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RESEARCH NOTE

Phloematic mobility of ^{10}Boron in kiwifruit (*Actinidia deliciosa)* mixed shoots

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Abstract

C. Sotomayor, R. Ruiz, and L. Muñoz. 2012. Phloematic mobility of ^{10}Boron in kiwifruit (*Actinidia deliciosa)* mixed shoots. Cien. Inv. Agr. 39(3): 563-567. Boron (B) is an essential micronutrient showing restricted mobility in plants, except in species that synthesize polyols. The ability of kiwifruit to mobilize B through phloem transport has not yet been confirmed, despite its ability to synthesize the polyol myo-inositol. This study examined kiwifruit plants in which boric acid enriched with the stable ^{10}B isotope was applied to the distal leaves of mixed shoots with flowers. At 24, 72 and 144 h, both ^{10}B-treated and un-treated leaves and flowers from treated shoots were sampled and subsequently analyzed via mass spectrometry to determine the resulting ^{11}B/^{10}B ratios. Control leaves and flowers showed a natural ratio, varying between 3.82 and 4.05. In contrast, the ratios in treated leaves were 1.57 at 24 h,h 2.06 at 72 h (both of which are significantly different from the control) and similar to the control again after 144 h, at 3.83h. In the flowers from shoots with treated leaves, the ratios were 1.31 at 24 h, h1.48 at 72 h (both different from the control) and 3.67 at 144 h (similar to the control). These results indicate that the B solution was absorbed through the leaves and then rapidly re-translocated to B-demanding sinks, such as flowers, within the first 72 h. This finding clearly indicates that kiwifruit can transport boron through the phloem, which is essential knowledge for the correction of leaf and flower B deficiencies through foliar applications.

Key words: Boron, foliar absorption, kiwifruit, phloem mobility, stable isotope.

Introduction

Boron (B) is an essential micronutrient for normal plant growth and development in higher plant species and is known to affect a series of important physiological processes, particularly cell division and the formation of cell walls and membranes, phloem generation, the metabolism of carbohydrates and auxins, RNA synthesis, the absorption and transport of cations (especially Ca), and processes associated with plant reproduction, such as flowering and fruit setting (Bolanos *et al.,* 2004). More recently, published studies have

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attributed specific functions to B that are essential for the structural stability of cell walls and the maintenance of membrane functionality (Yu *et al*., 2002). B is absorbed by plant roots through mass flow in the form of undissociated boric acid. Then, following the transpiration stream, it is transported through the xylem (Basso *et al.,* 1986). In vascular plants, B moves passively from the roots along with the transpirational flow and accumulates in sinks such as leaves and shoots, where the retranslocation of B is restricted, as it becomes fixed in the apoplast (Sattelmacher, 2001). B has traditionally been considered to be relatively immobile in the phloem of many dicotyledonous species, and thus, a continual re-supply of this element was thought to be needed to achieve normal plant growth (Tanaka and Fujiwara, 2008). However, in the last several years, B has been found to be quite mobile in certain fruit species, such as in cherries, almonds, apricots, peaches, olives, grapes, pears, apples and plums (Konsaeng *et al*., 2005).

There are two naturally occurring, stable isotopes of boron, with approximately 80.1% corresponding to ^{11}B and 19.9% to ^{10}B (Barth, 1997). Foliarly applied boric acid enriched with ^{10}B allows the detection of the movement of B into different plant tissues using mass spectrometry to compare the resulting isotope content against the naturally occurring $^{11}B/^{10}B$ isotope ratio (Huang *et al*., 2008). Studies based on comparison of $^{11}B/^{10}B$ isotope ratios have demonstrated that in some fruit species, such as apples and almonds, foliar applications of B in autumn temporarily increase the B concentrations in leaves, but that toward the end of autumn and in early winter, B is mobilized towards the cortex (Brown and Hu, 1996). In spring, B is re-mobilized from the cortex towards the flowers and increases fruit setting (Kerrien *et al.,* 1997). In vegetable species, which translocate a considerable portion of their photoassimilates through the phloem in the form of sugar-alcohols, B is freely transported from mature organs to growing tissues (Liakopoulos *et al*., 2005). In some species of the *Malus*, *Pyrus*, and *Prunus* genera, it has been found that B mobility in the phloem depends on the formation of stable complexes with sorbitol. Tree species that produce sorbitol include the European and Asian pears, apples, peaches, cherries, apricots, plums, almonds, and quinces (Tanaka and Fujiwara, 2008).

