NUTRITIONAL STUDIES ON LOBLOLLY PINE

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(WITH TWO FIGURES)

Introduction

The investigation herein reported was undertaken as part of a cooperative project on the biology of forest trees at Duke University. It represents an attempt to add a contribution to knowledge of the mineral nutrition of certain forest trees, in the hope that ultimately it may be possible to correlate the findings with studies of soil composition and with the local distribution of these tree species. Loblolly pine (*Pinus taeda* L.) was selected as a species that is relatively cosmopolitan, and yellow poplar (*Liriodendron tulipifera* L.) as a species that is relatively specific in environmental requirements. The present paper is a preliminary report on loblolly pine only.

For the purposes of this investigation, sand cultures were regarded as the most desirable method. Of the extensive work with sand cultures by many investigators within the past few years, little has been concerned with woody plants, and very little with forest trees. MITCHELL'S (**4**) experiments on Scotch pine and white pine in the Black Rock Forest represent the most fruitful work of the kind that has been done with forest trees.

Experimental methods

Loblolly pine was grown in sand cultures for periods as long as 29 months. The first experiment was started in February, 1934, when 50 seedlings, 8 months old, were transplanted from a sand-peat mixture to washed white quartz sand in ordinary 10-inch unglazed pots. Of these seedlings 30 were transplanted in January, 1935, to sand in 3-gallon glazed crocks with drainage at the bottom. Nutrient solutions were added at first twice and later three times per week; at all other times the moisture content was maintained by the addition of distilled water. From time to time individual plants were removed from the series for study. Four of the 22 plants that remained on July 14, 1936, are shown in figure 1.

Other series were grown for shorter periods of time, employing similar methods except that glazed self-draining pots were used from the start.

Another series of loblolly pine was grown in sand culture for 14 months under slightly different conditions. The same type of crocks and the same solutions were used, but the solutions were applied daily by a drip-method of renewal. Addition of nutrients was not continuous, but 10 pots set up in series received each day 18 liters of solution, which dripped from capillary tubes. The arrangement of apparatus is shown in figure 2. In this series, one

FIG. 1. *Pinus taeda* L. grown for 29 months in sand culture. For two trees on left, nitrogen was supplied as calcium nitrate; for two trees on right, as ammonium sulphate.

FIG. 2. Drip-cultures at the end of 14 months. Capacity of each carboy is 18 liters. Uniform rate of drip is maintained by means of bottles that serve as constant-level reservoirs. Solution reaches individual cultures by dripping from capillary tubes. For series on left, nitrogen was supplied as calcium nitrate; for series on right, as ammonium sulphate.

set of 10 pots received its nitrogen from calcium nitrate, the other set from ammonium sulphate.

Since the experiments were primarily exploratory in nature in all series, the composition of solutions was varied from time to time in an attempt to determine the optimum conditions for growth. Concentrations of salts were varied as well as the hydrogen ion concentration, the latter by means of the addition of NaOH and H_2SO_4. The composition of two of the solutions that were successfully employed is given in tables I and II.

TABLE I

COMPOSITION OF SOLUTION A

SALT	GRAMS PER LITER	CONCENTRATION IN P.P.M.	
	gm.	p.p.m.	p.p.m.
$Ca(NO_3)_2 \cdot 4H_2O$	1.148	Ca 195	N 136
KH_2PO_4	0.643	K 184	P 146
$MgSO_4$	0.283	Mg 57	S 76
H_3BO_3	0.001	B 0.20	
$MnSO_4$	0.001	Mn 0.40	
$FeCl_3$	0.002	Fe 0.70	

TABLE II

COMPOSITION OF SOLUTION B

SALT	GRAMS PER LITER	CONCENTRATION IN P.P.M.	
	gm.	p.p.m.	p.p.m.
$(NH_4)_2SO_4$	0.734	N 156	S 156
KH_2PO_4	0.870	K 249	P 198
$MgSO_4$	0.167	Mg 33	S 45
$CaCl_2$	0.324	Ca 117	Cl 204
H_3BO_3	0.001	B 0.20	
$MnSO_4$	0.001	Mn 0.40	
$FeCl_3$	0.002	Fe 0.70	

Results and discussion

The several experiments, extending over periods of varied lengths up to 29 months, proved to the satisfaction of the writer that (1) loblolly pine can be grown successfully in sand culture, and that (2) it can utilize nitrogen in the form of either nitrates or ammonium compounds. These two points will be discussed in some detail.

The pine trees grown in sand culture compared favorably with trees grown in soil as to size and general appearance, with the exception of color as discussed below. One difficulty that was experienced in sand culture, however, was the inequality of growth of the different individuals in a series. All of the plants grew, but the amount of growth varied greatly. When the number of individuals in a series must be limited, this factor is important. MITCHELL (**4**) refers the difference in his seedlings to difference in seed weight, and cites correction factors. In the present experiments an attempt was made to obviate the difficulty by using seedlings several months old and selecting them for uniformity of size and general appearance. The results indicate, however, that such selection is not sufficient. Figure 2 shows that the size of plants after several months of identical cultural treatment varied greatly. This difference was obviously the result of differences in plants and not of differences in treatment. In experiments with plants that can be propagated vegetatively, it would probably be better to work with rooted cuttings than with seedlings.

