A Nutritional Study of Slash Pine Seedlings Grown in Sand Culture

BY
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Abstract. In a greenhouse experiment conducted to determine the relationship of nitrogen, phosphorus, and potassium supply to slash pine seedling yield and composition, 30 combinations of N, P, and K were supplied to the seedlings for a four-month growing period. Seedling yield and composition were found to be closely associated with nutrient supply. Fresh weight, dry weight, and height growth were indicated by regression analyses to be maximum when the nutrient supply of N and K is greater than 125 ppm, but less than 625 ppm. Maximum response to phosphorus was not reached at 125 ppm, the highest concentration supplied. The concentration of N, P, or K in the seedling tissue increased as the supply of the respective element was increased.

THIS PAPER reports on a greenhouse study which explores several aspects of the mineral nutritional properties and requirements of slash pine seedlings (*Pinus elliottii* Engelm.). The study reveals some of the relationships of mineral nutrient supply to tissue, and to shoot-root ratios. Information is presented on the relation of nutrient content of the tissue to yield as well as on some of the roles of these nutrients in plant growth.

An impressive amount of work has been done in the past on plant nutrition in general, a portion of which can be applied to forest tree nutrition. The principles and methods established by Liebig (1840), Mitcherlich (1924), Hoagland (1938, 1944), and others have contributed materially to the development of research in tree nutrition.

Mitchell (1934), one of the first to study such problems in a timber species, reported on the nutritional response of Scotch pine (*Pinus sylvestris* L.) and eastern white pine (*Pinus strobus* L.). Later workers developed similar studies for a few other pine species. These studies generally indicate the range of nutrient supply concentrations that provide acceptable growth; they also establish the relationship between nutrient supply and the nutrient concentration in the tissue (Table 1). The results of this past work show that nutritional requirements of different pine species are related, and optimum growth for the different species occurs under somewhat similar supply regimes. One deviation from this general trend is the low phosphorus requirement for satisfactory growth of loblolly pine and Virginia pine found by Fowells and Krauss (1959). The apparent difference in requirement could be the result of different techniques. In the Fowells study nutrient solutions were supplied to the plant more frequently than in most of the other studies.

Other workers have attacked the mineral nutrition problem from other angles. Leyton and Armson (1955) analyzed the foliage of Scotch pine and found that height growth was correlated with the concentration of nutrients in needles from various parts of the tree. They also found that the

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TABLE 1. Composition of nutrient solutions in studies of mineral nutrition of pine.

Source of information	Species	Optimum supply range			Nutrient concentration in seedling shoot at optimum supply		
		N	P	K	N	P	K
		Ppm			*Percent*		
Mitchell (1939)	Eastern white pine (*Pinus strobus* L.)	300	350	150	3.51	0.62	1.59
Bensend (1943)	Jack pine (*Pinus banksiana* Lamb.)	230	......	......	2.75	......	......
Woodwell (1958)	Loblolly pine (*Pinus taeda* L.)	75 to 600	40 to 600	25 to 300	......	......	......
	Pond pine (*Pinus serotina* Michx.)	25 to 600	40 to 600	25 to 125	......	......	......
Fowells and Krauss (1959)	Loblolly pine and Virginia pine (*Pinus virginiana* Mill.)	25 to 100	1	......	1.7 to 2.3	0.14 to 0.16	......

concentration varied in tissues of different ages and from different locations in the tree. Cain (1953, 1959), working with apple trees, found that the percentage contents of phosphorus and potassium in the leaves decreased with increases in nitrogen content. His findings indicate that most interactions can be explained by nutrient dilution or redistribution.

Materials and Methods

Thirty glazed three-gallon crocks filled with acid-washed quartz sand of uniform particle size were set up so that they could be watered from black carboys underneath the greenhouse bench. Compressed air forced the nutrient solutions simultaneously into all the treatment crocks. When the air pressure was cut off, the nutrient solutions drained into the carboys.

