

Final Report

Increasing yield and quality in tropical horticulture with better pollination, fruit retention and nutrient distribution

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Project:

Increasing yield and quality in tropical horticulture with better pollination, fruit retention and nutrient distribution (PH16001)

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Public summary

This project aimed to increase the productivity and competitiveness of Australia's horticultural industries by optimising crop pollination efficiency. A key objective was to increase yield and quality through better understanding of cross-pollination effects on fruit and nut quality.

The project involved 20 experiments on 15 avocado, macadamia, strawberry, mango, almond and lychee farms. These determined the effect of distance to another cultivar on the paternity and quality of avocado, macadamia, strawberry, mango and lychee fruit. They also determined the levels of self-paternity and cross-paternity in almond cultivars. Experiments investigated crop-nutrition effects on yield, size, quality and paternity of avocado, macadamia and strawberry fruit. A pioneering experiment determined whether whole-tree macadamia yields could be increased by cross-pollination. Experiments were coupled with assessments of hyperspectral scanners for predicting nutrient concentrations in avocado, macadamia, strawberry, mango and lychee plants during pollination and fruit development. Further experiments assessed hyperspectral scanners for predicting the time-to-ripeness of avocado fruit and predicting quality attributes of strawberry, mango and lychee fruit.

Avocado growers may consider inter-planting pollinisers such as Shepard more closely with Hass trees. Cross-pollination declined with increasing distance from polliniser trees, limiting the opportunities for fruit production. Cross-pollinated fruit were often larger than self-pollinated fruit. Avocado processors could test hyperspectral scanners as they predicted time-to-ripeness up to 2 weeks away with an error of only 1 day.

Macadamia growers might need to inter-plant different cultivars more closely, as macadamia was heavily reliant on cross-pollination. Self-pollination provided less than 0.2 tons of nut-in-shell per hectare. Self-pollinated nuts had lower kernel recovery than cross-pollinated nuts. The presence of self-pollinated nuts reduces the prices paid for nut consignments.

Strawberry growers might introduce more beehives onto farms, as fruit size was strongly determined by the number of filled seeds per fruit. Small fruit have become a problem during late parts of the subtropical strawberry season, likely because: (a) low numbers of stigmas are produced on late-season flowers; or (b) inadequate pollen is deposited on stigmas of late-season flowers.

Mango fruit were produced by a mixture of self-pollination and cross-pollination. Mango cross-pollen was sometimes only transported effectively across three or four orchard rows. Hyperspectral imaging showed excellent capacity for estimating mango fruit quality. This technology has potential for use by growers, processors and retailers in optimising the quality of marketed fruit.

Almond growers may consider planting new self-fertile cultivars, because most of their nuts were produced by self-pollination rather than cross-pollination. These cultivars might sustain nut production in the face of uncertain beehive supply, although further research is required to understand whether self-fertile cultivars require bees to deposit the self-pollen.

Lychee fruit were produced by a mixture of self-pollination and cross-pollination. Cross-pollinated fruit were often larger or redder than self-pollinated fruit. Hyperspectral scanning predicted brix and acidity of lychee fruit using images of either the skin or flesh.

The project produced a podcast, video, factsheet, poster and 13 newsletter articles for growers, as well as 17 scientific papers. The podcast, video and factsheet are available to macadamia growers on the AMS Resources website. The 13 newsletter articles were published in *Talking Avocados*, *Australian Macadamia Society News Bulletin*, *Australian Berry Journal*, *Mango Matters*, *In A Nutshell*, *Australian Nutgrower* and *Living Lychee*.

Keywords

almond; avocado; lychee; macadamia; mango; pollination; pollinator; self-incompatibility; self-sterility; strawberry.

Introduction

This project aimed to increase the productivity and global competitiveness of Australia's horticultural industries by optimising crop pollination efficiency. Key objectives were to increase farm-gate profitability by increasing yield and quality through better understanding of crop nutrition during pollination and better understanding of the effects of cross pollination on fruit and nut quality.

Cross-pollination, compared with self-pollination, was known previously to improve fruitlet retention, nutrient accumulation and fruit yield in many temperate crops. There was also emerging evidence that cross pollination has major impacts on fruit and nut quality and on the health benefits of fruit and nuts for consumers. For example, cross pollination could result in higher nutritional value and longer shelf life of strawberries and could increase the proportion of healthy oils in almond kernels. It was less clear to what extent improved pollination could increase productivity, profitability and product nutritional quality in many tropical crops. Furthermore, there were no previous studies that demonstrated conclusively whether or not whole-tree yields were pollen-limited in mass-flowering tree crops such as avocado and macadamia.

The project built upon previous research by Plant & Food Research that showed how boron and carbohydrate concentrations in avocado flowers were related positively to fruit set. Limitations of previous work were that nutrient measurements were destructive snapshots of flower nutrient profiles because there had been no methods available for real-time monitoring of nutrient accumulation at key points during flower and fruitlet development. The project also had linkages with a project led by Griffith University and funded by the Australian Centre for International Agricultural Research. That project helped to develop a canarium nut processing industry in Papua New Guinea, including developing hyperspectral imaging methods for real-time assessment of the oil composition and shelf-life of canarium kernels. The current project, therefore, aimed to develop hyperspectral imaging tools to quantify nutrient concentrations in real time, which would allow fertiliser applications in a more-timely fashion to improve pollination success and increase fruit and nut yield. The project also built on previous Hort Innovation research (MC302 and MC98027) that identified lower yields of macadamia trees in the middle, compared with at the edge, of single-cultivar blocks. Those results suggested that yields were constrained by limited dispersal of cross-pollen into the middle of wide blocks of a single cultivar. The current project aimed to take one step further by determining clearly whether whole-plant yields were limited by the amount of cross-pollen received by plants.

The focus crops of the project were avocado, macadamia, strawberry, mango, almond and lychee. Research was conducted on 15 commercial farms in three states: Queensland, Victoria and South Australia.

The project aimed to support capacity building in Australian horticulture by developing international collaborations among pollination and horticultural science groups in Australia, New Zealand and Germany. Key research partners were Griffith University, University of the Sunshine Coast, Plant and Food Research, and Technische Universität München. The project also aimed to support capacity building by training postgraduate students in the Australian horticulture sector. Five students were associated with the project, investigating pollination biology and crop nutrition of avocado, macadamia, strawberry, mango and lychee.

The project also aimed to produce a series of newsletter articles, workshops and presentations for growers and beekeepers, as well as scientific papers. These described (a) the impact of cross pollination on yield, quality and nutrient composition of fruit and nuts, (b) crop-nutrient recommendations for farms, and (c) hyperspectral scanner methods for assessing crop nutrition during flowering and fruit development. One podcast, two videos, one factsheet, two posters, 13 newsletter articles, 18 workshops and presentations, and 17 scientific papers were produced by the project team.

Methodology

The project involved the establishment of 20 pollination and crop-nutrition experiments on 15 avocado, macadamia, strawberry, mango, almond and lychee farms in Queensland, Victoria and South Australia. Three experiments on strawberry plants were also conducted in a research glasshouse facility at the University of the Sunshine Coast in Queensland. The methodology was advised, formulated and modified by the Project Steering Committee, comprised of representatives from each of the contributing organisations including Hort Innovation. The experiments were scheduled according to an Annual Operating Plan. The project produced 30

newsletter articles, podcasts, videos, factsheets, workshops and presentations specifically for growers and beekeepers. The project activities, outputs and anticipated outcomes were guided by the Monitoring & Evaluation Plan for the project.

An overview of methods for each of the 20 experiments is provided here. Detailed descriptions of the methods are provided in Appendix 1. Examples of project outputs, such as newsletter articles for growers, are provided in Appendix 2. Examples of project impacts, such as changes to grower practice, are provided in Appendix 3.

Avocado experiment 1: Avocado pollen parentage and fruit quality

This experiment determined the effect of distance to another cultivar on the paternity and quality of avocado fruit. Hass fruit were harvested from transects in orchards at Goodwood and North Isis, near Childers, Queensland. Each transect consisted of three or four trees: one row, two rows, three rows and (at Goodwood) ten rows from another cultivar. Fruit were harvested from each tree, and each fruit and seed was weighed. We determined fatty acid composition and mineral nutrient concentrations of the flesh. Each seed was used for paternity analysis through genotyping, to identify how many fruit were cross-pollinated and how many fruit were self-pollinated at different distances to another cultivar. Fruit size, fatty acid composition and nutrient concentrations were compared between cross-pollinated and self-pollinated fruit.

Avocado experiment 2: Avocado boron nutrition, pollen parentage, fruit quality and yield

This experiment determined the effect of boron crop-nutrition on yield, size, quality and paternity of avocado fruit. Hass trees at Eastridge avocado orchard, near Childers, were assigned to one of three levels of boron, applied prior to flowering. Insect visitors to flowers were surveyed, and fruit set was monitored. A sample of flowers was collected from each tree during peak flowering, and leaf samples were collected from flowering to final fruit set. All fruit were harvested and counted at maturity. Sixteen fruit per tree were sampled and weighed collectively, and tree yield was calculated. Fruit were later weighed individually, dissected, and the seed weighed. The flesh was used to assess fatty acid composition and mineral nutrient concentrations. Flower and leaf samples were used to measure mineral nutrient concentrations. Seeds were genotyped to assign paternity and determine how many fruit were cross-pollinated and how many fruit were self-pollinated.

