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NASA CR 109873

ROLE OF GRAVITATIONAL STRESS IN LAND PLANT EVOLUTION: THE GRAVITATIONAL FACTOR IN LIGNIFICATION

by

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PREPARED UNDER
GRANT NO. NGR 12-001-053
WITH THE
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

UNIVERSITY OF HAWAII
HAWAII BOTANICAL SCIENCE PAPER
No. 19

JUNE 1970

SEMI-ANNUAL REPORT

Part I

June 1970

UNIVERSITY OF HAWAII

Honolulu, Hawaii

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NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Grant No. NGR-012-001-053

Preface

Evolution of the upright land plant can be associated with adaptive changes in the permeability of cells to water and their resistance to crushing forces. Although the appearance of the vascular plant involves many changes, lignification has often been identified as the major innovation in wall chemistry permitting the upright habit. Many species bear out the relationship of wall-permeating lignins to erectness and flexural strength, but the ubiquity of lignin in vascular forms can in no way account for all of the chemo-mechanical properties of plants. Silica has a role in many cell forms: *Equisetum spp.*; leaves of cereals and grasses; the stone cells of fruit; and epidermal hairs of many stems and leaves (e.g. nettles).

Further evidence for a supportive role for silica derives from diatoms, radiolarian skeletons, and (recently) from its reported appearance in early stages of bone healing, before deposition of hydroxyapatite is well initiated.

In diatoms, $GeO_3^{=}$, Group IV an analog of $SiO_3^{=}$, is a silica antagonist. Whether or not it acts as such in all cases remains an important point better resolved by experiment than conjecture.

The report which follows represents the first of the studies supported under the Principal Investigator's research programs to earn a graduate degree. It consists of a basic investigation of silicate in plant growth, and the toxicity of germanate and GeO_2.

Appended to the study is a brief report of work in progress showing that *Elodea*, a water plant derived from land angiosperm ancestry, can be induced to grow significantly out of water only when provided with relatively large amounts (10^{-3}M) of orthosilicate. Experimentation with this system has great promise as it suggests that a form limited by genetic loss to a buoyant medium could achieve another solution to its support requirements — silicification — within its existing genetic structure.

This thesis is part of a research program supported by National Aeronautics and Space Administration Research Grant NGR-012-001-053.

METABOLIC AND MORPHOLOGICAL EFFECTS OF SILICON AND GERMANIUM ON PLANTS

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN BOTANY

AUGUST 1970

By

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INTRODUCTION

Silicon has been discussed as an essential and non-essential element for plant growth since 1862, when Julius Sachs questioned whether or not silicic acid is an indispensable substance for those plants that contain silicon (17). Since the time of Sachs, methods of plant physiology have improved greatly and evidence pro and con of Sachs' question has accumulated. However, the role of silicon in plant growth has not yet been completely defined, and, with advancing technology, investigations are continuing. Interest in the possible metabolic role of silicon in plants seems to be legitimately founded because of the quantity of naturally occurring silicon. After oxygen, silicon is the most abundant element, and silica is the most abundant of all oxides (3). Compounds of silicon constitute 87% of the earth's crust (25).

EXAMINATION OF LITERATURE

Distribution of Silicon in Plants

Silica is deposited in epidermal cells and cell walls of many species of plants. It is also common in spines, spicules, needles (modified leaves), vessels and fibers. In some plants, silicon is not recognizable in any specialized structures, but, is instead diffusely distributed throughout the organisms. Amounts of silicon in roots and shoots vary. In tomato, radish, green onion, chinese cabbage and wheat silica content is either equal in the roots and shoots or higher in the roots than in the shoots (2, 15). In rice and oats, the silicon content of the aerial parts is higher than that of the roots (5, 15).

Jones, Milne and Wadham (7) found that all outer cell walls of the epidermis (except cork), subepidermal cell walls, vessels, fibers and tracheids of oat contain silicon. The silicon was deposited in association with other cell wall constituents. Yoshida, Ohnishi and Katagishi (28, 29), in a similar study of rice plants, found silicon in the epidermis, vascular bundles, bundle sheath and sclerenchyma of the leaf blade, in the outer epidermis, vascular bundles and walls of parenchyma cells of the leaf sheath and in all tissues of the root. In the leaf blade, there was a very thin layer of cuticle and just inside this a layer of silica. Beneath the cuticle and silica layers,

the silicon was intimately associated with cellulose in the epidermal cells. The authors attributed a limiting of water loss to this arrangement of substances.

Plant silicon content varies also with age. Younger rice plants and plant parts have less silicon than older plants and plant parts (15). Jones, Milne and Wadham (7) found that portions of six week old oat plants are silicified, but this silicification does not interfere with the enlargement of immature cells. This suggests that silicon is not a rigid deposit when it is first laid down.

Silicon Uptake and Chemical Forms

Silicon is usually supplied in nutrient media as meta-silicate which hydrolizes in water to give orthosilicic acid, $Si(OH)_4$ (12). This form is readily absorbed by diatoms and other plants. Silicon occurs in this form in soil solution and natural water below pH 9. Other evidence of the form of silicon during uptake comes from studies of xylem sap (1, 2). In the xylem, silicon is always in the form of monosilicic acid.

Silicic acid, however, represents only a small portion of plant silicon. Jones and Milne's study of oat (6) found most of the plant silicon to be non-crystalline and isotropic. Rates of dissolution (10, 27) and infrared absorption studies (27) show that silica gel is the form of amorphous silica present in diatoms and higher plants. Silica gel (hydrated amorphous silica, $SiO_2 \cdot nH_2O$ or polymerized

silicic acid) constitutes 90% to 95% of the total silicon in rice, while silicic acid constitutes 0.5% to 8.0%. Colloidal silica constitutes 0.0% to 3.3% (30).

There is good evidence that uptake of silicon is not merely passive diffusion of a non-polar substance across root hair membranes. Barber and Shone (1) found that silicon entered barley roots at a rate 2 to 3 times greater than water lost in transpiration. Xylem sap studies have shown concentrations of silicon higher than the concentrations of the extermal solutions for bean (1) and rice (15). This evidence suggests that there is active uptake of silicon by plant roots.

Effects of Silicon and Lack of Silicon on Plant Growth

Between 1926 and 1940, various workers established silicon to be responsible for increased growth in both monocotyledons and dicotyledons (4, 13, 14, 16, 18, 21). During this time, the question was raised as to whether the effect of silicon was direct or indirect. (It had been found that silicon played a role in resistance to fungal attack.)

