

Article

Boron Effects on Fruit Set, Yield, Quality and Paternity of Macadamia

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Abstract: Many tree crops experience sub-optimal yields and low fruit quality due to inadequate pollination, low fruit set, and poor crop nutrition. Boron (B) is a critical crop nutrient for fruit set because B levels affect pollen germination and pollen tube growth. However, the relationship between floral B concentration and fruit set is not well understood. The aim of this study was to determine the effect of B applications on the initial fruit set, yield, quality, and paternity of macadamia (*Macadamia integrifolia*). Cultivar ‘816’ trees received one of three treatments: (a) 0 g, (b) 15 g, or (c) 30 g B per tree prior to flowering. Boron application increased the B concentration of macadamia flowers. Application of 15 g B increased fruit set at 3 weeks after peak anthesis, but this higher initial fruit set was not translated into higher fruit set at 6 or 10 weeks after peak anthesis or higher yield. Boron application increased B concentrations in kernels but did not affect nut-in-shell (NIS) mass, kernel mass, kernel recovery, kernel oil concentration or incidence of whole kernels. Cultivar ‘816’ was highly outcrossing, with 97–98% cross-paternity among kernels from all treatments. Our results indicate that higher B concentration in macadamia flowers can be associated with an increased initial fruit set. However, high B levels did not affect yield, nut quality, or the proportion of self-pollinated fruit at maturity. The heavy dependence on outcrossing highlights the importance of inter-planting different cultivars and managing bee hives to sustain the productivity of macadamia orchards.

Keywords: crop nutrition; kernel; *Macadamia integrifolia*; nut; pollination; *Proteaceae*; self-incompatibility; self-sterility



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1. Introduction

Global crop yields are currently trending below anticipated food demand [1,2]. Tree crops contribute over 600 million tons of the 10,600 million tons of annual global food production [3–5], and fruit number and fruit size are key components of tree crop yield [6–8]. Tree yields can be constrained by the percentage of flowers that are pollinated and fertilised, the percentage of fruit that develop to maturity, and the mass of the fruit components that contribute to yield [9–12]. Mass-flowering trees, which include many of the world’s tropical and subtropical tree crops, typically produce many more flowers than mature fruit [9,13–15]. Many of the flowers do not set fruit and many of the fruit abscise during the early fruit development period [9,14,16–18]. The final size of the remaining fruit that reach maturity can be affected by environmental conditions [19,20], crop nutrition [21–23] and fruit paternity [15,24–27].

Macadamia (*Macadamia integrifolia*, *M. tetraphylla* and hybrids) is a subtropical nut crop that produces up to 3500 racemes annually per tree [28–30]. Each raceme possesses between 100 and 300 flowers [15,31]. Low and inconsistent macadamia yields are often attributed to low levels of initial fruit set, poor fruit retention, and variations in nut and kernel size [15,17,27,32–35]. Macadamia has a very low fruit:flower ratio [17,33], which is common among species of the Proteaceae family [9,36,37]. Typically, less than 2% of macadamia flowers develop into mature fruit [15,16,32,33,38]. Most macadamia cultivars are partially self-incompatible, with greater pollen tube growth in the style and higher initial fruit set after cross-pollination by a different cultivar than after self-pollination by the same cultivar [35,39–42]. Higher initial fruit set following supplementary cross-pollination of macadamia flowers is often translated into higher final fruit set [17,33]. Supplementary cross-pollination of individual racemes also increases nut-in-shell (NIS) mass by 11.6–21.8%, kernel mass by 18.4–31.3%, and kernel recovery (i.e., the percentage of NIS mass that is comprised of the kernel) by 1.5–3.3% [17,33]. Supplementary cross-pollination of whole trees can also increase NIS yield by 29–97%, kernel yield by 29–109% and kernel oil concentration by 0.5% [15]. Sub-optimal macadamia yield and nut quality have therefore been attributed to insufficient pollinator activity and under-supply of cross-pollen [15,34,35,43]. However, plant nutrition may also play a critical role in macadamia fruit set and retention [44,45].

Boron (B) is an essential plant micronutrient that has multiple functions in pollination and fruit set [46–48]. Both the male and female reproductive tissues have higher demand for B than vegetative tissues [49–51]. Inadequate B levels affect microsporogenesis, resulting in decreased pollen production, pollen size and pollen viability [52,53]. Boron also promotes pollen germination and pollen tube growth [54–56], while B deficiency decreases the flexibility of pollen-tube cell walls and can inhibit pollen tube growth [56–58]. Borate cross-linked pectin is a major component of the female transmitting tissue, which guides pollen tubes to the ovules [47]. Insufficient B levels can result in impaired development of the transmitting tissue, preventing fertilisation [47]. Increasing B concentration in flowers increases initial fruit set, fruit retention, final fruit set or yield of many tree crops, including almond (*Prunus dulcis*), apple (*Malus domestica*), avocado (*Persea americana*), olive (*Olea europaea*) and sour cherry (*Prunus cerasus*) [14,59–62]. However, the relationship between floral B concentration and fruit set in macadamia is not well understood.

