THE RELATION OF THE CONCENTRATION OF MACRONUTRIENTS IN THE SUBSTRATE AND IN THE FOLIAGE TO CELL WALL THICKNESS AND CELLULOSE CONCENTRATION IN THE XYLEM OF SLASH PINE (*Pinus elliotti*) [1]

E.Malavolta[2]
J.R.Sarruge[2]
H.P.Haag[2]
R.Vencovsky[3]
C.F.O.Santos[4]
O.Valsechi[5]
L.C.Scoton[6]
R.S.G.Coelho[6]

RESUMO

Foram conduzidos experimentos fatoriais cultivando *P. elliottii* em solução nutritiva destinados a estudar o efeito de macronutrientes na composição mineral, grossura da parede celular e concentração da celulose no xilema.

Os sintomas de deficiência dos elementos foram descritos e registrados.

O crescimento foi estimulado por N, P, K, Ca e Mg, sendo a maior resposta devida ao N.

Sòmente o enxôfre aumentou significativamente o teor de celulose.

A grossura da parede celular diminuiu com altos níveis de N, Ca, Mg e S; os primeiros diminuiram o comprimento das fibras.

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[2]Departamento de Química.
[3]Departamento de Genética.
[4]Departamento de Botânica.
[5]Departamento de Tecnologia Rural.
[6]Bolsistas da FAPESP.

INTRODUCTION

WHITE & LEAF published in 1957 a bibliographical listing of seven hundred abstracts dealing with the subject of forest fertilization. LEYTON (1958a), when discussing growth and mineral nutrition of forest trees, was restricted largely to the consideration of leaf analysis as a guide to the diagnosis of mineral deficiences, as well as to the interpretation of field observations; particular attention was given to the diagnostic value of ratios of concentration of elements. Broader aspects of the mineral requirements of forest plants were taken up by LEYTON (1958b). In a review of the mineral nutrition of trees, REUTHER et al (1958) focused exclusively fruit bearing plants. KRAMER & KOZLOWSKI (1960) presented an excellent mis-au-point of several angles of the problèms of mineral nutrition and salt absorption by trees; foliar diagnosis as a method of evaluating the fertility of forest soils was revie - wed; its values and limitations were pointed out; this subject was again treated briefly by KRAMER (1960). Soil and fertilizer requirements of *P.radiata* were discussed in detail by RAUPAC (1967).

In the available literature little could be found with regards to systematic work on the nutrition of a given species; most of the papers on this subject had, on the other hand, well defined aims. It was not possible to find a single one designed to encompass several aspects of the mineral nutrition of forest trees as symptoms of deficiency, growth, chemical composition and wood characteristics; the need for this type of work was raised by FOWELLS & KRAUSS (1959), by HACSKAYLO (1960) and by others.

Due to the great economic importance to Brazil and other countries presented by *P.elliottii* it was decided to undertake some basic studies of its nutrition with the following goals: "to find out the effects of nitrogen, phosphorus, potassium, calcium, magnesium and sulfur on growth, aspect, foliage composition, cellulose concentration, thickness of cell wall and length of fibers". Results obtained in two separate set of experiments are summarized in the next sections.

MATERIALS AND METHODS

Nitrogen, Phosphorus and Potassium Experiment.

Plant material - one-year-old seedlings of slash pine were used. They were previously grown in small pots filled

with soil at the Estação Experimental de Tupi (Tupi Experimental Station) at Piracicaba, São Paulo, Brazil. The composition of a sample of this starting material is given in TABLE 2-1.

TABLE 1 - Macronutrients concentration in the various parts of the slash pine seedlings at the start of the experiment.

PART	Nº	PERCENT DRY WEIGTH					
		N	P	K	Ca	Mg	S
ROOTS	1	1.12	0.25	0.60	0.26	0.13	0.14
	2	1.12	0.26	0.56	0.28	0.14	0.13
	3	1.26	0.27	0.64	0.24	0.15	0.15
	4	1.23	0.27	0.60	0.28	0.12	0.15
STEM	1	1.68	0.26	0.68	0.22	0.17	0.14
	2	1.54	0.28	0.60	0.20	0.16	0.14
	3	1.54	0.31	0.68	0.22	0.16	0.16
	4	1.54	0.28	0.68	0.22	0.15	0.17
NEEDLES	1	2.10	0.20	0.76	0.46	0.15	0.16
	2	1.99	0.19	0.76	0.38	0.14	0.15
	3	2.10	0.21	0.80	0.50	0.14	0.15
	4	2.14	0.23	0.84	0.48	0.14	0.16

Treatments - A 3 x 3 x 3 factorial with three levels each of nitrogen, phosphorus and potassium was used with two replicates. The concentration of these macronutrients in parts per million, were the following:

	N	P	K
Level 1	25	5	25
Level 2	50	10	50
Level 3	100	20	100

Other nutrients were supplied at constant levels (in ppm): Ca 100; Mg 50; S 50; B 0.5; Cu 0.05; Fe 5.0; Mo 0.01; Zn 0.05.

Six months after the treatments had begun the level one for each elements was dropped to zero in order to permit the manifestation of the symptoms of deficiency.

Experimental Tecnique - For each treatment two clay pots internally covered with a layer of inert, water proof black paint were filled with 7 kg of pure quartz sand; the average diameter of the quartz particles was such that they pass sieve nº 10, being retained by nº 16; these particles are capable of retaining 19.66 per cent of moisture by capillarity. Two retangular pieces of plywood on top of the pot prevented excessive evaporation. Through openings in their bottoms both pots communicated with a 4 liter jar of nutrient solution located at a lower level. Compressed air entering an external side arm went into the nutrient solution forcing it to raise through an inner plastic tubing and hence into the clay pots. Turning off the compressed air allowed the solution to flow by gravity into the glass jar. This operation was repeated three times a day: 7 a.m., noon, and 5 p.m.; each time, the nutrient solution was allowed to be in contact with the root system during a period of 15 minutes. Every other week the solution was discarded, distilled water being put in the jar;compressed air was admitted to force it into the pots; when brought back to the jar it brought some of the salts which were adhering to the sand particles; these operations were repeated with fresh portions of water until a test made in an aliquot showed only a trace of chloride. Later in the course of the experiment a system of timer and solenoid valves was introduced in order to make the supplying of nutrient solution to the plants fully automatic. Details of the experiment set up were given by MALAVOLTA et al. (1963).