Sorbitol is a polyol (a six-carbon alcohol) that possesses a specific function, as the end product of photosynthesis in many temperate fruit species, especially in Rosaceae. In these species, a polyol-B-polyol complex is formed in the photosynthetic tissues and is then transported by the phloem to sinks such as regions of active growth in the vegetative and reproductive meristems (Brown and Hu, 1996; Tanaka and Fujiwara, 2008).

In kiwifruit (*Actinidia deliciosa* cv. Hayward), the accumulation of B in older leaves and its relatively low concentration in roots and stems appear to indicate that this species lacks a mechanism for excluding B. Furthermore, the occurrence of high concentrations of this element in mature leaves in comparison with younger leaves could be evidence of potential immobility of B in the phloem of this species (Sotiropoulos *et al*., 2004).

On the other hand, Bieleski *et al*. (1997) determined that *Actinidia* contain unusually high levels of inositol (a sugar-alcohol), reaching 20% of the total soluble carbohydrates in leaves during the growing season and up to 40% of the soluble carbohydrates in parts of the fruit half-way though their development. In kiwis, inositol has been specifically identified in the form of the isomer myo-inositol. Klages *et al*. (1997) suggested that myo-inositol could be transported via the phloem from leaves to fruit.

It can therefore be proposed that myo-inositol, as a polyol (sugar-alcohol), should facilitate the movement of B through the symplastic route, as these sugars (e.g., galactitol/ dulcitol, mannitol, sorbitol, inositol) can easily form complexes with boric acid without enzymatic action (Jutamanee *et al*., 2002). In this way, B can be transported

to active sinks such as apical meristems and can, thus, accumulate in meristematic regions or in growing fruit of *Actinidia deliciosa*.

Sotomayor *et al*. (2010) applied boric acid to the distal leaves of mixed shoots of 'Hayward' kiwis and achieved significant increases in the concentration of boron in nearby flowers within 24 h. This effect lasted for 96 h. These results demonstrated the mobility of B through the phloem between leaves and flowers on the same shoot.

In the present study, it is hypothesized that isotopic boron applied to leaves will be absorbed and mobilized through the phloem towards the flowers and developing fruit within a short, but determined period of time. The specific objectives of this study are to determine the mobility of boron through the phloem of kiwi shoots through the use of boric acid enriched with ^{10}B and to identify the resulting $^{11}B/^{10}B$ ratios within sink organs (flowers and fruit).

Materials and methods

This experiment was conducted in a 20-yr-old 'Hayward' kiwi orchard planted with 'Matua' and 'Tomuri' pollenizers in Nogales in the V Región of Chile (32º44'06'' S and 71º14'12'' W) during the 2008/2009 growing season, over a period of just six days. Following a split plot design in randomized blocks with five replications, individual plants with a similar size and health were selected, each of which was marked on 5 mixed determinate shoots with both leaves and flowers. In November 2008, a solution of 0.5 g L^{-1} of boric acid **(**a concentration very similar to that commonly used in the field) enriched with 99% ^{10}B was sprayed to the run-off point on 3 leaves distal to the flowers on mixed shoots when they were in the process of opening. In the controls, only water was sprayed. Leaf and flower samples from the treated plants were collected at 0, 24, 72 and 144 h after application of the solution. The same sampling procedure was conducted in the controls only at the beginning (0 h) and at the end of the experiment (144 h). All of the samples were carefully washed, dried, ground, and subsequently dissolved in hot HNO_3 for digestion at 120º C.

The isotopic content of the samples was determined by the Chilean Nuclear Energy Commission using an Agilent 7500 Mass Spectrometer with an argon plasma source with a maximum potential of 2,500 W and a conventional sample loading system with a Babington nebulizer, sampler and skimmer cones, quadrupole analyzers and an electron multiplier detector. The obtained isotopic compositions were used to calculate the $^{11}B/^{10}B$ ratios for each of the treatments. The data were analyzed via analysis of variance and a Tukey means separation test, at a p-value of 0.05.

Results and discussion

The two naturally occurring stable isotopes of B are found in nature at a proportion of $^{11}B/^{10}B$ of ±4.02 on average (Barth, 1997). Any variation from this value would indicate an abnormally high proportion of ^{10}B, for example, due to enrichment through exogenous action, as in the present experiment. According to the control samples collected from this kiwi orchard, the ratio fluctuated between 3.82 and 4.05 (Table 1), which corresponds well with the expected naturally occurring ratio.

