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Boron Effects on Fruit Set, Yield, Quality and Paternity of Hass Avocado

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Abstract: Boron plays a critical role in pollination and fertilization and can affect fruit set and yield. We applied 0 g, 15 g (manufacturer recommendation) or 30 g boron pre-flowering to Hass avocado trees to determine the effects on fruit set, fruitlet paternity, yield, fruit size, mineral nutrient concentrations and fatty acid composition. The boron applications did not significantly affect the initial fruit set at 3 or 6 weeks after peak anthesis or the proportions of self-pollinated fruitlets or mature fruit. Approximately 88–92% of the mature fruit were self-pollinated. However, applying 30 g boron per tree reduced the fruit set at 10 weeks after peak anthesis by 56% and the final yield by 25%. Attaining > 90% of the maximum yield was associated with foliar boron concentrations being below 104 mg/kg at 6 weeks after peak anthesis and between 39 and 68 mg/kg at 28 weeks after peak anthesis. Applying 15 g boron per tree increased the fruit mass by 5%, fruit diameter by 2%, flesh mass by 9%, flesh boron concentration by 55%, and the relative abundance of unsaturated fatty acids by 1% compared with control trees. Applying the recommended amount of boron provided a good yield of high-quality avocado fruit but applying boron at double the recommended rate reduced the yield.

Keywords: boron; fatty acids; flowers; fruit size; mineral nutrients; *Persea americana*; plant nutrition; pollination; self-compatibility; self-fertility

1. Introduction

The human population is expected to reach 9.5 billion by 2050, and the demand for nutritious food is expected to increase by 56% over the same period [1]. Tree crops provide more than 600 million tons of food per year, mostly in the form of fruits and nuts [2–4]. However, tree crop yields are often limited by adverse climatic conditions, suboptimal crop nutrition, pests, diseases, poor pollination, premature fruit drop and low fruit quality [5–11]. The fertilisation of flowers and the initial set of fruitlets can be limited by insufficient pollen deposition, pollen tube arrest in the style, late-acting incompatibility in the ovule and inbreeding depression among self-fertilised embryos [12–14]. A subsequent phase of premature fruit drop is observed in mass-flowering tree crops such as mango, macadamia and lychee, even after successful pollination and initial fruit set [7,15–17]. Nutrient and carbohydrate partitioning within the tree during pollination, fertilisation and early fruit development can influence the initial fruit set and premature fruit drop, which can affect yield [6,7,15,18–23]. The high respiratory load of the large number of flowers and declining carbohydrate reserves have been considered causes of tree die-back [24].

Nutrient allocation within the tree during the later stages of fruit development also affects the quality of mature fruit, as the levels of nutrients such as boron, calcium, magnesium and nitrogen can influence the size, taste and postharvest quality of fruit [6,18,25].

Fruit of avocado (*Persea americana*, Lauraceae) are gaining popularity due to their rich flavour and health benefits [4,26]. Avocado fruit are enriched in monounsaturated fatty acids and have high levels of dietary fibre [26–28]. However, avocado production is constrained by poor pollination, flower abscission, premature fruit drop, inconsistent yield and poor fruit quality [8,29–35]. Avocado cultivars are categorised into two flowering types, type A and type B, according to the phenology of the opening of the female and male phases of the hermaphrodite flowers [36]. The flowers of type A cultivars such as Hass typically open in the female phase in the morning, close in the late morning and open in the male phase in the afternoon of the following day [36]. The flowers of type B cultivars such as Shepard typically open in the female phase in the afternoon, close in the late afternoon and open in the male phase on the morning of the following day [36]. Therefore, avocado orchards are usually established with a mixture of type A and type B cultivars to maximise the amount of pollen deposited on stigmas. However, avocado fruit set can occur through either self-pollination or cross-pollination [36,37]. Avocado trees are normally propagated clonally by grafting budwood from the scion cultivar onto seedling rootstocks [38]. Self-pollination occurs when pollen from one avocado cultivar is transferred to the stigma of the same cultivar, whereas cross-pollination occurs when pollen from one cultivar is transferred to the stigma of a different cultivar [14,36]. Avocado flowers are arranged in panicles, and these panicles can be determinate (terminating in a floral bud) or indeterminate (transitioning back to a vegetative apex) [39,40]. A mature avocado tree can have thousands of panicles that each contain 100–500 individual flowers, and so a tree can bear millions of flowers in one flowering season [40,41]. The initial fruit set is considered a limiting factor for avocado yield [42]. The panicle type and size, pollen limitation, poor pollen germination and fertilisation failure all influence the initial fruit set [8,31], and there is also a heavy premature fruit drop during the first 2 months after anthesis [31,36,39]. As a result, as little as 0.002–0.02% of avocado flowers develop into mature fruit [35,36].

Boron is an essential nutrient for fertilisation because it facilitates pollen germination and pollen tube growth [19,43,44]. High boron levels in pistils can increase the rates of pollen germination and pollen tube growth [19,45]. Boron concentrations in the styles of avocado flowers are positively correlated with the initial fruit set in boron-deficient trees [46,47]. Boron is largely transported through the xylem in most plants, although it is partially phloem-mobile in avocado and can be translocated from leaves to developing tissues such as flowers and fruit [48,49]. Boron application could improve the fruit set following self-pollination, because self-pollen tubes often grow more slowly than cross-pollen tubes and so they may take longer to reach the ovule, reducing the chances of self-fertilisation and fruit set [14,50]. However, no study has determined the effect of boron application on the proportions of self-pollinated avocado fruitlets and mature fruit. Furthermore, no study has determined relationships between avocado yield and floral or foliar boron concentrations.

