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Research Article

Pollination Requirements of Almond (*Prunus dulcis*): Combining Laboratory and Field Experiments

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Abstract

Almond (Prunus dulcis (Mill.) D. A. Webb; Rosales: Rosaceae) is a cash crop with an estimated global value of over seven billion U.S. dollars annually and commercial varieties are highly dependent on insect pollination.Therefore, the understanding of basic pollination requirements of the main varieties including pollination efficiency of honey bees (Apis mellifera, Linnaeus, Hymenoptera: Apidae) and wild pollinators is essential for almond production. We first conducted two lab experiments to examine the threshold number of pollen grains needed for successful pollination and to determine if varietal identity or diversity promotes fruit set and weight. Further, we examined stigma and ovules of flowers visited by *Apis* and non-*Apis* pollinators in the field to study the proportion of almond to non-almond pollen grains deposited, visitation time per flower visit, and tube set. Results indicate that the threshold for successful fertilization is around 60 pollen grains, but pollen can be from any compatible variety as neither pollen varietal identity nor diversity enhanced fruit set or weight. *Andrena cerasifolii* Cockerell (Hymenoptera: Andrenidae) was a more effective pollinator on a per single visit basis than *Apis* and syrphid flies. Nevertheless, *Apis* was more efficient than *A. cerasifolii* and syrphid flies as they spent less time on a flower during a single visit. Hence, planting with two compatible varieties and managing for both *Apis* and *non-Apis* pollinators is likely to be an optimal strategy for farmers to secure high and stable pollination success.

Key words: flower visitors, pollination effectiveness, pollination efficiency, pollen grain, tube set

Numerous horticultural crops benefit from animal pollination (Klein et al. 2007) and agriculture's dependency on pollinators is increasing globally as more land is planted with pollinator-dependent crops (Aizen et al. 2008). Local declines in honeybee (*Apis mellifera* L.; Apidae; hereafter *Apis* bees) populations (Pettis and Delaplane 2010, Potts et al. 2010) have stimulated interest in alternative pollinators and their contributions to crop production. These insect pollinators (usually wild bees such as bumble bees and solitary bees but also non-bee species like flies, hereafter referred to as non-*Apis* pollinators) can improve fruit and seed set for many crops in different regions of the world (Garibaldi et al. 2013, Bartomeus et al. 2014, Mallinger et al. 2015, Rader et al. 2016). While wild bees and flies can improve fruit set generally, we rarely know, for instance, the amount and kind of pollen being delivered by different pollinator groups to any given plant species (but see Free and Williams 1972, Gyan and Woodell 1987, Snow and Roubik 1987, Howlett et al. 2017), the consequences of such differences, or the eco-physiological mechanisms behind them. For example, different pollinators may deliver different amounts of pollen, which often influence pollen tube growth (Ter-Avanesian 1978) or seed set and fruit/seed mass (Dogterom et al. 2000). Also, for plant species which require outcrossed pollen, the pollen quality (viability [Maita and Sotomayor 2015] and compatibility) may vary in effectiveness at producing pollen tubes. Lastly, even after successful pollination, longer lasting effects (e.g., endogenous hormone production by the ovary) during fruit and seed development may occur as a result of pollen variety (Colbert and Oliveira 1990, Abbas et al. 2012).

In this study, we focus on the important cash crop, almond (*Prunus dulcis* (Mill.) D. A. Webb; Rosaceae), valued at over seven billion U.S. dollars globally and grown mostly (~80%) in California

(Almond Board of California 2013, USDA 2017). Almond farmers in California rely on managed pollinators, especially the European honeybee (Thorp 1996). Most commercially grown almond varieties require cross-pollination, although a few self-compatible varieties are available (Dicenta et al. 2001). Even so, the pollen needs to be transferred from the stamen to the pistil of a flower, which requires insect or hand pollination. Free pollination services by non-*Apis* pollinators may also be available in almond orchards, if there is at least 10% of seminatural vegetation in a 1 km radius of the orchard (Klein et al. 2012). In this study, we are interested in pollination effectiveness and efficiency of different pollinator groups. We define pollination effectiveness as the tube set resulting from a single flower visit by a target pollinator and efficiency as the time a single target pollinator spends on the flower. A short flower visit should result in more visited flowers and therefore higher pollination efficiency than a long flower visit. The product of effectiveness and efficiency should therefore reflect pollinator success.

To estimate pollinator success, we measured pollination effectiveness and efficiency of the target bee species *Apis mellifera* L., *Andrena cerasifolii* Cockerell, the most abundant wild bee in almond in our study area (Klein et al. 2012), and syrphid flies.

