

# Cross-pollination Enhances Macadamia Yields, Even With Branch-level Resource Limitation

Brad G. Howlett<sup>1</sup> and Samantha F.J. Read

*The New Zealand Institute for Plant & Food Research Limited, Private Bag 4704, Christchurch Mail Centre, Christchurch 8140, New Zealand*

Maryam Alavi

*The New Zealand Institute for Plant & Food Research Ltd, Private Bag 92169, Auckland Mail Centre, Auckland 1142, New Zealand*

Brian T. Cutting

*Plant & Food Research Australia, Brisbane, Queensland, Australia*

Warrick R. Nelson

*The New Zealand Institute for Plant & Food Research Limited, Private Bag 4704, Christchurch Mail Centre, Christchurch 8140, New Zealand*

Robert M. Goodwin and Sarah Cross

*The New Zealand Institute for Plant & Food Research Ltd, Private Bag 3230, Waikato Mail Centre, Hamilton 3240, New Zealand*

Trevor G. Thorp

*Plant & Food Research Australia, Melbourne, Victoria, Australia*

David E. Pattermore

*The New Zealand Institute for Plant & Food Research Ltd, Private Bag 3230, Waikato Mail Centre, Hamilton 3240, New Zealand, and School of Biological Sciences, University of Auckland, New Zealand*

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**Abstract.** *Macadamia* is partially self-incompatible and cross-pollination is considered important to improve yields. However, questions remain regarding the importance of self- vs. cross-pollination, and subsequently whether managed pollinators are useful in commercial orchards. Pollinators play a key role in cross-pollination, but for self-pollination, the protandrous florets might also benefit from the movement of potentially more viable self-pollen among florets, racemes, and trees through pollinator movement. There is also a lack of information on pollination deficits throughout orchards and whether by increasing the intensity of cross-pollination, final nut yield is limited by within-tree resource allocation. Using caged and bagged racemes on three cultivars, we found strong evidence for self-pollination, but no evidence that hand moving self-pollen within racemes, between racemes, or between trees improved final nut set. In all cases, hand cross-pollinated racemes yielded significantly more nuts. Hand cross-pollinated racemes also produced significantly more developed nuts than open-pollinated racemes (all racemes were exposed to pollinators). However, by increasing the intensity of hand cross-pollination per tree, we showed that resource allocation probably overinflates these measures of pollination deficit in macadamia. Despite this, our findings point to an opportunity to increase yields through additional cross-pollination, as high-intensity hand cross-pollination of flowering racemes within trees still resulted in increased nut set. Although self-pollination can occur in macadamia, to optimize yield potential, strategies to maximize cross-pollination should be adopted.

*Macadamia* is partially self-incompatible (Sedgley, 1983), although self-pollinated nut set is known to occur in at least some cultivars (Meyers, 1997; Vithanage et al., 2002; Wallace et al., 1996). Cross-pollination has been considered important to increase final nut set (defined here as 3 months following fruit initiation) in trees across many cultivars (Meyers, 1997; Wallace,

1999). However, when comparing separate trials, the consistency of the increase in final nut set was found to be variable even with the use of the same pollen donor and receiver cultivars (Wallace et al., 1996). Much of the current knowledge of *Macadamia* spp. floral biology and breeding systems has been outlined in a review by Trueman (2013); however, the breadth and scale of published

research conducted across the many cultivars remains poor.

It is unclear whether self-pollination can occur within florets; pollen needs to be moved between florets by pollinators; pollination deficits are widespread within blocks of different cultivars; the amount of cross-pollination across an increasing number of racemes (hand cross-pollination intensity) influences potential nut yields. Such information can be useful for evaluating whether current pollinator management strategies are adequate, and for developing strategies to boost pollination if required.

Although self-pollination is known to occur in macadamia and may even be responsible for producing economic yields within isolated single cultivar blocks, published data showing the extent of self-pollination across multiple cultivars are largely lacking. A study by Meyers (1997) found that self-pollination of racemes resulted in limited nut set across some, but not all cultivars. In a block containing 27 rows of cultivar A16, self-pollination never exceeded 20% in a row (across 11 rows assessed), including the center row (13 rows from the nearest alternative cultivar) (Vithanage et al., 2002). Previous studies also demonstrate lower nut weight through self-pollination compared with hand cross-pollination (Meyers, 1997; Trueman and Turnbull, 1994; Wallace et al., 1996).

Despite the capability of at least some cultivars to produce fully developed nuts through self-pollination, there is a lack of studies exploring whether this pollination would likely benefit from an animal pollen vector, or (arguably less likely) wind or gravity (see Wallace, 1999). *Macadamia* presents viable pollen on or near the stigma (Trueman, 2013) but the floret is protandrous, with the stigma becoming receptive 1 or 2 days after anthesis (Sedgley et al., 1985) and reaches peak receptivity between 3 and 7 days after anthesis (Meyers, 1997). Therefore, autogamous pollination (within floret) could potentially occur if the pollen stays viable until the stigma becomes receptive. Alternatively, stigmas may receive self-pollen of variable age from other florets within the raceme, other racemes within the same tree, or from other trees of the same cultivar. This knowledge is important for evaluating whether pollinators are needed to transfer pollen to a floret, as wind pollination has been considered of minimal importance (Wallace, 1999). Wallace et al. (1996) explored how much final nut set in bagged racemes of cultivars A4 and 246 was affected by the transfer of self-pollen from other trees of the same cultivar compared with that from bagged racemes with no pollination. Few to no final nuts developed in these treatments. Evidence for the potential benefit of self-pollen movement between florets, to our knowledge, has not been obtained.