Avocado experiment 3: Hyperspectral modelling of avocado carbohydrate levels

This experiment developed hyperspectral methods to predict foliar carbohydrate concentrations, which can be related to pollination success and fruitlet retention in avocado trees. Hass leaves were collected in orchards at Eastridge and Goodwood. Leaf samples were used to obtain hyperspectral images and to measure carbohydrate concentrations. Models were tested for predicting concentrations of starch, sucrose, glucose, fructose, myoinositol, perseitol and mannoheptulose.

Avocado experiment 4: Hyperspectral modelling of mineral-nutrient levels in avocado leaves

Leaves from *Avocado experiment 2* (above) were used to develop hyperspectral models of foliar mineral-nutrient levels. This would allow rapid prediction of crop nutrition during flowering and fruit development. Images of both the abaxial (bottom) and adaxial (top) leaf surfaces were captured using a hyperspectral imaging system. We tested models for predicting foliar nitrogen, phosphorus, potassium, aluminium, boron, calcium, copper, iron, magnesium, manganese, sodium, sulphur and zinc concentrations.

Avocado experiment 5: Hyperspectral modelling of avocado ripening

We analysed hyperspectral images of 316 Hass and 160 Shepard fruit to determine whether the images could be used to predict time-to-ripening. Hass fruit were stored for 8 days at 4°C and Shepard fruit for 7 days at 7°C before imaging. Fruit were then moved to room temperature to allow the onset of ripening. We captured an image of the skin daily until the fruit reached full ripeness. We then developed models to predict the time-to-full-ripeness of each avocado fruit.

Avocado experiment 6: On-farm hyperspectral prediction of avocado crop nutrition

This experiment assessed the potential for on-farm hyperspectral imaging to predict mineral nutrient concentrations in avocado leaves. The experiment was conducted at Eastridge avocado orchard, near Childers. Hass trees were imaged using a handheld hyperspectral imager on four occasions from flowering to fruit maturity. Leaves were also transferred to the laboratory, imaged again under laboratory conditions, dried, and then used for nutrient analysis. Spectral data from the hyperspectral images was used to further develop models that predicted mineral nutrient concentrations in avocado leaves.

Macadamia experiment 1: Macadamia pollen parentage and nut quality

This experiment determined whether large nuts and small nuts differ in their paternity, which would indicate that variation in nut size is caused by having a mixture of cross-pollinated and self-pollinated nuts in orchards. Nuts were collected from orchards at Alloway and Sandy Creek, near Bundaberg, Queensland. Nuts were collected from cultivars 816, A4 and Daddow. Nuts from each harvest were sorted by size, to allow comparison of the smallest nuts and the ten largest nuts. Nut-in-shell (NIS) mass was recorded for each nut before it was cracked and its kernel mass was recorded. Kernels were crushed and separated into three subsamples for: (1) genotyping; (2) fatty acid analysis; and (3) mineral nutrient analysis. The percentages of large nuts and small nuts on each tree that arose from cross-pollination and self-pollination were calculated.

Macadamia experiment 2: Macadamia boron nutrition, pollen parentage, fruit quality and yield

This experiment determined the effect of boron crop-nutrition on yield, size, quality and paternity of macadamia nuts. Cultivar 816 trees in the Alloway orchard were assigned to one of three boron treatments, applied prior to flowering. Insect visitors to flowers were surveyed, and fruit set was monitored. A sample of flowers was collected from each tree during peak flowering, and leaf samples were collected from flowering to final fruit set. All fruit were harvested and weighed. A subsample of fruit from each harvest was weighed, dehusked, and dried. Mature nuts from each tree were then subsampled. Nut-in-shell (NIS) mass was recorded for each nut. Each nut was cracked, and its kernel mass recorded. Fruitlets and mature kernels were used for genotyping and assessment of mineral nutrient concentrations. A subsample of each mature kernel was used to determine oil concentration. Flower and leaf samples were also analysed for mineral nutrient concentrations. Yield of each tree was calculated, as were the levels of cross-paternity and self-paternity.

Macadamia experiment 3: Cross-pollination of whole macadamia trees

This experiment determined whether whole-tree yields of macadamia are limited by the amount of cross-pollination. Trees of cultivars 816 and Daddow were selected in the Sandy Creek orchard. Twenty trees of each cultivar were selected in the first row adjacent to the other cultivar, and twenty trees of each cultivar were selected in the twenty-fifth row away from the other cultivar. We supplementary cross-pollinated racemes on ten trees in each row. Racemes on other trees received no supplementary pollination. We pollinated approximately 60% of the racemes on each tree. We surveyed insects that contacted flowers. We harvested and weighed all fruit under each tree from February to June. A subsample of the fruit from each harvest was weighed, dehusked, and dried. Twenty nuts from each tree were used to assess nut quality and paternity. Each nut and kernel was weighed. A subsample of each kernel was extracted for genotyping, and the levels of cross-paternity and self-paternity were calculated for each tree. The yield of each tree was calculated.

Macadamia experiment 4: Hyperspectral modelling of macadamia carbohydrate levels

This experiment developed hyperspectral methods to predict carbohydrate concentrations in macadamia trees, as these concentrations can be related to fruitlet retention. We collected 244 Daddow leaves from the Alloway orchard. We imaged the samples using a hyperspectral scanner and analysed their non-structural carbohydrate and soluble sugar concentrations. Models were developed to predict the concentrations of starch, sucrose, glucose and fructose.

Macadamia experiment 5: Hyperspectral modelling of macadamia foliar-nutrient levels

This experiment evaluated the capacity of hyperspectral imaging to predict mineral-nutrient concentrations in macadamia leaves. The experiment compared the accuracy in predicting nutrient concentrations between images of the top and the bottom surfaces. We targeted nutrient assessments around the periods of pollination, premature fruit drop, and nut maturity. We selected thirty cultivar 816 trees at Alloway and collected leaves from flowering to final fruit set. We captured two images from each sample: (1) the top surface; and (2) the bottom surface. We used a subsample of the leaves at each sampling time to analyse mineral nutrient concentrations. Models were developed to predict nitrogen, phosphorus, potassium, calcium, copper, manganese, sulphur and zinc in macadamia leaves.

Strawberry experiment 1: Pollination effects on strawberry fruit quality

This experiment compared the effects of cross-pollination versus self-pollination on strawberry fruit quality. Plants of three cultivars, Redlands Joy, Sugar Baby and Ruby Gem, were raised on the Sunshine Coast,

Queensland. Redlands Joy plants were arranged into four treatments: (1) autogamous pollination; (2) self-pollination; (3) cross-pollination with Sugar Baby; and (4) cross-pollination with Ruby Gem. All flowers on each plant were pollinated until the last flower opened, and all mature fruit were sampled. We measured the length, diameter, mass, colour and firmness of each fruit. Each fruit was cut vertically into two halves. The number of seeds was counted on the first half, which was then used to measure mineral nutrient concentrations. The other half was used to measure total soluble solid concentration (TSS), acidity, and sugar concentrations. Two fruit from each plant were stored at 4°C to determine shelf life. We calculated plant yield, and assessed the effects of cross-pollination versus self-pollination on fruit quality.

Strawberry experiment 2: Nutrient partitioning and quality of self- and cross-pollinated strawberry fruits under varying calcium-nutrition levels

This experiment assessed differences in nutrient accumulation and quality between fruit arising from self-pollination and cross-pollination on the same plant. The plants were provided with six sprays of calcium at (a) 1, (b) 2 or (c) 4 kg elemental Ca ha⁻¹ spray⁻¹. Successive flowers on each plant were either self-pollinated with Redlands Joy pollen or cross-pollinated with Ruby Gem pollen, creating a mixture of self-pollinated and cross-pollinated flowers on each plant. A subsample of fruitlets from ten of the 20 replicate plants per treatment was harvested every 7 d after the last pollination for analysis of nutrient concentrations. The length, diameter, mass and colour of each fruitlet was recorded. All fruit from the remaining ten replicate plants per treatment were harvested at fruit maturity. We measured length, diameter, mass, colour, firmness, seed number, mineral nutrient concentrations, TSS, acidity, sugar concentrations and shelf life of mature fruit.

