Final Report

Pollination as a controlling factor in almond yield

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Content

Summary

Almond trees are known to require pollination to produce nuts. Less is known regarding how pollination interacts with resource constraints to determine quantity and quality of production. This project was established to examine how determinants of resource availability such as light environment and leaf area influence flowering and fruiting at spur level. We compared different pollination treatments to establish how light, leaf area and flower number influence the response to elevated pollination. If increased pollination creates a resource demand greater than the tree can sustain, then increased nut production at spur level may not translate into increased whole tree yield. Further, the cost of producing more nuts might be expressed by a reduction in nut size, which would diminish crop value. Therefore we also examined the relationship between number and size of nuts produced, and tested whether it was possible to elevate production of nuts at the whole tree level (rather than spurs or branches) by using a whole-tree application of pollen.

Seventy five percent of flowering spurs had between 2 and 5 flowers, but 94% of spurs produced 3 or fewer nuts. We found that spurs producing more than one nut produce fruit of the same size (on average) as spurs that produce only one, i.e. there is no number versus size trade off. Among spurs with 1 or more flowers, those that support many leaves (November) generally produced fewer flowers in that same growth season (August). There is <u>not</u> a general positive relationship between light environment and flower number at spur level. Instead the response depends strongly on the part of the crown (aspect) that the spur is in.

Among open pollinated spurs the probability of producing one or more fruit depends strongly on the number of flowers. Each flower could be thought of as a ticket in a lottery (the pollination lottery) where the best way to "win" (i.e. to produce a nut) is to have more tickets (flowers). When the pollination rate is elevated spurs more often achieve the outcome of one or more nuts. Thinking of the chance of effective pollination being a lottery is consistent with the observation that although bee activity is high in the orchards, only a small percentage of visits is likely to be useful in the sense that cross-pollination is achieved. Most visits are expected to transfer self-pollen from flowers in the same tree or the same row. In this sense a flower needs to be "lucky" to be visited by a bee that recently picked up pollen from flowers on a tree of a pollinator variety in a neighbouring row.

The total mass of nuts produced per spur was greater for spurs that were higher in the canopy and therefore in a better light environment. There is a benefit from increased pollination for kinds of of spur (i.e. low and high light, many or few flowers), but the greatest benefit from increased pollination was for spurs with few flowers (i.e. would have a high probability of failure if pollination were not guaranteed) but high light (providing the resource opportunity to increase nut set when pollen is available).

Only 10% of spurs in the control group flowered multiple times over the three year period. Spurs that had pollination guaranteed were even less likely to flower in the next year or two. Spurs which flowered again in a subsequent year were those with a lower nut set from the first flowering event.

Whole tree pollen spraying elevated pollination and this was achieved in spite of the relative lack of experience in the industry regarding how to achieve this outcome. In all four experiments the pollen-sprayed trees had higher median yield than the control (normal pollination practice) trees, with the benefit being between 10% and 16% increase, depending on the experiment. Given that the marginal benefit from improved pollination is around four times greater than the current level of spending on pollination, there is considerable room for increased spending on pollination with the expectation that benefits will exceed costs.

Keywords

almonds, pollination, honeybees, flowers, light, pollen

Introduction

It is well understood that pollination is a critical requirement for almond production. For this reason the provision of honeybee hives for pollination is standard practice in commercial production. Experiments conducted in the period 2012-2014 indicated that current standard pollination practice in Australia is likely to lead to under-pollination in many orchards (Cunningham 2014, Cunningham et al 2016). Improvements to pollination practice are predicted to lead to an increase in production. Indeed it is already well established that hand pollination of flowers on branchlets increases nut set relative to the level achieved by standard commercial pollination practice (Cunningham et al 2016). These experiments, however, did not consider the way in which resource constraints effect the response to increased pollen supply.

The project reported here was established to examine how determinants of resource availability such as light environment and leaf area influence flowering and fruiting at spur level. We then compare different pollination treatments to establish how light, leaf area and flower number influence the response to elevated pollination. If increased pollination creates a resource demand greater than the tree can sustain, then increased nut production at spur level may not translate into increased whole tree yield. Further, the cost of producing more nuts might be expressed by a reduction in nut size, which would diminish crop value. Therefore we also examined the relationship between number and size of nuts produced, and tested whether it was possible to elevate production of nuts at the whole tree level (rather than spurs or branches) by using a whole-tree application of pollen.

This work was conducted as part of a co-ordinated research program on almond productivity. Across the whole program collaborators include Victorian Department of Environment and Primary Industries (DEPI: Dave Monks and Cathy Taylor) Plant and Food Research Australia (Andrew Granger), SARDI (Dane Thomas) and another CSIRO team (Everard Edwards). This particular component involves closest co-operation with the DEPI team because we share a trial site, co-ordinating of field activities, and sharing data. Whereas this component is concluding now after 3 years of work, the other projects are continuing over longer terms. The leader for this project, Saul Cunningham, was employed by CSIRO when the project was contracted, but moved to the Australian National University in mid-2016. The project was completed under the CSIRO contract, but with Saul Cunningham conducting analyses and writing reports as an external contractor.

Throughout the report we have highlighted sections of text that report key results by placing them inside a box.

Methodology

Study Site

The focal trees for our research are at the CMV orchard at Lindsay Point, Victoria (latitude 34° 4'39.26"S, longitude 141° 0'19.59"E), shown in Figure 1. The experimental trees used in the main experiment extended from row 18 to row 43, and down the row to tree number 32 (each row was about 58 trees long, with tree number increasing toward the NE). In year two we extended down the row to tree number 48 to accommodate additional treatments (Fig. 5). The study was conducted over three seasons with year one extending from flowering in August of 2014 and harvest of nuts on February 2015. Across all three years of the study honey bee hives were provided in the orchard in accordance with normal pollination practice, which in this orchard is 6.5 hives per hectare.

Figure 1: *Location of the research trees in the CMV orchard at Lindsay Point, Victoria.*

The main experimental area is laid out to accommodate a design with different irrigation and fertilisation treatments. There are six replicates in the design, each comprised of a column of 4 blocks, each with a different treatment. The position of the treatments within the replicate were assigned randomly. For the experiments reported here, only trees in the "normal" treatment blocks (coded blue in Fig. 2) were used.

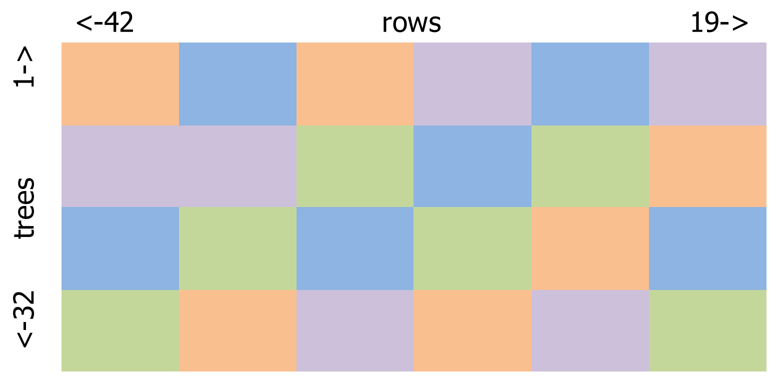

Figure 2*: The layout of the main experiment, with each replicated block in the trial represented by a different colour, arranged in six replicates aligned with rows. Blue is normal orchard management, Green is normal water reduced N, Purple is reduced water, normal N, Orange is reduced water and N (See Monks and Taylor project for design details).*

Each block consists of two rows of eight Non-Pareil trees, and two rows of eight trees of the two pollinator varieties (e.g. Carmel and Monterey). The experiments reported here focused exclusively on Non-Pareil trees. The design of each block is shown in figure 3. Two trees in one row were assigned to a treatment with hand pollination of a sample of spurs, followed by a pollen spray over the whole tree (HPS). Two trees in the other row were assigned to have hand pollination of a sample of spurs, but no pollen spray (HP). Three trees were assigned to the treatment "open pollination" (OP) where they received no hand pollination or whole-tree pollination, and therefore can be thought of as controls. Therefore for the spur-level survey we assessed 18 OP trees (6*3), 12 HP trees (6*2) and 12 HPS trees (6*2).

Non Pareil	Carmel	Non Pareil	Monterey
x	x	x	x
x	x	HP	x
HPS	x	OP	x
x	x	x	x
x	x	OP	x
HPS	x	OP	x
x	x	HP	x
x	x	x	x

Figure 3: *Layout of trees in each experimental block of the main experiment. Each cell contains one tree. X marks trees that are present but not assigned to any treatment. HPS denotes trees with spurs hand pollinated and also a whole-tree pollen spray. HP trees had spurs hand pollinated, but no pollen spray. OP trees were open-pollinated according to normal orchard practice, with no pollen spray or hand pollination.*

Spur selection

On each tree we established two vertical transects of six labelled flowering spurs (a spur is a fruit-bearing shoot coming off a branch), approximately evenly spaced, and with the highest spurs at approximately 2.8 m and the lowest spurs approximately 1 m above ground level. Therefore there were 12 spurs (2 transects of 6) in each tree assessed. Trees in this trial were approximately 6.5 m tall (average in 2015). The spur transects were selected in defined quadrants of the canopy, treating the line of the row as the mid-line (Fig. 4). We selected the location for each transect using a balanced random strategy so that there was equal representation of each part of the tree (i.e. north, south, east and west) across each treatment group.

In years two and tree we examined spurs from the previous year(s). If the spur was not flowering again (which was usually the case) a new spur was selected, to ensure that we continued to assess 12 spurs per tree each year.

