

Is boron transport to avocado flowers regulated by carbohydrate supply?

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Low carbohydrate reserves have been cited as a cause of alternate bearing in avocado, and boron deficiency has been associated with poor tree growth and low productivity. Our recent work has demonstrated that perseitol, an important transport sugar in avocado, can complex with boron to make boron phloem mobile. This suggests that boron transport from avocado leaves could be regulated by carbohydrate supply. We tested this hypothesis using branch girdling treatments to manipulate the supply of carbohydrates from leaves to flowers and examined if these treatments would also affect the supply of boron from leaves to flowers. Branch girdling treatments were applied at different phenological stages to 'Hass' avocado trees with high (ON) and low (OFF) crop loads. Girdling treatments applied to ON cropping trees increased the rate of boron loss from leaves during winter and increased the boron content of flowers at mid-bloom in spring. Leaf D-Mannoheptulose concentration, the storage form of perseitol, declined during winter at a similar timing to that of leaf boron. These results are consistent with boron supply to flowers being linked to carbohydrate flows to inflorescences. Few differences were found between leaf carbohydrate concentrations in ON and OFF cropping trees. However, leaf boron concentrations were consistently lower in ON cropping trees, which suggests that in ON cropping years fruit are a major competing sink for boron and could result in less boron being available to support flower and early fruit development in the following spring.

Es de transporte de boro en aguacate flores regulado por suministro de carbohidratos?

Las bajas reservas de carbohidratos han sido citadas como causal de la frutacion alterna en el aguacate, y la deficiencia de boro ha sido asociada con problemas de crecimiento de las plantas y baja productividad. Nuestro reciente trabajo ha demostrado que el perseitol, principal transportador de azucares en el aguacate, puede formar complejos con el boro para hacerlo mobil en el floema. Esto sugiere que el transporte del boro en las hojas de aguacate podrian ser reguladas por el suministro de carbohidratos. Nuestra hipotesis fue que el anillado de las ramas, una tecnica utilizada para manipular el suministro de carbohidratos a las flores, tambien afectara al abastecimiento de boro. Tratamientos de anillado de ramas fueron aplicados en distintos estados fenologicos para manipular el suministro de carbohidratos y de boro a las flores de arboles de aguacates 'Hass' con altas (ON) y bajas (OFF) cargas de frutos. Escasas diferencias fueron encontradas entre las concentraciones de carbohidratos en hoja de arboles de altas y bajas cargas. Sin embargo, las concentraciones de boro fueron consistentemente mas bajas en hojas de arboles de alta carga (ON), lo que sugiere que los frutos podrian ser importantes competidores acumulando boro. La concentracion de boro en hoja decrecio en invierno en tiempo similar a la D-Mannoheptulose, la forma de almacenaje del perseitol. Los tratamientos de anillado aplicados a arboles de alta carga (ON) aceleraron la perdida de boro de las hojas y tambien aumento el contenido de boro de las flores en el periodo inmediatamente previo a la antesis. Esto condice con que el suministro de boro este asociado al abastecimiento simultaneo de carbohidratos a las inflorescencias.

Keywords: carbohydrates, boron, girdling, flowering, alternate bearing

Introduction

Typically 'Hass' avocado trees produce more than 1 million flowers but just 0.1% of these are needed to produce an economic crop. A slight variation in percent fruit set can shift trees from bearing practically no fruit, an OFF cropping year, to producing too many fruit, as is often observed in an ON cropping year. Research by Plant & Food Research in New Zealand, in collaboration with the New Zealand Avocado Industry Council (AIC) and science groups in California and Spain, aims to determine causal factors behind poor fruit set and the resulting major fluctuations in yield of avocados. A particular focus of our research concerns the partitioning of the 7-carbon sugar D-Mannoheptulose and its polyol form, perseitol (Liu et al. 2002). These are major forms of non-structural carbohydrates found in unusually high amounts in avocado and perseitol is a component of phloem sap transported from leaves into growth sites, including flowers and developing fruit.

This research has demonstrated that boron can become phloem mobile in avocado by forming a complex with perseitol (Minchin et al. unpublished), which suggests that boron transport from avocado leaves could be regulated by carbohydrate allocation patterns. It is known that avocado flowers with high starch content are more likely to set fruit than flowers with low starch content (Alcaraz et al. 2010) but no studies have examined the boron content of avocado flowers themselves. In this paper we provide a summary of work we have done using branch girdling to alter carbohydrate allocation patterns in order to test if the supply of perseitol from leaves to flowers could mediate supply of boron to flowers and thereby affect fruit set.

