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Calcium—Boron Interaction

Demonstrated by Lemna Minor on Clay Suspensions

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Calcium—Boron Interaction

Demonstrated by Lemna Minor on Clay Suspensions

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Lemna minor L., a member of the duckweed family, has characteristics which recommend it highly as a laboratory plant for demonstrating facts of plant nutrition. It reproduces by budding. The resulting fronds can readily be counted. Thus, one can easily take periodic measure of the rate of vegetative reproduction. The reproduction of cells and not just their enlargement, becomes the criterion of growth. The small size of this plant (*Lemna minor* is among the smallest of flowering plants) is an asset when space is restricted but large numbers are required.

HISTORICAL

Growth Requirements of Lemna.

Though in their normal environment, the duckweeds are usually subjected to reduced light, they have been grown satisfactorily under laboratory conditions with wide variation in light intensity and length of day. The underside of the plant should be protected from light (17).** Optimum temperature seems to be near 25° C although the plants bud actively at much lower temperatures (9).

The nutrient requirements for Lemna are similar to those of other green plants (18). It responds readily to different culture media by varying the size and shape of its fronds and speed of asexual reproduction (13). Prepared mineral nutrient solutions have not been as satisfactory for reproduction as soil-water systems (4). In previous studies, the amount of boron used in nutrient solutions for *Lemna minor* has varied from 0.04 to 0.5 p.p.m. (11, 18). Nutrient solutions of pH values ranging from 3.85 to 5.46 permitted growth (18).

Calcium-Boron Relationships.

Naftel (15) reported that excessive liming of Norfolk loamy sand gave serious injury to crops while large applications of basic slag caused no injury.

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**Figures in parentheses refer to References cited in the back.

He suggested that minor elements in slag, especially the boron, prevented "overliming" injury. Injury was related to pH of 6.8 and above, to the low exchange capacity, and to the leaching to which the soil was naturally subjected.

Cook and Miller (5) suggest that boron availability is influenced by pH and by the presence of calcium and magnesium, especially their carbonates.

Wolf (21) found that a change in pH, without a corresponding change in the content of calcium or magnesium, had little effect upon the absorption by plants of boron from solution.

Symptoms of the deficiencies of boron and of calcium are very similar (16). Both affect the highly intensive metabolic processes of the meristematic region. It is interesting to note that neither is essential for the growth of *Aspergillus niger.* A relationship between boron and calcium was observed by Brenchly and Warington (2). In some way boron either enabled the plant to absorb more calcium in a given time or to utilize it more effectively in metabolism after it was once within the plant. Much less calcium was taken up by *Vicia faba* from a boron-deficient solution (20).

The calcium content of soybean leaves has been found roughly proportional to the amount of boron in the nutrient solution. Sub-optimal quantities of boron gave an inverse relationship (14).

Marsh and Shive (12) found, within limits, no correlation between total calcium and total boron in corn. However, a relation was found between boron and soluble calcium. Eliminations of boron from the substrate, resulting in a boron deficiency, destroyed the metabolic possibilities of the calcium even when calcium was present in the plant tissue.

For proper plant growth, Jones and Scarseth (10) suggested some approximate calcium to boron ratios. Their ratio is wide for tobacco (12,000:1) and narrow for beets (100:1).

If this balance is disturbed by a small uptake of calcium, a low tolerance of boron will result. A similar relationship has been suggested between boron and the potassium-calcium ratio of tobacco plants (19). With increasing boron in the soil, plant calcium remained more or less constant while potassium increased. Considerable improvement in yield was obtained when the calcium-boron ratio was reduced from 900:1 to 300:1.

PLAN OF THE INVESTIGATION

Lemna minor was grown on a suspension of electrodialized Putnam clay into which nutrient cations were titrated. The procedures and advantages of using this type of medium have been set forth by Albrecht (1).

The clay has a pH of about 3.2 when dialysis is complete, but upon standing this value will increase slowly due to break-down of the clay minerals. The acid clay used in this investigation had a pH of 3.6. After nutrients were added, the pH of the suspensions varied from 4.5 to 8.3, depending upon the amount of the calcium treatment. Its exchange capacity was 68 milliequivalents (m.e.)

per 100 grams.