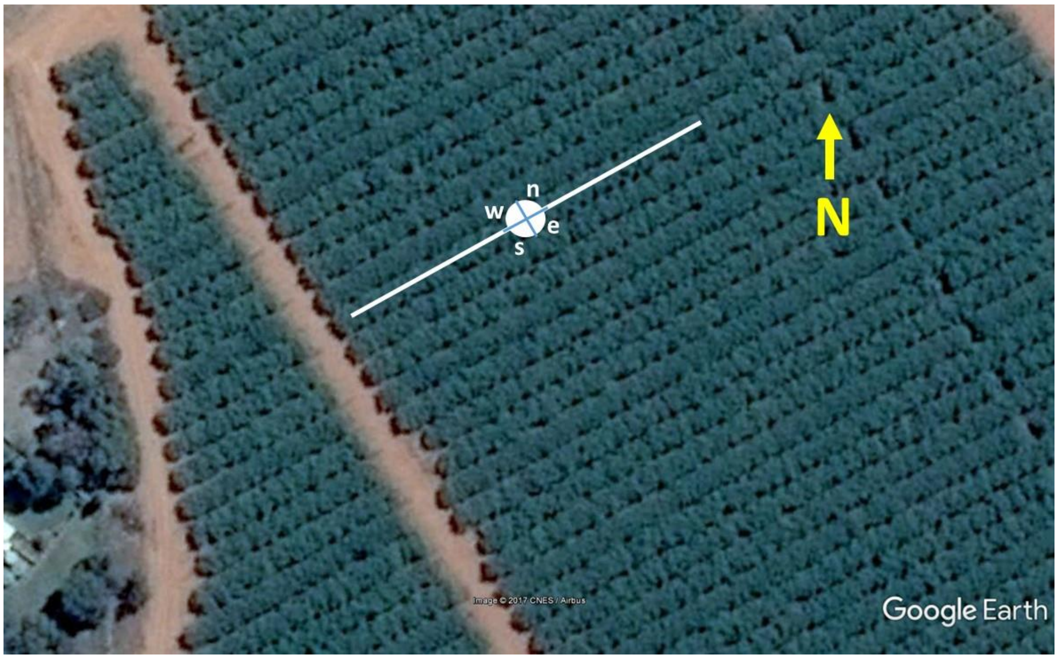

Figure 4: *Flowering spurs were surveyed on trees in the research block, selecting them from four different quadrants of the crown. This figure shows the four different quadrants and their aspect, relative to the direction of the row, and true north (in yellow). For convenience we labelled the quadrants n, s, e and w, even though they were not aligned perfectly with the compass directions.*

Hand pollination of spurs

Tagged spurs on trees selected for the hand pollination treatment were monitored daily during the flowering period. When flowers opened they were hand pollinated by applying pollen from the anthers of freshly picked flowers from Peerless trees, because these have the highest possible level of pollen compatibility with Non-Pareil. This was repeated daily so that as new flowers opened on each spur they then received hand pollen.

Flower, leaf, light and fruit assessment

All spurs were surveyed for the number of flowers produced during the flowering season. We also surveyed the number of leaves supported, and the length of the longest leaf, with this assessment occurring in

November. A study by Hereema et al (2008) established that leaf number multiplied by length of the longest leaf is a very good predictor of total leaf area, although the latter is much more painstaking to measure. We therefore calculated leaf number*length of longest, and refer to this at "leaf area score".

The light received by a spur over the course of a season would vary as the leaves grow, as the sun angle changes, and as the branches droop with the weight of nuts. Nevertheless the typical amount of light received will be strongly determined by position in the canopy. As part of the larger almond productivity project, Monks and Taylor characterised the relationship between spur height and light received, by extensive measurement of light environments for spurs on trees in this experiment block. They then developed a statistical model which estimates the expected light environment as a function of spur height in the tree and which is appropriate to the particular orchard design and management of this location. We have used this model to estimate light environment for our spurs. The model is as follows:

$$light = 0.2688*x - 0.4058,$$

where x is spur height above the ground in meters.

and light is proportion of incoming PAR

Mature fruit were collected at the February harvest, then transported back to Canberra and placed in a -20°C freezer for 4 days to kill any insect pests. After this they were removed and allowed to dry and return to room temperature for processing. All fruit were hulled and kernels were cracked out, but kept in labelled bags so that they could be related to the labelled spur. Every kernel was weighed after oven drying at 65°C for 6 days, at which point weight loss had stabilised. We refer to this as dry kernel weight.

Whole-tree pollen application

In addition to the spur-level hand pollination experiment we also conducted a whole-tree pollen spray experiment. We used bee-collected pollen from hives placed near a part of the orchard that had a high level of Peerless flowering, and a low level of other varieties. This means the bee collected pollen is likely to be rich in Peerless variety pollen. Pollen balls were knocked out of the pollen baskets of incoming bees at the hive using pollen traps on the hive entry. This pollen was kept in a refrigerator at -20°C.

Bee-collected pollen was suspended in a solution (below) based on a recipe used successfully for spraying kiwi fruit pollen (Hopping and Simpson 1982). The suspension was sprayed over the whole canopy of the target trees using hand sprayers operated from a hydralift cherry picker to access all parts of the canopy. In year 1 we used 2 litre Cambrian hand operated sprayers with a 1 m extension nozzle. In years 2 and 3 we used mounted Solo hand sprayers. Trees were sprayed from multiple directions so styles would be hit by pollen regardless of which way any individual flower was facing. Trees were first sprayed from the bottom of the tree upwards, then horizontally from the side of the tree and then from above the tree downwards.

We sprayed twice, a few days apart, with the goal of first application when trees were 45-65% in flower (approximately 4 litres applied), and second when trees were 90-100% in flower (approximately 6 litres applied).

Pollen Suspension

1 l distilled water

7 g bee-collected pollen

100 mg of calcium nitrate tetrahydrate

100 mg of Boric acid

100 mg of carboxymethyl cellulose

50 mg of gum Arabic

The HP trees (Fig. 3) were used as the "control" for comparison (i.e. having received no whole tree pollen spray) to which we compared the HPS trees (which received the whole tree pollen spray). The fact that we had hand-pollinated approximately 40 flowers per tree in the HP treatment is a trivial contribution to whole tree yield in the context of approximately 50,000 flowers across the whole tree.

We harvested almonds from each tree separately to allow a comparison of whole tree yield. The fruit collection area on the ground was delineated by the point half way between trial and non-trial trees. Any visible windfall was raked back from non-trial trees, while windfall from trial trees was raked towards the trial tree. After the trees were shaken, a leaf rake was used to move fruit into windrows. Again, raking was done to delineate between sampled trees.

Fruit from trial trees was then individually harvested and fruit deposited into a bin for weighing. Several of the empty bins were weighed and an average tare weight was derived. This was then used in calculating the total weight of the almonds. After the gross weight was recorded, a subsample of fruit was collected by overfilling a 10 litre bucket. This sample was used to check if there was variation among trees in moisture levels of fruit or percent crack-out. Having determined that there was no such variation we have elected to report fresh weight of unprocessed fruit, because this is exactly what was collected in the field.

Boron was included in the pollen suspension because it is expected to help maintain pollen viability, and so improve the likely effectiveness of the spray. However, boron may have an effect on tree productivity independent of the effect on pollen. To test for this possibility we modified the "control" treatment for two of the whole tree spraying experiments (Table 2). In year 2 we extended the "whole-tree spray" experiment into trees immediately adjacent to and downhill from the main experiment. The design for these trials are described in figure 5. The control treatment in this block was for trees to be sprayed with the boron solution (as per pollen suspension recipe) but with no added pollen. In year 3 the same strategy was applied to whole tree spray trees and controls in the main block. Separate sprayers were used for the pollen and the water only treatments so as to avoid any contamination.

			rep 1				rep 2				rep 3				rep 4				rep 5				rep 6			
	43	42	41	40	39	38	37	36	35	34	33	32	31	30	29	28	27	26	25	24	23	22	21	20	19
	m	n	c	n	m	n	c	n	m	n	c	n	m	n	c	n	m	n	c	n	m	n	c	n	m
33	X	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x
34	X	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x
35	X	N	x	x	x	Y	x	x	x	N	x	x	x	Y	x	x	x	N	X	x	x	Y	x	x	x
36	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x
37	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x
38	X	N	x	x	x	Y	x	x	x	N	x	x	x	Y	x	x	x	N	X	x	x	Y	x	x	x
39	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x
40	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x
41	X	x	x	Y	x	x	x	N	x	x	x	Y	x	x	x	N	x	x	X	Y	x	x	x	N	x
42	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x
43	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x
44	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x
45	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x
46	X	x	x	Y		x	x	N		x	x	Y		x	x	N		x	X	Y		x	x	N	x

Figure 5: *Layout of trees in rows 19 to 43, tree number 33-46, with two experimental treatments. N trees received a spray of boron solution, but no pollen. Y trees received a whole tree pollen spray, in boron solution. Only Non-Pareil trees were used (n) but pollinator varieties occur in alternate rows. Note that the main block (Fig. 2) ends at tree 32 and is therefore adjacent to this block, at the top of this diagram. Along the top of the diagram the letter represent each variety, m, Monterey; n, Non-Pareil; c, Carmel.*

Outcomes Evaluation and Discussion

Flowering

Based on our survey of 1550 spurs, there is a wide range in the number of flowers found on a flowering spur, from 1 to 15 (Fig. 6).

Flower counts between 2 and 5 are most frequent, accounting for just over 75% of all flowering spurs.