The aim of this study was to determine the effect of B applications on initial fruit set, yield, quality and paternity of macadamia fruit. We hypothesised that B application would increase initial fruit set and yield, possibly by increasing the number of self-pollinated flowers that developed into fruit. Thus, we expected that B application would increase the proportion of self-pollinated fruit at harvest. We also hypothesised that B application would affect NIS mass, kernel mass and kernel quality as a result of changes in the proportions of self-pollinated and cross-pollinated fruit.

2. Materials and Methods

2.1. Study Site

The study was conducted in a commercial, irrigated, macadamia orchard at Alloway (24°56'6" S 152°21'16" E), near Bundaberg, Queensland, Australia. The study region has a humid subtropical climate with wet summers and dry winters. Bundaberg had total rainfall of approximately 543 mm during the study period of June 2018 to June 2019 and mean daily minimum–maximum temperatures of 9.0–25.3 °C in the coldest month (August) and 21.2–32.0 °C in the warmest month (January) [63–65] (Figure S1). The site has a yellow/brown kandosol soil [66,67]. The orchard contained cultivars 'A4', 'A16', 'A29', 'A38', 'A203', 'A268', 'Daddow', 'Own Venture', '246', '344', '660', '741', '814', '816', '835', '842' and '849', with the trees being either 13 or 16 years old. Tree spacing was 10 m between rows and 2 m within each row. Each cultivar was planted in a block that occupied five contiguous rows.

2.2. Experimental Design, Sample Collection and Processing

We selected thirty trees in the middle row (i.e., the third row) of a block of cultivar ‘816’ trees that were 13 years old. Cultivars in the neighbouring blocks (i.e., three rows away from the experimental row) were ‘842’ and ‘A4’. The experimental trees had a mean (\pm SE) trunk circumference at 10 cm above the graft union of 63 ± 1 cm and height of 8.0 ± 0.1 m ($n = 30$). Each experimental tree was separated from other experimental trees by at least one non-experimental buffer tree. The thirty experimental trees were divided into ten plots along the row, with each plot containing three experimental trees as well as buffer trees. The row had no B applied either to the soil or the foliage between June and October 2018, except as stated here. Each of the three experimental trees within a plot was assigned randomly to one of three B treatments: (a) 0 g per tree; (b) 15 g per tree; or (c) 30 g per tree of elemental B, applied as 0 g per tree, 5 g per tree or 10 g per tree of elemental B, respectively, on each of three occasions: 27 June 2018, 25 July 2018 and 26 August 2018. The treatments were applied on each occasion by spraying the orchard floor under each experimental tree with 5 L of an aqueous solution of Yara Soluble BoronTM (Yara, Karratha, Australia). The control trees received 5 L of water on each occasion. The intermediate application rate (15 g B) was based on the manufacturer’s recommendations.

We tagged five racemes on each experimental tree immediately prior to flowering and then counted the number of flowers on each raceme during peak anthesis on 12 September 2018. The number of fruit retained on each of the five tagged racemes was counted at 3, 6 and 10 weeks after peak anthesis. Fruit set at each time point was calculated as:

$$\text{Fruit set (\%)} = \frac{\text{Number of fruit remaining on the raceme}}{\text{Number of flowers on the raceme at peak anthesis}} \times 100\% \quad (1)$$

We counted the numbers of (a) honeybees, (b) stingless bees, (c) syrphid flies, (d) other insects, and (e) other animals such as birds that contacted a flower within a 5-min period in a 1 m³ quadrat on the illuminated side of each experimental tree between 0800 h and 1100 h on three days during peak anthesis. The main foragers were European honey bees (*Apis mellifera*) (Figure S2).

We collected one sample of flowers, consisting of two racemes including their rachises, from each tree at peak anthesis. We also collected one sample of four young fully-expanded leaves from each tree at each of 0, 6 and 10 weeks after peak anthesis and at the commencement of harvesting (i.e., 26 weeks after peak anthesis). At least 300 mg of each flower sample, and of each leaf sample at 0, 6, 10 or 26 weeks after peak anthesis was used for mineral nutrient analysis.