Fluorescent and incandescent lights provided 15 hours of illumination per day extending from 6:00 a.m. to 9:00 p.m.

Measurements and observation - Every other week in the course of the experiment the following measurements were taken: height and diameter of stem near the basal portion. Plants examined daily to check appearance and course of symptoms of deficiency. At the end of the experiment (18 months after starting) all plants were removed from the crocks and their roots washed free of sand. Measurements were made of height, diameter of stem and fresh weight. Number of branches were registered. The material was then separated into several parts: roots, stem, branches, primary needles and secondary needles. A basal portion of the stem was cut out to be used in determination of length of fibers and thickness of cell wall.

A small part of the root system was saved for the examination of incidence of mycorrhizal formations. All plant parts were then dried at 70ºC in an oven with forced ventilation. The remaining material was ground and stored in plastic capped vials. A sample of stem was taken, the bark removed, in order to serve for technological analysis.

Methods of Mineral Analysis - The samples of the seedlings used in the experiment were subject to chemical analysis by conventional methods.

Methods of Technological Analysis - Methods recomended by Technical Association of the Pulp and Paper Industry, N. York, N.Y., U.S.A., were used throughout.

Measurements of length of fibers and of thickness of cell wall - To serve as references, samples of the one year old seedlings used at start of the experiment were measured. The material was collected from two parts of the stem: lower and middle region; from each part a block 1 cm long was used for maceration in glacial acetic acid and hydrogen peroxide (100 volumes). After complete maceration, five samples of the material from each plant were distributed in five slides and stained with safranine and mounted in balsam. Five elements (fiber-tracheids and tracheids) in each slide were then measured. The same technique was used in the case of the sample collected at the end of the experiment.

Calcium, Magnesium and Sulphur Experiment

Plant material - Six month old seedlings, obtained as mentioned in page 3 were used; the trial, however, was discontinued due to lack of uniformity of the plant material. In another trial one year old seedlings were employed; they had previously been grown in small pots containing a mixture of soil and manure. After the experiment was started, lack of uniformity within duplicates was evident; the trial nevertheless was carried to completion. The composition of the starting material is given in TABLE 2.

Treatments - A 3 x 3 x 3 factorial with three levels each of Ca, Mg and S, was used with two replicates. The initial concentration of these elements in parts per million were the following:

Level	Ca	Mg	S
1	12.5	6	6
2	25	12.5	12.5
3	50	25	25

Other nutrients were supplied at constant levels (in ppm): N-50, P-10, K-50, Cu-0.02, Fe-5.0, Mn-0.5, Mn-0.5, Mo-0.01, Zn-0.05. Three months after the experiment was started the level 1 in each treatment was dropped to zero.

TABLE 2 - Initial characteristics of the one year seedlings used.

Characteristic	Roots	Stem	Needles
Dry weight, gm(*)	1.80-2.50	1.10-1.50	1.95-2.10
N per cent(**)	1.22	1.70	2.13
P	.28	.29	.20
K	.60	.66	.81
Ca	.28	.22	.45
Mg	113	.13	.16
S	117	.15	.16
Cellulose	--	40.0	--

(*) range of variation among 20 individual seedlings.

(**) average of 4 determinations, each one consisting of 5 seedlings.

Experimental technique - See page 298. Illumination was discontinued 6 months after the experiment started according to suggestion presented by Dr. Carl E.Ostron who thought the extra lighting unnecessary. Nutrient solution was supplied twice a day only.

Measurements and observations - Every other week in the course of the experiment the following measurements were taken: height and diameter of stem near the basal portion. Plants were examined daily to check appearance and course of symptoms of deficiency. At the end of the experiment (12 months after starting) all plants were removed from the crocks and their roots washed free of sand. Measurements were made of height, diameter of stem and fresh weight. Number of branches were registered. The material was then separated into several parts: roots, stem, branches, primary needles and secondary needles. A basal portion of the stem was cut out to be used in the determination of lenght of fibers and thickness of cell wall. A small part of the root system was saved for examination of incidence of mycorrhizal formations. All plant parts were then dried at 70ºC in an oven with forced ventilation. The remaining was ground and stored in plastic capped vials. A sample of stem was taken, the bark removed in order to serve for technological analysis.

Methods of mineral analysis - See page 299

Methods of technological analysis - See page 299

Measurements of length of fibers and of thickness of cell wall - The same technique described previously (see page 299) was followed. An effort was made, however, to sample only wood produced after the treatments started.

RESULTS AND DISCUSSION

Nitrogen, Phosphorus and Potassium experiment

Initial characteristics of the seedlings

Composition - In order to determine the effects of the several treatments on the composition of the seedlings at end of the experiment, both chemical and technological determinations were made in the initial material. Twenty plants were joined in 4 groups of 5 seedlings each. After obtaining the fresh and dry weights of roots, stems and needles, the analysis were made in each one of these parts.

The dry weights of the parts subject to chemical analysis varied between the following limits:

roots..... 1.76 - 2.10 gm

stem ..... 0.93 - 1.30

needles ..... 1.75 - 1.93

TABLE 2 summarizes the results of the chemical determinations of macronutrients. These results show that the plant material was sufficiently uniform in composition thus allowing for these data to be taken as initial reference. Among the three parts analysed, higher concentrations of N, K and Ca were found in the needles. The macronutrients concentration in this part of the plant is of the same order as found in other species. These results are in close agreement with those obtained by MELLO et al (1960) insofar nitrogen, calcium and magnesium are concerned; levels of phosphorus and potassium in the two year old plants analysed by those investigators were however, lower than those given in TABLE 2; since the plants analysed by MELLO et al (1960) were grown in the same soil, this difference is perhaps due to a reduced uptake or dilution of these two elements in the older plants.

Technological analysis were carried out in two samples, each one made out of 10 stems, bark removed. The results are given in TABLE 3.

TABLE 3.1- Technological composition of samples of the seedlings of slash pine used in the experiment.