Work since this time has further suggested that the beneficial effects of silicon for plants in general may be indirect rather than resulting from a direct requirement of the plants for silicon. Silicon has been shown to be beneficial to plants in general as well as plants that develop deficiency symptoms in the absence of silicon. Various individual plants and groups of plants have been shown to require

silicon for healthy growth, but silicon has not been proven to be essectial for the healthy growth of higher plants in the strict sense of the word.

Recently, it has been shown by Tanaka and Park (19) that silicon does not affect the growth rate of rice, and by Woolley (23) that tomato, radish, green onion and chinese cabbage do not have increased growth in the presence of silicon. In contrast to the work of Tanaka and Park (19), Okuda and Takahishi (15) found that silicon increased both root and shoot growth in rice. Reduced fertility and necrotic spotting have also been found in rice plants grown in silicon deficient media, along with wilting and withering of the leaves (15) called "weeping willow" effect. "Weeping willow" effect can be explained by the increased rate of transpiration found in silicon-deficient rice plants by Yoshida, Ohnishi and Kitagishi (28). As was suggested by the authors, silica gel associated with the cellulose in epidermal cell walls could function in reducing water loss. Plants grown in the absence of silicon would lack this protection and wilt more easily under water stress.

It has been demonstrated that silicon is related to the metabolism of other inorganic nutrients. Vlamis and Williams (20) and Okuda and Takahashi (15) found that the necrotic spotting on silicon deficient rice plants was due to iron or manganese toxicity (respectively) enhanced by the lack of silicon. In a similar study on barley, Williams and Vlamis (25), using manganese-54, detected radioactivity in spots

in the same locations as necrotic spots on leaf blades of plants grown in silica-deficient Hoagland solutions. In solutions supplied with silicon there was no necrotic spotting, and radioautographs showed manganese to be more evenly distributed in the leaf blades.

Inhibition of Silicon Metabolism

Lewin (9) has shown silicon uptake to be an aerobic process dependent on respiration, in diatoms. Silicon uptake by diatoms can be inhibited by respiratory inhibitors such as arsenate, azide, cyanide, iodoacetate and fluoroacetate at the concentrations which they inhibit respiration. Kelly (8) has shown that organic substances (sugars, starches, and intermediates of the tricarboxilic acid cycle) which stimulate respiration also stimulate uptake of silicon in diatoms. That silicon uptake is dependent on respiration is consistent with the evidence that shows the uptake of silicon to be active rather than passive.

Germanic acid has been considered a specific inhibitor of silicic acid metabolism by Lewin (9, 11) and Werner (22, 24). These authors, from their research have suggested that only plants requiring silicon are sensitive to germanium and germanium inhibition can be reversed by addition of silicon.

Werner has examined the effects of germanium on the silicon metabolism of the diatom *Cyclotella cryptica* (22, 23). He found that germanium does not interfere with the uptake of silicon, but rather,

affects its metabolism once silicon is inside the cell. Germanium inhibited chlorophyll and protein synthesis as well as breakdown of carbohydrates in the dark.

The symptoms of germanium suppression in higher plants are similar to those of silicon deficiency. These are: reduced shoot and root growth, wilting, withering, spotting and reduced chlorophyll content.

Werner (24) has reported that germination is not affected by germanium, but, later growth is inhibited. His premise is that during germination the endosperm supplies nutrients. When the energy reserve of the endosperm has been exhausted and the seedling begins to rely on the external environment for nutrients, germanium becomes an effective inhibitor.

PURPOSE OF THIS STUDY

This research was designed to examine various aspects of the literature in relation to several plant types and then to select one plant for intensive study.

Initially, experiments were carried out on cucumber and radish as representatives of the dicotyledons and wheat and barley as representatives of the monocotyledons. These preliminary experiments were intended to examine the silicon requirements of two members of the grass family, which have been shown to require silicon for normal growth, and two members of a group of plants that generally do not require silicon. Also, in the initial experiments, the author intended to examine germanium inhibition of silicon metabolism. According to the literature (9, 11, 22, 24), plants which do not require silicon are not affected by germanium. This was tested with cucumber and radish.

Barley was chosen for intensive study for several reasons. Preliminary experiments had shown barley to be sensitive to silicon deficient solutions, and to succumb readily to germanium. Seeds of Mariot barley germinate overnight and grow to a height of about 7 cm. in 3 days, showing effects of germanium inhibition in this time.

It was intended to examine various aspects of the effects of silicon and germanium on barley. These are: the effects of different concentrations of silicon and germanium, the reversibility of germanium inhibition, preconditioning to germanium inhibition, necrotic spotting with germanium and the effects of various chemical forms of silicon and germanium.

MATERIALS AND METHODS

Only plastic was used in preparing the solutions and the germination procedure, regardless of whether the plants were to be grown in silicon or not. This was done to control completely the amount of silicon to which the plants were exposed. In some experiments, 1 M (Na,K)OH was used to leach silicon out of seeds. The seeds were soaked in the solution until the radicles had emerged or for several days of growth. The solution was changed 2 or 3 times to make the leached-out silicon unavailable to the seedlings.

Plants were grown under one of three experimental techniques. These were: 1) on ash-free filter paper in plastic petri plates, 2) in plastic pots containing either plastic oven crystals ($1^1/_2$ mm. square plastic chips) or vermiculite, or 3) in water culture apparatuses consisting of polystyrene cups and plastic petri plate halves with holes punched in the surface. No nutrients, other than the ones being tested, were added.

The first technique was used for short range experiments such as to measure germination. The second and third were used for longer range experiments. The third proved the most accurate because exposure to the air was limited. Less exposure to air results in less evaporation and less fungal infestation.

Shoot growth was measured as total living shoot length (the sum of the measurements of the living portion of the leaves of each shoot). Root growth was measured as the average length of the longest root.

RESULTS AND DISCUSSION

Effects of Na_2SiO_3, Na_2GeO_3 and GeO_2 on Germination and Later Growth of Barley

200 plants per treatment were either 1) germinated and grown in varied concentrations of Na_2SiO_3, Na_2GeO_3 and GeO_2, or 2) germinated in water and transferred to Na_2GeO_3 and GeO_2 24 hours after germination. Percent germination and growth were recorded daily over a period of 8 days. Results are recorded in Table I. (There was no increase in germination after 2 days.)