Moreover, the pollination success depends on several factors, most importantly on the quality and quantity of delivered pollen (Aizen and Harder 2007). There may also be variation in the efficacy (how often a target variety results in pollination success) of the different varieties of potential pollen donors because most almond varieties are obligate outcrossers (Badenes and Byrne 2012). Lastly, the almond crop is likely to have a threshold number of pollen grains to complete fertilization as Weinbaum (1985) found that approximately seven pollen grains were not sufficient to assure pollination success. However, the actual threshold number of required pollen grains remains unclear. Therefore, we also established the threshold number of pollen grains needed to effect fertilization in almond (defined as at least one pollen tube reaching the ovules), and we studied the numbers of almond- to non-almond pollen grains deposited on stigmas by different pollinator groups and the effects of individual varieties and of mixing different varieties of pollen (two to five pollen varietal combinations as a measure of pollen diversity) on nut production, and nut mass.

Materials and Methods

We conducted both field and lab experiments. All field studies were conducted in Colusa and Yolo County in the Sacramento Valley in California (38°42′ to 38°57′ N and 121°57′ to 122°14′W) in March 2008. We selected two orchards with 32–54% of surrounding natural habitat in a 1 km radius that showed high flower-visitation frequencies by wild pollinators and included the main commercial almond varieties of the region Nonpareil, Peerless, Monterey, Mission, and Padre.

Determination of Threshold Number of Pollen Grains

We selected approximately 50 healthy almond branches of the variety Nonpareil that were up to 80 cm long with closed flower buds 7–10 days from opening, in each of two orchards in Capay Valley, Yolo County in California (38°42′ to 38°57′ N and 121°57′ to 122°14′W). Branches were bagged with Delnet mesh-bags in late February 2008 to exclude pollinators (Kremen et al. 2002, Greenleaf and Kremen 2006). When flowers were just ready to open, they were collected and stored in the laboratory at room temperature for 24 h. Anthers and petals of nearly opened flowers were removed and each remaining pistil and peduncle was deposited upright in 2 ml centrifuge-tubes containing 0.5 ml tap water (Ben-Njima and Company 1995).

Pollen of the compatible variety Ne Plus was collected from bagged flowers of a different field, not belonging to the study site. Pollen was stored in centrifuge-tubes at −30°C for approximately 72 h until needed. Pollen grains were counted under magnification on the microscope slide and attached to the stigma by pressing it gently on the microscope slide. We attached 0, 1, 10, 30, 60, or >100 pollen grains to a single stigma and replicated each treatment 20 times. As it was difficult to attach exactly 100 pollen grains it is likely, that pollen grain numbers varied between 100–110 attached grains. Afterwards, pistils were stored for 72 h in their water-filled centrifuge-tubes at room temperature (20°C). The number of pollen tubes that had grown to the bottom of the style was counted in stained pistils by means of fluorescence microscopy (see "Pollination Effectiveness and Visitation Efficiency"). Final sample sizes for the treatments were, respectively, n = 19, 20, 20, 19, 18, 17. In cases with lower sample sizes than 20, some flowers died for unknown reasons and could not be analyzed further.

Pollen Delivery to Stigmas by Different Pollinator Groups

To determine the pollen composition delivered by different pollinator groups in a single flower visit, we sampled pollen from stigmas using scanning electron microscope (SEM). We bagged almond branches with Delnet mesh-bags prior to flower opening to exclude pollinator visits. After flower opening, we cut single branches, removed the bag, and offered them to various flower-visiting insects foraging on trees of the same almond variety of the offered branch (modified wand technique) (Ben-Njima and Company 1995, Kremen et al. 2002, Brittain et al. 2013b). The investigated species included *Apis* pollinators, aphidophagous syrphid flies (mainly *Syrphus* spp., *Toxomerus* spp. and *Platycheirus* spp., determined by morphospecies), and *A. cerasifolii*. We grouped the flower visitors in the categories of *Apis* pollen forager, *Apis* nectar forager, *A. cerasifolii*, and syrphid flies.

