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RESEARCH PAPER

Interacting effects of pollination, water and nutrients on fruit tree performance

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ABSTRACT

Pollination is critical to fruit production, but the interactions of pollination with plant resources on a plant's reproductive and vegetative features are largely overlooked. We examined the influences of pollination, irrigation and fertilisation on the performance of almond, *Prunus dulcis*, in northern California. We used a full-factorial design to test for the effects of pollination limitation on fruit production and foliage variables of whole trees experiencing four resource treatments: (i) normal water and nutrients, (ii) reduced water, (iii) no nutrients, and (iv) reduced water and no nutrients. In each of these combinations, we applied three pollination treatments: hand-cross pollination, open-pollination and pollinator exclusion. Pollination strongly affected yield even under reduced water and no nutrient applications. Hand-cross pollination resulted in over 50% fruit set with small kernels, while open-pollinated flowers showed over 30% fruit set with moderate-sized kernels. Pollinator-excluded flowers had a maximum fruit set of 5%, with big and heavy kernels. Reduced water interacted with the open- and hand-cross pollination treatments, reducing yield more than in the pollinator exclusion treatment. The number of kernels negatively influenced the number of leaves, and reduced water and no nutrient applications interacted with the pollination treatments. Overall, our results indicate that the influences of pollination on fruit tree yield interact with the plant availability of nutrients and water and that excess pollination can reduce fruit quality and the production of leaves for photosynthesis. Such information is critical to understand how pollination influences fruit tree performance.

INTRODUCTION

There is strong evidence that the majority of wild and cultivated plant species benefit from or rely entirely on the transportation of pollen grains by bees, other insects, birds and mammals (Burd 1994; Klein *et al.* 2007; Ollerton *et al.* 2011). Furthermore, at the within-plant or whole plant scales, many crops exhibit decreased crop production in response to decreases in the number and types of pollinator (e.g. Ricketts 2004; Klein 2009; Carvalheiro *et al.* 2010; Garibaldi *et al.* 2013), and these results have been extrapolated to field and landscape scales (Ricketts *et al.* 2004), as well as regional (Morandin & Winston 2006) and global scales (Klein *et al.* 2007; Garibaldi *et al.* 2013; Kennedy *et al.* 2013). In natural populations, pollination may also strongly limit reproduction, particularly in fragmented landscapes (Aquilar *et al.* 2006; Slagle & Hendrix 2009).

While pollination clearly affects reproductive yields of many wild and crop plants (Klein *et al.* 2007; Ollerton *et al.* 2011), the degree to which it regulates yield in real-world cropping systems is debated (Ghazoul 2007; Kremen *et al.* 2008). The emphasis on the role of pollination in determining yield has been criticised on the basis that it does not account for possible post-pollination processes affecting the amount and quality of fruit maturation (Niesenbaum 1993; Bos *et al.* 2007; Ghazoul 2007). For example, water and nutrient limitation can strongly affect early fruit abortion (Pías & Guitián 2006) and variation in losses to pests and diseases may ultimately be more important than pollination in determining realized yields (Brown & McNeil 2006). Furthermore, the interactions between pollination and post-pollination processes are rarely considered for wild plants (Bierzychudek 1981; Casper & Niesenbaum 1993; Niesenbaum 1993) and are almost unstudied in crop plants (Bos *et al.* 2007; but see Groeneveld *et al.* 2010).

Given widespread concerns about colony losses of *Apis mellifera* L., a major crop pollinator worldwide (Neumann & Carreck 2010; Ellis 2012), as well as evidence for declines of other pollinators at landscape and regional scales (Potts *et al.* 2010), a greater understanding of the importance of pollination processes for reproduction and yield is critically needed.

To date, studies of pollination limitation in tree crops have examined only a subset of flowers per plant or invoke pollinator limitation without supplementary hand-cross pollination of the flowers (see studies cited in the review of Klein *et al.* 2007). Instead, yields should be analysed at the whole plant scale to avoid the confounding effects of resource allocation among individual flowers or branches (Zimmerman & Pyke 1988; Knight *et al.* 2006; Wesselingh 2007). Thus, experiments are needed that compare hand-pollinated yields to those in which pollinators are totally excluded (Kearns & Inouye 1993), with and without resource applications. Furthermore, high fruit yield may come at the expense of the trees' vegetative performance, such as quantity of foliage. The indirect influences of pollination in combination with plant resource variation on vegetative features of crops such as leaf quality and quantity is not yet investigated.

