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Effect of nitrogen and boron fertilisation on juvenile stone pine growth. A pot experiment

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Abstract. At the establishment stage of a forest plantation, soil nutritional limitations can lead to insufficient plant growth and predispose plants to biotic and abiotic damages. Adequate plant nutrition contributes to proper plant establishment and subsequent growth. Nitrogen (N) and boron (B), among others, are essential nutrients for plant vigour and growth, and their requirements need to be established. However, there is a lack of information concerning post-transplant fertilisation of stone pine (*Pinus pinea* L.).

The main objective of this work was to evaluate the effect of N and B applications on the vegetative growth of stone pine during the first three years in a conditioned environment. Morphological characteristics such as height growth (HGI), root collar diameter (RCDI), total branches length (TBL) and terminal buds length (BL) were evaluated for each plant, as well as the aboveground and root biomass production. The results showed that N fertilisation caused an increase in all growth variables suggesting that the application of N at a juvenile stage (after transplanting) of this specie will improve its development and obtain a reserve of this nutrient to be used by the plant at later stages of development, especially in soils with low fertility. Boron application had no effect on the evaluated parameters.
Key words: *Pinus pinea*; fertilisation; vegetative growth; biomass.

Efeitos da fertilização com azoto e boro no crescimento de pinheiro-manso juvenil instalado em vasos

Sumário. Na fase de plantação florestal, as limitações nutricionais do solo podem causar um crescimento insuficiente e uma maior predisposição das plantas para sofrerem danos bióticos e abióticos. Uma adequada nutrição vegetal contribui para um melhor estabelecimento das jovens plantas e seu posterior crescimento. Entre outros, o azoto (N) e o boro (B) são nutrientes que promovem o vigor e o crescimento das plantas e as suas necessidades devem ser calculadas. Contudo, ainda há pouca informação sobre a fertilização, envolvendo estes nutrientes, a realizar ao pinheiro-manso (*Pinus pinea* L.) após o transplante.

Este trabalho teve como objetivo avaliar o efeito da aplicação de azoto e de boro, no crescimento vegetativo do pinheiro-manso nos primeiros três anos, em ambiente controlado. Parâmetros morfológicos, como o crescimento em altura (HGI), o aumento do diâmetro da base do tronco (RCDI), o comprimento total dos ramos (TBL) e o comprimento dos gomos terminais (BL), foram avaliados em cada planta, assim como a produção de biomassa da parte aérea e das raízes. Os resultados revelaram que a fertilização azotada originou um incremento em todas as variáveis observadas, levando a crer que a aplicação de azoto numa fase juvenil (após transplante) desta cultura será benéfica - melhorando o seu desenvolvimento - e poderá ajudar a constituir uma reserva deste nutriente, a ser utilizada pela planta numa fase posterior, especialmente em solos de baixa fertilidade. A aplicação de boro não exerceu qualquer efeito sobre os parâmetros considerados.
Palavras-chave: *Pinus pinea* L.; fertilização; desenvolvimento vegetativo; biomassa.

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Effets de la fertilisation à l'azote et au bore sur la croissance du pin parasol juvénile installé en pots

Résumé. Lors de la phase de plantation forestière, les limitations nutritionnelles du sol peuvent entraîner une croissance insuffisante et une plus grande prédisposition des plantes à subir des dommages biotiques et abiotiques. Une nutrition adéquate des plantes contribue à un meilleur établissement des jeunes plantes et à leur croissance ultérieure. Entre autres, l'azote (N) et le bore (B) sont des nutriments fondamentaux à la vigueur et à la croissance des plantes et leurs besoins doivent être calculés. Cependant, il existe encore peu d'informations sur la fertilisation à effectuer chez le pin parasol (*Pinus pinea* L.) après transplantation.

Ce travail visait à évaluer l'effet de l'application de N et de B sur la croissance végétative du pin parasol au cours des trois premières années, dans un environnement contrôlé. Les paramètres morphologiques tels que la croissance en hauteur (HGI), l'augmentation du diamètre de la base du tronc (RCDI), la longueur totale des branches (TBL) et la longueur des bourgeons terminaux (BL) ont été évalués pour chaque plante, ainsi que la production de biomasse de pousses et de racines. Les résultats ont révélé que la fertilisation azotée a conduit à une augmentation de toutes les variables observées, laissant croire que l'application de N dans la phase juvénile (après le repiquage) de cette culture sera bénéfique - améliorant son développement - et constituera une réserve de cette culture, qui sera utilisé par la plante à un stade ultérieur, en particulier dans les sols peu fertiles. L'application de B n'a eu aucun effet sur les paramètres considérés.

