ICP-MS Measurement of $^{11}B/^{10}B$ Isotope Ratios in Grapevine Leaves and the Investigation of Possible Boron Isotope Fractionation in Grapevine Plants

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The correlation between the $^{11}B/^{10}B$ ratio in grapevine leaves and that in the growth medium was established in a series of hydroponic experiments with grapevine cuttings for different cultivar/rootstocks combinations. The hydroponic growth medium was alternately spiked with boric acid containing B with natural isotope composition and B enriched in ^{10}B, so as to vary the $^{11}B/^{10}B$ ratio. B isotope ratios in grapevine leaves were determined by quadrupole-based ICP-MS after digestion and complete matrix removal through microwave digestion and isolation of matrix-free B species using ion exchange separation. It was found that the B isotope ratios in the leaves were not identical to those in the growth medium, but that a change in the ratio in the growth medium induced a similar change in the leaves. For a particular cultivar/rootstock combination, a characteristic B isotope ratio was found that was different from the ratio in a group of plants with a different cultivar/rootstock combination.

INTRODUCTION

Various isotope fractionation processes occurring in geochemical systems may cause isotope abundances for some elements, in particular for the light elements, to differ from place to place on Earth. Isotope ratios of certain elements may therefore characterise a particular soil, and this ratio may also be reflected in the agricultural product ensuing from the soil, thus enabling provenance determination of the product through chemical analysis. An important application of isotope ratio determinations is therefore in food authentication. A comprehensive review (Förstel, 2007) of isotope ratio mass spectrometry reveals that stable isotope ratios, especially those of the light elements $^{2}H/^{1}H$, $^{11}B/^{10}B$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{18}O/^{16}O$, but also of two heavy elements, $^{87}Sr/^{86}Sr$ and $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{207}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, have been used to determine the authenticity and provenance of food products such as wine (Coetzee & Vanhaecke, 2005), coffee (Bellato *et al*., 2003; Serra *et al*., 2005), fruit juices (Martin *et al*., 1995), cheese (Pillonel *et al*., 2003), vegetables (Marentes *et al*., 1997a,b), lamb meat (Piasentier *et al*., 2003), honey (Rossmann *et al*., 1992), and Scotch whisky (Parker *et al*., 1998).

B isotope ratios ($^{11}B/^{10}B$) seem to be particularly useful for provenance determination in agricultural products because the B isotopic composition of soils and sediments may vary from place to place on Earth. Different geochemical processes are responsible for this phenomenon. Natural waters, including interstitial water, may be enriched in ^{11}B because of mechanisms such as the preferential adsorption of ^{10}B onto clay minerals and the fractionation that takes place during the precipitation of salts from brine waters (Wieser *et al*., 2001). The $^{11}B/^{10}B$ ratio in sea water, for example, is much higher than that in typical soils and may be related to this process. Another important fractionation mechanism is the pH-dependent isotopic exchange (Vengosh *et al*., 1994) between boric acid, $B(OH)_3$, and the borate ion, $B(OH)_4^-$. This exchange can lead to a slight enrichment of ^{11}B in boric acid species and a slight depletion in borate species. Plant roots absorb B in the form of boric acid through the roots. It therefore is possible that the $^{11}B/^{10}B$ ratio in plants could be slightly higher than that in the provenance soil because of this exchange process and the enrichment of ^{11}B in interstitial waters through differential adsorption. The effects will both be pH dependent. On the other hand, $B(OH)_4^-$ is incorporated in corals without measureable fractionation, thus converting this material into a chronological archive, B isotope analysis of which can be deployed for assaying the paleo-pH (determined by CO_2 concentration in the atmosphere) of sea water (Kasemann *et al*., 2009). The variation in B isotope composition in soils and, by assumption, also in the plants associated with the soils, allows the use of $^{11}B/^{10}B$ ratios in food for provenance determination or as indicators of geographical origin. B is an