Unlike the findings of Werner (25) for *Secale cereale* and *Sinapsis alba*, germanium can inhibit germination of barley. The lowest concentrations of germanium used (10^{-4} M Na_2GeO_3 and 20 mg. GeO_2) inhibited germination. The highest concentration of Na_2SiO_3 used (10^{-2} M) had the same percent inhibition as the lowest concentration of Na_2GeO_3 (10^{-4} M). Germination in germanium also affected later growth. The shoots of plants germinated in water and transferred to germanium solutions were from 1.1 to 6.5 times longer than those germinated and grown in germanium. Spotting appeared on plants germinated in germanium 2 days before it appeared on plants germinated in water and transferred to germanium.

The roots of plants germinated in 2×10^{-4} M Na_2GeO_3 had 60% ±4% fewer root hairs than the control plant roots and 40% ±4% fewer than the 10^{-4} M concentration plant roots. At concentrations higher than

2×10^{-4} M, there were very few root hairs, and those present were one-fourth the length of the control root hairs. Roots of plants germinated in GeO_2 also showed an almost complete absence of root hairs, with those present one-fourth or less the length of the control root hairs.

It is obvious from this experiment that germanium does inhibit germination in barley. Germanium was imbibed by the seeds and affected metabolism during the first stages of growth.

Table I. Effects of Silicon and Germanium on Germination and Later Growth of Barley.

$[Na_2GeO_3]$ M	germination at 2 days (%)	inhibition of germination (%)	*ave. shoot length at 8 days (cm.)	*ave. root length at 8 days (cm.)
Plants germinated and grown in germanium				
2×10^{-3}	4	95	0.0	0.3
10^{-3}	51	48	0.0	0.3
5×10^{-4}	80	19	1.0	1.0
2×10^{-4}	81	18	3.0	2.1
10^{-4}	89	10	9.2	5.8
control	99	0	9.4	7.1
mg. GeO_2/ 15 ml. H_2O				
40	88	11	1.4	0.2
30	85	14	1.7	0.2
20	87	12	3.7	0.5
Plants germinated in water and transferred to germanium solutions				
2×10^{-3}	99	0	6.5	3.3
10^{-3}	99	0	7.4	4.2
5×10^{-4}	99	0	8.2	7.6
2×10^{-4}	99	0	8.7	10.4
10^{-4}	99	0	10.2	10.7
mg. GeO_2/ 15 ml. H_2O				
40	99	0	6.5	4.9
30	99	0	10.9	4.8
20	99	0	11.2	5.0
Plants germinated and grown in silicon				
$[Na_2SiO_3]$				
10^{-2}	99	0	8.7	3.1
10^{-3}	99	0	13.1	9.9
10^{-4}	99	0	11.0	8.2

*Standard Error equal to 11% of mean.

Effects of Na_2GeO_3, GeO_2 and Na_2SiO_3 on Germination and Later Growth of Wheat

It would be expected that the reaction of wheat to germanium and silicon would be similar to that of barley as both are grasses and have a higher than average silicon content. Three replicates of 40 wheat seeds per petri dish were germinated in varied concentrations of silicon and germanium. The seed was old and less viable. (Only 70% of the control seeds germinated.) This may have made it more susceptible to germanium inhibition than new seed would have been. The effects of silicon and germanium on the germination and growth of wheat are shown in Table II.

Of the seeds which were recorded as having germinated, 27% had no growth after the radicles had emerged 2 to 5 mm. Most of these were seeds treated with the higher concentrations of germanium. Root measurements of these seeds were included in the average root length data.

The percent germination of wheat seeds was significantly reduced by germanium, as was also the case with barley. Both shoot and root growth were inhibited as was the development of root hairs. The wheat plants, however, did not develop spots on the leaves.

Table II. Effects of Silicon and Germanium on Germination and Later Growth of Wheat.

$[Na_2SiO_3]$ M	germination at 2 days (%)	inhibition of germination (%)	ave. shoot length at 8 days (cm.)	ave. root length at 8 days (cm.)
10^{-2}	41	20	4.7	1.9
10^{-3}	65	5	6.1	3.2
10^{-4}	67	3	7.3	3.3
control	70	0	7.0	3.5
$[Na_2GeO_3]$ M				
2×10^{-3}	17	53	1.7	0.4
10^{-3}	15	55	3.9	0.3
5×10^{-4}	24	46	4.7	1.7
2×10^{-4}	39	31	5.3	1.4
10^{-4}	47	23	5.3	2.7
mg. GeO_2/ 15 ml. H_2O				
40	32	38	0.6	0.4
30	27	43	0.2	0.3
20	34	46	0.7	0.2

Effects of Na_2GeO_3, GeO_2 and Na_2SiO_3 on Germination and Later Growth of Cucumber

According to Lewin (9, 11) and Werner (22, 24), germanium is a specific inhibitor of silicon metabolism. Only plants requiring silicon are inhibited by germanium. If this is a true generalization, it should be possible to determine whether an organism requires silicon by its inhibition or lack of inhibition in the presence of germanium.

To test the silicon requirement of cucumber, 100 seeds were germinated and grown in each 10^{-4} M and 10^{-3} M Na_2GeO_3 and 20 mg. GeO_2/15 ml H_2O. Another 100 seeds were germinated and grown in 10^{-4} M or 10^{-3} M Na_2SiO_3. After 5 days, none of the shoots of the plants in germanium were longer than 2.5 cm. None of the roots were longer than 1.0 cm. The control plants had an average shoot length of 4.0 cm. and an average root length of 18.0 cm. The average shoot length of the 10^{-4} M and 10^{-3} M Na_2SiO_3 grown plants was 15.7 cm. and 13.3 cm. respectively. Many of the plants treated with germanium had morphological abnormalities. There was a lack of chlorophyll and the margins of the leaves were split. This was not true for the water control or silicon grown plants.

An attempt was made to precondition cucumber seeds to germanium by soaking them in 10^{-5} M or 10^{-4} M Na_2SiO_3 for 4 hours prior to germination in germanium. The preconditioning treatment was not effective. At 5 days the shoot and root lengths of germanium grown plants were the

same as the previous experiment in which there was no preconditioning. The same morphological abnormalities were present. Figure 1 shows the reduced growth and split leaf margins which occur in the presence of germanium.

It can be concluded from these experiments with cucumber that germanium is not necessarily a specific inhibitor of silicon metabolism. The results indicate that germanium may be toxic in itself. It can inhibit growth and cause morphological abnormalities in plants which do not have a specific requirement for silicon.