Mots-clés : *Pinus pinea* L.; fertilisation; développement végétatif; biomasse.

Introduction

Due to the economic importance of its delicious and highly nutritious edible seeds, the stone pine is one of the most important contributors to non-wood forest products in the Mediterranean region (MUMCU KÜÇÜKER and BASKENT, 2017). Stone pine is a forest species with multiple uses, being the production of edible nuts the most valued in Portugal (CORREIA, 2013), combining high economic value, environmental protection and landscape. According to the 6th forest inventory (ICNF, 2019), the area of stone pine in Portugal increased between 2005 and 2015, occupying approximately 193,000 ha and representing 6% of the total Portuguese forest area.

According to MARCELO *et al*. (2022), cross-referencing the information on the occurrence of stone pine (ICNF, 2019) with the lithological complexes indicated by SILVA (1982) and the FAO soil units represented by CARDOSO *et al*. (1973), stone pine stands are mainly located in nutriente-poor soils, associated with sedimentary formations, preferring sandy to sandy loam textures, with good aeration and drainage, and low compaction, with an acid to slightly acid reaction, i.e. they predominate in our country on Podzols, Regosols, Cambisols and Arenosols (50.8%).

Adequate fertilisation increases crop yield and improves quality of nut-bearing species, as it promotes root development and aerial plant growth, thus contributing to the efficient use of soil and water (MARSCHNER, 2012), also improving the development of symbiotic bacteria and mycorrhizal fungi (FREIRE *et al.*, 2019). LUIS *et al.* (2009) observed an increase in survival of *Pinus Canariensis* under drought conditions when comparing fertilised and unfertilised seedlings. FREIRE *et al.* (2019) reported that the most productive pine stands corresponded to agroforestry systems associated with fertilised pastures.

Fertilisation is, after watering, the most influential practice on plant growth, acting on physiological processes such as growth regulation, energy flow and synthesis of molecular organic complexes (MENGEL and KIRKBY, 2001; MARSCHNER, 2012). FINCK (1982) noted that although fertilisation plays a lesser role in forestry than in agriculture, the application of nutrients to young trees in the nursery promotes their growth after transplanting.

There is limited scientific knowledge about the effects of fertilisation on young or mature plantations or forests of stone pine. Studies with this species are challenging because pine cone development takes about 4 years from differentiation to harvest, and 1, 2 and 3-year-old cones occur simultaneously on the same tree, making the evaluation of any management practice more complex than in other species (LOEWE *et al.*, 2016).

Few authors have studied the effect of fertilisation on *Pinus pinea* immediately after plantation. These studies showed that the use of a slow-release fertiliser increased height growth during the first two years (CAÑELLAS *et al.*, 1999). The impact of micronutrients on *Pinus pinea* has been also poorly studied. MARCELO *et al.* (2017) carried out a fertilisation study in a 6-year-old stone pine plantation, and they demonstrated that B fertilisation affects the nutrient needles composition, in agreement with the observations of VALE *et al.* (1999).

Nitrogen is the main component of plant proteins and some hormones, and plays a prominent role in many metabolic and physiological processes, such as photosynthesis, amino acid synthesis, respiration, and tricarboxylic acid (TCA) cycle (FOYER *et al*., 2011). For these reasons, it is an important nutrient for the growth and development of plants and, in general, the main limiting factor, due to its multiple changes and loss processes to which it can be subjected in the soil. N fertilisation during nursery cultivation can be an effective solution to improve field performance. More N is translocated from perennial tissues to new apical growth tissues in seedlings with rich N reserves, especially when seedlings are planted in low fertility soils (CERASOLI *et al*., 2004; SALIFU and TIMMER, 2003, OLIET *et al.,* 2009, VILLAR-SALVADOR *et al.,* 2012, cited by LI *et al.,* 2015).

When the supply of N from the root medium is insufficient, N from older leaves is mobilised to feed the younger plant organs, proteins are hydrolysed (proteolysis), leading to chloroplast collapse and thus to a decrease in chlorophyll content. When N is available, a high growth rate occurs and reserves from perennial tissues are not overused for meristematic tissue formation (MENGEL and KIRKBY, 2001).