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essential nutrient for all plants. $^{11}B/^{10}B$ ratios were determined in coffee beans from a number of coffee-growing regions, each showing a characteristic abundance ratio (Wieser *et al*., 2001). $^{11}B/^{10}B$ ratios were also determined in commercial produce from different geographical production areas in North and Central America (Vanderpool & Johnson, 1992). Experiments done with various vegetables showed that the plants did not acquire the same B isotopic composition as the applied ^{10}B-enriched hydroponic nutrient solution. Boron isotope ratios were found to differ for different plants – from 4.162 ± 0.003 for cabbage to 4.013 ± 0.008 for whole-wheat flour. This could suggest that B isotopes were fractionated during incorporation in plants. B isotope fractionation (Marentes *et al*., 1997b) in plants may occur during the uptake of nutrients from the soil through root membranes, during protein-facilitated transport processes (Hu & Brown, 1997; Matoh & Kobayashi, 1998), and during metabolic process in which membranes (or membrane-like) structures are involved. An example of the latter is cell wall deposition, as B is an important component in cell wall structures. It is known that the cell membranes in plants are much more permeable to $B(OH)_3$, and much less permeable to $B(OH)_4^-$. Therefore the plant material was found to be enriched in ^{11}B. These observations were made on plants such as wheat, corn and broccoli (Marentes *et al*., 1997b).

The grapevine may acquire B from interstitial ground waters surrounding the plant roots, or from migration from older parts of the plant itself (Oertli, 1993). Boron isotope fractionation may thus occur during membrane transport in nutrient uptake by the roots, at translocation or adsorption sites, or during metabolic processes in the plant itself. Isotope fractionation is a phenomenon caused by chemical reactions and physical processes that may proceed with different efficiency (small differences in the reaction rates and/or states of equilibrium) depending on the molecular mass of the species involved. In the case of light elements, the mass difference is relatively large and isotopic effects are readily observed (Mather & Porteous, 2001).

In previous work (Coetzee & Vanhaecke, 2005), it was proposed that B isotope ratios could be used to differentiate wine according to geographical origin. Wines from three different wine-producing regions in South Africa and two regions in Europe were classified according to area of production. The assumption was made that the $^{11}B/^{10}B$ ratio in a wine will reflect the isotope ratio of B in the provenance soil. This was, however, never proven. It was thus also assumed that no measureable isotope fractionation occurs during the nutrient uptake process or during B migration in the grapevine. Both these assumptions need to be addressed as part of a method validation process. The main objective of this pilot study was therefore to ascertain the correlation between the $^{11}B/^{10}B$ ratio in grapevine leaves and that of the provenance soil, and to investigate the possibility of B isotope fractionation in the grapevine that could cause the ratio in the plant to differ from that in the soil. A series of hydroponic experiments on grapevines with different rootstocks and different cultivars was performed to achieve this aim. The hydroponic growth medium was alternately spiked with boric acid containing B with natural isotope composition and B enriched in ^{10}B, so as to vary the $^{11}B/^{10}B$ ratio. The B isotope ratios in vine leaves were determined by quadrupole-based ICP-MS after complete matrix removal through microwave digestion and extraction of pure B using a column ion exchange separation.

MATERIALS AND METHODS

Samples and sampling

Grapevine cuttings, namely Pinot noir on rootstock Richter 110 and Chardonnay on 101-14 and Ramsey, were obtained from the ARC-Nietvoorbij, Stellenbosch. The cuttings were planted in quartz sand and grown hydroponically in a greenhouse facility provided by the Department of Botany and Biotechnology at the University of Johannesburg. Each cutting was supplied with a plastic dropper through which controlled amounts of commercial hydroponic nutrient solution were pumped using an automatic irrigation system. The droppers were set to supply nutrient solution to each plant for approximately 10 minutes every two hours. No nutrient solution was supplied at night time. The 25% Hoagland's nutrient solution (Ross, 1974) was maintained at pH 6 and a conductivity of 2 000 µS/cm throughout the duration of the experiment. Temperatures and humidity were monitored and controlled to remain constant. The plants were sprayed with the general fungicides, Funginex (Effecto) and Folicur 250 EW (Bayer), every two weeks. The B content of these products was determined and found to be below the detection limits (14 µg/L for B) of the ICP-MS instrument and therefore low enough as not to affect the experimental results.

