Enhancing almond pollination efficiency

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Enhancing Almond Pollination Efficiency

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Media Summary

Pollination by bees is a critical component of commercial almond production. Every year more than 170,000 bee hives are brought into Australian almond orchards at flowering, making pollination a significant input to production. It is important to manage pollination well, to ensure high productivity and efficient use of resources. Further, it is important for growers to know what pollination strategies are available, given the risks to supply of bee hives if a significant new bee disease (such as *Varroa* mite) arrives in Australia. Our research showed that although bees are capable of flying many kilometres, they are less active on trees that are more 400m from the hive. This reduced activity level corresponds with a decrease in the proportion of flowers that are turned into nuts, by about 20%. In general, reducing the number of hives per hectare below 6.7 reduced pollination effectiveness, and therefore the rate at which flowers are turned into nuts. However, we found that for a given hive density the nuts per flower outcome is better when hives are spread around the orchard in small placements, ensuring that no trees are too far from hives. This means that for the same number of hives, one can get a better pollination outcome by adopting the "spread them around" strategy. These insights into bee pollination strategies are being discussed with almond growers and beekeepers to make sure that we can guarantee the best use of our bees, and the most productive almond orchards.

Technical summary

This project focused on the influence of pollination strategy in almond orchards on bee activity (which drives pollination) and the flower to fruit conversion ratio (which is substantially determined by pollination). In particular we examined whether adjustment to the pollination strategy might help to support higher yields.

It has been suggested that pollen traps and enpollination felt fitted at hive openings could raise pollination effectiveness of bees. There is concern from beekeepers, however, that there would be negative impacts on bee hive health. We examined the effect of these hive modifications and found that pollen traps had a strong negative effect on brood production and a more variable effect on number of bees that was negative across most hives. In contrast, the effects of enpollination felt on brood and bees were small. An unreplicated trial in which hives with enpollination felt were applied to an orchard did not, however, suggest the enpollination felt increased fruit set compared to normal practice.

The first season was focused on observations of bee activity in transects extending from hives out to one to two hundred meters distant. We established that there was no correlation between the observed bees-per-flower-per-minute and fruit set. This reflects that, we may not have observed a large enough gradient in bee activity and further, that this measure of bee activity provides only a poor window into the actual amount of pollen movement. We also examined data on seven years of variation in almond yield and related it to records of the weather during flowering. This exploration of yield variation suggests that "bee weather" is not a strong predictor of yield.

To get a more informative insight into patterns of bee activity, we next focused on recording the rate at which pollen is removed from anthers, comparing flowers on trees ~400m from hives to those near hives. These data show that pollen removal is significantly lower on trees far from hives. Having established that bee activity declines with distance from hives at this scale, we then examined if reduced activity corresponded with reduced fruit set. This experiment was conducted over many orchards, and over two seasons. We found a significant decline in fruit per flower at increasing distance from hives. The analysis shows fruit set declining by about one fifth, from 36% fruit set near hives declining to 28% at 850 meters.

Standard practice in many large orchards is 6.7 hives per hectare (hph) achieved with placements of approximately 120 hives hundreds of meters apart. Our next experiment examined a range of pollination strategies using lower hph and smaller numbers of hives per placement. Again the experiment was conducted over many orchards and two flowering seasons. We found a significant positive relationship between conversion of flowers to fruit and hive density. The fitted relationship indicates on average 33% fruit set at 2.8 hph increasing up to 46% at 6.7 hph. Combining the experiments on distance from hives (using large placements) with the hive density effect (using smaller placements), we found that the best fruit per flower outcomes were gained with a high hive density (approximately 6.7 hph) but using smaller placements with shorter distances between them.

Hand pollinations were conducted on trees in both the pollination strategy experiments. These confirmed that hand application of pollen from a compatible variety does raise average fruit set up to 55%, which is above that typically seen in open pollination. This confirms that fruit set for most flowers is pollen limited. However, fruit set from hand pollination was more variable than fruit set from open pollination, which indicates that hand pollination of flowers can have unintended negative effects, and although it raises the average outcome, it underestimates the maximum.

Our experiments used assessments of fruit set in October, but harvest typically commences in February. For 275 trees that flowered in 2013 we counted fruit set in October and February. This analysis found that only 6% of fruit were lost between surveys, so that fruit count in October is a very strong determinant of the pattern of fruit set at harvest. We also examined the influence of branchlet diameter and flower density on fruit set and determined that these architectural and resource constraints on fruiting have some influence, which need to be understood if we are to further increase productivity.

1   Preface

Cross pollination is known to be a critical factor for almond production, and consequently standard management is to provide managed honey bees at densities that are high relative to many other crops. It is important to ensure that pollination is provided in the most effective manner because good pollination provides the foundation for good yield, but also because it is a significant input cost that needs to be cost efficient. This project focused on the influence of pollination strategy on bee activity (which drives pollination) and the flower to fruit conversion ratio (which is substantially determined by pollination). In particular we examined whether adjustment to the pollination strategy might help to support higher yields. This project was conducted from July 2011 to the end of June 2014.

This final report brings together all the key outcomes of the study into one document. Although most of the content is new, some elements have been reported in previous progress reports. We also conducted a literature review in the first year of the project, which we summarise here.

The literature provides extensive and strong evidence that nut yield in almond orchards would be increased in most circumstances if one could increase the delivery of cross pollen to flowers. This has been shown by hand pollinations, moving bouquets of flowers, and enpollinating bees as they leave the hive. However, these insights have not yet been developed into methods for economical orchard scale improvements to the rate of cross-pollination. Because it is difficult to directly increase cross-pollination, simply increasing the frequency with which bees contact flowers has been viewed as an alternative possibility for increasing nut yield. Although there are some conflicting data, it is likely that most bee visits transfer no cross-pollen, so that increasing the number of effective visits would only be expected to have a weak effect on nut yield. Clearly very low visitation can reduce nut set, but after a certain threshold is reached, higher bee density alone might not further improve yield. There are few data to identify what this threshold bee density or visit rate may be, but it is understood that it would vary from year to year because of variation in the weather during pollination and variation in the quantity and synchrony of flowering among cultivars. These insights from the literature helped us to design the experiments described in this report and suggest testable predictions.

The complete literature review and bibliography is available from Dr Saul Cunningham.

2   Effect of pollen traps and felt on bee hive performance

2.1   Introduction

Pollen traps and enpollination felt have been suggested as hive modifications that might improve pollination effectiveness by influencing the probability of cross pollination. The principle behind pollen traps is that by stripping pollen from bees before they enter the hive, the demand in the hive for pollen will increase, perhaps causing foragers to focus more on pollen collecting behaviour. The principle behind enpollination felt is to brush some pollen off arriving bees and on to departing bees, increasing the diversity of pollen loads on departing foragers. Higher diversity of pollen types (i.e. pollen of different almond varieties) on foraging bees may raise the cross pollination rate. To understand their potential for commercial use it is necessary to know not only whether they have a beneficial effect on pollination outcomes, but whether or not they have any effect on the health of hives. Here we present data relevant to the latter question.

2.2   Materials and Methods

Thirty six hives were placed in one location in a Riverland almond orchard on July 29 2012 (i.e. early in the flowering period). Twelve hives had pollen traps attached to the entrance, and 13 hives were fitted with enpollination felt. The remaining 11 were left unmodified. For each hive we counted the amount of brood and bees in terms of the total area of frames covered. These hives were left for 13 days (i.e. for most of the flowering period), at which point brood and bee counts were repeated. Our analyses focus on the effect of treatment (trap or felt) on the change in amount of brood and bees over the time period.
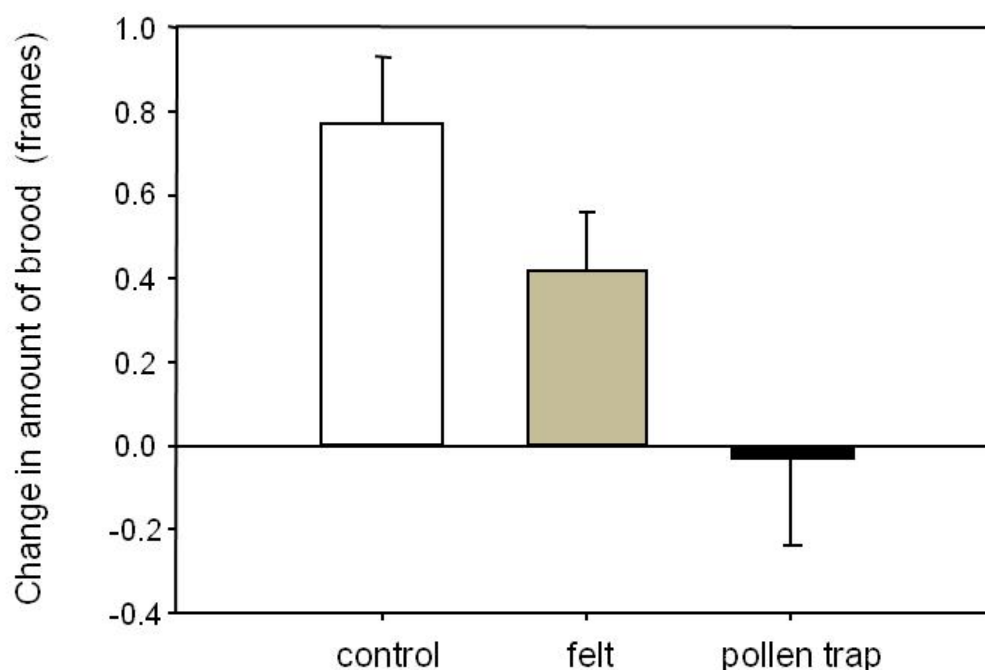
Figure 1: Means and standard error for change in amount of brood, comparing control and two treatments (felt and pollen trap).

2.3   Results

The amount of brood grew by, on average, 0.77 frames in the control and 0.42 in the felt treatment, but actually decreased by 0.03 with the pollen trap (Fig. 1). Analysis of variance shows that this effect is highly significant (P=0.007, df 2,33). By looking at the overlap in standard error bars we can say that the felt effect was not quite statistically significant (P>0.05) but the pollen trap effect was (Fig. 1).

In contrast, there was no treatment effect on the number of bees (P=0.561). However, one hive had an extreme result (one pollen trap hive had a large increase) which makes the robustness of this analysis questionable. If we drop this single data point, and re-analyse the remaining data there is a near significant treatment effect (P=0.054) and the pattern is for a small increase in the control, a bigger increase in the felt, and a decrease in the pollen trap group.

2.4   Discussion

In summary, the pollen trap had a strong negative effect on brood production and a more variable effect on number of bees that was negative across most hives. In contrast, the effects of enpollination felt on brood and bees were so small that they were not significantly different from the controls.

In 2013 we followed up with a trial of enpollination felt fitted hives on a block of almonds near Renmark SA. This experiment was a pilot only, because the strategy was not repeated across replicate orchards. We used 48 strong hives (12-13 frames of bees) arranged in four groups of 12, spaced evenly around the perimeter of 7.5 ha block (i.e. 6.4 hives per ha). Flower and fruit counts were done on 12 trees in the block, using the same strategy described for the other experiments (see 6.1 methods). Fruit set from open pollinated flowers was 29.4% (1SE 3%). This puts it in a low range compared to results discussed later in this report for open pollination from hives without enpollination felt or other fittings (e.g. Fig. 12). In short, this unreplicated trial did not suggest the enpollination felt increased fruit set compared to normal practice. It is worth noting though that fruit set from hand pollination was also relatively low (40% vs. 55% across other experiments, Fig. 12) which may indicate that this set of trees were below average productivity for reasons not related to pollination.

3 Observing bee activity

3.1 Introduction

Pollination is the result of an interaction between flowers and visiting insects. The frequency of bees at flowers is expected to be one important driver of the pollination outcome. One cause of under-pollination could be that bees are not moving around orchards in a manner that supports best pollination outcomes. It may be that bees are less likely to travel long distances from hives, therefore under servicing some trees. Also, it could be that bees favour movements within rows of the same cultivar, this leading to a shortage of cross pollination events. Nevertheless, tasking individual bee movements is fraught with difficulty, so our ability to get a strong insight into patterns of bee movement may be limited.

3.2 Materials and methods

In 2011 we observed bee activity in 6 different blocks (assigned letter codes A-F) all within 50km of Renmark, South Australia, though with two being over the border in Victoria. By agreement with landholders, we reduced the density of managed bees in and around the experimental block, and placed our own hives in the middle (8 hives, each of 8 frames, at least 4 of brood). The goal was to create areas of orchard with lower bee density than normally occurs under standard commercial practice, and also to have a replicated transect of trees at different distances from hives of known strength (i.e. those we managed). We then established three transects centred on the middle of the block, where hives were placed. One transect was the row with hives, the other two transects formed an X (i.e. two diagonals) intersecting in the middle.