Avocado tree productivity is determined not only by the quantity of mature fruit but also by their size and quality [29,30,33]. The commercial value is typically based on the fruit mass or diameter, with standardised cartons often used for transporting avocado fruit of different sizes [42,51,52]. Consumers are increasingly attracted to avocado fruit because of the healthy fatty acid profile, with a high ratio of unsaturated to saturated fatty acids in the fruit flesh [26,28,53]. Foliar application of boron to other mass-flowering tree crops such as almond, apple and citrus can increase the fruit size and improve fruit quality by intensifying the colour, increasing the total soluble solid concentrations and acidity and reducing the incidence of postharvest disorders [54–56]. Foliar or soil boron applications both increase fruit size in boron-deficient avocado trees [57–59], and high boron levels in avocado fruit are associated with improved postharvest quality, including a reduced incidence of flesh disorders and vascular browning [25,60]. However, there is currently limited knowledge on the effects of soil boron applications on avocado flesh nutrient concentrations and fatty acid composition.

Here, we investigated the effects of pre-flowering boron application on the initial set, paternity, yield and quality of Hass avocado fruit. We aimed to determine: (1) how boron application prior to flowering affects floral and foliar nutrient concentrations, fruit set, yield and the proportion of self-pollinated fruit; (2) relationships between fruit yield and boron concentrations in flowers or leaves; (3) how boron application prior to flowering affects fruit size and quality. We hypothesised that boron application would increase boron concentrations in the flowers and leaves, and as a result would increase fruit set, yield and the proportion of self-pollinated fruit. We expected a positive relationship between fruit yield and boron concentrations in flowers or leaves. Furthermore, we hypothesised that boron application would increase the fruit size and quality.

2. Materials and Methods

2.1. Study Site

We conducted the experiment in Eastridge avocado orchard (25°13′25′′ S 152°18′54′′ E), near Childers, Queensland, Australia. The orchard has a red clay-loam soil and contains the cultivars, Hass, Shepard, Carmen Hass and Maluma Hass, in large, single-cultivar blocks. We selected and tagged thirty trees on 26 July 2018 in the 82nd and 83rd rows of a 132-row block of 5-year-old Hass trees. The nearest other cultivar was Shepard, which was located at least 190 m from the experimental trees. The tree spacing was 10 m between rows and 5 m within rows. The study region had total rainfall of 545 mm and mean daily minimum–maximum temperatures of 7.7–26.0 °C in the coldest month (August) and 23.0–32.8 °C in the warmest month (February) during the study period of 26 July 2018 to 13 May 2019 [61] (Figure S1).

2.2. Experimental Design and Sample Collection

The thirty experimental trees were located in ten plots, with each plot containing three experimental trees as well as non-experimental buffer trees. Each experimental tree was separated from other experimental trees by at least one buffer tree. The plots had no boron applied between June and October 2018, except as stated below. We randomly assigned each of the three experimental trees within a plot to one of three boron treatments: (a) 0 g, (b) 15 g, or (c) 30 g of total boron per tree, applied as 0, 5 or 10 g of elemental boron per tree, respectively, on each of three occasions: 26 July 2018, 6 August 2018 and 16 August 2018. The treatments were applied by spraying the orchard floor under each tree with 5 L of an aqueous solution of Yara Soluble Boron™ (Yara, Karratha, Australia) on each occasion. The control trees received 5 L of water on each occasion. The intermediate application (15 g boron per tree) was based on the manufacturer recommendations.

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 m^3 quadrant on the illuminated side of each experimental tree. Flower visitors were counted in both the morning (08:00–11:30 h) and the afternoon (12:30–16:00 h) on three days during peak anthesis: 22 August 2018, 23 August 2018 and 24 August 2018.

We counted the number of flowers per panicle on three panicles from each experimental tree on 22 August 2018, during peak anthesis. The number of fruitlets retained on each tagged panicle was counted at 3, 6 and 10 weeks after peak anthesis. We also collected four branchlets of a panicle (including the flowers and stalk) from each tree at peak anthesis, and ten fruitlets from the canopy of each tree at 6 and 10 weeks after peak anthesis. A sample of four young fully expanded leaves was also collected from each tree at 0, 6, 10 and 28 weeks after peak anthesis.

We harvested sixteen mature fruit per tree in May 2019 (36 weeks after peak anthesis) using a stratified sampling design, with each tree divided into eight sectors, of which four sectors were on each side of the canopy. Two fruit were taken per sector, one from the inside and one from the outside of the canopy. These 16 fruit were weighed collectively and then stored in a cold room at 4 °C. We also harvested and counted all of the remaining fruit on each tree. The tree yield was calculated by multiplying the total number of fruit harvested per tree by the mean fruit mass determined from the 16-fruit sample.

Eight of the 16 fruit per tree were selected randomly after 7–21 days and weighed individually, and their length and diameter were recorded. These unripe fruit were dissected, and the seed and flesh (excluding the skin) were weighed. The other eight fruit per tree were stored at 4 °C for 22–28 days and then at 21 °C until ripe. Ripeness was measured using an FR-5120 fruit hardness tester with an 8-mm head (Lutron Electronic, Taipei, Taiwan). A fruit was considered ripe if the force required to punch a hole of approximately 1 mm in depth was less than 20 N. We recorded the mass, length, diameter and seed mass of each ripe fruit. We also recorded the mass of each fruitlet collected at 6 and 10 weeks after peak anthesis. We stored the samples of 6-week-old and 10-week-old fruitlets, mature fruit flesh and seeds at −18 °C until further processing.