We harvested all fruit under the canopy of each tree on eight occasions during autumn and winter 2019, i.e., representing the harvest period for cv. ‘816’ at the study site. All fruit from under each tree were weighed after each harvest. We then collected and weighed a subsample of fruit from each tree after each harvest. The subsampled fruit were dehusked, the nuts were dried at 37 °C for 2 d, 45 °C for 2 d and 57 °C for 2 d [68], and the total mass of the dried nuts were recorded. We then subsampled 20 dried nuts from each tree for assessment of nut quality, mineral nutrient concentration, and paternity. To make up the subsample of 20 nuts, the number of nuts selected from each of the eight harvests was in proportion to the total mass of nuts collected from each of the eight harvests. However, the nuts within each of the eight harvests were selected randomly. We recorded NIS mass for each nut. Each nut was cracked manually using a T.J.’s nutcracker (T.J.’s, Morayfield, Australia) and its kernel mass was recorded. Kernel recovery was calculated as the percentage (w/w) of NIS that was comprised of the kernel. Whether each kernel remained as a whole kernel or split into halves upon cracking was recorded. The yield of each tree was then calculated on both a dried NIS and kernel basis.

The 20 kernels that were subsampled from each tree were each dissected into three representative sub-samples of at least: (a) 500 mg for determination of oil concentration; (b) 300 mg for mineral nutrient analysis; and (c) 50 mg for paternity analysis.

2.3. Determination of Oil Concentration

We determined the oil concentration of each kernel by measuring its specific gravity using a pan immersed in a 95% (*v/v*) ethanol solution [69,70]:

$$\text{Oil concentration (\%)} = 284.7 - 212.57 \times \text{specific gravity} \quad (2)$$

where:

$$\text{Specific gravity} = \frac{0.7995 \times \text{mass in air}}{(\text{mass in air} - \text{mass in 95\% ethanol})} \quad (3)$$

2.4. Mineral Nutrient Analysis

We determined nitrogen (N) concentration of flowers, leaves, and a random subsample of ten of the 20 kernels per tree by combustion analysis using a LECO 928 Macro Determinator (LECO, Saint Joseph, MI, USA) [71,72]. We determined aluminium (Al), boron (B), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), potassium (K), sodium (Na), sulphur (S) and zinc (Zn) concentrations by inductively coupled plasma–atomic emission spectroscopy after nitric and perchloric acid digestion [73,74]. Mineral nutrient contents of each kernel were calculated by multiplying each mineral nutrient concentration by the kernel mass.

2.5. Paternity Analysis

We analysed the paternity of each of the 20 subsampled kernels per tree. We also collected a leaf from each experimental tree, from which an approximately 70-mg subsample was used to confirm its cultivar identity. Each kernel or leaf subsample was combined with disposable 0.1 mm and 2.3 mm diameter zirconia/silica beads (Daintree Scientific, St Helens, Australia) and frozen in liquid nitrogen before grinding into a powder using an MM2000 TissueLyser (Retsch, Haan, Germany) [75]. DNA extraction followed the glass-fibre DNA-extraction protocol for plants [76].

We used unique, homozygous, single nucleotide polymorphism (SNP) markers that were identified from macadamia cultivars [15] to determine the pollen parent of each kernel. High-throughput genotyping to assign paternity was performed using the Agena MassARRAY platform (Agena Bioscience, San Diego, CA, USA), using methods described previously [15]. A self-pollinated kernel resulted in all assays providing a homozygous signal, whereas a cross-pollinated kernel resulted in at least the maternal assay providing a heterozygous signal. The proportion of nuts with cross-paternity was then calculated for each experimental tree.

2.6. Statistical Analysis

We tested the effects of B on foraging activity and fruit set using generalised linear models (GLMs) (SPSS version 26, SPSS Science, Chicago, IL, USA) with a Poisson distribution and log link function. Fixed effects were B treatment, plot and day for foraging activity and B treatment and plot for fruit set. We tested the effects of B on yield, NIS mass, kernel mass, kernel recovery, whole kernel incidence, kernel oil concentration, and leaf or kernel nutrient levels using GLMs with a Gaussian distribution and identity link function. Fixed effects were B treatment and plot. We compared differences between B treatments using a pairwise comparison procedure with sequential Šidák's corrections when significant differences were detected. Pearson's correlation coefficients were calculated between NIS yield, kernel yield or nut quality parameters and floral or foliar mineral nutrient concentrations at 0, 6, 10 or 26 weeks after peak anthesis. Correlations, and differences between means, were regarded as significant at $p < 0.05$.