Bee activity was observed on trees in the transect; each observation being one minute long, focused on a cluster of approximately 100 open flowers. Observations were confined to the hours between 9am to 3:30pm, when air temp was above 13C, and the wind less than 5m per second and it was not raining. We also counted samples of flowers on these same trees, and then returned in October (when fruit were forming) to count fruit, establishing a fruit per flower ratio for the sample.

3.3 Results

In this summary all the patterns described are based on significant statistical models, unless stated otherwise. As expected, bee foraging density varied with air temperature and time of day. Interestingly, the time of day effect was different in the hive rows compared to the other rows. In the hive rows visitation dropped off as the day progressed, regardless of temperature, whereas for trees outside of the hive row there was not a significant decline. This is consistent with the idea that bees foraged heavily in the hive row early in the day, but as pollen was removed they foraged less or moved out more widely. Outside of the hive row bees were probably more spread out, and so the problem of pollen stripping would have been less intense.

This pattern supports the idea that a lot of bees simply fly up and down the hive row, not contributing to cross-pollination between trees.

In three of our six blocks we saw patterns of bee visitation that show we created a gradient in bee density that was higher near hives and lower further away. In B and A blocks there were significant relationships with distance-from-hive (also taking into account some other variables, like temperature). At E block there were more bees in the hive row (but not a distance effect throughout the block).

What about the other three sites? In D and F blocks there was no strong pattern linking bee density to location in the orchard. In C block we saw *fewer* bees in the hive row – but this might be related to the pollen stripping effect mentioned above.

It is possible that absence of a distance-from-hive effect in three blocks was to do with the proximity of commercial hives. We know that commercial hives were placed quite close to the experiment and at high density in C block. Although these hives didn't arrive until late in the flowering they may have had an effect on the later bee observations. We also know that at F block there were significantly more bees near commercial hives (i.e. a distance-from-commercial-hives effect).

Because we already observed that bee density in the hive row shows a different pattern over time compared to the other trees, we tested for patterns with this in mind. Given that we had three blocks where bee density declined with distance-from-hive, we might expect a similar distance-from-hive effect on fruit set. We *did* find this at B block (i.e. more fruits near the hive, except in the hive row itself), but not at the other two blocks with a bee density gradient (A and E).

The other effects we found that related fruit set to distance-from-hive suggested a *disadvantage* to being near hives. F block had *fewer* fruits at trees closer to our hives, and E and C blocks had fewer nuts nearer commercial hives. These were all relatively weak effects, but may relate to the pollen stripping problems already discussed. In other words, at higher bee densities there might be a lot of foraging on only a few trees, not leading to effective cross pollination.

We focused first on distance from hive because this was the bee gradient we tried to establish, but we also considered the possibility that there was an association between fruit set and visit rate that did not depend only on the distance-from-hive gradient.

At the most general level, you might expect a relationship between visitation and fruit set at the level of whole blocks, but this was not so (Fig. 2). For example, visitation rates were much lower at A, but nut set was in the middle range. E and F had the highest rates of visitation, but the lowest nut set.
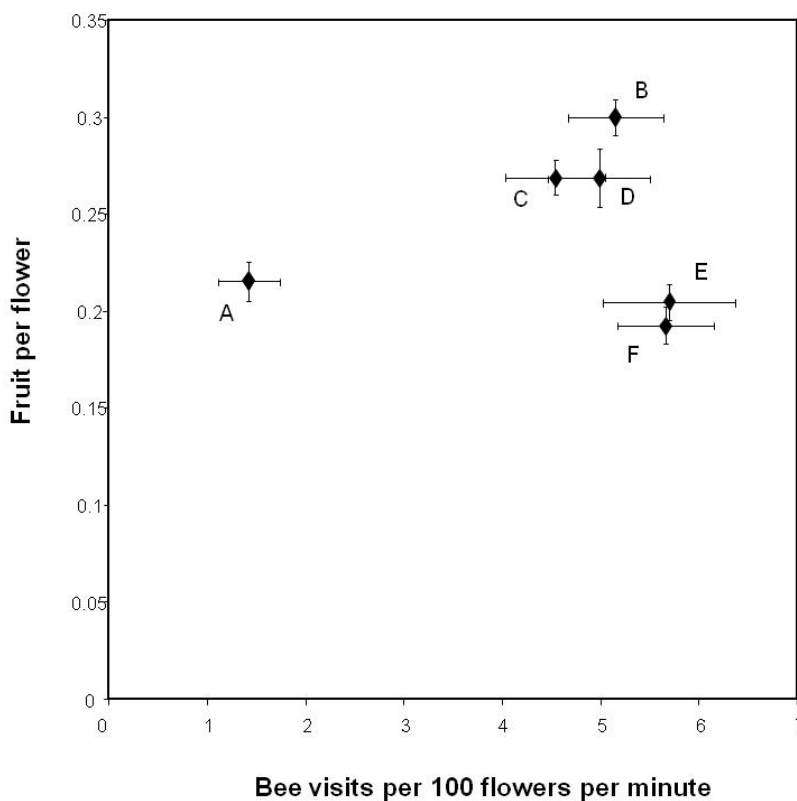
Figure 2: *The relationship between visitation rate (x axis) and the conversion of flowers into fruits (y axis). Each point is the mean value for each of the six sites, with 1 SE.*

We examined fruit set per tree and tested for an effect of bee visitation at the same tree, and whether or not the tree was in the hive row, treating each site separately. For three sites there were effects of bee visitation, or of being in the hive row. At B block (where we already documented a distance-from-hive effect) there was a positive effect of bee visits and a negative effect of being in the hive row. At F block, fruit set was positively linked to visitation outside the hive row, but negatively associated in the hive row. At D block there was a negative effect of being in the hive row, but no correlation with visitation.

Another subtle pattern in fruit set may be linked to bee movement: when pooling across all sites there is a significant tendency for more fruits near the edge of the block (see Table 1). The distance class at the edge has 1.4 more fruits per flower sample than the far distance class. This means fruit set is 14% higher at the edge than the middle. The difference in the unadjusted fruit count is even greater than that predicted in the model. Although we did not see a general pattern for more bees near edges, it may be that bees near edges are more likely to have recently visited a different variety (i.e. to have arrived from a different row), and are therefore more likely to effect cross pollination. Edge trees, however, are also different in terms of light environment and root competition, making attribution to a pollination effect difficult. It is also important to recognise that this edge pattern would have weakened any distance-from-hive effect on nut set, because edge trees are always relatively far from the central hives, so the edge effect runs in the opposite direction to the expected distance-from-hive effect.

Table 1: Mean flower and fruit counts for samples in four distance from edge classes (m) . "Model adjusted" is the fruit count predicted by a model fit to the data, taking into account variation in flower number and site.

Distance from hive	N	Flowers	Fruits	Fruits/flower	Model adjusted fruits
0-30m	216	49.3	12.1	0.25	11.3
31-60m	268	46.5	10.7	0.25	10.9
61-90m	168	49.1	9.8	0.23	10.5
>90m	139	49.0	9.5	0.22	9.9

3.4   Discussion

As intended in the original experimental design, we did create distance-from-hive effects in bee density and we also saw evidence of a relationship between low bee density and nut set. However, these patterns were only found in some blocks and were often weak effects (even though significant) because they identify small differences in a quite variable system. The more important lessons from the experiment come from some of the other observations.

Bees strongly favour moving within the row that hives were placed in, until late in the day when the pollen and nectar run low.

High bee density was in many instances associated with poor fruit set. This tells us that the link between observed bee activity and fruit set is very variable. Observed bee activity is a very poor predictor of what really matters, which is the frequency of cross-pollination events. The importance of cross-pollination (rather than activity per se) is underlined by the pattern of poor nut set in the hive row, and by the pattern of better fruit set near edges of the block.

Both the poor fruit set in the hive row and the better fruit set near edges make sense, but they both contribute to "washing out" the simple distance-from-hive effect that we otherwise expected.

Because it was difficult to create low bee density in the blocks (given the background level of commercial pollination), all of our data might be at a relatively "high" bee density (i.e. bee numbers may not be the limiting factor). If we were able to reduce bee density further, we may have had more relationship between bee density and nut set.

This first field season of study was crucial in establishing that focusing on observation of bee behaviour was unlikely to be an efficient or informative method for understanding and improving pollination. We also determined that the processes that influence pollination outcomes are likely to be influenced by variation on a larger scale than were the focus of this first set of experiments. In other words, rather than looking for distance effects with survey over 1 or 2 hundred meters, it was necessary to conduct experiments at a larger scale, considering effects that might be apparent at >200 meters.

4   Does poor weather for bees reduce yield?

4.1   Introduction

Our discussions with growers and beekeepers revealed a commonly held idea that some flowering seasons where dominated by bad weather for bees, and that this might cause lower yields for those years. This idea makes intuitive sense, because bees are known to be less active and forage closer to the hive in cold or wet weather. Indeed our own observations of bee activity indicate some of these effects (above). However, it is also possible that bees compensate for periods of poor conditions by more efficient foraging in good periods, in which case poor average conditions may have little effect if there are still some opportunities for bee flight.

4.2   Materials and Methods

To test the importance of weather during flowering as a predictor of yield, we examined yield data from a number of blocks (5-7, depending on the year) that were part of an experimental trial conducted near Loxton and Berri in South Australia, and which was monitored for many years (data provided by the Almond Board). This trial had a consistent regime of fertiliser and water application. We used data from a nearby weather station (Renmark Aerodrome) to examine correlations between yield and weather.
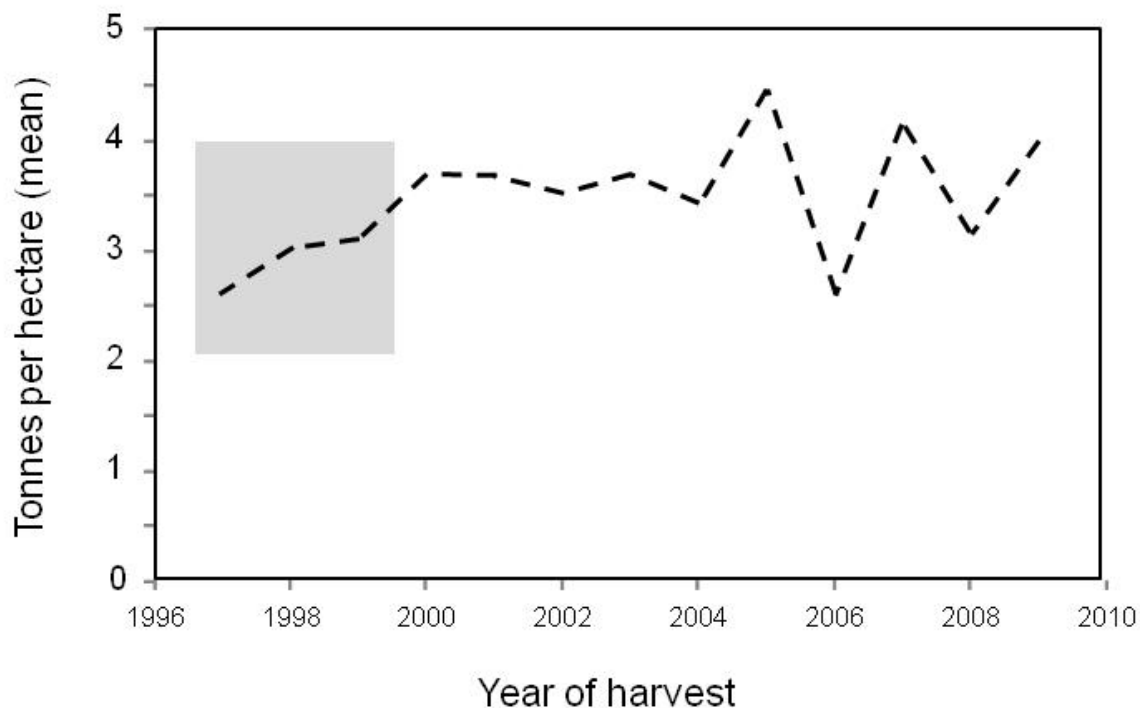
Figure 3: Tonnes per ha, mean across blocks (5 blocks 1997-2000, 6 blocks 2001-2, 7 blocks 2003-9). We dropped the first 3 points (grey box 1997-9) because trees were young and still showing a maturity-based increase in yield, then analysed the remaining 7 years, including the dramatic fluctuations from 2004 to 2009.

