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# Magnesium Nutrition of Two Species of Sunflower

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*Abstract.* *Helianthus annuus* L., the common cultivated sunflower, and *Helianthus bolanderi* Gray subspecies exilis Heiser, a form endemic on serpentine soils, were grown in culture solutions with widely varying Mg levels. For comparable development, *H. bolanderi* exilis required higher levels of Mg in the solution than *H. annuus*, both in the range of visual deficiency symptoms and in higher ranges.

With 0.5 mM Ca in the solutions, *H. annuus* reached a plateau in yield in the range of 0.25 to 2.0 mM Mg in the solution, then declined in weight markedly as Mg was increased further. This decline was probably caused by the marked "luxury" consumption of Mg. Conversely, *H. bolanderi* exilis increased steadily in yield as Mg was raised up to 10 mM and tolerated up to 50 mM Mg in the solution with only about 30% decline in yield. The increasing yield of *H. bolanderi* exilis in solutions of increasing Mg level is attributable to the steadily rising Mg content of the plants. A high level of external Mg is apparently needed to bring internal Mg to the sufficiency range. This characteristic of the species also serves to obviate luxury consumption of Mg, thus contributing to its tolerance of very high external levels of this ion.

Although in the past Mg levels used in nutrient solutions often ranged up to 10 to 20 mM, in recent decades most nutrient cultures have been 2 mM or less in this ion (2). Successful growing of numerous plant species at the latter level indicates its adequacy for many experimental plants. This is true in spite of a wide range in frequency of change of solutions, volume of solution per plant, season, and other factors affecting the Mg supply needed. In fact, to induce deficiency, Mg must be reduced in most cases to levels under 0.2 mM.

Although the customary 2 mM Mg is adequate for many plants, higher levels may be necessary for growing certain species. In particular, those native to high magnesium soils derived from serpentine or dolomite have been presumed to have unusually large requirements for this element on the basis of high contents of Mg in both the soil and the plants (7). This has not been tested experimentally, although it has not appeared too likely because serpentine endemics usually grow well on non-serpentine soils (4). Further, most studies of plant growth on soils of this type have stressed the low availability of calcium and its limitations on development, rather than emphasizing the Mg requirement (9, 10, 11).

In this study 2 sunflower species, *Helianthus annuus*, the common cultivated form, and *Helianthus bolanderi* exilis, an endemic on serpentine soils, were used. The principal objectives were to compare their responses in dry matter yield to widely varying Mg supplies, and to compare their Mg requirements using plant tissue analyses.

## Materials and Methods

Seeds of *Helianthus bolanderi* Gray subspecies exilis Heiser were originally collected about 4.3 km east of Middletown (Lake County), California. These were then grown in our greenhouse and selected for *H. bolanderi* exilis (hereafter called exilis in this paper), since the wild form was believed to be a mixture of *H. bolanderi* exilis (serpentine endemic) and *H. bolanderi* (serpentine tolerant). Seeds of *Helianthus annuus* L. var. Russian Mammoth (hereafter called annuus in this paper), were obtained commercially from Olds Seed Company, Madison, Wisconsin. Seedlings were grown in greenhouse flats containing unsterilized silica sand. The flats were watered with tap water and after germination sprinkled occasionally with 1/10 strength nutrient solution F (table I).

The seedlings, when 8 to 10 cm tall (8–10 days old in the case of annuus and 4 to 5 weeks old in the case of exilis), were transferred to solution cultures in 2-quart Mason jars which had been painted on the outside with 2 coats of black asphaltum varnish and then wrapped with aluminum foil. One seedling of annuus or 1 or 2 seedlings of exilis were grown in each jar. The solutions were aerated through capillary tubes (8). Four or 6 replicates were grown for each treatment.

