Can phloem supply of nutrients at flowering affect the cropping potential of avocado?

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Abstract

The phloem is a key structure in the supply of carbohydrates to developing plant tissues, including the flower. Inadequate supply of nutrients via the phloem could result in poor flower quality and low fruit set. Phloem sap composition is regulated by nutrient availability from the mature leaf in addition to strategies for loading, translocation and unloading of these nutrients along the supply pathway. There has been a wide range of assessments of phloem nutrient supply strategies for many different plant types, with the aim of understanding possible limitations of fruiting potential and subsequent production. In the case of avocado, it has been reported that there are unusual carbohydrates which are synthesised within the mature leaf and subsequently transported via the phloem. To investigate the role of the phloem in the supply of nutrients to the avocado flower, and how this may affect the processes of pollination and fruit set in different regions/environments, we have monitored the phloem sap content (carbohydrates and nutrients) at the flower over a 24-hour period during flowering in 'Hass' avocado orchards in New Zealand, Australia and California. We found that three major carbohydrates, sucrose, perseitol and mannoheptulose, plus boron appear to be key nutrients supplied by the phloem for flowering and fruit set. Early in this area of research, we have identified some possible relationships between boron, perseitol and 'heavy' and 'light' flowering and have begun investigating the effects of changing temperature on the potential supply of these compounds to flowers.

Introduction

The phloem is the main conduit for supplying carbohydrates and many of the essential mineral nutrients required for the growth and development of plant tissues. Carbohydrates are the main constituent of the phloem, although the types of carbohydrates transported and their relative amounts differ between plant species. The type of carbohydrates and their amounts found within phloem sap has also been found to change diurnally (Klages et al, 2001). Sucrose is the most abundant carbohydrate found within the phloem of herbaceous plants, however polyols and raffinose series sugars are also found to be translocated in the phloem of some species for example mannitol in celery (Noiraud et al, 2001) and raffinose series sugars in cucurbits (Zhang et al, 2010). Although sucrose is also a key component of the phloem in perennial plants, there are many examples where phloem comprises similar or greater portions of carbohydrates other than sucrose, for example mannitol in olives (Liakopoulos et al, 2005), sorbitol in apples (Klages et al, 2001), and in avocado where perseitol and mannoheptulose have been found in the leaf phloem in similar or greater amounts than sucrose (Liu et al, 2002; Cowan, 2004).

The methods used within the literature to collect phloem sap for assessment vary from collecting exudate from excised stylets of aphids that were feeding from the phloem, allowing the phloem sap to bleed from a cut surface into a collecting vessel, and using a pressure chamber to force out the sap into syringes (Cowan, 2004; Turgeon & Wolf, 2009). Most species (including avocado) will not naturally bleed large amounts of phloem sap from a wound so the addition of EDTA (ethylenediamine tetraacetic acid) is required to stop the plants natural wound response from blocking the exudation. Each of these techniques has well known advantages and disadvantages highlighting the difficulty of carrying out research on the phloem transport system (Rennie & Turgeon, 2009).

The studies so far carried out on avocado have reported the contents of phloem sap as it is exported from the leaf or subsampled phloem exudate from woody tissue. These investigations found that perseitol and mannoheptulose are phloem mobile (Liu et al, 2002; Cowan, 2004) and present in the

phloem in similar amounts to that of sucrose. We have looked at the content of phloem sap collected from the inflorescence stem of avocados during flowering. Given the potential importance of carbohydrate and boron supply in pollination and fruitset, the aim of this work was to establish a phloem sampling method that would allow us to examine phloem carbohydrate and boron content as it is delivered to the flower. Phloem sap was collected from four different experiments providing an insight into the effects of temperature, tree cropping status and region on phloem sap contents.

Materials and Methods

Phloem sap was collected from trees at approximately 50% flowering. To collect phloem sap, the flower, open in its female form was cut from the inflorescence under 5mM EDTA solution leaving the pedicle in place on the tree. A 1.5ml Eppendorf tube containing 5mM EDTA in agarose at pH 7 (Klages et al. 1998) was inserted over the cut surface of the pedicle and held in place on the tree using Blutak© (Figure 1). The Eppendorf tubes were left in place for 24 hours. Since EDTA can be phytotoxic to plant tissue at high concentrations, a preliminary experiment was carried out using a range of EDTA concentrations, enabling us to select 5mM as the optimum concentration for phloem collection.

