Distribution of Foliar-applied Boron Measured by Spark-source Mass Spectrometry and Laser-probe Mass Spectrography

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ABSTRACT

The distribution of foliar-applied boron (11B]boric acid) in radish (Raphanus sativus L.) was studied using for analysis of the stable isotopes a technique allowing a high sensitivity: spark-source mass spectrometry. Boron was recovered in the untreated aerial parts and in the roots; however, the greatest fraction was in the treated leaf. It was possible with a laser-probe mass spectrometer to show that boron was not superficially located in the treated area but was present in tissues at all levels of depth considered.

Many investigations on the uptake and the translocation of nutrients applied to leaves have been carried out with the help of radioactive isotopes (2); however, for elements such as boron, the lack of a convenient radioactive isotope makes similar studies very difficult. Here, two novel techniques are used to determine the distribution of foliar-applied boron: (a) in intact plants by measurement of the isotopic ratio 11B/10B using a highly sensitive technique, spark-source MS; and (b) in the tissues of boron-treated leaves, with a microanalytical method that we have recently developed using the laser-probe mass spectrometer (4). The example of boron is considered because this micronutrient is applied frequently by foliar spraying, particularly when the soil is potentially capable of fixing large amounts of this element (7). The redistribution of boron after foliar application has already been studied by Thellier (6, 10) with the help of an enriched stable isotope and a nuclear reaction (n,α).

MATERIALS AND METHODS

EXPERIMENTAL PROCEDURE

The experiments were performed on radish (Raphanus sativus L. cv. 18 days). The plants were grown in a greenhouse on a sandsphagnum peat moss mixture (1:2, v/v) supplied with a complete commercial fertilizer and micronutrients. When they were 31 days old, 20 mm H2BO3 with Tween 20 (0.1% v/v) was applied to the middle of the lamina of one of the pair of first leaves at a temperature of 25 C and a RH of 70%.

The distribution of boron in the entire plants was determined with plants receiving 2 droplets each of 20 μl boric acid enriched with 11B (90.5 ± 0.2 atom %). The localization of boron in the treated part was studied with leaves receiving 3 contiguous droplets each of 2 μl of nonenriched boric acid. In the latter case, a small volume of liquid was applied in order to have a limited and well-defined treated area. With both methods of boron application, the treated leaf was sectioned 24 h after the foliar application and was washed rapidly twice for a total of 60 s in 1 liter distilled H2O and then the different parts, including the treated leaf, hypocotyl, and epicotyl, were freeze-dried.

The samples for microanalysis were cut immediately after the washing from the treated leaves to include the area initially in contact with the droplets. They were rapidly immersed in isopentane cooled with liquid N2, then freeze-dried in an apparatus VirTis (model 10-010TD) and preserved in a desiccator until analysis.

SPARK-SOURCE

The preparation of plant samples and analyses were carried out according to a previously described method (3). The plant tissues were wet ashed with suprapure H2SO4 (Merck) and the dry residue was mixed with graphite (Ringsdorff RWS) for the preparation of the electrodes. The apparatus used for analyses was a model MS7 (AEI) with photographic detection, usually enabling isotopic ratio measurements to be obtained with coefficients of variation of 5 to 10%.

LASER-PROBE MASS SPECTROGRAPHY

Principle. A light pulse of 1010 to 1014 w is focused on a precisely determined point on the specimen to be analyzed. The target tissue undergoes a sudden rise in temperature (106 K) and is locally volatilized with the formation of a shallow crater. The volatilized atoms are ionized, accelerated, separated by interaction with a magnetic field, and finally detected on a photographic plate. After development, the photographic plate shows a number of lines which are characteristic of the different chemical elements and their isotopes. It is possible to perform analysis to greater depths, through the total thickness of the leaf, by repeated laser pulses at the same point, the crater being depressed progressively (8). Different applications of this technique have been previously considered with plant or animal specimens (4, 5, 8).