The solutions used (table I) were modified from Hoagland and Arnon solution No. 2 (3). Variation in Mg concentrations in the culture solutions was obtained by substitutions of $MgSO_4$ for $Na_2SO_4$. In experiments with high Mg levels, the Ca level in the solutions was reduced to 0.50 mM to accentuate the Mg:Ca ratio, and in such cases $NaNO_3$ was used to maintain the nitrate level. For extremely high

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Mg treatments half rather than full levels of other ions, except for micronutrients and Fe, were used to keep osmotic concentrations as low as feasible. All solutions were prepared using distilled water. The solutions were changed once every week, and the volumes were maintained between changes by addition of distilled water as required.

All plants were grown in the greenhouse during the spring and summer months, under natural light and greenhouse temperatures (about 16–18° at night and 18–29° in the day during the spring, and around 16–21° at night and 24–35° in the day during the summer). The plants were harvested about 30 days after transferring to the culture solutions. At this time the plants had either approached maturity or had started showing severe deficiency or toxicity symptoms. Leaves, stems, and roots of individual plants were harvested separately and analyzed for Mg, Ca, K, and Na.

The tissue samples were ashed in a muffle furnace at 500° and then taken up in dilute HCl. Ca and Mg were determined with $Na_2EDTA$, after Lewis and Melnick (5), but substituting Napthol Blue (Mallinckrodt #5630) as calcium indicator and Calmagite (Mallinckrodt #4283) as Ca plus Mg indicator. The suitability of the EDTA method was confirmed by comparing with the results obtained by a conventional precipitation of Ca as the oxalate and titration with standard potassium permanganate in hot sulfuric acid solution, and for Mg by a colorimetric molybdivanadate method. Potassium and sodium were determined by using a Beckman DU

Table I. *Composition of Culture Solutions (mMoles per Liter)*

Micronutrients were supplied as follows (μmoles per liter): Fe, 90 (as NaFeEDTA); B, 46; Mn, 9.1; Zn, 0.76; Cu, 0.32; Mo, 0.52. The pH of the solutions was adjusted to 5.5 using 0.1 N NaOH. The osmotic potentials of the solutions in atm were as follows: A-F: 0.60; N-R: 0.96; S-V: 1.30 and W-X: 2.0.

| | $NH_4H_2PO_4$ | $KNO_3$ | $Ca(NO_3)_2$ | $NaNO_3$ | $MgSO_4$ | $Na_2SO_4$ | NaCl |
|---|---|---|---|---|---|---|---|
| A | 1 | 6 | 4 | 0 | 0.005 | 1.995 | 0.1 |
| B | 1 | 6 | 4 | 0 | 0.025 | 1.975 | 0.1 |
| C | 1 | 6 | 4 | 0 | 0.05 | 1.95 | 0.1 |
| D | 1 | 6 | 4 | 0 | 0.2 | 1.8 | 0.1 |
| E | 1 | 6 | 4 | 0 | 0.4 | 1.6 | 0.1 |
| F | 1 | 6 | 4 | 0 | 2.0 | 0.0 | 0.1 |
| N | 1 | 6 | 0.5 | 7 | 2 | 8 | 0.1 |
| O | 1 | 6 | 0.5 | 7 | 4 | 6 | 0.1 |
| P | 1 | 6 | 0.5 | 7 | 6 | 4 | 0.1 |
| Q | 1 | 6 | 0.5 | 7 | 8 | 2 | 0.1 |
| R | 1 | 6 | 0.5 | 7 | 10 | 0 | 0.1 |
| S | 0.5 | 3 | 0.5 | 3.5 | 5 | 25 | 0.1 |
| T | 0.5 | 3 | 0.5 | 3.5 | 10 | 20 | 0.1 |
| U | 0.5 | 3 | 0.5 | 3.5 | 20 | 10 | 0.1 |
| V | 0 5 | 3 | 0.5 | 3.5 | 30 | 0 | 0.1 |
| W | 0.5 | 3 | 0.5 | 3.5 | 40 | 10 | 0 1 |
| X | 0.5 | 3 | 0.5 | 3.5 | 50 | 0 | 0.1 |

Table II. *Cation Composition in the Low Mg Range*

Ca level in the solutions was 4.0 mM. All analyses are the averages of determinations on 2 of the 4 replicate plants.