To allow us to account for solute exudation from cells at the cut surface that do not originate from the phloem, each sample collected under EDTA was paired with a second sample which was cut under distilled water and collected in agarose without the EDTA present. Following sap collection for 24 hours, samples were collected, frozen and stored at -80^{o}C until analysis, except the samples from Australia which were freeze dried before transport back to New Zealand for analysis.

Experiment 1
'Hass' trees located at Te Puke Research Centre in the Bay of Plenty, New Zealand (37.81544^{o} S, 176.32623^{o} E) were selected as being 'heavy' or 'light' flowering. Six apical flower stems on an inflorescence on a branch on each tree were selected.

Experiment 2
At the University of California South Coast Research and Extension Centre, Irvine, USA (33.66483^{o} N, 117.84193^{o} W) in the flowering season following the New Zealand one, 'Hass' 'heavy' or 'light' flowering trees were selected and phloem was collected from these as in the experiment in Te Puke.

Experiment 3
In September 2010 two orchards close to Toowoomba, Australia: Key Road (27.35996^{o}S, 152.06130^{o}E) and Shearer Road (27.37929^{o}S, 152.16452^{o}E) were selected that had different temperature ranges during flowering. The Shearer Road site was selected as it was deemed to be a standard orchard that experienced classical signs of irregular cropping, whereas the Key Road site was selected because the trees sampled all underwent heavy pruning regimes. 'Heavy' or 'light' flowering trees were selected in each orchard; at the Shearer Road orchard the 'light' flowering trees were assumed to be in an 'on' cropping stage of irregular cropping (Figure 2B) whilst at the Key Road orchard the 'light' lowering trees were a result of heavy pruning (Figure 2D).

Experiment 4
The final experiment was set up to test the hypothesis that increasing tree temperature will alter phloem sap composition, and potentially increase sap boron and perseitol content (Mary Lu Arpaia, pers. comm.). Four-year-old flowering 'Hass' trees in 40L pots were used for phloem collection. These trees were moved in and out of tunnel houses to raise and lower their temperature during the flowering season. Phloem sap samples were collected at the inflorescences under the different temperature regimes.

Results and discussion

Three experiments were set up in Te Puke, New Zealand; California, USA; and Toowoomba, Australia to allow measurements to be made of the phloem sap carbohydrate and boron contents delivered to the flowers of heavy and light flowering trees (experiments 1,2 and 3 respectively). Sucrose, perseitol and mannoheptulose were found to be the key carbohydrates found within the phloem. Values for the amounts of total carbohydrates (Figure 3), perseitol (Figure 4) or boron (Figure 5) did not differ greatly between the trees sampled in Australia or New Zealand. However both total

carbohydrate and perseitol content of the phloem sap collected in California were several orders of magnitude lower than that from sap collected in Australia or New Zealand. The reason for this is unclear as it is unlikely to be due to a two-fold reduction in sap concentration or flow rate, but it is perhaps associated with a difference in the length of time the phloem sap will exude from the cut surface when tissue was removed. A shorter exudation time would give less solute content in the collection vial.

There did not appear to be a significant effect of flower intensity on the phloem sap total carbohydrate content collected at flowering. In three of the four sets of heavy versus light flowering trees investigated there was no effect of flowering intensity upon the amount of sap total carbohydrate collected (ANOVA; $P>0.05$; Figure 3). The exception was the Shearer Road site at Toowoomba which had three times the total carbohydrate in the heavy flowering trees compared with the light flowering trees (ANOVA; $P<0.05$). However, heavy flowering was associated with increased sap perseitol content in all experiments, with a significant increase shown in the Shearer Road trees and the Te Puke trees (ANOVA; $P<0.05$; Figure 4). Likewise, the heavy flowering trees all showed increased boron content of the phloem sap (ANOVA; $p<0.05$; Figure 5). If phloem sap boron content is dependent upon the availability of perseitol in the phloem sap, further work should examine the mechanism leading to increased perseitol in the phloem sap. For example, it would be informative to ascertain whether the relationship between phloem contents and flowering intensity is caused by the larger inflorescence's acting as a strong sink for the perseitol and 'pulling' it into the flower, or whether a high perseitol content promotes development of the inflorescence.