The basic nutrient supply solution contained: calcium as $CaCl_2$, 100 ppm; magnesium as $MgSO_4$, 75 ppm; boron as H_3BO_4, 0.5 ppm; manganese as $MnCl_2 \cdot 4H_2O$, 0.5 ppm; zinc as $ZnSO_4 \cdot 4H_2O$, 0.05 ppm; copper as $CuSO_4 \cdot 5H_2O$, 0.02 ppm; molybdenum as H_2MoO_4-H_2O, 0.05 ppm; and chelated iron (Sequestrene, Geigy), 5.0 ppm. Nitrogen, phosphorus, and potassium were added to the supply solutions at five elemental concentrations or levels (Table 2). Space and materials limited the number of selected treatments to 30 of the 125 possible combinations. Nitrogen was added to the solution in the form of NH_4NO_3; phosphorus as $NaHPO_4 \cdot H_2O$; and potassium as KCl.

The culture solutions were replaced at four-week intervals; each time the system was flushed to remove excess salts. Water lost by evapotranspiration (two or three liters per month) was replaced periodically by pouring enough distilled water over the seedlings to replenish the reservoir supply to 18 liters.

The study began on January 12, 1959, when seed of near uniform size were planted and continued until the seedlings were harvested on May 25, 1959. A good, even stand developed in all crocks and on February 1 the seedlings were thinned to 12 per pot. No seedling mortality occurred throughout the duration of the study.

Irrigation was with distilled water until February 2 when one-third of the assigned nutrient supply was added to each treatment carboy. The two additional one-third

TABLE 2. Nutrient concentration by treatment level.

Element	Levels				
	0	1	2	3	4
	Ppm				
Nitrogen (N)	0	5	25	125	625
Phosphorus (P)	0	1	5	25	125
Potassium (K)	0	5	25	125	625

TABLE 3. Summary of treatment data.

Treatment number	Nutrient supply Nitrogen	Phosphorus	Potassium	Shoot-root ratio	Dry weight	Fresh weight	Elongation	Nitrogen in shoot	Nitrogen in root	Phosphorus in shoot	Phosphorus in root	Potassium in shoot	Potassium in root
	Parts per million				*Grams*		*Millimeters*	*Percent*					
1	125	25	25	3.25	0.707	4.39	46.1	3.02	1.85	0.111	0.189	0.508	0.586
2	5	1	5	1.55	0.310	1.63	18.0	1.39	0.85	0.052	0.104	0.303	0.527
3	125	25	125	2.44	0.902	5.63	48.0	2.93	1.49	0.234	0.378	0.391	0.596
4	125	5	25	2.94	0.831	5.20	45.4	2.71	1.83	0.111	0.228	0.459	0.478
5	125	125	625	3.11	0.925	5.12	50.0	2.82	1.56	0.248	0.424	0.606	0.679
6	25	125	25	2.42	0.562	3.42	33.3	2.50	1.33	0.312	0.319	0.459	0.557
7	5	125	625	.82	0.690	3.26	38.0	1.32	0.73	0.273	0.560	0.645	0.703
8	625	125	625	3.28	0.715	4.03	25.8	3.28	2.60	0.274	0.176	0.450	0.557
9	25	5	125	2.08	1.070	5.68	67.8	2.03	0.86	0.185	0.189	0.508	0.704
10	0	25	125	1.27	0.302	1.60	11.1	1.09	0.77	0.280	0.417	0.615	0.704
11	25	1	25	1.67	0.498	2.98	31.1	2.11	1.10	0.080	0.078	0.410	0.478
12	5	25	125	1.42	0.541	3.03	37.5	1.28	0.73	0.267	0.560	0.635	0.830
13	625	25	625	2.82	0.550	3.13	28.5	2.89	1.86	0.176	0.306	0.742	0.742
14	125	5	125	2.55	1.080	5.70	57.3	2.63	1.51	0.222	0.117	0.586	0.635
15	625	125	125	3.26	0.465	2.41	16.2	2.92	2.70	0.404	0.156	0.450	0.664
16	125	125	0	2.32	0.145	0.57	6.7	4.19	......	0.430	0.228	0.087	0.117
17	25	5	25	2.15	0.650	3.91	47.3	2.43	1.41	0.189	0.306	0.489	0.733
18	125	25	625	2.84	0.713	4.06	29.5	2.60	1.80	0.215	0.358	0.655	0.831
19	125	0	125	1.75	0.366	1.53	13.8	1.99	1.10	0.130	0.156	0.342	0.449
20	25	25	25	2.23	0.538	3.37	31.9	......	1.19	0.356	0.310	0.499	0.567
21	5	5	625	1.45	0.250	1.46	16.5	1.36	0.97	0.241	0.345	0.332	0.890
22	125	25	5	2.85	0.272	1.36	14.5	2.57	2.29	0.176	0.287	0.244	0.166
23	125	125	125	3.02	0.720	4.75	43.8	2.55	1.85	0.319	0.241	0.606	0.459
24	0	0	0	1.54	0.193	.80	6.5	0.94	0.57	0.026	0.026	0.147	0.087
25	625	125	25	3.51	0.667	3.60	28.1	3.19	2.82	0.215	0.163	0.459	0.283
26	125	1	125	2.41	0.375	1.70	22.2	2.02	1.31	0.091	0.104	0.410	0.557
27	625	5	5	3.01	0.253	1.22	14.4	4.15	3.19	0.306	0.185	0.166	0.117
28	25	25	125	1.64	0.400	2.37	23.3	1.50	0.87	0.426	0.560	0.684	0.703
29	25	125	125	2.26	0.796	4.49	42.3	2.15	1.01	0.358	0.560	0.469	0.391
30	625	1	625	2.55	0.465	2.23	18.2	3.12	1.89	0.072	0.104	0.929	0.586

