Substitution of Germanium for Boron in Plant Growth

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Summary. The observation was confirmed that the addition of germanium dioxide (soluble form) to the nutrient solution can delay for a short time the appearance of boron deficiency symptoms on the shoots of sunflower plants (Helianthus annuus L.). This appeared to be true, however, only under growing conditions in which the plants had a low boron requirement. The delay in the appearance of boron deficiency symptoms by administering germanium was demonstrated in sunflower plants ranging in age from 7 to 20 days. This effect was noted whether the germanium was administered prior to or at the time the plants were transferred to minus-boron nutrient solution.

It is proposed that germanium does not truly substitute for boron in metabolic processes of the plant but rather functions through increasing the mobility of soluble boron within the plant and in binding nonmetabolic polyhydroxyl sites thus serving in a sparing role for the limited quantity of soluble boron in the growth centers.

Various investigators have considered the possibility that the physiological action of boron may be related to the capacity of the borate ion to form complexes with polyhydroxyl compounds. Although earlier workers failed to do so (1, 3), Skok (5) was able to demonstrate that several other elements that form similar complexes could temporarily alleviate boron deficiency in sunflower plants. Of the elements examined, germanium appeared to function the most efficiently in this respect. Other than serving in a complexing role, those experiments offered no specific clue as to how germanium substituted for boron. The purpose of this investigation was to explore this particular point in more detail.

Materials and Methods

Sunflower (Helianthus annuus L., var. Mammoth Russian) achenes were sown in quartz sand moistened with tap water. After 1 week uniform seedlings were selected, the roots washed free of sand and the plants transferred to nutrient solution in quart soft glass jars wrapped in black cloth to exclude light. The basic nutrient solution and the conditions of the controlled-environment room in which the plants were grown have been previously described (5). Unless otherwise specified, plus-boron plants were given 500 µg of boron supplied as boric acid. Germanium dioxide (GeO₂, soluble form, sp gr 4.7) was added to the nutrient solution to give a germanium level of 3630 µg per plant.

The method selected for estimating soluble boron was one previously employed (4). Freshly harvested leaf blades were weighed and then homogenized in a refrigerated monel-metal Waring blendor cup for 5 minutes with twice their weight of distilled water. That part of the homogenate which passed through 2 layers of cheese cloth was centrifuged at 34,800 × g for 30 minutes at 3° to remove all particulate matter except perhaps some of the microsomal component. Complete exclusion of the microsomal fraction was not considered important since previous work had shown this component to be extremely low in boron (6). Aliquots of the supernatant fluid was dialyzed in cellulose tubing (Visking Company) against 10³ volumes of distilled water at 3° for 24 hours to remove dialyzable boron. Dialyzable (soluble) boron in the supernatant fluid was calculated as the difference between total and non-dialyzable. The determination of dialyzable boron by this method was found to be an acceptable procedure in previous experimentation (4). Normally, a minimum of 25 g of leaves harvested from at least five plants were included in each sample. Duplicate biological samples were analyzed for each experimental treatment.

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All samples were dried at 90° and ashed in a muffle furnace at 550°. Boron analyses were performed according to the curcumin procedure of Dible et al. (2). In preliminary experimentation using this analytical method it was discovered that the curcumin reagent would react with germanium to produce a color reaction similar to that for boron. It was found, however, that it took over 40 units of germanium to yield a color reaction equivalent to that produced by 1 unit of boron. In spite of the interference of germanium in boron analysis, the authors could not demonstrate a statistically significant difference in the boron content of the supernatant fraction of leaves from plants administered a given level of boron irrespective of whether they had also been supplied germanium (e.g. see table I, series 1 vs. series 4). This method was, therefore, deemed acceptable for use in this investigation. Statistical analyses were carried out according to established procedures (7).

Data and Discussion

Preliminary experiments confirmed Skok's (5) observations that, under the environmental conditions he indicated, the addition of germanium to the nutrient solution of 7-day-old seedlings could delay the appearance of boron deficiency symptoms on the shoots. It was further observed, however, that when plants were grown under higher light intensities there was little or no delay in the appearance of boron deficiency symptoms by plants administered germanium. It appeared that more rapid growth of the plants occurred at the higher light intensities, and it is presumed that they therefore had a higher boron requirement. A high plant boron requirement under the growing conditions used in Eaton's experiments (3) may explain his inability to demonstrate a beneficial effect of germanium substitution for boron.