Cuttings were allowed one month after planting to develop new shoots and leaves before the start of the experimental cycle. Leaf samples, selected from the new growth, were then taken every week for five weeks (Period 1) from each grapevine, while the plants were given nutrient solution containing boric acid with "natural" B isotope ratio – referred to as "normal" growth medium. The leaf lengths were measured before harvesting so as to obtain leaves of approximately the same growth stage and age during each sampling period. Sampling was specifically done at 10:00 am.

During growth Period 2, immediately following Period 1, the plants were supplied with nutrient solution spiked with boric acid enriched in ^{10}B to lower the $^{11}B/^{10}B$ ratio – referred to as "spiked" growth medium. The B isotope standard, NIST SRM 952 (containing boric acid with isotopic composition: 95% ^{10}B and 5% ^{11}B), was used for this purpose. The amount of ^{10}B-enriched boric acid added was calculated to yield a ca. 0.7% decrease in the $^{11}B/^{10}B$ ratio in the spiked growth medium. A 0.7% change in B isotope ratio could be determined accurately by quadrupole-based ICP-MS and did not require excessive amounts of expensive NIST SRM 952 to prepare the spiked growth medium. No samples were taken for one week to give the plants sufficient time to produce new growth and to reach equilibrium with regard to the new growth medium. Sampling was then done weekly for five weeks.

The above cycle was repeated three times, alternating between normal and spiked growth medium and lasting 26 weeks.

TABLE 1
Microwave digestion programme for vine leaf samples.

Step 1:	10 min, up to 180°C, ramp power up to 1000 W
Step 2:	10 min, constant at 180°C, power at 1000 W

Microwave-assisted acid digestion procedure

The grapevine leaf samples were dried overnight at 70°C. About 0.5 g of the dried mass of each sample was weighed accurately using a Sartorius BP121S, four-decimal analytical balance. The samples were then digested with 2 mL 14 M HNO_3 (Merck, SP) and 1 mL 30% H_2O_2 (Merck, SP), using a Milestone ETHOS microwave digestion apparatus according to the programme summarised in Table 1.

Isolation of matrix-free B by ion exchange separation

Because of unavoidable matrix interference during the measurement of the ^{10}B and ^{11}B isotopes with ICP-MS, even after microwave digestion of the vine leaves, it was necessary to separate B from all the other elements and matrix components still present. An ion exchange procedure (Aggarwal & Palmer, 1995) using Amberlite IRA 743 (Saarchem) was adapted and optimised for this purpose. The pH of a microwave-digested leaf sample was adjusted to 10.00 with 3 M ammonium hydroxide to convert all of the B present in the sample to the borate ion form, $B(OH)_4^-$. The sample was then loaded onto a column packed with conditioned resin in its cationic form, and washed with 30 mL de-ionised water. Thereafter, the total B was eluted with 100 mL of 2 M HNO_3. The resin was regenerated by the addition of 6 M HCl in order to be reused in further separations.

Instrumentation

A Thermo X-series 2 quadrupole-based ICP-MS instrument with a conical spray chamber and impact bead, a Meinhard concentric nebuliser, nickel cones, and a dual mode electron multiplier detector (in counting mode) was used for determining B isotope ratios in the leaf samples. Sample uptake was accomplished by means of a CETAC 5 A5X-510 automatic sampler and a peristaltic pump. After mass calibration and detector cross-calibration, instrumental conditions for the ICP-MS were optimised for maximum B intensity by following a manual tuning procedure using Thermo Tuning Solution A containing the elements ^7Li, ^9Be, ^{59}Co, ^{115}In, ^{138}Ba, ^{140}Ce, ^{206}Pb and ^{238}U at 10µg/L. Detector dead time was determined, specifically for B, and this dead time was used in the instrument set-up to apply the appropriate count rate corrections during the measurements. Dead time corrections (Vanhaecke *et al*., 1998) are critically important for accurate isotope ratio determinations and are dependent on analyte mass. The operating conditions and data acquisition parameters optimised for the B isotope ratio determinations are summarised in Table 2.

TABLE 2

Optimised operating conditions and acquisition parameters for the measurement of boron isotope ratios in grapevine leaf samples.