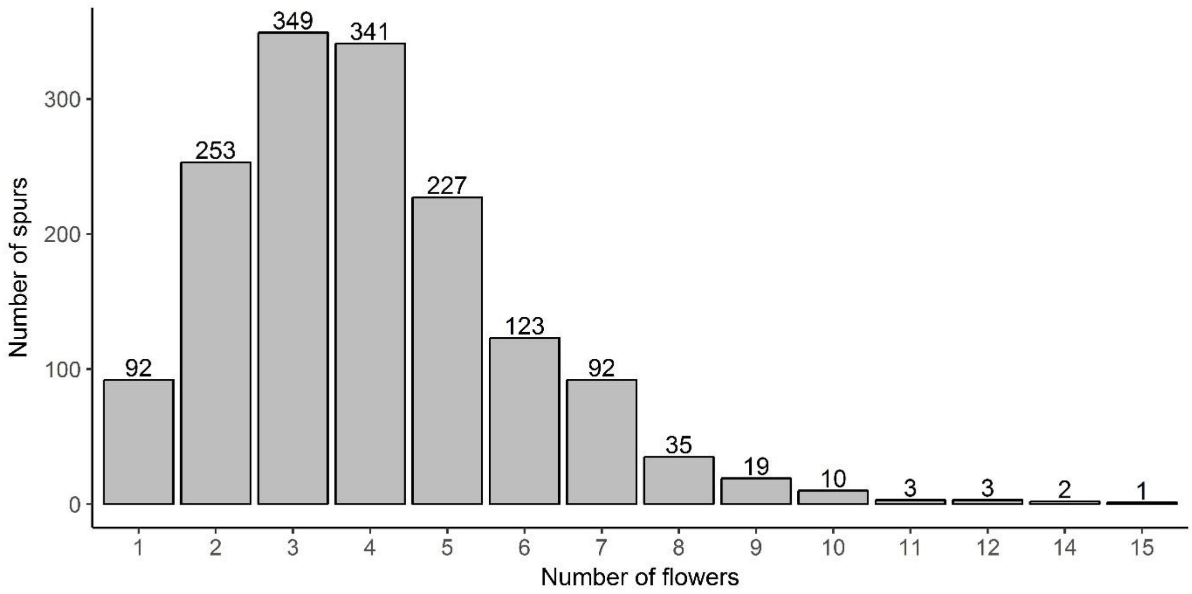

Figure 6: *Histogram of the number of spurs in each flower number category (non-zero spurs only, we did not systematically sample non-flowering spurs). Contains data from all years.*

Leaf per spur

Leaf area scores for flowering spurs ranged from zero to >2500, but 87% of spurs were in the range zero to 500. Average leaf length varied among years (table 1). There was a reduction in leaf length for each year such that the year 3 leaves are, on average, 11.7 cm shorter than those in year 1. This pattern corresponds with a decline in yield in general, even on control trees (Table 2). The cause of this correlated pattern is unknown, but may reflect the same general change in growing conditions.

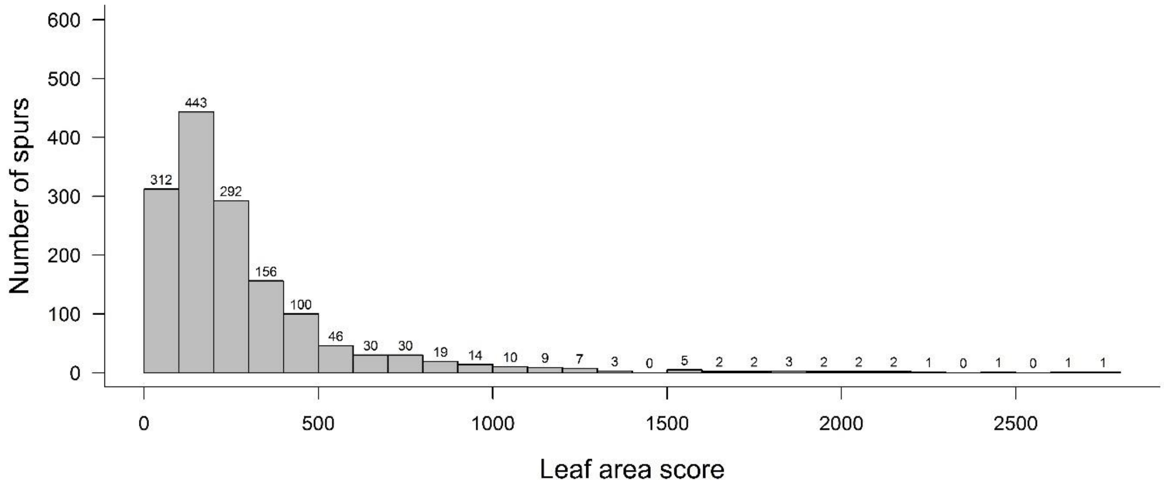

Figure 7: *Histogram of the frequency of spurs in different leaf area score categories. Contains data from all years. Flowering spurs only.*

Table 1: *Average length of the longest leaf on flowering spurs surveyed, over three years.*

Year	Avg. leaf length, cm	1 SD	N (number of spurs)
1	46.7	13.3	277
2	39.9	12.9	441
3	35.0	12.0	542

Nuts per spur

Most flowering spurs (78%) produced one or more nuts. Spurs producing one nut was the most frequent category, but two or three were reasonably common (Fig. 8).

Across all flowering spurs, 94% produced 3 or fewer nuts.

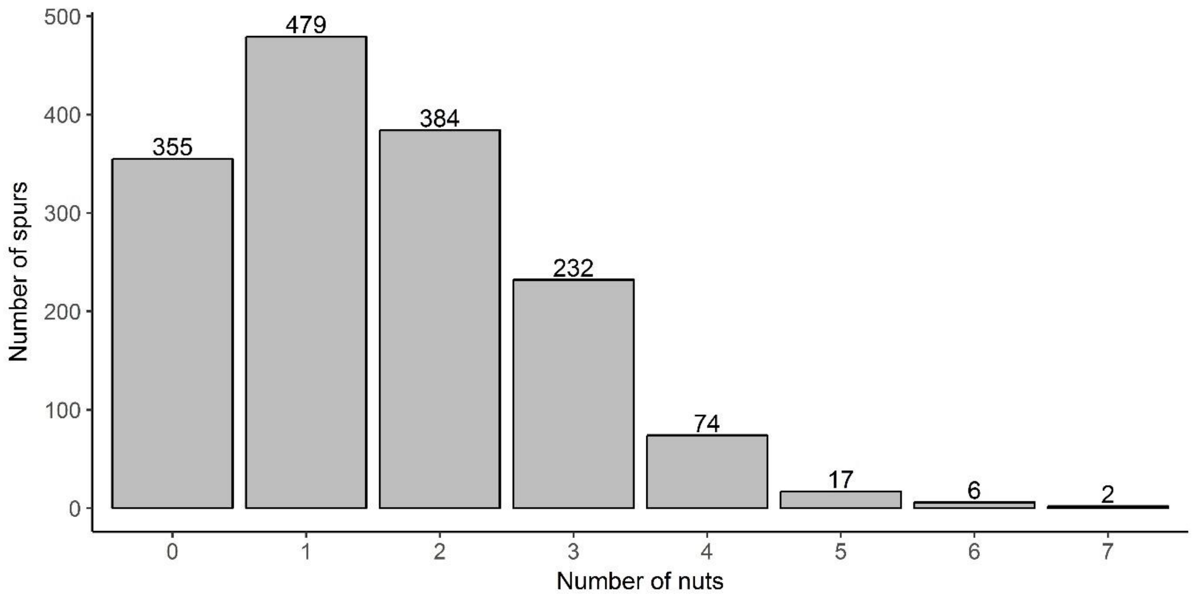

Figure 8: *Histogram of the number of spurs in each nut number category (only considering spurs that flowered). Contains data from all years.*

One of the important goals for this project was to establish if there is a cost to production of more nuts that is expressed in terms of nut size. In other words, if a spur produces more nuts, do they tend to be smaller? We addressed this by first calculating the mass (dry) of nuts supported by each spur. Typical mass of one nut is close to 1 g, but there is of course variation among nuts. By graphing the total mass of nuts on a spur against the number of nuts (Fig. 9) you can see the expected positive relationship. We did this for each of the three years separately in case the pattern varied by year. We also calculated a "line of no trade off". This shows the pattern that would be expected if the average mass of nuts on single nut spurs was exactly the same as the mass of nuts on multi-nut spurs. The figure does reveal that each year was a bit different in typical nut mass, so that nuts were smaller in year 3 than in the previous two years.

Our data over three years indicates that spurs producing more than one nut produce fruit of the same size (on average) as spurs that produce one nut, i.e. **there is no number vs. size trade off.**

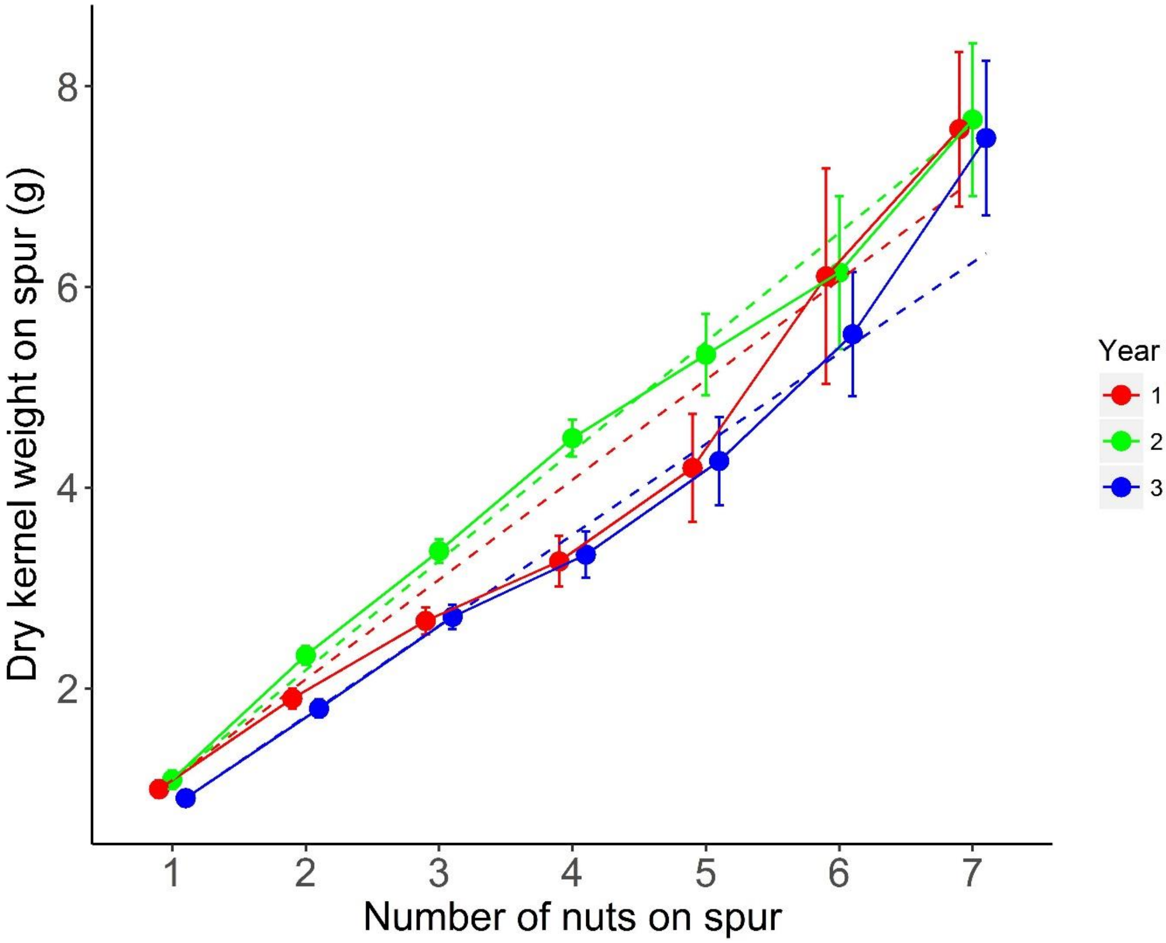

Figure 9: *Dry kernel weight as a function of number of nuts on a spur, across three years of the survey. The dashed lines are the 'lines of no trade-off' i.e. the expected dry weight if the mean weight of nuts was maintained at the 1 nut per spur level.*

Predicted values of a linear model ($p < 2.2\ e^{-16}$, $R^2 = 0.7869$, AICc = 1976.312). The error bars are 95% confidence intervals. The full model was: dry kernel weight ~ number of nuts*year of survey.