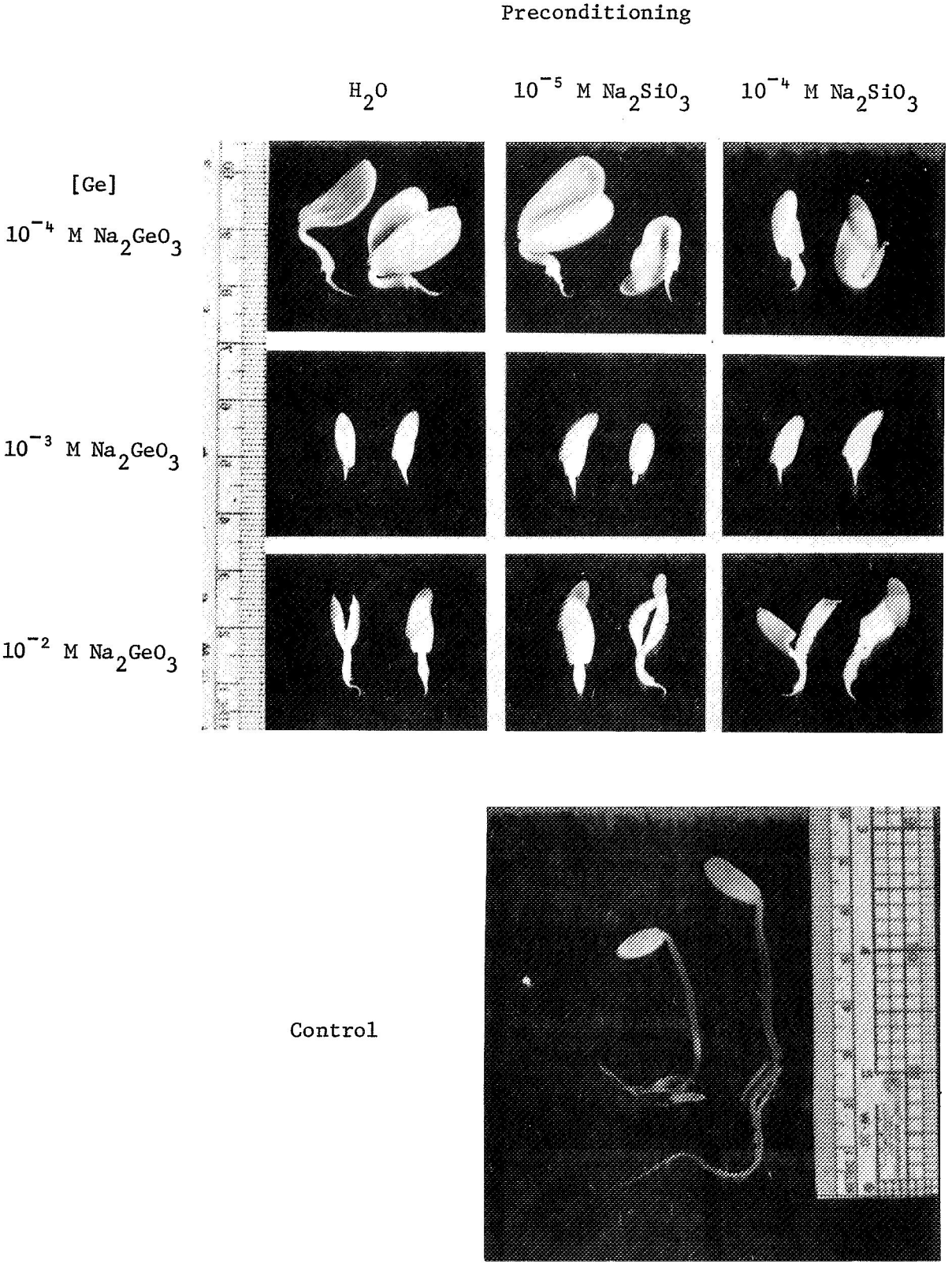

Fig. 1. Effects of Germanium on Cucumber, a plant which does not require Silicon. Pictures were taken when plants were 5 days old.

Effects of Na_2GeO_3, GeO_2 and Na_2SiO_3 on Germination and Later Growth of Radish

To further test the specificity of germanium inhibition, radish seeds were germinated and grown in varied concentrations of silicon and germanium. The results were not as dramatic as those of the cucumber experiments, partially because radish is a slower growing plant. After 9 days, shoot height increased 1.7 cm. in the control plants, 1.8 cm. in plants grown in 10^{-4} M Na_2SiO_3, and 1.1 cm. in plants grown in 10^{-4} M Na_2GeO_3 and 30 mg. GeO_2/15 ml. H_2O. There were no macroscopic morphological abnormalities in the germanium grown plants. At 17 days the germanium grown plants were dead. Shoots of the plants grown in silicon were 5.4 cm. long as compared to 5.1 cm. long in the control plants.

As with cucumber, silicon did not appreciably increase growth, but, it was found that germanium inhibited growth.

The Effect of pH on Germination of Barley Seeds

Sodium germanate and sodium silicate in solution are basic. To test how much pH affects germination of barley seeds, germination in basic solutions was examined. Percent germination was recorded for 0.01, 0.003, 0.001, 0.0003, 0.0001, 0.00003 and 0.00001 M NaOH, corresponding to a range of pH from 9 to 12. At pH 12 there was a 16% ±5% inhibition of germination, and after 5 days shoot growth was 80% ±8%

of that of the control plants. Shoots of plants grown at pH 11 averaged 0.1 cm. longer than those of the control and there was no inhibition of germination. At pH less than 11 there was no inhibition of germination or growth.

These results indicate that pH had little or no affect on barley seed germination, since the highest concentration of Na_2GeO_3 used was 2×10^{-3} M, corresponding to pH 11, and the highest concentration of Na_2SiO_3 used was 10^{-2} M corresponding to pH slightly greater than 11.

Differences Between Plants Grown in Oven Crystals and Vermiculite

Both oven crystals and vermiculite were used as substrata for the growth of plants. Oven crystals are plastic and served as a silicon-free substratum. Vermiculite contains natural silicon. Another difference between the two was water holding power. A pot of vermiculite will hold 50 ml. of water from 8 to 40 hours longer than oven crystals (depending on the relative humidity). It was attempted to keep the water content of both substrata the same through watering plants growing in oven crystals more often.

There were great differences in shoot height and root length between plants grown in the two substrata. Table III and Figure 2 show these differences. It is possible that the better growth of cucumber and radish in vermiculite is a result of uptake of potassium salts.