Two series were used. In series A, the total calcium was varied through increasing degrees of saturation of a constant amount of clay and increasing pH values. Each of these degrees was then used in four series of varied boron levels. Series A is spoken of as the calcium-hydrogen series with variable total calcium.

In series B, the total amount of calcium in the medium was held constant but at different degrees of saturation of the clay by calcium, giving different concentrations of clay. In this series the boron was held at a constant concentration.

Calcium-Hydrogen Series with Variable Total Calcium.

The amounts of nutrients added to the acid-clay suspensions to give the A series of different systems of media in which both calcium (with hydrogen as its reciprocal) and boron were varied are given in Table 1. The final volume of each system was 200 ml. of a 2 percent clay suspension.

Constant Total Calcium.

In another series the total calcium was held constant, while the percent calcium saturation was varied (series B, Table 1); the clay concentration, of necessity, was also a variable since the volume of each system was 200 ml. The boron concentration was the same in all the systems of this series: namely, 0.001 p.p.m.

The plants were handled by placing a C-shaped wire, fitted into a glass tube handle, under the frond without damaging the root. Ten fronds were started in each system. These were taken from stock cultures of minus-boron.

The containers used were wide-mouth, soft glass, pint, Mason fruit jars. The culture media were aerated continuously, using fritted glass aspirators and small vibrator pumps, with occasional stirring to help keep the clay in suspension. The tendency for the clay to settle out increased during the time plants were grown on the suspension. Systems more highly saturated with calcium were more difficult to keep in suspension.

Temperatures were maintained between 20° and 30° C by sinking the fruit jars into a large box of soil. The soil surface was flooded with water on hot days. The soil prevented light from reaching the fronds from below. A muslin screen suspended above the cultures gave protection from direct sunlight and more even distribution of light.

Measurements of Growth and Reproduction.

Since *Lemna* reproduces by budding, it is possible to express the rate of growth and development in terms of the time required for the average frond to reproduce itself (generation time). The number of fronds was determined as growth progressed and the generation time and growth constant calculated from the following equations, $\mathrm{Log}_{10} N_a - \mathrm{Log}_{10} N_b = K\,(t_a - t_b)$ where N_b equals the number of fronds at time b; N_a equals the number of fronds at time a; $t_a - t_b$ equals the time interval (days); and K equals the growth constant. The

TABLE 1--CHEMICAL COMPOSITIONS OF THE CLAY SUSPENSIONS ON WHICH LEMNA MINOR WAS GROWN

Compound Added	Quantities Added (m.e. per 100 Grams of Clay and p.p.m. of Suspension) — Series A: Calcium-Hydrogen with Variable Total Calcium	Series B: Constant Total Calcium	Series B: Calcium Saturation Percent
Magnesium m.e. (Oxide)	6.0	6.0	
Ammonium m.e. (Hydroxide)	5.0	5.0	
Potassium m.e. (Hydroxide)	3.2	3.2	
Calcium m.e. (Hydroxide)	0.0	6.25	9
	12.5	12.50	18
	25.0	18.75	28
	50.0	25.00	37
	75.0	37.50	55
	Free $CaCO_3$	50.00	74
		62.50	92
		75.00	110
Phosphorus p.p.m. (Monocalcium Phosphate)	15.5	15.5	
Sulphur m.e. (Ammonium Sulphate)	5.0	5.0	
Iron m.e. (Chloride)	0.5	0.5	
Copper p.p.m. (Sulphate)	0.025	0.025	
Zinc p.p.m. (Sulphate)	0.050	0.050	
Manganese p.p.m. (Sulphate)	0.100	0.100	
Boron p.p.m. (Boric Acid)	0.001	0.001	
	0.100		
	1.0		
	5.0		

generation time (GT) is the time required for a frond to reproduce itself and is obtained from the equation,

$$GT = \frac{\text{Log}_{10}{}^{2}}{K}.$$

The buds were counted as fronds when they approached the size of the parent frond.

Chemical Analyses. The plants were ashed in a muffle furnace at 500° C. The boron in the ash was estimated with a D.C. arc spectrograph using tin as an internal standard.* Semiquantitative methods were used. Calcium was determined

*These determinations were made by Prof. Edward E. Pickett of the Spectrographic laboratory.

by titration of the oxalate with permanganate. Potassium and sodium were estimated by the flame photometer.

RESULTS

Frond Development.