2.3. Paternity Analysis

We used the syngamous tissue (i.e., the embryo and possibly endosperm) from each of the 6-week-old and 10-week-old fruitlets and approximately 70 mg fresh mass of each mature seed for genotyping. DNA extraction followed the glass–fibre plate DNA extraction protocol for plants [62]. Disposable 2.3 mm and 0.1 mm zirconia–silica beads and liquid nitrogen were added to samples prior to shaking on a TissueLyser II system (Qiagen, Hilden, Germany). Multiplex PCR reactions were carried out for each DNA sample to amplify the regions with unique single-nucleotide polymorphisms (SNPs) that were identified from the cultivars, Hass, Shepard and Maluma Hass [37,63]. The cultivars Hass and Carmen Hass share the same DNA sequences in these regions and may be isogenic or nearly so [63]. High-throughput genotyping to assign paternity to each fruitlet and mature seed was performed via the Agena MassARRAY platform (Agena Bioscience, San Diego, CA, USA) using 28 recently designed assays for avocado [37,63].

2.4. Mineral Nutrient Analysis

We used a representative subsample of at least 300 mg from the flowers, leaves and the flesh or seed of mature fruit to analyse the concentrations of 14 nutrients. The concentrations of carbon (C) and nitrogen (N) were determined by combustion analysis using a LECO CNS 928 analyser (TruSpec®, LECO Corporation, St. Joseph, MI, USA) [64,65]. 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 were analysed via inductively coupled plasma–optical emission spectroscopy (ICP–OES) (Vista Pro®, Varian Inc., Palo Alto, CA, USA) after digestion with a 5:1 mixture of nitric and perchloric acids [66,67].

2.5. Fatty Acid Analysis

One half of the flesh from each of the eight ripe fruit per tree was mashed finely to extract oil. We derivatised fatty acid methyl esters from the extracted oil and determined the fatty acid composition via gas chromatography–mass spectrometry (PerkinElmer Clarus 580 GC coupled to a PerkinElmer Clarus SQ8S MS) using the methods described previously [68,69]. We calculated the relative abundance of individual fatty acids in each sample by dividing the peak area of each individual fatty acid by the total peak area of all fatty acids in the sample and multiplying it by 100%.

2.6. Statistical Analysis

We analysed the effects of boron application on the fruit set; yield; fruit size (mass, length, diameter, seed mass and flesh mass); mineral nutrient concentrations in the flowers, leaves and mature fruit; relative abundances of fatty acids; and levels of self-paternity using generalised linear models (GLMs) with a Gaussian distribution and identity link function.

We tested the effects of boron application on the number of flower visitors and number of mature fruit using GLMs with a Poisson distribution and log link function. The boron treatment and plot were included in the models as fixed effects. Post hoc sequential Šidák tests were performed when differences were detected among the means. Relationships between the yield and boron concentration in flowers or leaves at 0, 6, 10 and 28 weeks after peak anthesis were assessed by determining the best-fit linear, quadratic, cubic, logistic, peak, sigmoidal, exponential, hyperbolic, rational or logarithmic equations. Statistical analysis was performed using SPSS 26.0 (IBM, Armonk, NY, USA) and curve fitting was performed using SigmaPlot 14.0 (Systat Software Inc., San Jose, CA, USA). Differences among treatments, or correlations, were considered significant at $p < 0.05$.

3. Results

3.1. Flower Visitors

Honey bees were the main flower visitors (Figure 1). We also observed some stingless bees, syrphid flies and other insects, including ants, beetles and wasps (Figure 1). We did not observe any non-insect flower visitors.

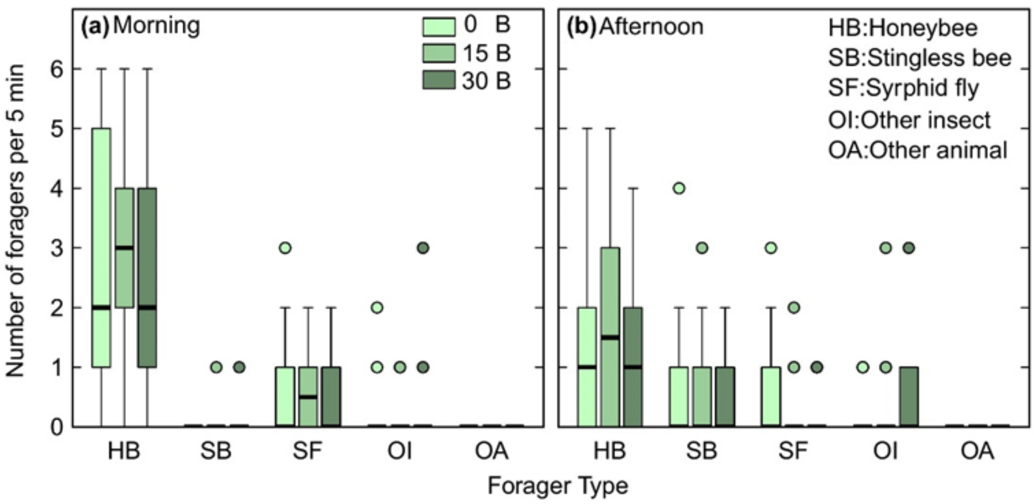

Figure 1. Numbers of flower visitors during 5-min periods in the (**a**) morning and (**b**) afternoon in a 1 m^3 section of Hass avocado trees treated with 0, 15 or 30 g boron (B) per tree prior to anthesis. Medians are presented with 25th and 75th percentiles (boxes), 10th and 90th percentiles (whiskers) and outliers (circles). Medians within a flower visitor type do not differ significantly among boron treatments within the morning or the afternoon (generalised linear models, $p > 0.05$, $n = 30$).