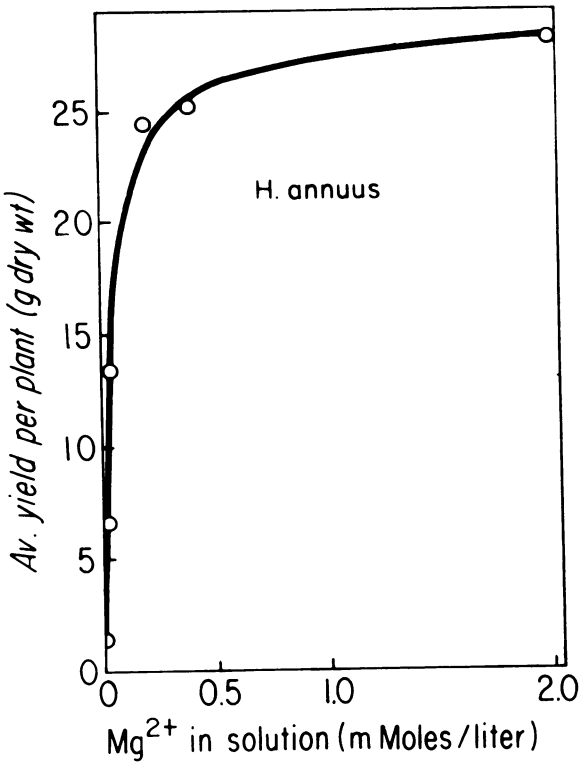
| Mg in Sol. | Plant Spp. | Avg. Yield of shoot | Mg | Ca | K | Na |
|---|---|---|---|---|---|---|
| *mM* | | *g dry wt* | | *mEq per 100g dry wt* | | |
| 2.00 | Annuus | 23.8 | 43.5 | 69.7 | 35.2 | 2.3 |
| | Exilis | 2.20 | 26.7 | 65.2 | 40.5 | 5.7 |
| 0.40 | Annuus | 20.2 | 20.9 | 86.1 | 41.3 | 4.2 |
| | Exilis | 0.86 | 17.3 | 66.3 | 51.9 | 7.0 |
| 0.20 | Annuus | 19.8 | 15.0 | 93.9 | 44.5 | 4.3 |
| | Exilis | 0.44 | 9.0 | 61.6 | 47.9 | 10 8 |
| 0.05 | Annuus | 12.0 | 9.0 | 86.6 | 47.1 | 4.5 |
| | Exilis | 0.08 | 6.0 | 75.0 | 43.7 | 9.0 |
| 0.025 | Annuus | 6.22 | 6.9 | 97.8 | 47.8 | 4.6 |
| | Exilis | 0.10 | 6.5 | 84.0 | 46.2 | 7.0 |
| 0.005 | Annuus | 1.35 | 6.3 | 91.5 | 53.0 | 6.3 |
| | Exilis | 0.06 | 5.7 | 78.5 | 47.5 | 9.4 |