Strawberry experiment 3: Levels of self- and cross-pollination on Australian strawberry farms

This experiment assessed the levels of self-pollination and cross-pollination, and variations in fruit quality, at increasing distances from inter-cultivar boundaries on a strawberry farm. The site was Strawberry Fields at Palmview, Queensland. The cultivars were Red Rhapsody and Sundrench. Insect visitors were surveyed during flowering. A total of 48 experimental plants was located along six transects. Each transect consisted of eight plants, with one plant at each of one row, three rows, 10 rows and 20 rows from the other cultivar. Mature fruit were sampled approximately 4 weeks after counting flower visitors. We measured length, diameter, mass, colour, firmness, TSS, acidity and sugar concentrations of each fruit. The number of filled and unfilled seeds was counted on each fruit. Ten filled seeds were removed from each fruit for genotyping. We analysed the paternity of 1403 Red Rhapsody seeds from 143 fruit and 1432 Sundrench seeds from 144 fruit and we calculated the percentages of filled seeds on each fruit that arose from self-pollination and cross-pollination.

Strawberry experiment 4: Hyperspectral imaging for estimating leaf, flower and fruit macronutrient concentrations and predicting yield

This experiment tested how well hyperspectral imaging could estimate macronutrient concentrations in strawberry plant parts and predict fruit yield. We used samples from 100 Redlands Joy plants. We collected leaf, flower, unripe fruitlets and ripe fruit throughout the flowering and fruiting periods. Fresh flowers and leaves were imaged, dried, and used for nutrient analysis. Unripe fruitlets and ripe fruit were imaged, stored fresh at -20°C, and then also used for nutrient analysis. The total number of ripe fruit samples was 620. Sixty composite samples, each of three unripe fruitlets, were used at each of 7, 14 and 21 d after pollination. Three hundred leaves and 120 flowers were used. We predicted fruit yield and fruit mass using hyperspectral images or macronutrient concentrations. Relationships were tested between yield or mass and leaf hyperspectra, or between yield or mass and leaf nitrogen, phosphorus, potassium or calcium concentrations.

Mango experiment 1: Levels of self- and cross-pollination in an Australian mango orchard

This experiment assessed the levels of self-pollination and cross-pollination, and variations in fruit quality, at increasing distances from an inter-cultivar boundary in a mango orchard at Goodwood, Queensland. The cultivars were Kensington Pride and Calypso. Each of the six transects per cultivar consisted of four trees: one row, two rows and three rows from another cultivar and in the middle row of the block (eight rows for Kensington Pride, and seven rows for Calypso). Insect visitors were surveyed during flowering. Ten mature fruit per tree were harvested and weighed, and their length and diameter were recorded. Fruit colour was assessed when the fruit became ripe. A total of 213 Kensington Pride seeds and 235 Calypso seeds was dissected and the embryos were genotyped to determine the percentages of cross-pollinated and self-pollinated fruit on each tree and to assess the effects of cross-pollination on fruit quality.

Mango experiment 2: Hyperspectral imaging for estimating mango fruit quality

This experiment tested how well hyperspectral imaging could estimate the quality and mineral nutrient status

of mango fruit. The skin and flesh of each ripe fruit from *Mango experiment 1* (above) were imaged using a hyperspectral camera. Spectral data was used to develop models that non-destructively predict TSS, acidity and mineral nutrient concentrations.

Almond experiment 1: Levels of self- and cross-pollination in Australian almond orchards

This experiment assessed the levels of self-pollination and cross-pollination, and related variations in nut quality, in almond orchards at Lindsay Point, Victoria, and Amaroo, South Australia. Insect visitors were surveyed during flowering. Mature fruit were sampled from six trees of each study cultivar. The potentially self-fertile cultivars, Carina, Capella, Mira, Maxima, Rhea and Vela, and the older commercial cultivars, Peerless and Nonpareil, were sampled at Lindsay Point. Cultivars Carmel, Nonpareil, Monterey and Price were sampled at Amaroo. The fruit were hulled, and nut-in-shell mass, nut diameter and nut length were recorded for each fruit. Each nut was shelled and its kernel mass, kernel diameter and kernel length recorded. Each kernel was crushed and subsamples were taken for: (1) fatty acid analysis; and (2) genotyping. We calculated the percentages of fruit on each tree that arose from self-pollination and cross-pollination, and we assessed the effects of different pollen parents on nut size and nut quality.

Lychee experiment 1: Levels of self- and cross-pollination in an Australian lychee orchard

This experiment assessed the levels of self-pollination and cross-pollination, and variations in fruit quality, at increasing distances from an inter-cultivar boundary in a lychee orchard at Welcome Creek, near Bundaberg, Queensland. The cultivars were Kaimana and Kwai Mai Pink, which were separated by a block of Fay Zee Siu trees. Each of the six transects per cultivar consisted of four trees: (a) one row, three rows, five rows and seven rows (i.e. the middle row) into the Kaimana block from the Fay Zee Siu block; and (b) one row, three rows, six rows and ten rows (i.e. the middle row) into the Kwai Mai Pink block from the Fay Zee Siu block. Ten mature fruit per tree were harvested and their skin colour, total mass, seed mass and skin mass were recorded. The seeds were genotyped to determine the percentages of self-pollinated and cross-pollinated fruit on each tree and to assess the effects of cross-pollination on fruit quality.

Lychee experiment 2: Hyperspectral imaging for estimating lychee fruit quality

This experiment tested how well hyperspectral imaging could estimate the quality and mineral nutrient status of lychee fruit. The fruit were sampled from *Lychee experiment 1* (above). We captured an image of the skin and flesh of each fruit using a hyperspectral imager. Spectral data was used to develop models that non-destructively predict TSS, acidity and mineral nutrient concentrations.

Results and discussion

Avocado experiment 1: Avocado pollen parentage and fruit quality

Trees in the middle of wide Hass blocks were not receiving as much Shepard pollen as Hass trees that were closer to Shepard trees, as the percentage of cross-pollinated Hass fruit decreased from 63% to 25% with increasing distance from a cross-pollen source. This limits the opportunities for effective Hass pollination, as it limits the period each day that female-phase Hass flowers are open at the same time as nearby male-phase flowers. Self-pollinated Hass fruit in this experiment did not differ significantly in fruit mass, flesh mass or seed mass from Hass fruit that were cross-pollinated by Shepard. However, self-pollinated fruit had 9% lower calcium concentration than cross-pollinated fruit, which could make them more prone to fruit disorders and give them a shorter shelf life. Self- and cross-pollinated fruit did not differ significantly in the relative contributions of palmitic, palmitoleic, stearic, oleic, elaidic or linoleic acid to their fatty acid composition.

Avocado experiment 2: Avocado boron nutrition, pollen parentage, fruit quality and yield

Boron applications affected the yield and size of Hass avocado fruit, without affecting fruit paternity. The main foragers on avocado flowers near Childers were honey bees and, to a lesser extent, stingless bees and syrphid flies. Boron concentrations were elevated in the flowers of trees that had been treated with the highest boron concentration of 2 g/L prior to flowering. Leaf boron concentrations were not elevated during flowering, but they were greatly elevated by 6 and 10 weeks after flowering. Early fruit set did not differ significantly between boron treatments. However, fruit set at 10 weeks after peak anthesis was lower on trees that had received the highest boron concentration. This lower fruit set then translated into reduced yield on trees that received the highest boron concentration. However, the individual mass of mature fruit was higher on trees that were treated with boron than on trees that received no boron. Boron increased fruit diameter rather than length. Flower and leaf nutrient concentrations at peak flowering were not correlated significantly with yield, but very

high leaf boron concentrations at 6, 10 or 28 weeks after peak flowering were associated with reduced yield. Boron application did not affect the percentage of Hass fruit that arose from self-pollination versus cross-pollination. Cross-pollinated mature fruit were significantly heavier and had greater diameter than self-pollinated mature fruit, unlike in the previous experiment. Fruit diameter is one of the major parameters that affect the value of each avocado fruit because the fruit are packed into 5.5-kg cartons that usually contain either 14, 16, 18, 20, 23, 25, 28 or 30 fruit. An increase in fruit diameter can provide increased financial returns to growers because premiums are paid for the cartons that contain larger fruit.

Avocado experiment 3: Hyperspectral modelling of avocado carbohydrate levels

Hyperspectral imaging methods were developed that successfully predicted the concentrations of sucrose, glucose, fructose, myoinositol, perseitol and mannoheptulose in Hass leaves. Carbohydrate concentrations can be related to pollination success and fruitlet retention in avocado trees, and so these methods could be used to monitor carbohydrate concentrations in real-time.

Avocado experiment 4: Hyperspectral modelling of mineral-nutrient levels in avocado leaves

We also developed hyperspectral imaging methods that successfully predicted foliar nitrogen, phosphorus, potassium, aluminium, boron, calcium, copper, iron, magnesium, manganese, sodium and zinc concentrations in Hass leaves. These methods have the potential to provide real-time leaf nutrient analysis, without delays in obtaining nutrient results from a chemical laboratory.

Avocado experiment 5: Hyperspectral modelling of avocado ripening

We developed models that predicted the time-to-full-ripeness of Hass and Shepard fruit that were initially between 1 day and 14 days from being ripe. The prediction of ripening time was highly accurate, being between 0.9 and 1.5 days from the true value. There is great potential to apply hyperspectral imaging technology to reliably predict how long it takes for each avocado fruit to become fully ripe.