3. Results

3.1. Floral and Foliar Mineral Nutrient Concentrations

Boron (B) application generally increased B concentrations in flowers and leaves (Figure 1). Floral B concentrations were almost twice as high in trees that had been treated

with 30 g B than in trees that received no B (Figure 1a). Foliar B concentrations in trees treated with 30 g B were higher during peak anthesis than in control trees, and they continued to increase at 6, 10 and 26 weeks after peak anthesis. Trees that had been treated with 15 g B also had increased foliar B concentrations at 6, 10 and 26 weeks after peak anthesis (Figure 1b). The floral and foliar concentrations of N, P, K, Al, Ca, Cu, Mg, Mn, Fe, Na, S and Zn varied only slightly or not significantly among treatments (Figures S3–S6).

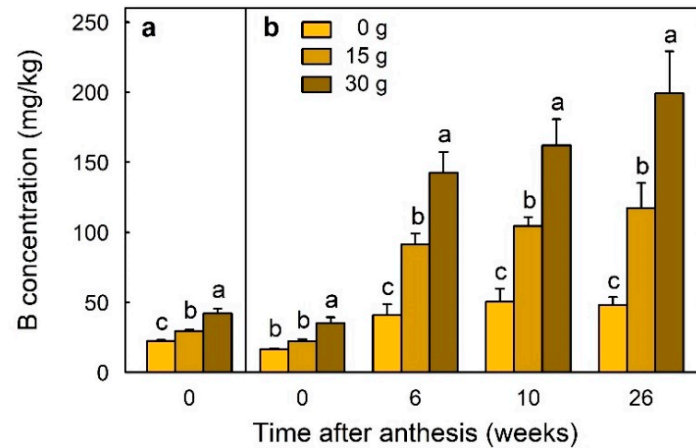


Figure 1. (a) Floral and (b) foliar boron (B) concentrations of macadamia cultivar ‘816’ trees treated with 0, 15 or 30 g B prior to flowering. Means (+SE) with different letters within a time point are significantly different (generalised linear models, $p < 0.05$, $n = 10$ trees).

3.2. Fruit Set and Yield

Initial fruit set at 3 weeks after peak anthesis was highest on trees that received 15 g B prior to flowering (Figure 2). Fruit set at 6 and 10 weeks after peak anthesis did not differ significantly among B treatments.

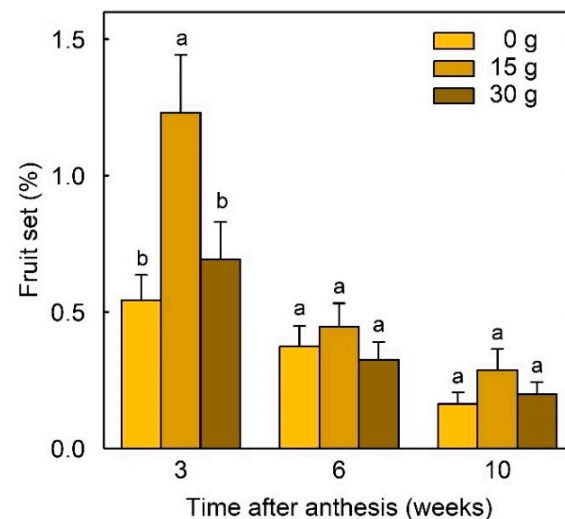


Figure 2. Fruit set on racemes of macadamia cultivar ‘816’ trees treated with 0, 15 or 30 g B prior to flowering. Means (+SE) with different letters within a time point are significantly different (generalised linear models, $p < 0.05$, $n = 50$ racemes).

Nut-in-shell and kernel yield did not differ significantly among B treatments (Figure 3). Nut-in-shell yields were 5.74 ± 0.30 , 6.00 ± 0.27 and 6.25 ± 0.28 kg/tree and kernel yields were 2.47 ± 0.14 , 2.57 ± 0.13 and 2.69 ± 0.14 kg/tree following treatment with 0, 15 or 30 g B, respectively.

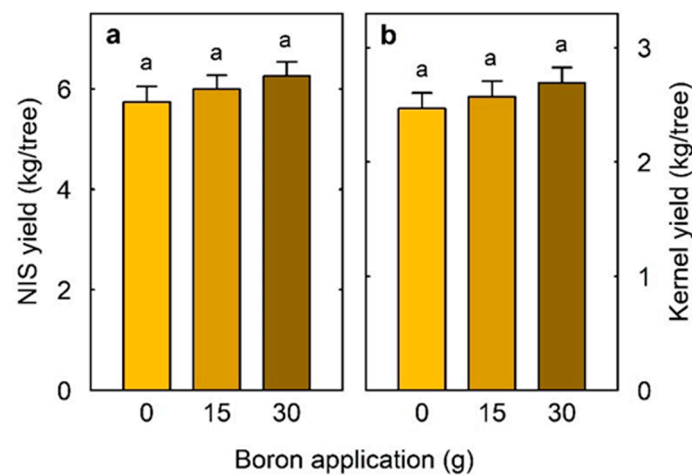


Figure 3. (a) Nut-in-shell (NIS) yield and (b) kernel yield of macadamia cultivar ‘816’ trees treated with 0, 15 or 30 g B prior to flowering. Means (+SE) do not differ significantly (generalised linear models, $p > 0.05$, $n = 10$ trees).