Cross-pollination has been demonstrated to increase final nut set across several cultivars compared with self-pollination (mostly measured at the raceme level) (Meyers, 1997;

Wallace et al., 1996). A broad assessment of whether pollination is optimal across cultivars, even within a region, does not appear to have been examined. Such information is useful in assessing whether strategies to boost cross-pollination are useful and the scale at which this might be required. Moreover, we are not aware of studies that have assessed whether increased nut set within cross-pollinated racemes can be sustained within trees with increased hand cross-pollination intensity. Limits to resource allocation within a tree may limit final nut set when cross-pollinated florets or racemes are preferentially retained at the expense of self-pollinated flowers. If carbohydrates and/or certain minerals are limiting, trees will not set a full crop even with adequate pollination. Pollen tube growth, fertilization, and fruit set require adequate amounts of carbohydrate and boron in particular (Alcaraz et al., 2013; Boldingh et al., 2016; Tanaka et al., 2013). Therefore, simple measures of pollination deficit may overestimate the importance of hand cross-pollination because of limits to resources allocated to develop nuts. There is evidence of physiological limitation of nut yields, in that just 0.3% of original floret numbers result in a harvestable nut (Ito, 1980). Only 4% of immature nuts present at 3 weeks after pollination developed into harvestable nuts (Wallace et al., 1996).

To gain further understanding of the contribution of cross-pollination, self-pollination, the occurrence of pollination deficits within blocks, and whether cross-pollination intensity affects final nut set, we conducted field trials in Queensland, Australia. We assessed whether the transfer of self-pollen by hand

between florets, racemes, and trees (typical of geitonogamous pollination) affected nut set compared with no movement (bagged), hand cross-pollinated (between different cultivars), and open (exposed to pollinators) racemes. We also assessed whether nut set was affected by pollination deficits across blocks by comparing open-pollinated (non-bagged) racemes with open racemes that also received supplemental hand cross-pollination. To test for resource limitation, we varied cross-pollination intensity by varying the number of hand cross-pollinated racemes on single trees.

## Materials and Methods

Experiments were conducted to assess the capacity for self-pollination at the raceme level, the prevalence of pollination deficits in blocks as measured within racemes, and the influence of cross-pollination intensity (varying the number of racemes cross-pollinated in trees), on final nut set yields within racemes and standardized unit volume of canopy. In each case, multiple trials were conducted to assess different cultivars. A summary of the experimental design information for each experiment is provided in Table 1.

All experiments were conducted at orchards within 30 km of Bundaberg, Queensland (24.8670° S, 152.3510° E), except for a single trial assessing pollination intensity for cultivar 842 that was conducted on an orchard within 10 km of Gympie, Queensland (26.1834° S, 152.6657° E). Trees were between 7 and 15 years old and orchards were stocked with managed honeybee hives. Trials comparing final nut set counts between cross-pollinated racemes (hand-pollinated using pollen from another cultivar and also left exposed to pollinators) and open-pollinated racemes (not hand-pollinated but left exposed to pollinators) were conducted at least two rows from the nearest alternative cultivar, whereas for the other trials the minimum distance was four rows.

**Bagging and cage technique.** Bags were constructed to prevent insect visitation using a very fine mesh (hole diameter <0.5 mm) fabric and sealed along their length and base using polyethylene tape that was stapled every 3 to 4 cm. Their size (30 × 15 cm) allowed them to envelop flowering macadamia racemes completely when tied around the peduncle. Wire cages were also used to minimize the potential rubbing of pollen between florets within a raceme. These were used in two treatments: caged no pollination (no hand selfing treatment to maximize the chance of autogamous pollination), and caged cross-pollination (to assess whether the cage might influence nut yield). These cages were constructed from wire mesh (gap diameter 2.5 cm) to create cylinders, 10 cm in diameter and 20 cm in length. Wire mesh platforms were secured within the upper and lower sections of the cage (at ≈10 cm above the cage base) through which the treatment raceme could be threaded and secured. The cages were then encased inside mesh bags.

The cages were placed over inflorescences to minimize pollen movement between florets that could occur if bags rubbed against the raceme.

**Hand-pollination.** Racemes were hand-pollinated as described by Trueman and Turnbull (1994), Wallace et al. (1996), and Penter et al. (2007). Pollen was collected inside a glass test tube (30 cm length, and 2.5 cm in diameter) for the trials assessing hand cross-pollination vs. open-pollination. Similarly sized disposable clear acetate tubes were used for the trials assessing capacity for self-pollination and intensity of cross-pollination. An earlier unpublished assessment found that these tubes were of similar effectiveness as glass tubes in collecting loose pollen from racemes onto tube walls. These tubes were used to conduct many single-raceme hand cross-pollen transfers while minimizing the collection of excessive self-pollen.

Selected racemes for pollen collection contained more than 50% fully open florets with undisturbed pollen present on the pollen presenter. Pollen was collected by placing a tube over a fully flowering raceme at peak pollen dehiscence so that the pollen presenters of florets (holding dehiscent pollen) within the raceme had contact with the sides of the tube. The tube was then twisted to collect pollen onto the tube wall. The process was repeated on additional racemes until a consistent dusting of pollen could be observed across the surface of the tube (usually five racemes). Pollen was then transferred within the hour (on ice to maintain pollen viability) to the pollen-receiving cultivar from which the final nut set was to be assessed. This was achieved by twisting the tube to allow the pollen to be picked up by the pollen presenters (containing the stigmas) of the treatment raceme.