When insects landed on a flower, they were allowed to carry out their activity and other insects were prevented from visiting (Greenleaf and Kremen 2006). After an insect left, we placed the pistils under dry conditions in small centrifuge-tubes (0.6 ml) in a cooling box filled with icepacks and froze them shortly after at −30°C until further observations. The upper part of the pistil was cut and placed into a centrifuge tube filled with 30 µl ethanol (70%). We treated the samples with a vortex mixer for approximately 30 s to remove the pollen grains from the pistil which sank to the bottom of the vial after 30 min. We then applied 10 µl of ethanol sample of pollen grains to a 25 mm^2 piece of silicon wafer, mounted for scanning electronic microscopy examination. The employed instrument was a Hitachi TM-1000 tabletop SEM (Hitachi High-Technologies Corporation, Japan). Samples were air-dried at room temperature (around 20°C). Preliminary tests revealed no difference in sample quality with or without sputter-coating with gold (Degrandi-Hoffman et al. 1992, Arzani et al. 2005), and therefore samples were not sputter-coated. Both the total number of pollen grains and identification of pollen based on reference pollen samples was determined for the following number of samples in each pollinator group: *Apis* pollen foragers n = 17; *Apis* nectar foragers n = 10; syrphid flies n = 13; *A. cerasifolii* n = 15.

Pollination Effectiveness and Visitation Efficiency

In addition to measuring the amount of pollen delivered to stigmas, we also measured the "pollinator effectiveness", defined as the proportion of flowers in a sample with at least one pollen tube reaching the ovary after a single visit by a pollinator. We also measured visitation efficiency, which is defined as the amount of time a target flower visitor spent on one single flower, i.e., fast visits are more efficient than long visits as more flowers per time unit can be visited.

The procedure for exposing fresh flowers to individual pollinators in the field was identical to that for pollen delivery to stigmas by different pollinator groups (see "Pollen Delivery to Stigmas by Different Pollinator Groups"), except we also collected information during each visit on stigma contact and time spent on stigma of each flower. The pistils of the visited flowers were removed and placed in an upright position in 2 ml centrifuge-tubes containing 0.5 ml tap water. Pistils were stored for 72 h at 20°C without direct impact of sunlight to permit pollen tubes to grow. Then, we removed the water and froze the pistils at –30°C for 7–14 days before fixation. Pistils were fixed in FAA (10:7:2:1 ethanol (95%): H_2O: formalin: acetic acid) and stored at 4°C for 24 h before softening of tissue (Cousin and El Maataoui 1998). Subsequently, tissue softening was accomplished by boiling the pistils in 5 ml of 5% sodium sulphite (Na_2SO_3) for 20 min and rinsing in tap water for 30 min before staining (Alonso et al. 2005). For staining, pistils were incubated for 12–24 h in the staining solution which consisted of 0.1% aniline blue dye dissolved in 0.1 N K_3PO_4 (potassium phosphate). We stored the samples at 4°C until the solution became colorless (Currier 1957). Stained pistils were gently squashed between slide and cover slip and were kept moist with distilled water while we scored the pollen tubes using fluorescence microscopy (Axio Imager 373, Qimaging Color camera, Zeiss, Germany).

Scoring included determining the number of germinated pollen grains on the stigma, and the number of pollen tubes grown down the style to the ovary. The investigated species were the same as in the pollen delivery study. The sample sizes differed for each insect: *Apis* pollen- and nectar-foragers, each $n = 26$, syrphid flies $n = 23$, *A. cerasifolii* $n = 25$.

Pollen Varietal Effects

Flowers on 50 two-year-old trees of the variety Nonpareil were selected and hand-pollinated to test for effects of different varieties and combinations of varieties of pollen with respect to pollen tube growth to the ovary. Pollen from the almond varieties Ne Plus ($n = 19$), Wood Colony ($n = 20$), Monterey ($n = 15$), and Carmel ($n = 20$) was tested. Additionally, the following two to five varietal combinations were tested: Ne Plus/Wood Colony ($n = 22$), Ne Plus/Wood Colony/Monterey ($n = 20$), Ne Plus/Wood Colony/Monterey/Carmel ($n = 20$), and Ne Plus/Wood Colony/Winters/Carmel/Aldrich ($n = 15$). Pollen was obtained from groves other than those of the study sites. Each flower received 100 pollen grains in total. For the pollen variety combination treatments, the 100 pollen grains were equally split between the different varieties. The trees were placed in fine-meshed net tents, thus preventing pollinator visits to flowers, and the fruit set was examined 3 wk after hand-pollination and nut weights were determined for the developed fruits.

Statistical Analyses

Data analyses were conducted using R 3.3.2 (R Core Team 2016). Each data set was first tested for normality using QQ-Plots and a Shapiro-Wilk test (Shapiro and Wilk 1965, Royston 1982, Royston 1995). As none of the data sets were normally distributed (all *P*-values <0.0036), we conducted nonparametric tests.