Avocado experiment 6: On-farm hyperspectral prediction of avocado crop nutrition

Hyperspectral imaging successfully predicted the foliar concentrations of phosphorus, potassium and calcium under field conditions. Imaging in the laboratory also successfully predicted the foliar concentrations of nitrogen, potassium and calcium. Prediction of foliar nutrient concentrations under field conditions provided 'good' predictions that might allow hyperspectral imaging to be developed as a rapid tool to fast-track fertiliser scheduling in avocado orchards. 'Excellent' prediction accuracy was possible with laboratory-based imaging.

Macadamia experiment 1: Macadamia pollen parentage and nut quality

This experiment aimed to determine whether large macadamia nuts are cross-pollinated and small macadamia nuts are self-pollinated. However, we found very few self-pollinated nuts. The levels of self-paternity were as low as 3%, 2% and 0% for cultivars Daddow, 816 and A4, respectively. Small nuts had higher kernel concentrations of most mineral nutrients. However, small nuts had consistently lower kernel potassium concentrations than large nuts, which suggests that potassium crop-nutrition might be important in determining nut size.

Macadamia experiment 2: Macadamia boron nutrition, pollen parentage, fruit quality and yield

The main foragers on macadamia flowers near Bundaberg were honey bees. A few stingless bees, syrphid flies and other insects were also observed. Boron concentrations were almost twice as high in the flowers of trees that had been treated with the higher boron concentration of 2 g/L prior to flowering than in trees that received no boron. Leaf boron concentrations were also elevated during flowering, and they were greatly elevated by 6 and 10 weeks after flowering. Initial fruit set at 3 weeks after peak flowering was higher on trees that received 1 g/L boron prior to flowering. However, fruit set at 6 and 10 weeks after peak flowering did not differ significantly among boron treatments. Nut-in-shell (NIS) yield, kernel yield, NIS mass, kernel mass, kernel recovery, kernel oil concentration, and the incidence of whole kernels also did not differ significantly among boron treatments. Almost all nuts arose from cross-pollination, with levels of self-paternity being only 2–3%. The large scale of this experiment meant that we had enough nuts to compare cross-pollinated and self-pollinated nuts. Cross-pollinated nuts (of cultivar 816) had significantly higher NIS mass and kernel mass than self-pollinated nuts except when the cross-pollen parent was 741. Cross-pollinated nuts had significantly higher kernel recovery than self-pollinated nuts when the pollen parent was cultivar A203 or A4 but not 741 or 842. These results provided a breakthrough in our understanding of the effects of cross-pollination on macadamia nut production. This was one of the first studies to demonstrate conclusively that macadamia nut size and quality are affected by cross-pollination. We also found that cross-pollination influences the mineral-nutrient

composition of macadamia nuts, greatly increasing boron levels and greatly decreasing aluminium levels. These effects may confer a slight health benefit on cross-pollinated nuts when compared with self-pollinated nuts. We also found that almost all fruitlets at 6 weeks after peak flowering either had an undeveloped embryo or were self-pollinated. However, many of the largest fruitlets at this stage were cross-pollinated. Almost all fruitlets remaining at 10 weeks after peak flowering were cross-pollinated. The fruitlets with undeveloped embryos or self-fertilized embryos abscised during the period of premature fruit drop, leaving almost only the fruitlets with cross-fertilized embryos. This highlights the need for bees to transfer cross-pollen between macadamia cultivars rather than simply self-pollen among flowers of the same cultivar.

Macadamia experiment 3: Cross-pollination of whole macadamia trees

Supplementary cross-pollination of whole macadamia trees increased NIS yield by 1.22 t/ha in the middle of an 816 block and by 0.62 t/ha in the 816 row next to a Daddow block. Supplementary cross-pollination increased NIS yield by 1.10 t/ha in the middle of a Daddow block and by 0.88 t/ha in the Daddow row next to an 816 block. These values represented increases in NIS yield of 97%, 29%, 40% and 29%, respectively. Most fruit arose from cross-pollination, as in previous macadamia experiments. Fruit of cultivar 816 that were fathered by Daddow had 28–33% higher NIS mass than self-pollinated 816 fruit. They also had 2.4–2.8% higher kernel recovery than self-pollinated fruit. Fruit of cultivar Daddow that were fathered by 816 did not have significantly higher NIS mass than self-pollinated Daddow fruit. However, they had 2.1–2.6% higher kernel recovery than self-pollinated fruit. This experiment demonstrated, for the first time, that pollen limitation can occur in a mass-flowering tree. The extent of pollen limitation depended on proximity to trees of another cultivar because macadamia flowers appeared heavily dependent on cross-pollination for the production of nuts. The mating system of macadamia was highly outcrossing, with 84–100% of nuts arising from cross-pollination even at 200 m (25 rows) from another cultivar. Improved pollination led to increased kernel yield of 0.31–0.59 tons per hectare, which equated to higher farm-gate income of approximately \$4,750–\$9,050 per hectare. The heavy reliance of macadamia flowers on cross-pollination and the strong pollen-parent effects on nut mass and kernel quality demonstrate the high value that pollination services can provide to horticultural food production.

Macadamia experiment 4: Hyperspectral modelling of macadamia carbohydrate levels

We developed hyperspectral imaging methods that successfully predicted the concentrations of starch, sucrose, glucose and fructose in macadamia leaves. Carbohydrate concentrations can be related to macadamia fruitlet retention during spring, and these methods may be used to monitor carbohydrate and sugar concentrations in real-time.

Macadamia experiment 5: Hyperspectral modelling of macadamia foliar-nutrient levels

Hyperspectral imaging also had the capacity to predict nitrogen, phosphorus, potassium, calcium, copper, manganese, sulphur and zinc concentrations in macadamia leaves. Prediction accuracies were generally higher using spectral data from the upper surface than from the bottom surface of leaves. Rapid estimation of crop nutrition could aid growers to increase orchard productivity and minimise fertilizer costs by allowing more-timely fertilizer management.

Strawberry experiment 1: Pollination effects on strawberry fruit quality

Redlands Joy strawberry plants were self-compatible, with yield not differing significantly between plants whose flowers had been self-pollinated versus cross-pollinated. Individual fruit mass, length, shape, firmness, and time to maturity did not differ significantly between self- and cross-pollinated fruit. However, cross-pollinated fruit were darker than self-pollinated fruit. Cross-pollinated fruit also had a higher °brix:acid ratio than self-pollinated fruit and would, therefore, taste sweeter than self-pollinated fruit. Hand self-pollinated and hand cross-pollinated fruit were also redder than fruit that arose from autogamous (i.e. unassisted) self-pollination. This suggests that pollination deficits, due to a shortfall in pollinator-mediated transfer of pollen from anthers to stigmas, might result in fruit that appear less red to consumers. Furthermore, pollination deficits appeared to reduce the firmness, shelf life and protein content of fruit. A pollination deficit was confirmed in the autogamy treatment, with autogamously self-pollinated flowers producing a lower percentage of filled seeds than fruit from hand-pollination treatments. Fruit mass, length and diameter were positively related to the number of filled seeds. These results suggested that the production of marketable strawberry fruit is heavily dependent on achieving a high number of successfully-pollinated stigmas on each flower.

Strawberry experiment 2: Nutrient partitioning and quality of self- and cross-pollinated strawberry fruits under varying calcium-nutrition levels

This experiment showed that cross-pollinated fruit were heavier and longer than self-pollinated fruit when both types of fruit were present on the same Redlands Joy strawberry plants. Cross-pollinated fruit were also wider, darker, redder, or faster to reach maturity than self-pollinated fruit, depending on the level of calcium nutrition. Cross-pollinated fruit were firmer than self-pollinated fruit at the highest calcium level, and they had longer shelf life than self-pollinated fruit at both the intermediate and highest calcium levels. Cross-pollinated and self-pollinated fruitlets occasionally differed in mass, length or diameter at either 1 or 2 weeks after first pollination. More than half of the fruit mass accumulation occurred during the final week (i.e. the fourth week) of fruit development, during which the fruit became darker and developed their red colour. Cross-pollinated and self-pollinated fruitlets differed little in the concentration or content of nitrogen, potassium, phosphorus, aluminium, boron, calcium, copper, iron, magnesium, manganese, sodium or zinc during the first 3 weeks after pollination. We had hypothesized that pollen-parent effects on fruit mass and fruit size were due to early differences in nutrient accumulation between cross-pollinated and self-pollinated fruitlets in the first 3 weeks of fruit development. Instead, growth differences between cross-pollinated and self-pollinated fruit occurred independently of nutrient accumulation, such that nutrient flow into the fruit followed water transport rather than regulated water transport. Our findings concur with current scientific understanding of nutrient transport processes through the xylem and phloem.

