THE GROWTH AND ANATOMICAL FEATURES OF NUTRIENT-DEFICIENT SEEDLINGS

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As the tree improvement and genetic programs supply better planting stock, a more suitable environment must be provided if their full potential is to be realized. This will require much more information than we now have on how nutrient deficiencies affect the growth and anatomy of forest trees. The importance of anatomical studies has been shown by Church (1949) and Harper (1913). They found that one of the first effects of defoliation is a marked reduction in latewood formation. Furthermore, the assumption that anatomical features exert an influence on the physical and mechanical properties of wood through alterations in specific gravity is generally accepted.

This paper reports the effects of various nutrient deficiencies on the growth and anatomical properties of silver maple (*Acer saccharinum* L.) seedlings. The nutrient elements chosen for investigation were calcium (Ca), boron (B), magnesium (Mg), and phosphorus (P). These were selected primarily because of their physiological functions: Calcium and boron are involved in the meristematic activities. Magnesium is a prime constituent of the chlorophyll molecule, and phosphorus is engaged in energy transfer and is a structural component of deoxyribose nucleic acid and ribonucleic acid.

Total height, diameter, and dry weight of the stem were measured to study growth effects. Maximum root length was also measured as an indication of the absorbing capacity of the seedlings. The anatomical features investigated were the vessels representing the conductive cells, the fibers representing the support cells, and the width of secondary xylem.

Methods

To limit genetic variation, seeds of equal size and weight were obtained from a single silver maple tree, and then only those seeds that germinated within a middle 24-hour period were used in the study.

After the seeds had germinated in a tray lined with moist filter paper, the selected materials were transferred to polyethylene-lined pots containing sterilized vermiculite and were supplied for 2 weeks with deionized water only. Then 25 seedlings were placed in a growth chamber, and each group of five seedlings received one of five nutrient solutions. One, a modified Hoagland's solution (Davis 1949, Hoagland 1948, and Went 1957) containing all nutrients, was given to the controls. The other four solutions were prepared in a similar manner except that each had one of the four study elements withheld. In the growth chamber, day length was maintained at 15 hours, temperature was held at 27° C, and the solutions were constantly aerated.

After 12 weeks in the chamber, the seedlings were observed for foliar symptoms denoting the various deficiencies, and growth data were recorded. To study the anatomical features, a 1-inch-long section was cut from each stem directly above the root collar, embedded in celloidin, and sectioned on a sliding microtome. The 10-micron-thick sections were then stained with Safranin Y and Delafield's Haematoxylin and mounted with a synthetic resin. The embedding and stain procedures used are outlined in Sass (1958).

Significance was tested by means of the analysis of variance, using a completely random design. Tukey's HSD (Tukey 1949) was used as a mean separation test to determine which treatments were significantly different .

Foliar Symptoms

Calcium deficiency was characterized by necrotic marginal areas on the older leaves. The young leaves were pale green and distorted, and had curled margins.

The foliage of the magnesium-deficient plants exhibited interveinal necrosis and marginal chlorosis. The plants themselves were characterized by resetting.

Areas of chlorosis throughout the leaf surface were noted on the boron-deficient plants. The leaves had a silvery appearance and a thickened texture.

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In the early stages of phosphorus deficiency, the older leaves were dark green while the younger leaves were a somewhat lighter shade. As the deficiency progressed, the older leaves became necrotic and the younger ones turned dark green with light green veins.

External Features

The data show that the phosphorus-, magnesium-, and calcium-deficient seedlings were all significantly lower in stem dry weight than the controls (table 1). A significant reduction in maximum stem height occurred in the calcium-deficient seedlings, while both calcium- and boron-deficient series tad significantly reduced root lengths. Only the magnesium-deficient series differed significantly from the control in stem diameter.

Anatomical Features

All four nutrient-deficient series had significantly larger fiber diameters than the controls (table 2). All the nutrient-deficient series except magnesium had significantly larger vessel diameters with a corresponding significant reduction in vessel distribution. For the width of secondary xylem, only the magnesium- and calcium-deficient series differed significantly from the controls.

Discussion

The foliar symptoms found in this study serve as evidence that the seedlings were in fact subjected to the various nutrient deficiences. For the most part the symptoms that occurred coincide with the findings of other workers (Childers 1954, Hacskaylo 1960, Hobbs 1944, Mitchell 1939, and Pessin 1937).

Nutrient deficiences primarily cause disruptions of physiological processes which, in turn, may alter growth and anatomical features. Therefore, the results obtained in this study must be considered as secondary effects. We can only speculate as to what the biochemical effects are since little information of this sort is available on forest trees. There is also a question as to how well the effects of nutrient deficiencies on seedlings can be considered as representing those that might occur on larger trees, since juvenile wood is expected to differ from mature wood. ' Nevertheless, it has been established that early anatomical differences can be used to forecast future trends. With these thoughts in mind, let us consider the effects of each nutrient deficiency in turn.