4.3   Results

Annual yield variation across these blocks ranged from approximately 2.5 to 4.5 tonnes per hectare (Fig. 3). In the period 2004 to 2009 yield varied dramatically, in a pattern consistent with biennial bearing. We then plotted the mean yields for the years 2000-9 in a two dimensional space defined by the air temperature during the flowering month (proportion of days above 15°C in August) and rainfall (mm rain in August, Fig. 4). If good weather for bees was an important predictor of yield then one would expect the high yield points to be in the dry and warm corner (bottom right) and the poor yield seasons in the cool and wet seasons (top left, Fig. 4). In fact, the data show no tendency to fit the weather-driven pattern: one of the best years was cool and wet and one of the worst years was warm and dry. We explored range of different weather variables, but the result was consistent.
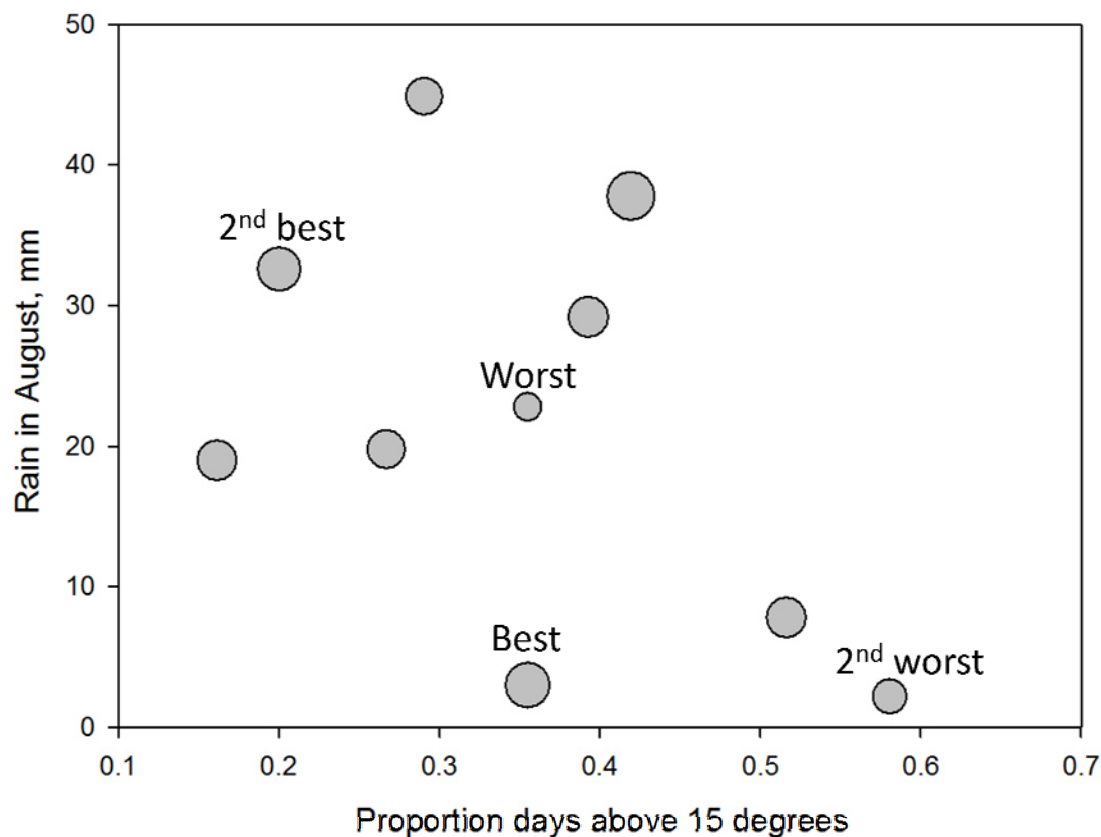
Figure 4: *Each circle represents the mean yield for one year (from figure 4) scaled so that bigger circles represent higher yield. The data are placed in a two dimensional space defined by the proportion of days above 15 degrees in August (month of flowering) and the rainfall for August. Labels show the relative ranking for each year.*

4.4   Discussion

This simple exploration of yield variation suggests that "bee weather" is not a strong predictor of yield. It may be that bee weather has a measurable effect on yield if we could first account for other factors (like biennial yielding or the impact of weather on flowering), but it is not a sufficiently strong driver of yield to dominate the overall pattern.

Our review of the literature uncovered one study that *claimed* to have found a significant association between weather during flowering and yield, which they attributed to a reduced bee activity effect (Lobell DB, Cahill KN, Field CB 2007 *Climatic Change* 81, 187-203). This study examined a 23 year time series of weather data and almond yield in California finding that high January rainfall was detrimental to yield. The authors raised the "bee behaviour" hypothesis, but the main flowering month for California almonds is in fact February, and the data showed no effect of weather in February on almond yield. We suggest therefore that the data actually provide no support for the "bee weather" hypothesis, and is in this way consistent with our analysis (above). It is more likely that the influence of rainfall in the month prior to flowering (in California) is either through effects of pathogens (e.g. increase prevalence of fungus and mold), or through influence on the synchrony of flowering across cultivars.

5 Pollen removal from anthers as an indicator of bee foraging

5.1 Introduction

Having established that direct observation of bee activity is not a good predictor of pollination outcomes, we were interested in examining other methods for surveying bee activity. We decided to focus on the rate at which pollen is removed from flowers, because pollen foraging is one of the primary activities of bees on flowers and is a component of the pollination process (i.e. the movement of pollen from one flower to another).

5.2 Materials and Methods

Study site and hive placement

We conducted experiments in four almond orchards in 2013 (blocks G, H, L and N: Table 3). The study sites were situated within a 35 km radius around Robinvale (latitude 35°35' S and longitude 142°42' E), northwest Victoria, Australia. These orchards shared the same pollination strategy, with an approximate hive density of 6.7 hives per hectare. Hives were arranged in placements of 120 hives on average, and the distance from one placement to another was approximately 700m.

Sample collection

We collected flowers along 6 pairs of transects (total number transects = 12), where a pair included one "far" and one "near" transect, relative to the same hive placement (far transects were on average 451 m away from the nearest hives and the near transects 36 m). Each transect was a set of 15 trees along which we collected one flower per tree per survey. We put the stamens from each flower in a separate vial of ethanol (total number of flowers: n = 711). We chose flowers randomly, provided that they had at least one stamen with an open anther (to make sure they had truly begun anthesis) and one with a closed anther (to make sure they were not past the end of anthesis). Once a flower was selected, we sampled all of the stamens with open anther sacs (i.e. rejecting the anthers that were closed or old and dry). For each transect, we collected samples four times per day (9-9:30 am, 11-11:30 am, 1-1:30 pm and 3-3:30 pm).

Preparing flower samples for pollen count

For each sample, we discarded the filaments and counted the number of anthers collected. We then performed an adapted version of Erdtman's acetolysis method (Erdtman, 1943). Acetolysis dissolves all organic material but sporopollenin, which forms the outer pollen wall. All steps were performed in a fume hood and behind a sliding window. As a starting point, we discarded the ethanol in which the samples had been kept, and we left the anthers overnight in 50 µl of concentrated glacial acetic acid. The next day, we added 500 µl of acetolysis mixture to each tube. The mixture consists of concentrated acetic anhydride and sulphuric acid (9:1). The tubes were then heated up at 100°C for 30 min. We performed a crushing step half way through the heating process to ensure complete dissolution of pollen bearing structures. We then centrifuged the tubes (2 min at 10,000 rpm), discarded the supernatant acetolysis mixture and replaced it with water. The water rinsed off the highly corrosive mixture stoped the reaction and allowed for safe subsequent manipulation of the samples. A second centrifugation (same conditions) enabled us to eliminate the rinsing water. Finally, the pollen grains were resuspended in 30 µl of distilled water. The whole procedure took place in the original *Eppendorf* tube to avoid any loss of pollen due to transfers from one tube to another (Fig. 5).

Pollen counting

We transferred the whole pollen grain suspension to a hemocytometer using a micropipette. It filled the two counting chambers of the hemocytometer by capillary action (Fig. 5). We used an Olympus SZX2-TR30 microscope at 30x magnification, and an Olympus DP25 digital camera to acquire pictures. We adjusted lighting until the pollen's colour contrasted sharply with that of the background and any contaminants. The hemocytometer slide had two separated counting chambers. We took a picture of each chamber, and thus obtained two different pictures per flower sample. Each picture corresponded to a volume of 1.564 µl, which was between 2 and 8% of the total pollen suspension.

We used *ImageJ*, a Java-based image analysis software provided by the US National Institutes of Health (Rasband, 2012) and an adapted version of Costa and Yang macro (Costa and Yang, 2009) to analyse the pictures. We firstly tested the accuracy and consistency of our novel image analysis method. To do so, we manually counted 27 pictures and compared the manual to the automatic counts by Pearson correlation coefficient. For this test, we chose images which represented a wide range of density and contrast characteristics and contained many contaminants in suspension because these images were the most likely to produce an inaccuracies in the image-analysis count.
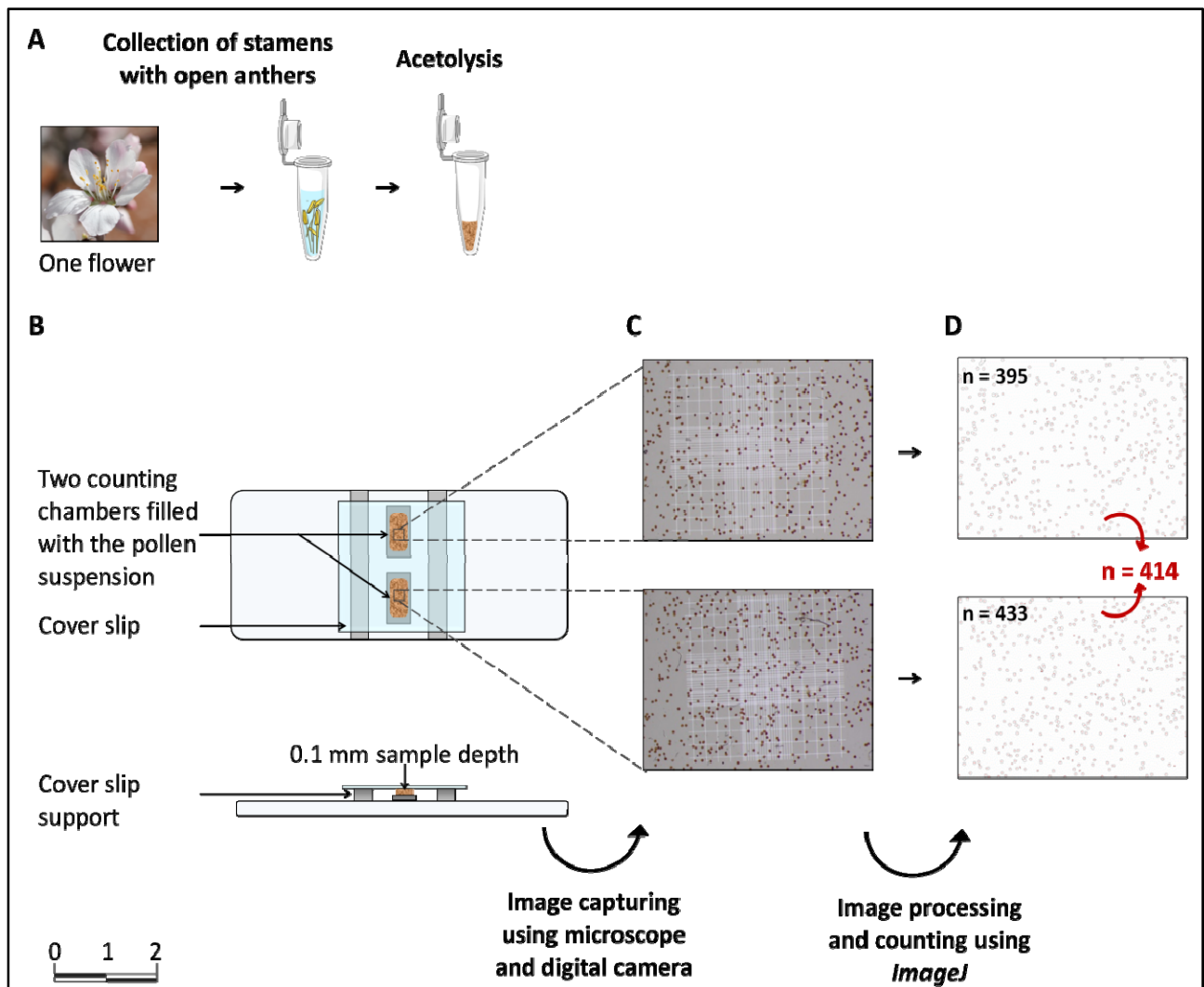
Figure 5: Major steps performed to obtain each flower's number of pollen grains; (A) the stamens from one flower are collected and processed to discard all pollen bearing structures; (B) the whole pollen suspension, obtained from one flower, filled up both chambers of the hemocytometer; (C) unprocessed digital images of each chamber; (D) images after ImageJ analysis (threshold adjusted, background subtracted and inverted, particles counted). The final number of pollen grains per flower is the mean of both picture's count (red number). Scale bar = 2 cm for (A) and (B) and 2 mm for (C) and (D).