Operating Conditions. The light pulse consisted of a mixture of UV (λ = 0.347 μm) and red (λ = 0.694 μm), these particular wavelengths having recently been shown (4) to be most effective for plant analysis. The diameter of the impact varied from 150 to 200 μm and the depth eroded by each laser pulse varied from 10 to 45 μm. Generally, five spectra were recorded for each profile. The detection limit was reduced by cumulating five mass spectra obtained for the same depth zone. Calcium was analyzed simultaneously and, being considered constant, allowed verification of the reproducibility of the ionization of the sample. At the end of the analysis, the laser craters were visualized using a scanning electron microscope type Cameca MEB-07 (Fig. 1).
RESULTS AND DISCUSSION

Results of an experiment reported in Table I show clearly that the values obtained by spark-source MS of the isotopic ratio $^{11}\text{B}/^{10}\text{B}$ in the different parts of the plant differ significantly from the value of the natural abundance ($^{11}\text{B}/^{10}\text{B} = 4.1$), thus demonstrating the penetration and translocation of foliar applied boron.

The enrichment of each part (in $\mu$g/g or in $\mu$g) was easily calculated from the initial content of boron determined in a nontreated plant by reference to the Ca analyzed simultaneously with boron and also by neutron radioactivation. The values in per cent recovered in each plant part are reported in Table II and show that the greatest fraction of boron deposited on leaves is recovered in the treated leaf, corroborating the previous report by Martini and Thellier (6) concerning the slow translocation of this element from leaves. However, these authors used another technique which did not allow a quantitative evaluation in the treated part because the concentration of boron was too high in this zone.

**Table I. Abundance Ratios of Isotopes ($^{11}\text{B}/^{10}\text{B}$) in Different Parts of Radish Plants 24 h after Application of $^{11}\text{B}$Boric Acid on First Leaf**

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>$^{11}\text{B}/^{10}\text{B}$ Abundance Ratios in Following Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Treated leaf</td>
<td>$0.8 \pm 0.1$</td>
</tr>
<tr>
<td>Epicotyl</td>
<td>$3.3 \pm 0.3$</td>
</tr>
<tr>
<td>Hypocotyl</td>
<td>$2.7 \pm 0.3$</td>
</tr>
</tbody>
</table>

The mean value of the uptake was 74.2% of the amount applied.

**Table II. Distribution of Boron in Radish Plants 24 h after Application of $^{11}\text{B}$Boric Acid on First Leaf**

The mean value of the uptake was 74.2% of the amount applied.

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Boron Distribution in Following Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Treated leaf</td>
<td>$83.7$</td>
</tr>
<tr>
<td>Epicotyl</td>
<td>$7.0$</td>
</tr>
<tr>
<td>Hypocotyl</td>
<td>$9.3$</td>
</tr>
</tbody>
</table>

It was possible to specify the distribution of boron as a function of the depth at the very place where the droplets were deposited using laser-probe mass spectrophotography. Natural boron was not detectable by this technique in the leaves of reference plants, so it was not necessary to use enriched boron in these experiments. The detection of boron in the treated part would indicate a high content of this element in the analyzed tissues (>0.05% of the total number of atoms detected). Figure 2 reports the boron spectra recorded at three depths. Spectra in intermediary zones are not indicated because they are similar to those shown in Figure 2. Results were confirmed by analyses carried out at different points on the treated area. They revealed that boron presented an homogenous distribution with respect to the depth along the analyzed profile and was not superficially located, contrary to previous results with copper using the same technique (8). This leads to the conclusion that boron had entered through the leaf surface and was present within the tissues perhaps partly bound as borate polysaccharide complexes (7, 9), taking account of the low rate of translocation from the treated leaf. In the laser-probe analysis, the following elements appeared with boron on the photographic plates: C, N, O, Na, Mg, Al, Si, P, S, Cl, K, Ca.

The use of an enriched stable isotope analyzed by spark-source MS has allowed precise measurement of the distribution in entire plants of foliar-applied boron. This method might be applicable to other physiological studies using stable isotopes. Moreover, the laser-probe mass spectrophotograph appears suitable as a technique for cartographic or in-depth localizations. In the case of foliar absorption, it is possible to study the distribution of the applied element in the treated part, an aspect that is important for a better understanding of the physiological significance of the nontranslocated fraction, which often represents the greatest proportion of the uptake (1, 2).

**LITERATURE CITED**

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**Fig. 1.** Scanning electron micrograph showing the laser impact on a radish leaf after boron analysis.

**Fig. 2.** Analysis of boron at different depths from the surface in the treated part of a radish leaf with a laser-probe mass spectrophotograph. The thickness of the leaf blade at the site of the analysis was 150 μm.
DISTRIBUTION OF FOLIAR-APPLIED BORON


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