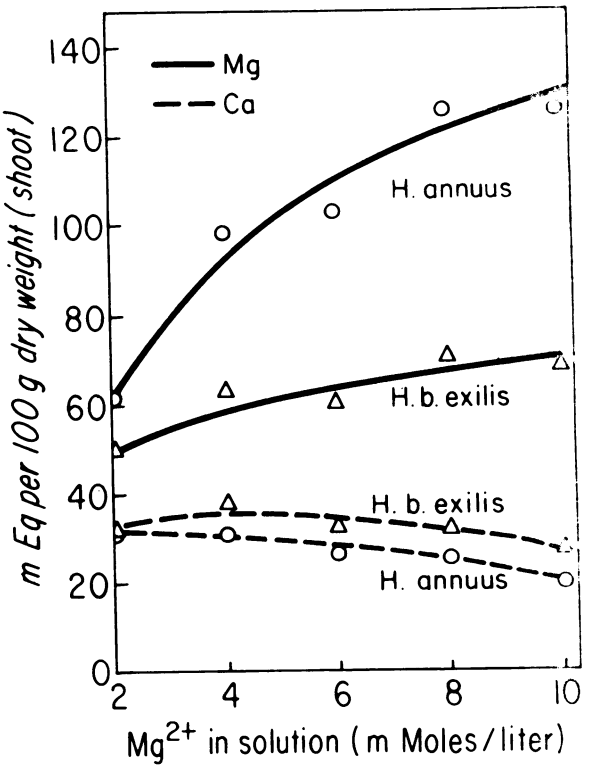
Flame Spectrophotometer, comparing with standard chloride solutions ranging from 0 to 4 mM (K) and from 0 to 2.5 mM (Na).

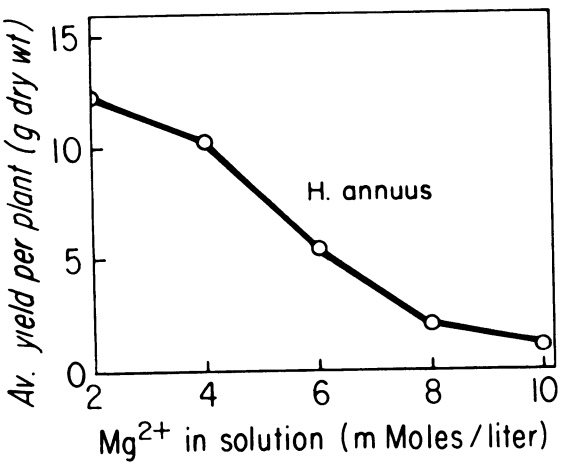
## Results

*Magnesium Deficiency.* Annuus and exilis were grown in culture solutions A through F (table I) at 0.005 to 2.0 mM Mg and 4.0 mM Ca. Yields of the 2 species are plotted against Mg level in the culture solution in Fig. 1 and 4. The cation contents of the shoots are given in table II.

Magnesium deficiency symptoms were more severe in exilis than in annuus, particularly in the range of 0.005 to 0.05 mM. This difference was reflected also in the yields of the 2 species. In this range, annuus showed a sharp increase in yield in contrast with little increase in exilis. At higher



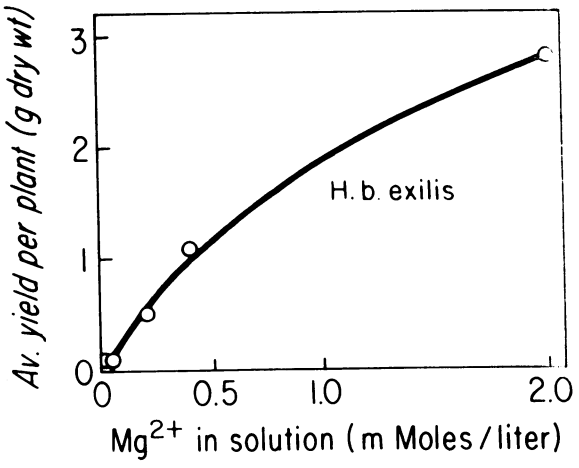




FIG. 1. (upper left) Yield of annuus as affected by 0.005 to 2.0 mM Mg in the culture solutions. Ca level: 4.0 mM.
FIG. 2. (lower left) Yield of annuus as affected by 2 to 10 mM Mg in the culture solutions. Ca level: 0.5 mM.
FIG. 3. (upper right) Levels of Mg and Ca in the shoots of annus and exilis as affected by 2 to 10 mM Mg in the culture solutions. Ca level: 0.5 mM.
FIG. 4. (lower right) Yield of exilis as affected by 0.005 to 2.0 mM Mg in the culture solutions. Ca level: 4.0 mM.