increments were added at consecutive five-day intervals. The plants were irrigated twice a day with the nutrient solutions until early May when higher temperatures made an additional daily application necessary.

Prior to harvest seedling heights were measured and average values computed. Immediately after harvest the average fresh weight per seedling was determined. The shoots and roots were then separated at ground line and ovendried at 72°C to constant weights. The modified Kjeldahl method was used for determining total nitrogen content of the tissue samples (Jackson 1958). Because only a small amount of plant tissue was available, a single wet oxidation extract was prepared for phosphorus and potassium determinations. Phosphorus contents were determined by the molybdenum-blue colorimetric method and potassium percentages by flame photometry (Jackson 1958). Chemical findings based on percentage of dry weight pertain to the succulent non-lignified portion of the shoot and total root tissue. A summary of all seedling data is presented by treatment in Table 3.

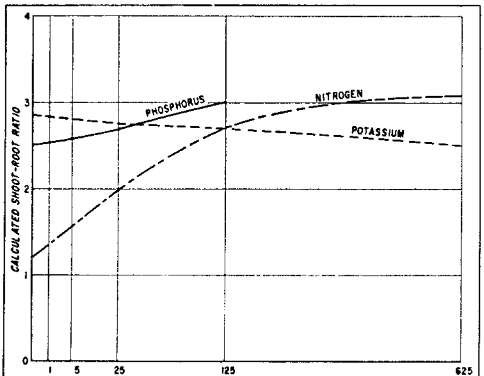

FIGURE 1. *Shoot-root ratios in relation to nitrogen, phosphorus and potassium supply. The regression equation was solved and plotted holding two of the nutrients constant and varying the third. Nitrogen and potassium were held at 125 ppm and phosphorus at 25 ppm.*

Results and Discussion

Growth response to nutrient supply. Seedling growth—as represented by shoot-root ratio, dry weight, fresh weight, and elongation—was influenced in varying degrees by available supplies of nitrogen, phosphorus, and potassium. Regression analyses of growth data obtained from the 30 treatment observations supplied the following equations:

A. $\text{Shoot-root ratio} = 1.345 + 0.1640\,(\sqrt{N}) - 0.014(\sqrt{K}) - 0.0043\,(N) + 0.0038\,\sqrt{NP}$

B. $\text{Fresh weight (grams)} = 0.8548 + 0.2982\,(\sqrt{N}) + 0.1282\,(\sqrt{P}) + 0.0615\,(\sqrt{K}) - 0.0113(N) - 0.0009(K)$

C. $\text{Total elongation (mm)} = 5.5436 + 2.2834\,(\sqrt{N}) + 0.8575(\sqrt{P}) + 2.6995\,(\sqrt{K}) - 0.0949\,(N) - 0.0866\,(K)$

D. $\text{Dry weight (grams)} = 0.0565 + 0.0401\,(\sqrt{N}) + 0.0174\,(\sqrt{P}) + 0.0480\,(\sqrt{K}) - 0.0014\,(N) - 0.0014\,(K)$

where: $N =$ parts per million of nitrogen in the nutrient solution

$P =$ parts per million of phosphorus in the nutrient solution

$K =$ parts per million of potassium in the nutrient solution.