The general effects of boron and calcium upon reproduction and growth of *Lemna* as demonstrated by these studies include: (1) the necessity for a liberal supply of calcium absorbed on the clay; (2) the detrimental effects of boron at concentrations above 1 p.p.m. even with liberal amounts of calcium present; and (3) the alleviative effect of high calcium levels on boron toxicity.

Reproduction was most rapid on systems which supplied the highest levels of calcium (Figs. 1, 2, 3) while plants established on suspensions treated with 12.5 m.e. (about 20 percent saturation of the clay) or less of calcium either made no growth or began to die. On these low-calcium systems the larger, more mature plants died first. Smaller, younger fronds and buds were more hearty. A possible explanation may rest on the fact that colloids of the young fronds and buds were better equipped to compete with the clay colloid in absorbing and holding nutrient ions.

Since the systems with the highest levels of calcium were required in this study for the more rapid reproduction, it is well to note that *Lemna* does not possess extensive root surfaces for nutrient absorption. Consequently, it does not compete well with the clay for adsorbed cations.

The frond division and development varied according to the degree of calcium saturation of the clay in a manner similar to the variation in calcium activity for this type of clay and similar to the way calcium is taken up by tomatoes, for example, from a clay of the Montmorillonite type (6). As shown in Figure 1, frond division was not apparent until the saturation of the clay with calcium reached 37 percent. Plants did not show a marked response until the calcium saturation reached 74 percent.

The length of time required for frond division decreased with each increment of added calcium, even to the point when calcium was added in excess of the exchange capacity. The plants growing at the highest calcium levels were of a lighter green color, suggesting a deficiency of some minor element, perhaps iron or manganese.

Increasing the concentration of boron in the nutrient medium had an opposite effect on frond division and development to that of calcium. The reproduction was most rapid on those systems supplying the lowest boron and the highest calcium levels. The time required for frond division increased sharply at the highest boron level for all calcium levels, except in the series containing free calcium carbonate (Figure 3). But even in this series, the larger increments of boron increased the generation time slightly.

Evidently boron impurities were present in sufficient quantities for maximum duckweed growth so that this experiment was essentially a measure of