Following observations by Arpaia and colleagues (unpublished) that phloem sap perseitol content increased in orchard grown avocado trees following periods of temperature increases of at least 10^{o}C over a number of days, a fourth experiment was set up. Four-year-old potted avocado trees were moved from cooler temperatures outside into a warmer plastic house. Phloem sap was collected from inflorescences both before moving the plants into the plastic house and again after the plants had been in the plastic house for at least seven days. The maximum temperature of the plastic house was about 6-9^{o}C higher than that of the outside.

Using a Wilcoxon signed-rank test to compare changes in phloem sap total carbohydrate, perseitol and boron content, increasing the temperature that trees experienced in the plastic house significantly increased the sap boron and perseitol content ($P<0.05$), but had no effect upon the total carbohydrate content of the phloem sap. Using changes in temperature to alter the phloem sap composition allowed a comparison between the perseitol and boron content of the sap. The relationship between perseitol and boron content of the phloem sap followed the pattern where an increase in boron was correlated with an increase in perseitol content (Figure 6). These results support the theory that perseitol is required to transport boron to the avocado flowers via the phloem, and that a reduction in the supply of perseitol to the flower may lead to a reduction in flower boron content which could potentially affect the ability of the flower to be pollinated and set fruit (Iwai et al, 2006; Wimmer & Goldbach, 2007). Furthermore, our results shown here suggest that further work is merited on the relationship between tree temperature and fruit set behaviour with avocado.

Conclusions

- We found that three major carbohydrates, sucrose, perseitol and mannoheptulose, plus boron were key nutrients supplied by the phloem at flowering.
- We also made some preliminary observations of relationships between flowering intensity and the supply of perseitol and boron to the flower.
- Using trees exposed to alternating temperature regimes, we showed that the phloem sap boron content was correlated to the amount of perseitol within the phloem sap.

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Figure 1. Small Eppendorf tubes were attached to the cut end of avocado inflorescences at mid-bloom to collect samples of phloem sap. This allowed us to quantify the type and amounts of carbohydrate and boron being transported to the flowers.