**Capacity for self-pollination.** Trials were conducted to assess the potential for self-pollination within three cultivars, 741, 816, and A268, as described in Table 1. Eight pollination treatments were conducted for each cultivar to assess: self-pollination within florets (two treatments), within the raceme, between racemes within a tree, and between trees of the same cultivar and compared with two cross-pollination (two treatments and open pollination) (Table 1). All treated racemes were covered by bags (removed after 20 days following treatment) to prevent exposure to known insect pollinators (bees, flies, beetles) (Howlett et al., 2015) except for open-pollinated racemes.

A randomized block design was applied for each trial with the number of replicates for each outlined in Table 1. In each block, eight trees were selected, and on each tree, one of the eight treatments was applied. We chose to treat just one raceme per tree to minimize the risk of cross-contamination between treatments and to ensure similar length, age, orientation, and position of racemes. Late budding stage racemes (2–3 d before flowering) were selected and marked with tape. For cultivars 741 and 816, three replicate blocks

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<sup>1</sup>Corresponding author. E-mail: brad.howlett@plantandfood.co.nz.

Table 1. Design information for three experiments conducted on macadamia trees across blocks. Cultivars in bold were measured for fully developed nut set following the application of pollen from a donor cultivar (bracketed). Selective raceme treatments were bagged and caged in Expt. 1 but these were removed 20 days after treatment application.

Experimental information	Expt. 1	Expt. 2	Expt. 3
	Capacity for self-pollination	Pollination deficits in racemes	Intensity of hand-cross pollination
No. trials	3	52	2
Experimental design	Random block	Paired	Random block
Cultivars assessed for yield in each trial (pollen donor cultivar)	<b>741</b> (816)  <b>816</b> (741)  <b>A268</b> (A203)	<b>741</b> (246), (344), (788), (816), (842), (849), (A16), (A203), (A268), (A38), (A4), (Daddow) <b>Daddow</b> (246), (344), (741), (788), (816), (842), (849), (A16), (A203), (A268), (A38), (A4) <b>A268</b> (246), (344), (741), (788), (816), (842), (849), (A16), (A203), (A4), (Daddow) <b>A203</b> (246), (344), (741), (788), (816), (842), (849), (A16), (A268), (A38), (A4), (Daddow) <b>842</b> (246), (344), (741), (816)	<b>741</b> (816)  <b>842</b> (344)
No. treatments	8	2	5 for 741, 4 for 842
Treatments	1. Open pollination (exposed to pollinators) 2. Caged none (no hand-pollination caged and bagged) 3. Bagged none (no hand-pollination, bagged) 4. Within raceme (hand and self-pollination bagged) 5. Between racemes (hand self-pollination within tree, bagged) 6. Within cultivar (hand self-pollination between trees, bagged) 7. Cross (hand cross-pollination and bagged) 8. Crossed caged (cross-pollination caged and bagged)	1. Cross (hand cross-pollination) exposed to pollinators 2. Open pollination (exposed to pollinators)	1. Control (no cross-pollination) 2. One raceme cross-pollinated 3. Three racemes cross-pollinated 4. Fifteen racemes cross-pollinated 5. One hundred racemes cross-pollinated (741 only)
Replicates	Ten of each treatment both trials (741 and 816), 8 of each for trial A268	20	Ten of each treatment (741 and 842) except 6 for 100 cross-pollinated racemes

were placed within a tree row for each of three tree rows and one block in a fourth row. For 'A268', three sets of two replicate blocks were placed within a tree row across four tree rows. Blocks were separated by two or more trees.

Racemes were initially bagged (and caged) at budding stage (containing pale green to cream-colored buds), monitored every 1 to 2 d and treated when in full flower (more than 90% of florets fully open). Each cylinder used for hand-pollination was used once to avoid unintentional pollen transfer between treatments.

Following the application of each treatment, bags were reapplied and the racemes left for 20 days to ensure stigmas were no longer receptive (pollination has been measured to occur up to 12 days post-anthesis) (Meyers, 1997). Bags were then removed, and marked racemes left to develop nuts. Nut set was counted on 25 Jan. 2016 (cultivars 741 and 816) and 26 Jan. 2016 ('A268').

*Pollination deficits in racemes.* Five macadamia cultivars, 741, Daddow, A203, A268, and 842, were chosen to assess how cross-pollination by different cultivars might influence the final number of nuts set within individual racemes (Table 1). Each trial

consisted of 20 trees of the same cultivar. For each tree, two fully flowering racemes located in similar positions (height, aspect, branch location) but on separate branches, were marked with tape. Chosen racemes were 120 to 200 cm above ground and positioned on the same side on the outer tree canopy facing into the row. One was randomly assigned to be cross-pollinated (coin toss). This raceme was hand-pollinated using the pollen of a single assigned cultivar. The second marked raceme was not hand-pollinated and represented the open-pollinated control. All raceme treatments were left uncaged to allow access for potential pollen vectors. Final nut set was counted at between 130 and 140 d after pollination (treatments conducted 10–20 Sept., final nut set counts 25–30 Jan.).