These regression equations account for 85, 45, 40, and 46 percent of the variation, respectively, after reducing from about the mean.

Shoot-root ratios were greatly influenced by nutrient supply, and were essentially controlled by nitrogen availability (Fig. 1). This finding agrees with the work of Mitchell (1939) and Fowells (1959). Two explanations appear applicable to the high degree of correlation found between the supply of nitrogen and shoot-root ratios. One is that when supply of the element is high, carbohydrates are rapidly utilized in the aerial portion of the tree and less solutes are available for translocation into the roots. The second approach exploits the effect of nitrogen on the production of growth regulators and their subsequent control of shoot and root growth. Avery *et al.* (1936) showed that the production of indoleacetic acid (IAA) is highly correlated with the supply of available nitrogen. Further work by Bosemark (1954) indicates that at low concentrations, IAA promotes growth of both the shoots and the roots, but that the optimal concentration of IAA is much lower for roots. Thus, as the supply of nitrogen is increased, the concentration of IAA is increased and the tops are stimulated to a greater degree than the roots.

The regression analyses indicate that optimum growth would occur when supply concentrations of nitrogen and potassium are greater than 125 ppm but less than 625 ppm. The maximum point in the phosphorus supply-response curve was not reached at the highest concentration of phosphorus supplied (125 ppm) (Fig. 2).

Nitrogen exerts considerably more influence on fresh weight production than either potassium or phosphorus (Fig. 2a). This finding agrees with Black (1957), who states that an increase in nitrogen supply is usually associated with greater succulence. This is caused by a tendency for plants with high nitrogen supplies to produce new, large, thin-walled cells rather than to deposit carbohydrates on the walls of older cells.

On the other hand, the production of dry weight and stem elongation seems to be more closely associated with the supply of potassium than either nitrogen or phosphorus (Fig. 2b and 2c). The metabolic functions of potassium, while not fully understood, might explain its influence on dry weight production and elongation inasmuch as over 90 percent of the dry material of the plant is a product of photosynthesis.

Growth response in a greenhouse study is a function of the total volume of the supply solution, the concentration of the solution with respect to the individual nutrients, the frequency with which the nutrient supply is made available to the plant, and the chemical nature of the solution itself with respect to osmotic pressure and pH. It is evident that greenhouse supply systems are not constant; that is, as the solution is made available to the plant the concentration of the solution slowly decreases. If the supply solution is large and frequently replaced, the reduction may be negligible. In this study, the maximum percentage reduction in concentration occurred in a treatment designed to supply five ppm of nitrogen. It was deduced that the concentration was reduced roughly to 4.3 ppm by the end of the 30-day period. The range of osmotic pressure and pH of