Table 1. Ratio of $^{11}B/^{10}B$ in leaves and flowers on mixed kiwi shoots treated with ^{10}B-enriched boric acid compared with un-enriched controls at 0 and 144 hours.

Treatment (Hours)	Leaves on mixed shoots	Flowers on mixed shoots
Control at 0	3.93 a	3.82 a
Treatments at 24	1.57 c	1.31 b
Treatments at 72	2.06 b	1.48 b
Treatments at 144	3.83 a	3.67 a
Control at 144	4.02 a	4.05 a

Different letters in each row indicate significant differences by the Tukey test at P = 0.001.

Table 1 shows when there was no application of boron through spraying, the ratio at 0 and 144 h did not change, following the pattern in nature, and these plants can therefore be considered as controls. Twenty-four hours after application of the ^{10}B-enriched solution to the leaves, they had already presumably absorbed a significant amount of the applied ^{10}B, and a significant ^{10}B level was also found in the nearby flowers. This result demonstrates the capacity of kiwi shoots to transport B through their phloem (^{10}B in particular in this case) over short distances within the shoot. At 72 h, there was a significantly different ratio of $^{11}B/^{10}B$ in the treated leaves compared with the controls, though this difference was smaller than was observed at 24 h, which would indicate that ^{10}B was being re-exported to other tissues and that ^{11}B continued to arrive at normal levels through the xylem. However, in the flowers, the ratio was still significantly different after 72 h, which would indicate that the ^{10}B received by this organ had been incorporated structurally. At 144 h, the ratio in the leaves and flowers had returned to a more normal level, likely associated with the arrival of more ^{11}B from other structural parts of the plant and through the roots and due to the dilution of ^{10}B in nearby sinks.

These results isotopically demonstrate the mobility of B in the phloem of kiwi shoots, confirming the results of Sotomayor *et al*. (2010), who demonstrated significant increases in B in flowers after treatment of nearby leaves with standard boric acid. However, these findings contradict the proposal of Sotiropoulos *et al*. (2004), based on studies using nutrient solutions, that foliar B mobility in kiwi would be low.

Based on the results of the experiment described herein, it can be inferred that in kiwi, there is effective movement of the phloem towards certain active sinks (flowers), most likely due to the presence of myo-inositol, a sugar-alcohol that forms complexes with B and can therefore travel through the symplast. These findings are consistent with those of Klages *et al*. (1997) and Jutamanee *et al*. (2002), who demonstrated that this transport sugar is present in kiwis. These results allow us to conclude that foliar B applications are effective in kiwis, resulting in the absorption of boron, which is subsequently re-distributed to the flowers and fruit in the case of deficiencies or low levels of this micronutrient.

Resumen

C. Sotomayor, R. Ruiz y L. Muñoz. 2012. Movilidad floemática de Boro^{10} en brotes mixtos de kiwi (*Actinidia deliciosa).* Cien. Inv. Agr. 39(3): 563-567. El B es un microelemento cuya movilidad en las plantas es restringida, excepto en aquellas que contienen polioles. En el caso del kiwi no se conoce con exactitud la movilidad del B vía floema, pese a que dispone del poliol mio-inositol. Se realizó un experimento con plantas de kiwi, cv. Hayward, en que hojas distales de brotes mixtos (con hojas y flores) fueron tratados con aspersiones de ácido bórico, enriquecido al 99% con el isótopo estable B^{10}. A las 24, 72 y 144 h se muestreó hojas tratadas y no tratadas y flores del mismo brote con y sin tratamiento de B^{10}. Se realizó en cada ocasión análisis por espectrometría de masa y se estableció la relación B^{11}/B^{10} en hojas y flores. Las hojas y flores testigo mostraron la relación natural entre 3,82 y 4,05, sin embargo, en las hojas tratadas, la relación a las 24 h fue de 1,57, a las 72 h de 2,06 (ambas significativamente diferentes al testigo) y a las 144 h de 3,83 (similar al testigo). En las flores del brote con hojas tratadas a las 24 h la relación fue de 1,31, a las 72 de 1,48 (ambas significativamente diferentes

al testigo) y a las 144 de 3,67 (similar al testigo). Esto demostraría la efectiva absorción de la solución por las hojas y la redistribución del B^{10} hacia sumideros como las flores, dentro de las primeras 72 h, demostrando que en kiwi la movilidad floemática del B es efectiva, permitiendo avalar aplicaciones foliares, para solucionar deficiencias de boro en flores y frutos.

Palabras clave: Absorción foliar, boro, isótopo estable, kiwi, movilidad floemática.

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