Goldhamer and Viveros 2000). Third, bloom timing is somewhat hard to predict and to synchronize with the emergence of solitary bees in the early spring, and matching bee nesting activity for the duration of the short almond bloom period is critical. *O. lignaria* management practices must be refined to accommodate this component of providing future pollination services. The ability to transport *A. mellifera* hives and feed them artificial diets prior to bloom is an advantage that the use of these bees has over the use of *O. lignaria*. Lastly, other challenges beyond optimizing pollination exist (environmental or managerial) for nut production. To maximize crop yield and quality, almond trees require sufficient water. Not only do water deficiencies affect almond trees in the year in which the stress occurs, but they can also negatively affect the nut yield and quality, particularly nut size, in subsequent years (Goldhamer and Viveros 2000, Esparza et al. 2001). During the nut maturation period, there was more precipitation in 2014 than in 2013, but whether this difference was enough to influence June nut drop and ultimately nut yield is not known.

Our study and others by our research group demonstrate the use of a second managed pollinator to complement *A. mellifera* as an integrated crop pollination (ICP) strategy (Isaacs et al. 2017). It presents an opportunity to relieve pressure on the *A. mellifera* industry for having to accommodate such a huge almond industry in the very early spring and also on the ability of almond growers to increase their overall yield. If more broadly incorporated into standard orchard management, further considerations will have to be met to maintain and protect a managed, univoltine solitary bee species such as *O. lignaria*. Bee managers and pollination providers must be trained and kept abreast of management enhancements to reduce the cost of using *O. lignaria* as an almond pollinator and increasing these commercial populations. Almond growers throughout California will continue to protect their crop year-round with applications of insecticides, fungicides, and herbicides. The routes of bee exposure to pesticides, their residuals and metabolites, surfactants, and their interacting synergistic effects, need to be examined to safeguard commercially-managed *O. lignaria* and *A. mellifera*, to assure no sublethal effects on nesting and reproductive success, and to protect progeny developing in nests and colonies within orchard environments (Thompson and Wilkins 2003, Ladurner et al. 2005, Ladurner et al. 2008, Artz and Pitts-Singer 2015, Fine et al. 2017).

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References Cited

Aizen, M. A., and L. D. Harder. 2009. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Curr. Biol. 19: 915–918.

Almond Board of California. 2016. Almond almanac 2016 annual report. http://www.almonds.com/sites/default/files/misc/Almanac/2016_almond_almanac.pdf.

Artz, D. R., M. J. Allan, G. I. Wardell, and T. L. Pitts-Singer. 2013. Nesting site density and distribution affects *Osmia lignaria* (Hymenoptera: Megachilidae) reproductive success and almond yield in a commercial orchard. Insect Conserv. Divers. 6: 715–724.

Artz, D. R., M. J. Allan, G. I. Wardell, and T. L. Pitts-Singer. 2014. Influence of nest box color and release sites on *Osmia lignaria* (Hymenoptera: Megachilidae) reproductive success in a commercial almond orchard. J. Econ. Entomol. 107: 2045–2054.

Artz, D. R., and T. L. Pitts-Singer. 2015. Effects of fungicide and adjuvant sprays on nesting behavior in two managed solitary bees, *Osmia lignaria* and *megachile rotundata*. Plos One. 10: e0135688.

Biddinger, D. J., N. K. Joshi, E. G. Rajotte, N. O. Halbrendt, C. Pulig, K. J. Naithani, and M. Vaughan. 2013. An immunomarking method to determine the foraging patterns of *Osmia cornifrons* and resulting fruit set in a cherry orchard. Apidologie 44: 738–749.

Bosch, J., and M. Blas. 1994. Foraging behavior and pollinator efficiency of *Osmia cornuta* and *Apis mellifera* on Almond (Hymenoptera, Megachilidae and Apidae). Appl. Entomol. Zool. 29: 1–9.

Bosch, J., and W. P. Kemp. 2000. Development and emergence of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). Environ. Entomol. 29: 8–13.

Bosch, J., and W. P. Kemp. 2001. How to manage the blue orchard bee as an orchard pollinator. Sustainable Agricultural Network, Handbook No. 5, Beltsville, MD.

Bosch, J., W. P. Kemp, and S. S. Peterson. 2000. Management of *Osmia lignaria* (Hymenoptera: Megachilidae) populations for almond pollination: methods to advance bee emergence. Environ. Entomol. 29: 874–883.

Bosch, J., and W. P. Kemp. 2002. Developing and establishing bee species as crop pollinators: the example of *Osmia* spp. (Hymenoptera: Megachilidae) and fruit trees. Bull. Entomol. Res. 92: 3–16.