Characteristic	Sample nº 1	2
Moisture, percent	66.3	65.9
Dry matter, percent	33.6	34.0
Cellulose, Cross & Bevan, percent	39.5	39.8
Cellulose, % Cross & Bevan	68.3	67.3
Lignin, percent	31.9	31.7
Pentosan, percent	16.5	16.9
Ashes, percent	1.7	1.7
Extract by alcolhos-benzene, percent	12.1	12.1
Extract by 1% NaOH, percent	39.4	39.6
Extract by cold water, percent	16.1	14.5
Extract by hot water, percent	21.8	19.2

Length of fibers and thickness of cell wall - The average results of the measurements of length of fibers and thickness of cell wall are given in TABLE 3. Statistical analysis has shown that samples were uniform; the experimental error for the measurements of length fibers was low whereas that corresponding to cell wall thickness was rather high. Length of fibers was statistically different as a function of the part of stem taken for measuring.

Symptoms of deficiency - As already mentioned, on 3/2/1963 the level 1 for N, P and K was dropped to zero ppm in order to permit symptoms of deficiency to develop. No abnormality in the foliage had been observed previously. Apparently most of the plants receiving N_1 and 1, were in the threshold region between hidden hunger and true deficiency. This assumption is made because shortly after omitting N and P from the nutrient solutions clear cut symptoms of deficiency began to show up. This was not the case insofar as K is concerned; abnormalities in the foliage showed up only after that element was omitted from the nutrient solution for several months. The symptoms observed in this experiment confirm a general pattern observed in other species of pine (HACSKAYLO, 1960; ADDOMS , 1937; HOBBS, 1944).

Nitrogen deficiency - Initial symptoms of lack of nitrogen were first observed on 4/7/1963. The following description deals only with those abnormalities found in plants receiving no nitrogen in the presence of both P and K.

The minus N plants were usually stunted. The primary older needles gradually lost their green color which was replaced by a yellowish one. Later on, paling also occurred in young needles thus giving the entire plant a yellowgreen color. Next, the lower needles developed a brownish color in their tips, which looked burned; as a last stage of the deficiency the entire showed a distinct reddish color; when this took place in the older needles, the young ones began to show a reddish discoloration on their tips. The affected needles detached from the plant after wilting.

Phosphorus deficiency - Symptoms of phosphorus deficiency were first observed on 4/12/1963 as a slight yellowing in the tips of older primary needles; this was soon followed by the appearance of a brown color in the same region, the remaining part of the needle keeping a dark green color. These symptoms appeared in the secondary needles at the botton of the plant one month later. The final stage of phosphorus defi

TABLE 3.2 - Measurements of length of fibers and cell wall thickness in seedlings used as starting material.

ELEMENT	Sample Nº	LENGTH (μ)		THICKNESS OF CELL WALL (μ)	
		Plant 1	Plant 2	Plant 1	Plant 2
		LOWER PART OF THE STEM			
Tracheids	1	745	879	1.96	3.00
	2	622	679	2.72	2.06
	3	822	859	2.40	2.78
	4	757	757	1.68	2.59
	5	807	819	2.40	2.72
Fiber-tracheids	1	920	833	2.14	2.32
	2	885	938	2.11	2.32
	3	964	847	2.27	2.17
	4	892	786	1.90	1.97
	5	1.040	890	2.06	2.06
		MIDDLE PART OF THE STEM			
Tracheids	1	689	1,011	2.72	3.00
	2	829	982	3.21	1.65
	3	947	945	2.67	2.78
	4	1,093	1,263	2.11	2.59
	5	732	939	2.16	2.72
	1	1,173	1,027	3.18	2.85
	2	1,119	1,187	2.85	2.50
	3	1,125	987	2.40	2.11
	4	1,037	1,230	2.57	2.91
	5	1,036	1,186	2.83	2.70

ciency was the establishment of a characteristic brown color taking the entire needle.

Potassium deficiency - Symptoms of potassium deficiency were first observed on 11/4/1963. They were apparent only in the older, primary needles which developed light yellow brownish tips, which later became necrotic.

Growth Data - Table 3.3 gives the main effects of the levels of element an several plant characteristics.

TABLE 3.3 - Main effects of treatment levels on growth characteristics of slash pine.

ELEMENT	Levels	Height (cm)	Diameter of stem (mm)	CHARACTERISTIC: Dry weights: Roots	Stem	Branches	1 ary needles	2 ary needles	Total	Shoot/ root
Nitrogen	0	65	15	43	21.3	1.53	24.6	8.6	98.9	1.4
	1	83	22	54	40.1	3.37	56.5	11.6	174.4	2.3
	2	93	26	77	65.3	5.28	70.0	22.7	240.7	2.1
Phosphorus	0	70	18	45	30.4	2.44	38.6	11.1	127.9	1.8
	1	85	22	60	54.9	3.36	58.7	15.8	193.5	2.1
	2	86	23	68	50.3	4.38	53.8	16.0	192.5	1.9
Potassium	0	72	21	51	43.7	3.00	49.7	13.0	160.6	2.1
	1	84	21	59	47.3	3.62	54.6	14.6	181.0	2.0
	2	79	20	64	44.6	3.58	44.8	15.2	172.3	1.7
L.s.d.	5%	6.8	3.6	5.6	8.0	1.53	16.8	8.1	22.2	0.51
	1%	8.7	4.1	7.2	10.2	1.96	21.5	10.5	28.4	0.65

Raising the level of nitrogen in the nutrient solution caused a corresponding increase in all growth characteristics studied, with the possible exception of the shoot/root ratio. It seems clear, therefore, that the curve describing the effect of nitrogen on growth did not reach a plateau under the experimental conditions.

The following regression equations describe, respectively, the effect of level of nitrogen in substrate (x = 0, 1, 2) on dry matter produced and on height; in both cases only the linear component was significant, at the 0.1% level of probability.

$$Y_w = 100.43 + 70.91x$$

$$Y_h = 66.11 + 19.17x$$

The shoot/root ratio did increase when the concentration of N varied from 0 to 50 ppm; supplying this element at 100 ppm brougth a slight decrease in said ratio, which might not be statistically significant. The favourable effect of N on shoot growth is explained by several alternative or complementary hypothesis; according to one, increasing the supply of nitrogen causes a rise in the level of indolacetic acid in the plant tissues; growth of shoots is promoted whereas that of the root system is less stimulated since its auxin requirement is lower.

The effect of phosphorus supply on growth was not so marked as that of nitrogen. The intermediate level of this element in the nutrient solution was apparently, sufficient for maximum development.