Commercial almond production is an excellent model system for testing effects of pollination on crop production and foliage variables in different water and nutrient applications because results are likely to be applicable to many fruiting trees, such as apples, cherries, pears, peaches and plums, all with main varieties depending on insect pollination. Almond trees are generally considered to be drought-tolerant (Torrecillas *et al.* 1996), but growers are highly dependent on irrigation and nutrient inputs to produce high yields of top quality (Castel & Fereres 1982; Micke 1996). Moreover, almond production in California is highly dependent on honeybee management to set a commercial crop, but declines in honeybee colonies and an increase in production acreage have resulted in honeybees becoming a limiting resource for almond in the USA (Thorp 1996; Kremen *et al.* 2008; Neumann & Carreck 2010; Ellis 2012). Furthermore, California, as one of the largest irrigated agricultural areas in the world, is facing water shortages, and almond growers may be forced to reduce their annual water use (Blake 2008). Almond was therefore selected to test experimentally the effect of pollination on fruit yield and foliage while taking into account the interactions of pollination, water and nutrient applications in a full-factorial design.

MATERIAL AND METHODS

Almond breeding and pollination system

Almond [*Prunus dulcis* (Mill.) D.A. Webb] is a subtropical obligate outcrossing tree that is intensively grown in large monocultures in California, where the climate is similar to its place of origin in the Middle East (Pakistan to Syria and Turkey; Wang *et al.* 2005). Almond flowers must be pollinated by a compatible variety to produce a fruit (Delaplane & Mayer 2000). Bees, and possibly other insects, are the principal vectors for transferring the sticky pollen between flowers of different varieties, and there is little to no transfer by wind (Klein *et al.* 2007). The mature fruit consists of one (the almond nut) or occasionally two (double kernel) kernels per fruit, surrounded by a shell nested inside a hull. The most popular variety for nut production in the USA is Nonpareil, which comprises around one-third of all marketable nuts produced in California (Micke 1996). Compatible pollen donor varieties for Nonpareil include Padre, Mission, Wood Colony and Carrion. Almond trees flower from early February to mid-March in California, and fruits ripen from July to September, depending on variety, weather and age of the trees.

Experimental site

The experiment was carried out from January to August 2008. The site was located in the Sacramento Valley, the northern portion of California's agriculturally intensive Central Valley, in Colusa County, near the border with Yolo County (122º2′1.925″W, 38º55′19.372″N, World Geodetic System 1984). Colusa County produces around 5% of California's marketable almonds. Precipitation in this area is low, with an annual amount of 268.7 mm (Lapham *et al.* 1907; WRCC 2010). Rainfall occurs primarily from October through May, with a peak in January; no precipitation typically occurs from June to September. We selected young, productive trees for this experiment, because kernel quantity and quality of the whole tree can be measured, and accumulated resources are limited in smaller trees (Morgan *et al.* 2006). The 3.2-ha study site consisted of Nonpareil trees that were grafted onto peach rootstock [*Prunus persica* (L.) Batsch] in 2005 and were planted in 2006 (third leaf planting). The trees were approximately 1.75-m tall and had not been harvested prior to this study. The trees were planted 4-m apart within rows and 6.4 m between rows.

Several 'polleniser' varieties compatible with Nonpareil were available in surrounding orchards located 100–300 m away from the experimental trees. These included a 16-year-old, 4.7-ha orchard with Mission and Carrion varieties and a 13-year-old orchard with Nonpareil and Wood Colony. Honeybee hives were placed in the orchards surrounding the experimental study site. The eight hives closest to the experimental trees were 300–350 m away and were part of the commercial pollination management system used by the grower. Padre pollen was placed every second day at 10:00 and 14:00 h at the nest entrance of the honeybee hives to maximise the amount of compatible pollen transported by honeybee workers to Nonpareil trees.

Experimental design and setup

We used a full-factorial experimental design to test for the effects of pollen limitation on fruit production and foliage variables of whole trees experiencing four resource treatments: (i) normal water and nutrients; (ii) reduced water/normal nutrients; (iii) no nutrients/normal water; and (iv) reduced water and no nutrients. In each of these resource input combinations, we applied three pollination treatments: supplemental hand-pollination to maximise cross-pollination; open-pollination with flowers exposed to bees freely foraging in the field; and pollinator exclusion, accomplished by caging trees during flowering. The 12 treatment combinations were randomly assigned to individual trees and replicated five times in adjacent rows (n = 60 experimental trees).

Pollination, nutrient and water manipulations

Hand-pollination was carried out from 20 to 28 February using Padre pollen that had been harvested before bud opening and stored at −20 °C to maintain viability. Prior to application, pollen was thawed and used immediately (up to a maximum of 2 h post-thaw) to ensure viability. We hand-pollinated all open flowers using small brushes every 2–3 days until about 90% of all buds had opened. The last 5–10% of flowers that opened late in the blooming season were frequently characterised by deformed or missing female or male parts.