A second difficulty was the yellowing of leaves that was apparent from time to time in both nitrate and ammonium series. Usually it showed during the first few months after the seedlings were set out, and in most instances the plants subsequently recovered. The yellowing was much less noticeable in the drip cultures. In the light of MITCHELL's (**4**) experience it seems reasonable to suppose that the cause was insufficient nitrogen. Mitchell states that the optimum concentration of nitrogen is 300 p.p.m., whereas in the solutions listed above, the concentration of nitrogen was only about half of that. In his experiments, however, the nutrient solution was added only twice during the 15 weeks of the experiments, whereas in the present experiments nutrient solution was added two or three times a week, and in the series of drip-cultures, every day. It is obvious, then, that the total amount of nitrogen supplied to the plants was considerably more than the concentrations indicate, in comparison with those of MITCHELL's solutions. However, the fact that the pines of the drip-series showed much less yellowing than the others lends some evidence to the theory that the abnormality was caused by an insufficient supply of nitrogen. It was obviously not caused by deficiency of boron, manganese, or iron; iron was added in several forms and in several concentrations during the experiments, without apparent effect. Alteration of the hydrogen ion concentration from time to time during the course of the experiments had no appreciable effect on the yellowing. As stated above, most of the plants recovered and developed a healthy green color. Similar yellowing has been observed in soil cultures, and occasionally in the field.

Some of the seedlings developed a peculiar twisting and curling of the leaves that produced an appearance of knotting. This was observed in some but not all plants of every series, and in some plants more generally than in others. Eventually the leaves straightened and assumed a normal appearance. The abnormality was not restricted to these experiments, but was observed in even more acute form in some soil cultures that were being conducted by another worker in the adjoining greenhouse. It has since been observed occasionally in pine trees growing in the field. The first explanation that suggested itself was that the sheath of the fascicle had become dry and hard, with the result that the normal elongation of leaves was mechanically restricted. In order to test this theory, several plants were placed for several weeks under bell jars, so that the humidity in which they were growing was greatly increased. Under this treatment leaves were as "knotted" as ever, disproving the theory.

These experiments showed conclusively that loblolly pine is capable of utilizing nitrogen in the form of either nitrates or ammonium. Alteration of the acidity over a wide range showed the best development with calcium nitrate as the source of nitrogen when the solution was decidedly acid (3.8 to 5 pH); with ammonium sulphate as the source of nitrogen when the solution was more nearly neutral (6 pH). The observations are entirely in keeping with those of TIEDJENS (**6**), TIEDJENS and BLAKE (**7**), TIEDJENS and ROBBINS (**8**), NIGHTINGALE (**5**), and DAVIDSON and SHIVE (**2**) on apple, peach, and other plants. It is altogether probable that under field conditions in the Piedmont of North Carolina loblolly pine receives most of its nitrogen in the form of ammonium compounds, for little nitrification occurs. In many forest stands of loblolly pine, as determined by COILE (**1**), the hydrogen ion concentration of the A horizon varies from 5.4 to 6.5 pH.

Although mycorrhiza were present when the seedlings were set out, they did not develop to any considerable degree in sand cultures. Field-grown plants show long white roots that are free from fungous infection and short, stubby, much-branched lateral roots that are infected with a tight mass of mycelium that penetrates the epidermis. Pines of the several experimental series also showed long white roots and short, branched lateral ones; however, the latter were not nearly so stubby as those of field-grown plants and microscopic observations showed no trace of fungous infection—any mycelium that was present was entirely external. Since rapid and apparently healthy growth was obtained in sand culture, it would seem likely that in field-grown loblolly pine the occurrence of mycorrhiza is incidental rather than highly beneficial.

The root systems developed in sand culture are worthy of mention, as their distribution is in rather striking contrast to that of loblolly pine roots under field conditions. In nature, most of the absorbing roots occur in the uppermost layers of the soil. In sand culture, however, the root systems practically fill the pots, and the absorbing roots are much more generally distributed. It is possible that the difference is attributable to the difference in aeration, and that the superficial character of the root systems of loblolly pines in many forest soils is caused by the deficiency of aeration in underlying horizons.

During the course of the present experiments, the trees were not dormant through a long period of time, such as the winter. In all of the series they showed during a year several periods of growth and several periods of dormancy, but never a long-continued winter dormancy. It is altogether possible that this condition is attributable to illumination. Although the pines were not intentionally given a longer photoperiod, other experiments in adjoining greenhouses were lighted regularly. It is possible that sufficient light reached the pines to prevent winter dormancy. WITHROW and BENEDICT (9) have shown that for certain plants an intensity of 0.3 foot candle is sufficient to produce a response. KRAMER (3) has demonstrated that it is possible to keep loblolly pines growing actively throughout the winter under greenhouse conditions.

In the light of these experiments the writer feels justified in pursuing the study of mineral deficiencies in loblolly pine by the use of sand cultures; employing either nitrates or ammonium salts as sources of nitrogen.

Summary

1. Loblolly pine seedlings were grown successfully in sand cultures for periods up to 29 months.

2. They proved capable of utilizing nitrogen in the form of either nitrate or ammonium—the former more successfully in acid reaction, the latter more successfully in a more nearly neutral reaction.

3. The seedlings showed great individual variation in rate of growth even though they were selected on the basis of uniformity of size.

4. The root systems were deeper than those that are usually developed in the field.

5. Mycorrhiza were not formed.

6. Many plants of both the nitrate and the ammonium series showed, at times, a yellowing of leaves that may have been caused by an insufficient quantity of nitrogen. Apparently similar conditions were observed in soil cultures and to a lesser degree in the field.

7. Plants were grown throughout the year without long-continued periods of dormancy. This continuous activity may have been associated with a photoperiod longer than that of winter days.

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