Parameter	Setting value
Operating conditions	
Forward power	1400 kW
Nebuliser flow rate	0.71 L/min
Auxiliary gas flow rate	0.70 L/min
Plasma gas flow rate	15.00 L/min
Sample uptake rate	1.00 mL/min
Acquisition parameters	
Detector dead time	35 ns
Dwell time	10 ms
Sweeps per reading	3000
Number of replicates	5
Readings per replicate	1

$^{11}B/^{10}B$ isotope ratio measurements

Determination of the B isotope ratio, $^{11}B/^{10}B$, by means of ICP-MS is complicated by a large mass discrimination effect (because of the relatively large mass difference between the two B isotopes) and the drift in the mass discrimination during measurement, which may lead to a concomitant drift in the measured isotope ratio. This problem was solved by using an external standard approach, wherein samples and the isotopic standard (B reference standard NIST SRM-951) were measured alternately in the following 'bracketing' measurement sequence: standard-sample1-standard-sample2-standard. This method assumes that the mass discrimination drift is linear with respect to time between the analyses of the standards bracketing a sample measurement. Mass discrimination correction is then achieved by applying the equation:

$$R^{s}_{i-corr} = R^{s}_{i} \times \frac{R^{std}_{ref}}{\left(R^{std}_{i-1} + R^{std}_{i+1}\right)/2}$$

The isotope ratios of the standard, R_{i-1}^{std} and R_{i+1}^{std} are measured as closely as possible in time before and after the measurement of the i^{th} sample with isotope ratio R_i^s. R_{ref}^{std} is the reference value for the isotope standard.

RESULTS

The B isotope ratios measured in the leaves harvested from 12 grapevine cuttings grown hydroponically over a period of 26 weeks are summarised in Table 3. During this time, the plants were alternately exposed to growth media containing boric acid with natural $^{11}B/^{10}B$ ratios ("normal" growth medium) and modified $^{11}B/^{10}B$ ratios ("spiked" growth medium). Leaf samples were harvested weekly during six five-week periods, starting with five weeks using normal growth medium. Each five-week period with normal growth medium was followed by a five-week period using spiked growth medium. The cycle was repeated three times. The $^{11}B/^{10}B$ isotope ratio of the normal and spiked growth media supplied during these periods was experimentally determined as 4.036 ± 0.003 and 4.009± 0.001 respectively. Four plants, three test plants and one control plant, of each of the following cultivars were grown: Pinot noir (rootstock Richter 110), Chardonnay (rootstock 101-14), and Chardonnay (rootstock Ramsey). The control plants were only given normal growth medium throughout the test period.

The results for the first period (normal growth medium) show that the average $^{11}B/^{10}B$ ratios were the same for the four plants within each group (three test plants plus one control), but differed for the groups. A group in this context is defined by rootstock type. For Group A (Pinot noir, rootstock 110), the average B isotope ratio (4.038 ±0.008) was close to the

TABLE 3
$^{11}B/^{10}B$ ratios in grapevine leaves after alternately exposing the plants to normal and spiked growth medium, over a growth period of 26 weeks.