Materials and Methods

Experiments were conducted using large (>7 years old) 'Hass' avocado trees growing on 'Zutano' seedling rootstocks at three orchards near Tauranga, New Zealand (37° 41' S; 176° 12' E).

Leaf samples were collected from Orchard 1 at monthly intervals from July until August in the following year, from 3 ON-cropping and 3 OFF-cropping trees. Samples were analysed for carbohydrate and boron content. All samples were taken from the mid-point along terminal, current season's shoots from well-illuminated parts of the canopy, 2-3 m above the ground. Samples for carbohydrate analyses were extracted using 80% ethanol and analysed using DIONEX ICS-3000 Reagent-Free™ IC (RFIC™) system with a CarboPac PA20 column. Samples for boron analysis were extracted using nitric acid-perchloric acid digestion, followed by inductive coupled plasma-optical emission spectroscopy (ICP-OES: Integra XL, FBC, Hampshire IL, USA).

Branch girdling treatments to alter carbohydrate allocation patterns were established at Orchard 2; one pair of branches of similar size, aspect and crop load was tagged on each of 18 trees selected to be in either their ON- or OFF-cropping year. One of the paired branches on each tree was girdled on 15 April (autumn) and the other paired branch was left intact with no girdle. At Orchard 3, four similar branches were selected on each of 15 trees, each judged to have moderate crop loads. Girdles were applied to a different set of branches on 30 March, 14 May and 23 September so that each tree had one branch girdled at each date, with the remaining fourth branch on each tree left as an un-girdled control. In both projects, girdles were made by removing a full circle of bark (approx. 6 mm wide) at the base of the terminal two-year-old growth section on each branch. Leaf samples were collected when girdles were applied and again at flowering in spring, for analysis of carbohydrates and boron as previously described. Flower samples were collected in spring.

Branch girdling treatments at Orchard 2 were analysed using a split-plot design (crop load*girdling) using SAS software (SAS OnlineDoc® 9.2 Cary, NC: SAS Institute Inc., 2008). The interaction crop load*girdling was not found to be significant for all variables and so has not been included. Paired t-Tests (MINITAB® R 15.1) were then used to compare treatment means from girdled branches with the non-girdled (intact) controls.

Results and Discussion

Leaf starch concentrations at Orchard 1 increased rapidly in spring, to be at their maximum values during early summer (December/January), before quickly declining in late-summer (Figure 1). Leaf boron concentrations also increased during spring and summer then declined during winter, well after the rapid decline in starch concentrations. Seasonal changes in the 6-carbon sugars sucrose, glucose and fructose were relatively minor in all tissues sampled (data not shown). As with previous studies (Liu et al. 1999), we found the 7-carbon sugar D-Mannoheptulose and its polyol form perseitol to be the major carbohydrates in all avocado tissues sampled. Leaf D-Mannoheptulose concentrations increased substantially during summer, before gradually declining during winter to be at their lowest values in spring, just before flowering (Figure 2). Leaf boron concentrations followed a similar pattern to that of leaf D-Mannoheptulose. Leaf boron concentrations were consistently higher in leaves from OFF-cropping trees than from ON-cropping trees, while D-Mannoheptulose concentrations were similar on both groups of trees.

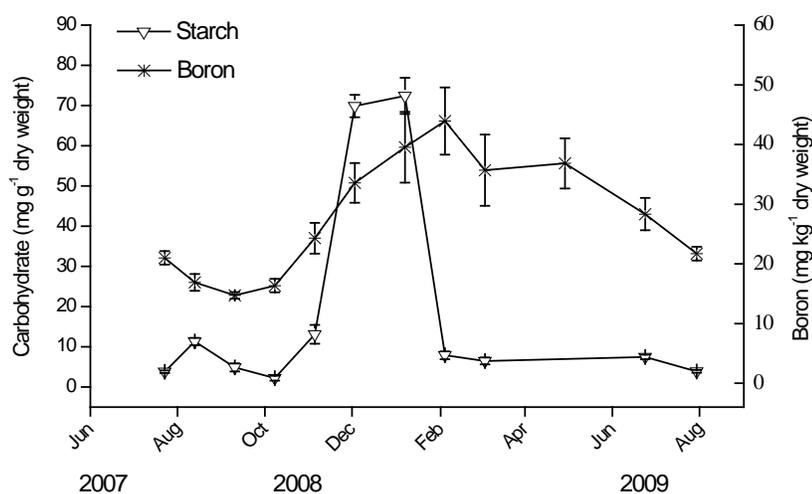


Figure 1. Seasonal changes in starch and boron concentrations in leaf tissue from 'Hass' avocado trees. Flowering occurred during October/November. Values are tree averages \pm 1 SE.

