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Effect of fertilization on the mineral composition of stone pine needles

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Abstract. Leaf analysis is a suitable technique to evaluate the nutritional status of forest stands since it reflects both the availability of nutrients in the soil and the ability of the trees to absorb them. This study aims to assess the effect of some nutrients on the needle mineral composition of *Pinus pinea* L. one year after their application to the soil. The experiment was established on a stone pine planted in 2009, located in the Portuguese Region of Ribatejo on a soil with acid reaction, medium levels of organic matter and extractable potassium, and low levels of extractable phosphorus, manganese, zinc, copper and boron. Eight experimental treatments consisting of different combinations of nitrogen, phosphorus, potassium, micronutrients, and limestone were considered. The needles were collected in March from the middle part of the growths of the former year. Fertilization induced significant differences ($p \leq 0.05$) on the needle levels of nitrogen, phosphorus, sulphur, manganese, zinc and boron. The application of phosphorus and boron increased the foliar levels of these nutrients, but liming induced lower levels of manganese and zinc. Concerning nitrogen, the levels of soil organic matter may be responsible for the appropriate nitrogen levels in the needles. The study will continue for further years in order to confirm the obtained results.

Keywords. Leaf analysis – Mineral nutrition – *Pinus pinea* L. – Soil analysis.

I – Introduction

In Portugal, stone pine (*Pinus pinea* L.) stands occupy about 176,000 ha, corresponding to 6% of the total forest area of the country (ICNF, 2013). This species assumes a great social and economic importance because of the commercial value of pine nuts, a seed with high quality and currently the main product of this forestry sector.

There are, however, strong annual variations regarding the production of pine cone, which worries producers, being necessary to study the factors that determine these fluctuations, namely genetic aspects, pests and diseases, and water and nutrients availability of the stands. It is also necessary to know the effect of agronomic practices, such as irrigation and fertilization, on the productivity and quality of pine cone and pine nut, bearing in mind the preservation of natural resources and biodiversity.

In what concerns fertilization, if it is performed in a rational way, it will improve the soil fertility and consequently the nutritional status of stone pine stands, which will help to increase their resistance to biotic and abiotic stress factors leading, probably, to higher and more regular yields of pine cone and pine nut. Therefore studies on nutrition and fertilization of stone pine stands must be carried in order to establish fertilizer recommendations to these stands. The required experimental work shall be based on the response of the trees to nutrient applications involving different situations (climatic, soil and management, among others) and conducted over an extended period of time in order to obtain representative results, since they depend on the covered experimental conditions (Calama *et al.*, 2007; Piqué and Martín, 2007).

The evaluation of the nutritional status of stone pine stands should be performed through leaf analysis because the mineral composition of the leaves reflects both the nutrient availability in the soil and the ability of the trees to use these nutrients (Brockley, 2001). With that purpose, a fertilizer trial was installed in a young even-aged plantation, as a first step to establish the response of stone pine to the application of several nutrients to the soil. This paper reports the first experimental results of the above referred experiment, concerning the effect of the application of some nutrients on the mineral composition of the needles, one year after their application to the soil.

II – Materials and methods

1. Location and soil characteristics

The field experiment was established in a stone pine planted in 2009 with a 8 m x 6 m layout (156 trees per hectare) with plants from a nursery of the region. It is located at Vale Porquinho, in the Portuguese Region of Ribatejo. The trees were not grafted.

On average, annual mean temperature and rainfall are 16.6 °C and 722 mm, respectively, the last occurring mainly in autumn and winter.

Soil samples taken before the establishment of the trial, in 2008, show, on average, on the surface layer (0 to 0.2 m) sandy loam texture (Bouyoucos hydrometer), acid reaction ($\text{pH}_{\text{H}_2\text{O}} = 5.0$, potentiometric determination in a 1:2.5 soil-to-water (v/v) suspension), medium levels of organic matter (29 g/kg, organic carbon x 1.724, wet oxidation) and extractable potassium (78 mg/kg K_2O , Egner-Riehm method), very low levels of extractable phosphorus (<23 mg/kg P_2O_5 , Egner-Riehm method), manganese (2 mg/kg Mn, AAAC-EDTA method), zinc (0.5 mg/kg Zn, AAAC-EDTA method), and copper (0.25 mg/kg Cu, AAAC-EDTA method), low levels of extractable boron (0.22 mg/kg B, hot water extraction) and very low potential exchange capacity (4.29 cmol(+)/kg, ammonium acetate method).

2. Experimental design and treatments

The experiment was arranged into complete randomized blocks with three replications, assigned to plots with eight trees each.

The experimental treatments were as follows: T1 - control (without fertilization); T2 - N; T3 - NP; T4 - NPK; T5 - NPK + limestone; T6 - NPK + MnZnCuB; T7 - NPK + MnZnCuB + limestone; T8 - NPK + B + limestone. Before planting, in spring 2008, phosphorus, potassium and limestone were applied to the soil. In March 2014, all the treatments were implemented. The nutrient levels used are: N - 40 kg/ha; P - 87 kg/ha; K - 100 kg/ha; Mn - 4 kg/ha; Zn - 2 kg/ha; Cu - 1,5 kg/ha; B - 2 kg/ha; Limestone - 8000 kg/ha (2008) and dolomite limestone - 4340 kg/ha (2014). All the fertilizers were applied to the soil. Nitrogen was applied as ammonium nitrate (20.5% N), phosphorous as calcium superphosphate (18% P_2O_5), potassium as potassium chloride (60% K_2O), manganese, zinc and copper as sulphate (27% Mn, 22% Zn and 25% Cu) and boron as Borax (11% B).