Table III. Shoot and Root Growth in Oven Crystals and Vermiculite.

Plant	Substratum			
	oven crystals		vermiculite	
	ave. shoot height (cm.)	ave. root length (cm.)	ave. shoot height (cm.)	ave. root length (cm.)
cucumber	7.2	3.1	10.1	10.5
radish	4.3	5.3	8.7	9.7
wheat	8.2	4.7	17.7	9.4
barley	11.0	11.2	16.3	11.7

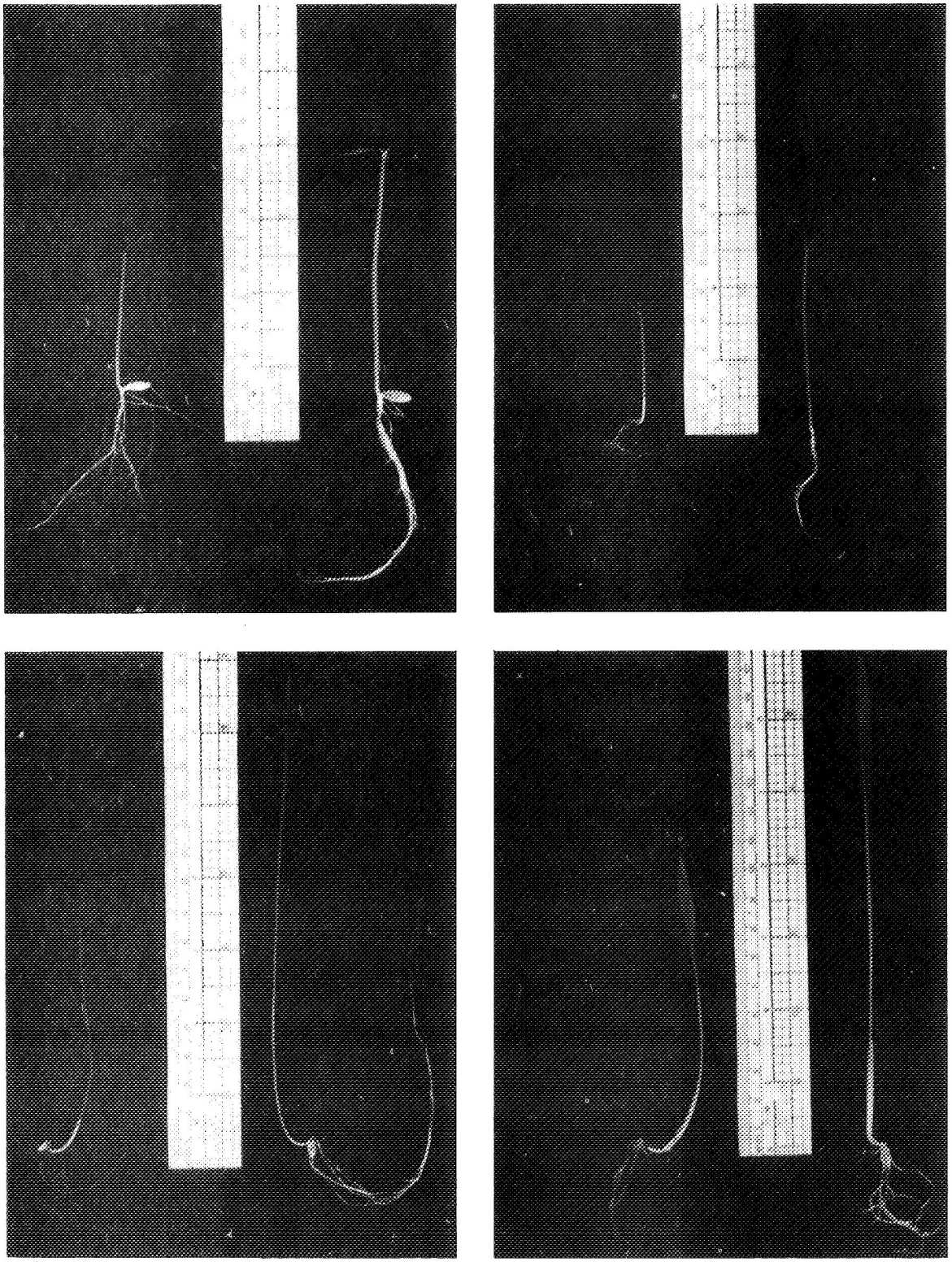

Fig. 2. Differences between plants grown in Oven Crystals and Vermiculite. Plants were 10 days old when photographed. The plant in the left in each photograph was grown in oven crystals, the one on the right in vermiculite.

Upper left: Cucumber
Upper right: Radish
Lower left: Wheat
Lower right: Barley

Silicon Deficiency Symptoms in Barley and the Effects of Germanium Inhibition

Silicon deficiency symptoms in barley were comparable to those reported by other (15, 28). In the absence of silicon, root and shoot growth is reduced and the leaves show wilting, withering, reduced chlorophyll and necrotic spotting. These effects, especially the spotting, are enhanced by germanium.

The only germanium-enhanced deficiency symptom affected by the substrate was wilting. Spotting of the leaves, withering, reduced chlorophyll and reduced growth were present regardless of the substrata when the plants were given germanium. In vermiculite there was less wilting than there was in oven crystals. This difference was attributed to the greater water holding power of vermiculite.

In addition to reports that shoot height is decreased, it was noted in this research that shoot expansion is affected by both silicon and germanium concentration, and, also that germanium causes a lag in the development of the second leaf.

Germanium decreases shoot expansion in two ways. Shoots of plants grown in germanium are initially narrower than shoots of water controls. Then, with the development of spotting, the shoots wither decreasing their initial shoot width.

Germanium spotting begins at the tip of the first leaf blade before the blade has grown to its maximum height. It then continues

down to the base where the blade emerges from the coleoptile. The upper edge of the margin of the coleoptile also turns brown. Successive leaf blades emerge already spotted.

Spotting first appears as patches of slightly lighter, indented areas. These patches turn yellow and finally dark brown. The brown areas enlarge and unite forming larger patches. This joining of spots can be seen especially well at the margins of the leaves. At first there may be only a few discolored areas. A few days later, the entire margin is brown. This sequence is shown in Figure 3.