The first step of the macro consisted in removing the background of each picture and replacing it with a dark monochromatic background. But because all our images did not have the exact same background colour, we could not automatically carry out this step. We therefore designed a new preliminary step to standardize their colours. This step converted the pictures from and RGB to L*ab colour space. In this new colour space, each pixel is characterized by the value of its luminosity. This value was low for the dark pollen grains and high for the light background and remaining contaminants. Our preliminary step analysed the distribution of these light intensity values and determined the light intensity threshold separating the background and the pollen grains for each image. The value of this threshold enabled the subsequent automatic background removal from each picture using *ImageJ*. The exact number of pollen grains per image was then calculated using Costa and Young macro and *ImageJ*. We counted the number of pollen grains for each image, and as a result, we had two different counts per flower. The mean of these two counts was used for the subsequent statistical analyses.

Data analysis

For each transect, our experimental scheme provided us with three types of data: (1) number of open anthers (2) number of pollen per open anther and (3) number of pollen per flower. We focused analysis on the number of pollen grains per flower.

We compared the mean number of pollen per flower and graphed each pair of them so one could directly see the difference between "near" and "far". The pattern of pollen removal over the course of the day clearly emerged from the visual analysis. A series of two-paired t-tests on our 6 pairs of transects supported the conclusions drawn from the visual analysis.

5.3   Results

Image analysis software

The manual and automatic counts were very highly correlated (*Pearson's r = 0.986, n = 27*) therefore we considered this method to be reliable. Furthermore, the software count was 200 times faster than the manual count. Indeed, the macro enabled us to analyse 1422 images in slightly less than 2 hours. A manual count would have taken more than 400 hours. Finally, repeated manual counts of the same pictures proved that the software was not only faster but also more accurate than visual analysis of the pictures.

Number of anthers per flower, pollen per anther, and pollen per flower

The number of pollen grains per flower was related to the number of anthers sampled, creating a triangular scatter plot (Fig. 6). The number of open anthers per flower was typically less than 32 (only two of 711 flowers exceeded this). The number of pollen grains per flower ranged between 501 and 34,500 for 99% of the flowers. The diagonal upper threshold in this scatter (solid line, Fig. 6) exists because the maximum number of pollen per flower is constrained by the number of pollen in an unopened anther, multiplied by the number of anthers (x axis).

 Some of the values were above this upper threshold. This was due to biological variability and error introduced by the counting method. The equation of this line was: *y = 1,482 x*, where the slope corresponded to the average maximum number of pollen per anther. This number (1,482) provides an estimate for the typical number of pollen grains per unopened anther, or, in other words, the number of pollen grains released every time a new anther opens.
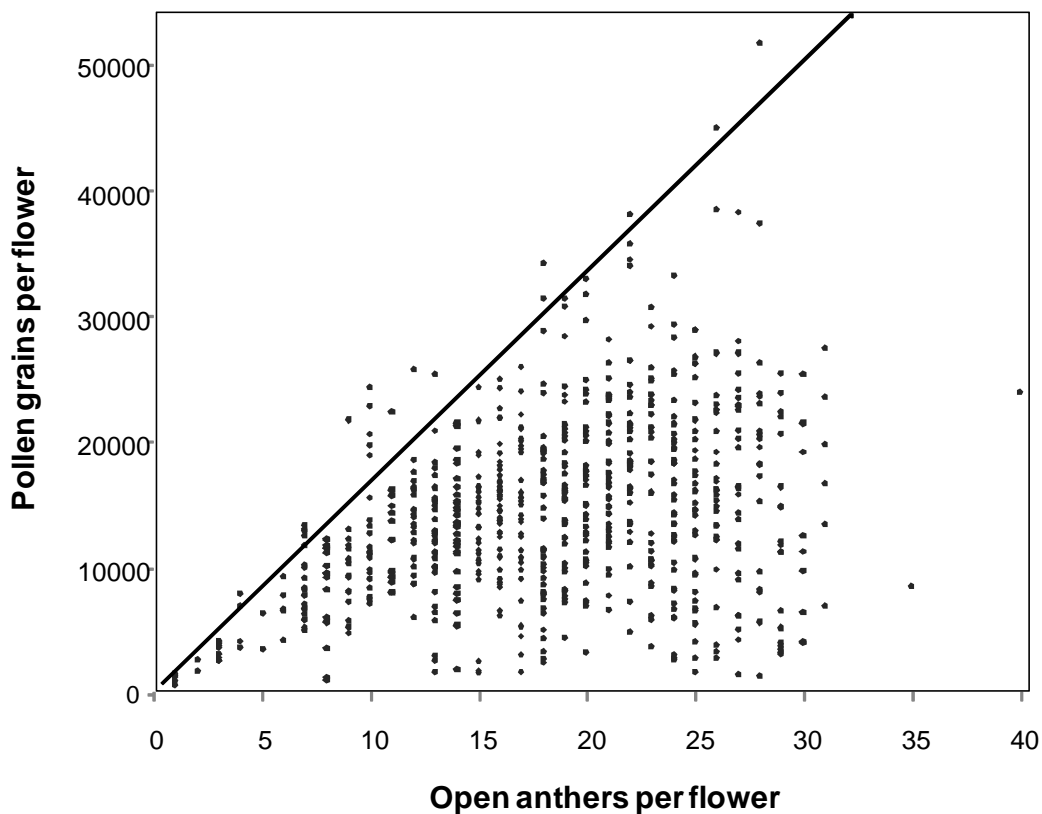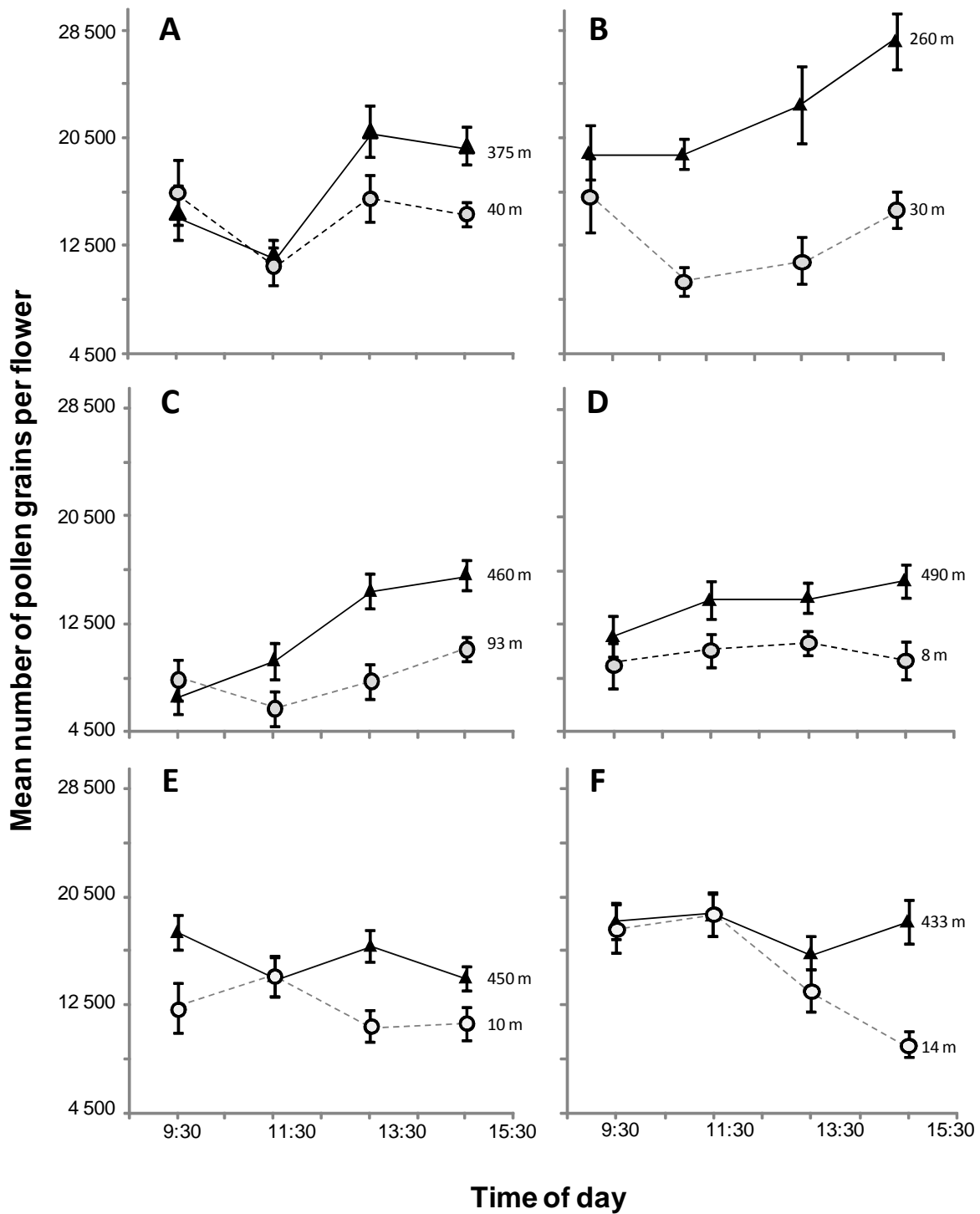
Figure 6: Number of pollen per flower as a function of number of open anthers per flower. Each point represents a flower (n = 711). The solid line was fit by eye to represent the typical upper limit of number of pollen per flower.

Comparing morning and afternoon of the same transect

The number of pollen grains per flower is the result of two opposing forces. On the one hand, there is the bees' foraging activity: it removes pollen from the open anthers and reduces the number of pollen grains per flower. On the other hand, there is the continuous process of anther opening: each anther releases approximately 1,500 new pollen grains and thus increases the mean number of pollen grains per flower. The number of open anthers also increased throughout the day (Table 2). The average flower had about 19 open anthers, which represent two thirds of its total number of anthers. Figure 7 enables us to assess the relative effect of those two opposing forces on the number of pollen per flower, along the course of a day.

In all of the "near" transects, the number of pollen grains per flower at the end of the day was similar to at the beginning of the day. In fact, in 5 of the 6 transects the number was slightly lower at the end of the day (Fig. 7). This means that at a short distance from the hives (less than 93 m), the bees' removal of pollen balanced or even exceeded the release of pollen due to anther dehiscence. In contrast, in 4 of the 6 "far" transects, the number of pollen grains per flower was significantly higher at the end of the day compared with the beginning. In other words, at the far transects (>400 m from hives) pollen gathering by bees was usually insufficient to keep up with pollen released by flowers (Fig. 7). The number of pollen grains per anther showed the same pattern; close to the hives, bees removed pollen faster than anther dehiscence released it, but the opposite happened at >400 m from the hives (Table 2).

Figure 7: Number of pollen grains per flower as a function of time; solid lines represent the "far" transects, dotted lines represent the "near" transects. Symbols (triangles = far and circles = near) show mean number of pollen grains per flower (± 1 SE) at each sampling time. For each point, n = 15. Labels indicate the distance between each transect and their nearest hive placement. We expanded the scale of the vertical axis so it does not start at 0. Each graph represents one pair of transect; (A) Block L , pair 1; (B) Block L, pair 2; (C) Block N, pair 1; (D) Block N, pair 2; (E) Block G pair 1; (F) Block H, pair 2.