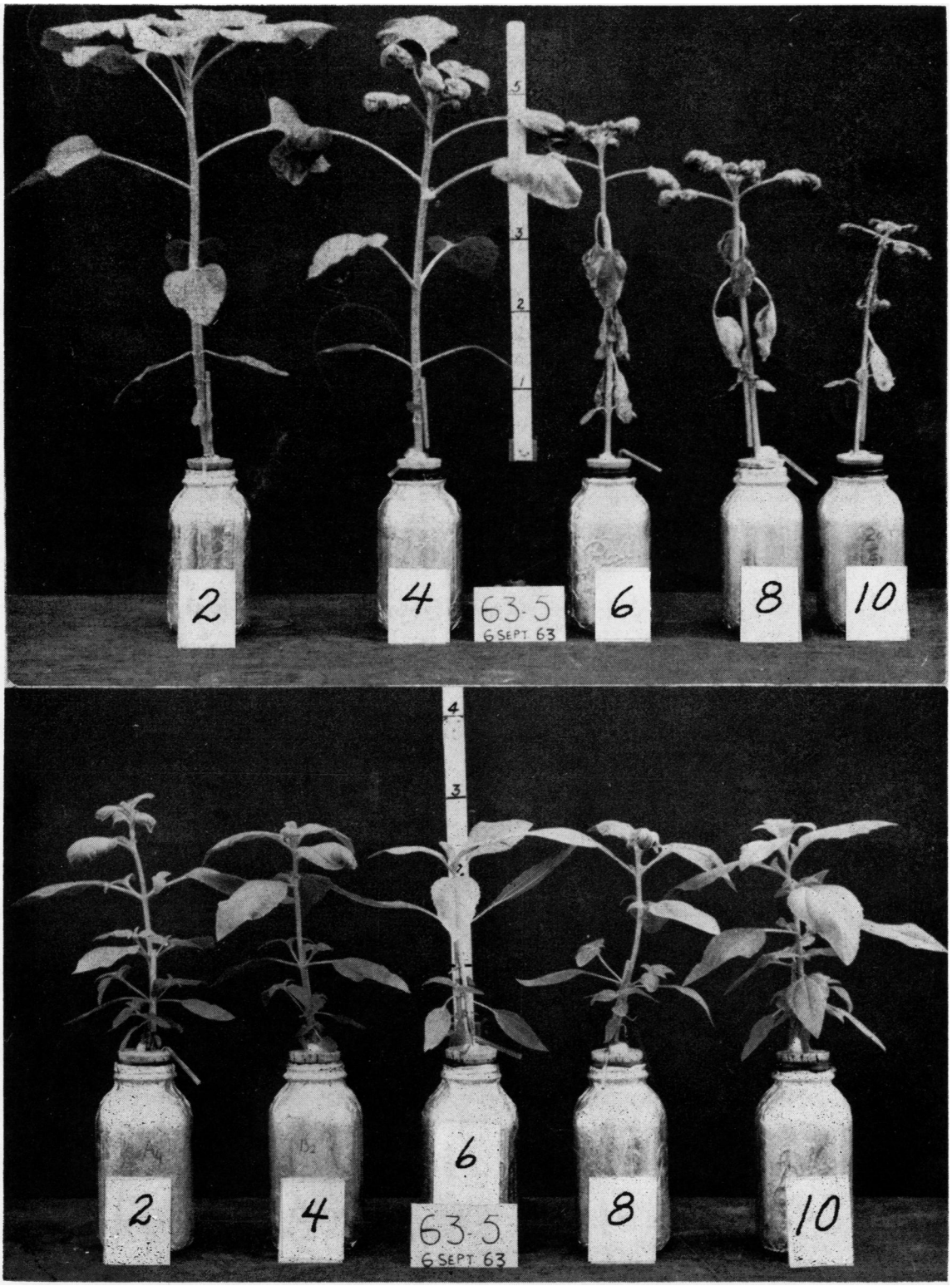



FIG. 5. Effects of 2 to 10 mM Mg on the growth of annuus (upper) and exilis (lower). Ca level: 0.5 mM. Scale in the background of photos is in decimeters.