*Intensity of hand cross-pollination.* Trials on '741' and '842' were conducted to assess how the number of hand cross-pollinated racemes within trees affected nut set across all hand-pollinated racemes. For '741', we also tested whether this influenced nut set on open-pollinated racemes that were not also hand cross-pollinated. Within separate single cultivar orchard blocks, four treatments were

applied to '842' trees and five treatments to '741'. Treatment descriptions and experimental design are outlined in Table 1.

Hand-pollinated racemes, with exception of the 100x, were marked with tape. Moreover, for '741' (not '842') a further six fully flowering racemes that were not hand-pollinated were marked on each tree to assess final nut set. Hand-pollinations and marked racemes for all but the 100x treatment were located on a single tree branch with basal diameter between 15 and 30 cm of similar height and orientation on each tree. For the 100x treatment, hand cross-pollinations were randomly conducted on flowering racemes across the entire tree to a height of 2 m. Following the hand cross-pollination treatment, 15 cross-pollinated and six open-pollinated flowering racemes were marked on a single branch to assess final nut set.

To conduct the hand cross-pollinated treatments on '741', tubes were used once for all treatments with the exception of 100x, in which each tube was used to pollinate two racemes. Nut set assessments were conducted on 26 Jan. 2016.

In addition, nut set counts were conducted on four sides (north, south, east, and west) of

Table 2. Summaries of the statistical analyses from three experiments. Generalized Linear Mixed Models were used to assess data for Expts. 1 and 3, and a Hierarchical Generalized Linear Mixed Model for Expt. 2.

Expt. 1: Capacity for self-pollination						
Cultivar	Dispersion estimate	Dispersion SE	F statistic	d.d.f	Residual df	F prob
741	1.039	0.182	6.67	65.0	63	<0.001
816	0.653	0.120	1.99	60.1	66	0.071
A268	0.036	0.083	2.58	37.5	42	0.028

Expt. 2: Pollination deficits in racemes						
Cultivar	Wald statistic for interaction	df for interaction	Approximate Pr for interaction	Wald statistic for treatment	Residual df	Approximate Pr for treatment
741 – Multiple pollenizers	35.30	11	<0.001	285.3	536.1	<0.001
Daddow – Multiple pollenizers	21.46	11	0.029	172.65	428	<0.001
A268 – Multiple pollenizers	19.79	10	0.031	199.5	354	<0.001
A203 – Multiple pollenizers	6.598	11	0.831	60	374.1	<0.001
842 – Multiple pollenizers	3.41	3	0.333	34.10	112.02	<0.001

Expt. 3: Intensity of hand cross-pollination on raceme nut counts						
Cultivar	Dispersion estimate	Dispersion SE	F statistic	d.d.f.	Residual df	F prob
842 – Cross-pollinated racemes	1.128	0.112	18.02	38.8	230	<0.001
741 – Cross-pollinated racemes	1.735	0.185	2.66	34.5	206	0.063
741 – Untreated racemes	0.717	0.07	2.88	40.7	247	0.034

Expt. 3: Intensity of hand cross-pollination on canopy quadrat nut counts						
Fixed effect	Dispersion estimate	Dispersion SE	F statistic	d.d.f.	Residual df	F prob
Location treatment	1.537	0.066	5.19	1087.1	1094	0.001

d.d.f = degrees of freedom; Pr = probability.

each tree. At each side, a fold-out quadrat forming a 0.75-m<sup>3</sup> cube (Howlett et al., 2018) was held at a height of 1.5 m, and all nuts contained within its volume were counted. Final nut set counts and surveys were conducted on 26 to 27 Jan. 2016.

**Data analysis.** In all trials, we assessed how the treatments affected final nut set counts using Generalized Linear Mixed Models in GenStat 17 for self-pollination and hand cross-pollination intensity trials. The final nut set counts were modeled as process with Poisson distribution for which the dispersion parameters were also estimated; however, we found that the dispersion parameters were reasonably close to 1. Because the blocks were randomized over the experiments and cultivars, each experiment-cultivar pair was conducted in one block. Therefore, we did not consider block effects in nested-structured random effects in the analysis. For each experiment, Wald test was used for the overall treatment effect and Fisher's unprotected least significant difference test was used for multiple comparisons. For the analyses, the mean and associated 95% confidence intervals were calculated on the link scale (logarithmic) and back-transformed for presentation.

For self-pollination trials, the fixed effect was treatment (seven treatments) for each of the three cultivars, and the random effect was tree identity number. For the intensity of cross-pollination trials, the fixed effect in the model was the number of racemes crossed within trees (four treatments for cultivar 842 and five treatments for cultivar 741), and the random effect was tree identity number. We also compared final nut set counts contained within cubic quadrats for '741' between control trees (no hand cross-pollination conducted) and trees in which 100 racemes were hand cross-pollinated,