For the pollinator exclusion treatment, we covered individual trees from shortly before blooming started in February to the end of bloom in early March with 1.5 m^2 × 2-m tall cages constructed of aluminium tubing and cloth with a mesh size of 0.8–1.0 mm. We fixed the cages to the ground with hooks and weighted the edges down with stones. To test whether wind could carry pollen grains through the mesh, we conducted the following experiment. An almond branch with more than 50 flowers whose anthers were dehiscing was held between an electric fan and a new, unused cage free from pollen grain contamination. Inside the cage four microscope slides were placed at the same height as the flowers, to intercept any pollen grains that might have passed through the mesh. No pollen packets or single pollen grains of almond could be detected with light microscopy on the microscope slides, although using the same technique without a cage many pollen grains were caught. Cages were removed after blooming was completed, just before trees began to develop leaves.

In winter (October 2007 to January 2008) trees were not irrigated and fertilised. The experimental water and nutrient treatments were conducted from January to August 2008. The following nutrients were applied every month by hand when irrigated: 521.6 g nitrate, 344.7 g potassium, 244.9 g sulphur, 158.8 g calcium, 158.8 g phosphorus, 54.4 g magnesium, 27.22 g boron, 27.22 g iron, 27.22 g manganese, and various micronutrients including zinc, cobalt, molybdenum (amounts when given are per tree, for that growing year). No nutrients were applied to trees in the no nutrient treatment.

Water reduction of the typical irrigated volume (108 l every third day) for this region and age of the trees was accomplished by manipulating the irrigation system of tubing and emitters at each tree. For the water reduction regime, three out of the four emitters at each tree were closed, reducing water to 27 l (75% reduction) every third day. The fungicide Rovral was applied at the rate of 0.0844 g m^{-2} before rain during blooming to avoid fruit fungal infections.

Fruit and leaf quantity and quality

To quantify fruit set at different developmental stages, we counted the total number of withered flowers on each main branch of each experimental tree from 28 February to 10 March, and we then counted developing fruits four times every 3–4 weeks (9 April, 16 May, 16 June and 2 July). On 2 July, we harvested and counted all fruits per whole tree for the last time and then kept 48 fruits per tree in the lab for further measurements. Fruits were randomly selected from the main branches (12, 16 or 24 fruits per branch, corresponding to trees with four, three or two main branches). Freshly harvested fruits were dried on the ground for 7 days while protected from bird and mammal predation with metal cages. After fruit drying, the hulls were removed and shells cracked. We characterised kernel quality by counting the number of unfilled, single and double kernels and the number of kernels damaged by arthropod pests or fungal and bacterial diseases. We measured the length (in mm) and weight (in mg) of each of the 48 kernels per tree. On the same dates as developing fruits were counted, we counted the number of leaves, starting at the tip of the main branches for 20 cm and noted the length (in mm) and colour (dark green, light green, yellow green) of ten randomly selected leaves per main branch of each tree. Leaf loss was calculated as the proportion of leaves that dropped between full development of the leaves (4 weeks after blooming, 9 April) and fruit harvest (2 July).

Data analyses

The effect of the treatments on the following response variables were analysed: fruit set and its decrease over time (which corresponds to fruit abortion), estimated total number of harvested kernels, mean kernel weight based on the 48 kernels per experimental tree harvested for detailed measurements, and estimated total yield per tree at harvest (number of kernels x mean kernel weight). To quantify the vegetative response to treatments, we also analysed effects on the number of leaves, proportion of leaves lost from 4 weeks after blooming until harvest, and the proportion of yellowing leaves.

Fruit set over time was modelled using generalised linear mixed models (GLMM) with a binomial distribution and a logit link. We accounted for non-independence of multiple measurements per tree and for extra-binomial variance by including tree and observation, respectively, as a random factor in analyses. Total number of harvested kernels, mean kernel weight and yield were analysed for differences among pollination and resource treatments using generalised linear models (GLM). The number of harvested kernels and yield were ln-transformed to reduce variance heterogeneity. For analyses of number of kernels and yield, the number of flowers was included as a covariate in the models, since this is a pre-treatment variable that varies from tree to tree (but not significantly between treatments; results not shown). The ln-transformed number of flowers was centred on its mean (6.77) to make model interpretation easier. For analysis of mean kernel weight, the number of harvested kernels was included as a covariate.