Table 2: *Mean numbers of anthers and of pollen per anther at each time of the day. Each number is the mean for the 6 "near" or the 6 "far" transects . Sample size for every mean: n = 90 (standard error of the mean is in parenthesis).*

	Near transects		Far transects	
Time of day	Anthers	Pollen per anther	Anthers	Pollen per anther
9:30 am	16.8 (0.64)	849 (77.7)	18.0 (0.81)	909 (79.4)
11:30 am	17 .3 (1.21)	771 (69.8)	16.8 (0.78)	944 (65.9)
1:30 pm	18.7 (0.84)	709 (56.3)	20.6 (0.24)	902 (49.8)
3:30 pm	19.6 (0.79)	672 (66.3)	20.1 (0.65)	1002 (91.5)

Comparing "near" and "far" pairs of transects

Our experimental design enabled us to compare the change in pollen count along the course of the day. We collected data that provide 24 paired contrasts (4 times a day * 6 transects: Fig. 7). In 16 of these 24 situations, the mean number of pollen per flower was higher in the "far" than in the "near" transect. In the 8 other contrasts, there was no significant difference between the "far" and "near" mean numbers of pollen per flower (judged by overlap in SE bars, Fig. 7) and all of those 8 comparisons were in the morning; (9:00 and 11:30am) before much bee activity had occurred. From 1:30pm onwards, across all of our paired transects, the number of pollen grains per flower from the "far" transects was always significantly higher than the one from "near" transects. The average number of open anthers also increased during the day (Table 2). To assess the effect of distance from hive on bee activity the most important comparison is the situation at the end of a whole day of foraging activity. As expected, distance did not have any effect on the mean number of pollen grains per flower at the beginning of the day (no significant difference between "near" and "far" at 9:30 am; $t_5 = 1.33$, $P = 0.241$). In contrast, the difference between the number of pollen grains in the paired "far" and the "near" transects (*mean = 6905, SD =1347, N = 6*) was significant at the end of the day ($t_5 = 5.13$, *two-tail p = 2.57*, $P = 0.0037$). On average, flowers from the "far" transects had 37% more pollen than the "near" ones at the end of the day. The number of pollen per anther showed the same pattern (Table 2); while similar at the beginning of the day, it was significantly different at 3:30 pm ($t_5 = -2.61$, *two-tail p = 2.57*, $P = 0.0024$).

5.4   Discussion

The data show unambiguously that there is less pollen collecting activity on trees that are 400m or more from hives compared to those trees near hives. This approach provides a much clearer picture of the pattern of bee activity than we were able to determine by direct observation. This is probably because direct observation of bee activity provides an insight only at a moment in time (the observation period) whereas the focus on pollen removal tracks the *effect* of bee activity over the whole blocks of time. Also, pollen removal is more directly related to pollination than a record of bees being "present" or "visiting". Nevertheless, even pollen removal is many steps removed from what we really want to know about pollen movement, which is the frequency of cross pollination. Although this experiment clearly demonstrates a

deficit of bee activity far from hives, it may not translate into an effect on fruit set, because there may be a poor relationship between pollen removal from anthers and the rate of cross pollination, or because the level of activity may be sufficient for best pollination even at the reduced rate. The next step therefore is to examine if the reduced bee activity at trees far from hives is correlated with reduced fruit set on trees far from hives.

6   Effect of distance from hive on fruit per flower

6.1   Introduction

Given that bee activity (estimated by pollen removal rates) is lower far from hives, the next step was to determine if this led to a corresponding decline in fruit set, because of insufficient pollination. We conducted this experiment against the background of the normal pollination practice in the large orchards in Victoria, which is a hive density of approximately 6.7 hives per ha, achieved with hive placements typically of ~120 hives, spaced up to 700 m apart. As a result, most trees are within about 400 m of a hive placement. This approach means placements are larger in number and further apart than is commonly used in many other orchard crops. There is variation in the size of placements and the distance between hives, because of logistic constraints (including site access, safety issues, and room for hive placement). Some parts of the orchard might be further than 400 m from hives because the shape of the planting and pattern of the roads makes it hard to do otherwise, when using large placements. There can also be variability in the number of hives at a placement because the preferred number of hives were not available (smaller loads) or because two loads are combined (extra large placements). Across the two years of our study we counted 94 hive placements that were identified as the nearest placements to our experimental survey transects. They ranged in size from 72-338 hives, with a mean of 166 in 2012 (N=45), and 144 in 2013(N=49).

6.2   Materials and Methods

We surveyed at 276 trees in 2012 and 305 trees in 2013 (Table 3) selected to represent a range of situations in terms of distance from hive placements. In the pursuit of getting the contrast of interest we deliberately sought out opportunities where trees were relatively far from hive placements. In a few instances the orchard managers and pollination agent were able to help the experiment by moving hives to create locations that were far from hive placements. Trees were usually assessed in transects of 5, but a few transects varied from this number because trees were lost or extra trees were recorded. We give each transect a single distance measure reflecting the distance to the nearest placement of hives. To capture spatial variation for our analysis we assigned clustered transects that were near one another into nine spatial blocks (Table 3), each block being under one farm manager.

At each tree we counted flowers on four tagged branchlets (mean=53 per branchlet), which we returned to in October to assess the conversion of flowers into fruit. We also hand pollinated a much smaller sample of flowers (mean 6.1 per branchlet) on a single tagged branchlet per tree. Our analysis focused on the effect of distance from hives (in meters) on the percentage of flowers converted into fruit. While the focus is on open pollinated flowers we also analysed the conversion of hand pollinated flowers into fruit. The hand pollinations helped us to estimate the gap between the achieved fruit set and what might be possible with a higher pollination rate. They also helped to determine if some trees might have low flower-fruit conversion ratios even when pollination is high, perhaps because of other constraints.

We analysed the fruit per flower data using a Generalised linear model, with a binomial error distribution (as preferred for proportional outcomes) fit using the PQL procedure in the "R" statistical analysis program. Our random model includes transects nested within block (to capture spatial variation) and the percentage of flowers converted into fruit on hand pollinated flowers (in case this shows inherent differences among trees, perhaps controlled by resources). Although the spatial factors proved important in the analysis, the inclusion of the hand pollinated flowers as a random term actually made little difference.

Table 3: *Number of trees assessed per block over two seasons of the distance from hives experiment.*

| Block	2012	2013
G	35	40
H	0	30
I	12	0
J	10	65
K	28	10
L	75	56
M	38	29
N	39	35
O	39	40

6.3   Results

Our analysis indicated a significant effect of distance from hive placements on the percentage of flowers converted into fruit (Wald=6.89; df=1, 105.6; P=0.010). To assess the pattern of the relationship we examined the estimated slope and intercept from the full model, and graphed the slope predicted by the fixed model (ignoring the random effects, Fig. 8). Although the underlying data is of course variable, there is a strongly significant effect, with a decline in fruit per flower at increasing distance from the hive. The fitted model shows 36% fruit set at 20m, declining to 28% at 850 meters (i.e. the range we explored) or in relative terms, a 22% decline over that range (=36-28/36).

Hand pollinated flowers on these trees had consistent higher fruit set than open pollinated flowers (mean 55%, n=581, SE=1.2). We examined whether fruit set from hand pollinated flowers also varied as a function of distance from the hive placements, using the same random model, and found no significant effect. This indicates that increasing the pollen supply can raise fruit set, at least to a subset of flowers, and that this benefit does not depend on distance from hives.

6.4   Discussion

This decline in fruit set with increasing distance from hives is consistent with the observed decline in bee activity estimated from pollen removal. While the pollen removal data show a clear decline from a relatively small sample of transects, the fruit set result is much more variable. Our analysis shows a statistically significant effect, but against the background of a lot of variation. This is not surprising, given the manifold influences on fruit set that we know we have not controlled in this experiment. Together the data show that reduced bee activity far from hives (>400 m) is associated with reduced fruit set on average at trees far from hives. The most likely explanation for this associated is that bees are less common on flowers far from hives, and that this decline in overall activity means a decline in the frequency of cross pollination events.
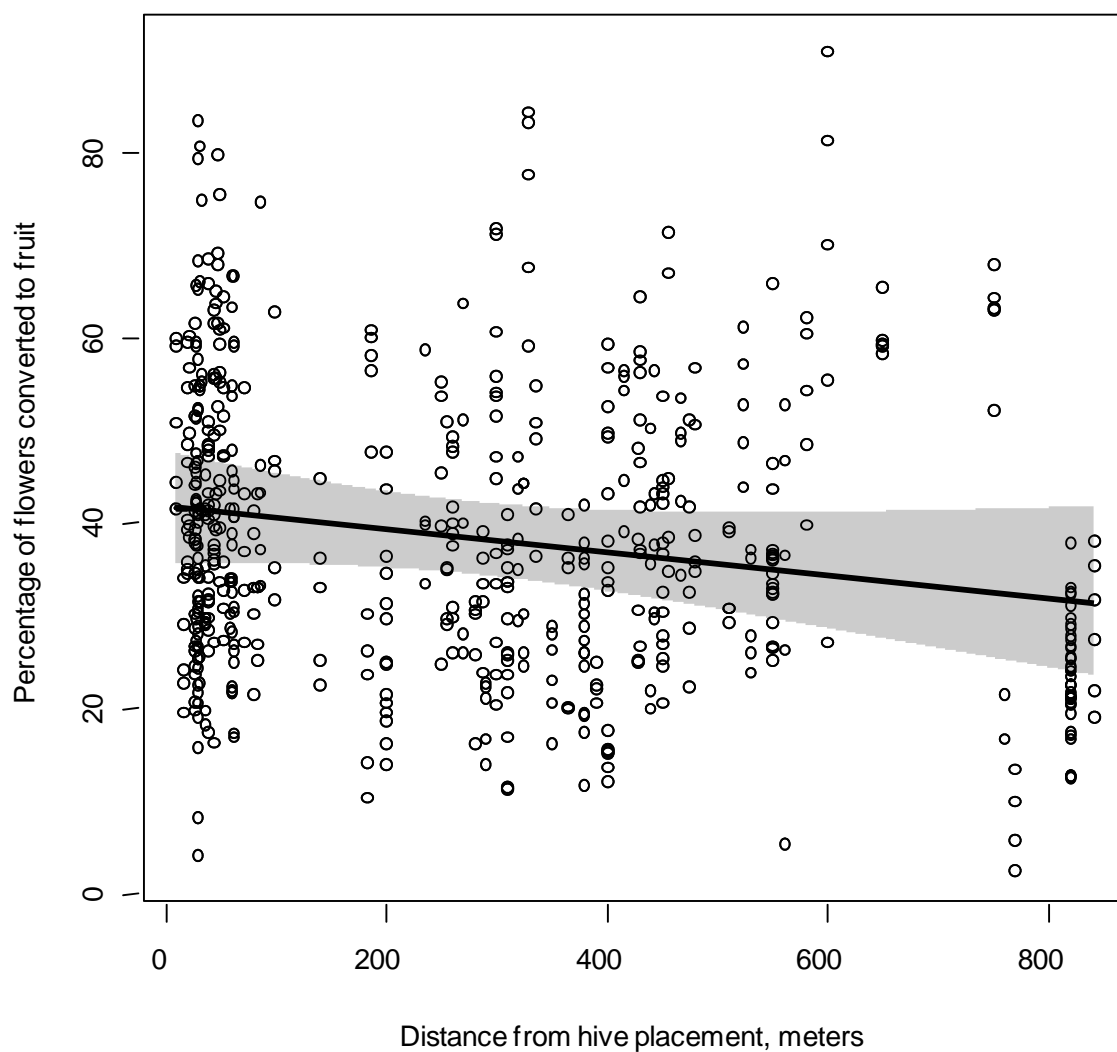
Figure 8: The effect of distance from hive on fruit set (flowers per fruit) for open flowers. Circles show the underlying data points – each circle is a tree. The solid line shows the regression line fit to the data (for the figure, no random terms were included in the model), which has a significant downward slope (i.e. fruit set decreases on trees that are further from the hive placement) and the grey envelope shows the 95% confidence interval.