To test for significant effects of different numbers of pollen grains deposited on the number of developed pollen tubes and between the number of pollen grains and the number of pollen tubes reaching the ovary, we conducted Kruskal–Wallis tests (Kruskal and Wallis 1952). To test for effects of the number of pollen grains on tube set, we conducted Pearson's chi square test and Fisher's exact test for pairwise comparison due to lower numbers of observations (Fisher 1954). Further, we conducted Spearman's rank correlation coefficient (Spearman 1904, Spearman 1906) to test for a correlation between the number of pollen tubes developed and the number of pollen tubes reaching the ovary.

To test for significant differences in the type and number of pollen grains delivered to stigmas by the different insect pollinators, we conducted Kruskal–Wallis tests (Kruskal and Wallis 1952).

We used generalized linear models (GLM) (Nelder and Wedderburn 1972) to analyze the variables (pollinator species group, time spent on a flower, touching the stigma) that could influence the development of pollen tubes. As the response variable we used the *number of pollen tubes reaching ovary* and as explanatory variables we used the *pollinator species group*, *time spent on a flower* and *whether the stigma was touched* by the pollinator during the flower visit (with no interaction terms). We tested both a negative binomial (function glm.nb from the "MASS" package, Venables and Ripley 2002) and a Poisson generalized linear model (function glm). We used the functions simulateResiduals and plotSimulatedResidulas from the "DHARMa" package (Hartig 2017) to plot both a qqplot of expected and observed residuals and a plot of the standardized residuals against the predicted values. Further, we tested for uniformity of the residuals (function testUniformity from the "DHARMa" package, Hartig 2017) to check if assumptions are met and to compare both models. The negative binomial model was selected because this model showed uniformity of the residuals (one-sample Kolmogorov–Smirnov test, $D = 0.045$, $P = 0.93$) in contrast to the Poisson model analyzed by comparing the plot of standardized residuals against the predicted values and test statistics. We tested this model with a likelihood ratio test (LRT) (function anova) and conducted post hoc Tukey's contrasts (function glht from the "multcomp" package, Hothorn et al. 2008) to test for differences between the pollinators in the number of pollen tubes reaching ovaries after one single visit.

To analyze the effect of the time spent on flowers on tube sets within each pollinator group, we conducted Kruskal–Wallis tests (Kruskal and Wallis 1952). Wilcoxon–Mann–Whitney tests (Wilcoxon 1945, Mann and Whitney 1947) were used to investigate pairwise differences in the time spent on a flower by comparing different pollinator groups to one another. To investigate pairwise differences in tube set between each pollinator combination, we used Fisher's exact test due to low sample sizes (Fisher 1954).

To analyze the relationship between pollen identity or pollen diversity and fruit set, we used Pearson's chi square tests, respectively. Further, to test for significant effects of pollen identity or pollen diversity on nut weight, we conducted Kruskal–Wallis tests (Kruskal and Wallis 1952).

Results

Determination of Threshold Number of Pollen Grains

The number of detected pollen tubes increased with the number of deposited pollen grains almost reaching saturation with about 100

grains (Kruskal–Wallis test, $P < 0.001$, chi-squared = 77.74, df = 5) and a tube set of 88.24% (Fig. 1). The number of pollen tubes reaching the ovules increased with an increasing number of detected pollen tubes (Spearman's rank correlation, S = 51,414, rho =0.79, $P < 0.001$). We found that about 100 pollen grains significantly increased tube set compared to 30 pollen grains (Fisher's exact test, odds ratio = 7.83, $P = 0.01$), whereas 60 pollen grains were statistically comparable to the 100 pollen grains (Fisher's exact test, odds ratio = 4.56, $P = 0.12$).

Pollen Delivery to Stigmas by Different Pollinator Groups

We found no significant differences in almond pollen deposition between the different pollinator groups (Kruskal–Wallis test, $P = 0.45$, chi-squared = 2.638, df = 3). On average, the pollinators deposited between 16.53 and 24.13 almond pollen grains. However, the deposition of non-almond pollen grains varied significantly between pollinator groups (Fig. 2) (Kruskal–Wallis test, $P = 0.005$, chi-squared = 12.83, df = 3): *Apis* collecting pollen and syrphid flies deposited two or fewer foreign grains per visit, whereas *Apis* foraging for nectar and *A. cerasifolii* deposited on average four foreign pollen grains.