Nut-in-shell yield (Table 1) and kernel yield (Table 2) were not correlated significantly with either floral or foliar B concentrations. Nut-in-shell yield was correlated with floral N, K and Cu concentrations (Table 1) and kernel yield was correlated with floral N and Cu concentrations (Table 2). Nut-in-shell and kernel yield were negatively correlated with floral Fe concentration (Tables 1 and 2). Nut-in-shell yield was correlated occasionally with foliar N, K, Ca, Mg or Zn concentration (Table 1) and kernel yield was correlated occasionally with foliar K, Ca, Mg or Mn concentration (Table 2).

Table 1. Relationships between nut-in-shell (NIS) yield and floral or foliar mineral nutrient concentrations at 0, 6, 10 or 26 weeks after peak anthesis in macadamia cultivar ‘816’ trees treated with 0, 15 or 30 g B prior to flowering.

Nutrient	Correlation with NIS Yield									
	Sample Type and Time After Peak Anthesis									
	Flowers (0 Weeks)		Leaves (0 Weeks)		Leaves (6 Weeks)		Leaves (10 Weeks)		Leaves (26 Weeks)	
	r	p	r	p	r	p	r	p	r	p
B	0.242	0.197	0.214	0.257	0.116	0.543	0.340	0.066	0.277	0.138
N	0.410 *	0.024	0.195	0.302	0.050	0.076	0.362 *	0.049	0.138	0.467
P	0.327	0.078	0.289	0.122	0.050	0.792	0.225	0.232	−0.189	0.316
K	0.383 *	0.037	0.224	0.235	0.182	0.337	0.407 *	0.025	−0.112	0.557
Al	−0.303	0.103	0.008	0.967	−0.234	0.212	0.068	0.721	0.893	−0.026
Ca	−0.283	0.130	−0.028	0.881	0.018	0.927	−0.006	0.976	0.428 *	0.018
Cu	0.408 *	0.025	0.325	0.080	−0.089	0.640	−0.145	0.446	0.061	0.749
Fe	−0.415 *	0.023	0.080	0.722	−0.305	0.102	−0.258	0.169	−0.001	0.998
Mg	−0.130	0.495	0.244	0.194	0.104	0.585	0.162	0.392	0.579 **	0.001
Mn	0.041	0.832	0.242	0.198	0.067	0.724	0.063	0.741	0.279	0.135
Na	−0.230	0.221	−0.066	0.728	−0.083	0.664	0.117	0.538	0.118	0.536
S	0.331	0.074	0.099	0.603	0.133	0.484	0.240	0.201	0.349	0.060
Zn	−0.200	0.289	0.361 *	0.050	−0.196	0.297	0.112	0.557	−0.079	0.677

Significant correlations (r) are indicated by bold font and asterisks (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$; $n = 30$ trees).

Table 2. Relationships between kernel yield and floral or foliar mineral nutrient concentrations at 0, 6, 10 or 26 weeks after peak anthesis in macadamia cultivar ‘816’ trees treated with 0, 15 or 30 g B prior to flowering.

Nutrient	Correlation with Kernel Yield									
	Sample Type and Time After Peak Anthesis									
	Flowers (0 Weeks)		Leaves (0 Weeks)		Leaves (6 Weeks)		Leaves (10 Weeks)		Leaves (26 Weeks)	
	r	p	r	p	r	p	r	p	r	p
B	0.230	0.221	0.220	0.243	0.115	0.545	0.335	0.070	0.317	0.088
N	0.373 *	0.042	0.139	0.465	0.287	0.124	0.329	0.076	0.111	0.559
P	0.328	0.077	0.233	0.215	0.049	0.796	0.213	0.259	−0.241	0.199
K	0.333	0.072	0.144	0.448	0.123	0.516	0.403 *	0.027	−0.207	0.273
Al	−0.300	0.107	0.001	0.994	−0.261	0.163	0.028	0.885	0.005	0.979
Ca	−0.257	0.170	−0.062	0.745	−0.003	0.986	−0.077	0.687	0.472 **	0.008
Cu	0.421 *	0.021	0.293	0.116	−0.029	0.880	−0.156	0.410	0.100	0.599
Fe	−0.400 *	0.029	−0.113	0.553	−0.297	0.111	−0.296	0.113	0.005	0.979
Mg	−0.127	0.502	0.197	0.297	0.062	0.743	0.099	0.604	0.616 ***	<0.001
Mn	0.115	0.544	0.253	0.177	0.118	0.535	0.072	0.704	0.364 *	0.048
Na	−0.272	0.145	−0.074	0.698	−0.137	0.472	0.128	0.500	0.104	0.583
S	0.298	0.110	0.053	0.779	0.105	0.579	0.147	0.438	0.291	0.119
Zn	−0.147	0.439	0.314	0.091	−0.165	0.383	0.068	0.722	−0.074	0.699