Sections made through spotted areas on leaf blades showed that the spotting is not a result of fungal attack. Cells in spotted areas were found to contain an accumulation of dark material. Immersion of spotted blades in phloroglucinol in HCl to determine if the dark material is lignin gave negative results. Spotted leaves were also tested with cupric chloride. Under this treatment, spotted areas turned grey, indicating a phenolic compound was present.

The lag in the development of successive leaves can be explained to be a result of the reduced growth of previous leaves. Because the first leaves of plants grown in germanium are shorter, narrower and have less chlorophyll than control plants, there is less area photosynthesizing and producing materials necessary for growth. Consequently, the second leaves are delayed in developing. Germanium may also interfere directly with the formation of sucessive leaves.

Recovery from germanium inhibition was successfully accomplished in both silicon and water. Plants were germinated in a .5 M NaOH and .5 M KOH (equal parts) solution and transferred to water cultures of 10^{-3} M Na_2GeO_3. The solutions were changed to 10^{-3} M Na_2SiO_3 or water after 2, 4, or 6 days. The results are shown in Figure 3. Necrotic spotting was arrested by transfer to either the silicon solution or water. New leaves developing after treatment with silicon or water had no spotting or discoloration. At 10 days, the average total shoot growth of the plants transferred after 2 days was not less than that of the silicon and water controls. Plants transferred to silicon after 4 and 6 days had 11% ±3% and 23% ±4% lower shoot growth respectively. Plants transferred to water after 4 and 6 days had 11% ±5% and 27% ±4% lower shoot growth respectively.

The effects of varied concentrations of germanium on barley shoot growth are shown in Figure 4. Plants were germinated in a .5 M NaOH and .5 M KOH (equal parts) solution and transferred to water culture after 2 days of growth. Necrotic spotting appears first in the 10^{-3} M concentration (on the third day), second in the 10^{-2} M concentration (on the fourth or fifth day) and last in the 10^{-4} M concentration (on the eighth or ninth day). Spotting was most dramatic in the 10^{-3} M concentration.

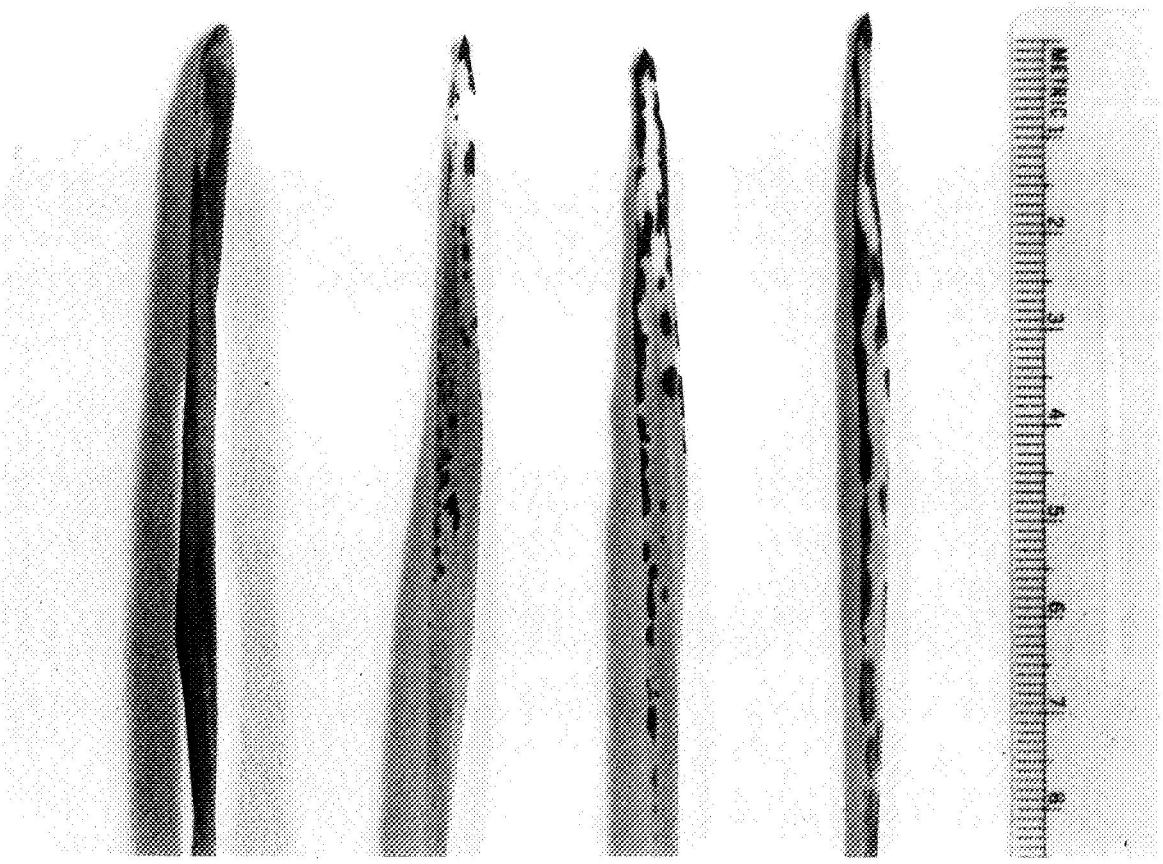

Fig. 3. Recovery from Germanium inhibition in Barley. Pictures were taken when plants were eight days old.

Treatment from left to right: 6 days in Si, 2 days in Ge and 4 days in Si, 4 days in Ge and 2 days in Si, 6 days in Ge.