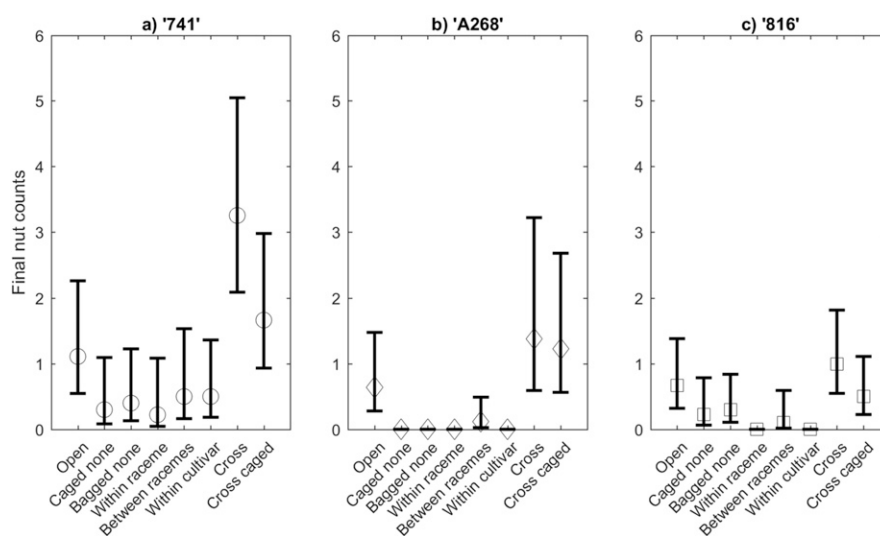


Fig. 1. Mean final nut set per raceme (circle) and 95% confidence limits (error bars) of three macadamia cultivars a) 741, b) A268, and c) 816 following different pollination treatments: no hand-pollination open to pollinators (open), no hand-pollination caged and bagged (caged none), no pollination bagged (bagged none), within-raceme hand self-pollinated bagged (within raceme), between racemes within-tree hand self-pollinated bagged (between racemes), between-tree within-cultivar hand self-pollination bagged (within cultivar), between racemes of different cultivars hand cross-pollinated bagged (cross), and between racemes of different cultivars hand cross-pollinated caged and bagged (cross caged).

with the fixed effect being quadrat side location, treatment, and their interaction, and the random effect being replicate number.

For hand cross-pollination vs. open pollination on single-raceme trials, a Hierarchical Generalized Linear Mixed Model was used (GenStat 17) to assess data for each cultivar. The fixed effect was cultivar treatment and the random effect was replicate. The fixed effect was modeled by Poisson distribution with estimated dispersion parameter, and the random effect was modeled by Gamma

distribution at logarithmic scale. Both Wald's test and likelihood test reported consistent results for the treatment (and interaction with pollenizer where applicable) effect. Following the analysis, means and associated 95% confidence intervals were calculated on the link scale and back-transformed for presentation.

## Results

A summary of analyses for all three experiments: 1) capacity for self-pollination, 2)

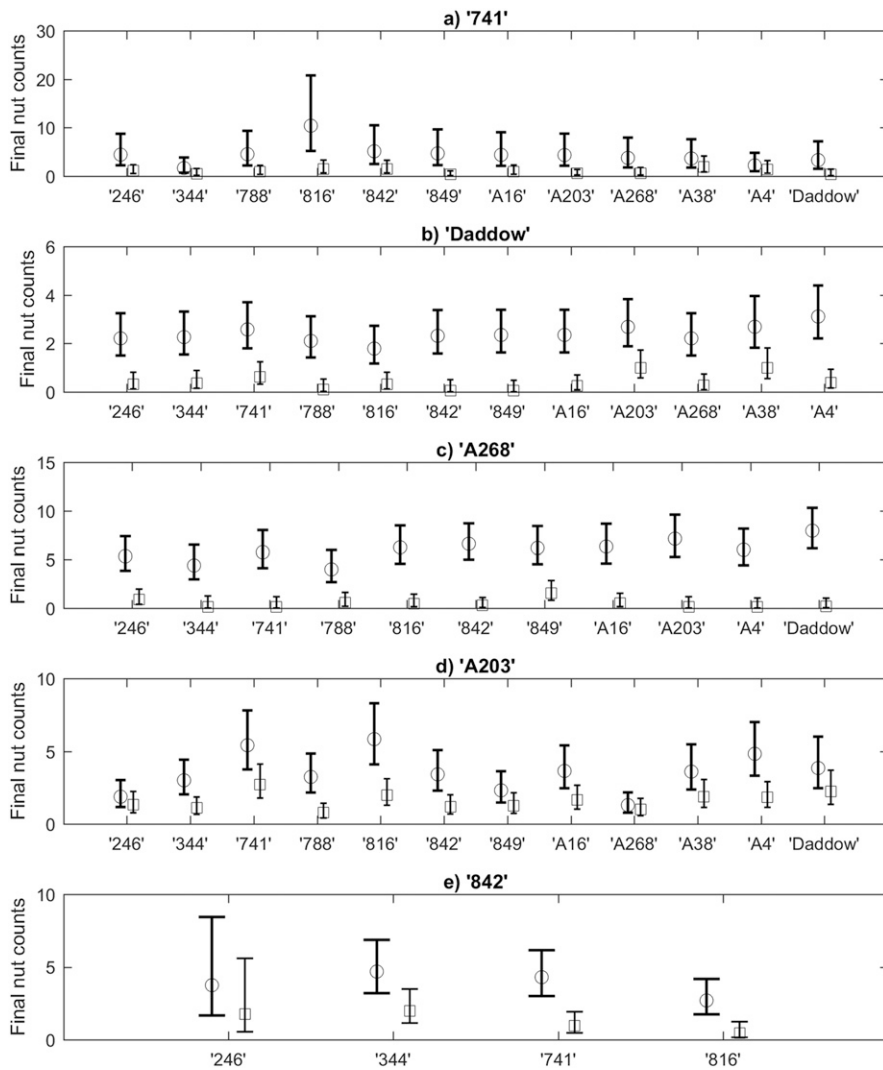


Fig. 2. Mean final nut set per raceme for macadamia cultivars comparing hand cross-pollination (circles) and open pollination (squares) across five cultivar trials a) 741, b) Daddow, c) A268, d) A203, and e) 842, each using a different pollen donor cultivar. Error bars are 95% confidence intervals of the means.

pollination deficits in racemes, and 3) intensity of hand cross-pollination on raceme nut counts are provided in Table 2.