Sampling week	Group A: Pinot noir (Richter 110)				Group B: Chardonnay (101-14)				Group C: Chardonnay (Ramsey)			
	1	2	3	Control	1	2	3	Control	1	2	3	Control
Period 1: Normal growth medium Reference $^{11}B/^{10}B = 4.036 \pm 0.003$												
1	4.048	4.051	4.038		4.038	4.040	4.041		4.005	3.912	3.974	
2	4.031	4.035	4.049		4.091	4.064	3.995		3.942	3.968	3.979	
3	4.035	4.041	4.041	4.028	4.057	4.040	4.078	4.051	3.991	4.004	4.012	4.001
4	4.024	4.032	4.045		4.079	4.068	4.049		3.995	4.017	4.003	
5	4.025	4.032	4.039		4.052	4.031	4.030		3.991	4.007	3.997	
AVE	**4.033**	**4.038**	**4.042**		**4.063**	**4.049**	**4.039**		**3.985**	**3.982**	**3.993**	
STD DEV	0.010	0.008	0.005		0.021	0.016	0.030		0.025	0.043	0.016	
Period 2: Spiked growth medium Reference $^{11}B/^{10}B = 4.009 \pm 0.001$												
6				4.022	4.004	3.982	3.971	4.051	3.897	3.949	3.942	4.001
7	3.950	3.961	4.037		4.019	3.996	3.994		3.959	3.969	3.982	
8	4.036	4.026	4.029		4.005	3.978	3.967		3.866	3.922	3.942	
9	4.030	4.039	4.028	4.021	4.015	3.991	3.993	4.039	3.959	3.955	3.961	3.993
10	4.025	4.031	4.024		4.011	4.003	3.992		3.965	3.969	3.969	
AVE	**4.010**	**4.014**	**4.030**		**4.011**	**3.990**	**3.983**		**3.929**	**3.953**	**3.959**	
STD DEV	0.040	0.036	0.005		0.006	0.010	0.013		0.045	0.019	0.017	
Period 3: Normal growth medium Reference $^{11}B/^{10}B = 4.036 \pm 0.003$												
11	4.020	4.026	4.027		4.045	4.054	4.038		3.994	4.005	4.007	
12	4.031	4.032	4.033		4.039		4.023		3.982	4.002	4.007	
13	4.038	4.032	4.049	4.032	4.099	4.082	4.001	4.057	3.965	3.985	3.978	3.986
14	4.034	4.031	4.037			4.053	4.047		3.997	3.969	3.965	
15	4.037	4.039	4.030		4.069	4.053	4.064		3.971	3.976	3.998	
AVE	**4.032**	**4.032**	**4.035**		**4.063**	**4.061**	**4.035**		**3.982**	**3.987**	**3.991**	
STD DEV	0.007	0.005	0.009		0.027	0.014	0.024		0.014	0.016	0.019	
Period 4: Spiked growth medium Reference $^{11}B/^{10}B = 4.009 \pm 0.001$												
16	4.018	4.025	4.018	4.025	3.998	3.982	4.01	4.051	3.963	3.967	3.942	4.007
17	4.011	4.032	4.021		4.013	4.018	4.016		3.962	3.991	4.018	
18	3.986	4.001	3.967		3.987	3.981	4.005		3.97	3.96	3.953	
19	4.022	4.029	4.005	4.035	3.985	4.009	3.978	4.059	3.963	3.942	3.943	4.005
20	4.036	4.023	4.018		3.992	3.997	4		4.001	3.966	3.957	
AVE	**4.015**	**4.022**	**4.006**		**3.995**	**3.997**	**4.002**		**3.972**	**3.965**	**3.963**	
STD DEV	0.018	0.012	0.023		0.011	0.016	0.015		0.017	0.018	0.032	
Period 5: Normal growth medium Reference $^{11}B/^{10}B = 4.036 \pm 0.003$												
21	4.021	4.033	4.048	4.028	4.033	4.041	4.076	4.052	3.994	3.983	3.98	4.011
22	4.058	4.029	4.033		4.032	4.057	4.065		4.009	4.025	4	
23	4.031	4.032	4.029		4.052	4.056	4.077		4.004	3.985	3.974	
AVE	**4.037**	**4.031**	**4.037**		**4.039**	**4.051**	**4.073**		**4.002**	**3.998**	**3.985**	
STD DEV	0.019	0.002	0.010		0.011	0.009	0.007		0.008	0.024	0.014	
Period 6: Spiked growth medium Reference $^{11}B/^{10}B = 4.009 \pm 0.001$												
24	4.015	4.014	4.017		4.002	3.999	4.004			3.961	3.962	
25	4.018	4.012	4.017	4.033	4.007	3.992	3.996	4.051				3.997
26	4.017	4.005	4.014		4.005	4.004	4.004					
AVE	**4.017**	**4.010**	**4.016**		**4.005**	**3.998**	**4.001**			**3.961**	**3.962**	
STD DEV	0.002	0.005	0.002		0.003	0.006	0.005					

All Data	Group A	s	Control	s	Group B	s	Control	s	Group C	s	Control	
AVE Normal	**4.035**	0.008	**4.028**	0.005	**4.050**	0.018	**4.051**	0.006	**3.988**	0.021	**4.000**	0.008
AVE Spiked	**4.015**	0.021			**3.997**	0.012			**3.959**	0.027		
DIFFERENCE	**0.020**				**0.053**				**0.029**			

TABLE 4
Average B isotope ratios for the cultivar/rootstock combinations, Groups A, B and C, for different growth media, and P values for ANOVA and t-test comparisons.