Significant correlations (r) are indicated by bold font and asterisks (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p < 0.001$; n = 30 trees).

3.3. Nut Quality

Individual NIS mass, kernel mass, kernel recovery and kernel oil concentration, and the incidence of whole kernels, did not differ significantly among B treatments (Table 3). There were very few significant correlations between these nut quality parameters and floral or foliar mineral nutrient concentrations (Tables S1–S5).

Table 3. Nut-in-shell mass (NIS) (g), kernel mass (g), kernel recovery (%), whole kernel incidence (%), kernel oil concentration (%) and kernel mineral nutrient concentrations (mg/100g) of macadamia cultivar ‘816’ trees treated with 0, 15 or 30 g B before flowering.

	B Application (g)		
	0	15	30
<i>Nut mass and quality</i>			
Nut-in-shell mass	6.57 ± 0.08 a	6.67 ± 0.08 a	6.71 ± 0.08 a
Kernel mass	2.83 ± 0.04 a	2.87 ± 0.05 a	2.89 ± 0.05 a
Kernel recovery	42.86 ± 0.43 a	42.83 ± 0.42 a	42.58 ± 0.40 a
Whole kernels	73.50 ± 2.70 a	74.50 ± 2.30 a	75.50 ± 1.90 a
Kernel oil concentration	76.90 ± 0.40 a	77.50 ± 0.30 a	77.50 ± 0.40 a
<i>Nutrient concentrations</i>			
B	0.718 ± 0.034 a	0.912 ± 0.036 b	0.823 ± 0.035 c
N	1339 ± 20 a	1314 ± 19 a	1352 ± 19 a
P	213 ± 6 a	207 ± 7 a	202 ± 7 a
K	392 ± 28 a	365 ± 83 a	380 ± 13 a
Al	0.149 ± 0.008 a	0.143 ± 0.008 a	0.151 ± 0.009 a
Ca	62.97 ± 2.08 a	58.73 ± 1.70 a	63.48 ± 2.20 a
Cu	0.385 ± 0.010 a	0.403 ± 0.012 a	0.378 ± 0.012 a
Fe	1.78 ± 0.08 a	1.80 ± 0.07 a	1.78 ± 0.07 a
Mg	122 ± 4 a	110 ± 4 b	116 ± 4 ab
Mn	0.548 ± 0.027 a	0.471 ± 0.017 b	0.509 ± 0.019 ab
Na	1.16 ± 0.07 a	1.06 ± 0.07 a	1.22 ± 0.07 a
S	134 ± 4 a	127 ± 2 a	130 ± 3 a
Zn	0.997 ± 0.034 a	0.918 ± 0.036 ab	0.868 ± 0.041 b

Means ± SE within a row with bold font and different letters are significantly different (generalised linear models, $p < 0.05$, n = 200 nuts for NIS mass, kernel mass, kernel recovery and kernel oil concentration, n = 10 trees for whole kernel incidence, n = 100 nuts for mineral nutrient concentrations).

Kernel B concentration was 27% higher in trees that received 15 g B and 15% higher in trees that received 30 g B, than in control trees (Table 3). Total B content was also higher in kernels from B-treated trees than control trees (Table S6). The concentrations (Table 3) and total contents (Table S6) of most other kernel nutrients did not differ significantly among B treatments.

3.4. Nut Paternity

Boron application did not affect the levels of cross- and self-paternity among kernels at harvest (Figure 4). Most kernels arose from cross-pollination. The levels of cross-paternity were $97.4 \pm 1.2\%$, $98.3 \pm 0.9\%$ and $96.8 \pm 1.2\%$ on trees treated with 0, 15 or 30 g B, respectively.