How do leaf area and light environment effect flower number at spur level?

Spur-level analyses are based on GLMM modelling of our large data set of approximately 1500 spurs. Here we present figures that show the functional relationships that explain a significant amount (i.e. P>0.05) of the among-spur variation, plotted with 95% confidence intervals and visually simplified by removing with all the underlying data points. The modelling statistics are recorded under the figures, in a smaller font.

If well-resourced spurs produce both a high flower number and a high leaf number one would see a positive association between leaves and flowers. Alternatively, investment in flowers could trade-off with investment in leaves at spur level, because there is limited room to arrange the different buds, or because the resources for flowers and leaves come from the same limited pool.

Among spurs with 1 or more flowers, those that support many leaves (November) generally produced fewer flowers in that same growth season (August) (Fig. 10).

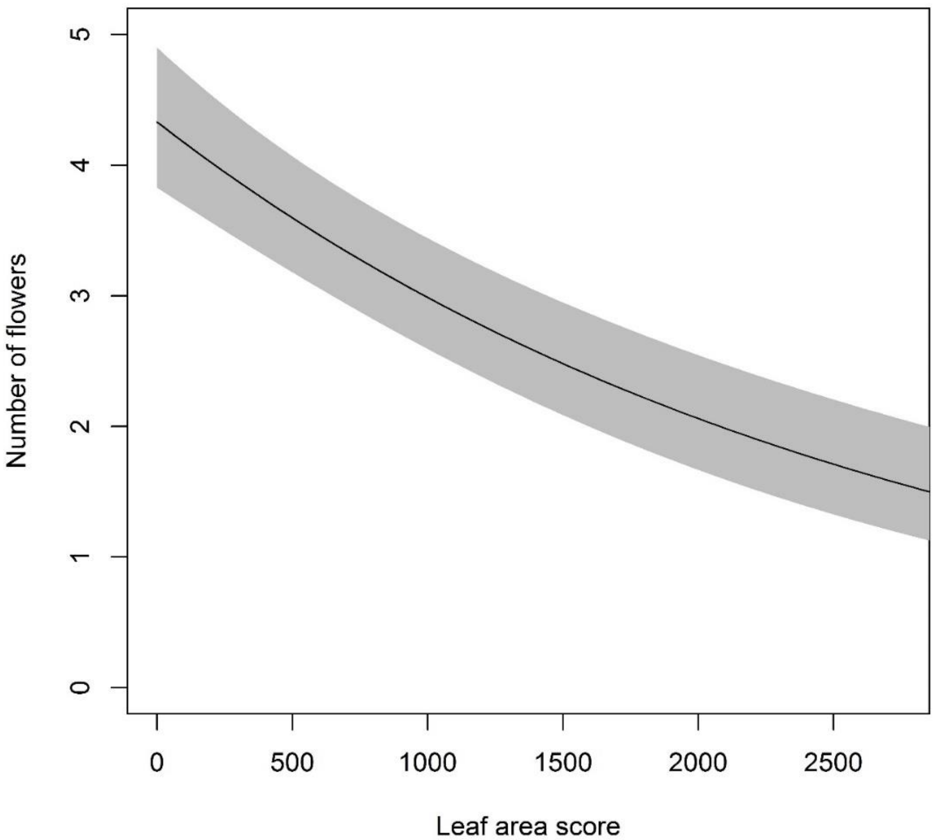

Figure 10: *The number of flowers on a spur (flowering spurs only) against the leaf area score.*

(p = 1.314e^{-14} , R^2 = 0.109, AICc = 5923.465). The model is as follows: Number of flowers ~ leaf area score + random(row/tree/spur) + random(year). The random effects of row nested within tree nested within spur act as spatial controls. Spur also acts as a temporal control because we are using repeated measures across the years. Year is to control for variation between years.

Spurs higher in the canopy receive more light, and therefore may have greater resources to invest in flowering or nut production. We found that the effect of light environment on spur level flower production depended strongly on the location of the spur in the tree relative to the alignment of the row of trees and the compass orientation.

Spurs on the west side of the tree (which is expected to receive more light than south or east) show a positive relationship between potential light and flower number (Fig. 11). In contrast, for other orientations there is a negative relationship, with the strongest negative on the north side.

In summary, there is not a general positive relationship between light environment and flower number at spur level. Instead the response depends strongly on the part of the crown (aspect) that the spur is in.

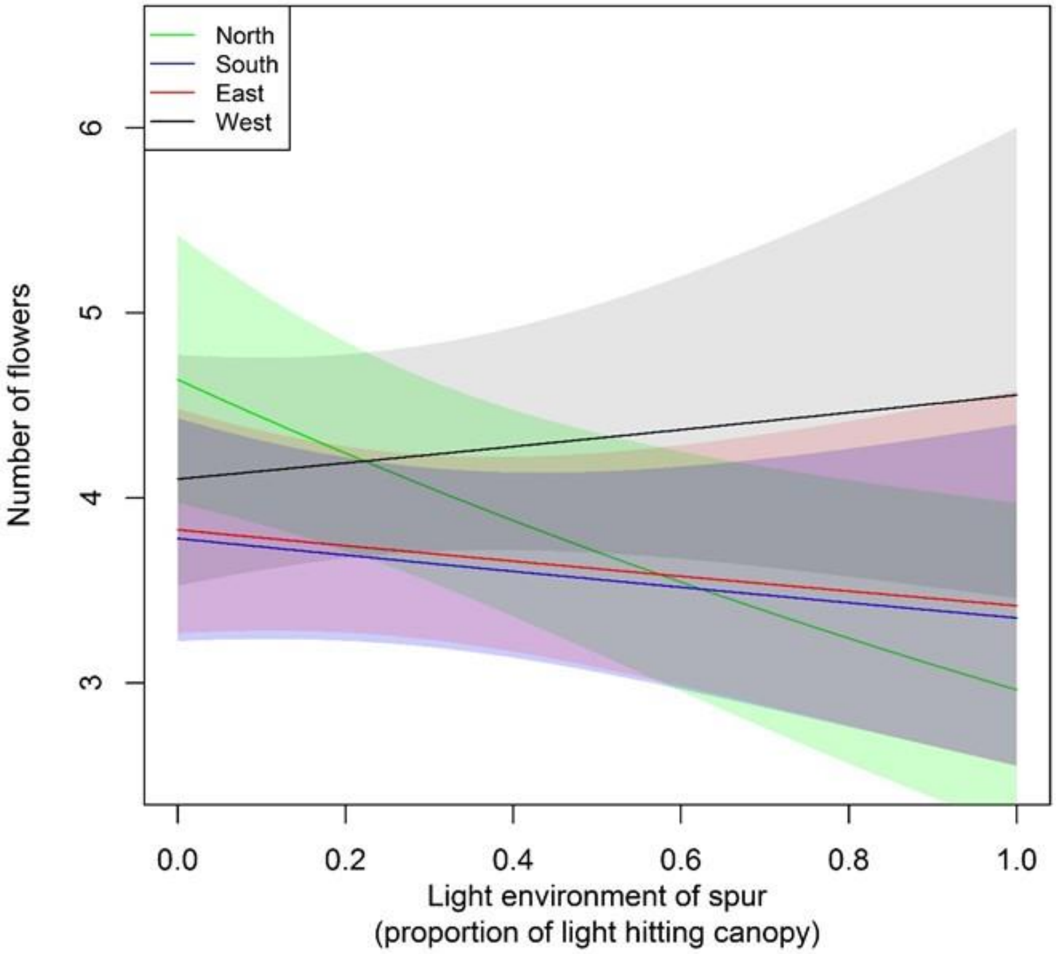

Figure 11: *The number of flowers on a spur against the proportion of light received, comparing each quadrant of the tree (North, South, East, West).*

($p = 8.927e^{-5}$, $R^2 = 0.083$, AICc = 6205.487). The model is as follows: Number of flowers ~ proportion of light + quadrant + proportion of light:quadrant +random(row/tree/spur) + random(year). The random effects of row nested within tree nested within spur act as spatial controls. Spur also acts as a temporal control because we are using repeated measures across the years. Year is to control for variation in each year.

How do flower number, leaf score and light environment effect nut production at spur level?

There is a general positive relationship between flower number and the total mass of nuts that are produced by a spur (Fig. 12). Comparing among treatments, the relationship is steepest for the control group, and flatter for the two treatments where pollination was elevated.

This suggests that when the pollination rate is elevated spurs will often achieve the outcome of one or more nuts, whereas for open pollinated spurs (controls) the probability of producing one of more fruit depends strongly on the number of flowers. Each flower could be thought of as a ticket in a lottery (the pollination lottery) where the best way to “win” (i.e. to produce a nut) is to have more tickets (flowers).