Treatment effects on number of leaves, the proportion of leaves lost and the proportion of yellow leaves were analysed using GLM. Average number of leaves per branch was analysed using a GLM for normal data, with the response variable untransformed. Leaf loss was analysed with a GLM for binomial data as a proportional variable. A quasi-binomial GLM (with a logit link function) was used to model the tree-level leaf colour outcome, identified as the most frequent leaf colour recorded on the tree, with a binary variable (1 = yellow, 0 = light or dark green).

We removed interactions that did not contribute at least marginally to the model ($P \geq 0.10$). Non-significant main effects were retained. For individual variables, F (or χ^2 for GLMM) and *P*-values in the text are from comparisons between the model with all main effects and significant interactions and the model with the tested variable dropped. All analyses were performed using *R*, version 2.8.1 for Windows (R Development Core Team 2011). Mixed models were fit using *lmer* (package lme4).

RESULTS

Fruit production

Models explaining observed fruit set across time, with just main effects, showed significant overall effects for pollination and time ($\chi^2_{df=1} = 105.63$, $P < 0.001$; $\chi^2_{df=1} = 99.31$, $P < 0.001$), but not for nutrients ($\chi^2_{df=1} = 1.618$, $P = 0.203$) and water ($\chi^2_{df=1} = 0.184$, $P = 0.668$). Hand-pollination across all resource input treatments resulted in ≥50% fruit set, while open-pollinated flowers set ≥30% fruits, and pollinator-excluded flowers set ≤5% fruits with mostly big and heavy kernels (Fig. 1, Table 1). Very low fruit set was found in the pollinator exclusion treatments (interaction: time x pollination: $\chi^2_{df=1} = 8.705$, $P = 0.012$; Fig. 1). Reduced water interacted with pollination (interaction: water x pollination: $\chi^2_{df=1} = 8.493$, $P = 0.014$; Fig. 1A, C *versus* B, D), with fruit set declining over time for open- and hand-pollinated treatments, but not the pollinator exclusion treatment (interaction: water x time: $\chi^2_{df=2} = 30.689$, $P < 0.001$; Fig. 1A, C *versus* B, D). Four- and three-way interactions ($\chi^2_{df=2} = 1.86$, $P = 0.393$; $\chi^2_{df=3} = 10.651$, $P = 0.155$), as well as the interactions between nutrients and pollination ($\chi^2_{df=2} = 2.227$, $P = 0.329$) and between nutrients and water ($\chi^2_{df=2} = 0.484$, $P = 0.487$) were not significant.

In total 93% of the variance in the number of harvested kernels was explained by pollination treatment and number of flowers (explained *versus* null-model deviance). The number of harvested kernels was significantly and positively related to the number of flowers ($F_{1,54} = 12.07$, $P = 0.001$) and differed between pollination treatments ($F_{2,54} = 162.75$, $P < 0.001$), with higher numbers in the hand-pollination and lower numbers in the pollinator exclusion treatment compared to the open-pollination treatment. Reduced water and nutrients did not significantly influence the number of harvested kernels (water: $F_{1,54} < 0.01$, $P = 0.99$; nutrients: $F_{1,54} = 0.36$, $P = 0.55$; Table 2A).

About 79% of the variance in mean kernel weight was explained by pollination limitation. Mean kernel weight depended on pollination treatment ($F_{2,53} = 42.81$, $P < 0.001$), with lower values in the hand-pollination and higher values in the pollinator exclusion treatment compared to the open-pollination treatment. The number of harvested kernels did not significantly add to the model containing the pollination treatment ($F_{1,53} = 0.65$, $P = 0.42$), but was significant in a model without pollination ($F_{1,53} = 45.78$, $P < 0.001$; Fig. 2). Kernel weight was only marginally and negatively influenced by water reduction ($F_{1,53} = 3.77$, $P = 0.0574$; Table 2B, Fig. 2), while nutrient reduction had no effect ($F_{1,53} = 2.68$, $P = 0.107$; Table 2A).

In terms of almond yield, 91% of the variance was explained with a model including both pollination and resource treatments (Fig. 3). The effect of pollination was significant ($F_{2,53} = 197.39$, $P < 0.001$), with higher yield in the hand-cross pollination and lower yield in the pollinator exclusion treatment compared to the open-pollination treatment. Reduced water negatively influenced yield ($F_{1,53} = 2.98$, $P = 0.015$), but only in open- and hand-cross-pollination treatments (pollination x water $F_{2,51} = 2.86$, $P = 0.067$). Water and fertilisation treatments had no significant influence on yield (water:

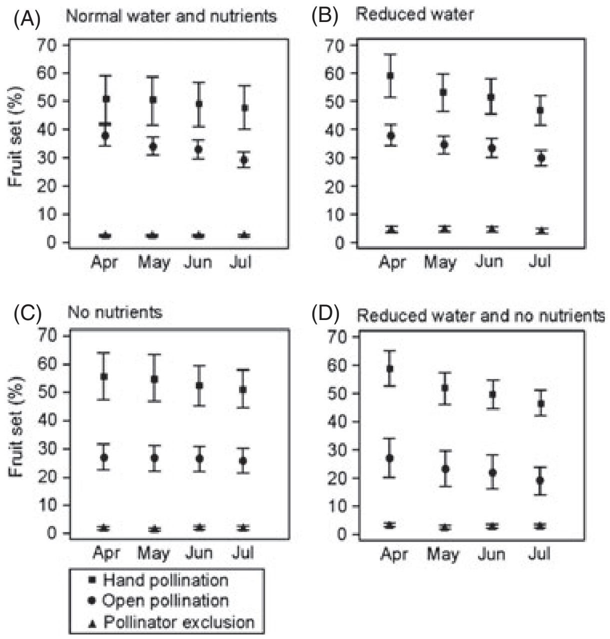

Fig. 1. The percentage of developing fruits to flowers (% fruit set at different fruit development stages, ±SE) of trees receiving different pollination treatments after 4 weeks (mid-April), 8 weeks (mid-May), 12 weeks (mid-June), and at harvest (beginning of July) for (A) trees that received the normal amount of water and nutrient application; (B) trees that received reduced water, but normal nutrients; (C) trees that received no nutrients but normal water application; (D) trees that received reduced water and no nutrient applications. GLMM testing for the effects of pollination treatment ($P > 0.0001$), water treatment ($P = 0.0717$), nutrient treatment ($P = 0.2273$) and time ($P = 0.0399$) on percentage fruit set: overall model ($P > 0.0001$, $R^2 = 0.78$, with one significant interaction of pollination with water treatment: $P = 0.0169$).

Table 1. Parameter estimates of GLMM with the response variable 'proportion of developing fruits to flowers' (treatment contrasts were used, with open-pollination and resource controls as reference treatment). Group means can be obtained by adding the estimate of the intercept to the estimate of treatment of interest, *e.g.* 'hand- *versus* open-pollination'. Interactions are indicated with 'x' between variables, and z represents the z-test statistic.

explanatory variables	estimate	±SE	z	*P*
intercept (open-pollination/normal resources)	−0.65	0.22	−2.887	0.0039
time	−0.05	0.01	−3.776	<0.0002
hand- *versus* open-pollination	1.00	0.30	3.391	0.0007
pollinator exclusion *versus* open-pollination	−3.44	0.30	−11.314	<0.0001
reduced *versus* normal water	−0.30	0.30	−1.017	0.3040
no nutrients *versus* normal nutrients	−0.23	0.17	−1.375	0.1693
time x reduced water *versus* time x normal water	−0.08	0.01	−5.880	<0.0001
time x pollinator exclusion *versus* time x open-pollination	0.06	0.03	2.452	0.0142
time x hand-pollination *versus* time x open-pollination	−0.012	0.01	−0.824	0.4101
reduced water x pollinator exclusion *versus* reduced water x open-pollination	1.26	0.42	2.998	0.0027
reduced water x hand-pollination *versus* reduced water x open-pollination	0.52	0.41	1.262	0.2070

Table 2. Results of GLM explaining the response variables (A) number (No.) of kernels harvested, (B) mean kernel weight and (C) yield (number of harvested kernels × mean kernel weight). Potential explanatory variables were hand-pollination, open-pollination, pollinator exclusion, reduced water and no nutrients. Full models included all two- and three-way interactions but non-significant interactions were deleted in the final models. Treatment contrasts used open-pollination and resource controls being the reference treatment. Group means can be obtained by adding the estimate of the intercept to the estimate of the treatment of interest, *e.g.* 'hand- *versus* open-pollination'. Interactions are indicated with 'x' between variables and *t* represents the Student's *t*-test.