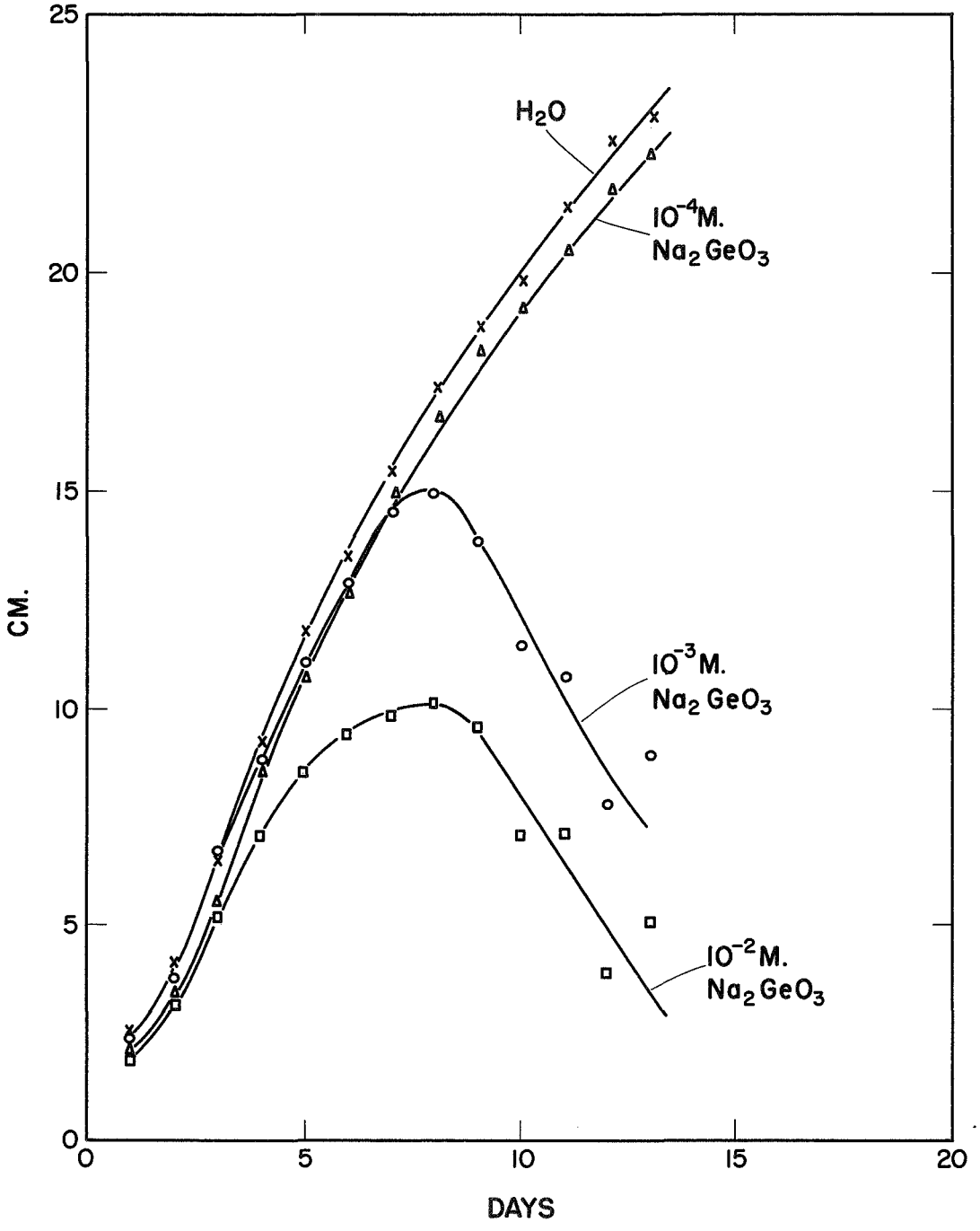

Fig. 4. Barley Shoot Growth under Varied Concentrations of Germanium.

The Silicon Requirement of Barley

Plants grown in 10^{-4} M, 10^{-3} M and 10^{-2} M Na_2SiO_3 were compared with control plants grown in silica-free water to examine the silicon requirement of barley. There were 10 plants per treatment and the experiment was repeated 3 times. Plants were grown in water culture. The average results of the 3 experiments are shown in Figure 5. There was little variation in results between the 3 individual experiments. The 10^{-2} M Na_2SiO_3 solution clearly inhibited shoot growth on and after the third day of the experiment. At 13 days, shoot growth in 10^{-2} M Na_2SiO_3 was 63% ±4% that of shoot growth in the control plants. Shoot growth in 10^{-3} M and 10^{-4} M Na_2SiO_3 was 10% ±4% and 3% ±2% higher than that of the control plants respectively.

It was found that 10^{-3} M Na_2SiO_3 was optimum for root growth as well as shoot growth. At 13 days average root length in the control solution was 79% ±4% of average root length in 10^{-3} M Na_2SiO_3.

Leaf blade expansion was also affected by silicon concentration. The widest portion of the first and second blade was measured for 21 days. The widest measurement is recorded in Table IV.