The effect of P level on dry matter production and on height is defined by two regression equations given below;both linear and quadratic components when significant at the 0.1% level.

$$Y_w = 128.07 + 98.88x - 33.30\ x^2$$

$$Y_h = 70.08 + 22.02x - 7.08\ x^2$$

Shoot/root ratio was affected by the level of phosphorus the same way described for nitrogen. This is in agreement with the observations of MC GEE (1963) with slash pine and of INGESTAD (1962) with several species.

In a general way, the influence of potassium on growth

judging through its effect in importance characteristics such as height and total dry weight, was even less marked than that of phosphorus. This could be explained assuming a low demand for that element by slash pine. Alternatively it is possible that potassium reserves in the seedlings were sufficient to meet the requirements for growth during the limited experimental period.

K has a significant effect only at the height of the plants, as described by the following equation:

$$Y_h = 72.13 + 16.92\ x - 5.25\ x^2$$

the linear component proved to be significant at the 1% point.

Clearly, raising the level of K in the substrate, consistently decreased the shoot/root ratio. Putting it another way, root development was helped when the potassium supply increased. This is a fairly common situation found in many plants. In cassava plants grown under low potassium concentration in the medium MALAVOLTA et al (1955) observed that the shoot/root ratio, was much larger than that found in normal plants; this finding was explained on the assumption that lack of potassium prevented leaf carbohydrate from being translocated to the root system which therefore could not grow at an adequate rate.

TABLE 3.4 presents the several cases of interactions which were found and their level of significance; it is clear that the most frequent one was the NP. The only variable not showing this effect was the diameter of stem which seems to respond directly to the level of a given element.

TABLE 3.4 - Interactions among elements as revaled by the analysis of variance.

PLANT CHARACTERISTIC	INTERACTION	
H e i g h t	NP**	PK**
Dry weight		
Roots	NP***	NK*** PK***
Stems	NP**	
Branches	NP**	PK**
Needles	NP*	
Total	NP*	

* Significant 5% ; ** Significant 1% ; *** Significant 0.1%

Chemical Data

Mineral composition - The main effects of treatments on the macronutrients content of the various plant parts are summarized in TABLE 3.5 and in TABLE 3.6.

Comparing the initial composition of the seedlings with that at termination of the experiment a considerable dilution of both N and P is apparent. This was not the case insofar as K is concerned; the K content of roots and stem from plants kept as level 1 or 2 of the element in the substrate is nearly the same as found in the original seedlings; K percent in the needles - corresponding to plants receiving the element in the nutrient solution is however much higher; to this increase in K content there was not a parallel rise is growth (see Growth Data); this suggests therefore a case of luxury consumption.

When the level of a given element in the substrate was raised, its content in all parts of the plants was also increased. Figure 3.1 shows the close relationship between level of element in substrate and its content in the needles. Some interactions among elements may be mentioned. The well known depressive effect of K on Mg uptake is evident in the composition of the root system and of primary and secondary needles. The influence of the K status of the plant on its N content which was described by GOOR (1962) is not apparent. It seems, on the other hand, that whenever the N content increased the K percent in the dry matter decreased; this suggests that at least in the case of the composition of roots and needles there is a negative correlation between those two elements. A similar situation was found by INGESTAD (1962). TABLE 3.7 shows how the level of certain elements in the substrate has affected the content of other in the foliage; only the statistically significant results are given.

In the case of slash pine, as well as in that of other species, a visual identification of deficiencies in not always easy. Chemical analysis is, therefore, a valuable help for diagnostic purposes. A decision should be made as to whish kind of needle reflects better the nutritional status of the entire plant with respect to each one of the element under study. A simple approach to solve this question would be to substract the content of the element in the needle corresponding to level zero from that corresponding to the maximum level of supply; TABLE 3.8 gives the results obtained this way. I is clear that both types of needles supply equally good information.

TABLE 3.5 - Main effects of treatment levels on the macro-nutrients content of roots and stems.

ELEMENT	Level	PER CENT, DRY WEIGHT					
		N	P	K	Ca	Mg	S
		R O O T S					
Nitrogen	0	0.31	0.16	0.61	0.26	0.15	0.82
	1	0.54	0.11	0.49	0.36	0.16	0.87
	2	0.72	0.09	0.42	0.37	0.16	0.85
Phosphorus	0	0.48	0.06	0.59	0.35	0.16	0.82
	1	0.53	0.12	0.50	0.30	0.14	0.83
	2	0.56	0.18	0.43	0.34	0.16	0.85
Potassium	0	0.52	0.10	0.31	0.33	0.16	0.83
	1	0.55	0.14	0.57	0.37	0.17	0.86
	2	0.53	0.12	0.64	0.29	0.14	0.83
L.s.d.	5%	0.05	0.02	0.05	0.05	0.02	0.11
	1%	0.06	0.02	0.06	0.06	0.03	0.14
		S T E M S					
Nitrogen	0	0.22	0.05	0.37	0.35	0.15	0.55
	1	0.34	0.05	0.42	0.23	0.15	0.58
	2	0.53	0.05	0.37	0.26	0.16	0.61
Phosphorus	0	0.29	0.02	0.42	0.27	0.15	0.58
	1	0.41	0.05	0.36	0.28	0.15	0.58
	2	0.38	0.07	0.39	0.28	0.17	0.58
Potassium	0	0.39	0.05	0.25	0.26	0.16	0.61
	1	0.35	0.05	0.16	0.27	0.14	0.52
	2	0.34	0.05	0.47	0.30	0.15	0.61
L.s.d.	5%	0.04	0.01	0.05	0.04	0.01	0.07
	1%	0.05	0.01	0.07	0.05	0.01	0.08

TABLE 3.6 - Main effects of treatment levels on the macronutrient composition of needles.