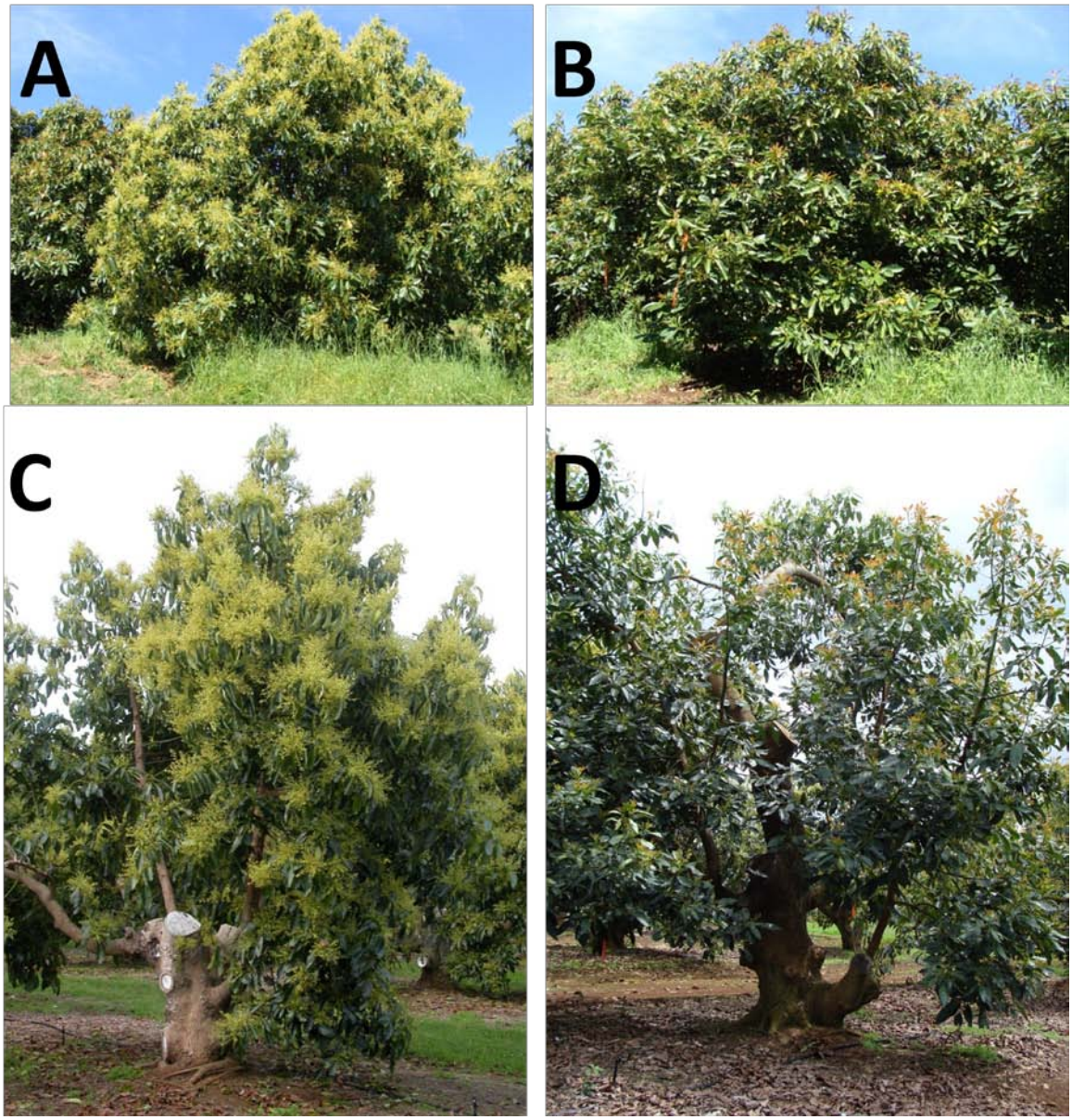

Figure 2. Examples of ‘heavy’ (A,C) and ‘light’ (B,D) flowering ‘Hass’ avocado trees near Towoomba, Australia. Phloem sap was collected from these trees to determine the effect of crop load in the previous season on the quantities of carbohydrates and boron that were being delivered to flowers.