response variable	explanatory variables	estimate	± SE	*t*	*P*
(A) no. kernels	intercept (open-pollination/normal resources)	5.24	0.17	31.34	<0.0001
	no. flowers	0.49	0.14	3.47	0.0010
	hand- *versus* open-pollination	0.80	0.18	4.38	<0.0001
	pollinator exclusion *versus* open-pollination	−2.41	0.18	−13.04	<0.0001
	reduced *versus* normal water	0.00	0.15	0.01	0.9930
	no nutrients *versus* normal nutrients	−0.09	0.15	−0.60	0.5501
(B) kernel weight (mg)	intercept (open-pollination/normal resources)	1082.78	43.21	25.06	<0.0001
	no. harvested kernels	0.10	0.13	0.81	0.4224
	hand- *versus* open-pollination	−172.14	47.91	−3.59	<0.0001
	pollinator exclusion *versus* open-pollination	387.66	44.81	8.65	<0.0001
	reduced *versus* normal water	−59.11	30.44	−1.94	0.0574
	no nutrients *versus* normal nutrients	49.89	30.47	1.64	0.1075
(C) yield	intercept (Open-pollination/Normal resources)	5.49	0.14	38.99	<0.0001
	no. flowers	0.68	0.11	6.53	<0.0001
	hand- *versus* open-pollination	0.54	0.18	2.97	0.0046
	pollinator exclusion *versus* open-pollination	−2.26	0.18	−12.18	<0.0001
	reduced *versus* normal water	−0.47	0.19	−2.51	0.0153
	no nutrients *versus* normal nutrients	0.03	0.11	0.25	0.8012
	hand-pollination x reduced water *versus* open-pollination x reduced water	0.22	0.26	0.84	0.4036
	pollinator exclusion x reduced water *versus* open-pollination x reduced water	0.63	0.27	2.35	0.0228

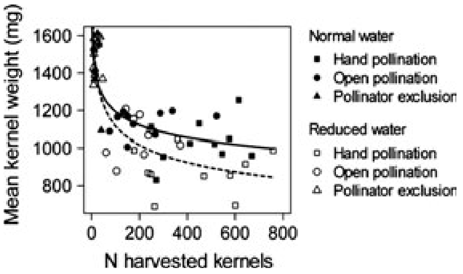

Fig. 2. The relationship between number of harvested kernels and mean almond kernel weight per tree. The broken line indicates the relationship for trees treated with reduced water and the solid line indicates the relationship for trees receiving normal water amount. Fitted model is log(y) = 7.49 − 0.089*log(x) for controls, and log(y) = 7.65 − 0.137*log(x) for water reduction, with main effects and interaction significant. Overall model R² = 0.65, *P* < 0.0001, n = 59.

$F_{1,54}$<0.01, $P = 0.99$; nutrients: $F_{1,54} = 0.36$, $P = 0.55$). Yield was significantly and positively related to the number of flowers ($F_{1,51} = 42.58$, $P < 0.001$; Table 2C), but the number of flowers was not influenced by any treatment combination (data not shown).

None of the examined fruits and kernels showed evidence of attack from pests or diseases. Over 98% of these fruits comprised single kernels; the remainder had double kernels. The ratio of double to single kernel fruits was not related to the pollination mode or resource availability (results not shown).

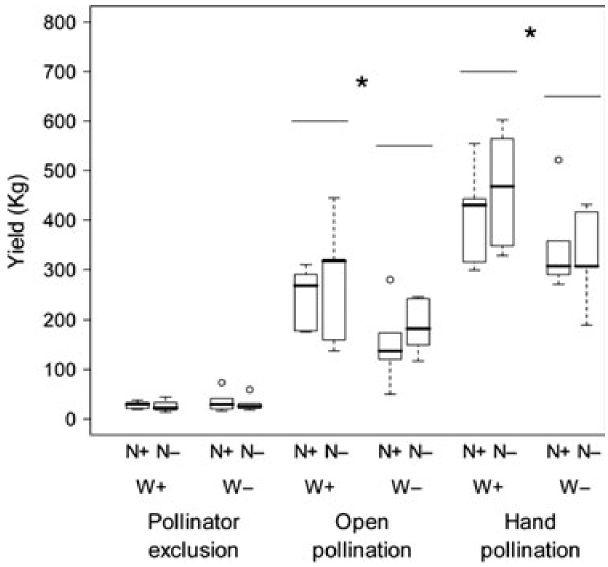

Fig. 3. Almond yield (number of harvested kernels × mean kernel weight) for trees receiving the different treatment combinations. Heavy horizontal line = median, boxes = 25–75% quartiles (upper and lower hinges), light horizontal lines = 1.5 × hinge. Points outside this interval are represented as open circles. Treatments are N+ = with nutrients, N− = no nutrients, W+ = normal water, W− = reduced water. Asterisks indicate significant differences between restricted water treatments and water controls (for statistics see results and Table 1A).

Tree foliage

The number of leaves that developed 4 weeks after blooming ended was influenced by pollination treatment ($F_{1,55} = 370.51$, $P < 0.001$), but not by water reduction or nutrient elimination (water: $F_{1,55} = 1.93$, $P = 0.170$; nutrients: $F_{1,55} = 1.97$, $P = 0.166$). The model explained 91% of variance in the number of leaves. Trees with pollinators excluded had more leaves, and trees in the hand-pollinated treatment had fewer leaves than the trees in the open-pollination treatment. The number of developed leaves after 4 weeks was negatively related to the number of kernels at harvest (Fig. 4A, Table S1A).