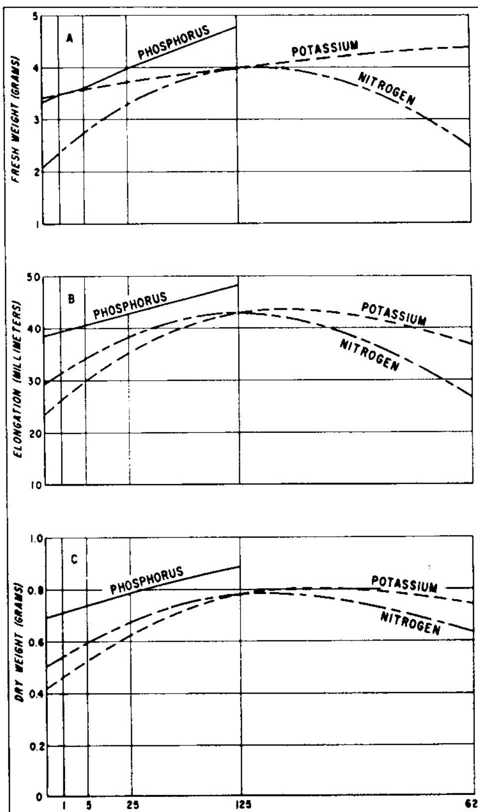

FIGURE 2. *Seedling yields in relation to the supply of nitrogen, phosphorus and potassium. A. Fresh weight. B. Elongation. C. Dry weight. The regression was solved holding two test elements constant and varying the third.*

the nutrient supply was too small to seriously affect the availability of salts and water. The pH generally ranged from 4.6 to 5.1 with no apparent correlation to treatment and specific conductance from 100×10^{-5} to 350×10^{-5} mho.

Composition of seedling tissue. The relation of the nutrient composition of seedling shoots and roots to nutrient supply was established on a dry weight basis by regression analysis. Likewise, the relation of shoot composition to root composition was determined by regression. The following equations were obtained after testing numerous forms of the supply variables and their interactions:

E. Nitrogen in shoot (percent) $= 1.4886 + 0.0108\ (N) - 0.000013\ (N)^2$

Nitrogen in root (percent) $= 0.8118 + 0.0079\ (N) - 0.000008\ (N)^2$

Nitrogen in shoot (percent) $= 3.3086 - 1.4944 \left(\frac{1}{\text{percent nitrogen in root}}\right) + 0.0801\ (\text{Percent nitrogen in root})^2$

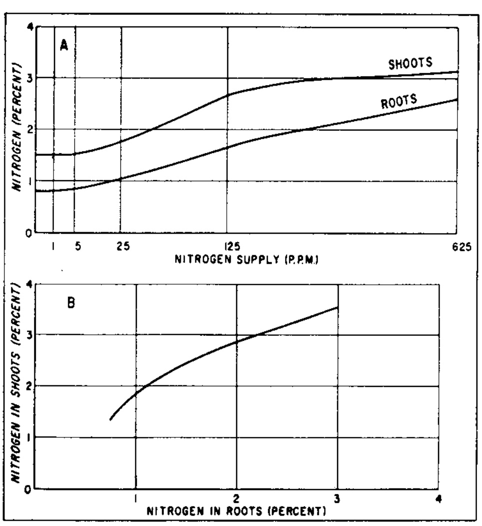

FIGURE 3. *A. Nitrogen percentage in shoots and roots in relation to nitrogen supply. B. Nitrogen percentage in shoots in relation to nitrogen percentage found in roots.*

F. Phosphorus in shoot (percent) $= 0.2617 - 0.1215 \left(\frac{1}{P}\right)$

Phosphorus in root (percent) $= 0.3151 - 0.1499 \left(\frac{1}{P}\right)$

Phosphorus in shoot (percent) $= 0.0730 + 0.4783$ (Percent phosphorus in root) $+ 0.000131\ (N)$

G. Potassium in shoot (percent) $= 0.3883 + 0.00132\ (K) - 0.000001\ (K)^2 - 0.1433 \left(\frac{1}{K}\right)$

Potassium in root (percent) $= 0.4308 + 0.001764\ (K) - 0.000002\ (K)^2 - 0.1763 \left(\frac{1}{K}\right)$

Potassium in shoot (percent) $= 0.1576 + 0.5836$ (percent potassium in root)

where: $N =$ parts per million of nitrogen in the nutrient solution
$P =$ parts per million of phosphorus in the nutrient solution
$K =$ parts per million of potassium in the nutrient solution.

The analyses of variance describing these equations indicate a large difference in the degree of control over composition exerted by the supply of the three nutrient elements. This difference is pointed out by the variation accounted for in each of the sets of equations after reducing from about the mean. The nitrogen equations (set E) accounted for 74 to 86 percent, the potassium equation (set F) 50 to 60 percent, and the phosphorus equation (set G) for only 35 to 40 percent of the variation.