Pollination Effectiveness and Visitation Efficiency

Overall, 96% of the single visits by *A. cerasifolii* resulted in at least one pollen tube reaching the ovary (Table 1). *A. cerasifolii* was significantly more effective as a pollinator than individuals of the other groups (GLM: 50% explained deviance with null deviance of 231.42 and residual deviance of 115.71, mean = 0.64 ± 0.22, LRT: chi-squared = 111.89, df = 3, $P < 0.001$). *Apis*, collecting pollen, was significantly more successful at approximately 65% of visits than flower visits by syrphid flies (22% of single visits resulted in at least one pollen tube reaching the ovary) (Tukey's contrasts, $P = 0.05$) and did not differ from results by flower visits of *Apis* collecting nectar (Tukey's contrasts, $P = 0.94$) (Table 1).

The amount of time spent per visit varied significantly among groups but did not differ between successful or unsuccessful visits for each pollinator group (Kruskal–Wallis tests, for *A. cerasifolii* chi-squared = 4, df = 13, $P = 0.99$, for *Apis* nectar chi-squared = 16.20, df = 19, $P = 0.64$, for *Apis* pollen chi-squared = 14.95, df = 13, $P = 0.31$, for syrphids chi-squared = 16.38, df = 18, $P = 0.57$). The time spent on a flower was five times lower by the pollen-collecting *Apis* than by *A. cerasifolii* (Wilcoxon–Mann–Whitney test, W = 113, $P < 0.001$) (Table 1). Syrphid flies spent almost twice as much time on flowers as *A. cerasifolii* and four times as much time as *Apis* nectar-collecting bees (Wilcoxon–Mann–Whitney test, for syrphids –*A. cerasifolii*, W = 403, $P = 0.01$, for syrphids—*Apis* nectar W = 41.5, $P < 0.001$).

The number of pollen tubes reaching ovules did not vary with the time spent on the flower (GLM: mean = –6.11 ± 0.8, LRT: chi-squared = 3.83, df = 1, $P = 0.0504$). Thus, pollinator group was the most important factor in the relationship between single visits and pollen tubes reaching ovules.

Pollen Varietal Effects

The pollen varietal identity was neither related to fruit set (Pearson's chi square test, chi-squared = 3.56, df = 7, $P = 0.83$) or nut weight (Kruskal–Wallis test, chi-squared = 3.11, df = 7, $P = 0.87$). Further, pollen varietal diversity was neither related to fruit set (Pearson's chi square test, chi-squared = 2.18, df = 4, $P = 0.70$) or nut weight (Kruskal–Wallis test, chi-squared = 1.15, df = 4, $P = 0.89$) (Table 2).

Discussion

This study aims at disentangling different aspects of pollination requirements of almond and shows that the threshold number of pollen grains for a successful fertilization is between 60 and about 100 pollen grains and that different pollinator groups deposit similar amounts of almond pollen on the stigma. However, differences in pollinator effectiveness and efficiency between pollinators occurred because *A. cerasifolii* was the most effective pollinator but *Apis* was

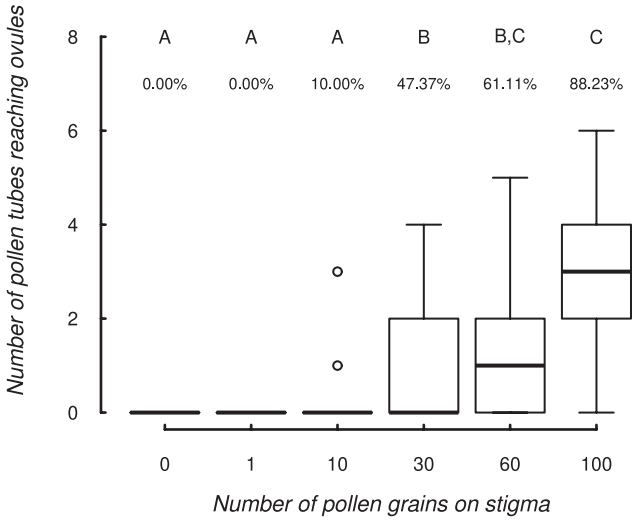

Fig. 1. Number of pollen grains and the resulting pollen tubes to ovules and tube set percentage. Different letters indicate significant differences by Pearson's chi square test and Fisher's exact test, (for pg 0, 1, 10, 30, 60, >100, *n* = 19, 20, 20, 19, 18, 17, respectively). The percent values show the mean tube set percentage resulting from the different number of pollen grains tested. The box and whisker plots show the median (horizontal line), quartiles (boxes), 5% and 95% percentiles (whiskers), and outliers of each data set (open circles).