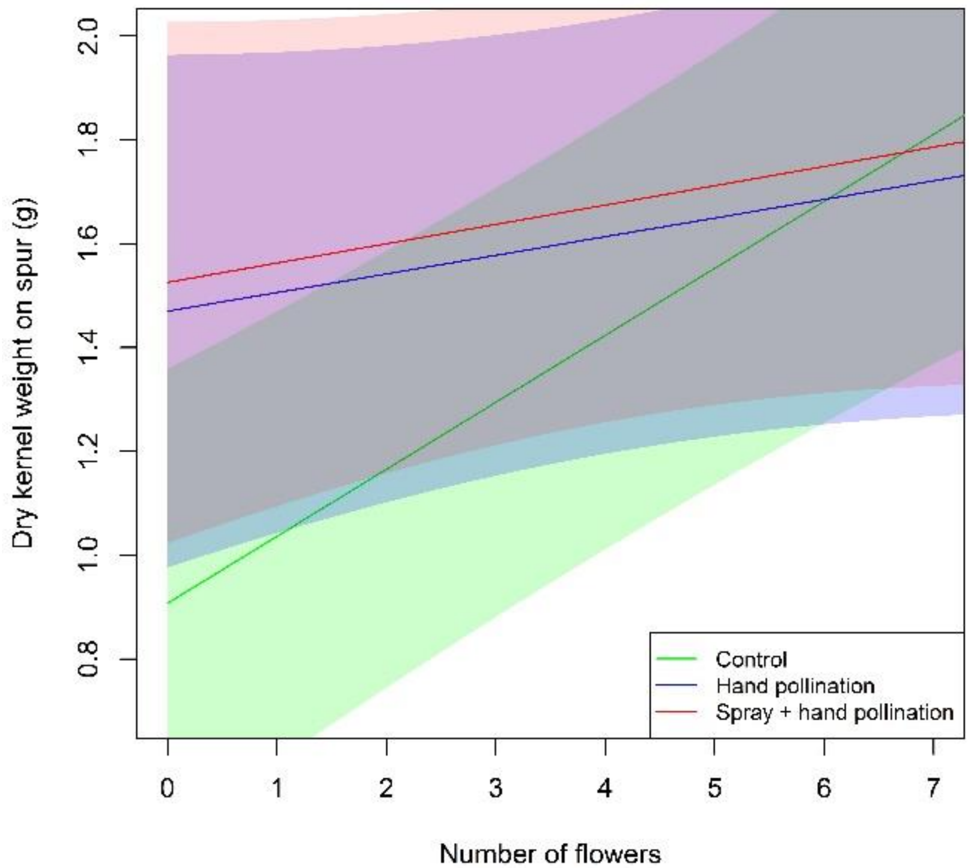

Figure 12: *The dry kernel weight per spur against the number of flowers on the spur, comparing different pollination treatments.*

($p = 6.793e^{-16}$, $R^2 = 0.377$, AICc = 5213.094). The model is as follows: dry kernel weight ~ number of flowers+ treatment + number of flowers:treatment +random(row/tree/spur) + random(quadrant) + random(year). The random effects of row nested within tree nested within spur act as spatial controls. Spur also acts as a temporal control because we are using repeated measures across the years. Quadrant is to control for the differing responses due to the different amounts of light received in each of the quadrants. Year is to control for variation in each year.

Whereas the negative relationship between leaf area and flower number in the same season is expected given that the energy benefit of leaves is realised after the number of flowers is fixed. However, one might expect that having a larger area of leaves may provide more carbohydrates to support nut development on the spur. This would then lead to a positive association between leaf area and nut mass per spur. In fact, in our data we see a <u>negative</u> relationship between leaf area and mass of nuts produced at spur level (Fig. 13).

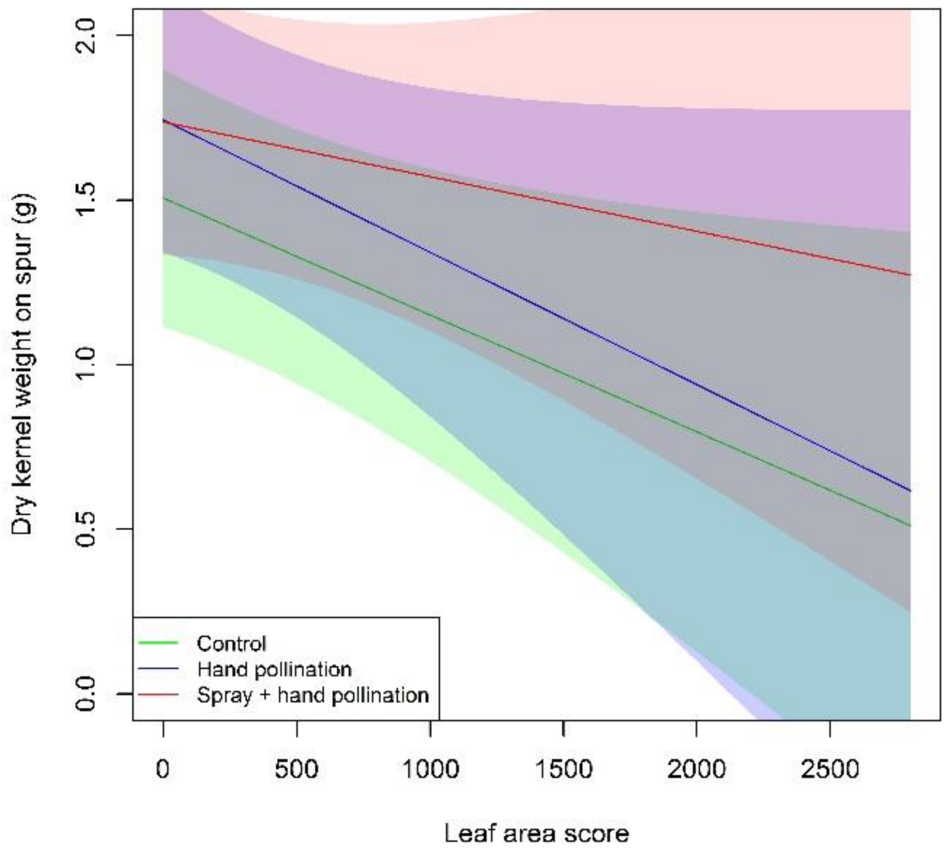

Figure 13: *The dry kernel weight per spur against the leaf score of the spur, comparing different pollination treatments.*

($p = 2.716e^{-3}$, $R^2 = 0.414$, AICc = 5061.799). The model is as follows: dry kernel weight ~ leaf score + treatment + leaf score:treatment +random(row/tree/spur) + random(quadrant) + random(year). The random effects of row nested within tree nested within spur act as spatial controls. Spur also acts as a temporal control because we are using repeated measures across the years. Quadrant is to control for the differing responses due to the different amounts of light received in each of the quadrants. Year is to control for variation in each year.

Whereas light environment at spur level was not generally associated with flower number (Fig. 11) there was a positive association between light environment and nut production at spur level. This pattern was true regardless of the location of the spur in the tree crown (i.e. Fig. 14). Further, it was true regardless of whether the spurs were open pollinated or had pollen supplementation (Fig. 15). The positive association between light environment and nut production was flatter for the spurs that received a whole tree spray as well as hand pollination (HPS) compared with open pollinated or hand pollination only.

The total mass of nuts produced per spur was greater for spurs that were higher in the canopy and therefore in a better light environment.

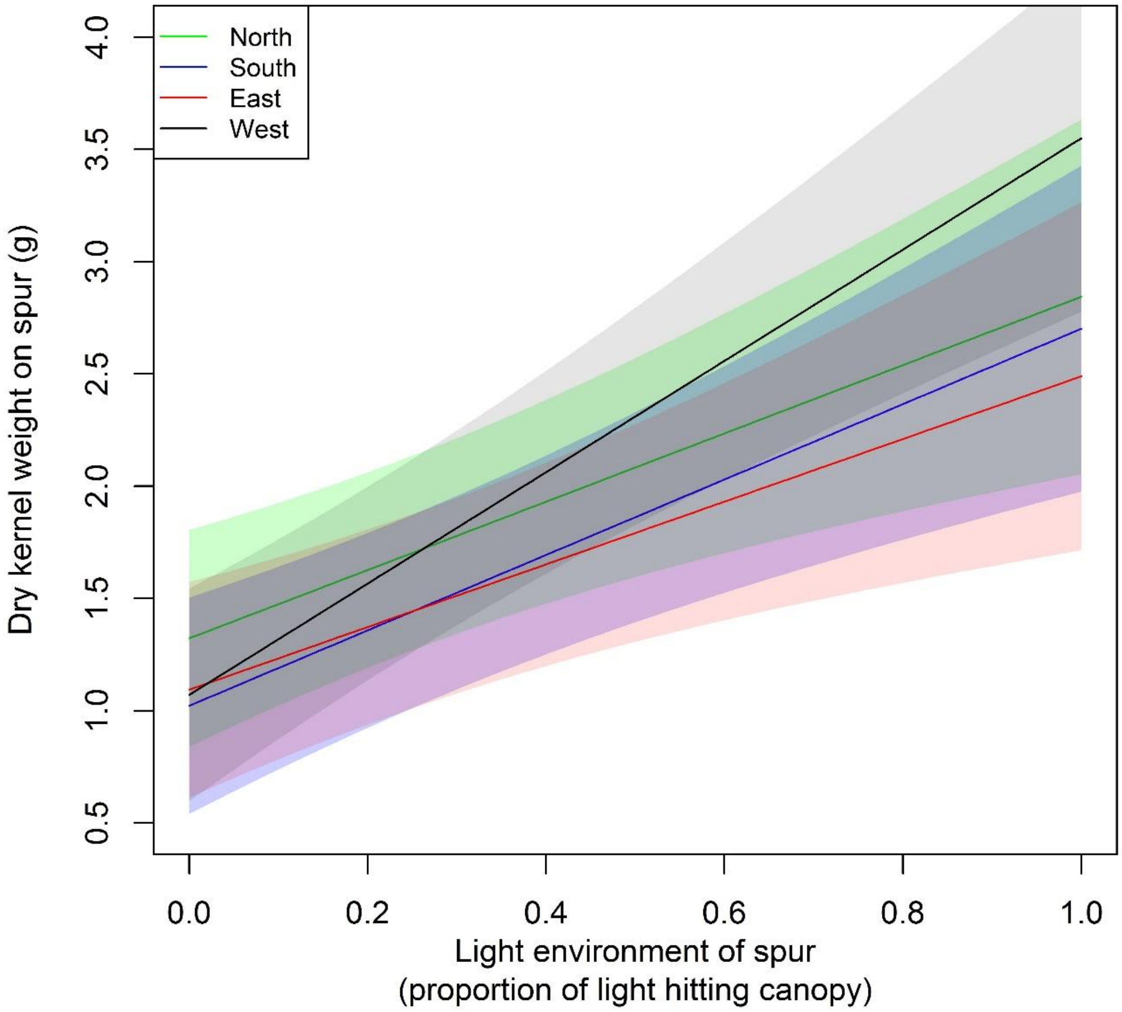

Figure 14: *The dry kernel weight on spur against the proportion of light received by that spur, comparing four different quadrants of the canopy.*

($p < 2.2e^{-16}$, $R^2 = 0.391$, AICc = 5162.669). The model is as follows: dry kernel weight ~ proportion of light + quadrant+ proportion of light:quadrant +random(row/tree/spur) + random(year). The random effects of row nested within tree nested within spur act as spatial controls. Spur also acts as a temporal control because we are using repeated measures across the years. Year is to control for variation between each year.