Period	P ANOVA for A,B,C	P t-Test for A,B	Group A	Group B	Group C
1 Normal	< 0.0001	0.094	4.038 ± 0.008	4.048 ± 0.021	3.986 ± 0.028
2 Spiked	< 0.0001	0.16	4.018 ± 0.029	3.99 ± 0.015	3.947 ± 0.031
3 Normal	< 0.0001	0.0008	4.033 ± 0.006	4.045 ± 0.013	3.987 ± 0.015
4 Spiked	< 0.0001	0.01	4.014 ± 0.018	3.998 ± 0.013	3.967 ± 0.021
5 Normal	< 0.0001	0.01	4.035 ± 0.011	4.054 ± 0.016	3.995 ± 0.016
6 Spiked	< 0.0001	< 0.0001	4.014 ± 0.003	4.001 ± 0.003	3.962 ± 0.021
All data					
Normal	< 0.0001	< 0.0001	4.035 ± 0.008	4.050 ± 0.018	3.988 ± 0.021
Spiked	< 0.0001	< 0.0001	4.015 ± 0.021	3.997 ± 0.012	3.959 ± 0.027

TABLE 5
Comparison of the difference in average $^{11}B/^{10}B$ ratios measured for the three plants in each group with the theoretical difference, after exposure to normal and spiked growth media.

Sample identification	$^{11}B/^{10}B$ isotope ratios Normal growth medium	Spiked growth medium	Difference in average isotope ratio
Reference value	4.036 ± 0.003	4.009 ± 0.001	0.027
Pinot noir (Richter 110)			
Plant 1	4.033 ± 0.006	4.013 ± 0.007	0.020
Plant 2	4.034 ± 0.008	4.014 ± 0.008	0.020
Plant 3	4.038 ± 0.008	4.016 ± 0.008	0.022
Chardonnay (101-14)			
Plant 1	4.057 ± 0.008	4.003 ± 0.008	0.054
Plant 2	4.053 ± 0.007	3.996 ± 0.008	0.057
Plant 3	4.045 ± 0.008	3.997 ± 0.008	0.048
Chardonnay (Ramsey)			
Plant 1	3.988 ± 0.007	3.961 ± 0.006	0.026
Plant 2	3.988 ± 0.008	3.960 ± 0.006	0.027
Plant 3	3.995 ± 0.007	3.963 ± 0.006	0.033

reference value of 4.036 for each of the four plants, whereas for Group B (Chardonnay, rootstock 101-14) it was slightly higher (4.048±0.021) and for Group C (Chardonnay, rootstock Ramsey) it was lower (3.986±0.028) (see Table 4). The results for the second period (spiked growth medium) show that the B isotope ratio for all plants dropped significantly, as was expected after exposure to growth medium with a lower $^{11}B/^{10}B$ ratio. The reference value of 4.009 was, however, not attained. The results for the third period (normal growth medium) show a return to initial ratios for all the plants. The pattern repeats itself through the cycles. The group averages and standard deviations for the combined data (B isotope ratios from all plants per group combined for normal and spiked growth periods) are also given in Table 3. The differences between the average isotope ratios for normal and spiked growth periods are close to the expected difference of 0.027 (i.e. 4.036 minus 4.009) for groups A and B, but significantly larger for Group C. It is clear that the spiked medium consistently produced lower $^{11}B/^{10}B$ ratios in the leaves.

ANOVA calculations were done on the isotope ratio results for each period by combining the data for all three plants per group to establish whether the differences in the group averages were statistically significant on the 95% confidence level. The group averages, standard deviations and P values are summarised in Table 4. P values were all <0.0001, strongly suggesting that the group averages were statistically different. Because the group averages for Group A and Group B apparently differed only slightly, a Student's t-test was also performed to establish whether the differences were significant. The P values are generally small and in the order of 0.01, and therefore small enough to indicate probable significant differences. ANOVA and t-tests were also done on combined data per group for the normal and spiked growth periods. The P values were all < 0.0001.