3.2. Floral and Foliar Nutrient Concentrations

Floral boron concentrations were higher in trees treated with 30 g boron per tree than 0 g boron per tree (Figure 2a). The foliar boron concentrations did not differ significantly at peak anthesis but were higher at 6, 10 and 28 weeks after peak anthesis in trees that received 15 or 30 g boron per tree than control trees (Figure 2b). The differences in the floral or foliar concentrations of other nutrients were negligible or not significant (Table S1).

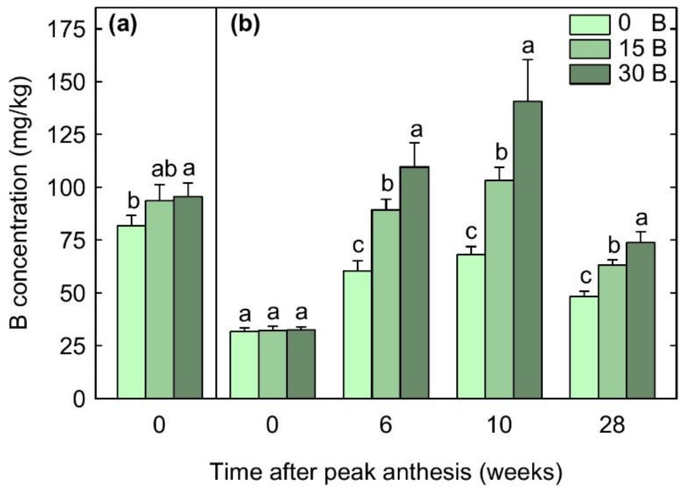

Figure 2. (**a**) Floral and (**b**) foliar boron concentrations of Hass avocado trees treated with 0, 15 or 30 g boron (B) per tree prior to anthesis. Means (+SE) with different letters within a time point are significantly different (generalised linear models, $p < 0.05$, $n = 10$ trees).

3.3. Fruit Set and Yield

The boron application did not significantly affect the fruit set at 3 or 6 weeks after peak anthesis, but 30 g boron per tree reduced the fruit set at 10 weeks after peak anthesis by 56% when compared with control trees (Figure 3).

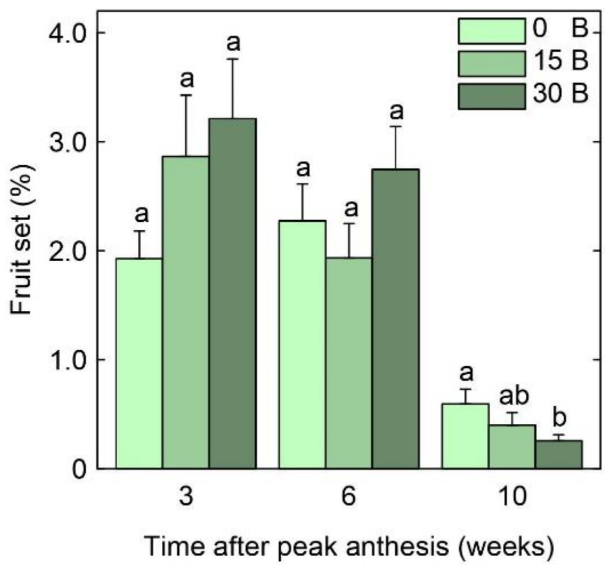

Figure 3. Fruit sets of Hass avocado trees at 3, 6 and 10 weeks after peak anthesis. Trees were treated with 0, 15 or 30 g boron (B) per tree prior to anthesis. Means (+SE) with different letters within a time point are significantly different (generalised linear models, $p < 0.05$, $n = 30$ panicles).

The application of 30 g boron per tree reduced the yield by 25% (Figure 4a) and the number of mature fruit by 26% (Figure 4b) when compared with control trees.

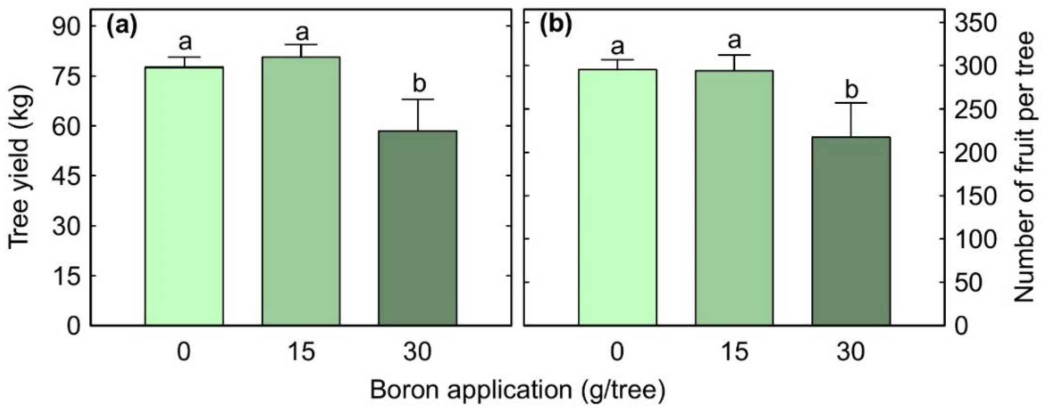

Figure 4. (**a**) Yield and (**b**) number of fruit harvested from Hass avocado trees treated with 0, 15 or 30 g boron (B) per tree prior to anthesis. Means (+SE) with different letters are significantly different (generalised linear models, $p < 0.05$, $n = 10$ trees).