7 Effect of hive density on fruit per flower

7.1 Introduction

Our analyses of distance effects on bee activity and fruit set were conducted against the background of a relatively standardised practice using 6.7 hives per hectare with typical placements using approximately 120 hives. But this, of course, is only one of many possible strategies for placing hives in orchards. Although there is a tendency to focus on the "hives per hectare" as the summary of the pollination strategy, there are in fact other important variables. As we have already indicated, one can also vary the number of hives per placement and the distance between placements. These three factors are interdependent. To explore this interdependence we simulated a range of scenarios using an imaginary 1000ha site. We represented the distance between hives by treating the hive placement as the middle of a circle, with the diameter representing the inter-placement distance. We then estimated approximately how many circles could fit the site, based on the area of the circle and the area of the orchard. The method is approximate because in a real orchards, circles do not neatly piece together to cover a square, and furthermore real orchards have areas without trees such as access roads, and areas with poor soil. Nevertheless, this simulation is useful to describe the underlying pattern of inter-relation between the three variables (hives per hectare [hph], hives per placements, and inter-hive distance; Fig. 9). In real orchards the presence of areas with no trees means that for a fixed hph and number of hives per placement one would expect a greater distance between placements.
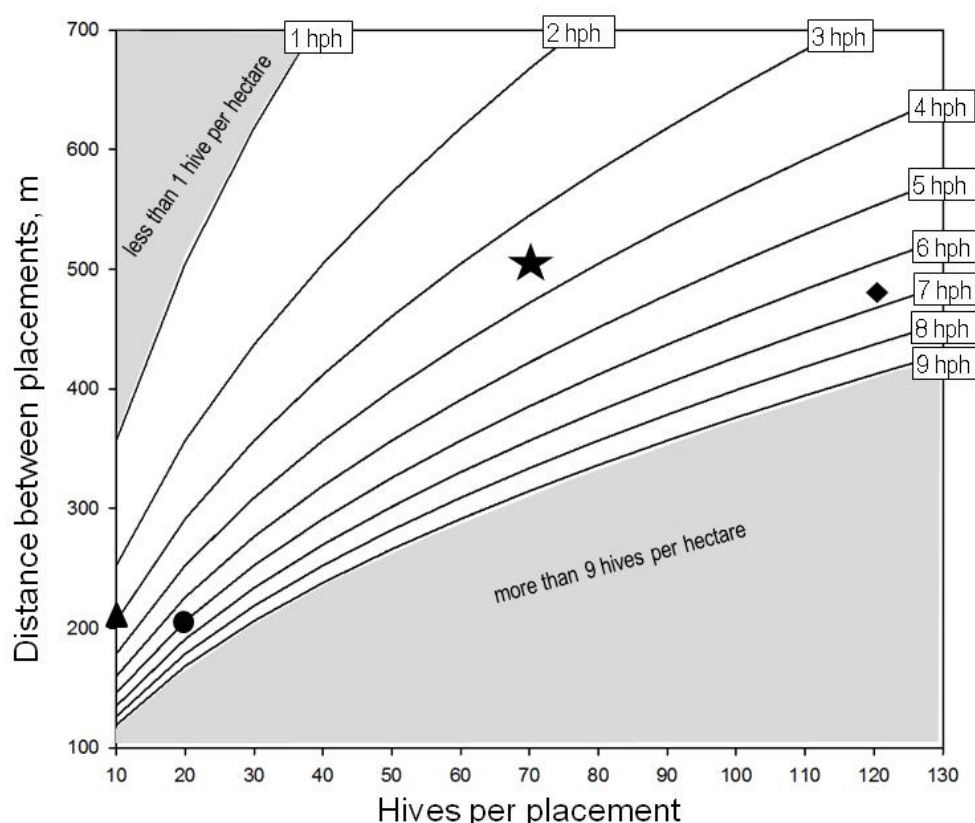
Figure 9: *This figure describes the relationship between hives per placement, distance between placements, and hive density, in a modelled system. The diamond marks the hives per placement and hives per hectare strategy typical across many large orchards in Victoria. The other three shapes mark some examples of the hive arrangements that contributed to our "effect of density" experiment. The star is 70 per placement, 3.6 hph. Circle is 20 per placement, 6.7 hph, and Triangle is 10 per placement and 3hph.*

If the hph is kept constant, then an increase in the number of hives per placement (Fig. 9, x axis) requires an increase in the distance between placements (Fig. 9, y axis), thus the lines of constant hph slope upwards. Or, to explore another comparison among scenarios, if one reduces the distance between hive placements while keeping the number of hives per placement constant, then one is increases the hives per hectare.

7.2   Materials and Methods

The goal of our hive density experiment was to examine the effect on fruit set of a range of other strategies, focusing on lower hives per hectare densities and smaller numbers of hives per placement. We were able to collect data on these strategies by survey of trees in orchards that were already using lower hive per hectare strategies (i.e. <6.7) as their standard practice, and also by applying different pollination strategies to blocks within the large Victorian orchards, by co-operation with orchard managers and the pollination provider. For these experiments we selected blocks that were naturally isolated from other blocks by areas of open grass (i.e. unplanted areas approximately 100 m across). By doing so our aim was to apply a distinctive pollination strategy on these isolated blocks, with the expectation that the spatial isolation would reduce the influence of hives applied to the rest of the orchard. Over the two years of this experiment we examined pollination outcomes on 15 blocks, with hive per hectare densities, ranging from 2.9 up to 6.8. The blocks used in this experiment were usually smaller than the blocks used to assess spatial variation in the "distance from hives" experiment. Where these blocks were located within one of the blocks already assigned a code (Table 3) we used the existing letter followed by a number to denote the smaller block (e.g. G.2 is a block within the already named G block).

Table 4: Number of trees assessed in the hive density experiment, and hive density (hives per ha) per block. Note that most have small placements relatively close together, except blocks P and Q which had smaller placements than the standard across the larger Victorian orchards, but at similar distance between hives.

Block	Area (ha)	Year	Trees surveyed	Hives per ha	Mean hives per placement
L.2	1.5	2013	15	5.33	8
L.1	6.4	2013	15	3.13	7
G.1	15	2012	16	6.26	21
G.2	13	2012	16	6.26	21
G.3	14	2012	26	6.26	21
P	175	2013	10	3.64	74
Q	175	2013	10	3.64	74
R	115	2012	35	3.72	9
S	115	2012	30	3.72	9
K.1	4.2	2013	15	2.86	6
T	17.8	2012	60	3.37	12
T	17.8	2013	20	5.06	18
N.1	4.7	2013	15	6.81	15
N.2	8.7	2013	18	3.67	16
N.3	5.3	2013	12	6.79	18

We used the same survey approach already described for the "distance from hives" experiment: i.e. trees surveyed in transects, branchlets tagged for assessment of flower to fruit conversion, and hand pollination on a smaller sample of flowers. We also used a similar model for statistical analysis; i.e. a Generalised linear model, with binomial error distribution, a random model including transects nested within block (to capture spatial variation) and the percentage of flowers converted into fruit on hand pollinated flowers (in case this shows inherent differences among trees, perhaps controlled by resources).

7.3   Results

We documented a positive relationship between the fruit set (percentage conversion of flowers to fruit) and the number of hives per hectare applied to each block (Fig. 10), which is strongly statistically significant (Wald=11.5; df=1, 30; P=0.002). The fitted relationship indicates on average 33% fruit set at 2.8 hives per ha increasing up to 46% at 6.8 hives per ha, or in relative terms a 39% increase over the range we explored (=46-33/33).
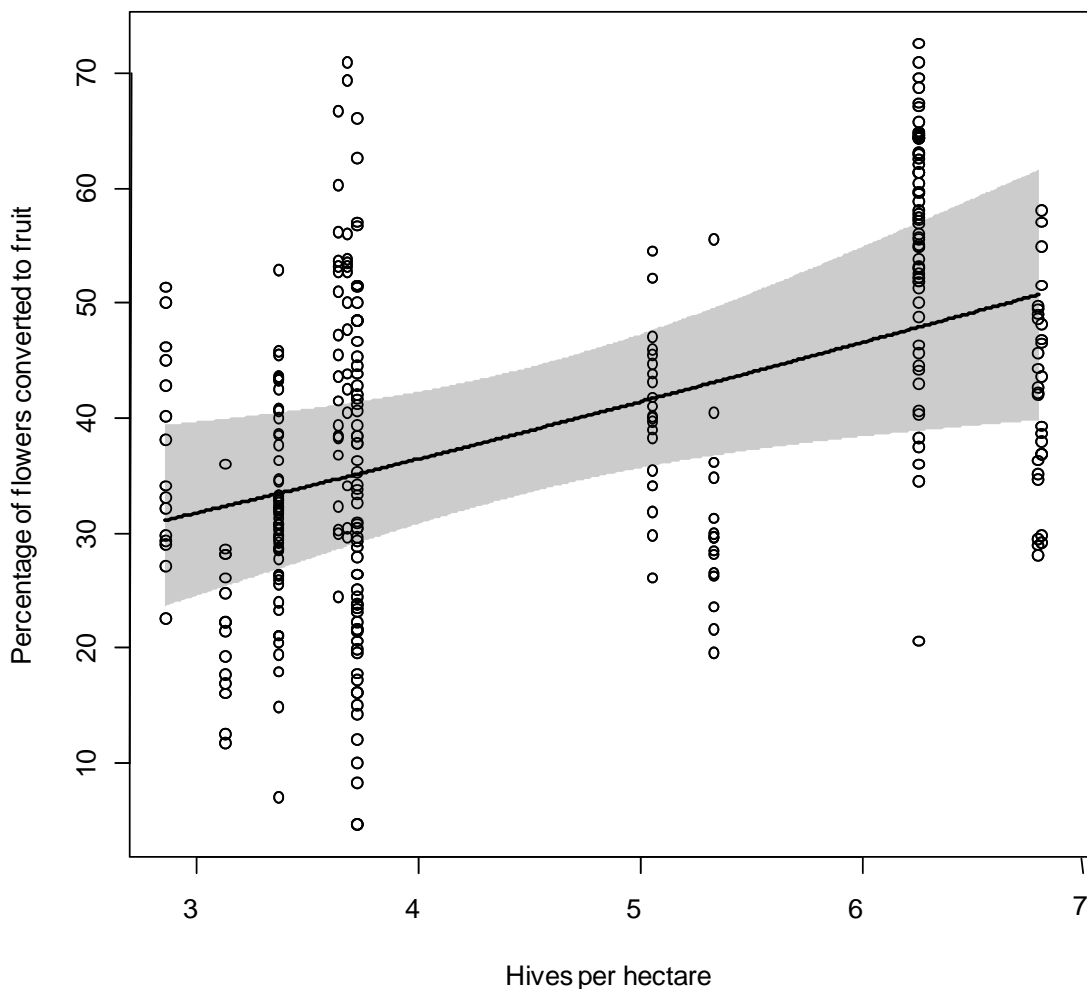
Figure 10: *Effect of hive density on fruit set (flowers per fruit) for open flowers. Circles show the underlying data points – each circle is data from one tree. The solid line shows the regression line fit to the data (for the figure, no random terms were included in the model), which has a positive upward slope (i.e. higher hive density is associated with higher fruit set) and the grey envelope shows the 95% confidence interval.*

As in the "distance from hives" experiment, hand pollination increased fruit set substantially. In this case, however, we also detected that the outcome from hand pollination was significantly positively associated with hive density (P=0.011). We believe this is because the hand pollination benefit is being additional to the existing fruit set gradient we see in open pollination trees. In other words, a single hand pollination per flower, such as we applied may not take pollination up to a fixed maximum, but instead add a constant increase on top of the underlying level of pollination by bees. The fact that this effect is apparent in the "hive density" study but not the "distance from hive" study may reflect that the underlying gradient was steeper in the density study, and therefore an effect is easier to detect.

7.4   Discussion

Integration of hive density and distance effects

The "distance from hive" and "effect of hive density" experiments were conducted using different designs, suited to the different questions. But nevertheless, they were conducted using the same underlying flower survey and hand pollination methods, in the same two flowering seasons (2012 and 2013) and mostly using the same orchards or orchards in the same region of Victoria (the only exceptions being 2 blocks in the hive density experiment, which were located in NSW). Given the largely shared circumstances it is fair to report the experimental outcomes in one integrated comparison (Fig. 12). Because we have analysed each experiment separately we cannot report study-wide confidence intervals in this figure. Nevertheless, the analyses reported above show that there is strong statistical support for a distance effect (e.g. the diamonds in Fig. 12) and for the hive density effect (dotted line, Fig. 12). There is also a clear effect of hand pollination leading to higher fruit set (mean 52.8%, n=302, se=1.8%) across both experiments.

Taken together, the studies indicate that the best fruit per flower outcomes were gained with a high hive density (approximately 6.7) but using smaller placements (and therefore with shorter distances between them) than in the regional norm. In the "distance from hives" study the density of hives calculated across the whole orchard was 6.7 per ha using large placements strategy, but fruit set outcomes were lower than that achieved with a similar hive density and smaller placements, and worst for hives at a greater distance from hive placements (e.g. 400-850 m).