It is important to note that the isotope ratios of the control plants, which were only supplied with normal growth medium, remained constant during the whole period of 26 weeks. The standard deviation of ca 0.1% RSD for N=27 achieved for the B isotope ratios in the control samples is, at the best precision level, typically obtainable with quadrupole-based ICP-MS. It therefore supports the reliability of the results obtained for the test plants throughout the experimental cycle. The fact that no systematic trend in B isotope ratios is discernable in the weekly measurements after a change in the growth medium implies that the plants reached equilibrium with regard to the change in the $^{11}B/^{10}B$ ratio in less than a week after the introduction of the growth medium. Leaves were harvested one week after the introduction of a new growth medium. The $^{11}B/^{10}B$ isotope ratios for the three individual plants of each group were statistical identical for each growth period over 26 weeks, as were the ratios for the control plants.

Table 5 compares the difference in the average $^{11}B/^{10}B$ ratio measured for the three plants in each group with the theoretical difference, after exposure to normal and spiked growth media. The differences for the Pinot noir (Richter 110) and Chardonnay (Ramsey) groups are close to the theoretical difference, but the

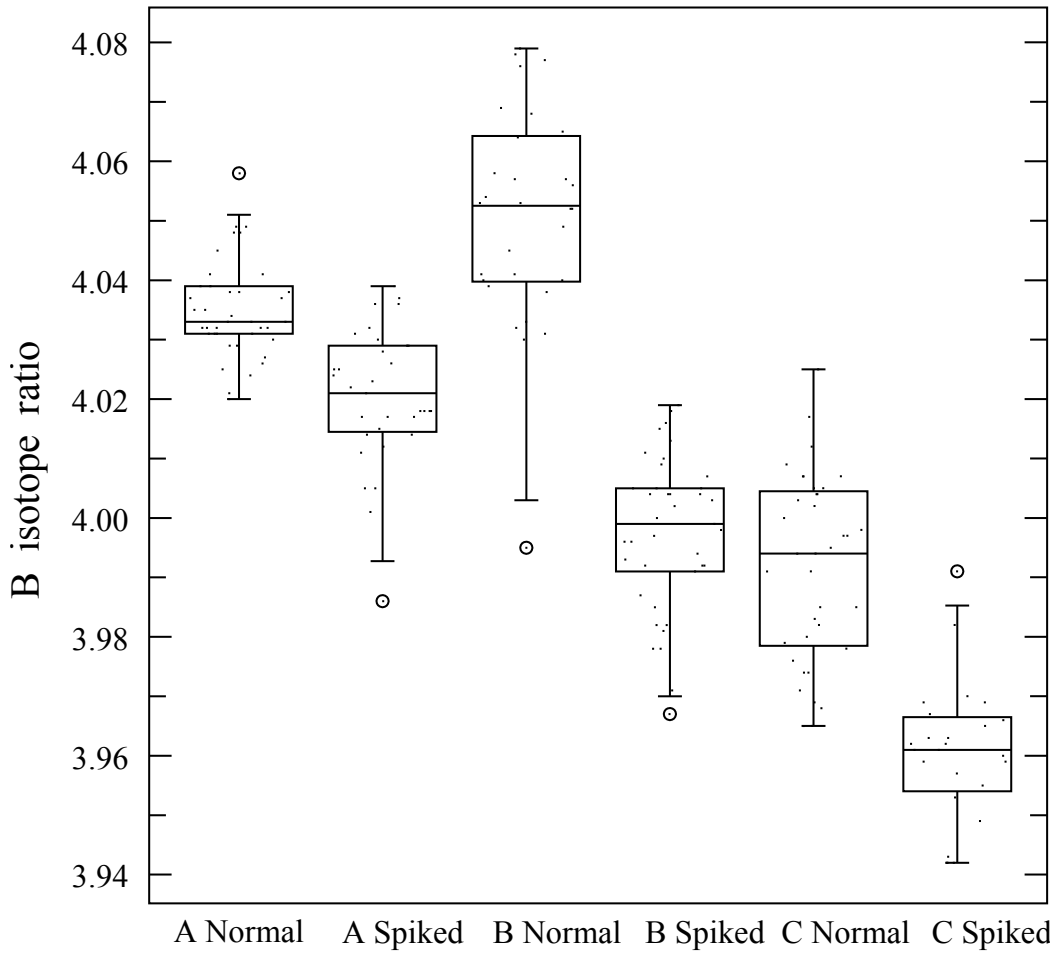

FIGURE 1
Box plots of the spread in average B isotope ratios of the combined data for normal and spiked growth periods for the three cultivar/rootstock combinations, Groups A, B and C.