3.4. Levels of Self- and Cross-Paternity

The boron application did not significantly affect the levels of self- and cross-paternity among fruitlets at 6 or 10 weeks after peak anthesis or among mature fruit (Figure 5). Most mature fruit (88 ± 4%–92 ± 2%) arose from self-pollination. All cross-pollinated fruitlets and fruit were pollinated by cultivar Shepard.

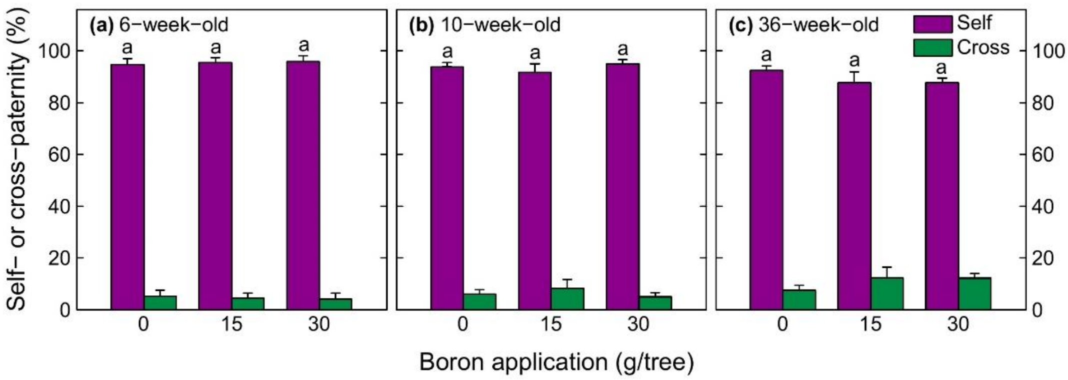

Figure 5. Percentages of self-pollinated and cross-pollinated (**a**) 6-week-old fruitlets, (**b**) 10-week-old fruitlets, and (**c**) 36-week-old fruit (i.e., mature fruit) in the canopies of Hass avocado trees treated with 0, 15 or 30 g boron (B) per tree prior to anthesis. Means (+SE) within a time point do not differ significantly (generalised linear models, $p > 0.05$, $n = 10$ trees).

3.5. Relationships between Yield and Boron Concentrations

The yield did not have a significant relationship with floral or foliar boron concentrations at peak anthesis (Figure 6a,b). However, the yield showed significant quadratic relationships with foliar boron concentrations at 6, 10 and 28 weeks after peak anthesis (Figure 6c–e). The attainment of at least 90% of the maximum yield was associated with foliar boron concentrations at 6 or 10 weeks after peak anthesis being lower than 104 or 117 mg/kg, respectively (Figure 6c,d). Greater than 90% of the maximum yield was associated with foliar boron concentrations at 28 weeks after peak anthesis being between 39 and 68 mg/kg (Figure 6e).