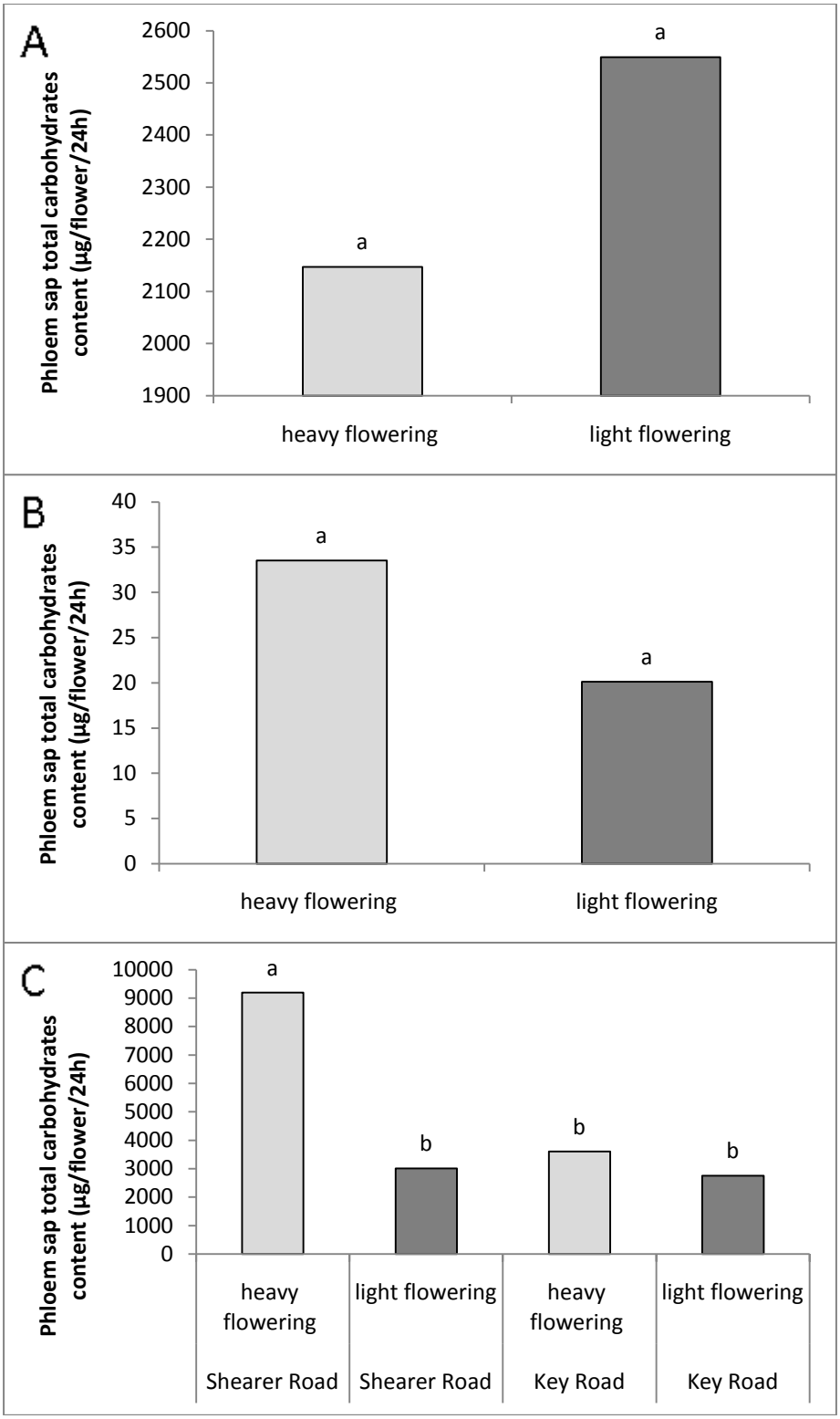

Figure 3. Total carbohydrate content of avocado sap collected from phloem tissue supplying the inflorescences at mid-bloom. Sap was collected from trees grown at Te Puke, New Zealand (A), California, USA (B), and Toowoomba, Australia (C). Bars with different letters represents significant differences between the means ($P<0.05$).