If one were to adjust pollination strategies with the goal of achieving the best flower to fruit conversion, one would keep hph to at least 6.7, but use fewer hives per placement (i.e. <120 hives) and therefore reduce the distance between placements and the average distance between trees and hives. This conclusion is well supported by the experiments reported in this study. However, our experiments only cover a limited range of possible scenarios. For example, we have no data on hph above 6.7, and we were not able to explore all possible hive placement strategies for achieving 6.7 hph (see Fig. 9). These experiments have focused on the benefit side (i.e. improved fruit set as a driver of yield) but not included the cost side, i.e. the costs of hives and the labour associated with spreading them around the orchard. The best management outcome must, of course, consider the cost/benefit ratio. It is worth noting, however, that a number of the blocks used in our study with different hive arrangements were operating according to commercial practice adopted by the farm manager, rather than experimental practice for our research. So this underlines that a range of strategies that include smaller placements have been judged commercially viable by those farm managers.

As well as managing for a high mean fruit set, growers would benefit from reduced variability. We examined the variability in fruit set for open pollinated flowers in these experiments to see if it was affected by the treatments. To simplify, we reduced each data set to two categories per experiment and then examined the inter-quartile range of variation in fruit set (Fig. 13). This revealed that the differences in variability between the treatments are quite small: in other words, our treatments had relatively little effect on variability. We also examined the variability in fruit set from hand pollinated flowers, and found that there was consistently much greater variability for hand pollinated flowers than for open pollinated flowers.
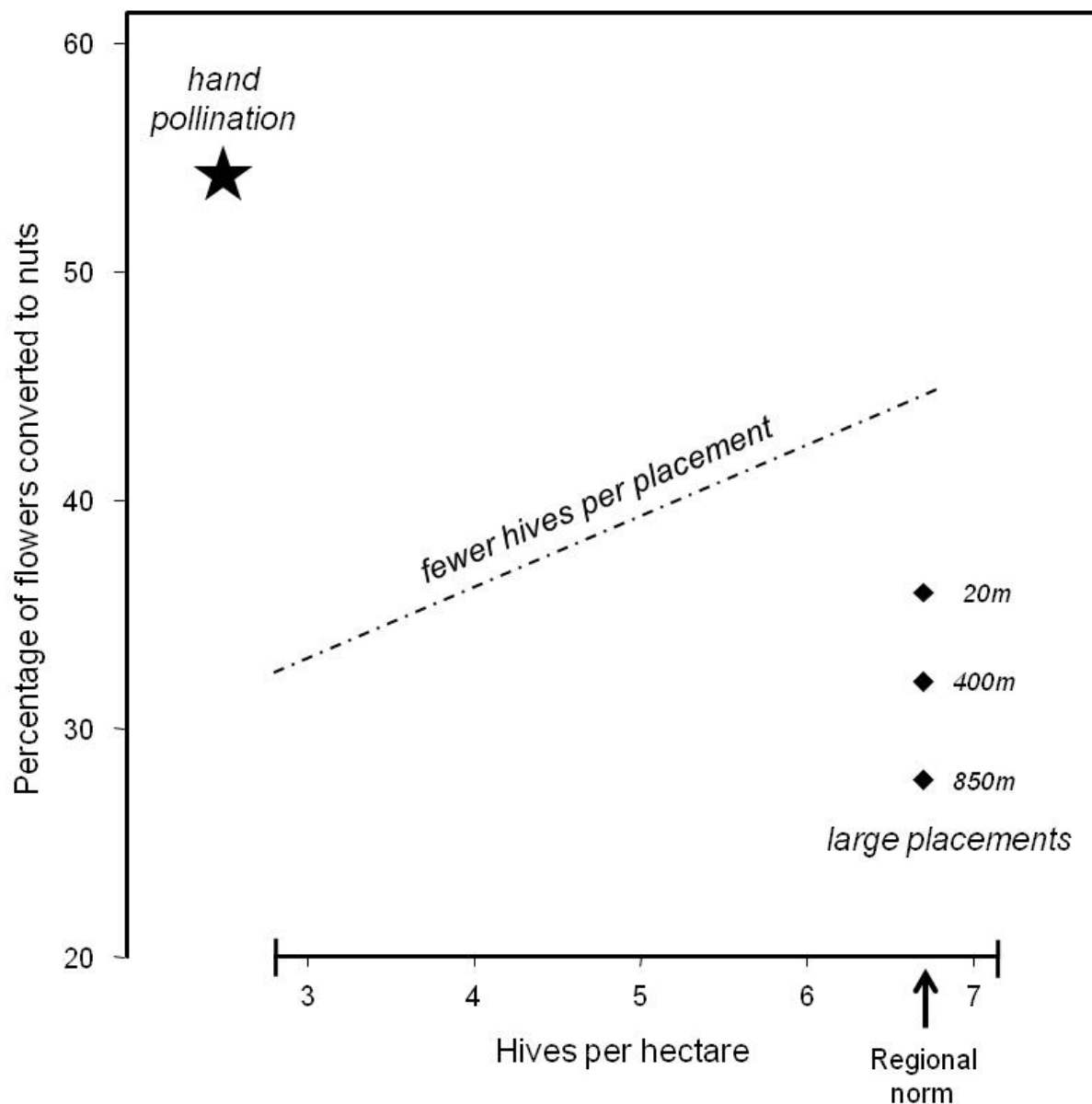
Figure 12: Summary of the model outputs from the "distance from hive" experiment (diamonds, labelled large placements) and the "hives per hectare" experiment (dotted line) different experiments. Hand pollination value is the mean across both experiments.

If hand pollination were simply supplementing open pollination, and bringing fruit set up closer to the maximum possible, one would predict that fruit set from hand pollinated flowers would be less variable than for open flowers. So in that sense, the result is surprising. The likely explanation is that the hand pollination method that we used must be only partially effective, raising fruit set on average, but leaving many flowers with low fruit set. It may be that hand pollination sometimes interferes with fruit set either because the handling of the flowers causes mechanical damage, or because the transfer of a larger than normal amount of pollen to the stigma causes problems in fertilisation. Either way this result is a reminder that while hand pollination experiments help to establish if fruit set is, on average, raised by increased pollen delivery (yes, in our studies), it is should not be treated as an estimate of the outcome of "optimal" pollination. This is in keeping with the observation that fruit set from hand pollination responded to hive density, which would not be expected if the hand pollination truly brought fruit set to optimal regardless of the background level of pollination.
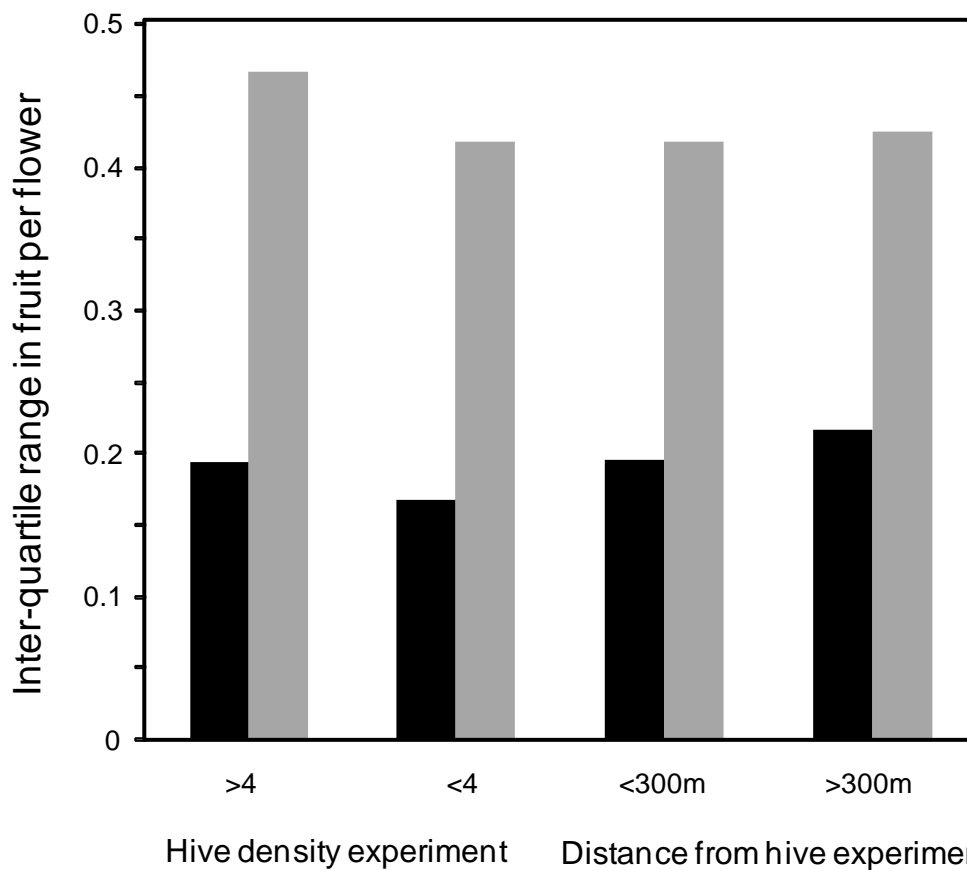
Figure 13: *Inter-quartile range in fruit per flower (as a proportion) , comparing open pollinated flowers (black columns) and hand pollinated flowers (grey columns) across four different treatments.*

# 8 Does October fruit set predict harvest?

8.1 Introduction

All the experiments reported to date rely on fruit counts made in October. At this time fruit are clearly developing, and some of the aborted fruit have dropped. We chose this date because it is expected to most strongly reflect the influence of pollination. Fruit losses after October are more likely to reflect other impacts including pests, diseases, extreme weather and resource limitation. Nevertheless, there are still 5 months between our counts and harvest and it is useful to know if the patterns we describe for October fruit set are strongly predictive of fruit set at harvest.

8.2 Materials and Methods

To explore this question, we counted fruit twice for 275 trees (1107 branchlets); first in October 2013, and second as harvest commenced in February 2014.

8.3 Results & Discussion

The mean percentage change in fruit count was a reduction of only 6.5% (i.e. about 6 in 100 fruit are lost between October and February). This should not be confused with a difference in fruit to flower conversion rate, which is very much smaller. Given that so few fruit are lost in this period, it is unsurprising then that fruit count in October is a very good prediction of the fruit count in February (Fig. 11, r squared, 79%). We can conclude then that the patterns we describe on the basis of fruit count in October are very strong determinants of the pattern of fruit set at harvest.
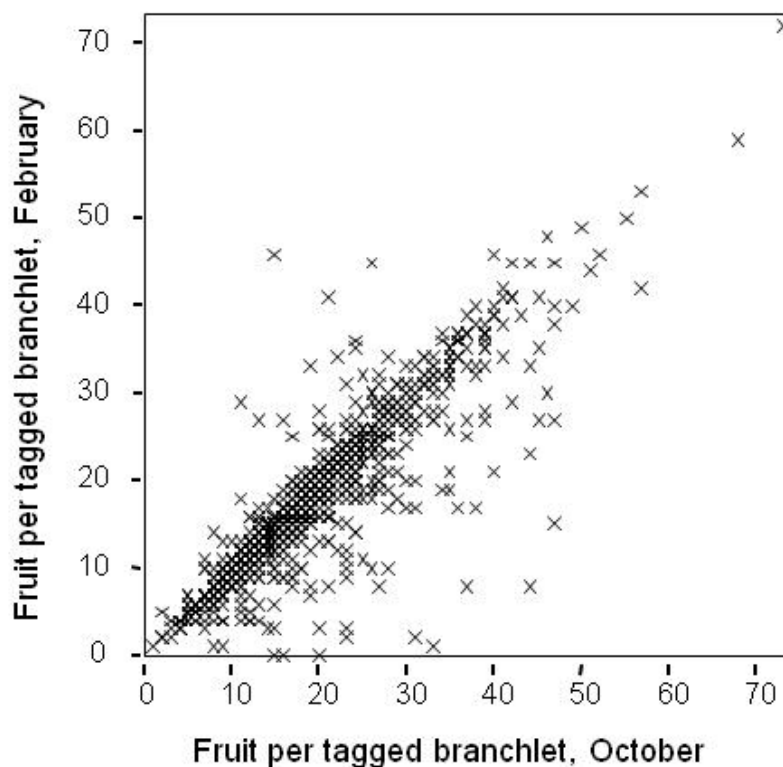
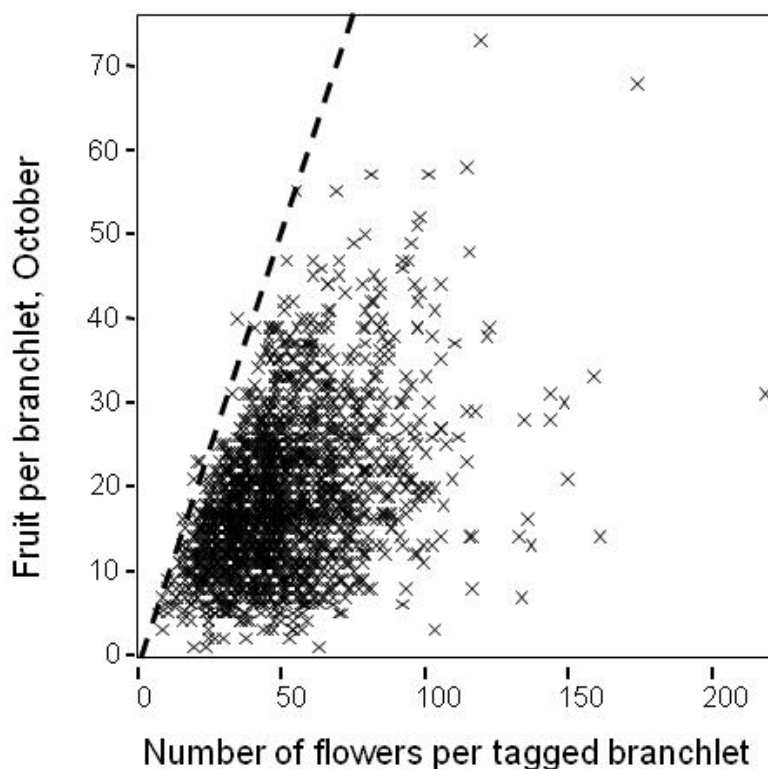***Figure 11:*** *The relationship between fruit count in October and fruit count in February.*

9 Effect of branchlet size and flower density on fruiting

9.1 Introduction

The focus of the experiments has been the effect of pollination on the conversion of flowers to fruit. We have shown a number of strong effects linked to the arrangement of hives. In addition to the pollination driven effects it is expected that fruit set will also be influenced by other factors, including the density of flowers on branchlets and the size of branchlets, because this influences the potential for the plant to resource developing fruit, and then to hold the weight of fruit as they expand. These kinds of constraint are expected to be part of the focus of future research, but here we report some preliminary data.