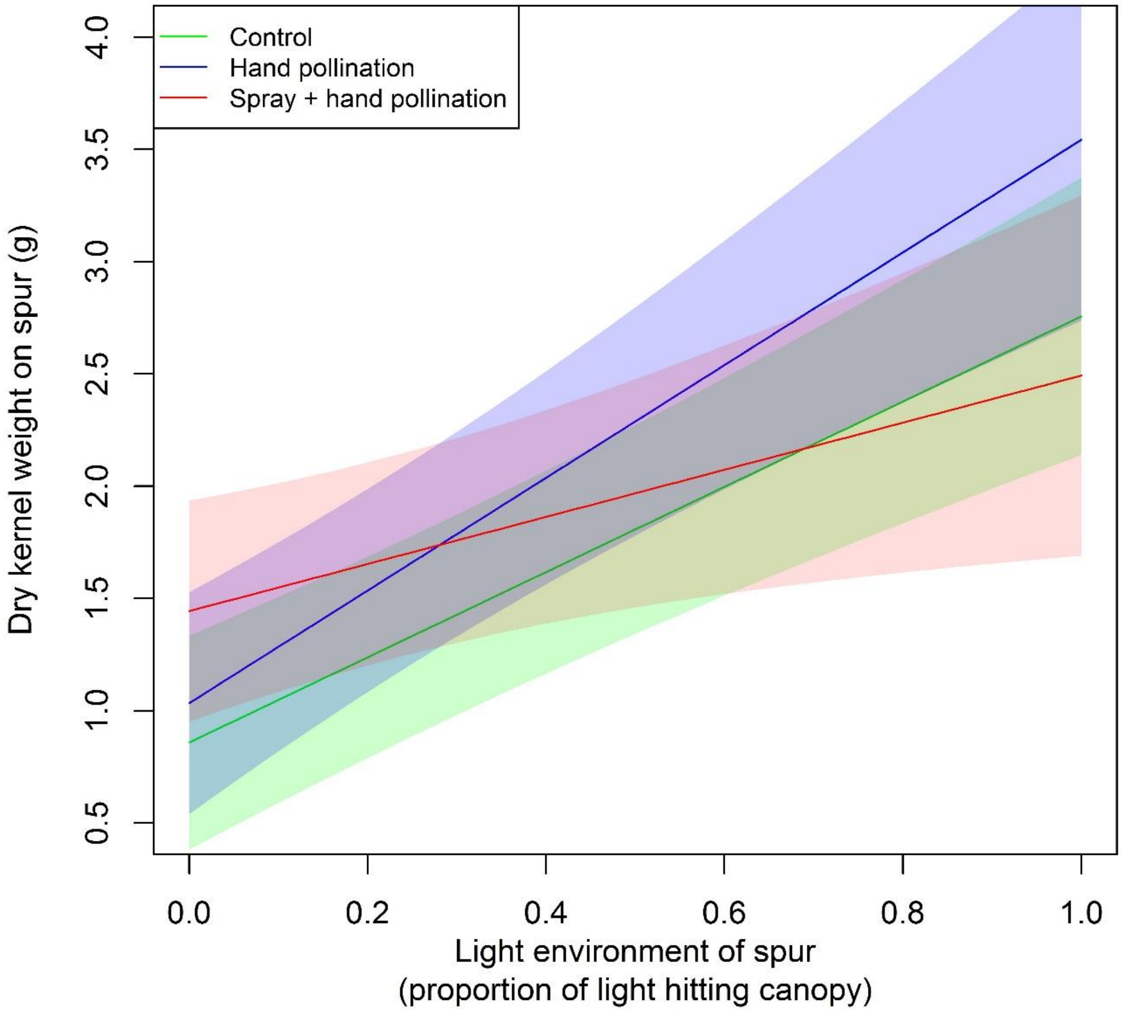

***Figure 15**: The dry kernel weight on spur against the proportion of light received by that spur, comparing across pollination treatments.*

($p < 2.2e^{-16}$, $R^2 = 0.366$, AICc = 5147.455). The model is as follows: dry kernel weight ~ proportion of light + treatment+ proportion of light:treatment +random(row/tree/spur)+ random(quadrant)+ random(year). The random effects of row nested within tree nested within spur act as spatial controls. Spur also acts as a temporal control because we are using repeated measures across the years. Quadrant is to control for the differing responses due to the different amounts of light received in each of the quadrants. Year is to control for variation between each year.

Finally, note that we have not focused on the evidence that hand pollination works, because it is well established (Cunningham 2014, Cunningham et al 2016). Nevertheless these analyses show that spur level nut production is highest for hand pollination plus the whole tree spray (HPS) and then second highest for hand pollination (HP) and lowest in open pollination (control) in both the analysis of the effect of flower number (Fig. 12) and the effect of leaf area (Fig. 13). For the analysis of the effect of light (Fig. 15) it became apparent that whereas the benefit of HP was consistent over the controls, for HPS the benefit diminished at high light. This may be evidence that pollen spraying at the whole tree level leads to resource constraints at the spur level. In other words, the cost of increased fruit set on spurs all over the tree as a result of whole tree pollen spraying means that the focal spurs we examined were less able to boost nut production compared with the hand pollinated spurs on trees that did not have whole tree pollen spraying.

Spur level interactions between light, flowers and treatments on kernel weight

The analyses above show that flower number, light environment and the availability of pollen (i.e. HP and HPS treatments) all influence kernel production at spur level. It is important to examine how these factors interact to establish how one would aim to maximise production, and where the limits lie. We have summarised these effects in figure 16. Considering the control group one can see that both flower number and light environment contribute to kernel production in an additive way, so that the order from worst to best is as follows:

few flowers, low light< many flowers, low light< few flowers, high light< many flowers, high light

Interestingly, the biggest step up in production was achieved between the second top and the top group, emphasising the importance of the high light spurs to achieving high production. Considering the effect of hand pollination (without whole tree spraying) ……

…..there is a benefit from increased pollination for all four categories of spur (i.e. low and high light, many or few flowers), but the greatest benefit from increased pollination was for spurs with few flowers but high light. This is consistent with the idea that the low flower number greatly reduced the probability of nut set from open pollination (fewer tickets in the pollination lottery), whereas the high light environment provided the resource opportunity to increase nut set when pollen availability was guaranteed.

The benefit of hand pollination for any one category is generally not as great as the difference between the best category (many flower, high light) and the worst (few flowers, low light). This indicates that the benefit to be gained by further improving pollination in this system (noting that even the control group have current industry standard pollination practice) may be less than what could be gained by improving flower number or light environment of spurs. However, strategies to improve these things are complex and depend on whole of orchard design, pruning, and so on, whereas improved pollination might be possible by simply increasing hive density or arrangement (Cunningham et al 2016).

Comparing the effect of whole tree spraying on top of hand pollination of target spurs, it can be seen that whole tree spraying has increased the productivity of the less productive spurs (i.e. few flowers, low light and many flowers low light). In contrast, for the most productive spurs there is little additional benefit above hand pollination (few flowers, high light) and a decline in production from off target spurs in the many flowers, high light category). This result is another expression of the phenomenon seen in figure 15, which we interpret as evidence of a resource constraint being seen on spurs that are otherwise expected to be the most productive, but which are constrained by the cost of high production elsewhere on the tree because of whole tree spraying.

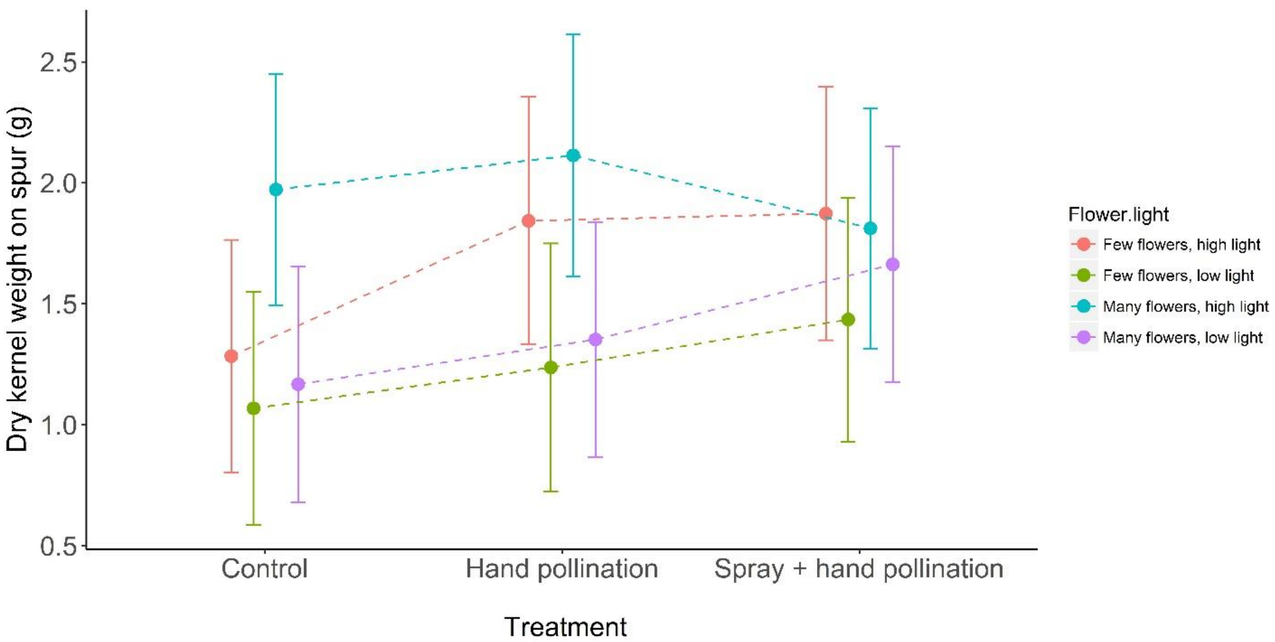

Figure 16: *The effect of treatment, light and the number of flowers on the dry kernel weight at the spur level. Light data were categorised into two levels: high and low (0.24 and above is 'high, below 0.24 is 'low') and flower number data were categorised into two levels: many and few (>3 is 'many' flowers, 3 and below is 'few' flowers). These two variables were combined to make an interaction variable with four levels (few flowers, high light; few flowers, low light; many flowers, high light; many flowers, low light).*