Strawberry experiment 3: Levels of self- and cross-pollination on Australian strawberry farms

We determined the paternity of 1403 seeds from 143 Red Rhapsody fruit and 1432 seeds from 144 Sundrench fruit at Strawberry Fields, Palmview, Queensland. Only 1–3% of Red Rhapsody fruit and 1–4% of Sundrench fruit showed evidence of some seeds being cross-pollinated, while all remaining fruit were fully self-pollinated. The percentages of fruit that were partly cross-pollinated did not differ significantly at 1, 3, 10 or 20 rows from a cross-pollen source. Firmness, number of filled seeds, and percentage of filled seeds did not differ significantly between partly cross-pollinated and fully self-pollinated fruit. Red Rhapsody fruit had 269 filled seeds per fruit, representing 96% of all seeds. Sundrench fruit had 262 filled seeds per fruit, representing 78% of all seeds. Fruit mass, length and diameter increased with the number of filled seeds per fruit in both cultivars. These results confirm that production of marketable strawberry fruit is heavily dependent on achieving a high number of successfully-pollinated stigmas on each flower. Reductions in fruit size during the latter parts of the strawberry production season may be caused by either: (a) a low number of stigmas produced on late-season flowers; or (b) inadequate pollen deposition on the stigmas of late-season flowers.

Strawberry experiment 4: Hyperspectral imaging for estimating leaf, flower and fruit macronutrient concentrations and predicting yield

Hyperspectral imaging showed great potential for estimating nitrogen, phosphorus, potassium and calcium concentrations in strawberry plants, with often-high prediction accuracies for leaves, flowers and unripe fruit, but not for ripe fruit. The technology may be used, in future, by strawberry growers to monitor crop nutrition in real time and manage fertilizer inputs during flowering and fruit growth.

Mango experiment 1: Levels of self- and cross-pollination in an Australian mango orchard

Two of the most common visitors to mango flowers at Goodwood, Queensland, were a rhiniid fly, *Stomorhina discolor*, and the small transverse ladybird beetle, *Coccinella transversalis*. The most common bees were stingless bees, Homalictus bees and honey bees. A total of 30% of Kensington Pride fruit were cross-pollinated and 70% were self-pollinated. A total of 32% of Calypso fruit were cross-pollinated and 68% were self-pollinated. Distance from a cross-pollen source did not affect the percentage of self-pollinated fruit in Kensington Pride. However, the percentage of cross-pollinated Calypso fruit decreased from 51% in the row next to a cross-pollen source to 16% at five rows from a cross-pollen source. Self-pollinated and cross-pollinated Kensington Pride fruit did not differ significantly in mass, length, diameter, mineral nutrient concentrations, or Brix:acid ratio. However, self-pollinated Kensington Pride fruit were less bright in colour than cross-pollinated fruit. Self-pollinated Calypso fruit had greater mass and diameter than cross-pollinated fruit. Self-pollinated and cross-pollinated Calypso fruit did not differ significantly in colour, mineral nutrient concentrations, or Brix:acid ratio. Our results show that Kensington Pride and Calypso flowers are self-fertile. We did not monitor yields across the orchard rows, but it was clear that mango cross-pollen was sometimes only transported effectively across three or four orchard rows. It remains possible that yields decline in the middle of wide single-cultivar mango blocks, as they sometimes do in macadamia and avocado orchards.

Mango experiment 2: Hyperspectral imaging for estimating mango fruit quality

Hyperspectral models developed using PLSR successfully predicted TSS (Brix) and the concentrations of calcium, boron and magnesium in mango flesh using either skin or flesh images. The models successfully

predicted acidity and Brix:acid ratio from flesh images only. Concentrations of phosphorus, potassium, iron, manganese, and sulphur were also predicted successfully from flesh images. Models developed using another method, SVMR, often had much higher prediction robustness than those using PLSR or ANN, when predicting TSS using either skin or flesh images. Hyperspectral imaging, particularly coupled with SVMR modeling, showed excellent capacity for estimating the quality of mango fruit. This technology has great potential for use by growers, processors, exporters and retailers in optimising the quality of mango fruit that are presented to consumers.

Almond experiment 1: Levels of self- and cross-pollination in Australian almond orchards

Nuts of the traditional almond cultivars, Carmel, Monterey, Nonpareil, Peerless and Price, resulted mainly from cross-pollination whereas 47–90% of nuts of the new self-compatible cultivars, Capella, Carina, Mira and Vela, resulted from self-pollination. Only 9–10 % of nuts from new cultivars bred for size (Maxima) and taste (Rhea) resulted from self-pollination. Cross-pollinated kernels of cultivar Mira had a slightly higher ratio of unsaturated to saturated fatty acids than did self-pollinated kernels. This result confirmed previous findings that cross-pollinated almond kernels might have slightly greater health benefits than self-pollinated kernels, although both types of almond kernels provide a healthy fatty acid profile.

Lychee experiment 1: Levels of self- and cross-pollination in an Australian lychee orchard

The levels of self-paternity and cross-paternity did not differ significantly with increasing distance from another cultivar in either the Kaimana or the Kwai Mai Pink block. Kaimana trees produced a mixture of self-fertilised and cross-fertilised fruit. At least 33% of Kaimana fruit were self-fertilised and at least 43% of Kaimana fruit were cross-fertilised. Kwai Mai Pink trees, in contrast, produced many more self-fertilised fruit than cross-fertilised fruit. At least 75% of Kwai Mai Pink fruit were self-fertilised and at least 19% of Kwai Mai Pink were cross-fertilised. The whole-fruit mass and flesh mass of Kaimana fruit were higher when they had been cross-pollinated by Souey Tung or Fay Zee Siu than when they were self-pollinated. Furthermore, seed mass was lower when they were cross-pollinated by Souey Tung than when they were cross-pollinated by Kwai Mai Pink or Fay Zee Siu. The whole-fruit mass, flesh mass and seed mass of Kwai Mai Pink did not differ significantly between fruit that were cross-pollinated by Wai Chee and fruit that were self-pollinated. Kaimana fruit that were cross-pollinated by Souey Tung had redder skin than self-pollinated fruit. Our results show that there is significant transfer of cross-pollen across multiple rows of lychee trees, but that many fruit still result from self-pollination. The self-pollinated fruit are often smaller and less red than cross-pollinated fruit. Further research is required to understand how to improve the rates of cross-pollination in lychee orchards.

Lychee experiment 2: Hyperspectral imaging for estimating lychee fruit quality

Hyperspectral models developed using PLSR successfully predicted TSS (Brix), acidity and sulphur concentration in lychee flesh using either skin or flesh images. Models were also developed that predicted calcium and manganese concentrations successfully, but only using flesh images.

Outputs

Table 1. Output summary

Output	Description	Detail
Grower article	The results are in: Most nuts come from cross-pollination. Stephen Trueman, Wiebke Kämper, Tarran Richards, Shahla Hosseini Bai, Steven Ogbourne, Joel Nichols, Helen Wallace. Macadamia growers: circulation 850.	Australian Macadamia Society News Bulletin. Winter 2019, Volume 47(2), 72–73.
Grower article	The latest results are in: Whole-tree yields can be increased with better cross-pollination. Stephen Trueman, Wiebke Kämper, Joel Nichols, Shahla Hosseini Bai, Steven Ogbourne, Helen Wallace. Macadamia growers: circulation 850.	Australian Macadamia Society News Bulletin. Spring 2019, Volume 47(3), 68–70.
Grower article	Cross-pollination or self-pollination – that was the question. Stephen Trueman, Wiebke Kämper, Tarran Richards, Steven Ogbourne, Joel Nichols, Helen Wallace, Shahla Hosseini Bai. Nut growers: circulation 1000.	Australian Nutgrower. Spring 2019, Volume 33(3), 19–21.
Grower article	The importance of macadamia pollination: an overview. Stephen Trueman, Helen Wallace. Macadamia growers: circulation 850.	Australian Macadamia Society News Bulletin. Winter 2020, Volume 48(2), 22–25.
Grower article	Better cross-pollination increases whole-tree yields. Stephen Trueman, Wiebke Kämper, Joel Nichols, Shahla Hosseini Bai, Steven Ogbourne, Helen Wallace. Nut growers: circulation 1000.	Australian Nutgrower. Spring 2020, Volume 34(3), 41–44.
Grower article	Stingless bees as pollinators for tropical fruits. Helen Wallace. Lychee growers: circulation 250.	Living Lychee. October 2020, Issue 83, 14–16.
Grower article	The proportion of self-pollinated Hass fruit increases at greater distance from another cultivar. Wiebke Kämper, Steven Ogbourne, David Hawkes, Stephen Trueman. Avocado growers: circulation 1400.	Talking Avocados. Autumn 2021, Volume 32(1), 65–67.
Grower article	Cross-pollination versus self-pollination: effects on nut size and kernel recovery. Stephen Trueman, Anushika De Silva, Wiebke Kämper, Joel Nichols, Shahla Hosseini Bai, Helen Wallace, David Hawkes, Trent Peters, Steven Ogbourne. Macadamia growers: circulation 850.	Australian Macadamia Society News Bulletin. Spring 2021, Volume 49(3), 44–45.
Grower	The advantages of self-fertile almonds. Wiebke Kämper, Grant Thorp, Michelle	In A Nutshell. Winter 2021, Volume 22(2), 18-21.