Leaf loss was reduced under pollinator exclusion, and increased under hand-pollination, when compared to open-pollination ($F_{2,55} = 5.57$, $P = 0.006$). Water reduction increased leaf loss ($F_{1,55} = 29.65$, $P < 0.001$; Fig. 4B). This effect was largest when nutrients were eliminated ($F_{1,50} = 19.90$, $P = 0.003$; Fig. 4B), and depended on pollination treatment ($F_{2,50} = 2.70$, $P = 0.0771$), with a lower effect of water reduction in the hand-pollination treatment than in the other pollination treatments. Nutrient reduction increased leaf loss in the exclusion and hand-pollination treatments, but not in the open-pollination treatment ($F_{1,50} = 39.53$, $P < 0.001$; Table S1B). The model explained 85% of the variance in leaf loss.

Trees with predominantly yellow leaves were found with a higher probability under hand- (13 out of 20 trees) and open- (ten out of 20 trees) pollination than in the pollinator exclusion treatment (zero out of 20 trees; contingency table analysis: $\chi^2_{df=2} = -26.2$, $P < 0.0001$). The probability of having mainly yellow leaves was especially high in hand-pollinated trees with no nutrients added (ten out of ten) than in trees with nutrients added (three out of ten trees) (interaction: $\chi^2_{df=2} = 14.9$, $P < 0.0001$). The probability of having mainly yellow leaves was also high in hand- and open-pollinated trees with reduced water (hand: eight out of ten; open: ten out of ten) than in trees with normal water treatment (hand: five out of ten; open: zero out of ten) (interaction: $\chi^2_{df=2} = 14.9$, $P < 0.0001$).

DISCUSSION

Our experiment shows that pollination strongly limits almond fruit set and yield and therefore supports general expectations and previous results of high pollinator dependency in almond (Thorp 1996; Delaplane & Mayer 2000). The strong pollination effect on yield even in conditions of reduced water input and nutrient reduction was in contrast to descriptions of California almond production as dependent on high water and nutrient inputs (Micke 1996).

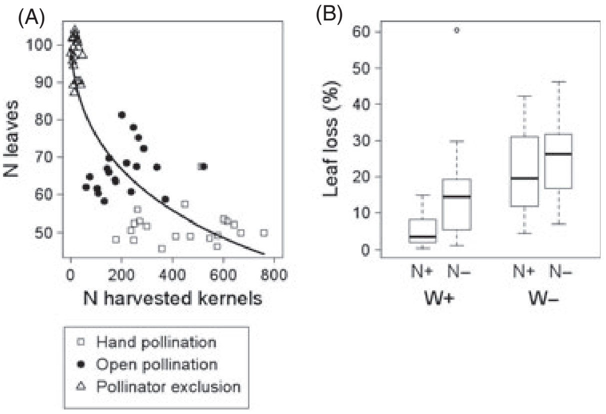

Fig. 4. (A) The relationship of number of leaves 4 weeks after blooming to number of harvested kernels per tree. Fitted model is $\log(y) = 4.64 - 0.031*(x+1)^{0.5}$, $R^2 = 0.72$, $P < 0.0001$, $N = 60$). (B) The proportion of leaves lost during the growing season and resource treatment. Heavy horizontal line = median, boxes = 25–75% quartiles (upper and lower hinges), light horizontal lines = 1.5 × hinge. Treatments are N+ = with nutrients, N− = no nutrients, W+ = normal water, W− = reduced water. Asterisks indicate significant differences between restricted water treatments and water controls. Both water ($P < 0.0001$) and interaction between water and nutrient reduction ($P = 0.0036$) are significant (see also Table S1B).

The negative effects of water reduction on yield, with only marginal negative effects on fruit set and mean kernel weight and no detectable effect on the number of kernels, in this study is supported by previous studies (Esparza *et al.* 2001) that showed negative effects of water stress on yield (kg nuts from several trees harvested only after 2 years of the first stress event), but not on bud development, fruit abortion and kernel weight (Girona *et al.* 1997; Romero *et al.* 2004; but see Goldhamer *et al.* 2006). Surprisingly, the initial benefit of pollination on yield components was not eliminated by reduced water and was not offset by the negative relationship between number and weight of kernels.