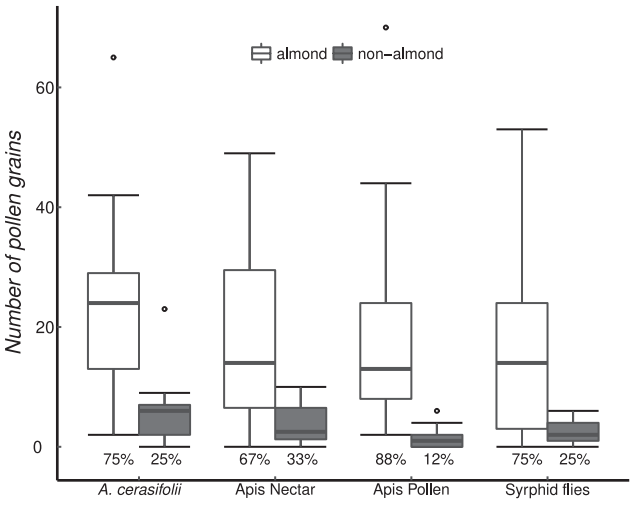

Fig. 2. Number of deposited almond- and non-almond pollen grains by each pollinator group. The percent values show the proportion of almond- to non-almond pollen deposited by each pollinator group. All box and whisker plots show the median (horizontal line), quartiles (boxes), 5% and 95% percentiles (whiskers), and outliers of each data set (open circles).

Table 1. Developed pollen tubes in style and ovules, time spent on flower, stigma contact after one single flower visit of the insect, pollen tube set, and overall pollinator success

Insect pollinator	Pollen tubes in style (mean ± SE)	Pollen tubes to ovules (mean ± SE)	Time spent on flower (mean ± SE)	Touched stigma (%)	Tube set (%)	Pollinator success $\left(\frac{1}{time\ spent\ on\ flower} \times tube\ set\right)$
A. cerasifolii	50.32 ± 11.792^{a}	5.68 ± 0.734^{a}	61.92 ± 9.360^{a}	80.00	96^{a}	1.55
Apis nectar	9.62 ± 1.333^{b}	1.27 ± 0.252^{b,c}	24.50 ± 5.509^{a,b}	53.85	62^{b}	2.53
Apis pollen	12.38 ± 1.385^{b}	1.54 ± 0.273^{b}	12.42 ± 2.597^{b}	88.46	65^{b}	5.23
Syrphid flies	5.44 ± 1.006^{b,c}	0.35 ± 0.149^{c}	117.87 ± 18.682^{a}	52.17	22^{c}	0.19

Different letters indicate significant differences by post-hoc Tukey's contrast of the GLM (pollen tubes to ovules), Fisher's exact test (tube set), and by Wilcoxon–Mann–Whitney test (pollen tubes in style, time spent on flowers) (n for tube set: Non-*Apis* bee, *Apis* n, *Apis* p, Syrphid flies, n = 15, 10, 17, 13, respectively and n for pollen tubes in style and to ovules and time spent on flowers: *A. cerasifolii*, *Apis* n, *Apis* p, Syrphid flies, n = 25, 26, 26, 23, respectively).
$^{a–c}P < 0.001$.

the most efficient. Pollen identity and pollen varietal diversity did not influence fruit set or nut weight.

Determination of Threshold Number of Pollen Grains

Our results indicate that a relatively high number of pollen grains (approximately 60) is needed to approach 60% success of pollen tubes reaching the ovary (Fig. 1), but the inability to reach 100% success even with about 100 pollen grains indicates that either even more pollen grains are needed to guarantee 100% success or stigma saturation is reached between 60 and 100 grains. The inability to reach 100% success in this study may be due to a nonlinear relationship between the number of pollen grains attached and pollination success. Possibly, pollen competition or genetics influence pollination success more significantly when higher numbers of pollen grains are attached and reduce the ability to reach 100% pollination success with the given number of pollen grains. Alternatively, access of pollen to the relatively small stigma surface in almond may be spatially limited and saturation may occur, resulting in the loss of stigmatic receptivity as observed in blueberry (Parrie and Lang 1992). Distinguishing between a pollen grain threshold above 100 pollen grains and stigma saturation will require testing at a finer scale between 60 and 100 grains and with more than 100 grains. Our results also show that while the probability for a successful fertilization increases with increasing number of detected pollen tubes in the style (Table 1), many tubes did not reach the ovary. Thus, using only the developed pollen tubes in the style is not an appropriate measure for pollination success, a common practice in many studies (Ben-Njima and Company 1995, Ortega et al. 2002). Altogether, the threshold of the number of pollen grains needed for successful pollination is at least between 60 and about 100 pollen grains.