The minor element, boron, is necessary in only minute quantities, but it is essential for vigorous growth. It affects cell division, nitrogen metabolism, carbohydrate metabolism, active salt absorption, hormone movement and action, and metabolism of pectic substances. It is a constituent of

Table 1. — Seedling means arranged in order of magnitude by nutrient-deficiency treatment'

Dry weight of stem		Height		Root length		Stem diameter	
Treatment	Mean	Treatment	Mean	Treatment	Mean	Treatment	Mean
	(Grams)		(Cm)		(Cm)		(Mm)
-B	0.481	-B	27.7	-P	36.0	Control	1.55
Control	.455	Control	27.5	Control	35.8	-B	1.50
-P	.296	-P	21.5	-Mg	34.0	-P	1.40
-Mg	.146	-Mg	20.9	-B	26.4	-Ca	1.29
-Ca	.074	-Ca	14.9	-Ca	10.0	-Mg	1.18

1/ Based on five trees for each treatment. Any two means not scored by the same line are significantly different.

Table 2. — Seedling means of various anatomical features arranged in order of magnitude by nutrient-deficiency treatment[1]

Fiber diameter		Vessel diameter		Vessel distribution[2]		Width of secondary xylem	
Treatment	Mean	Treatment	Mean	Treatment	Mean	Treatment	Mean
	(Microns)		(Microns)		(No.)		(Microns)
-P	16.0	-B	28.8	Control	13	-B	294
-Ca	15.1	-Ca	27.1	-Mg	12	Control	280
-B	14.9	-P	27.1	-P	9	-P	246
-Mg	13.3	-Mg	25.7	-B	9	-Mg	162
Control	10.8	Control	23.8	-Ca	6	-Ca	151

1/ Based on five trees per treatment. Any two means not scored by the same line are significantly different.

2/ Per 25,000 square microns.

membranes, serves to increase the solubility of calcium, and acts in precipitating excess cations. Boron is said to be necessary in the maintenance of conducting tissues. In this study, neither the height, diameter, nor dry weight of stem of the boron-deficient seedlings differed from those of the controls; but the root length did. The roots of the boron-deficient seedlings were more or less stunted. This corresponds to the results of Sommer and Sorokin (1928). The reduction in root growth may seriously affect the development of the seedlings by impairing the uptake of water and the accumulation of salts.

With regard to anatomical features, the boron-deficient seedlings had larger fibers and vessels and consequently fewer vessels per unit area than the controls. These changes may be the result of alterations in physiological processes, such as cell division, caused by the lack of boron. Warington (1926), working on the anatomical effects of boron deficiency, found an abnormal enlargement of the cambial cells.

Calcium is a major element required in large quantities for plant growth. It affects the activities of certain enzymes but is especially important in combining with pectin to form calcium pectate, a constitutent of the intercellular substance. It reduces the toxic effects of magnesium, sodium, and potassium, and neutralizes certain organic acids. At the growing points, calcium has an enzymatic function. The reductions in growth obtained in this study for the calcium-deficient seedlings agree with the results of other workers investigating calcium deficiencies (Hobbs 1944, Mitchell 1939, Pessin 1937, and Wyatt 1961). The growth losses may well result from the lack of calcium (1) to stimulate enzymatic action and possibly (2) to reduce the toxic effect of certain salts and to neutralize certain organic acids.

The calcium-deficient seedlings also differed from the controls in all four anatomical features. These results correspond to those of Davis (1949) who found in calcium-deficient *Pinus* taeda a decrease in cell division accompanied by an increase in cell enlargement and cell differentiation in the secondary xylem. Yenning (1953) found a reduction in cambial activity and amount of secondary xylem in calcium-deficient tomato (*Lycoperiscon* spp.) plants. These alterations of the anatomical features may well reflect calcium's enzymatic role in the meristematic regions of the plants.

Magnesium is active in the enzymatic system and is a possible carrier of phosphorus. The reduced stem diameter and dry weight of stem exhibited by the magnesium-deficient seedlings is probably a secondary effect since one of the prime functions of magnesium is as the central component of the chlorophyll molecule. Therefore, a deficiency of this element would greatly reduce the rate of photosynthesis. Pessin (1937) and Wyatt (1961) also showed a reduction in growth due to magnesium deficiency.

As to the anatomical features, the reduction in secondary xylem is understandable as this is in accordance with the general reduction in growth previously noted. However, the enlargement of the fibers occurring without an accompanying change in the size and distribution of vessels is not so easily understood. Possibly a deficiency of magnesium has a much greater effect on the support cells than on the conductive elements of the secondary xylem.

Phosphorus is necessary for photosynthesis, the synthesis of carbohydrates, and the transfer of energy within the plant. It is a structural component of nucleic acid, which includes RNA and DNA. The reduction in dry weight of the phosphorus-deficient seedlings occurred even though the stems were of approximately the same size as the controls. The phosphorus-deficient seedlings, however, had stems which were composed primarily of young succulent tissue with larger fiber and vessel diameters and correspondingly fewer vessels per unit area than in the controls. These anatomical alterations may well reflect the role of phosphorus in the organization of the cells and in the transfer of hereditary characteristics.

Conclusions

The results of this study indicate that nutrient deficiencies in forest trees may cause disruptions of certain physiological processes which, in turn, may result in reduction of growth and alteration of anatomical features.

Much work remains before the complete effects of nutrient deficiences on growth and anatomy of forest trees are fully understood. The interaction between nutrient elements is highly complex. Therefore, it is difficult to assign one particular anatomical or growth alteration to a deficiency of a single element. This information is vital, however, if the full potential of our tree improvement programs are to be realized outside the laboratory.

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