Although leaf water potential was not measured in this study, as in other work (Romero *et al.* 2004; Goldhamer *et al.* 2006; Rouhi *et al.* 2007), water stress was indicated as increased leaf loss occurring in the reduced water treatment. Such leaf loss is often observed in water-limited almond trees (Romero *et al.* 2004; Rouhi *et al.* 2007). The strong effect of reduced water on leaf loss, its marginal effect on mean kernel weight and the increased number of yellowed leaves in open- and hand-pollinated trees with reduced water indicate that when under water stress, almond trees may allocate resources selectively to maintain kernel quantity while reducing kernel quality (size) and delivery of resources to leaves.

The lack of any direct significant effects of the cut-off of nutrients on fruit set, yield or leaf loss suggests that the young trees may have already accumulated sufficient nutrients for fruit maturation from the previous summer's nutrient applications. Nevertheless, the significantly higher proportion of yellowed leaves at harvest on trees receiving no nutrients and reduced water, and the significant interaction between the water and nutrient treatments on leaf colour indicate that the trees were stressed in this treatment combination, especially when pollination took place. Trees from which pollinators were excluded were characterised by canopies consisting of dense, large and dark-green leaves, in contrast to hand-pollinated trees characterised by small, yellow-green leaves. These differences in foliage indicate that excess nutrients beyond those needed for nut production in the pollinator-excluded trees were used for canopy development. Thus, the positive effect of pollination on fruit production comes at the expense of vegetative performance features and may have long-term consequences for the tree.

We found a significant interaction of pollination and irrigation on yield resulting from decreased yield in hand- and open-pollinated treatments receiving reduced water, but no effect of reduced water on yield in the pollinator exclusion treatment, indicating a threshold of pollination is needed before the negative relationship between pollination quantity and water reduction on yield manifests itself. Two other studies analysed the interactions between pollination and plant resources on fruit set in woody plants (Niesenbaum 1993; Groeneveld *et al.* 2010). Niesenbaum (1993) focused, in two consecutive years, on a dioecious, understorey forest shrub whose reproduction was highly limited by light, but not by pollination, with no interaction effect between pollination and light. In contrast, Groeneveld *et al.* (2010) manipulated pollination, light, nutrient and water input and tested for the single and interaction effects of these variables on fruit set and number of harvested cacao pods after 1 year. They found that shade increased the number of aborted fruits, and the interaction of hand-pollination with shade, as well as the interaction of hand-pollination with nutrients, reduced the number of fruit abortions, but the interaction effects were not translated to losses or increases in fruit set or yield found in our study. To our knowledge, the present study is the first in which significant interactions between pollination and plant resources on fruit set and yield were found, highlighting the importance of studying pollination and plant resources in a full factorial design to understand their single and combined effects on plant performance in general and crop production in particular.

Almond yield was extremely low when pollinators were excluded, although these trees produced large kernels, while yield of hand-pollinated trees were high with small kernels. The kernel size in the different pollination treatments is likely caused by resource allocation and availability rather than pollination quality. In the pollinator exclusion treatment, kernels are assumed to result from self-pollination with low quality and quantity pollen. These results are contrary to studies showing that fruit or seed size and weight are often positively related to pollination quality and quantity (Ricketts 2004; Klein *et al.* 2007). It also indicates that intensive pollination management, such as simulated by our hand-pollination treatment, can result in low kernel quality (extremely small kernels).

Future experiments conducted over consecutive years are needed, particularly because high fruit set in year one resulting from supplemental pollination in the previous year may impose limits on reproduction in subsequent years (Bierzychudek 1981; Zimmerman & Pyke 1988). We found that foliage was reduced by water stress and indirectly by pollination in our 1-year study, but this may influence fruit set in the following year because the number and size of leaves influences rates of photosynthesis and hence resources available to develop new flowers (Esparza *et al.* 2001). Further, fruit load may be more strongly determined by the stress history of the trees rather than the current year's irrigation treatments (Esparza *et al.* 2001). Although the need to study pollination and resource limitation for several years in perennial plants is evident, the pollinator-dependent yield response determined with and without resource limitation of a single year can help growers to make *ad-hoc* decisions in years of pollinator and/or water shortages.

Our results suggest that for almond, pollination of the crop should be a high priority, but that other resources must be concurrently monitored and managed because of their well known effects (Kozlowski *et al.* 1991) and potential interactions that can influence overall plant performance. As a reduction of water and nutrients increased leaf loss and changed the colour of leaves in the same year when stress was imposed, future studies should investigate plant performance (vegetative and reproduction parameters) of almond experiencing pollination limitation in subsequent years after stress was imposed and after long-term limitations to plant resources.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Results of GLM explaining the response variable (A) number (No.) of leaves counted 4 weeks after blooming ended; and GLM for quasi-binomial response explaining the response variable (B) leaf loss (proportion of lost leaves starting from 4 weeks after blooming ended to harvest).

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