article	Wirthensohn, Stephen Trueman. Almond growers: circulation 1100.	
Grower article	Predicting the ripening time of Hass and Shepard avocado fruit using machine vision technology. Shahla Hosseini Bai, Wiebke Kämper, Helen Wallace, Stephen Trueman, Kourosh Khoshelham, Yifei Han. Avocado growers, processors and retailers: circulation 1400.	Talking Avocados. Spring 2021, Volume 32(3), 64-65.
Grower article	Levels of self-pollination & cross-pollination among fruit on a Queensland strawberry farm. Wiebke Kämper, Helen Wallace, Stephen Trueman, Cao Dinh Dung, Steven Ogbourne. Berry growers: circulation 2450.	Australian Berry Journal. Winter 2022, Edition 11, 78-81.
Grower article	Pollen parentage of nuts during premature nut drop: do self-pollinated nuts drop and cross-pollinated nuts remain? Stephen Trueman, Anushika De Silva, Wiebke Kämper, Joel Nichols, Shahla Hosseini Bai, Helen Wallace, Jack Royle, Trent Peters, Steven Ogbourne. Macadamia growers: circulation 850.	Australian Macadamia Society News Bulletin. Winter 2022, Volume 50(2), 19–20.
Grower article	Mango pollination: levels of self-pollination and cross-pollination among Kensington Pride and Calypso fruit. Wiebke Kämper, Joel Nichols, Chris Burwell, Stephen Trueman. Mango growers: circulation 1500.	Mango Matters. July 2022, Volume 48, 26–28.
Podcast	Pollination in macadamias. Helen Wallace. Macadamia growers: 1115 listeners.	Australian Macadamia Society: https://australianmacadamias.org/industry/news/pollination-in-macadamias-the-latest-research
Video	Cross-pollination for macadamias. Stephen Trueman. Macadamia growers: 255 viewers.	Australian Macadamia Society: https://australianmacadamias.org/industry/resources/cross-pollination-for-macadamias-august-2021-macgroups
Fact sheet	Pollination Fact Sheet. Macadamia growers: AMS website.	Australian Macadamia Society: https://australianmacadamias.org/industry/resources/pollination
Grower talk	Macadamia physiology. Stephen Trueman. Macadamia growers and processors: 25 attendees.	Macadamia Physiology Workshop, Hort Innovation / Australian Macadamia Society, Bundaberg. 2 August 2017.
Grower talk	Increasing yield and quality with better pollination and nutrient distribution. Stephen Trueman. Fruit and vegetable	Q&A Session for Avocado, Macadamia and Vegetable Growers, Bundaberg Fruit and Vegetable Growers / Hort Innovation, Bundaberg.

	growers: 30 attendees.	17 November 2017.
Grower talk	How much macadamia crop is cross pollinated? Helen Wallace, Wiebke Kämper, Tarran Richards, Stephen Trueman. Macadamia growers and processors: 20 attendees.	Macadamia SIAP meeting. Brisbane Hort Innovation office. 26 September 2018.
Grower talk	Don't forget the bees: why macadamia needs cross pollination. Helen Wallace, Wiebke Kämper, Tarran Richards, Stephen Trueman. Macadamia growers: 25 attendees.	Macadamia IPDM Annual Research Meeting. Cypress Room, RACV Royal Pines Resort, Gold Coast. 12 November 2018.
Workshop	Don't forget the bees: why macadamia needs cross pollination. Helen Wallace, Wiebke Kämper, Tarran Richards, Stephen Trueman. Macadamia growers: 30 attendees.	AusMac 2018 Conference: Beekeeping/Pollination Workshop. Royal Pines Resort, Gold Coast. 13 November 2018.
Grower and consultant talk	Don't forget the bees: why macadamia needs cross pollination. Helen Wallace, Wiebke Kämper, Tarran Richards, Stephen Trueman. Macadamia growers and consultants: 65 attendees.	Australian Macadamia Society – Annual Meeting of Key Consultants and Growers. Pacific Hotel, Spring Hill, Brisbane. 5 June 2019.
Invited conference presentation	Pollination and bees. Postharvest nut quality. Stephen Trueman. Macadamia researchers and consultants: 250 attendees with 30 Australian representatives.	International Macadamia Symposium, Lincang, China. 5 November 2019.
Poster	Dependence on cross-pollination in macadamia and challenges for orchard management. Wiebke Kämper, Stephen Trueman, Steven Ogbourne, Helen Wallace. Agricultural scientists: 700 attendees.	International Tropical Agriculture Conference, Brisbane Convention and Exhibition Centre, Brisbane. 11–13 November 2019.
Conference presentation	What is an effective pollination service? Why a better understanding of bee behaviour is critical for crop production. Helen Wallace. Beekeepers and researchers: 100 attendees.	Australian Native Bee Conference, The University of Queensland, Brisbane. 5–7 December 2019.
Grower talk and video	How important is cross-pollination for macadamias? Stephen Trueman. Macadamia growers: 130 attendees in teleconference and 742 viewers.	Green Farms Nut Co. Online Study Group, White River, South Africa. 4 August 2020. https://www.youtube.com/watch?v=co4Dme4IQs0
Grower talk	Stingless bees and lychee pollination. Helen Wallace and Wiebke Kämper. Lychee growers: 20 attendees.	Australian Lychee Growers Association R&D Updates for Growers, Zoom Conference. 9 September 2020.
Grower talk	Nut abscission and nut retention. Stephen Trueman. Macadamia growers: 14 attendees.	Australian Macadamia Society High Level Growers' Group, Hinkler Park Plantations, Bundaberg. 24 November 2020.
Grower talk	Bees as managed pollinators for avocado. Helen Wallace, Stephen Trueman, Shahla Hosseini Bai. Avocado	MacKay's Marketing, Malanda, Zoom conference. 13 August 2021.

	growers: 4 attendees.	
Grower talk	Cross-pollination effects on nut size and kernel recovery. Stephen Trueman. Macadamia growers: 13 attendees.	Australian Macadamia Society High Level Growers' Group, Bundaberg. 19 August 2021.
Beekeeper talk	Who is your daddy? Paternity testing in crops to understand pollination needs. Helen Wallace. Beekeepers: 125 attendees.	Queensland Beekeepers Association 2022 State Conference, Warwick. 23 June 2022.
Workshop	How can growers improve pollination for better yields? Stephen Trueman, Helen Wallace. Macadamia growers: 50 attendees.	Workshop 4A, AusMac 2022, Gold Coast. 8 November 2022.
Workshop	How can growers improve pollination for better yields? Stephen Trueman, Helen Wallace. Macadamia growers: 112 attendees.	Workshop 4B, AusMac 2022, Gold Coast. 8 November 2022.
Grower talk	Cross-pollination effects on macadamia nut size and kernel recovery. Anushika De Silva. 150 attendees.	AusMac 2022, Gold Coast. 9 November 2022.
Poster	Prediction of avocado leaf carbohydrate content using hyperspectral imaging. Trisha Pereira, Shahla Hosseini Bai, Mahshid Tootoonchy, Wiebke Kämper, Iman Tahmasbian, Michael Farrar, Helen Boldingh, Hannah Jonson, Joel Nichols, Helen Wallace, Stephen Trueman. 44 attendees.	International Avocado Brainstorming Meeting, Maroochydore. 27–31 March 2023.
Poster	Prediction of avocado leaf carbohydrate content using hyperspectral imaging. Trisha Pereira, Shahla Hosseini Bai, Mahshid Tootoonchy, Wiebke Kämper, Iman Tahmasbian, Michael Farrar, Helen Boldingh, Hannah Jonson, Joel Nichols, Helen Wallace, Stephen Trueman. 1147 attendees.	10 th World Avocado Congress, Auckland. 2–5 April 2023.