ELEMENT	Level	PER CENT, DRY WEIGHT					
		N	P	K	Ca	Mg	S
		1 ARY NEEDLES					
Nitrogen	0	0.50	0.13	0.85	0.46	0.23	0.18
	1	0.85	0.09	0.74	0.36	0.18	0.16
	2	1.24	0.08	0.77	0.33	0.18	0.16
Phosphorus	0	0.90	0.04	0.85	0.36	0.18	0.18
	1	0.78	0.10	0.77	0.40	0.20	0.16
	2	0.92	0.16	0.70	0.39	0.20	0.18
Potassium	0	0.90	0.09	0.37	0.39	0.22	0.19
	1	0,90	0.10	0.94	0.40	0.19	0.16
	2	0.80	0.11	1.04	0.35	0.17	0.16
		2 ARY NEEDLES					
Nitrogen	0	0.47	0.14	1.10	0.55	0.26	0.13
	1	1.12	0.09	0.78	0.44	0.21	0.15
	2	1.16	0.07	0.72	0.42	0.22	0.12
Phosphorus	0	1.05	0.04	1.06	0.42	0.21	0.12
	1	0.97	0.09	0.78	0.48	0.25	0.13
	2	0.92	0.16	0.75	0.49	0.25	0.14
Potassium	0	1.05	0.08	0.47	0.47	0.27	0.12
	1	0.98	0.10	1.06	0.50	0.24	0.14
	2	0.92	0.11	1.06	0.41	0.21	0.14
L.s.d.	5%	0.09	0.02	0.10	0.04	0.02	0.01
	1%	0.12	0.02	0.12	0.05	0.02	0.01

TABLE 3.7 - Effect of level of element in the substrate on the content of other elements in the needles.

Element in substrate	Element in needles	"Theta" linear	Value regression quadratic	Effect
K	S	6.52***	N.S.	Negative
P	S	4.60***	N.S.	Positive
N	S	6.18***	3.78***	Negative
N	Ca	6.24***	2.32*	Negative
N	K	4.47***	2.17*	Negative
P	K	4.89***	N.S.	Negative
P	Ca	2.72*	N.S.	Positive
P	Mg	2.69*	N.S.	Positive
N	Mg	3.73***	2.48*	Negative
K	Mg	2.56*	N.S.	Negative
N	P	10.65	4.10***	Negative
K	P	4.74	N.S.	Positive

TABLE 3.8 - Differences between the content of a given element in the two types of needles considering the concentration found when it was supplied at two extreme levels in the nutrient solution.

ELEMENT	CONTENT AT LEVEL 2- CONTENT AT LEVEL 0	
	1 ary needles	2 ary needles
Nitrogen (N)	0.78	0.89
Phosphorus (P)	0.12	0.12
Potassium (K)	0.67	0.63

A comparison can be made of levels of element content corresponding to deficiency considering data reported here and those found in the literature. This is shown TABLE 3.9 which deals only with needle composition. Despite the obvious limitations of such a kind of comparison, a fairly good agreement among the several data can be found.

TABLE 3.9 - Some levels of element content corresponding to deficiency.

PLANT SPECIES	PER CENT DRY WEIGTH			LITERATURE
	N	P	K	
Pinus elliottii	0.47-1.11	0.04-0.10	0.37-0.47	This paper
Pinus silvestris	0.70-1.60	0.06-0.09	0.30	Ingestad(1960)
Pinus strobus	0.70-1.30	-	-	Ingestad(1960)
		0.10	0.30	Ingestad(1960)
P. taeda				Ingestad(1960)
P. nigra			0.30	Ingestad(1960)

The relationship between the content of a given element in the needles and two of the growth characteristics is apparent through the examination of data shown in TABLE 3.10. In the case of N, when its content in the needles increased the same trend is observed in dry weight and height variations. This is shown in Figures 3.2 and 3.3. A different situation is found, however, with respect both to P and K. When the level of these elements in the substrate was increased from 1 to 2 the P and K content in the needles increased in each case. Growth, nevertheless, was not stimulated equally. It seems, therefore, that maximum growth under experimental conditions was attained with the treatment $N_2P_1K_1$ among the 27 used.

TABLE 3.10 - Relationship between needle composition and growth characteristics.

ELEMENT	Level suplied	Element % in needles	Dry weight (g)	Height (cm)
	0	0.47 - 0.50	100	64
N	1	0.85 - 1.11	174	83
	2	1.28 - 1.36	241	93
	0	0.04	128	70
P	1	0.09 - 0.10	197	85
	2	0.16	190	86
	0	0.37 - 0.47	160	63
K	1	0.94 - 0.09	179	84
	2	1.04 - 1.10	175	79

Cellulose concentration - TABLE 3.11 gives the relationship between level of element in the substrate and cellulose content in the dry matter of the stem. The statistical analysis was unable to detect significant effects of the elements on cellulose concentration. By comparing these results with those in TABLE 3.1 it is clear that a large increase in cellulose content took place due to the aging of the plants.

Length of fibers and thickness of cell wall - TABLE 3.12 gives the main effects of the levels of element in the substrate on lengh of fibers and on the thickness of the cell wall.

The statistical analysis of the data failed to show any significant effect, of the treatment of the length of both tracheids and fiber tracheids. It appears, however, that some trends do exist: heigher levels of N seem to decrease length of those elements; the intermediate concentrations of P and K

TABLE 3.11 - Relationship between level of element in the substrate and cellulose concentration in the stem.

ELEMENT	Level	Cellulose per cent
N	0	50.85
	1	54.44
	2	49.94
P	0	49.94
	1	50.79
	2	50.49
K	0	50.04
	1	51.21
	2	49.98

on the substrate, on the other hand, apparently caused a slight increase. ANOMYMOUS (1964), stated, when discussing work carried out with 12 and 16 year old loblolly pine, that tracheid length lowered as the result of nitrogen fertilization. HARTEY (1960) found no effect of the elements N, P, K, on the tracheid length of seedlings of *P.radiata*. In 5 year plants of loblolly pine grown in the field under a 3 x 3 x 3 NPK factorial experiment, LINNARTZ & THOMPSON (1964) have found, however, tracheid length was significantly increased by all levels of N and K in linear fashion. The absence of significant effect noted in the present experiments can be explained by several ways: too short experimental period; large individual variation, true characteristic of the species used.

TABLE 3.12 - Influence of level of nutrient on fiber elements and on the thickness of the cell wall.