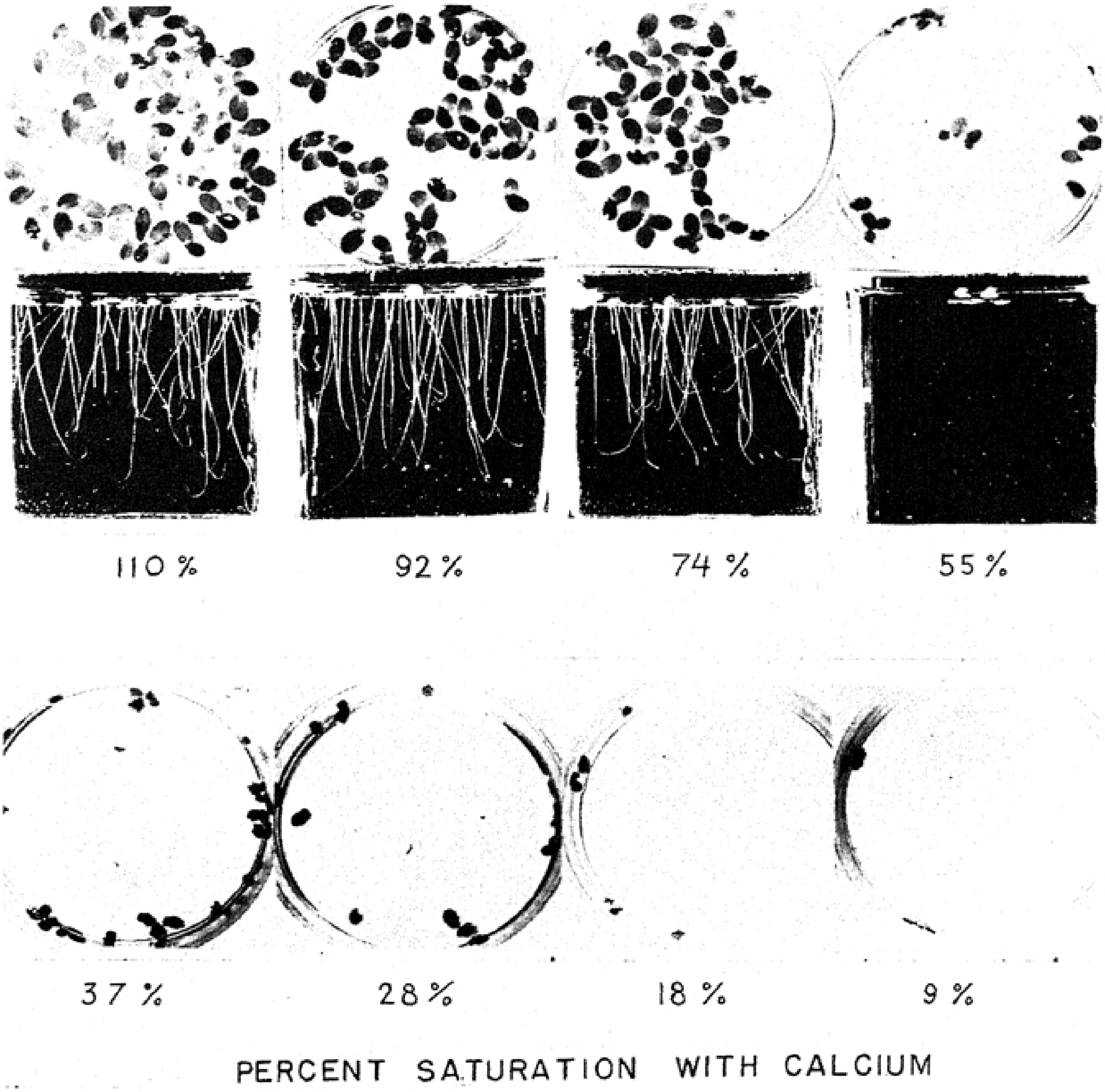

Fig. 1—Division of the fronds and development of the roots of Lemna minor according to the different percentages saturation of the clay by calcium. Series B. Constant total calcium.

plant performance at various toxic concentrations of boron. Later experience with the growth of alfalfa on this medium has also indicated boron impurities (8). The requirements of duckweed for boron must be low if such small boron additions (0.1 p.p.m.) can be demonstrated by decreased frond division.

The detrimental effect of the higher boron concentrations on reproduction was greatest at intermediate values of calcium saturation of the clay (Fig. 2). The presence of free calcium carbonate was partially effective in alleviating the effects of a high concentration of boron (Fig. 3). The literature reviewed presents evidence that high calcium levels will serve to counteract the effects of high boron. This conclusion seems to be borne out to some extent by this experiment.