Outcomes

Table 2. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
<p>Intermediate outcomes:</p> <p>Growers have improved awareness of: (a) the impact of cross pollination on fruit yield and quality; (b) on-farm practices for enhancing pollination; (c) crop nutrient management practices that impact on pollination success and fruit quality; and (d) hyperspectral tools to predict crop nutrient levels, quality and the health value of fruit and nut products.</p>	<p>Outcome: Improved management of European Honey Bee for pollination: 3. Understand impacts of growing systems on pollination success</p> <p>Outcome: Crop pollination requirements are understood and integrated into best practice: 1. Understand current and future pollination requirements 2. Develop adaptive and tailored strategies to meet pollination requirements 3. Integrate effective pollination into horticulture production systems 4. Understand environmental and climatic barriers to effective pollination</p> <p>Outcome: Alternate pollination options developed and available: 2. Develop and enable novel technologies to support pollination</p>	<p>The project improved grower awareness by communicating that:</p> <p>(a) Growing systems that use single-cultivar wide blocks are not optimal for ensuring pollination success, high yield and excellent product quality;</p> <p>(b) many cultivars of almond and macadamia have a cross-pollination requirement, whereas many cultivars of avocado, lychee, mango, and strawberry only require self-pollination;</p> <p>(c) fruit and nut quality can be improved by cross-pollination in both self-sterile and self-fertile cultivars;</p> <p>(d) effective pollination can be integrated into orchards by inter-planting different cultivars more closely;</p> <p>(e) crop nutrition can influence initial pollination success, but the over-riding influence on final yield is often the level of effective cross-pollination;</p> <p>(f) novel hyperspectral imaging technologies have great potential for predicting crop nutrition during pollination and fruit development and for predicting the stage of ripening of mature fruit.</p>	<p>The project team has compiled a list of publications and presentations that were provided for growers and beekeepers, and they quantified the total readership and attendance for these engagement activities:</p> <p>(1) Thirteen articles were written for growers, and these were published in <i>Talking Avocados</i>, <i>Australian Macadamia Society News Bulletin</i>, <i>Australian Berry Journal</i>, <i>Mango Matters</i>, <i>In A Nutshell</i>, <i>Australian Nutgrower</i> and <i>Living Lychee</i> (Table 1). The total circulation of these seven publications is 8550 readers. The cumulative circulation of the 13 articles was 14,350 readers.</p> <p>(2) One podcast, two videos, one factsheet, one poster, and 17 workshops or talks were provided to growers and beekeepers (Table 1). The number of listeners, viewers and attendees for the podcast, videos, workshops and talks currently comprises a cumulative audience of 2925 growers and beekeepers.</p>

<p>End-of-project outcomes:</p> <p>(a) Growers have increased the yield of first-grade fruit and nuts and have optimised the sustainable delivery of crop nutrients during flowering and fruiting; and (b) Growers, consultants, processors or retailers have adopted hyperspectral scanning tools to rapidly predict crop nutrient levels or fruit quality.</p>	<p>Outcome: Improved management of European Honey Bee for pollination: 3. Understand impacts of growing systems on pollination success</p> <p>Outcome: Crop pollination requirements are understood and integrated into best practice: 1. Understand current and future pollination requirements 2. Develop adaptive and tailored strategies to meet pollination requirements 3. Integrate effective pollination into horticulture production systems 4. Understand environmental and climatic barriers to effective pollination</p> <p>Outcome: Alternate pollination options developed and available: 2. Develop and enable novel technologies to support pollination</p>	<p>Improved grower awareness of <i>(a) the impact of cross pollination on fruit yield and quality;</i> <i>(b) on-farm practices for enhancing pollination;</i> <i>(c) crop nutrient management practices that impact on pollination success and fruit quality; and</i> <i>(d) hyperspectral tools to predict crop nutrient levels, quality and the health value of fruit and nut products</i></p> <p>has led to improvements and efficiencies in horticultural production that are increasing the yield of first-grade fruit and nuts, while minimising the excessive use of fertilisers. Furthermore, hyperspectral scanning tools are being developed at scale for predicting nut quality on farms and predicting kernel quality in processing facilities.</p>	<p>The project team has compiled published case studies that show how new growing systems have been adopted on the basis of improved understanding of pollination requirements, the need for tailored strategies to meet these pollination requirements, and the capacity to integrate effective pollination into horticulture production systems. These case studies (Appendix 3) show, for example, how growers have recognized the cross-pollination requirement of macadamia cultivars and developed tailored strategies to integrate effective pollination into their growing systems.</p> <p>In addition, almond growers are establishing orchards with new self-fertile cultivars from the Australian breeding program, which will underpin effective pollination in the face of uncertain future supply of honeybee hives.</p> <p>Hyperspectral imagers are currently being tested and developed in four Australian macadamia processing facilities for monitoring kernel quality. Similar techniques are being developed in conjunction with macadamia growers for on-farm detection of defective nut-in-shell (https://www.corematic.com.au/#projects).</p>
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Monitoring and evaluation

Table 3. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
<p>Intermediate outcomes:</p> <p>(1) 50% of growers in target industries have improved awareness of the impact of pollination on fruit yield and quality, or of crop nutrient management practices that impact on pollination success and fruit quality.</p>	<p>The project team compiled circulation and attendance data for the communication activities that were integrated into the project. These demonstrate the wide reach of the awareness-raising program across all six of the horticultural industries involved in the project:</p> <p>(1) Thirteen articles on pollination impacts or crop nutrient management practices were published for growers (Table 1), covering the industry bulletins for all target industries in the project; i.e. avocado, macadamia, strawberry, mango, almond and lychee. These articles were published in:</p> <ul style="list-style-type: none"> ➤ <i>Talking Avocados</i>, ➤ <i>Australian Macadamia Society News Bulletin</i>, ➤ <i>Australian Berry Journal</i>, ➤ <i>Mango Matters</i>, ➤ <i>In A Nutshell</i>, ➤ <i>Australian Nutgrower</i> and ➤ <i>Living Lychee</i>. <p>The total circulation of these seven publications is 8550 readers.</p> <p>The cumulative circulation of the 13 articles was 14,350 readers.</p> <p>(2) One podcast, two videos, one factsheet, one poster, and 17 workshops or talks were also provided to growers and beekeepers (Table 1). The number of listeners, viewers and attendees for the podcast, videos, workshops and talks currently comprises a cumulative audience of 2925 growers and beekeepers.</p>	<p>The 13 articles written for growers (Table 1) were published in bulletins that cover many horticultural industries:</p> <ul style="list-style-type: none"> ➤ <i>Talking Avocados</i> (avocado), ➤ <i>Australian Macadamia Society News Bulletin</i> (macadamia), ➤ <i>Australian Berry Journal</i> (strawberry, blueberry, raspberry and blackberry), ➤ <i>Mango Matters</i> (mango), ➤ <i>In A Nutshell</i> (almond), ➤ <i>Australian Nutgrower</i> (almond, chestnut, hazelnut, macadamia, pecan, pistachio and walnut) and ➤ <i>Living Lychee</i> (lychee). <p>The podcast, two videos, the factsheet, the poster, and the 17 workshops or talks provided to growers and beekeepers (Table 1) were focused largely on growers and beekeepers associated with the avocado, lychee and macadamia industries. This was the result of two main factors: (a) the timelines of project findings for the phase 1 study crops and, subsequently, the phase 2 study crops in the project; and (b) the postponement of some horticultural industry conferences during covid lockdowns and border closures. Further opportunities will arise for delivering project results and recommendations in podcasts, videos, workshops and talks for almond, mango and strawberry growers, including by those project team members who are involved in Project PH20001 (<i>Crop and varietal data to better understand the importance of pollination</i>).</p>

<p>End-of-project outcomes:</p> <p>(1) 30% of growers in target industries have improved on-farm practices to enhance pollination and nutrition; 20-70% increase in yield or 25% increase in first grade fruit or nuts of target crops; and</p> <p>(2) 10% of growers, consultants or processors have adopted hyperspectral tools.</p>	<p>(1) Almond and macadamia growers, in particular, have adopted improved on-farm practices to enhance pollination.</p> <p>Six case studies are provided (Appendix 3) that highlight the adoption of new growing systems to enhance pollination.</p> <p>Macadamia orchards had, for the most part, been established previously as single-cultivar blocks. We communicated our project findings that nut yields can be increased by 29–97% with better cross-pollination and that yields decline in the middle of wide single-cultivar blocks. Furthermore, we communicated that kernel recovery can be increased by 3.3–6.4% by cross-pollination compared with self-pollination. These findings have led to closer inter-planting of different cultivars and the use of polliniser trees to take into account the effects of cross-pollination and strategic polliniser selection on yield and nut quality.</p> <p>We also confirmed and communicated that the new self-fertile almond cultivars are producing most of their nuts by self-pollination. These findings provided evidence for growers that these new cultivars can underpin nut production in the face of uncertain honeybee hive supply. Adoption of these new cultivars is currently estimated to be 5-10% of new plantings.</p> <p>(2) Hyperspectral tools are being developed to monitor kernel quality in four major facilities that process the majority of macadamia production in Australia.</p> <p>Hyperspectral tools are also being tested by major growers for on-farm quality sorting of macadamia nut-in-shell (https://www.corematic.com.au/#projects).</p>	<p>Further communication is recommended in the macadamia industry, as there remains a false perception among some growers that macadamia trees can set a good crop of high-quality nuts by self-pollination. As a result, some growers are continuing to plant wide blocks of single cultivars. Furthermore, data is required to clearly identify the best balance in orchard design that allows convenient management of irrigation, nutrition, pests, diseases and harvesting on a single-cultivar basis while also providing adequate access to cross-pollen from a neighbouring cultivar. Growers may be more likely to integrate effective pollination into their growing systems if they can visualise how this can be achieved without compromising their other orchard operations.</p> <p>Ongoing communication is warranted in the almond industry to explain the advantages of self-fertile cultivars, as most new plantings continue to comprise self-sterile cultivars despite potential issues with honeybee supply. This communication would ideally be backed up by robust data that confirms whether or not the new self-fertile cultivars require honeybees to perform self-pollination, and whether yields of these cultivars can be improved even further with cross-pollination.</p> <p>Hyperspectral imaging is being widely developed in the macadamia industry, but some of the most promising findings from the project were on the very high potential to predict ripening time of avocado fruit. Further engagement is recommended with producers, processors and retailers to develop this technology for avocado fruit.</p>
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Recommendations