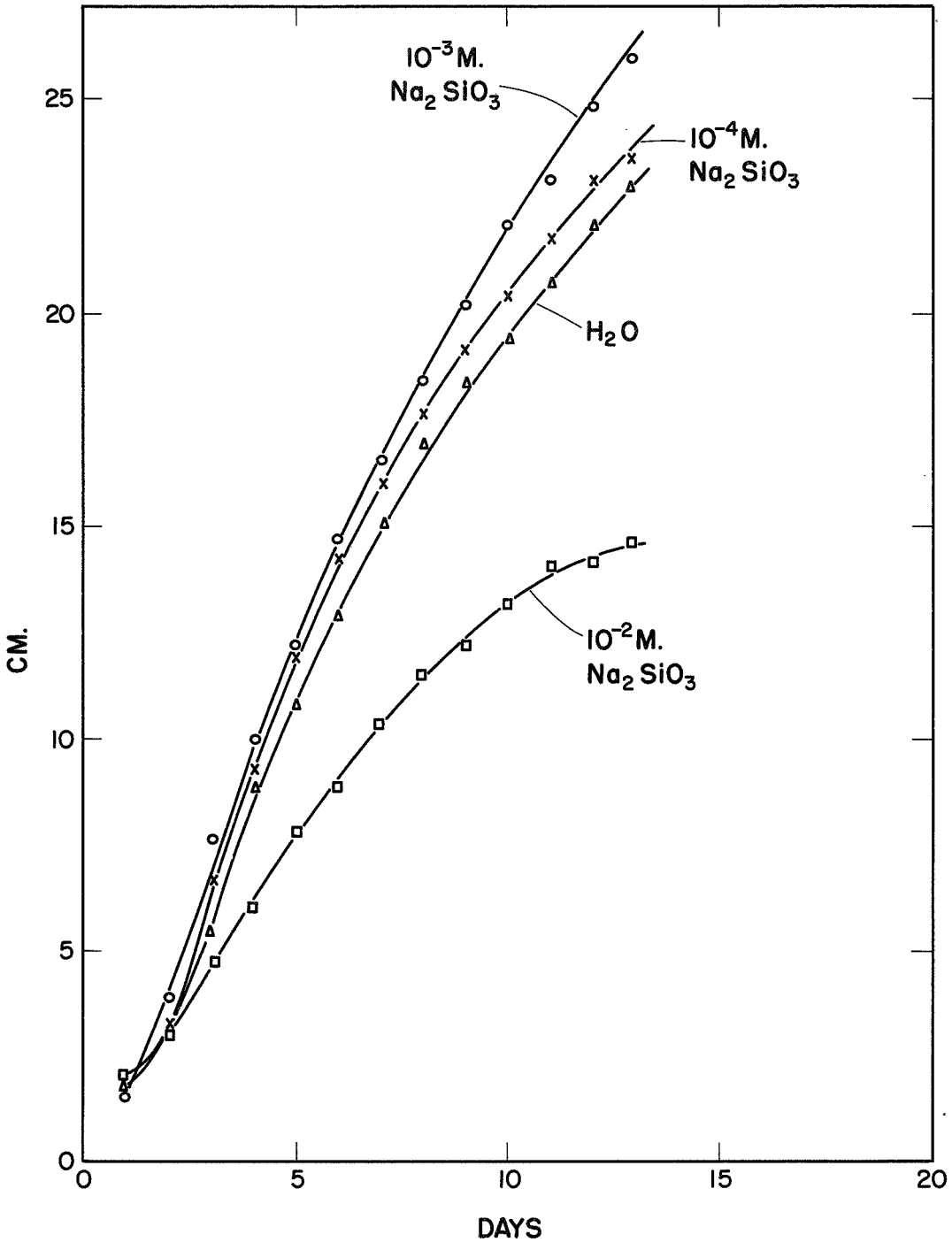

Fig. 5. Barley Shoot Growth under Varied Concentrations of Silicon.

Table IV. The Effect of Silicon on Barley Leaf Blade Expansion.

$[Na_2SiO_3]$ M	maximum leaf width of first blade (mm.)	age when leaf measured widest (days)	maximum leaf width of second blade (mm.)	age when leaf measured widest (days)
10^{-2}	3.5	11	1.7	19
10^{-3}	6.5	7	4.9	12
10^{-4}	4.9	8	4.5	15
control	5.8	7	4.4	13

Preconditioning Barley Seeds to Germanium

Barley seeds were germinated in either 10^{-3} M Na_2SiO_3 or water. After 2 days of growth, the seedlings were transplanted to water culture solutions of 10^{-3} M Na_2GeO_3. After 10 days of growth, the average shoot length of the plants germinated in silicon was 18.2 cm. ±6 cm. The average shoot length of the plants germinated in water was 17.0 cm ±4 cm. It was not possible significantly to precondition barley to germanium with silicon.

Various Forms of Silicon and Germanium

Both tetraethyl orthosilicate and sodium silicate were examined as sources of silicon for barley. The salt proved to be the better source of silicon. Plants grown in the ester had a drastic reduction in growth as compared to plants grown in the salt. Of the plants grown in the ester, 60% ±13% died before their shoot height reached 7 cm. The other 40% ±13% grew normally except for a 30% to 37% reduction in shoot height from the 10^{-3} M Na_2SiO_3 control. Tetraethyl orthosilicate is quite insoluble in water making it difficult to control concentrations. Another problem with the ester is that it is vulnerable to fungal attack and makes an excellent culture medium for some fungi and algae. The fungi also attack the roots of plants growing in the solutions and affect their growth.

The oxide and the sodium salt of germanium were used as a source of this element. Both forms were effective inhibitors of growth. The oxide is not as soluble as the salt and presented problems in controlling concentration. Hence, the salt was the better form and was used exclusively in the intensive work on barley.

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APPENDIX

Effect of Silica on the Growth of a Non-Lignified Aquatic — *Elodea*

Elodea canadensis is a representative of the aquatic angiosperms that have lost the capacity to synthesize lignin, hence are deficient in two vital features of land adaptness — water retention and compression resistance. This group, an assortment of families with differing affinities, characteristically contain a suitable peroxidase, but apparently fail in conversion of tyrosine and phenylalanine into precursor cinnamic acids.

By feeding high concentrations of H_2O_2 and phenylpropanoid precursors to *Elodea* segments, their mechanical properties were improved, but all living protoplasm was killed.

As an alternative to forced lignification, it was considered of interest to try silica feeding in aquatic media as means for introducing supportive material into the cell wall. Thus far, only preliminary studies have been carried out as follows:

> Six *Elodea* apical sections 1-cm. in length were placed in water of ca. 1-cm. depth in plastic chips in a polyethylene-lined vessel. This arrangement provides a silica-free environment. Sections were incubated 30 days, measured, and incubation continued for two additional weeks. Results were as follows at 30 days:

	length mm	Δ
1. No SiO_2, free floating	22	12
2. No SiO_2, anchored	23	13
3. 10^{-5}M Na_2SiO_3, free	25	15
4. 10^{-5}M Na_2SiO_3, anchored	35	25
5. 10^{-6}M Na_2SiO_3, free	26	16
6. 10^{-4}M Na_2SiO_3, free	24	14
7. 10^{-7}M Na_2GeO_3, free	24	14
8. 10^{-6}M Na_2GeO_3, free	20	10

Effect of anchorage alone (2 *vs* 1): $^{13}/_{12} = 1.085$

Effect of 10^{-5}M Na_2SiO_3 alone (3 *vs* 1): $^{15}/_{12} = 1.25$

Calculated effect of anchorage plus 10^{-5}M Na_2SiO_3

(2 X 3 *vs* 1): $1.085 \times 1.25 = 1.36$

Measured effect of anchorage plus 10^{-5}M Na_2SiO_3

(4 *vs* 1): $^{25}/_{12} = 2.03$

Thus, if this experiment is confirmed, there may be a marked synergistic effect.

After an additional 16 days, the water level had fallen to the plastic substratum. At this time, experimental solutions could be grouped with respect to survival as follows:

Alive per 6 apices

0	3	4	6
10^{-4}M Na_2SiO_3	free control	anchored control	anchored 10^{-5}M Na_2SiO_3
10^{-7}M Na_2GeO_3		10^{-6}M Na_2SiO_3	free 10^{-5}M Na_2SiO_3
10^{-6}M Na_2GeO_3			

In addition, it was noted that only plants in the anchored 10^{-5}M Na_2SiO_3 had developed branching and that 4 of the 6 plants were extended ca. 15 mm above the water line.

It seems most promising, therefore, that an evolutionary (genetic) loss in lignifying capabilities can be offset in part at least by an alternative and very different cell wall component, silica. Efforts to "convert" *Elodea* into a "land" plant via the silica by-pass will be continued.