Mg levels the relationship was reversed. The yield of annuus rose very little from 0.20 to 2.0 mM, but exilis continued to increase in yield over this range. The difference in the yield responses of the 2 species suggested that the Mg requirement of exilis was higher than that of annuus. In further support of this indication, there was a continuous increase in yield with increasing Mg in the shoots of the endemic species. On the contrary, in the cultivated species the internal Mg levels correlated with the yield only up to the 0.20 mM Mg treatment. Above this level, the yield of annuus leveled off but the Mg content in the shoots continued to increase, indicating "luxury" absorption of Mg.

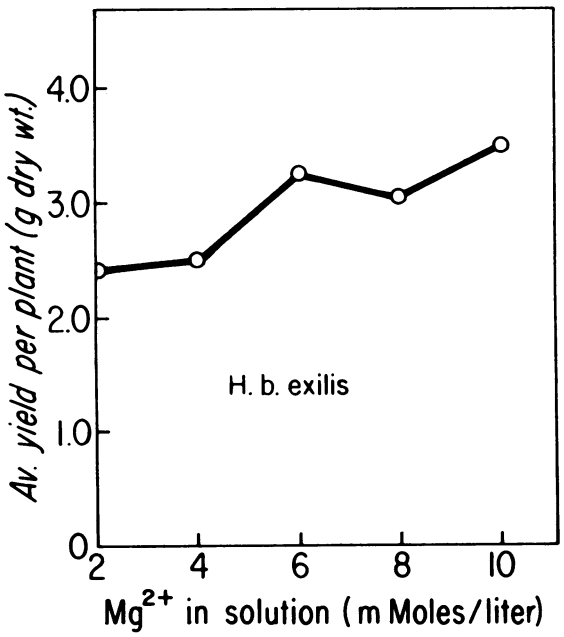
The chemical analyses further showed that the Ca levels were slightly higher in the shoots of annuus. Also there was a regular increase in K contents of annuus with decreasing Mg levels in the culture solutions, but K content did not vary consistently in the case of exilis. Exilis absorbed somewhat more Na than the cultivated species, but in both species the amounts of Na taken up were small in comparison with the other cations.

*Experiments With Intermediate Mg Levels.* In a series of experiments, the effects of intermediate levels of Mg on the growth and yield of the 2 species were investigated. When the Mg level was increased to 5 mM with Ca at 4 mM, there was a slight increase in the yield of exilis but no noticeable effect on the yield of annuus. To accentuate the Mg effect in subsequent experiments, Mg was increased to 10 mM and the Mg:Ca ratio increased further by lowering the Ca level to 0.5 mM. Before doing this we examined the effects of this lower level of Ca on the growth of annuus, at a normal Mg concentration (2 mM). This caused a reduction in yield of about 20 %, but the general vigor of the plants was not affected. The shoots did not show any Ca deficiency symptoms and the roots in all treatments looked very healthy.

Annuus and exilis seedlings were then grown in solutions of N through R (table I) containing 2 to 10 mM Mg at 0.5 mM Ca. Fig. 5 shows the plants photographed at the time of harvest. The yields and the contents of Mg and Ca in the shoots are plotted against Mg in the solution in Fig. 2, 3, and 6.