TABLE 6
Comparison of group $^{11}B/^{10}B$ ratio averages with the B isotope ratio in the growth media and P values for the t-test applied to the combined data.

Statistical parameter	Normal growth medium 4.036 ± 0.003			Spiked growth medium 4.009 ± 0.001		
	Group A	Group B	Group C	Group A	Group B	Group C
mean	4.035	4.050	3.988	4.015	3.997	3.959
s	0.008	0.018	0.021	0.021	0.012	0.027
n	39	30	39	36	39	32
P	0.74	0.084	< 0.0001	0.49	0.056	0.0002

$^{11}B/^{10}B$ ratio for the Chardonnay (101-14) group is about double the theoretical difference. It is difficult to explain the larger than expected difference in the latter case, or even to attribute it with any certainty to a real effect. A proper assessment would require many more test results and was beyond the scope and resources of this study.

Fig. 1 shows box plots of the spread in average B isotope ratios of the combined data for normal and spiked growth periods for the three groups. The pictorial presentation and statistical analysis in Tables 5 and 6 seem to suggest that each group of plants reacted differently to the supplied growth media with regard to the equilibrium B isotope ratio attained in the plant.

From Table 6 it can also be seen that, with the exception of Group A (P value 0.74 and 0.49 for normal and spiked growth media respectively), the B isotope ratio in the leaves was statistically different from that in the corresponding growth media. It therefore is not excluded that B uptake by the roots might introduce an isotope fractionation effect that is rootstock specific. Although this possible interpretation is supported by

the results obtained in previous studies (Marentes *et al*., 1997b) on other plant material, the test population in the current study was too small to warrant a definitive conclusion.

An earlier pilot study by the same authors (Coetzee & Vanhaecke, 2005) showed that B isotopic analysis of wines indeed shows potential to be used as a tool for objective provenance determination. In order to use such an approach with confidence and to avoid all related pitfalls, a profound insight into the link between the B isotopic composition shown by the wine and the characteristics of its origin is required. This study is a first step in this direction. Admittedly, it eventually will be required to study the final B isotopic signature in wine and how it is affected by various parameters, but the authors believe that this study, in which the B isotopic composition was studied in vine leaves, is a valuable first step. Self-evidently, leaves can be harvested more quickly after the start of the experiment than grapes or wine. In the experiments that were carried out a controlled setup (hydroponic cultivation) was opted for. The reality is indeed much more complex and the possible influence of factors such as the composition of irrigation water and its possible effect on B isotope ratios in the grapes, boron leaf sprays commonly used in vineyards with boron-deficient soils, and the possibility that B isotope ratios in the leaves may differ and will have to be investigated step by step in further work.

CONCLUSION

The study established that the $^{11}B/^{10}B$ ratio in grapevine leaves, and by assumption also in the grapes produced by the plant and in the wine produced from the grapes, broadly reflects that of the growth medium supplied to the roots or, by assumption, that of the provenance soil of a particular wine. The B isotope ratio in the leaf was not identical to that in the growth medium, but a change in the ratio in the growth medium induced a similar change in the leaves. For a particular cultivar/rootstock combination, a characteristic B isotope ratio was found in the plants of that description, which was different from the ratio in a group of plants with a different cultivar/rootstock combination. The fact that the B isotope ratio in plants with different cultivar/rootstock combinations was not the same when supplied with the same growth medium may suggest isotope fractionation, or at least does not effectively exclude isotope fractionation as a possible explanation. Further work with a sufficiently large sample size and an appropriate experimental design is necessary to provide a definitive conclusion.

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