**Capacity for self-pollination.** Final nut set from the self-pollination treatments varied between cultivar trials, with '741' producing the highest counts and 'A268' the lowest. All self-pollination treatments produced lower nut set counts than open and hand cross-pollination treatments (Fig. 1).

For '741', there was evidence of differing final nut set for the various treatments (Table 2). All self-pollination treatments resulted in some nut set (Fig. 1); however, there was no evidence of differences between the selfing treatments ( $P = 0.345$ ). Supplemental hand cross-pollination produced higher nut set than other treatments (Fig. 1), significantly greater than in all other treatments [caged hand cross-pollination ( $P = 0.047$ ), open ( $P = 0.006$ ), and all self-pollination treatments ( $P \leq 0.001$ )]. Mean nut set in the caged hand cross-pollination treatment was also higher than in the various self-pollination treatments ( $P \leq 0.026$ ), but not substantially different from that of the

open-pollinated racemes ( $P = 0.334$ ). Open racemes produced more nuts than racemes self-pollinated within raceme ( $P = 0.046$ ), but this higher nut set was not significantly different from that of the between racemes between trees of the same cultivar treatment ( $P = 0.190$ ), or of the within cultivar between trees ( $P = 0.158$ ), bagged with no pollination ( $P = 0.095$ ), or caged with no pollination ( $P = 0.055$ ) treatments.

For 'A268' there was evidence of a significant impact of the various treatments on the final nut set (Table 2). This cultivar produced just a single nut across all of the self-pollination treatments. There is the possibility that this nut may have developed through accidental cross-pollen contamination; however, we did not observe any obvious pathways for this to eventuate, such as through bag breakage. In contrast, the two hand cross-pollination treatments (bagged and caged) and the open-pollination treatments produced more nuts (Fig. 1). Nut set from the caged hand cross-pollinations were significantly higher than the open-pollinations ( $P = 0.047$ ), but not for the bagged hand cross-pollination treatment ( $P = 0.733$ ), whereas the difference between

the bagged hand cross-pollination and open treatments was just short of significant ( $P = 0.051$ ). Low nut counts for both treatments (crossed mean 1.50, maximum 2, minimum 0; open mean 0.85, maximum 3, minimum 0) and missing replicates (three for crossed; one for open) also may have affected the statistical result.

For '816', there was some evidence that treatments affected final nut set (Table 2). However, the potential significance of this result may have been limited by a combination of low numbers of final nut set within racemes (Fig. 1) and the relatively high number of racemes across treatments that did not produce any nuts (cross 2, caged cross 6, open 4, caged no pollination 8, bagged no pollination 8, within raceme 10, between raceme 9 and within cultivar 10). There was no evidence of significant differences between self-pollination treatments ( $P = 0.244$ ), with no nuts being set for two treatments (between racemes and within cultivar). As with '741', the bagged cross-pollination treatment for '816' produced significantly higher nut set than the various self-pollination treatments ( $P = 0.029$ ) (Fig. 1), but there was no evidence that they varied substantially from the results from the caged hand cross-pollination ( $P = 0.559$ ) or the open-pollination treatments ( $P = 0.335$ ).

**Pollination deficits in racemes.** Of the 52 trials conducted across '741', 'Daddow', 'A268', 'A203', and '842' to compare final nut set within treated racemes (hand cross-pollinated) and untreated racemes (open), our analyses suggested that in all but five trials, significantly greater final nut set was achieved in the treated racemes. For '741', the increase in final nut set on hand cross-pollinated racemes, above that of open-pollinated racemes, ranged from 13.6 to 1.6 times; for 'Daddow', from 43.7 to 2.7; for 'A268', from 54.1 to 4.0; for 'A203', from 4.3 to 1.3; and for '842', from 6.0 to 2.1 (Fig. 2). In all of the cultivars assessed, the effect of treatment on nut set was significant (Table 2). However, the effect of treatment (crossed vs. open) on nut set varied significantly between treatments for '741', 'Daddow', and 'A268', but not for 'A203' or '842' (Table 2). For '741', treated racemes produced significantly higher final nut set than untreated racemes ( $P < 0.001$ ) for all pollen donor cultivars except when the pollinizer was '344' ( $P = 0.130$ ) or 'A4' ( $P = 0.179$ ). For 'Daddow' and 'A268', cross-pollinated racemes from all assessed cultivar donors set significantly higher nut numbers than untreated racemes ( $P < 0.005$  for all cultivars) (Fig. 2). 'A203'-treated racemes produced significantly higher final nut set than untreated racemes for seven pollen donors ( $P = 0.030$ ), but not for the pollen donor cultivars A38 ( $P = 0.082$ ), Daddow ( $P = 0.152$ ), 849 ( $P = 0.172$ ), 246 ( $P = 0.445$ ), or A268 ( $P = 0.626$ ) (Fig. 2). Racemes treated with '842' produced significantly higher final nut set when treated with pollen from cultivars 741 and 816 ( $P > 0.001$ ), but not for '344' ( $P = 0.009$ ) or '246' ( $P = 0.275$ ) (Fig. 2).