The yields of annuus decreased rapidly with increasing Mg in the solution. At levels higher than 2 mM, the leaves showed symptoms of disorder very early. The lower leaves developed brown spots, especially on the margins, possibly caused by Mg toxicity. In the case of upper leaves, the margins and tips showed downward curling, presumably from Ca deficiency. These symptoms became more and more severe with increasing Mg levels, leading in the end to necrosis and dying of the lower leaves, more curling of the upper leaves, and a typical "rosette" condition of the youngest leaves. The roots in all the treatments higher than 2 mM Mg turned brown and became slimy to the touch, probably due to mixed effects of high Mg and low Ca.

In contrast to annuus, increasing Mg levels enhanced the yield of exilis. Further, in none of the treatments did its lower leaves show any brown or necrotic spots. Some of the young leaves developed a little downward curling, but in general the leaves



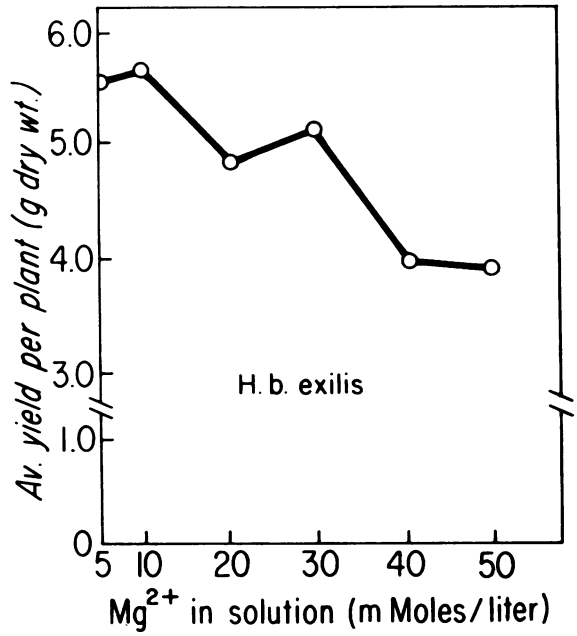




FIG. 6. (left) Yield of exilis as affected by 2 to 10 mM Mg in the culture solutions. Ca level: 0.5 mM.
FIG. 7. (right) Yield of exilis as affected by exceptionally high concentrations of Mg in the culture solutions. Ca level: 0.5 mM.

were healthy and did not show Ca deficiency symptoms. The roots of this species were normal in all treatments.

The chemical analyses (Fig. 3) showed that the cultivated species absorbed much more Mg than the endemic species. At Mg levels in the solution higher than 4 mM, annuus absorbed about twice as much Mg as exilis. In fact, the Mg contents of the shoots of annuus from the 2 mM treatment were about the same as those of exilis from the 10 mM treatment. Also, annuus absorbed less Ca than exilis. The K and Na levels followed a pattern similar to that observed in the Mg deficiency experiment (table II), indicating little influence of the treatments, so are not included here.

*Experiments Using High Mg.* The increase in yield of exilis under intermediate Mg levels led to an experiment with this species in which the range was increased to 5 to 30 mM Mg (solutions S through V, table I). After 2 weeks in the culture solutions, the upper leaves of the plants in the 20 and 30 mM treatments had begun to show symptoms of Ca deficiency but the lower leaves and the roots appeared to be quite normal. At this stage, additional plants from the seedling flat were transferred to solutions W and X containing 40 and 50 mM Mg respectively. The shoots of these plants soon developed symptoms similar to those in 20 and 30 mM levels, but the roots, though less vigorous in growth, were otherwise healthy and of normal color. The plants in the 40 and 50 mM treatments were harvested after a similar period of growth (36 days) as the ones in lower levels. The average yield per plant is plotted against Mg in the solution in Fig. 7. Yield declined only about 30 % even at the highest Mg concentration.

## Discussion

Clear differences in response to Ca-Mg regimes, both at Mg deficiency and at high Mg levels, are demonstrated in these 2 species of the same genus.