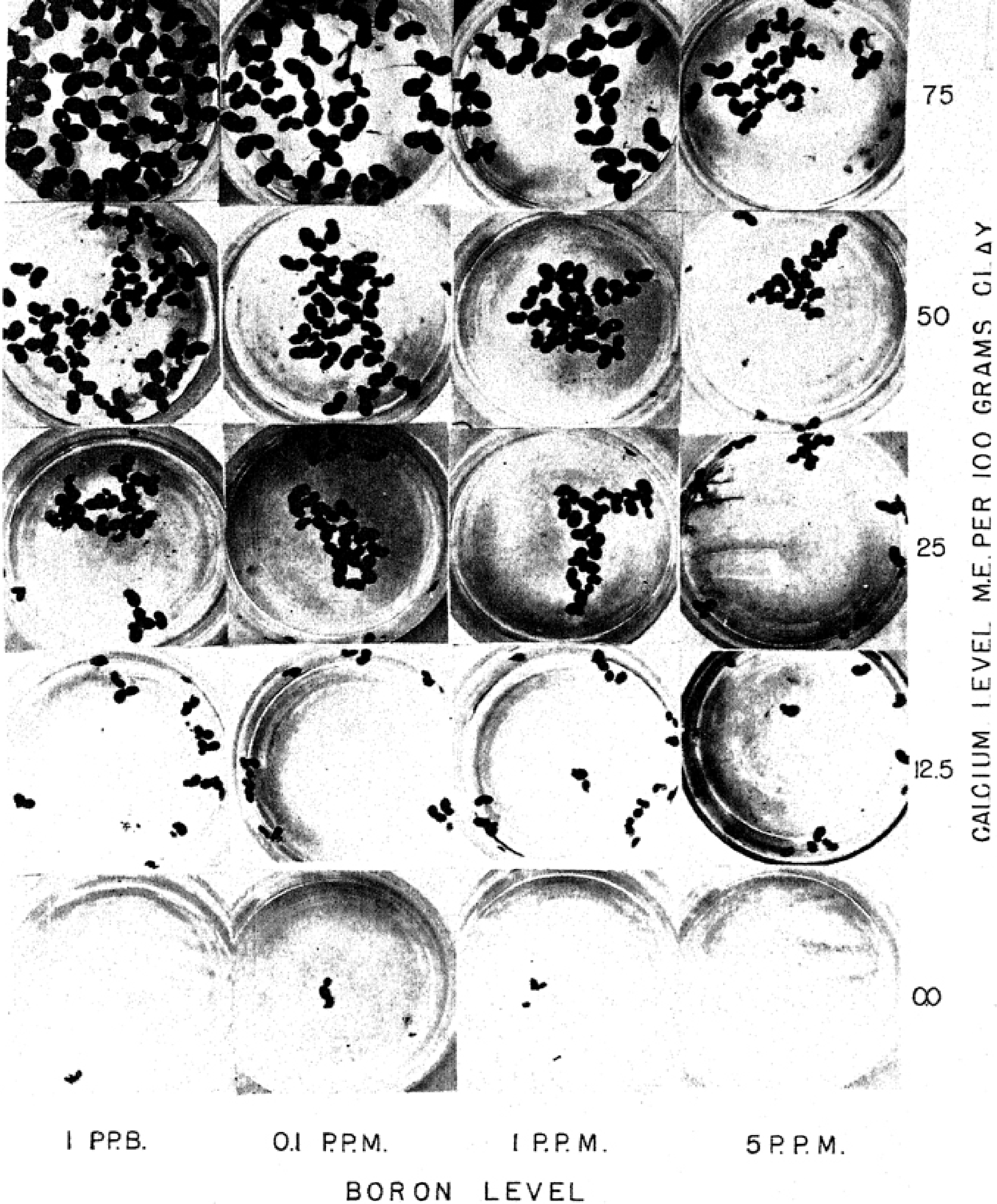

Fig. 2—Division and development of Lemna minor as influenced by the increasing calcium saturations of the clay combined with increasing concentrations of boron in the suspension. (Nineteen days after transplanting ten fronds into each container).

Root Development.

The influence of calcium and boron on duckweed root development was outstanding. Root development was a sensitive indicator and measure of calcium saturation of the clay (Fig. 1). Roots did not develop until the calcium