Pollen Delivery to Stigmas by Different Pollinator Groups

The overall number of almond pollen grains deposited by *Apis* and by non-*Apis* pollinators was similar (Fig. 2), a result that confirms the previous work of Thomson and Goodell (2001). Willmer et al. (2017) showed that mostly non-*Apis* bees were more effective pollen depositors than *Apis* in measurements of single-visit pollen deposition and that peak visit times differed between bees and non-bees, indicating temporal variation in pollen delivery by different groups of pollinators.

However, Thomson and Goodell (2001) argue that measuring pollen deposition is only sound when pollen removal is considered, because pollen deposition depends on the number of pollen grains provided and on pollen removal by previous pollinator visits. Thus, conclusions drawn from pollen deposition data only may be limited.

Despite similar amounts of pollen deposited by different groups, there were major differences in pollination effectiveness among species (Table 1), indicating that factors such as pollen quality or pollinator behavior influence effectiveness. If pollinator behavior results in movement from non-almond to almond flowers as appears to happen often with *A. cerasifolii*, nectar-feeding *Apis*, and syrphid flies, then high proportions of non-almond pollen may be deposited per visit (Fig. 2). However, even with low non-almond pollen deposited by *Apis* foraging for pollen, *Apis* may forage within one row of almond trees of the same variety and thus deposit more incompatible pollen (Brittain et al. 2013b) than wild bees that may be more likely to transfer between rows. Thus, even though the number of pollen grains deposited was similar between pollinators, differences in pollinator effectiveness indicate that other factors such as pollinator behavior are more important for pollination success.

Table 2. Effects of pollen identity and diversity on fruit set and nut weight

Variety	Fruit set (%)	Nut weight (g) (mean ±SE)
NePl	11 (n = 19)	0.79 ± 0.105
M	13 (n = 15)	0.90 ± 0.033
C	24 (n = 20)	0.89 ± 0.040
WC	14 (n = 20)	0.88 ± 0.117
NePl + WC	23 (n = 22)	0.87 ± 0.118
NePl+ WC+ M	10 (n = 20)	0.82 ± 0.065
NePl + M + C+ WC	25 (n = 20)	0.81 ± 0.040
NePl+ W+ C+ WC+ A	20 (n = 15)	0.84 ± 0.034

NePl = Ne Plus, C = Carmel, M = Monterey, WC = Wood Colony, A = Aldrige, W = Winters). We did not include the varietal combination M/WC in this table and in statistical analyses due to low sample size (n = 6).

Pollination Effectiveness and Visitation Efficiency

A. cerasifolii was the most effective pollinator because after one single visit there was the highest proportion of flowers with tubes reaching the ovary (Table 1). Similarly, Park et al. (2016) found that *Andrena* of the subgenus *Melandrena* was the most effective per visit pollinator of apples (*Malus domestica* Borkh.), another tree crop species in the Rosaceae. Vicens and Bosch (2000) likewise found an unmanaged bee species (*Osmia cornuta* Latreille) (Hymenoptera: Megachilidae) to be more effective on a per flower visit basis than the honey bee. In the field and under nonstandardized conditions, non-*Apis* pollinators may be particularly effective in delivering pollen, because they are often more tolerant to inclement weather conditions common in the almond bloom period from February to March (Soodan et al. 1989, Vargas and Romero, 2001), whereas *Apis* bees avoid foraging flights during these conditions (Brittain et al. 2013a). Furthermore, non-*Apis* pollinators are known to visit different parts within the tree than *Apis*, and enhance the likelihood that *Apis* will deliver outcross pollen (Brittain et al. 2013a, 2013b). Both of these factors potentially contribute to higher yields that have been observed when non-*Apis* pollinators are present in orchards along with *Apis* (Klein et al. 2012). Further, fruit set in various different crops increased with wild insect visitations (Garibaldi et al. 2013), highlighting the high pollination effectiveness of wild pollinators.