The predicted values from a linear mixed model are plotted with 95% confidence intervals ($p < 2.2e^{-16}$, $R^2 = 0.420$, AICc = 5162.338). The model is as follows: dry kernel weight ~ treatment+ light and flower interaction factor variable + treatment:light and flower interaction factor variable+ random(row/tree/spur)+ random(quadrant) + (1|year). The random effects of row nested within tree nested within spur act as spatial controls. Spur also acts as a temporal control because we are using repeated measures across the years. Quadrant is to control for the differing responses due to the different amounts of light received in each of the quadrants. Year is to control for variation between each year.

Between year effects on flowering and fruiting

Over the three year period of the spur survey we were able to establish the frequency with which spurs that flowered in y1 or y2 were then seen to flower a second time.

Approximately 10% of spurs in the control group (i.e. flowered at least once, open pollination only) flowered multiple times over the three year period. Spurs that had pollination guaranteed were even less likely to flower in the next year or two (Fig. 16).

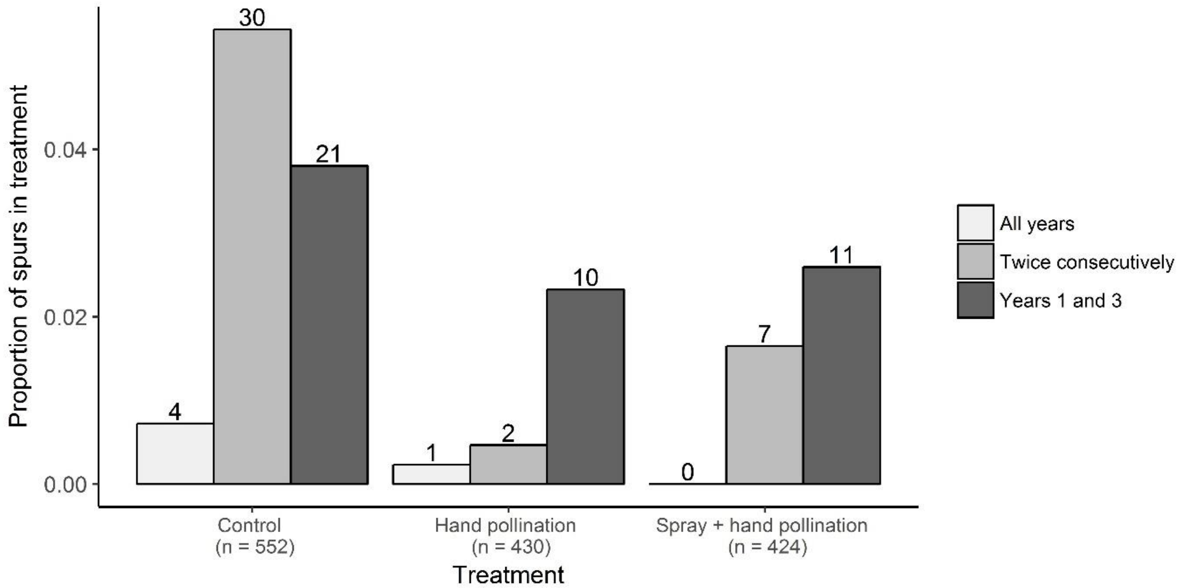

Figure 16: *Bar plot of the proportion of spurs that flowered in multiple years vs flowered once. "All years" = spurs that flowered in all years, "Twice consecutively" = spurs that flowered consecutively, but only twice, "Years 1 and 3" = spurs that flowered in year one, skipped a year and flowered in year three. Numbers above bars represent the number of spurs within that group. The spurs that flowered only once consist of the majority of the spurs and are omitted from this plot.*

The negative effect of pollen supplementation on the probability of a spur flowering multiple times is probably due to the spur level cost of developing and supporting fruit development. This interpretation is supported by the following observation explained in the box below.

Spurs which flowered again in a subsequent year were those with a lower nut set from the first flowering event (i.e. mean 0.5 nuts) whereas mean nut set for spurs that did not flower in the subsequent year had a nearly 3 times greater mean nut set (Fig. 17).

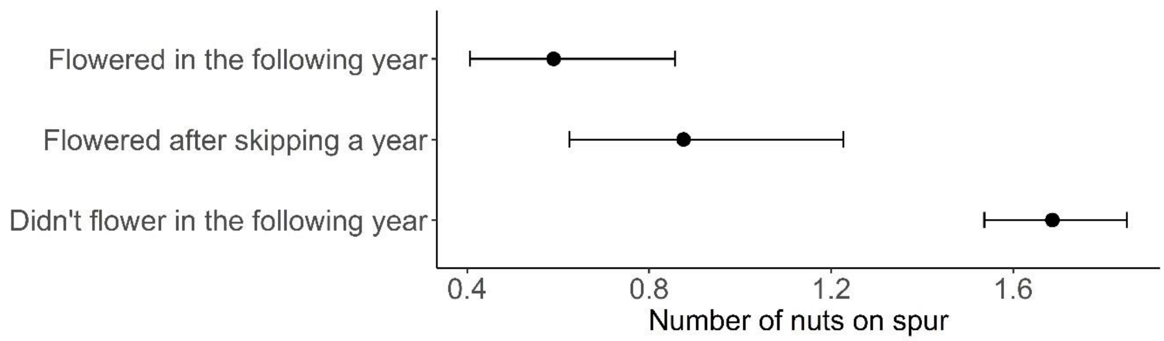

Figure 17: *The effect of treatment on whether a spur that produced a certain number of nuts goes on to flower in subsequent years.*

The predicted values are plotted with 95% confidence intervals ($p = 7.342e^{-14}$, $R^2 = 0.137$, AICc = 2994.429). The model is as follows: Number of nuts re-flower variable + random(row/tree/spur)+ random(quadrant). The re-flower variable consists of three levels (whether that spur flowers in the following year, whether that spur flowers in the 3rd year and not the 2nd, and whether that spur does not flower again). The random effects of row nested within tree nested within spur act as spatial controls. Spur also acts as a temporal control because we are using repeated measures across the years. Quadrant is to control for the differing responses due to the different amounts of light received in each of the quadrants. The model assumes a Poisson error distribution.

Whole tree harvest results

Results from the spur level analysis indicate that whole tree pollen spraying did have significant impacts on nut production. The HPS treatment was consistently above the HP only treatment in most measures, suggesting that there was a benefit that was additive with the hand pollination, but the real motivation for the whole tree pollen spray was to examine whole-tree effects. Interestingly the spur level analysis (Fig. 15) indicates the influence of resource constraints caused by high production on the non-surveyed spurs. Collectively, these observations suggest the following:

Whole tree pollen spraying elevated pollination rates. This was achieved in spite of the relative lack of experience in the industry regarding how to achieve this outcome.

To understand the effect of whole tree pollen spraying on whole tree yield we must focus on the individual tree harvest results. Whereas the spur level analysis benefits from data on many hundreds of individual spurs, the power of the whole tree harvest analysis is limited by the fact that we only have 12 trees in each treatment category for any given comparison. The limited number is because whole tree spraying is logistically challenging both in terms of the flowering season work (comprehensively spraying whole trees using a hydralift) and at harvest time (separately assessing yield of individual trees. Therefore it was anticipated that only very large effects could be determined to be significantly different.

Experiment	Year	Block	Control method	Sprayed with pollen	Control	Percent difference
A	1	Upper	No spray	59	51	16
B	2	Upper	No spray	41	37	10
C	2	Lower	Boron solution	54	49	10
D	3	Upper	Boron solution	21	19	10

Table 2: *The median yield (fresh weight whole fruit, kg) for individual trees in each treatment of four different experiments examining whole tree pollen spraying. We report fresh weight because it was what was measured in the field, and there were no systematic differences in water content or crackout by treatment.*

In all four experiments the pollen-sprayed trees had higher median yield than the control (normal pollination practice) trees, with the benefit being between 10% and 16% increase, depending on the experiment.

Variability among trees was quite high because in each sample there were one or two outliers. We have reported medians (rather than means) because the mean is less influenced by outlying large or small values. Because of the high variability and relatively small sample size we determined a statistically significant difference only for experiment A, which was the trial with the largest yield difference (16%) (U-test, U=37, N =12, 12, P=0.043).

It is important to note that of the four experiments only A and B had the same combination of location (upper versus lower block) and design (control with or without the boron spray). However, A, B and D were conducted on the same set of trees over sequential years, which means that one must also consider specific inter-year effects. For example, the elevated yield in experiment A (year 1) could have exacted a resource cost, such that the trees had fewer resources to respond to pollen spraying in experiment B (year 2). This could contribute to a reduced benefit from 16% in year 1, to only 10% in year 2. Because of the variation in design and potential for inter-year effects we decided it was not appropriate to analyse these experiments in a combined multiyear model.

The rational for the "boron control" experiments (C, D) was to establish if the benefit seen in experiment A could have been an effect of the boron in the pollen suspension, rather than the pollen itself. If the benefit was from boron instead of pollen, then the "boron control" experiments (C, D) would have the similar yield in the control and the pollen sprayed treatments.