Bosch, J., W. P. Kemp, and G. E. Trostle. 2006. Bee population returns and cherry yields in an orchard pollinated with *Osmia lignaria* (Hymenoptera: Megachilidae). J. Econ. Entomol. 99: 408–413.

Boyle, N. K., and T. L. Pitts-Singer. 2017. The effect of nest box distribution on sustainable propagation of *Osmia lignaria* (Hymenoptera: Megachilidae) in commercial tart cherry orchards. J. Insect Sci. 17. doi:10.1093/jisesa/iex008.

Brittain, C., N. Williams, C. Kremen, and A. M. Klein. 2013. Synergistic effects of non-*Apis* bees and honey bees for pollination services. Proc. Biol. Sci. 280: 20122767.

Brittain, C., C. Kremen, A. Garber, and A. M. Klein. 2014. Pollination and plant resources change the nutritional quality of almonds for human health. Plos One. 9: e90082.

California Department of Food and Agriculture (CDFA). **2014.** http://www.almonds.com/sites/default/files/content/attachments/2013_nass_acreage_report.pdf.

California Department of Food and Agriculture (CDFA). **2015.** https://www.cdfa.ca.gov/Statistics/.

California Department of Pesticide Registration. 2013. California Pesticide Information Portal. http://calpip.cdpr.ca.gov.

Dupont. 2006. Dupont Asana XL Insecticide. Technical bulletin. http://www.rrsi.com/wp-content/uploads/2014/05/Asana_XL_Tech_Sheet.pdf.

Esparza, G., T. M. DeJong, S. A. Weinbaum, and I. Klein. 2001. Effects of irrigation deprivation during the harvest period on yield determinants in mature almond trees. Tree Physiol. 21: 1073–1079.

Fine, J. D., D. L. Cox-Foster, and C. A. Mullin. 2017. An inert pesticide adjuvant synergizes viral pathogenicity and mortality in honey bee larvae. Sci. Rep. 7: 40499.

Flint, M. L. 2002. Integrated pest management for almonds, 2nd ed. University of California Agriculture and Natural Resources Publication, Davis, CA, p. 3308.

Fulton, M., L. K. Jesson, K. Bobiwash, and D. J. Schoen. 2015. Mitigation of pollen limitation in the lowbush blueberry agroecosystem: effect of augmenting natural pollinators. Ecosphere 6: 235.

Gbur, E. E., W. W. Stroup, K. S. McCarter, S. Durham, L. J. Young, M. Christman, M. West, and M. Kramer. 2012. Analysis of generalized linear mixed models in the agricultural and natural resources sciences. American Society of Agronomy, Madison, WI.

Goldhamer, D. A., and M. Viveros. 2000. Effects of preharvest irrigation cutoff durations and postharvest water deprivation on almond tree performance. Irrigation Sci. 19: 125–131.

Greenleaf, S. S., and C. Kremen. 2006. Wild bees enhance honey bees' pollination of hybrid sunflower. Proc. Natl. Acad. Sci. U. S. A. 103: 13890–13895.

Hooven, L., Sagili, R., and E. Johansen. 2014. How to reduce bee poisoning from pesticides. Pacific Northwest Extension Publication, PNW 591, Oregon State University, Corvallis, OR.

Isaacs, R., N. Williams, J. Ellis, T. L. Pitts-Singer, R. Bommarco, and M. Vaughan. 2017. Integrated crop pollination: combining strategies to ensure stable and sustainable yields of pollination-dependent crops. Basic Appl. Ecol. 22: 44–60.

Klein, A. M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Proc. Biol. Sci. 274: 303–313.

Klein, A. M., S. D. Hendrix, Y. Clough, A. Scofield, and C. Kremen. 2015. Interacting effects of pollination, water and nutrients on fruit tree performance. Plant Biol. (Stuttg). 17: 201–208.

Koh, I., E. V. Lonsdorf, D. R. Artz, T. L. Pitts-Singer, and T. H. Ricketts. 2017. Ecology and economics of using native managed bees for almond pollination. J. Econ. Entomol. doi:10.1093/jee/tox318.

Kraemer, M. E., and F. D. Favi. 2005. Flower phenology and pollen choice of *Osmia lignaria* (Hymenoptera: Megachilidae) in central Virginia. Environ. Entomol. 34: 1593–1605.

Kraemer, M. E., and F. D. Favi. 2010. Emergence phenology of *Osmia lignaria* subsp. *lignaria* (Hymenoptera: Megachilidae), its parasitoid Chrysura kyrae (Hymenoptera: Chrysididae), and bloom of *Cercis canadensis*. Environ. Entomol. 39: 351–358.

Kraemer, M. E., F. D. Favi, and C. E. Niedziela. 2014. Nesting and pollen preference of *Osmia lignaria lignaria* (Hymenoptera: Megachilidae) in Virginia and North Carolina orchards. Environ. Entomol. 43: 932–941.