The appearance of boron deficiency symptoms on the shoots could be delayed by applications of aqueous solutions of GeO₂ (0.052 mg GeO₂ per application) to the terminal bud of 7-day-old seedlings. Such applications to the cotyledons, however, were not effective. As previously observed (5), germanium applications in the nutrient solution resulted in a root system with a greater degree of branching. This characteristic root development occurred in all plants grown in nutrient solution containing germanium irrespective of whether boron was also present. Germanium applications to the

Fig. 1. Top view of 19-day-old sunflower plants 4 days after transfer from (A) plus-boron to minus-boron nutrient; (B) plus-boron to minus-boron, plus-germanium nutrient; and (C) plus-boron, plus-germanium to minus-boron nutrient. Note boron deficiency symptoms in young leaves of A but not in B or C.
terminal bud caused some necrosis of leaves of the bud but did not, within the term of the experiments, result in any malformation of the roots.

Plants which were grown in a nutrient solution containing an initial quantity of 500 µg of boron for 15 to 20 days prior to being transferred to minus-boron usually showed deficiency symptoms four days following this transfer. For plants which were supplied with germanium at the time of such transfer, the appearance of deficiency was delayed for an additional 3 to 4 days. If plants were supplied with boron and germanium during the initial 15 to 20 days growth period and then transferred to minus-boron solution, the appearance of deficiency symptoms was delayed for at least 4 days and in some experiments for a longer period (fig 1).

Variations of boron levels in the nutrient solution ranging from 50 to 500 µg during the initial growth period resulted in only small differences in the time of appearance of deficiency symptoms on the shoots following transfer to minus-boron. This was true even though the plants grown on the higher levels of boron contained considerably more total and soluble boron in their tissues. Additions of germanium to the nutrient solution at the time the plants were transferred to minus-boron nutrient resulted in similar delays in the appearance of deficiency symptoms regardless of what the previous boron level had been.

The fact that germanium alleviated the appearance of boron deficiency symptoms for only a short period suggested that this element probably did not truly substitute for the physiological requirement of boron in the plant; or if it did, it was in a very limited or inefficient manner. Two other obvious possibilities presented themselves as to how germanium caused the delay in the appearance of boron deficiency symptoms. One was that it resulted in a greater mobility of boron within the plant whereby the soluble boron in the older organs moved more readily to the meristematic sinks. In a previous investigation it was shown that although a rather large pool of soluble boron existed within the older leaves it was relatively immobile and was not redistributed rapidly enough to meet the requirement of a rapidly growing plant (4). The second possibility appeared to be that germanium might serve in a sparing role for boron in the meristems. It was possible that germanium could be fixed by polyhydroxyl compounds in non-metabolic complexes thereby permitting the limited quantity of soluble boron to be utilized in metabolic processes.

In an attempt to determine whether germanium increased boron mobility, experiments were conducted in which boron analyses were made of the supernatant fractions from fully expanded leaves prior and subsequent to transferring the plants to minus-boron nutrient solution. Data from a representative experiment are shown in tables I and II. In this experiment the first boron analyses were made of leaves from nodes 2 to 5 for 2 series of 15-day-old plants at the time of transfer to minus-boron solutions (Series No. 1, plus-boron plants and Series No. 4, plus-boron, plus-germanium plants). Four days later a second set of samples were harvested from equivalent nodes for analyses (Series No. 2, plus-boron plants transferred to minus-boron; Series No. 3, plus-boron plants transferred to minus-boron, plus-germanium and Series No. 5, plus-boron, plus-germanium transferred to minus-boron).