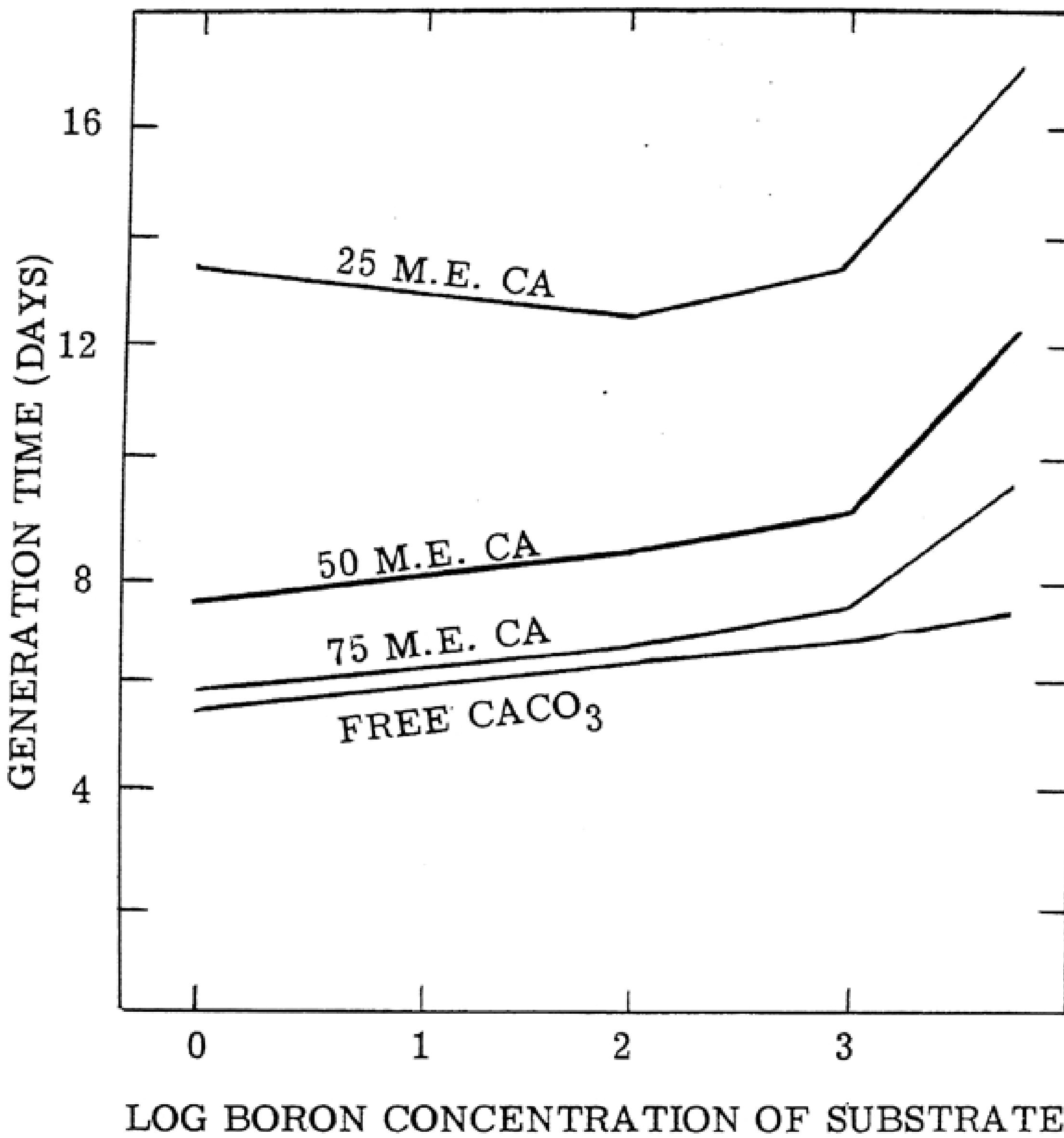

Fig. 3—Generation time of Lemna minor as related to the concentration of boron in the substrate.

saturation of the clay reached 74 percent in the series in which the total calcium was constant. However, at a higher concentration of clay some root development was noted at a calcium saturation as low as 37 percent (Fig. 3, 25 m.e. Ca per 100 grams clay). This is in agreement with the results of studies of tomato root development at various levels of calcium saturation (6), and with the observation that alfalfa root develpment is adversely influenced by a high sodium-low calcium environment (7).

Roots were also sensitive indicators of boron levels and served well to indicate the interaction between boron and calcium (Fig. 4 and Table 2). As an example of this interaction, only the lowest boron level permitted good root