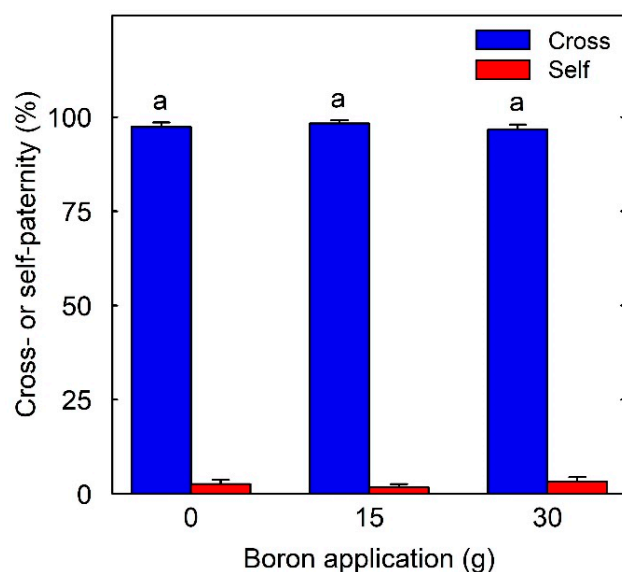


Figure 4. Levels of cross- and self-paternity among nuts of macadamia cultivar '816' trees treated with 0, 15 or 30 g B prior to flowering. Means (+SE) do not differ significantly among B treatments (generalised linear model, $p > 0.05$, $n = 10$ trees).

4. Discussion

Our results show that applying B to macadamia trees at the recommended rate of 15 g per tree elevated floral B concentrations and increased initial fruit set at 3 weeks after peak anthesis. Surprisingly, this effect did not translate into higher fruit set at later stages of fruit development, possibly because, contrary to our hypothesis, B application did not increase the retention of self-pollinated fruit. Instead, we found very low proportions of self-pollinated fruit at harvest, even at high B levels. This study highlights the necessity for cross-pollen transfer across commercial macadamia orchards to sustain orchard productivity.

Boron application prior to anthesis elevated the floral B levels, indicating that B was taken up rapidly by macadamia roots and deposited in the flowers. Boron is largely transported in the xylem in most plants, although it can be remobilised in species that contain abundant polyols in the phloem [77]. Boron is regarded as phloem-immobile in macadamia and so transport to the shoots and flowers occurs along the transpiration stream through the xylem [78]. Boron application increased the initial fruit set in the trees treated with 15 g B, perhaps due to an increased set of self-fertilised fruit. Self-pollen is already present on the macadamia stigma and upper style when the flower opens, although much of this self-pollen may be removed and cross-pollen deposited by insects before the stigma becomes receptive [18,46,79]. Many self-pollen tubes are arrested in the macadamia style before they reach the ovary [40]. Higher floral B levels may have accelerated the growth of pollen tubes, increasing the likelihood of self-fertilisation and improving the initial fruit set. In other crops like avocado, the probability that a flower develops into fruit is related to

the B concentration of the style [14]. High B levels facilitate pollen germination and pollen tube growth because B forms complexes with pollen cell-wall polymers, assisting pollen grains to germinate on the stigma and pollen tubes to grow through the transmitting tissue towards the ovary [55,80,81]. Self-pollen tubes often grow more slowly than cross-pollen tubes, thereby reducing the chances of self-fertilisation and fruit set [82]. Boron application can improve fruit set following self-pollination because high B concentrations can increase the speed of pollen germination and pollen tube growth [60,61,81].

Contrary to our hypothesis, the increased initial fruit set following B application was not translated into higher fruit set at 6 or 10 weeks after peak anthesis or into higher yield. In our study, only 2–3% of macadamia fruit at maturity were self-fertilised and so, if many self-fertilised fruit were set initially, then very few were retained beyond the period of premature fruit drop. Most macadamia fruit that set after flowering abscise in the first 10 weeks after anthesis [17,38]. Possible causes of fruit abscission include fertilisation failure due to pollen tube arrest or late-acting genetic incompatibility [40,83], early-acting inbreeding depression [83–85], and limited availability of maternal resources to support fruit retention [9,16,29,86,87]. Early-acting inbreeding depression occurs when genetically inferior embryos fail to develop, and it can occur at any stage of embryo development [83,85]. Selective abortion of self-fertilised fruit has been reported in many highly-outcrossing plants [9,10,84,88,89], including Proteaceae species [90,91]. The final fruit set could also have been limited by the availability of maternal resources such as carbohydrates, with cv. '816' trees in all B treatments possibly having produced an initial fruit set that was above their resource-provisioning capacity [9,16,29,86,87]. Strong pollen limitation of final fruit set and yield has been demonstrated recently on cv. '816' trees at another orchard in the same region [15]. Improved cross-pollination in that orchard elevated yields to 2.48–2.74 t ha⁻¹, whereas average yields on trees in the current study were 2.87–3.13 t ha⁻¹. Examination of the genotype of both abscised and retained fruit is needed to resolve the causes of fruit abscission in macadamia.