Practical application of the project findings

- Avocado growers may consider inter-planting Type-B polliniser trees such as Shepard more closely with their Hass trees. The rates of cross-pollination in Hass blocks decline with increasing distance from the polliniser trees, which limits the opportunities for fruit set. Furthermore, cross-pollinated Hass fruit are often larger than self-pollinated Hass fruit, giving them greater market value.
- Macadamia growers may often need to inter-plant different cultivars more closely, as macadamia is heavily reliant on cross-pollination for nut production. Self-pollination provides less than 0.2 tons of nut-in-shell per hectare. Self-pollinated nuts are often smaller and have lower kernel recovery than cross-pollinated nuts, and so the presence of self-pollinated nuts reduces the price paid for a nut consignment.
- Strawberry growers may consider introducing more beehives onto farms, as fruit size is directly related to the number of filled seeds on each fruit. The presence of many small fruit has become an issue during later parts of the subtropical strawberry season.
- Almond growers may consider planting new self-fertile cultivars from the Australian breeding program. Most nuts of these cultivars in commercial orchards were produced by self-pollination rather than cross-pollination. These cultivars may sustain nut production in the face of uncertain supply of honeybee hives.
- Avocado growers and processors could test hyperspectral scanners for predicting time-to-ripeness of fruit. The project scanner predicted ripening time up to 2 weeks away with a margin of error of only 1 day.

Possibilities of future RD&E that directly flow from the project findings

- This project, PH16001, demonstrated that macadamia yields are constrained by inadequate cross-pollination. Project PH20001 is showing that avocado yields are also constrained by inadequate cross-pollination. Future research is warranted to determine whether whole-tree yields are limited by inadequate cross-pollination in other crops, such as mango, that also have declining levels of cross-pollination at increasing distances from another cultivar.
- This project confirmed that self-fertile almond cultivars are producing most of their nuts by self-pollination. However, two important questions remain unanswered: (1) are honeybees needed for self-pollination of these cultivars or, alternatively, can these cultivars produce self-pollinated nuts autogamously without honeybees; and (2) are the yield and nut quality of self-fertile cultivars improved when they receive high levels of cross-pollination?
- The presence of many small strawberry fruit during later stages of the production season may be the result of: (a) late-season flowers producing few stigmas, i.e. a pre-pollination problem, or (b) late-season flowers receiving inadequate pollen on their stigmas; i.e. a pollination problem. Research is required to determine whether the production of small strawberry fruit could be alleviated by improving the abundance and distribution of pollinators on strawberry farms.
- The flowers of most macadamia and almond cultivars require cross-pollination for nut production, while the yield or fruit quality of other crops such as avocado and lychee benefit from cross-pollination. Largely unanswered question for these crops are: (a) what percentages of pollinators are carrying crop-pollen; (b) what percentages of pollinators are carrying cross-pollen; (c) how can pollinator placement be optimised to ensure maximum levels of cross-pollination; and (d) could pollen dusting onto bees be used to increase the levels of cross-pollination? Recent advances in metabarcoding and SABER analysis of pollen DNA, as well as pollen-dusting technology, make it possible to answer these questions.
- Yields decline in the middle of wide single-cultivar blocks of macadamia and avocado, and possibly other crops. However, it remains unclear how many rows can be planted with a single cultivar before the yield begins to decline. This issue is critical for growers to achieve the right balance between harnessing the benefits of cross-pollination in their growing systems while minimising the costs of irrigation, nutrition, pest control, disease management, and harvesting by growing multiple rows of a single-cultivar.
- Hyperspectral scanners are currently being tested at scale for assessing macadamia nut quality and kernel quality. Further RD&E is recommended to fast-track the use of these scanners by avocado processors or retailers so that they can deploy high-quality fruit onto retail shelves at exactly the right stage of ripeness.

This would reduce the amount of pre-purchase handling of fruit by consumers at retail outlets and increase consumer confidence in the ripeness and quality of avocado fruit.

Refereed scientific publications

Published papers

- Kämper, W., Trueman, S.J., Tahmasbian, I., Hosseini Bai, S., 2020. Rapid determination of nutrient concentrations in Hass avocado fruit by Vis/NIR hyperspectral imaging of flesh or skin. *Remote Sensing* 12, 3409.
- Richards, T.E., Kämper, W., Trueman, S.J., Wallace, H.M., Ogbourne, S.M., Brooks, P.R., Nichols, J., Hosseini Bai, S., 2020. Relationships between nut size, kernel quality, nutritional composition and levels of outcrossing in three macadamia cultivars. *Plants* 9, 228.
- Kämper, W., Thorp, G., Wirthensohn, M., Brooks, P., Trueman, S.J., 2021. Pollen paternity can affect kernel size and nutritional composition of self-incompatible and new self-compatible almond cultivars. *Agronomy* 11, 326.
- Kämper, W., Trueman, S.J., Cooke, J., Kasinadhuni, N., Ogbourne, S.M., 2021. Single-nucleotide polymorphisms that uniquely identify cultivars of avocado (*Persea americana*). *Applications in Plant Sciences* 9, e11440.
- Dung, C.D., Wallace, H.M., Hosseini Bai, S., Ogbourne, S.M., Trueman, S.J., 2021. Cross-pollination affects fruit colour, taste, firmness, shelf life and mineral nutrient concentrations of self-compatible strawberry. *PLoS ONE* 16, 0256964.
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- Kämper, W., Trueman, S.J., Ogbourne, S.M., Wallace, H.M., 2021. Pollination services in macadamia depend on across-orchard transport of cross pollen. *Journal of Applied Ecology* 58, 2529-2539.
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- Dung, C.D., Wallace, H.M., Hosseini Bai, S., Ogbourne, S.M., Trueman, S.J., 2022. Biomass and mineral nutrient partitioning among self-pollinated and cross-pollinated fruit on the same strawberry plant. *PLoS ONE* 17, 0269485.
- Hapuarachchi, N.S., Kämper, W., Wallace, H.M., Hosseini Bai, S., Ogbourne, S.M., Nichols, J., Trueman, S.J., 2022. Boron effects on fruit set, yield, quality and paternity of Hass avocado. *Agronomy* 12, 1479.
- Kämper, W., Dung, C.D., Ogbourne, S.M., Wallace, H.M., Trueman, S.J., 2022. High self-paternity levels and effects of fertilised-seed number on size of strawberry fruit. *PLoS ONE* 17, 0273457.
- Dung, C.D., Wallace, H.M., Hosseini Bai, S., Ogbourne, S.M., Trueman, S.J., 2023. Fruit size and quality attributes differ between competing self-pollinated and cross-pollinated strawberry fruit. *International Journal of Fruit Science* 23, 1-12.
- De Silva, A.L., Trueman, S.J., Kämper, W., Wallace, H.M., Nichols, J., Hosseini Bai, S., 2023. Hyperspectral imaging of adaxial and abaxial leaf surfaces as a predictor of macadamia crop nutrition. *Plants* 12, 558.
- Davur, Y.J., Kämper, W., Khoshelham, K., Trueman, S.J., Hosseini Bai, S., 2023. Estimating the ripeness of Hass avocado fruit using deep learning with hyperspectral imaging. *Horticulturae* 9, 599.
- Han, Y., Hossein Bai, S., Trueman, S.J., Khoshelham, K., Kämper, W., 2023. Predicting the ripening time of 'Hass' and 'Shepard' avocado fruit by hyperspectral imaging. *Precision Agriculture* (in press).
- Hapuarachchi, N.S., Trueman, S.J., Kämper, W., Farrar, M.B., Wallace, H.M., Nichols, J., Hosseini Bai, S., 2023. Hyperspectral imaging of adaxial and abaxial leaf surfaces for rapid assessment of foliar nutrient

concentrations in Hass avocado. *Remote Sensing* 15: 3100.

Submitted manuscripts

- Kämper, W., Nichols, J., Tran, T.D., Burwell, C.J., Byrnes, S., Trueman, S.J. Levels of self-fertilisation and cross-fertilisation, and their effects on mango fruit quality, at increasing distances from a cross-pollen source.
- Dung, C.D., Trueman, S.J., Wallace, H.M., Farrar, M.B., Gama, T., Tahmasbian, I., Hosseini Bai, S. Hyperspectral imaging for estimating leaf, flower and fruit macronutrient concentrations and predicting strawberry yields.

Intellectual property

No project IP or commercialisation to report.

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