When the nitrogen composition equations are solved and the results plotted for various supply levels, nitrogen concentration in the tissue is shown to increase with an increase in nitrogen supply (Fig. 3a). However, several individual treatments deviated noticeably from the described trend. Seedlings from two treatments, low in potassium supply and high in nitrogen supply, contained considerably more nitrogen in the shoots than the mean indicated. Nitrogen accumulation in potassium-deficient tissue was previously observed by Wall (1940). Two other treatments, adequately supplied with nitrogen but with less than adequate phosphorus, had much less nitrogen in the shoot than the mean indicated. This could be the result of a lack of the phosphorus-related energy necessary for active absorption of nitrogen.

In all observations, greater percentages of nitrogen were found in the shoots than in the roots (Fig. 3b). Nitrogen values for individual treatments ranged from 0.94 to 4.19 percent in the shoots and from 0.57 to 3.19 percent in the roots. In situations in which nitrogen was limiting to growth either from scarcity or overabundance, the distribution of nitrogen was more uniform throughout the plant. These findings support a hypothesis by Specht (1960) that plants possess a mechanism which controls the variability of element concentration in tissues. Specht suggested further that the degree of variability of an element in tissue is related to the concentration of that element in the tissue and therefore is related to the supply source.

The data indicate that as the supply of phosphorus increases the concentration of phosphorus in the tissue increases, but there is a leveling off of the phosphorus concentration (Fig. 4a). The regression trends indicate that the roots contain higher percentages of phosphorus than the shoots at all levels of supply. Actually, seedlings in eight treatments had a larger percentage in the shoot. Seven of these treatments had high nitrogen supplies (125 ppm or more) and all had sufficient phosphorus. On the other hand, seedlings inadequately supplied with nitrogen exhibited the highest percentage of phosphorus in the roots. When phosphorus in the shoots is plotted as a function of phosphorus in the roots, the nitrogen supply becomes a variable significant at about the 7 percent level (Fig. 4b). The phosphorus percentage values for individual treatments ranged from 0.026

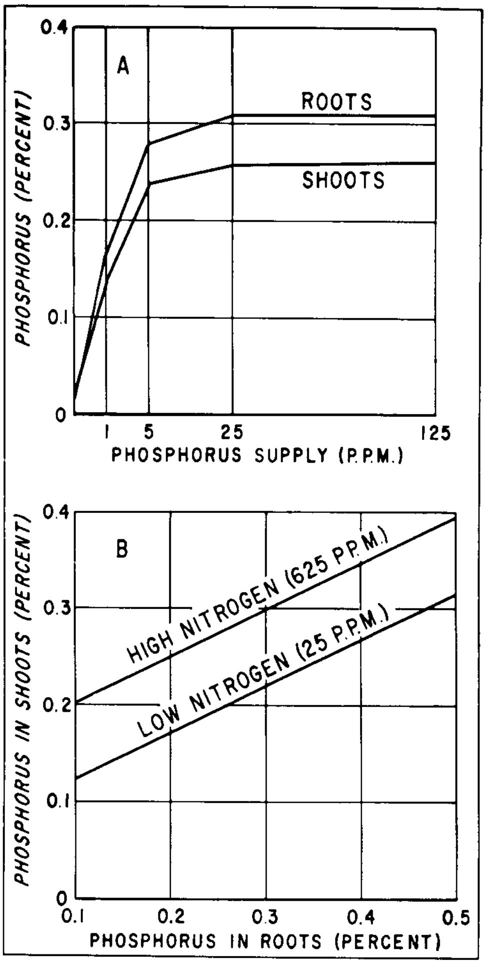

FIGURE 4. *A. Phosphorus percentage in shoots and roots in relation to phosphorus supply. B. Phosphorus percentage in shoots in relation to phosphorus in roots and nitrogen supply.*

to 0.430 percent in the shoots and from 0.026 to 0.560 percent in the roots.