The results seem to indicate that the whole tree spraying effect is primarily from pollen rather than boron, because the benefit in experiments A and B (no boron control) is similar to C and D (control includes boron).

Nevertheless, more research is recommended in this area because the statistical power of these trials is too low to establish a high level of confidence. It should also be noted that the yield of trees in year 3 was very low regardless of treatment. This probably reflects that is was a poor year for yield across the block in general. Interestingly the yield advantage of 10% with pollen spraying was consistent in spite of this.

Recommendations

1) Growers should maximize flower production as the foundation for boosting nut production, even though there appear to be "surplus" flowers (because spurs with more flowers make more fruit, on average, even under current pollination practice)

2) Orchard management strategies that decrease self-shading will support greater nut production when combined with ample pollination (because high light availability to spurs increased the conversion of pollinated flowers into fruit even though it did not increase flower number)

3) The industry should explore strategies to further boost pollination by more effective application of managed honeybee hives (because experiments strongly indicate that increasing pollination rates will increase yield without a corresponding cost in quality).

4) The industry should explore methods of whole tree pollen spraying to boost pollination (because pilot trials indicate likely yield benefit, but were not conclusive).

5) The industry should compare the cost effectiveness or different pollination improvement strategies (because different pollination strategies will have different costs of implementation)

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Appendix: Cost Benefit Analysis

Any change to practice for almond producers should be measured not only against the effect on the amount of crop produced, but the quality effects and the cost of achieving a benefit. This project and the previous related project (Cunningham 2014) clearly indicate the potential of increased pollination to increase yield, but whether or not this is a good strategy also depends on how much it costs to achieve an increase in pollination. To address this, we use the data collected from the whole-tree pollination experiments conducted during this project to answer the following questions.

- Does production of an increased number of nuts come at a cost in terms of size of nuts?
- Does increased production in one year come at a cost in future production in a subsequent year?
- How large is the production boost possible from improved pollination?
- Given this knowledge, what is the expected cost /benefit relationship for pollination boosted production?
- What pollination strategies could lead to the improved pollination benefit?

This research project has advanced our knowledge on a number of these issues, and some require more investigation. Here we summarise what we have learned regarding each question.

Does production of an increased number of nuts come at a cost in terms of size of nuts?

Spur level analysis shows that there is no nut size versus number trade off in the range 1-3 nuts on a spur. Given that 94% of spurs are in this category even when pollination is elevated, this result should give confidence that pursuing a high yield strategy is unlikely to reduce nut size.

There is some evidence for some trade-offs in that spurs that produce more nuts are less likely to flower again (Fig. 17). However, given that multiple flowering of spurs is relatively rare in the whole sample, it is unlikely that this effect has a large impact on yield. The fact that trees receiving whole tree pollen spray had higher yield than the controls for three consecutive years (Table 2) indicates that the cost of increased yield is not so great as to cause a large slump in the second year, such as for an alternate bearing crop. Nevertheless, to gain a better insight into the between-year cost would require further research.

How large is the production boost possible from improved pollination?

At the spur level the effect of improved pollination was consistently strong. However, to understand the yield benefit one must consider whole tree yields, where competition among spurs could be expressed as a limit to the pollination benefit. Our experiments indicate that even at whole tree level there appears to be the potential to lift yield by 10-16%. It is important to understand that the whole tree spraying experiment was conducted without any existing standard for such an approach in the almond industry. Therefore what we can say that the experiment indicates that an overall lift in yield appears possible, but there is insufficient experience yet to know if the 10-16% benefit range indicates a real threshold from resource constraints, or in fact that the method could be improved so that an even greater increase is possible.

Given this knowledge, what is the expected cost /benefit relationship for pollination boosted production?

The whole tree harvest experiment indicates that a 10-16% increase in yield from improved pollination is plausible, and we use this as the basis for our cost-benefit calculations. To express this benefit in dollars per ha we assumed 3.2 tonnes per ha as the average yield for a mature orchard such as this (Almond Board 2015) and $8,150 per tonne of dry kernel (Andrew Downs pers comm) as a current realistic sale price. Therefore:

Typical value of nuts per hectare = $26,080 (i.e. 3.2*8150)

Marginal value of 10 to16% increase = $2,608 to $4,173 extra per ha

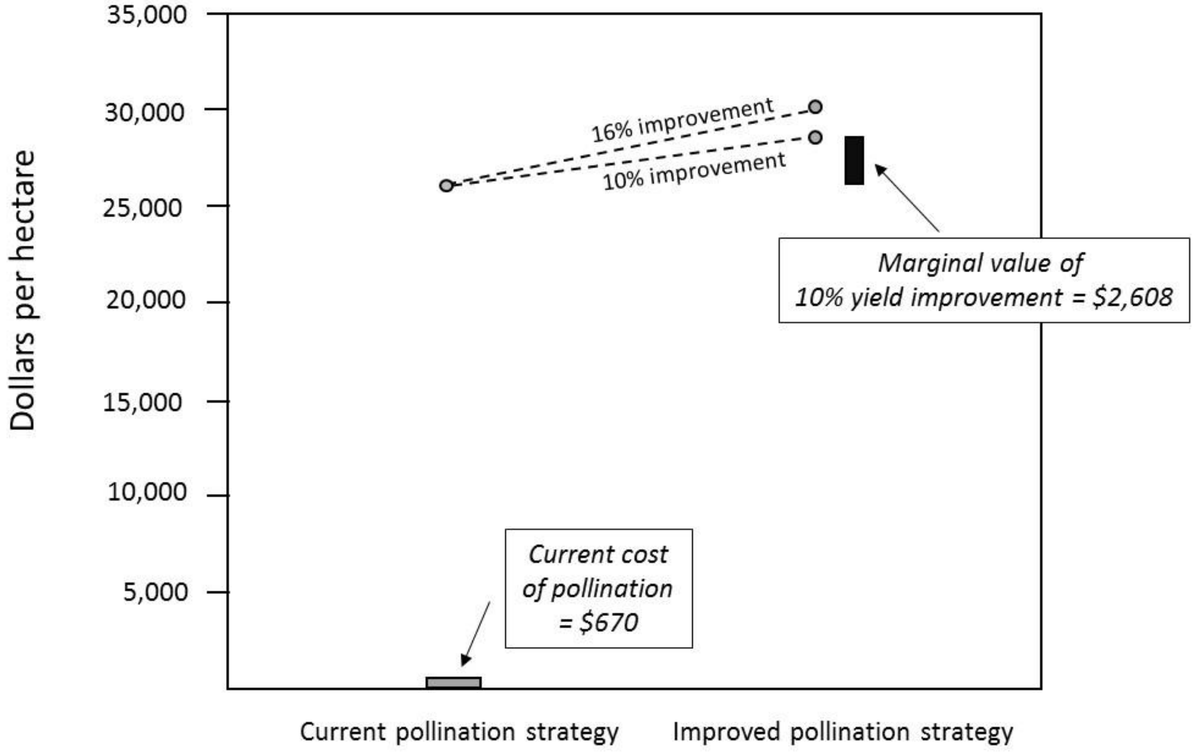

Figure 18: *The gray circles show current standard crop value in dollars per hectare (left side) compared with the projected increase possible from improved pollination (right side), which is in the range 10-16%. The black box indicates the size of the marginal benefit from a 10% increase. This can be compared to the current cost of pollination (gray box). The improved pollination strategy may include changes to hive number and arrangement or even a commercial whole-tree spraying approach.*

An increase in revenue of this order ($2,608 to $4,173 extra per ha) is clearly worth capturing if it can be achieved at reasonable cost. Currently the total expenditure on pollination is approximately $670 per ha (6.7 hives per ha at $100 each). Raising the density from 6.7 to 8 hives per hectare, for example, would only require a marginal increase in hive costs of approximately $130 per ha (i.e. assuming $100 per hive), which is a very small cost relative to the potential for increased yield. Or to put it another way, if increased hive density was a path to attaining the increase yield, then even assuming only the 10% increase was possible, one could increase hive density to 32 hives per ha before there was a risk of spending more on hives than there was benefit to gain.

Given that the marginal benefit from improved pollination is around four times greater than the current level of spending on pollination, there is considerable room for increased spending on pollination with the expectation that benefits will exceed costs (Fig. 18).

This study indicates increased nut production comes at some cost terms in terms of the future flowering from a given spur, but there is not a cost in terms of the size of nuts produced. Therefore increased production does not come a risk to quality. Whole tree pollen spraying followed by whole tree harvest indicates that 10-16% increase in yield from better pollination is likely to be achievable. Such an increase in revenue ($2608 to $4173 extra per ha) is large relative to the current expenditure on pollination (approximately $670 per ha) and therefore it is likely that affordable improvements in pollination are achievable and economically beneficial. Further study should focus on the most cost efficient methods for achieving this pollination led improved yield.

What pollination strategies could lead to the improved pollination benefit?

Previous research has shown that reductions in hive density below 6.7 hives per hectare leads to lower flower to fruit conversion which would be expected to cause yield loss (Cunningham et al 2016). Here we show that an increase in the density of hives from 6.7 hives per ha to 8 hives per ha has the potential to increase yield and profit. However, we know that hive arrangement as well as density are important. In our previous study we explored different hive arrangements, and concluded that higher flower to fruit conversion is achieved when hives are arranged more evenly around the orchard, i.e. avoiding large placements of one hundred or more hives. The orchard where the current study was conducted was typical in using a hive density of approximately 6.5 hives per ha, but in keeping with the recommendations of the hive arrangement study (Cunningham et al 2016), hives were arranged in placements of 10-20 hives rather than one hundred or more. The scope of this project did not include exploration of different possible pollination strategies and their costs, but the Cunningham et al (2016) experiments indicated that there was still room to improve the standard practices in terms of the number and arrangement of hives.

Our experimental treatment for whole tree spraying was not developed as a potential commercial practice per se, but its apparent success suggest that similar methods could be possible. The main question here is whether the labour costs of pollen spraying could ever make this strategy competitive against the current bee hive pollination approach. The labour costs would include not only the application of pollen to the tree, but also the gathering of the pollen in enough quantity and quality to service the orchard. We do not include a cost/benefit relationship for this approach here, as it is outside the scope of this project.