9.2 Materials and Methods

In 2013 we measured the length and diameter of branchlets selected for the flower and fruit counts. The trees examined here are the same sample as for the "distance from hives" and "hive density" experiments described above.

Figure 14: *The relationship between number of flowers per tagged branchlet and the number of fruit on that branchlet in October. The dotted line marks the upper limit, assuming that fruit number should not exceed flower number.*

9.3 Results and Discussion

As expected there is a general relationship between the number of flowers per branchlet and the fruit per branchlet (Fig. 14). But this relationship is weak overall, and determined largely by the obvious constraint that flower number sets the upper limit for the number of fruit (dotted line, Fig. 14). If instead you examine flower to fruit conversion as a function of the flower density, you do see a pattern that is more informative (Fig. 15). The overall relationship is negative, meaning that the higher flower to fruit conversion rate occurs when the density of flowers (number per cm) is low. Conversely, when flowers per cm exceed about 1, conversion ratios are restricted to lower numbers (Fig. 15). This result is consistent with a resource or architectural constraint limiting the number of fruit that can be carried per cm of branchlet.
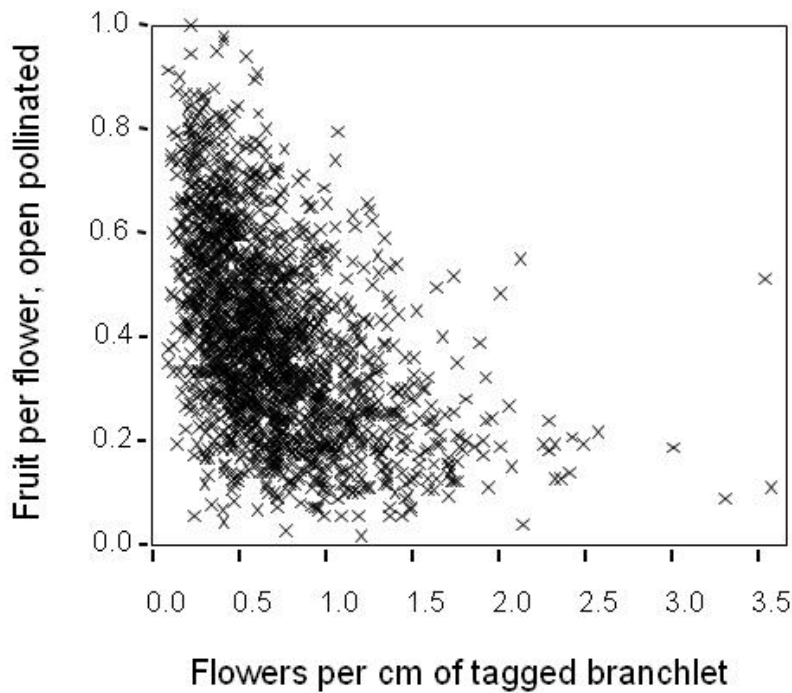
Figure 15: The relationship between number of flowers per centimetre of tagged branchlet and the fruit per flower ratio (under open pollination).

We also considered the possibility that branches with a greater cross-sectional diameter might be able to support more fruit, and therefore support a better conversion ratio of fruit to flowers. But the data indicate that there is no relationship between branchlet diameter and flower to fruit conversion (Fig. 16). It may be that any potential advantage of larger branches for support is outweigh in the data set because larger diameter branches are also older and therefore perhaps more shaded or showing other declining function. This preliminary exploration indicates the architectural and resource constraints on fruiting have some influence, which needs to be understood if we are to aim to further increase productivity.
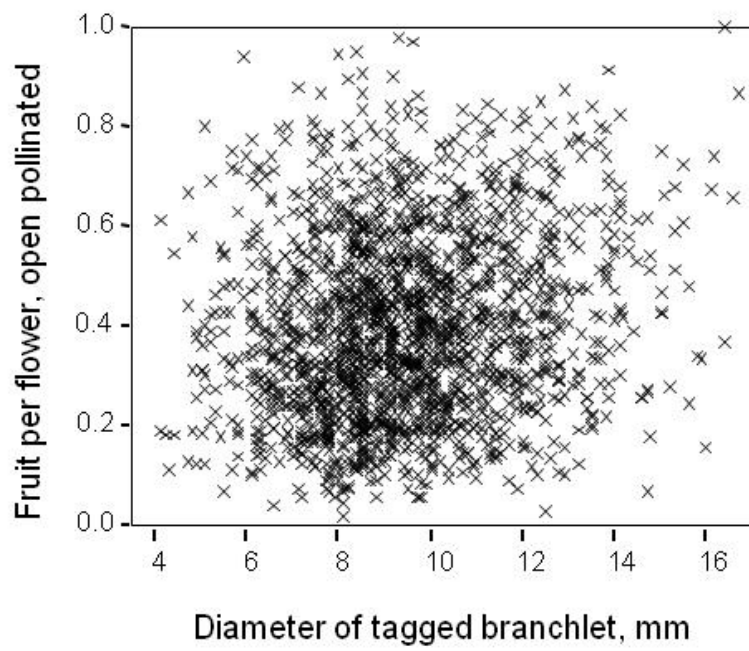
Figure 16: *The relationship between the diameter the tagged branchlets and the fruit per flower ratio (under open pollination).*

10   Technology Transfer

Dr Cunningham has communicated this research activity consistently over the life of the project. Most of these communications were specifically focused on almond pollination. Some others were on the larger issue of the importance of bees to pollination of horticultural crops. The following list highlights some of these communications

2011
- March : "Honeybee decline warrants concern, but not panic" The Conversation (on-line journal)
- October: Oral presentation at the annual almond industry conference

2012
- Briefing note to "In a Nutshell", the almond industry magazine
- June "Getting the best from our bees" article, "Australian Nutgrower" magazine
- September: Presentation in Australian Nut Industry research forum, Brisbane,
- October: Oral presentation at the annual almond industry conference

2013
- October: Oral presentation at the annual almond industry conference
- June: Oral presentation at the Almond industry R & D update workshop, Renmark

2014
- January: 20 minute interview on ABC Local, James O'Loghlin's National show. The topic was bees, crop pollination and bee health
- May: 20 minute interview on Radio 3RRR Melbourne. The topic was bees, crop pollination and bee health
- May: June: 10 minute interview on Radio Adelaide. The topic was bees, crop pollination and bee health
- May: "Explainer: Varroa mite, the tiny killer threatening Australia's bees" The Conversation (on-line journal)
- June: Dr Cunningham presented at the "ACARI" workshop in Mildura, speaking on the potential for the Almond Industry to adapt to a pollinators shortage should *Varroa destructor* establish in Australia. The draft report on Varroa contingency planning from Plant Health Australia draws strongly on the almond pollination research (i.e. this project) that has been communicated orally and in written milestone reports to HAL

Because the project has only recently drawn to a close a number of the important communications are still ahead of us. The following two are already scheduled for 2014.

- June: Oral presentation at the Almond industry R & D update workshop, Renmark
- August: Dr Cunningham will present the almond research at the International Horticultural Congress, in Brisbane

Further to this, a number of research publications for the scientific literature are now in preparation for submission to journals. Dr Cunningham will also, with the assistance of the Almond Board, develop some short communications (printed and on-line) that will communicate the key messages of the research project to almond growers and providers of pollination services.

11   Recommendations

The experiments described in this report provide a solid evidence basis for improving pollination outcomes for almond orchards. We have shown that bee activity on trees far from hives does decline, considering distances from hives that are common in orchards under normal management. Although this effect is strong, it is not easy to detect by direct observation of bee activity, but requires an assessment of pollen removal rates. Our experiments show that this decline in bee activity is associated with a decline in fruit set over a similar distance gradient, indicating a pollination deficit far from hives. We also show that fruit set is higher on average when smaller numbers of hive per placement are used, and higher hive per hectare densities are maintained. These specific results have been described in detail in each relevant section.

There had been concern that fruit set counted in October may not reflect fruit set at harvest, but our experiment shows that this concern is unfounded. Very few fruit are lost between October and harvest (February), so that maximising fruit set in October is clearly the foundation for a good harvest. Our data do suggest that there are limits to how many fruit a tree might support per unit length of branch, so that trees supporting very high flower density do not to convert all flowers to fruit. But the relationships between numbers of flower, fruits, and branch dimensions show a lot of variation within the bounds set by physical constraints. Our experiments suggest that much of the fruit set variation is related to variable pollination outcomes. Further work is required to establish the degree to which increased yield for almond trees is ultimately limited by resources available for flower and fruit, and this work should be the focus of future research into the potential to raise productivity.

The fact that we detected significant effects on fruit set linked to distance from hive and different hive arrangements indicates that there is significant scope for growers and pollination providers to find strategies for improved pollination outcomes. Ultimately, assessment of these hive placement strategies will depend on both the benefits in fruit set, which we have described here, and the cost of implementation, which must be established between the grower and the pollination provider. The consistent evidence for cross pollination as a constraint on fruit set also suggests that other strategies to raise supply of cross pollen could improve yield (e.g. mechanical pollination, alternative orchard design, hive modifications) but our experiment with enpollination felt indicate that this particular technique requires more research before it could be recommended.

With this background, we make the following recommendations:

(1) Growers and pollination providers should be made aware that hive densities of 6.7 hph provide better fruit set than lower hph, but that the way hives are arranged also has strong and important impacts on fruit set.

(2) Growers and pollination providers should examine their pollination strategies with the knowledge that for any given hive density, better fruit set is achieved when hives are in smaller placements (i.e. less than 100 hives) and spread around the orchard so that most trees are not more than a few hundred meters from hive placements.

(3) Growers and pollination providers can prepare for hive shortages (such as may occur if Varroa mite establishes) by understanding that changes to the hive arrangement strategy can be made to maintain good pollination outcomes even when using fewer hives

(4) Growers and pollination providers should determine the cost of different deployment strategies to ensure that the most profitable balance between costs (i.e. provision of hives) and benefits (i.e. good fruit set) can be achieved.

(5) Because this research project was only able to examine a limited range of scenarios, further research is required if to determine the optimal hive arrangement strategy,

(6) Further research is required to determine the extent to which resource limitation constrains the benefits from better pollination outcomes. This research should examine increased pollination over larger parts of whole trees, and consider the extent to which position in the canopy influences fruit development. It should also examine the extent to increasing the quantity of nuts could come at the expense of size. These considerations are important in determining the potential productivity increase.

(7) Other approaches to increasing the rate of cross pollination (i.e. further two optimising hive arrangement) would also support high fruit set, however no other methods are yet proven and ready to be implemented. Enpollination felt and mechanically blowing pollen on to flowers both have potential to increase the frequency of pollination events. Further research is required to determine if these strategies are likely to deliver cost effective improvements.