Seedlings in 23 treatments contained larger potassium percentages in the root than in the shoot. When the potassium concentration-potassium supply equations for shoots and roots are plotted, the trend lines cross in the region of high potassium supply (Fig. 5a). Figure 5b illustrates the trend established for concentration of potassium in the shoot in relation to concentration in the root and shows that shoot

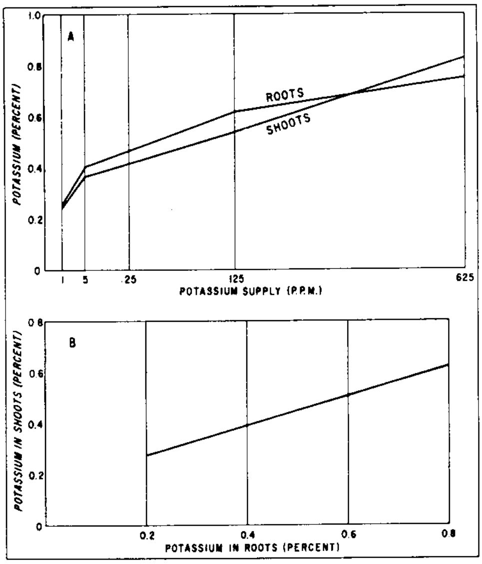

FIGURE 5. *A. Potassium in shoots and in roots in relation to potassium supply. B. Potassium percentage in shoots in relation to potassium in roots.*

and root concentration of potassium is approximately equal at 0.4 percent. The percentage potassium concentration for individual treatments ranged from 0.087 to 0.929 percent in the shoots and from 0.087 to 0.890 percent in the root.

The final phase of study relates seedling yield to chemical composition of the shoots. The equation for this analysis is as follows:

$$\text{H. Yield (grams dry weight)} = 0.6669 + 0.2159 \text{ (percent nitrogen)} + 3.260 \text{ (percent potassium)} - 2.729 \text{ percent potassium)}^2$$

This equation accounts for 60 percent of the variation about the mean and indicates that the concentration of potassium and nitrogen in the shoots can be correlated with yield. However, the phosphorus content of the shoot was not significantly correlated with yield and the indicated nitrogen relation is somewhat at variance with data presented in Figures 2 and 3. The discrepancy is caused by failure of the nitrogen in the shoot-yield curve to show a maximum point within the range of data.

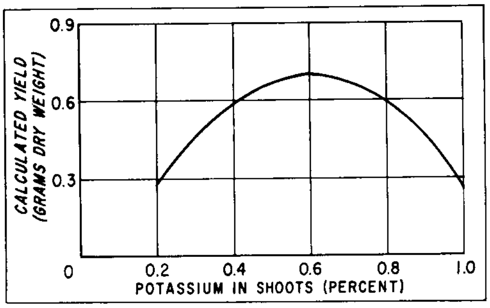

FIGURE 6. *Seedling yield in dry weight in relation to potassium in shoots.*

This is not too surprising because the equations fail to account for considerable portions of the variation and the possibility of curvilinear interactions was not examined in great detail.

Highest yields were obtained when the potassium concentration in the shoots ranged 0.5 to 0.7 percent (Fig. 6).

Conclusions

The following conclusions were drawn from study data:

1. The relative importance of nitrogen, phosphorus, and potassium varies in the production of different types of yield. Nitrogen was most influential as regards fresh weight yield, whereas potassium exerted the most influence on dry weight and seedling elongation.
2. Optimum growth response to nitrogen and potassium was indicated by regression analyses at supply concentrations greater than 125 but less than 625 parts per million. Growth response to phosphorus was still increasing at 125 parts per million, the maximum concentration of phosphorus supplied.
3. Variance in shoot-root ratios was largely a function of nitrogen supply. As the supply of nitrogen was increased, a greater percentage of the total dry matter of the plant was found in the shoot.
4. The percentage of nitrogen, phosphorus, and potassium found in the shoot and root tissue increased with each additional increment of nutrient supply.

5. The ratio of nutrient percentages present in shoot and root varies with each element. In all treatments, higher nitrogen percentages were found in the shoots than in the roots. Generally, higher percentages of phosphorus were found in the roots than in the shoots. Likewise, root tissue generally contained higher percentages of potassium.

6. Satisfactory dry matter yields are predicted when the potassium content of the shoot ranges from 0.5 to 0.7 percent of dry weight and other elements are present in adequate amounts.

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