ELEMENT	Level	Lenght (μ)	Thickness of wall(μ)
		T R A C H E I D S	
Nitrogen	0	1131.0	3.23
	1	1130.3	3.22
	2	1087.9	3.14
Phosphorus	0	1090.7	3.18
	1	1138.7	3.26
	2	1119.8	3.15
Potassium	0	1108.1	3.13
	1	1139.2	3.14
	2	1101.9	3.31
L.s.d.	5%	98.6	0.24
	1%	126.1	0.30
		FIBER TRACHEIDS	
Nitrogen	0	1243.0	5.04
	1	1229.2	5.02
	2	1158.1	4.46
Phosphorus	0	1171.5	5.15
	1	1258.8	4.70
	2	1204.4	4.70
Potassium	0	1200.1	5.70
	1	1215.4	4.28
	2	1215.2	4.58
L.s.d.	5%	118.1	1.06
	1%	151.0	1.36

With regards to the effect of treatments on the thi - ckness of cell wall, the following comments can be present. A definite trend exists showing that higher levels of N decrease cell wall thickness. Statistical analysis on the other hand, has shown that K affected tracheids differently from fiber tra cheids: while the cell wall thickness of the first was larger when K level was raised, that of the fiber tracheids decreased. This puzzling situation may be due to experimental error, howe ver, since the observed variations only barely fit the level of statistical signification.

Calcium, Magnesium and Sulfur Experiment

Initial composition of the seedlings - In order to de termine the effects of the several treatments on the composi - tion of the seedlings at end of the experiment, both chemical and technological determinations were made in the initial mate rial. Twenty plants were joined in 4 group of 5 seedlings each. After obtaining the fresh and dry weights of roots,stems and needles the analysis were made in each one of these parts.

TABLE 2-2 summarizes the results of the chemical de- terminations of macronutrients. These results show that the plant material was sufficiently uniform in composition thus allowing for these data to be taken as initial reference. Among the three parts analysed, higher concentrations of Ca,Mg and S were found usually in the needles. The macronutrients concentration in this part of the plant is of the same order as found in other species. These results are in close agree - ment with those obtained by MELLO et al (1960) insofar nitro - gen, calcium and magnesium are concerned; levels of phospho - rus and potassium in the two year old plants analysed by those investigators were however, lower than those given in TABLE 2- 2; since the plants analysed by MELLO et al (1960) were grown in the same soil, this difference is perhaps due to a reduced uptake of dilution of these two elements in the older plants.

It should be mentioned that the several characteris - tics of the seedlings used in this experiment are quite simi - lar to those corresponding to the plants used in the NPK facto rial. The same is true insofar fiber characteristics are con- cerned.

Symptoms of Deficiency - As mentioned in (Treatments, page 299), three months after starting the experiment, the le- vel 1 in each treatment was dropped to zero in order to permit

the symptoms of deficiency to show up and to allow for a better definition of response.

The symptoms which appeared afterwards are in agreement with a general pattern observed with different species in other works (HACSKAYLO, 1960; GOOR, 1963; WALKER & BEACHER, 1963).

Water colors were painted to register said symptoms.

Calcium deficiency - The very first indications of malnutrition were observed by the end of February, 1965. In the beginning the needles located in the tips of the branches kept their estremities green whereas the basal part was symptoms. In a more advanced stage the entire needle was yellowish green with red tips indicating initial necrosis. The needles lost their rigidity and bent downwards. The final stage of the deficiency was characterized by the death of terminal and a pical buds, drying of the needles which fell off.

Magnesium deficiency - The symptoms appeared one month after those corresponding to calcium deficiency; both primary and secondary needles showed a yellowish green discoloration, this being more intense in the upper third of the plant. Later on the needles showed a reddish brown colour extending from tip to their middle; these symptoms were more severe near the extremity of the branches.

Sulfur deficiency - These symptoms first showed up approximately at the same time those of calcium deficiency were registered. They were not so clear cut. Young needles were usually pale green in colour; the older ones showed indications of drying. A pink color was observed in the needle sheats, especially in those corresponding to the older ones.

Growth Data

TABLE 3.13 gives the main effects of the levels of elements on several growth parameters. The results of the statistical analysis are given in TABLES 3.14, 3.15 and 3.16.

TABLE 3.13 - Main effects of treatment levels on growth parameters.

ELEMENT	Level	height (cm)	diameter of stem (mm)	total dry weight (gm)
Ca	0	76.3	18.7	147.7
	1	100.9	20.9	180.0
	2	92.9	22.9	189.6
Mg	0	85.6	20.1	155.2
	1	96.6	20.7	186.8
	2	88.0	21.7	175.4
S	0	80.8	20.1	151.2
	1	104.6	21.7	201.0
	2	84.7	20.8	165.1
L.s.d.	5%	15.7	3.3	40.5
	1%	20.2	4.2	52.1

Height - The statistical analysis (TABLE 3.14) has shown: (a) the effect of Ca was significant at the 1 per cent level of probability; (b) no effect of Mg; (c) a significant effect, at the 1 per cent level due to S; (d) a significant interaction between Mg and S at the 5 per cent level. It was also found that the higher level of Ca in the substrate failed to give a response and that the effect of this element was quadratic mainly. The same was found to be true with respect to S. The lack of response to the higher rates of Ca could be due to a harmful effect of said elements, as suggested previously (MALAVOLTA et al, 1965). Working with younger seedlings of *P. silvestris*, INGESTAD (1962), in a short term experiment, verified that optimum growth was achieved with 40 ppm of Ca, 15 ppm of Mg and 20 ppm of S in the substrate, higher rates being toxic. The lack of absence to Mg level could be due either to lack of uniformity in the seedlings or to a high reserve, or both.

TABLE 3.14 - Analysis of variance for height data

Source of Variation	Degree of freedom	Sum of squares	Mean squares	F value
Ca	2	5,688.11	2,834.05	7.83 **
Mg	2	1,192.11	596.05	1.65
S	2	5,856.45	2,928.22	8.09 **
Ca x S	4	1,661.42	415.35	1.15
Ca x Mg	4	5,008.38	1,252.09	3.46 *
Ca x Mg x S	8	5,438.09	679.76	1.88
(Treatments)	(26)	(28,338.34)	(1,089.94)	(3.01) **
Residue	27	9,768.50	361.80	
Total	53	38,106.84		

TABLE 3.15 - Analysis of variance for diameter of stem data.

Source of Variation	Degree of freedom	Sum of squares	Mean of squares	F value
Ca	2	164,93	82.46	5.18 *
Mg	2	23.82	11.91	0.75
S	2	21.93	10.96	0.69
Ca x Mg	4	84.73	21.18	1.33
Ca x S	4	29.29	7.32	0.46
Mg x S	4	141.73	35.43	2.26
Ca x Mg x S	8	118.39	14.80	0.93
(Treatments)	(26)	(584.82)	(22.49)	(1.41)
Residue	27	430.00	15.93	
Total	53	1,014.82		

TABLE 3.16 - Analysis of variance for dry weight data.