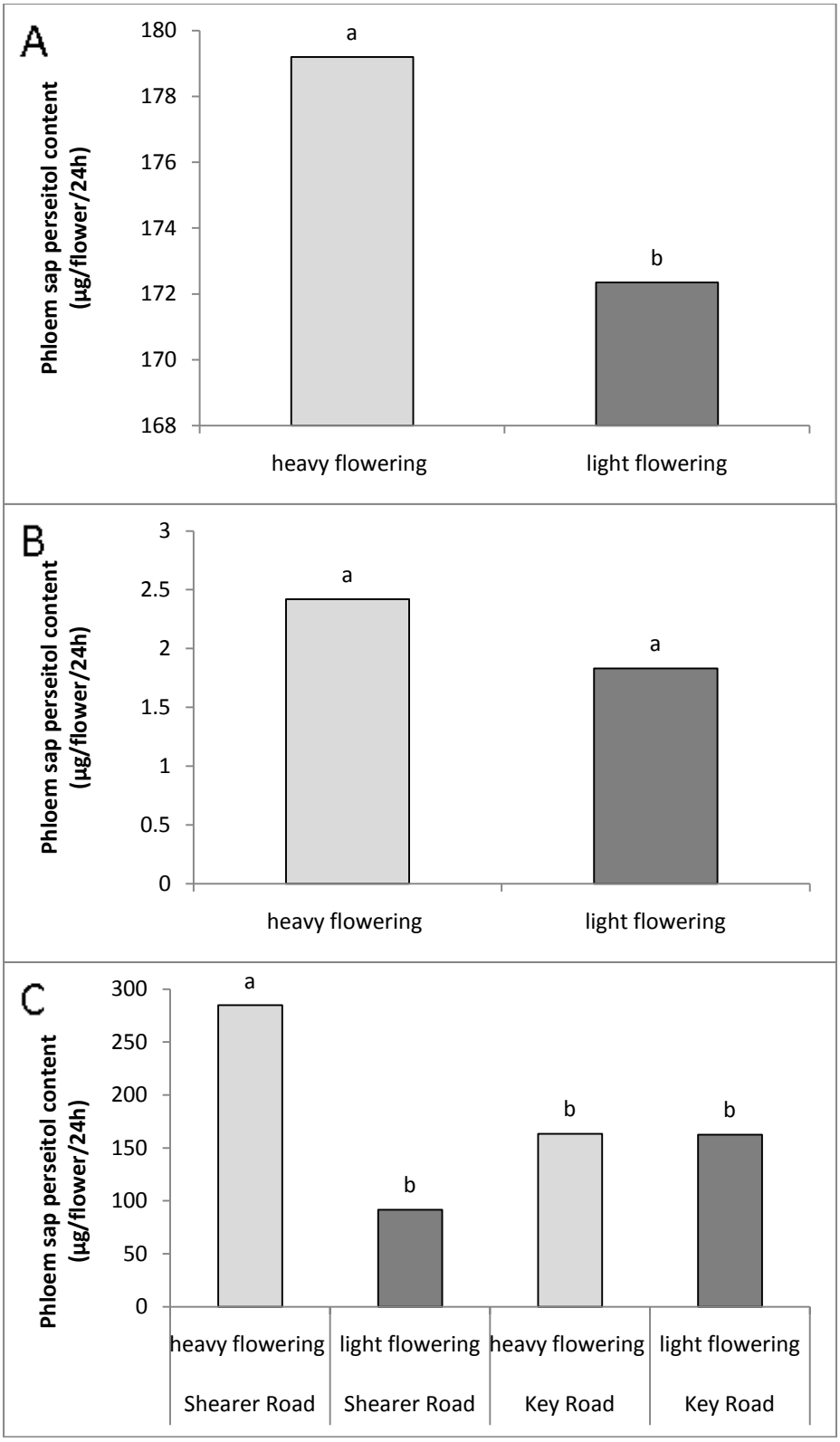

Figure 4. Perseitol content of avocado sap collected from phloem tissue supplying the inflorescences at mid-bloom. Sap was collected from trees grown at Te Puke, New Zealand (A), California, USA (B), and Toowoomba, Australia (C). Bars with different letters represents significant differences between the means ($P<0.05$).