In general, shorter visitation times are linked to higher visitation rates as the pollinator can visit more flowers in the same time, but high visitation rates do not necessarily translate into more pollination events. In this study, the time spent on a flower (visitation efficiency) did not influence the number of pollen tubes reaching the ovules in general and with regard to each pollinator group. Similarly, Javorek et al. (2002) also found that high visitation rates (which may be inversely related to time spent on flower) are not necessarily linked to higher effectiveness. Here, *Apis* spent relatively little time on almond flowers, a result similar to other studies (Rader et al. 2009, Albrecht et al. 2012), although other bees such as *Osmia* may spend even less time per flower (Bosch and Blas 1994).

Syrphid flies spent the longest times on a flower per single visit, but were not effective (Table 1). This is a similar result to studies of pollination in oilseed rape which found that increased syrphid fly density did not improve seed set when bee density was high (Jauker et al. 2012, Morandin et al. 2016). However, in cases where pollinator density is low, pollination performance of syrphid flies increases, and they may be efficient pollinators for oilseed rape (Jauker and Wolters 2008). Similar to our study, Rader et al. (2016) found that non-bee pollinators are less effective on a per visit basis than bees, but in contrast to our study, show higher visitation frequencies. Altogether, *A. cerasifolii* is the most effective pollinator followed by *Apis* and syrphids, and *Apis* is the most efficient pollinator followed by *A. cerasifolii* and syrphids. When combining both aspects to evaluate overall pollinator success, *Apis* is the most successful pollinator followed by *A. cerasifolii* because the time spent per flower was short enough to compensate for higher per single visit effectiveness of *A. cerasifolii* (Table 1). Further, when non-*Apis* bees are present, effectiveness of *Apis* increases (Brittain et al. 2013b). Syrphids were the least successful pollinators compared to *A. cerasifolii* and *Apis*.

Pollen Varietal Effects

As our and other studies (e.g., Dicenta et al. 2002) have not found differences in tube set or nut weight between different self-compatible varieties, there is no advantage of one compatible almond variety over another compatible variety. Further, the number of different varieties of pollen on a single stigma did not influence tube set or nut weight (Table 2), probably due to nonexisting competition between the different varieties (e.g., in terms of the velocity of pollen tube growth). Instead of the delivered pollen variety and the number of different pollen varieties on a single stigma, the way the pollen grains are deposited on the stigma surface (exact location), which is determined by the pollinator species, appears to be a more crucial factor for successful pollination. If the mechanistic process of fertilization plays a superior role, differences between the varieties and varietal diversity may be negligible. In a study investigating apple, pollen varietal diversity increased seed number in some but not all recipient genotypes (Kron and Husband 2006), which could be explained by inherent differences in fecundity across the recipient varieties. Such differences in fecundity among varieties may also occur in almond. In this study, we found that both the identity of a compatible pollen variety and varietal diversity are not important drivers for pollination success.

Conclusion

We found significant effects of the number of pollen grains and of pollinator species on the probability of pollination success in almond. However, there may also be other factors that can influence successful fruit production. For example, Klein et al. (2015) found that pollination success in almond interacts with the availability of water and nutrients. Further, we looked only at tube set rather than the resulting fruit set or fruit quality in each experiment except for pollen varietal effects. Between pollen tube growth and the time when the ovule starts to swell, fruit development failures can occur (Garratt et al. 2014). For example, poor genetic quality of pollen (quality limitation) or pollen limitation has been shown to constrain seed production (Aizen and Harder 2007).

We found that almond has a relatively high threshold of at least 60 pollen grains to be successfully pollinated and that wild pollinating species play a major role in reaching that threshold. *A. cerasifolii* was an even more effective pollinator than *Apis* on a per visit basis and deposited the most pollen grains per single flower visit. *Apis* was more efficient as it spent less time on a flower per single visit and could potentially therefore pollinate more flowers successfully at a given time, but they may also carry high loads of non-almond pollen or fail to deposit compatible pollen. Thus, both *Apis* and

non-*Apis* pollinators can contribute to successful almond pollination. We recommend planting at least two compatible varieties and managing both *Apis* and *non-Apis* pollinators as an optimal management strategy for almond farmers to secure high and stable pollination success (see also Brittain et al. 2013b). Further, a higher variability of almond varieties may present advantages such as a difference in susceptibility of the varieties to pests or pathogens. Such aspects were not the focus of this study and remain to be tested.

Additionally, further research could focus on how different pollinating species vary in the ratio of compatible to noncompatible almond pollen delivered, rather than on almond to non-almond pollen. Another interesting question for farmers that requires further research would be to address the resulting quality of fruits (i.e., contents of primary and secondary metabolites affecting the odor, taste and nutritional value) of almond after the respective flowers were pollinated by different insects.

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