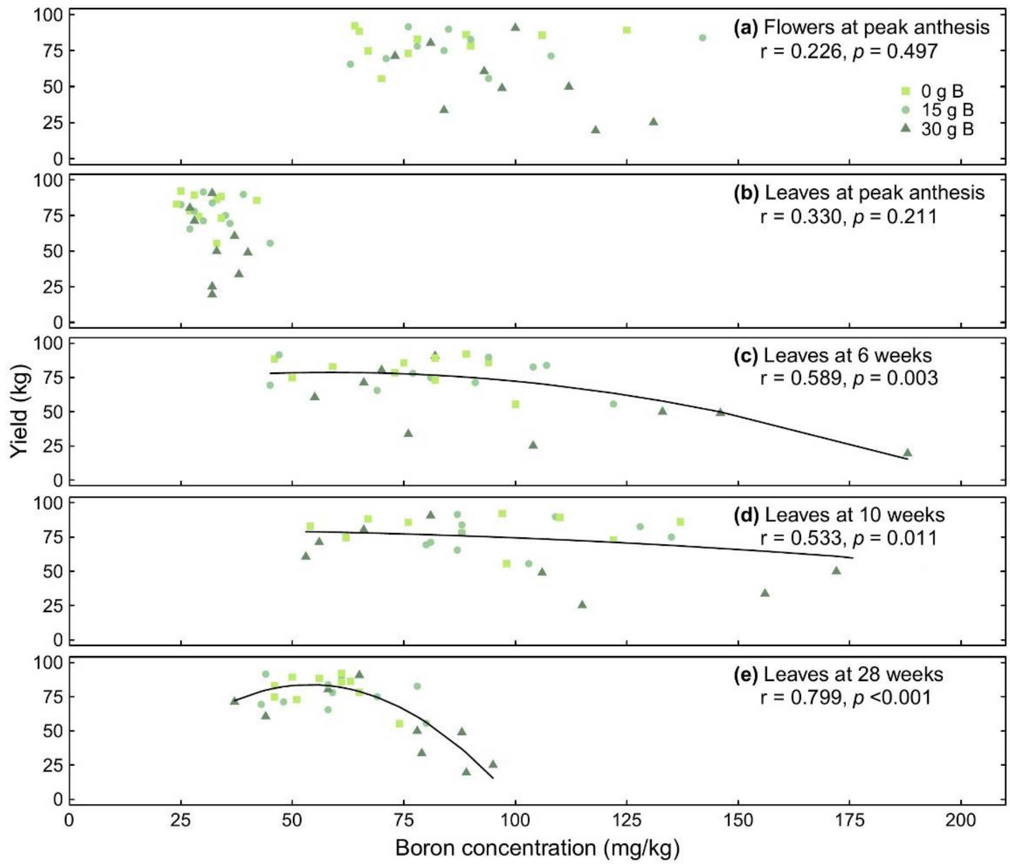

Figure 6. Relationships between final yields of Hass avocado trees treated with 0, 15 or 30 g boron (B) per tree prior to anthesis and boron concentrations of (**a**) flowers at peak anthesis; (**b**) leaves at peak anthesis; (**c**) leaves at 6 weeks after peak anthesis: yield = $65.60 + 0.45[B_6] - 3.79 \times 10^{-3}[B_6]^2$; (**d**) leaves at 10 weeks after peak anthesis: yield = $80.06 + 0.02[B_{10}] - 7.61 \times 10^{-4}[B_{10}]^2$; (**e**) leaves at 28 weeks after peak anthesis: yield = $-34.28 + 4.38[B_{28}] - 0.04[B_{28}]^2$, where $[B_6]$, $[B_{10}]$ and $[B_{28}]$ are the foliar boron concentrations at 6, 10 and 28 weeks after peak anthesis, respectively (n = 30 trees).

3.6. Fruit Size

The boron applications did not significantly affect the fruitlet mass at 6 or 10 weeks after peak anthesis (Figure 7a,b). However, boron-treated trees produced 5% or 6% heavier mature fruit than control trees (Figure 7c). The boron applications increased the mature fruit diameter by 2%, without affecting the fruit length significantly (Table 1). The boron applications increased the flesh mass by 8% or 9% without affecting the seed mass significantly (Table 1).

Table 1. Length, diameter, flesh mass and seed mass of mature fruit from Hass avocado trees treated with 0, 15 or 30 g boron per tree prior to peak anthesis.

Fruit Parameter	Boron Application (g/tree)		
	0	15	30
Length (cm)	9.94 ± 0.05 a	9.92 ± 0.06 a	9.97 ± 0.06 a
Diameter (cm)	7.24 ± 0.03 b	7.40 ± 0.04 a	7.39 ± 0.04 a
Flesh mass (g)	179.5 ± 2.9 b	195.4 ± 4.4 a	193.6 ± 3.8 a
Seed mass (g)	42.1 ± 0.8 a	40.1 ± 1.0 a	40.6 ± 1.0 a

Means ± SE within a row with different letters are significantly different (generalised linear models, $p < 0.05$, n = 160 fruit for length, diameter, and seed mass, n = 80 fruit for flesh mass).

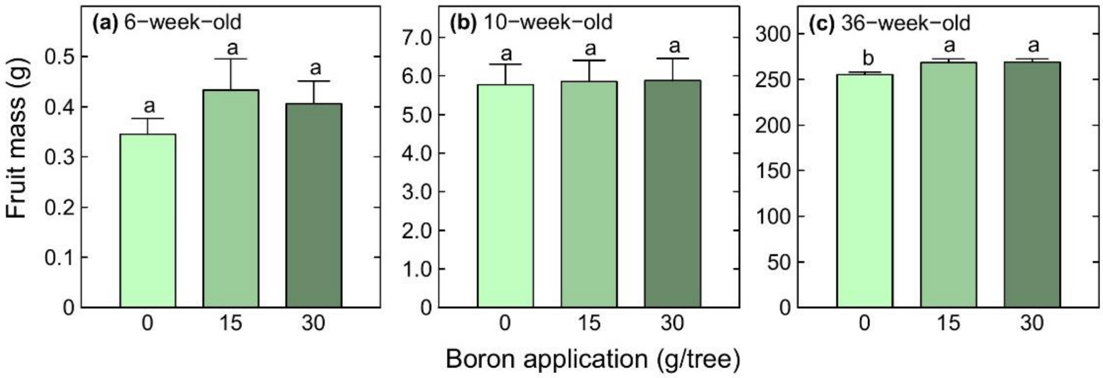

Figure 7. Masses of individual fruitlets or fruit at (**a**) 6 weeks, (**b**) 10 weeks and (**c**) 36 weeks after peak anthesis on Hass avocado trees treated with 0, 15 or 30 g boron (B) per tree prior to anthesis. Means (+SE) with different letters are significantly different (generalised linear models, $p < 0.05$, $n = 81–91$ 6-week-old fruitlets, $n = 99–100$ 10-week-old fruitlets, and $n = 160$ mature fruit).

3.7. Mineral Nutrient Concentrations

The application of 15 g boron per tree increased the boron concentrations in the flesh and seed by 55% and 96%, respectively (Table 2). The application of 30 g boron per tree increased the boron concentrations in the flesh and seed by 67% and 107%, respectively (Table 2). The application of 15 or 30 g boron reduced the aluminium concentrations in the flesh by 24% and 35%, respectively, and the application of 30 g boron reduced the calcium concentration in the flesh by 12% when compared with control trees (Table 2). The boron treatments did not significantly affect the concentrations of most other mineral nutrients (Table 2).

Table 2. Mineral nutrient concentrations * in the fruit flesh and seed from Hass avocado trees treated with 0, 15 or 30 g boron per tree prior to peak anthesis.