In addition to the final fruit set, NIS mass is a key component of macadamia yield. Macadamia growers are paid on the basis of NIS yield and kernel recovery, and macadamia processors receive higher prices for large kernels and for kernels that remain whole upon cracking rather than splitting into halves or pieces [92–95]. Cross-fertilised macadamia fruit often have higher NIS mass, kernel mass, kernel recovery and kernel oil concentration than self-fertilised fruit [15,27]. Boron application had no significant effect on any of these yield or quality parameters or on the incidence of whole kernels. This was possibly because, contrary to our hypotheses, B application did not affect the proportions of self- and cross-fertilised fruit. Our results indicate that cultivar '816' is highly dependent on cross-pollination for producing mature fruit and so techniques that aim to increase the levels of self-fertilisation might have limited capacity to increase yield or alter nut quality. Further research is warranted to determine whether all macadamia cultivars are highly outcrossing, and whether B application has the capacity to increase yield in any cultivars that display high levels of selfing.

Boron application increased B concentrations in macadamia kernels. Trees treated with the recommended rate of 15 g B produced kernels with 27% and 11% higher B concentration than trees treated with 0 g or 30 g B, respectively. Boron is important in bone formation and maintenance [96,97], and B supplementation can alleviate the symptoms of arthritis [98,99]. There are no clear recommendations for daily dietary B intake, although the World Health Organisation suggests an acceptable safety interval of 1–13 mg/day for adults [100]. Our results indicate that consumption of 38–50 and 500–650 macadamia kernels would amount to B intakes of between 1 mg and 13 mg, respectively.

The very high levels of cross-paternity among mature fruit of cultivar '816', irrespective of the B treatment, highlight the dependence of macadamia crop production on cross-pollination. Our results support previous findings that cultivars '816', 'A4', 'A16' and 'Daddow' are highly outcrossing in commercial orchards, even in the centre of single-cultivar blocks [15,27,67,101]. High levels of outcrossing have also been observed in

cultivars '246', '344', '800', 'A4' and 'A16' in a closely-interplanted multi-cultivar trial [102]. The amount of cross-pollination in orchards may depend on the travel distance of pollinators [15,27,103] and the flight range of pollinators in macadamia orchards is often small [104,105]. Cross-pollination in macadamia is generally achieved with the nearest cross-pollen cultivar [15,27,67,101,102]. Closer inter-planting of macadamia cultivars and strategic placement of beehives may be required to optimise cross-pollen flow in orchards, increase the frequency of cross-pollination, and improve nut yield and nut quality.

5. Conclusions

Our results clearly showed that B application at recommended rates during winter increased the initial fruit set of macadamia, but did not translate to a higher final fruit set or higher yield. Contrary to our hypotheses, B did not increase the proportion of self-pollinated fruit retained at maturity. Our results highlight the critical role of outcrossing for macadamia crop production and suggest that the productivity of orchards can be improved through strategic orchard design and pollinator management. Growers need to consider closely inter-planting different macadamia cultivars to increase the availability of cross-pollen and managing beehives to increase cross-pollen flow across orchards.

Supplementary Materials: Supplementary data is available online at <https://www.mdpi.com/article/10.3390/agronomy12030684/s1> and consists of the following. Figure S1: Meteorological data for the experimental area; Figure S2: Floral visitors at the experimental site; Figure S3: Floral and foliar concentrations of nitrogen (N), phosphorus (P) and potassium (K); Figure S4: Floral and foliar concentrations of aluminium (Al), calcium (Ca) and copper (Cu); Figure S5: Floral and foliar concentrations of magnesium (Mg), manganese (Mn) and iron (Fe); Figure S6: Floral and foliar concentrations of sodium (Na), sulphur (S) and zinc (Zn); Table S1: Correlations between nut-in-shell (NIS) mass and floral or foliar mineral nutrient concentrations; Table S2: Correlations between kernel mass and floral or foliar mineral nutrient concentrations; Table S3: Correlations between kernel recovery and floral or foliar mineral nutrient concentrations; Table S4: Correlations between incidence of whole kernels and floral or foliar mineral nutrient concentrations; Table S5: Correlations between kernel oil concentration and floral or foliar mineral nutrient concentrations; Table S6: Mineral nutrient contents of macadamia kernels.

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