Source of Variation	Degree of freedom	Sum of squares	Mean squares	F value
Ca	2	17,388.00	8,669.00	3.60 *
Mg	2	9,238.80	4.619.40	1.92
S	2	23,792.80	11,896.40	4.94 *
Ca x Mg	4	12,782.00	3,195.50	1.33
Ca x S	4	19,893.00	4,973.43	2.06
Mg x S	4	49,907.16	12,226.79	5.06 **
Ca x Mg x S	8	9,823.00	1,227.97	0.51
(Treatments)	(26)	(141,776.20)		2.26(*)
Residue	27	65,031.95		
Total	53	206,808.15		

The regression equations were the following, being Y = means and X = 0, 1, 2 that is, level of the element in the substrate.

for Ca $Y = 76.32 + 40.88X - 16.29X^2$

for S $Y = 80.87 + 45.63X - 21.84X^2$

Diameter of stem - The statistical analysis (TABLE 3.15) shows that only Ca, at the 5 per cent level of probability, had a significant effect. The linear component was significant at the 1 percent level.

The regression equation was found to be

$$Y = 18.73 + 2.10X$$

Total dry weight - As shown in TABLE 3.14 both Ca and S gave responses significant at the 5 per cent level; the interaction Mg x S was also significant at the 1 per cent level. Both the linear and quadratic components were significant at the 5 per cent level in describing the effect of Ca. The higher level of Ca in the nutrient solution failed to give a response. The influence of S was quadratic; in this case level 2 was significantly poorer, than level 1. In his work with seedlings of slash pine grown in nutrient solution, no effect of levels of Mg on growth was observed by DUMBROFF (1965).

The regression equation are:

for Ca $Y = 147.47 + 45.25X - 12.15X^2$

for S $Y = 151.34 + 92.63X - 42.84X^2$

TABLE 3.17 shows the significant interactions of elements on growth.

TABLE 3.17 - Interactions of elements on growth.

Characteristic	Interaction
Height	Mg x S *
Dry weight	Mg x S **

CHEMICAL DATA

Mineral composition - The main effects of the treatments on the macronutrient content of the various plant parts are given in TABLES 3.18 and 3.19.

The effects of the element in the substrate on the content of elements in the primary and secondary needles is summarized in TABLE 3.20 , which is self explanatory.

A comparison is made in TABLE 3.21 of the level of elements in needles corresponding to deficiency and maximum growth. The survey carried and by GOOR (1965).

TABLE 3.18 - Effects of treatment levels on the macronutrient content of roots and stems.

Element	Level	Per cent, dry matter					
		N	P	K	Ca	Mg	S
		Roots					
Ca	0	0.36	0.093	0.36	0.060	0.093	0.48
	1	0.34	0.088	0.37	0.078	0.066	0.38
	2	0.36	0.078	0.37	0.076	0.055	0.38
Mg	0	0.36	0.094	0.41	0.069	0.030	0.40
	1	0.38	0.081	0.32	0.067	0.090	0.46
	2	0.34	0.083	0.37	0.078	0.096	0.36
S	0	0.36	0.092	0.41	0.075	0.076	0.33
	1	0.35	0.075	0.36	0.073	0.066	0.43
	2	0.36	0.091	0.33	0.066	0.072	0.47
L.s.d.	5%	0.05	0.013	0.05	0.013	0.014	0.11
	1%	0.06	0.016	0.07	0.017	0.018	0.14
		Stem					
Ca	0	0.32	0.093	0.28	0.06	0.13	0.37
	1	0.27	0.087	0.28	0.13	0.09	0.31
	2	0.28	0.076	0.28	0.16	0.08	0.35
Mg	0	0.32	0.095	0.29	0.16	0.04	0.20
	1	0.27	0.080	0.26	0.09	0.12	0.31
	2	0.28	0.082	0.30	0.10	0.14	0.42
S	0	0.31	0.104	0.30	0.09	0.12	0.26
	1	0.27	0.071	0.26	0.12	0.09	0.39
	2	0.30	0.081	0.28	0.15	0.09	0.38
L.s.d.	5%	0.04	0.013	0.08	0.02	0.01	0.05
	1%	0.06	0.017	0.10	0.03	0.02	0.06

TABLE 3.19 - Effect of treatment levels on the macronutrient composition of needles.

Element	Level	Per cent, dry matter					
		N	P	K	Ca	Mg	S
		1 A R Y N E E D L E S					
Ca	0	0.82	0.069	0.46	0.18	0.13	0.13
	1	0.77	0.072	0.50	0.30	0.12	0.14
	2	0.76	0.072	0.55	0.42	0.14	0.13
Mg	0	0.80	0.075	0.50	0.38	0.05	0.14
	1	0.77	0.077	0.52	0.24	0.14	0.13
	2	0.78	0.062	0.49	0.28	0.20	0.12
S	0	0.80	0.076	0.49	0.34	0.13	0.10
	1	0.75	0.066	0.59	0.30	0.13	0.16
	2	0.80	0.072	0.44	0.25	0.13	0.14
L.s.d.	5%	0.07	0.008	0.08	0.03	0.017	0.014
	1%	0.09	0.010	0.11	0.04	0.022	0.018
		2 A R Y N E E D L E S					
Ca	0	0.86	0.063	0.42	0.14	0.14	0.15
	1	0.84	0.071	0.49	0.16	0.13	0.14
	2	0.89	0.071	0.50	0.31	0.15	0.14
Mg	0	0.90	0.071	0.50	0.27	0.06	0.16
	1	0.83	0.070	0.46	0.19	0.15	0.14
	2	0.86	0.066	0.47	0.16	0.20	0.15
S	0	0.89	0.073	0.53	0.22	0.14	0.13
	1	0.85	0.069	0.47	0.22	0.14	0.15
	2	0.85	0.064	0.43	0.18	0.13	0.16
L.s.d.	5%	0.11	0.008	0.08	0.10	0.02	0.022
	1%	0.14	0.010	0.11	0.13	0.03	0.028

TABLE 3.20 - Effects of elements in the substrate on the content of macronutrients in needles.