The effect of branch girdling treatments applied at Orchard 2 on carbohydrate distribution from leaves during the period from autumn to mid-bloom was dependent upon on crop load. With girdled branches on ON-cropping trees, leaf starch concentrations were similar (18.2 and 17.4 mg g⁻¹ in autumn and spring, respectively), whereas with OFF-cropping trees, leaf starch concentrations increased from 11.5 to 31.1 mg g⁻¹ between autumn and spring, as the leaves stored carbohydrate that would otherwise have been exported to fruit distal to (above) the girdle. On all branches, leaf perseitol and boron concentrations decreased between April and October, whereas D-Mannoheptulose concentrations remained relatively constant (data not shown).

Crop load did not affect the composition of flowers in spring, but girdling did increase the boron concentration in flowers at mid-bloom (Table 1). While leaf boron concentrations tended to be lowest in leaves on girdled branches (Figure 1), on these same girdled branches boron concentrations in flowers were on average higher, by almost 50%, than those on non-girdled control branches (Table I). Similar results were recorded at Orchard 3. Here branch girdling one month before flowering, during the inflorescence extension phase, increased the concentrations of both 7-carbon carbohydrates (D-Mannoheptulose and perseitol) and boron in developing flowers, but reduced the concentrations of the 6-

carbon sugars and starch (Table II). Although not always statistically significant, this was a consistent trend for all girdling treatments, to result in more boron and 7-carbon carbohydrates in flowers but less starch and 6-carbon sugars.

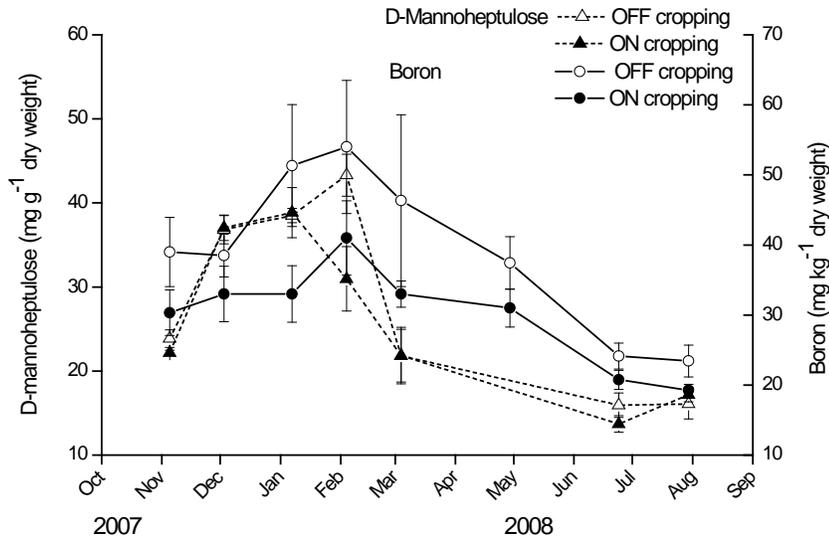


Figure 2. Seasonal changes in D-Mannoheptulose and boron in leaf tissue from spring flush shoots on OFF- and ON-cropping 'Hass' avocado trees. Flowering occurred during October/November. Values are tree averages \pm 1 SE.

Table I. Carbohydrate (starch, sucrose, D-mannoheptulose and perseitol) and boron concentrations in flowers on girdled and non-girdled branches on 'Hass' avocado trees at Orchard 2. Flower samples were collected at mid-bloom in October.

	Starch ¹ (mg g ⁻¹)	Sucrose (mg g ⁻¹)	D-Mannoheptulose (mg g ⁻¹)	Perseitol (mg g ⁻¹)	Boron (mg kg ⁻¹)
Girdled	18.5	34.9	22.9	17.2	56.0
Intact	20.7	39.9	23.1	18.4	38.2
<i>P-value</i>	0.257	0.118	0.844	0.312	0.005
<i>Significance</i>	NS	NS	NS	NS	**

¹ Significance: * $P < 0.01$; NS = not significant.