3. Leaf sampling and statistical analysis

Needle samples were collected in each one of the 24 plots of the experimental site in March 2015. A composite sample was prepared by mixing the needles of the eight trees of each plot. The needles were taken from the top third of the crown, from the middle part of the fully expanded growths of the former year.

The needle nutrients were determined as follow: nitrogen and sulphur through catalytic pyrolysis with elemental analyzer (Leco NS2000) and phosphorus, potassium, calcium, magnesium, sulphur, iron, manganese, zinc, copper and boron through radial and simultaneous ICP-OES in the solution obtained by the uptake in HCl of dry ashes 500°C.

Statistical analysis was performed through ANOVA in order to evaluate the effect of the experimental treatments on the mineral composition of the needles. Differences among means were established by Duncan multiple range test ($\alpha = 95\%$).

III – Results and discussion

The fertilization showed a significant mean effect on the needle levels of nitrogen, phosphorus, sulphur, manganese ($F_{[7;14]} = 3.05$, $F_{[7;14]} = 3.53$, $F_{[7;14]} = 2.91$ and $F_{[7;12]} = 4.19$, $p \leq 0.05$, respectively), zinc ($F_{[7;14]} = 4.29$, $p \leq 0.01$) and boron ($F_{[7;14]} = 12.09$, $p \leq 0.001$) and did not affect ($p > 0.05$) the others nutrients: potassium, calcium, magnesium, iron and copper (Table 1).

Table 1. Mineral composition of needles of *Pinus pinea* L. collected in March

Experimental treatments	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
	g/kg						mg/kg				
T1 - Control	12.3 ab	0.9 bc	5.4	1.9	1.4	1.6 ab	43	33 bc	24 a	3.5	4.6 c
T2 - N	12.0 ab	0.8 c	5.7	1.9	1.2	1.4 b	40	25 bc	23 ab	3.6	4.6 c
T3 - NP	11.0 b	1.7 a	6.1	1.8	1.2	1.5 ab	42	38 b	21 abc	3.6	5.0 c
T4 - NPK	13.0 a	1.5 ab	5.3	2.5	1.5	1.7 ab	41	42 b	24 a	3.7	4.1 c
T5 - NPK + Limestone	11.7 ab	1.4 abc	6.5	2.1	1.2	1.9 a	44	10 c	18 bc	3.4	4.6 c
T6 - NPK + MnZnCuB	13.0 a	1.9 a	5.8	2.4	1.3	1.8 a	41	72 a	25 a	3.7	21.0 a
T7 - NPK + MnZnCuB + Limestone	11.3 b	1.5 abc	5.9	2.3	1.2	1.6 ab	42	14 bc	16 c	3.2	14.3 b
T8 - NPK + B + Limestone	12.3 ab	1.5 abc	6.3	2.4	1.3	1.8 a	42	17 bc	18 c	3.2	15.6 ab

Mean values followed by different letters are significantly different ($p = 0.05$).

The application of phosphorus increased the needle levels of this nutrient, which may be explained by the low levels of extractable phosphorus in soil. This is in accordance with the results reported by Prietzel and Stetter (2010) in a long-term study with *Pinus sylvestris*, who also observed an increase of phosphorus levels in foliage during some years after the fertilization with phosphorus.

The needle levels of boron also increased with the application of the nutrient, being the higher value obtained in the experimental treatment that received macro and micronutrients (T6). The experimental treatments without boron showed low needle levels of this nutrient. In a plantation of *Pinus pinea* with symptoms of boron deficiency, Vale *et al.* (1999) also reported an increase on boron needle concentrations after six months of the application of this nutrient to the soil. Higher doses of the fertilizer resulted in higher boron needle levels, although decreasing over time.

Regarding the manganese needle concentrations, the higher levels were obtained with its application without liming (also in T6). The limestone induced the lowest levels of manganese as well as of zinc, according with their lower availability when soil pH rises (Tisdale *et al.*, 1985). Conversely, copper and iron needle levels were not affected by the applied fertilization.

In what concerns the needle levels of calcium and magnesium, they were not influenced by fertilization, despite the low levels in the soil.

The medium levels of extractable potassium in the soil may explain the absence of fertilization response to the application of this nutrient. Concerning the nitrogen, were not observed significant differences between the treatment without fertilization (T1) and others that are fertilized with nitrogen. The medium levels of the soil organic matter may be responsible for this result and the appropriate nitrogen levels in the needles.

IV – Conclusions

The results showed that the applied fertilization affected the mineral composition of the needles, and therefore the nutritional status of the trees.

This study is preliminary and should continue for further years in order to evaluate the effect of experimental fertilizations on pine nut production. Moreover, other fertilizer experiments (factorial) are necessary to study the most suitable fertilization of stone pine based on soil and leaf analysis.

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