Elem. in substr.	Elem. in needle	"F" linear	"F" quadr.	Effect
		1 A r y N e e d l e s		
S	N	0.01 N.S.	4.73 *	Neg.
Mg	P	16.77 **	11.34 **	Neg.
Ca	K	7.36 **	0.00 N.S.	Pos.
S	K	2.74 N.S.	13.91 **	Pos.
Ca	Ca	392.96 **	1.79 N.S.	Pos.
Mg	Ca	45.25 **	65.10 **	Neg.
S	Ca	44.80 **	1.45 N.S.	Neg.
Mg	Mg	460.61 **	14.72 **	Pos.
Mg	S	4.22 *	0.04 N.S.	Neg.
S	S	39.07 **	50.56 **	Pos.
		2 A r y N e e d l e s		
Ca	P	5.37 *	1.57 N.S.	Pos.
S	P	7.05 *	0.11 N.S.	Neg.
Ca	K	5.87 *	0.82 N.S.	Pos.
S	K	9.26 **	0.13 N.S.	Neg.
Ca	Ca	26.55 **	3.02 N.S.	Pos.
Mg	Ca	6.87 *	0.78 N.S.	Neg.
Mg	Mg	384.56 **	24.33 **	Pos.
Mg	S	0.35 N.S.	10.51 **	Neg.
S	S	14.42 **	0.86 N.S.	Pos.

TABLE 3.21 - Levels of elements corresponding to deficiency and maximum growth.

SPECIES	Per cent dry Wt.						LITERATURE
	Ca		Mg		S		
	Def.	Max. Gro.	Def.	Max. Gro.	Def.	Max. Gro.	
Pinus sp.	0.23	0.33	0.15	?	?	?	INGESTAD (1962)
P.elliottii	0.14-0.18	0.31-0.42	0.05-0.06	0.20	0.10-0.13	0.14-0.16	This paper

in slash pines plantations of Southern Brazil has shown, with regards to Ca and Mg (no data are given for S) the following:

Element	Ave. content	Range of variation
Ca	0.25%	0.03 - 0.50%
Mg	0.10%	0.05 - 0.17%

this suggests that the supply of both Ca and Mg is below the optimum needed for maximum growth, which is not surprising since it is well known that said soils are usually low in those elements due to their acidity.

Cellulose concentration - TABLE 3.22 gives the relationship between level of element in the substrate and cellulose concentration, as well as the least significant differences. Statistical analysis has shown a linear effect of S supply, significant at the 1% point. The coefficient of variation was found to be 5.5%.

TABLE 3.22 - Main effects of treatments on cellulose concentration (%)

Level	Element		
	S	Mg	Ca
0	48.10	50.89	49.86
1	51.06	48.76	50.90
2	50.71	50.21	49.10
L.s.d.	5%	2.50	
	1%	2.91	

Length of fibers and thickness of cell wall

TABLE 3.23 shows how the level of Ca, Mg and S in the substrate affected the length of fibers and the thickness of cell wall. No significant effect was found in the case of length of fiber, the variation coefficient, 19,7%, was eather high. With respect to the thickness of cell wall, both S and Mg significantly reduced this variable.

TABLE 3.23 - Influence of level of nutrient on length of fiber and thickness cell wall.

Level	Elements		
	S	Mg	Ca
	Thickness of cell wall (u)		
0	1.76	1.71	1.61
1	1.47	1.50	1.60
2	1.57	1.57	1.56
L.s.d. 5%	0.06		
L.s.d. 1%	0.07		
	Length of fiber (u)		
0	1369.1	1406.7	1365.6
1	1461.0	1381.5	1380.5
2	1356.8	1398.7	1440.8
L.s.d. 5%	250.7		
L.s.d. 1%	291.0		

SUMMARY

Sand culture experiments, using a sub-irrigation technique, were installed in order to find out the effects of the macronutrients N, P, K, Ca, Mg and S on growth, aspect, mineral composition, length of fibers, thickness of cell wall and cellulose concentration in slash pine. The aim was to obtain, under controlled conditions, basic information which could eventually lead to practical means designed to increase the rate of growth and to make of slash pine a richer source of cellulose.

Nitrogen, Phosphorus, Potassium Experiment

A 3 x 3 x 3 factorial design with two replicates was used. Nitrogen was supplied initially at the levels of 25, 50 and 100 ppm; phosphorus was given at the rates of 5, 10 and 20 ppm; potassium was supplied at the rates of 25, 50 and 100 ppm; six months after the experiment was started the first level for each element was dropped to zero. Others macro and all micronutrients were supplied at uniform rates. Fifteen hours of illumination per day were provided.

The experimental technique for growing the slash pine seedlings proved quite satisfactory.

Symptoms of deficiency of nitrogen, phosphorus and potassium were observed, described and recorded in photographs and water colors. These informations will help to identify abnormalities which may appear under field conditions.

Chemical analysis of the several plant parts, on the other hand, give a valuable means to assess the nutritional status of slash pine, thus confirming when needed, the visual diagnosis. The correctness of manurial pratices, on the other hand, can be judged with the help of the analytical data tabulated.

Under the experimental conditions nitrogen caused the highest increases on growth, as measured by increments in height and dry weights, whereas the effects of phosphorus and potassium were less marked.

Cellulose concentration was not significantly affec - ted by the treatments used.

Higher levels of N seemed to decrease both length of

fiber elements and the thickness of cell wall. The effects of P and K were not well defined.

Calcium, Magnesium, Sulfur Experiment

A 3 x 3 x 3 factorial design with two replicates was used. Calcium was supplied initially at the levels of 12.5 , 25 and 50 ppm; magnesium and sulfur were given at the rates of 6, 12.5 and 25 ppm. Other macro and micronutrients were supplied at uniform rates, common to all treatments. Three months after starting the experiment the first level for each element was dropped to zero.

Symptoms of deficiency of calcium, magnesium and sulfur were observed, described and recorded as in the case of the previous experiment.

Chemical analysis were made, both for mineral content and cellulose concentration.

Length of fibers and thickness of cell wall were measured.

Both calcium and magnesium increase height, sulfur failing to give significant response. Dry weight was benefically affected by calcium and sulfur.

The levels of calcium, magnesium and sulfur in the needles associated with deficiency and maximum growth are comparable with those found in the literature.

Cellulose concentration increased when the level of sulfur in the substrate was raised.

The thickness of cell wall was negatively affected by the treatments; no effect was observed with regards to length of fibers.

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