A significantly lower concentration of total and dialyzable boron in the supernatant was found after the plants had been on minus-boron (Series No. 2) or minus-boron, plus-germanium (Series No. 3) nutrient solution for 4 days (table I). A similar pattern was observed in plus-boron, plus-germanium plants transferred to minus-boron solution (Series No. 5). There was, however, no significant difference between minus boron (Series No. 2) and minus-boron, plus germanium (Series No. 3) plants in the concentrations of boron in the supernatant fractions. Thus, one might be inclined to conclude that germanium did not result in an increased mobility of boron. As pointed out in a previous publication (4), concentration values may be misleading with respect to boron redistribution. It is quite possible because of dry weight accumulation for a large change in boron concentration to occur without any significant shift occurring in the absolute amount of boron. The change in the total boron content of a leaf, therefore, is a better index of boron mobility. When this was considered, not only was there a significantly smaller amount of boron found in the leaves of plants switched to minus-

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**Table I. Boron Concentration in Supernatant Fraction of Mature Leaves**

<table>
<thead>
<tr>
<th>Series No.</th>
<th>Treatment</th>
<th>Boron per 10 ml supernatant (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>1</td>
<td>+B</td>
<td>25.69 ± 0.31</td>
</tr>
<tr>
<td>2</td>
<td>+B to −B</td>
<td>16.18 ± 0.65*</td>
</tr>
<tr>
<td>3</td>
<td>+B to −B+Ge</td>
<td>15.41 ± 0.03*</td>
</tr>
<tr>
<td>4</td>
<td>+B+Ge</td>
<td>27.56 ± 1.57</td>
</tr>
<tr>
<td>5</td>
<td>+B+Ge to −B</td>
<td>15.41 ± 0.03***</td>
</tr>
</tbody>
</table>

* Significantly different from No. 1 at 1% level.
** Significantly different from No. 1 at 5% level.
*** Significantly different from No. 4 at 1% level.
boron solutions, but those plants receiving germanium had a significantly still lower quantity (table II). Under the conditions of this experiment, there was not only a demonstrable mobility of boron in sunflower but the supplying of germanium in the nutrient solution increased the degree of mobility.

These data are, therefore, at variance with those of previous work with respect to boron mobility in sunflower (4). They do, however, substantiate the proposal previously made that it is very likely that boron mobility is relative, and that under environmental conditions of low requirement for this element, plants may show a significant degree of mobility. The same species under conditions in which the plant displays a high boron requirement may show visual deficiency symptoms before any appreciable redistribution of boron can occur. It was indicated earlier in this discussion that germanium appeared to delay the appearance of boron deficiency symptoms only when plants had a rather low boron requirement.

Although it was found that germanium resulted in a greater boron mobility when administered at the time the plants were transferred to minus-boron nutrient solution, such did not appear to be the case where germanium was given during the pretreatment period. Under these conditions there was no increased redistribution of boron from the older leaves (table II). These plants, nevertheless, exhibited a delay in the appearance of boron deficiency symptoms (fig 1). Therefore, it appears possible that germanium may also act in a boron sparing role. The delay in appearance of boron deficiency symptoms when germanium was applied to the terminal bud also appears to support this suggestion.

### Table II. Total Boron per Leaf in Supernatant Fraction of Mature Leaves

<table>
<thead>
<tr>
<th>Series No.</th>
<th>Treatment</th>
<th>Supernatant boron per leaf (µg)</th>
<th>Total</th>
<th>Non-dialyzable</th>
<th>Dialyzable</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+B</td>
<td>11.50 ± 0.14</td>
<td>0.77 ± 0.02</td>
<td>10.73 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+B to −B</td>
<td>10.75 ± 0.19</td>
<td>0.98 ± 0.01**</td>
<td>9.77 ± 0.15**</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>+B to −B+Ge</td>
<td>9.93 ± 0.23*</td>
<td>1.06 ± 0.04**</td>
<td>8.87 ± 0.17**†</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+B+Ge</td>
<td>5.97 ± 0.34</td>
<td>0.40 ± 0.01</td>
<td>5.57 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>+B+Ge to −B</td>
<td>5.89 ± 0.92</td>
<td>0.71 ± 0.04***</td>
<td>5.18 ± 0.87</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from No. 1 at 5 % level.
** Significantly different from No. 1 at 1 % level.
*** Significantly different from No. 4 at 1 % level.
† Significantly different from No. 2 at 5 % level.

### Literature Cited