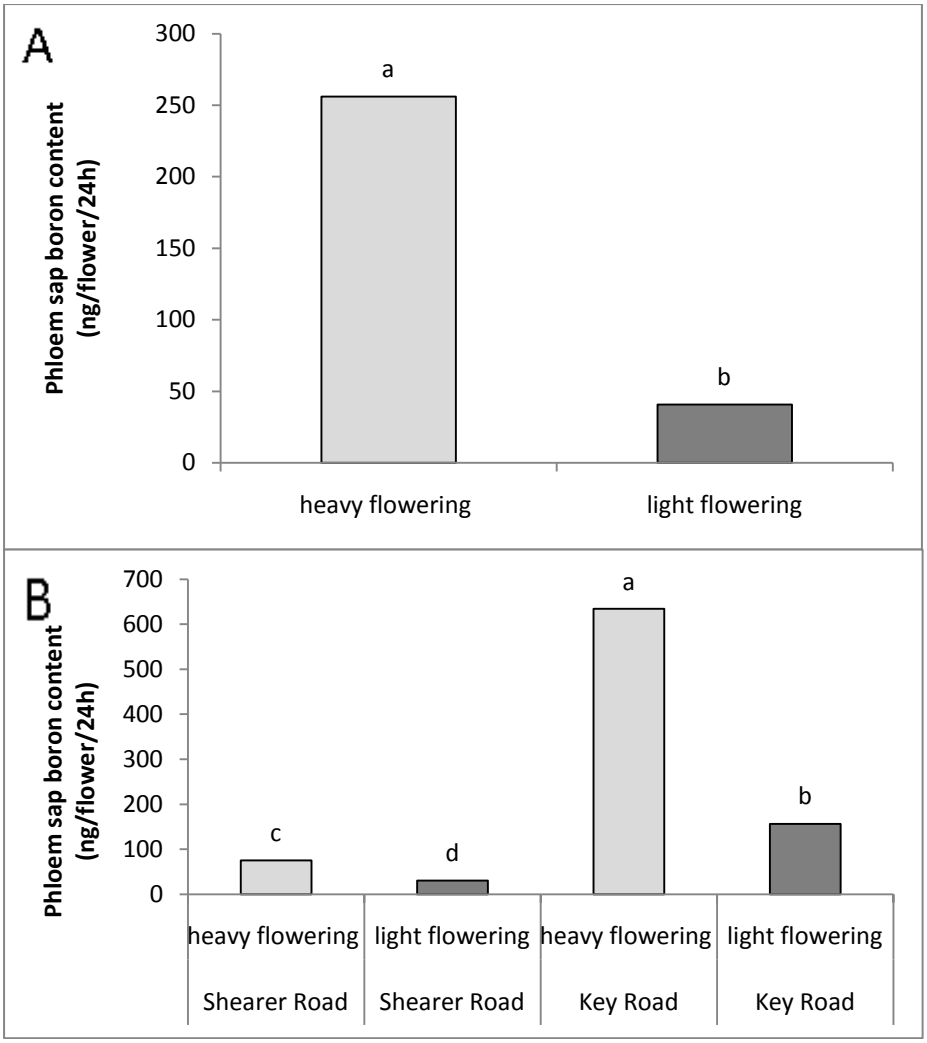

Figure 5. Boron content of avocado sap collected from phloem tissue supplying the inflorescences at mid-bloom. Sap was collected from trees grown at Te Puke, New Zealand (A), and Toowoomba, Australia (B). Bars with different letters represents significant differences between the means ($P<0.05$).

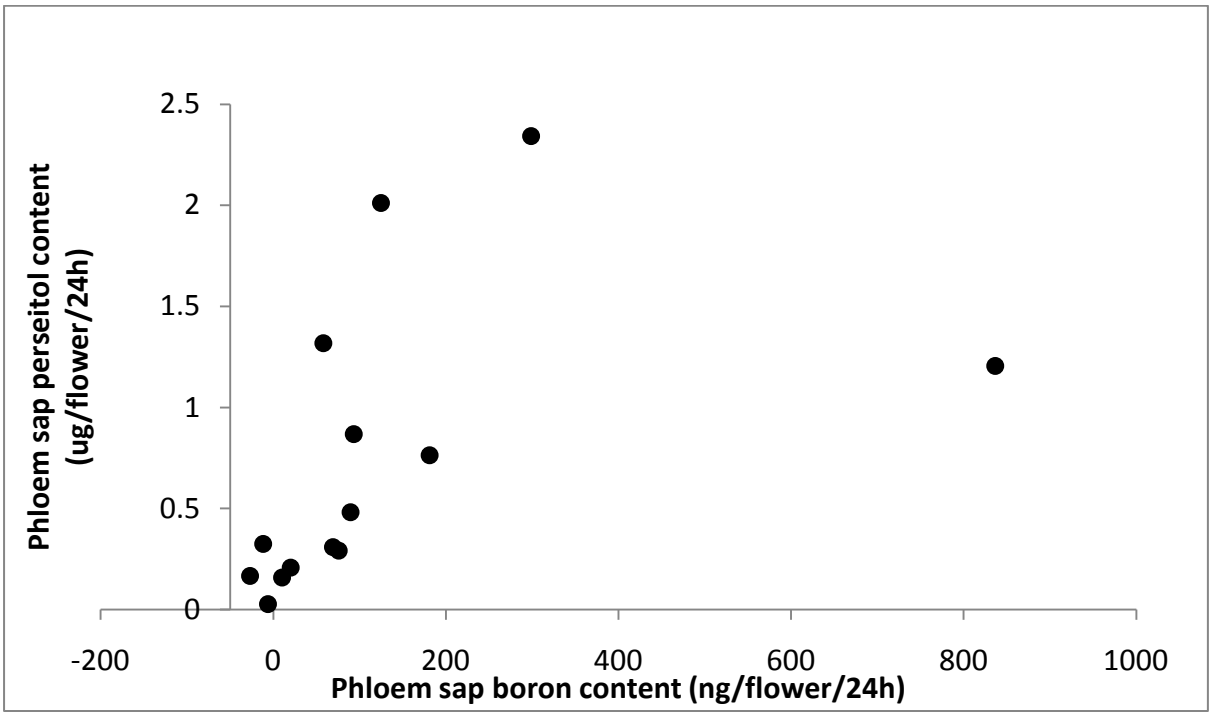

Figure 6. The relationship between boron and perseitol content measured in phloem sap collected in avocado trees that were grown for at least seven days either outside or in a plastic house before sampling (Experiment 4).