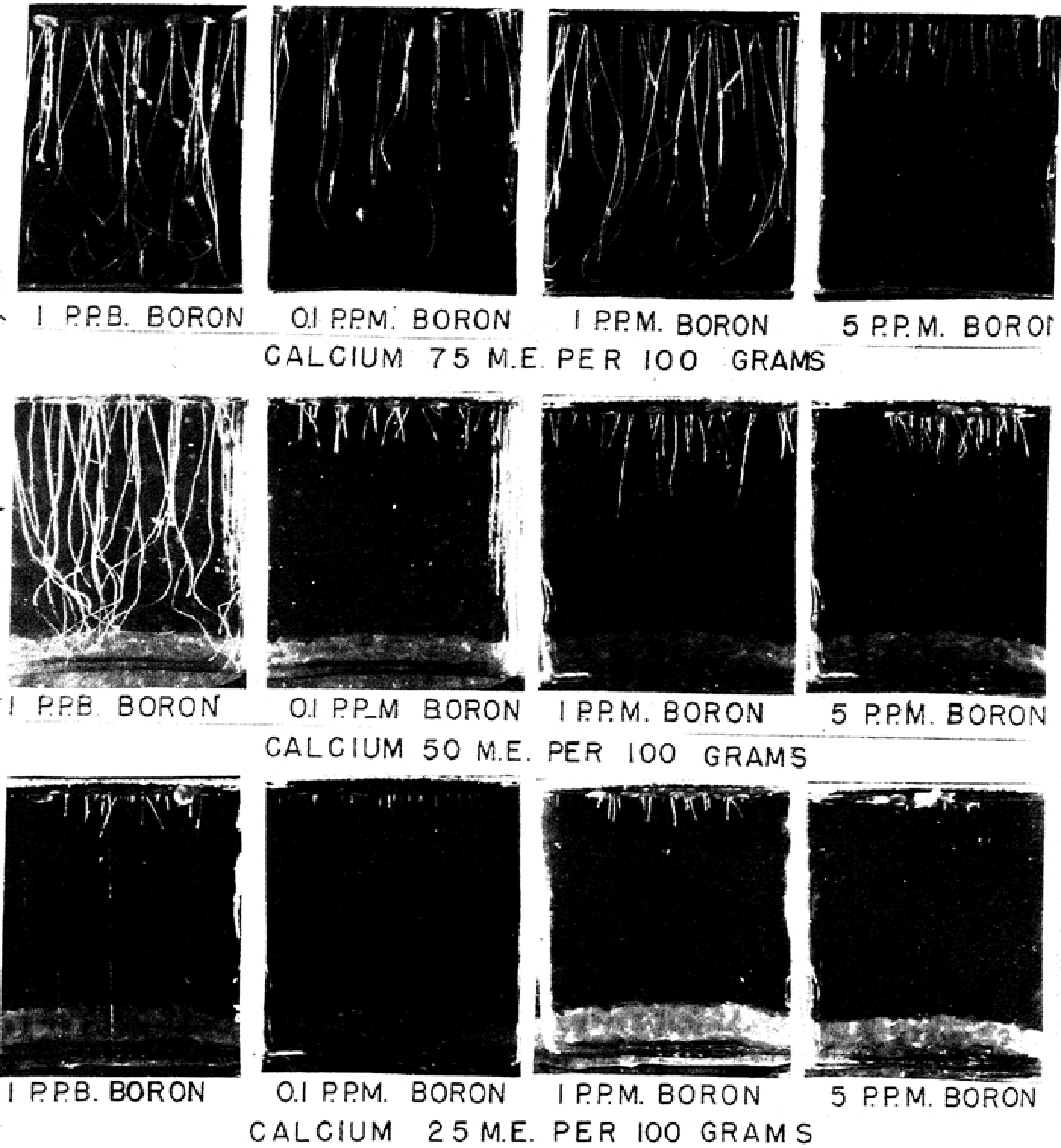

Fig. 4—Development of the roots of Lemna minor as related to four concentrations of boron combined with each of three different degrees of calcium saturation of the clay.

TABLE 2--LENGTH OF LEMNA MINOR ROOTS IN RESPONSE TO VARYING LEVELS OF CALCIUM AND BORON IN THE GROWTH MEDIUM

Calcium m.e./100 gm. Clay	Boron p.p.m. 0.001	0.1	1.0	5.0
	mm	mm	mm	mm
Free $CaCO_3$*	58	23	61	10
75	36	23	36	8
50	23	8	10	5
25	4	2	3	1

*Plants were grown on the free $CaCO_3$ systems at a later date but the same conditions prevailed as nearly as could be determined.

development at 50 m.e. (74 percent calcium saturation of the clay), while roots grew well on all except the highest boron level when calcium was increased to 75 m.e. (110 percent saturation of the clay). Root length as a function of calcium and boron is presented in Table 2.

Chemical Analyses of Plant Materials.

The results of plant analyses are given in Table 3. The percentages of total ash were higher than is normal for most plants. All except two treatments analyzed in this investigation contained more than 20 percent ash. The highest was 32.5 percent. These values are somewhat higher than those given for *Lemna* by Clark (3).

TABLE 3--CHEMICAL ANALYSES OF LEMNA ACCORDING TO VARIABLE LEVELS OF CALCIUM AND BORON IN THE GROWTH MEDIA

Calcium m.e./100 gm. Clay	Boron p.p.m.	Composition of Plant Ash %	Ca %	B p.p.m.	K %	Ca/B
25	0.001	21	1.4	40	3.8	357
	0.1	21	1.6	100	3.8	162
	1.0	23	1.7	100	5.9	168
	5.0	19	--	650	--	---
50	0.001	27	2.0	780	5.6	26
	0.1	23	1.6	980	4.9	16
	1.0	28	2.2	1050	6.4	21
	5.0	32	2.2	1790	--	12
75	0.001	29	2.0	1420	6.2	14
	0.1	30	2.2	2440	7.0	9
	1.0	31	2.0	4600	5.8	5
	5.0	22	2.6	3060	5.5	8
Free $CaCO_3$	0.001	26	1.7	880	5.6	20
	0.1	28	2.2	1300	6.5	17
	1.0	28	2.0	1690	7.7	12
	5.0	24	2.6	4710	5.5	6