Sample Type	Nutrient	Boron Application (g/tree)		
		0	15	30
Flesh	Boron (B)	2.46 ± 0.09 c	3.81 ± 0.13 b	4.10 ± 0.12 a
	Carbon (C)	15.3 ± 0.2 a	15.4 ± 0.2 a	15.7 ± 0.2 a
	Nitrogen (N)	443 ± 13 a	473 ± 13 a	480 ± 13 a
	Phosphorous (P)	56.6 ± 1.1 b	59.6 ± 1.4 ab	60.9 ± 1.2 a
	Potassium (K)	575 ± 11 a	570 ± 12 a	598 ± 11 a
	Aluminium (Al)	0.071 ± 0.010 a	0.054 ± 0.004 b	0.046 ± 0.003 b
	Calcium (Ca)	7.18 ± 0.21 a	6.65 ± 0.21 ab	6.33 ± 0.23 b
	Copper (Cu)	0.560 ± 0.030 a	0.650 ± 0.037 a	0.667 ± 0.036 a
	Iron (Fe)	0.624 ± 0.025 a	0.661 ± 0.026 a	0.605 ± 0.022 a
	Magnesium (Mg)	28.52 ± 0.39 a	28.21 ± 0.38 a	28.11 ± 0.38 a
	Manganese (Mn)	0.510 ± 0.020 a	0.520 ± 0.015 a	0.485 ± 0.016 a
	Sodium (Na)	8.12 ± 0.39 a	8.28 ± 0.47 a	7.87 ± 0.49 a
	Sulphur (S)	29.6 ± 1.3 a	29.0 ± 1.4 a	29.3 ± 1.2 a
	Zinc (Zn)	0.744 ± 0.015 b	0.799 ± 0.015 a	0.774 ± 0.015 ab
Seed	Boron (B)	1.36 ± 0.09 b	2.67 ± 0.16 a	2.82 ± 0.16 a
	Carbon (C)	20.5 ± 0.2 a	19.7 ± 0.3 a	20.1 ± 0.3 a
	Nitrogen (N)	483 ± 17 a	515 ± 16 a	516 ± 15 a
	Phosphorous (P)	65.4 ± 1.4 a	70.2 ± 1.7 a	65.0 ± 2.2 a
	Potassium (K)	571 ± 10 a	584 ± 12 a	554 ± 13 a
	Aluminium (Al)	0.050 ± 0.004 a	0.053 ± 0.004 a	0.051 ± 0.004 a
	Calcium (Ca)	8.45 ± 0.60 a	9.94 ± 0.98 a	8.28 ± 0.60 a
	Copper (Cu)	0.406 ± 0.016 a	0.400 ± 0.012 a	0.367 ± 0.015 a
	Iron (Fe)	1.06 ± 0.03 a	1.07 ± 0.03 a	1.02 ± 0.03 a
	Magnesium (Mg)	38.8 ± 1.2 a	39.1 ± 1.3 a	35.4 ± 1.3 a
	Manganese (Mn)	0.623 ± 0.032 b	0.799 ± 0.040 a	0.595 ± 0.027 b
	Sodium (Na)	0.490 ± 0.060 a	0.429 ± 0.052 a	0.530 ± 0.060 a
	Sulphur (S)	34.9 ± 1.1 a	35.3 ± 1.3 a	36.2 ± 1.4 a
	Zinc (Zn)	0.605 ± 0.019 a	0.646 ± 0.017 a	0.603 ± 0.019 a

* Nutrient concentrations are expressed in mg/100 g fresh mass, except the C concentration is expressed as %. Means ± SE within a row with different letters are significantly different (generalised linear models; $p < 0.05$; $n = 80$ fruit or seeds).

3.8. Fatty Acid Composition

The boron applications did not significantly affect the relative abundances of most fatty acids in the flesh (Figure 8a). However, fruit from trees that received 15 g boron had a lower relative abundance of the saturated fatty acid (SFA), palmitic acid. As a result, the fruit from trees that received 15 g boron had a 1% higher relative abundance of total unsaturated fatty acids (UFAs) than fruit from other trees (Figure 8b).

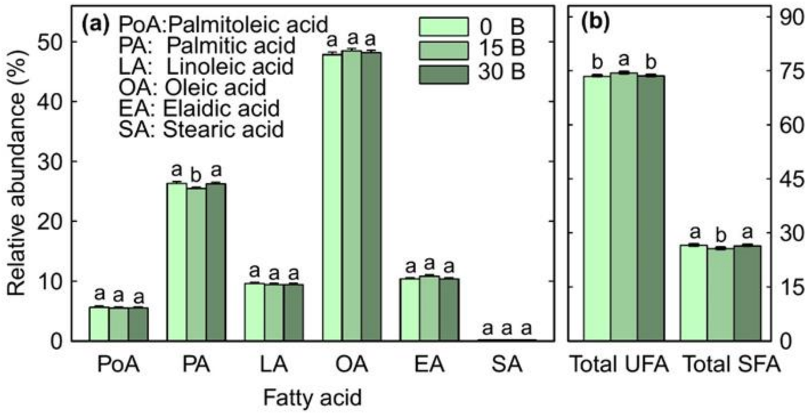

Figure 8. Relative abundances of (**a**) individual fatty acids and (**b**) total unsaturated fatty acids (total UFAs) and total saturated fatty acids (total SFAs) in ripe fruit from Hass avocado trees treated with 0, 15 or 30 g boron (B) per tree prior to anthesis. Means (+SE) with different letters within a fatty acid are significantly different (generalised linear models, $p < 0.05$, $n = 80$ fruit).