**Intensity of hand cross-pollination.** For the cultivar 842, varying the numbers of racemes crossed within trees had a significant impact on final nut set within the treated racemes when compared with control trees (no crosses made) (Table 2). Mean raceme final nut set also varied with the number of racemes crossed within a tree. Mean raceme final nut set was highest in treatments in which either a single raceme or three racemes were cross-pollinated (Fig. 3), but final nut set did not vary substantially between these treatments ( $P = 0.825$ ). However, the number of nuts in the racemes of these treatments were higher than on trees in which 15 racemes were pollinated (trees with 1 crossed vs. 15 crossed,  $P = 0.007$ ; trees with 3 crossed vs. 15 crossed,  $P < 0.001$ ) (Fig. 3). Final nut set in all crossed racemes irrespective of number of racemes pollinated per tree were higher than noncrossed racemes in control trees [control tree raceme (0x) vs. 1x ( $P < 0.001$ ), vs. 3x ( $P < 0.001$ ), vs. 15x ( $P = 0.007$ )].

For '741', we considered final nut set in control vs. cross for each hand cross-pollination intensity treatment raceme numbers, but not for '842' (we did not measure nut set in noncrossed racemes for '842'). For '741', varying the intensity of racemes pollinated within a tree resulted in a declining final nut set per raceme for the higher-intensity treatments (15x and 100x) (Fig. 3). Final nut set varied between crossed racemes and control racemes ( $P = 0.01$ ) but not strongly between control racemes across the various hand cross-pollination intensity treatments (all  $P > 0.100$ ).

The mean ( $\pm$  SE) total number of final nuts set for '741', within each cubic quadrat was  $116.8 \pm 15.9$  nuts per quadrat for the 100x-treated trees and  $69.3 \pm 5.2$  nuts per quadrat for the control trees. Overall, final nut set/raceme was significantly higher (70.2%) for the 100x-treated trees compared with control trees (no hand cross-pollinations) ( $P < 0.001$ ), particularly on the north and west sides (both  $P < 0.001$ ) (Fig. 4). Moreover, there was a significant effect of treatment and the side on which the quadrat was placed (north, south, east, or west) (Table 2; Fig. 4). There was moderate to no evidence to suggest significant difference among north, west, and east faces ( $P \geq 0.095$ ). For control trees, final nut set did not vary strongly between faces.

## Discussion

These results confirm that self-pollination can produce final nut set in some cultivars, but cross-pollination usually increases nut set. Our findings build on previous studies, but like Wallace et al. (1996), we did not find evidence that self-pollination through the hand-transfer of self-pollen influenced final nut set in self-pollinated racemes. We demonstrated that an increasing intensity of hand cross-pollination was associated with a decline in cross-pollinated nut set, a pattern indicative of within-branch resource limitation

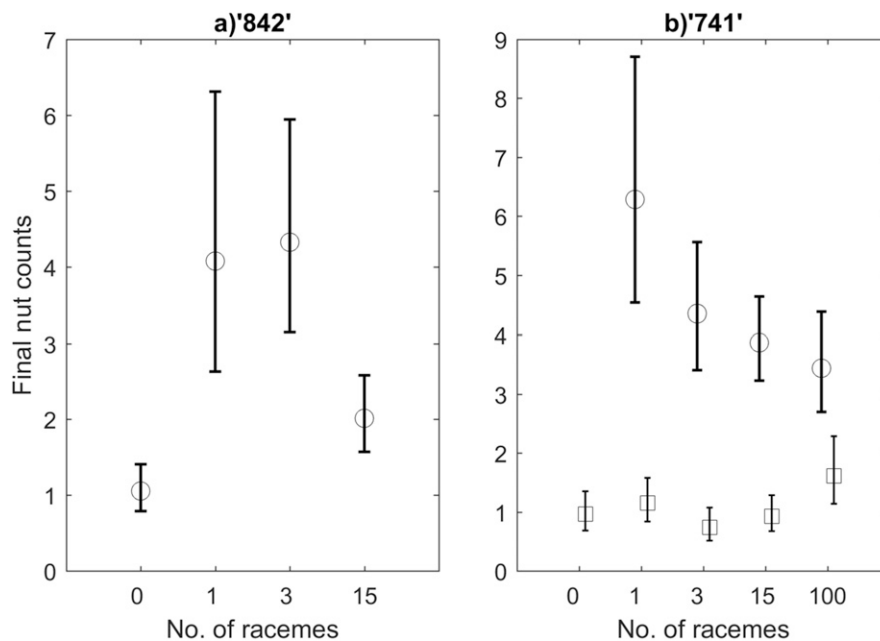


Fig. 3. Mean final nut set per raceme for two macadamia cultivars a) 842 and b) 741, each with varying hand cross-pollination intensity treatments (number of racemes crossed). Circles represent mean counts in hand-crossed racemes, squares are mean counts in untreated racemes on the same trees ('741' only). Error bars are 95% confidence intervals of the means.