Table II. Carbohydrate (starch, sucrose, D-mannoheptulose and perseitol) and boron concentrations in flowers on girdled and non-girdled (intact) branches on 'Hass' avocado trees at Orchard 3. Girdling treatments were applied on 30 March, 14 May and 23 September. All tissues were collected at mid-bloom in late-October.

Girdling treatment	Starch¹ (mg g⁻¹)	Sucrose (mg g⁻¹)	D-Mannoheptulose (mg g⁻¹)	Perseitol (mg g⁻¹)	Boron (mg kg⁻¹)
No girdle	23.4 ± 0.7	53.7 ± 1.8	14.3 ± 0.5	23.5 ± 1.1	46.6 ± 2.7
30-Mar	20.1 ± 0.7***	46.5 ± 1.3*	14.8 ± 0.5	23.4 ± 0.7	47.5 ± 3.0
14-May	21.5 ± 1.0	50.0 ± 2.1	14.4 ± 0.9	23.7 ± 1.2	51.0 ± 4.0
23-Sep	20.9 ± 0.8*	47.6 ± 2.6*	16.3 ± 0.8 ^(*)	27.7 ± 1.9*	56.4 ± 3.8***

¹ Significance: (*) P<0.10; * P < 0.05; *** P < 0.001 are significance of difference against the non-girdled (intact) control branch.

Conclusions

Several responses from this study demonstrate a close relationship between the supply/transport of the 7-carbon sugar D-Mannoheptulose, and its polyol form perseitol, and the supply of boron to avocado flowers via the phloem. On terminal shoots, leaf boron concentrations decreased at a similar rate and timing to the decline in D-Mannoheptulose, which was after leaf starch had already dropped to its lowest concentration. These are the shoot types that produce the next season's flowers and fruit. Branch girdling during winter increased boron concentrations and tended to increase D-Mannoheptulose in flowers. Leaf boron concentrations during winter tended to be lowest in leaves on autumn-girdled branches, while on these same girdled branches boron concentrations in flowers in spring were higher than in non-girdled control branches, sometimes by almost 50%. This result supports our hypothesis that perseitol forms a complex with boron and thus enables the export of boron from leaves to flowers via the phloem, following the same route as sugars (Minchin et al. 2011 unpublished).

Relatively high leaf boron concentrations were recorded in OFF-cropping trees compared with those in ON-cropping trees. Avocado fruit are an important sink for boron and it is likely that fruit have a relatively constant demand for boron to support the new cell division that continues throughout the time the fruit are on the tree (Schroeder 1953). It is therefore possible that the presence of fruit causes a shortage of boron in current season's leaves and that this limits the availability of boron for transport to flowers, which in turn reduces flower quality, lowers the probability of fruit set and thus contributes to alternate bearing. If this is correct then growers should be applying boron to meet the demands of the growing fruit so that there is sufficient boron accumulation in the leaves to supply the next season's flowers, rather than applying boron to the flowers themselves in spring.

Acknowledgements

We thank Colin Jenkins, Steve Bryant and the staff at Ngai Tukairangi Trust Orchard, Ron and Chris Bailey and Terry Davies, for assistance and access to their avocado orchards. We also thank Michael Blattmann, Tom Lear, Ella Maxwell and Sam Ong Eng Chye for assistance with fruit harvests and collection of tissue samples, Darienne Voyle for carbohydrate analyses, and Nihal De Silva for advice and assistance with statistical analyses. This project was partly funded by the New Zealand Avocado Industry Council, the New Zealand Foundation for Research Science and Technology (Contract No. C06X0708) and Horticulture Australia Limited (HAL), using voluntary contributions from The New Zealand Institute for Plant & Food Research Limited and matched funds from the Australian Government.

References

Alcaraz, M.L., Hormaza, J.I. and Rodrigo, J. (2010). Ovary starch reserves and pistil development in avocado (*Persea americana*). *Physiologia Plantarum* 140 (4): 395-404.

Liu X, Sievert JR, Arpaia ML and Madore MA 2002: Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado. *Journal of the American Society for Horticultural Science* 127(1): 108–114.

Schroeder, C.A. (1953). Growth and development of the Fuerte avocado fruit. *Proceedings of the American Society for Horticultural Science* 61: 103-109.