4. Discussion

We hypothesised that boron applications would increase boron concentrations in avocado flowers, and as a result would increase the initial fruit set by increasing the proportion of self-pollinated fruitlets. We found that boron applications elevated the boron concentrations in flowers and leaves but did not increase initial fruit set at 3 and 6 weeks after peak anthesis. Our paternity analysis demonstrated that boron applications did not affect the proportion of self-pollinated fruitlets. Most fruitlets were the result of self-pollination. The high proportion of self-pollinated fruitlets at our study site could be due to much greater deposition of self-pollen than cross-pollen, as the experimental trees were located in a wide block of Hass trees, and the nearest other cultivar, Shepard, was at least 190 m away. Our results confirm that Hass is a highly self-compatible cultivar. High rates of selfing have also been found among the fruitlets and mature fruit of Hass trees in Israel and Spain [70–72]. The self-compatibility of this cultivar is partly the result of the anthesis patterns of avocado cultivars. Hass flowers typically open in the female phase in the morning, close in the late morning and open in the male phase in the afternoon of the following day [36]. The flowers of the other cultivar at the site (Shepard) typically open in the female phase in the afternoon, close in the late afternoon and open in the male phase on the morning of the following day [36]. The synchronous opening of the female and male phases of these two cultivars promotes cross-pollination, potentially reducing self-pollination [36,73]. However, overlap between the female and male phases can occur within a cultivar, depending on climatic conditions such as the daylength and temperature [36]. As a result, the outcrossing rates in avocado trees often depend on the distance between cultivars of type A (e.g., Hass) and type B (e.g., Shepard), because cross-pollen transfer is greater when flowers of type A and B cultivars are in close proximity [37,72,74].

Applying the recommended amount of 15 g boron per tree did not affect the fruit set at 10 weeks after peak anthesis, mature fruit number or yield. However, applying 30 g boron per tree reduced the fruit set at 10 weeks after peak anthesis by 56%, which translated into a 26% reduction in mature fruit number and a 25% reduction in yield. Low fruit set in avocado has been associated with boron deficiency [46,47]. However, the reduced fruit set at 10 weeks after peak anthesis following the application of 30 g boron per tree could be the result of boron toxicity. Foliar boron concentrations above 104 or 117 mg/kg during spring at 6 and 10 weeks after peak anthesis, respectively, were associated with reduced yield. Foliar boron concentrations above 68 mg/kg during autumn, prior to harvest, were also associated with reduced yield. The optimal foliar boron concentrations during autumn in our study, 39–68 mg/kg, were similar to previously recommended foliar boron levels of 40–60 mg/kg during summer [75]. Our results confirm that there can be a narrow range between boron deficiency and toxicity in avocado trees [60].

The boron applications increased fruit mass by 5–6%, diameter by 2% and flesh mass by 8–9%. The higher fruit mass was due to higher flesh mass, as the seed mass did not increase significantly following boron applications. Growers are paid a premium for cartons containing larger fruit [42,51,52,76]. For example, carton sizes 40, 48 and 60 in California carry fruit of decreasing value, weighing from 270 to 325 g, 213 to 269 g and 178 to 212 g, respectively [42,51]. Therefore, our results indicate that boron application can increase the commercial value of avocado fruit by slightly increasing the fruit mass and fruit diameter.

The boron applications greatly increased the flesh boron concentration and slightly increased the relative abundance of total UFAs. The postharvest quality affects consumer perceptions when purchasing or repurchasing avocado fruit [77]. High boron concentrations in the flesh can reduce the incidences of flesh discoloration, vascular browning and body rot [25]. Furthermore, boron provides health benefits such as improving bone formation and maintenance, facilitating insulin action, increasing glucose metabolism and alleviating arthritis symptoms [78,79]. The recommended daily boron intake is between 1 and 28 mg/day for a 70 kg adult [80,81]. Our results indicate that consuming 0.13–0.23 fruit per day would provide the recommended minimum daily intake and 3.54–6.36 fruit per day would provide the recommended maximum daily intake of boron. The application of 15 g boron per tree also increased the relative abundance of total UFAs in the fruit. However, this increase was minor (1%) and the relative abundances of fatty acids in the fruit from all three boron treatments were within the standard ranges reported previously for Hass fruit [82]. Avocado consumption improves blood lipid profiles, supports weight management and promotes healthy ageing, while reducing the risk of metabolic syndrome [26–28].

5. Conclusions

The boron applications to avocado trees did not affect the initial fruit set or the proportions of self-pollinated fruitlets and self-pollinated mature fruit. Most fruitlets and mature fruit were self-fertilised, confirming that Hass is a highly self-compatible cultivar. Applying a higher-than-recommended rate of boron reduced the fruit set at 10 weeks after peak anthesis, and this translated into a reduced mature fruit number and yield. Foliar boron concentrations below 104 mg/kg during spring and between 39 and 68 mg/kg during autumn, were associated with achieving at least 90% of the maximum yield. Boron application at recommended rates increased fruit mass, fruit diameter, flesh mass, flesh boron concentrations and the relative abundance of total unsaturated fatty acids. Monitoring foliar boron levels and adjusting boron application schedules are recommended as strategies to produce a good yield of large and nutritious avocado fruit.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12061479/s1. Figure S1: Average daily (**a**) rainfall and (**b**) maximum (●–●) and minimum (○–○) temperatures at Bundaberg (24°53′55′′ S 152°19′20′′ E), Australia, for each week of the study period. Table S1: Mineral nutrient concentrations in flowers and leaves at 0, 6, 10 and 28 weeks after peak anthesis of Hass avocado trees treated with 0, 15 or 30 g boron per tree prior to flowering.

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