There was a suggestive trend toward increased calcium uptake with increased boron concentration. Only at the lowest calcium level tested was there an appreciable decrease of calcium in the plant. However, there was insufficient material for analysis at the lowest calcium levels and increased reproduction at the higher calcium levels probably accounts for the lack of calcium accumulation.

Boron in the plant increased with increasing calcium in the substrate, except where the presence of calcium carbonate evidently had an influence on boron accumulation at the three lowest boron levels. This effect of calcium carbonate was not evident at the highest boron concentration. Boron uptake by the plant increased as boron was increased in the substrate. The total uptake of boron was very high in almost all cases. Whether this is a characteristic of the plant or of the experiment is not known. It is interesting to observe that under some conditions, boron may be as high as approximately 2 percent of the ash without apparent damage to the plant.

The ratio of calcium to boron was very low in all except the lowest calcium treatments. These values were much lower than those which were considered optimum for the plants listed by Jones and Scarseth or by Swanback. Growth of *Lemna* was excellent when the Ca/B ratio was as low as 12 (free $CaCO_3$, and B 1 p.p.m.) and very poor when the ratio was again 12 (50 m.e. Ca/100 gm. and B 5 p.p.m.). It would seem that the Ca/B ratio in *Lemna minor* is not a good indicator of the toxicity of boron.

The average potassium contents of the plants increased with increasing calcium saturation of the colloidal clay. Not only was the percentage of potassium greater but total potassium was also much greater, since growth was greater at the high calcium levels. These results may be expected if high calcium levels make adsorbed potassium more active.

SUMMARY

A study of the relationship between the effects of calcium and boron on the reproduction rate of *Lemna minor* was undertaken using colloidal Putnam clay suspension as the medium on which nutrients were adsorbed. Calcium and boron were supplied at various saturation and concentration levels.

The effects of various degrees of calcium saturation of the clay and of various calcium-boron ratios were measured by the reproductive rate and root development of *Lemna minor*.

Chemical analyses of the plants for boron, calcium, and potassium were made to determine the effects of varying levels of calcium and boron in the nutrient media on their ratios in the plant.

On the basis of the results, the following statements may be made:

1. The duckweed plant is extremely sensitive to levels of calcium and of boron. Reproduction, root development, and chemical composition varied greatly with treatments and served to make *Lemna minor* a good indicator of plant nutrition.

2. Low tolerance by *Lemna minor* for boron was suggested by the detrimental effect of even 0.1 p.p.m. of boron in the nutrient medium. Boron must have been present as impurities in the electrodialized Putnam clay or this little plant collects it very effectively, because plants contained relatively large quantities of boron even when grown on the lowest boron treatments.

3. The highest rates of reproduction and growth of the plants were obtained at the highest calcium levels and low boron levels, showing the wide diversity in the amounts required for these two nutrients elements.

4. The percentage of ash in these plants was very high, amounting in one instance to more than 32 percent of their dry weight.

5. The percentage of boron present in the plants was also very high. Approximate values as high as 2 percent of the ash were obtained in some of the systems.

6. High calcium levels adsorbed on the clay tended to make other nutrient cations more available, according to the chemical data. Reproduction by the plants continued at a high rate for a greater length of time on the high calcium levels in the growth medium.

7. There was a suggestion that the higher calcium levels served to overcome the seemingly detrimental effects of excessive boron. This occurred in spite of the fact that the next to the highest calcium level gave the highest uptake of boron.

8. Free calcium carbonate in the substrate gave decreased boron uptake except at the highest boron level.

9. Increasing concentrations of boron in the substrate gave increasing concentrations of boron in the plants.

10. No relation was found between the Ca/B ratio in the plant and plant reproduction.

11. There was some support for the belief that free calcium carbonate in the soil reduces boron accumulation by plants and for the concept that high calcium reduces the effect of boron toxicity.

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