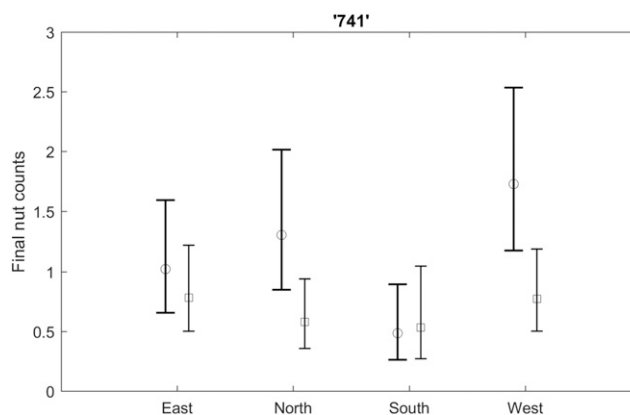


Fig. 4. Mean final nut set within the total area of cubic quadrats ( $0.75 \text{ m}^3$ ) of macadamia cultivar 741, comparing control trees (no hand-pollination) (squares) with trees with 100 hand cross-pollinated racemes (circles), and showing the effect of the side of the tree. Error bars are 95% confidence intervals of the means.

(Groeneveld et al., 2010). However, there was still a significant difference between cross- and open-pollinated nut set on racemes at the highest rate of hand cross-pollination.

Self-pollination in macadamia is known to result in the full development of some nuts within orchards that comprise many rows of a single cultivar (Meyers, 1997; Vithanage et al., 2002), and indeed many orchard blocks comprising a single cultivar could potentially be producing economically viable yields. Our bagging and caging experiments show that viable self-pollination is occurring even in the absence of managed pollinator species (honeybees or stingless bees). Pollination could be occurring within the bag through gravity, air movement, or the movement of very small unobserved insects (e.g., thrips) inadvertently trapped inside or moving

through the bag. This presumably low rate of active pollination resulted in comparable nut set to that of open racemes for cultivar 816. Therefore, for certain cultivars, some harvestable nut set could be achieved in isolated single cultivar blocks in the absence of large managed or wild pollinators ( $>0.5 \text{ mm}$  in body width).

However, our trials suggest that relying on self-pollination limits commercial yields for most cultivars, supporting the findings of other studies (Meyers et al., 1995; Penter et al., 2007). Our trials could not distinguish between within-floret (autogamy) and between-floret (geitonogamy) pollination within racemes, because we caged whole racemes rather than individual florets, but our results show that either autogamy or pollen vectors (e.g., very small insects or air

movement) other than managed pollinators are able to result in a low rate of nut set. In isolated orchard blocks comprising a single cultivar, the opportunity for cross-pollination is greatly reduced, and yield in these orchards is probably limited to the maximum achieved through self-pollination, regardless of the presence or absence of pollinator activity. Thus, efforts to increase pollination in these orchards through insect-pollinator management may not have a material effect.

Our findings also suggested widespread pollination deficits are likely within orchard blocks of several cultivars in the trial region (Bundaberg, Australia). This was demonstrated by the trials showing significantly lower nut set on open-pollinated racemes than on hand cross-pollinated racemes (both with exposure to pollinators). Our results are consistent with previous cross-pollination studies (e.g., Meyers et al., 1995), suggesting many cultivar combinations may not be equally effective in improving final nut set within racemes.

A common critique of pollination deficit studies of large trees (in which only a fraction of flowers are hand cross-pollinated) is that preferential allocation of resources by the plant to cross-pollinated flowers could exaggerate the potential tree-level increase in yield through improved pollination. It is known that there is a relationship between carbohydrate concentrations and retention of immature fruit, and fruit drop may be a response to competition for carbohydrates (McFadyen et al., 2011). Such competitive interactions between developing fruit may result in decreasing nut yields within racemes as the cross-pollination intensity is increased within a tree. By increasing the intensity of hand cross-pollination at the tree scale, we were able to quantify the magnitude of this resource-allocation limitation at the branch level in macadamia. This provides evidence as to whether the limit to total nut yield is due to pollination rather than a physiological limitation (such as through the availability of carbohydrate) in carrying these nuts to maturity.

Increasing the intensity of hand cross-pollinations did decrease the difference between cross- and open-pollinated racemes at a branch level, but a significant difference remained between the treatment both at branch and tree levels. This is strong

evidence for resource limitation at the branch level in macadamia, although we did not assess whether resource limitation also operated between branches. Our results demonstrate that, if researchers desire to quantify the possible gains from enhanced pollination, our recommendation is to conduct as many cross-pollination treatments as feasible on each macadamia tree to minimize this resource allocation bias.

In our trials, the 100x treatment resulted in greater than 50% of receptive racemes on the day receiving hand cross-pollination on a tree. Although this is not a complete hand cross-pollination of all racemes, the maintenance of a 70% increase in nut set with this high rate of hand cross-pollination indicates that a significant opportunity remains for growers to increase yields with improved pollination.

In our trial, the magnitude of the potential increase through hand cross-pollination might actually have been underestimated because of the additional effect of insect-mediated cross-pollination by moving pollen from hand cross-pollinated to open-pollinated racemes. At our study orchard, noncrossed racemes on hand cross-pollinated trees had an increased nut yield per raceme compared with control (0x) trees. This could arise from subsequent movement by insects of cross-pollen deposited by hand from one raceme to another, thus within-canopy cross-pollination.

Our results support the suggestion (Howlett et al., 2015) that steps to increase the potential for cross-pollination, such as mixed-cultivar orchards of known compatible cultivar combinations, and improving insect pollination activity, are likely to improve commercial orchard yields.

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