Magnesium deficiency symptoms in exilis were more severe at a given Mg level in the solution, showing that this species demanded a higher Mg supply than annuus. This is more definitely indicated by the yield responses of the 2 species. The yield of annuus leveled off at about 0.20 mM Mg (Fig. 1), but yield of exilis continued to rise with increasing Mg (Fig. 4). In exilis the plants were normal in appearance in the 2.0 mM Mg treatment, but were much smaller and showed obvious deficiency symptoms in the 0.40 mM treatment. Subsequent plant tissue analyses indicated that the requirement for Mg of this species is in the range of about 17 to 27 mEq per 100 g dry tissue (table II). In contrast, the plants of annuus were normal even in the 0.20 mM treatment. Only in the 0.05 mM treatment was yield reduced and were deficiency symptoms obvious. Looking at the Mg contents of the plants from these treatments, the Mg requirement of annuus appears to be about 9 to 15 mEq per 100 g. Wallace (12) reported that the levels of Mg are in the range of 30 to 50 mEq per 100 g dry matter for healthy and 20 to 25 mEq for Mg-deficient leaves of plants such as apple, black currant, kale, and potato. These levels are somewhat higher than the Mg contents required for normal appearance of the sunflower species in our experiments.

A probable reason for the lower Mg contents of exilis plants at a given Mg level in the solution, is a stronger depression of Mg uptake by Ca in this species than in annuus. This was noted by Walker *et al.* (11) in soil-grown plants. Preliminary short-term absorption studies in culture solutions (6) gave similar indications. This depression of Mg uptake may explain the drop in yield of exilis at high Ca levels as reported by Grover (1).

Mg levels up to 5 mM accompanied by Ca levels of 4 mM did not have a noticeable effect on the yield of annuus. This was to be expected, since the Mg:Ca ratio in the solutions scarcely exceeded unity. When the Mg level was raised to 10 mM and the Mg:Ca ratio was increased by reducing the Ca level to 0.50 mM, the effects of Mg became very noticeable. The lack of tolerance by annuus of such levels of Mg and consequently its depression in yield is probably due to luxury absorption of Mg (Fig. 3). In annuus there is a sharp increase in Mg content with each increment of additional Mg in the culture solutions. In contrast, over the same range, there is only a very moderate increase in Mg in exilis (Fig. 3). However, over this range the yields of exilis increased (Fig. 2), which meant that total Mg uptake per plant rose substantially.

The yield of exilis dropped off slowly when the Mg levels were increased beyond 10 mM. At one stage, it was thought that this drop in yield might be caused by high osmotic concentration in the culture solutions. Measurement of the osmotic potential of the solutions (heading, table I), showed that the value for the most concentrated solution was 2.0 atm. Such an osmotic concentration was found to cause little, if any, decrease in yield. The decrease in yield in the range of 10 to 50 mM Mg is thus attributed to high Mg effects, particularly in depressing the absorption and utilization of other cations. There is, for example, distortion of leaves at the higher Mg levels, indicating a Mg-induced Ca deficiency. However, it is noteworthy that the yield of exilis decreased only about 30 % as Mg in the solution increased from 10 to 50 mM, whereas the yield of annuus dropped about 90 % as Mg in the solution increased from 2 to 10 mM. This striking difference in the yield responses of the 2 species clearly shows the sensitivity of annuus and the exceptional tolerance of exilis to extremely high levels of Mg.

The results permit some suggestions concerning the nature of tolerance and intolerance to serpentine

and other high Mg soils. Some plants appear to be intolerant of these environments not only because of the low availability of calcium, but also because of luxury consumption of Mg The latter causes reduced growth apparently because of unfavorable cation balance. Tolerance of the high magnesium habitat, on the other hand, at least in the case of exilis, involves not just ability to withstand very high Mg (up to at least 50 mM) but also a higher requirement for Mg. Raising the Mg supply, stepwise from 0 to 10 mM, results in a gradual increase in Mg content of the plant tissue and a steady increase in yield. High external levels of Mg are necessary to bring the internal level to the sufficiency range. It follows that this same feature protects exilis against luxury consumption.

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