Ladurner, E., J. Bosch, W. P. Kemp, and S. Maini. 2005. Assessing delayed and acute toxicity of five formulated fungicides to *Osmia lignaria* Say and *Apis mellifera*. Apidologie 36: 449–460.

Ladurner, E., J. Bosch, W. P. Kemp, and S. Maini. 2008. Foraging and nesting behavior of *Osmia lignaria* (Hymenoptera: Megachilidae) in the presence of fungicides: cage studies. J. Econ. Entomol. 101: 647–653.

Le Conte, Y., M. Ellis, and W. Ritter. 2010. *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? Apidologie 41: 353–363.

National Oceanic and Atmospheric Administration (NOAA). 2017. Climate Data Online Search. https://www.ncdc.noaa.gov/cdo-web/datasets#GSOM.

Oldroyd, B. P. 2007. What's killing American honey bees? Plos Biol. 5: e168.

Park, M. G., R. A. Raguso, J. E. Losey, and B. N. Danforth. 2016. Per-visit pollinator performance and regional importance of wild *Bombus* and *Andrena* (*Melandrena*) compared to the managed honey bee in New York apple orchards. Apidologie 47: 145–160.

Peterson, S. S., and D. R. Artz. 2014. Production of solitary bees for pollination in the United States, pp. 653–681. *In* J. A. Morales-Ramos, M. G. Rojas and D. I. Shapiro-Ilan (eds.), Mass production of beneficial organisms: invertebrates and entomopathogens. Elsevier Science, London, UK.

Pisanty, G., O. Afik, E. Wajnberg, and Y. Mandelik. 2016. Watermelon pollinators exhibit complementarity in both visitation rate and single-visit pollination efficiency. J. Appl. Ecol. 53: 360–370.

Pitts-Singer, T. L., W. P. Kemp, D. Moreland, S. Peterson, J. S. Buckner, and M. Hagen. 2016. U.S. Patent No. US 9,301,501 B2. Bee Attractants.

Rust, R. W. 1974. The systematics and biology of the genus *Osmia*, subgenera *Osmia*, *Chalcosmia*, and *Cephalosmia*. Wasmann J. Biol. 32:1–93.

SAS Institute. 2008. SAS user's guide, version 9.4. SAS Institute Inc., Cary, NC.

Seitz, N., K. S. Traynor, N. Steinhauer, K. Rennich, M. E. Wilson, J. D. Ellis, R. Rose, D. R. Tarpy, R. R. Sagili, D. M. Caron, et al. 2016. A national survey of managed honey bee 2014–2015 annual colony losses in the USA. J. Apic. Res. 54: 292–304.

Souza, C. 2011. What's the buzz about pollination? http://californiabountiful.com/features/article.aspx?arID=845.

Steinhauer, N., K. Rennich, D. Caron, K. Delaplane, J. Rangel, R. Rose, R. Sagili, J. Skinner, J. T. Wilkes, M. E. Wilson, et al. 2016. Colony loss 2015- 2016: preliminary results. Bee Informed Partnership, https://beeinformed.org/results/colony-loss-2015-2016-preliminary-results/.

Thompson, H., and S. Wilkins. 2003. Assessment of the synergy and repellency of pyrethroid/fungicide mixtures. Bull. Insect. 56: 131–134.

Tombesi, S., B. D. Lampinen, S. Metcalf, and T. M. DeJong. 2011. Relationships between spur- and orchard-level fruit bearing in almond (*Prunus dulcis*). Tree Physiol. 31: 1413–1421.

Torchio, P. F. 1985. Field experiments with the pollinator species, *Osmia lignaria propinqua* Cresson, in apple orchards: V (1979–1980), methods of introducing bees, nesting success, seed counts, fruit yields (Hymenoptera: Megachilidae). J. Kansas Entomol. Soc. 58: 448–464.

Traynor, J. 1993. Almond pollination handbook. Kovak Books, Bakersfield, CA.

Tufts, W. P., and G. L. Philip. 1922. Almond pollination. University of California Extension Bulletin 346, Berkeley, CA.

USDA National Agricultural Statistical Service (NASS). 2017. 2016 California Almond Acreage Report. https://www.nass.usda.gov/Statistics_by_State/California/Publications/Fruits_and_Nuts/2017/201704almac.pdf.

Vanengelsdorp, D., J. D. Evans, C. Saegerman, C. Mullin, E. Haubruge, B. K. Nguyen, M. Frazier, J. Frazier, D. Cox-Foster, Y. Chen, et al. 2009. Colony collapse disorder: a descriptive study. Plos One. 4: e6481.

Ward, R., A. Whyte, and R. R. James. 2010. A tale of two bees: looking at pollination fees for almonds and sweet cherries. Am. Entomol. 56: 172–179.