Boron is a crucial micronutrient for the growth and development of all plants. It is mainly involved in carbohydrate metabolism and cell division. Many scientists have shown that B is essential for carbohydrate synthesis and for their translocation across the membrane to meristematic regions of roots and tops (MARSCHNER, 2012; ZHOU *et al.*, 2014; FREIRE *et al.*, 2019). Another major function of B in plant metabolism is cell division and the maintenance of cell wall structure (GUPTA and SOLANKI, 2013; VARENNES, 2003). Its deficiency leads to the death of vegetative growth tips, which constitute the meristematic cell line. The death of terminal buds directly affects the growth of lateral shoots, the tips of which can also be deformed as a rosette on the plant (GUPTA and SOLANKI, 2013). According to MENGEL and KIRKBY (2001), severe deficiency causes apical death and the whole plant exhibits reduced growth, with flowering and fruiting inhibition.

Boron deficiency is more common than deficiency of any other micronutrient and affects many species in the world (GUPTA, 1979; TEASDALE and RICHARDS, 1990). The uptake of B by plants and its subsequent transport within the plant follows the flow of water as a largely passive process. Plants in their first years of life are more susceptible to long drought periods because they have a limited root system occupying the surface soil layer that is exposed to drought, and therefore B supply is difficult. This explains why many plantations only suffer from B deficiency for a few years until they develop a root system deep enough to extend into the subsoil, making them less susceptible to drought (SCHLATTER and GERDING, 1984).

More information is needed to support fertilisation of young stone pine plantations. Although the results of pot experiments are not equivalent to those obtained in field conditions, they provide a comparative view of the effects of nutrient applications on plant growth and thus alert to the plant needs.

In this study, the pot experiment was carried out with *Pinus pinea* that was grown for three years receiving a gradient of B levels, with and without N application, to get single and combined effects of these two nutrients on the vegetative growth of very young plants of stone pine.

Materials and methods

Experimental design and soil characteristics

The study was conducted as a pot experiment under greenhouse conditions, using 6.9 dm^3 Kickbrauckman pots. The trial was established in February 2019, with one-year old stone pine (*Pinus pinea* L.) seedlings, and was set up using a randomised complete block design with a 2 x 5 factorial arrangement and three replications. Experimental treatments consisted of five B levels (B0, B1, B2, B3 and B4) applied to the pots, without (N0) and with N (N1).

The experimental treatments were as follows: T1 – N_0B_0 (control, without N and B); T2 – N_0 B_1; T3 - N_0 B_2; T4 - N_0 B_3; T5 - N_0 B_4; T6 – N_1 B_0; T7 – N_1 B_1; T8 – N_1 B_2; T9 – N_1 B_3; T10 – N_1B_4.

The application of B levels corresponded to the concentrations of: 0; 0.25; 0.5; 0.75 and 1 mg B/kg of soil per year, applied as borax ($Na_2B_4O_7.10H_2O$), fractionated into four doses along the year. To the experimental treatments with N (N1) was applied 872 mgN/pot/year as ammonium nitrate (NH_4NO_3).

Each pot containing 10 kg of soil received a complete mineral fertilisation (122 mg P, 622 mg K, 400 mg Ca, 124 mg Mg, 16 mg Fe, 5.3 mg Mn, 0.64 mg Zn, 0.3 mg Cu/pot/year).

Topdressing fertilisations were performed trimesterly with macronutrients (N, P, K and Mg) and B, and twice a year with the remaining micronutrients (Fe, Mn, Zn and Cu). Ca was annually supplied as solid $CaCO_3$.

The soil water content was maintained at approximately 50-60 % of water holding capacity, during the experiment, using deionised water.

The soil used in the trial came from the superficial layer (0-0.15 cm) of a *haplic Podzol*, collected at Herdade da Comporta, in Alentejo coast, Portugal (38º 20' 40.4'' N; 8º 43' 39.5'' W), with the adequate characteristics to respond to the intended effects: coarse texture, poor in nutrients and low buffer capacity. It is a sandy soil, moderately acid (pH-H_2O = 5,7), with low levels of organic matter (0.88 %), very low levels of P and K (7.7 mg kg^{-1} P_2O_5 and 14 mg kg^{-1} K_2O, ammonium lactate), very low levels of exchangeable cations (Ca 0.88; Mg 0.19; K 0.02 and Na 0.002 cmol(+)kg^{-1}, ammonium acetate 1M pH7), very low potential cation exchange capacity (2.21 cmol(+)kg^{-1}), medium level of base saturation (50.2 %), and a very low level of B (0.04 mg kg^{-1}, hot water).

The soil sample was previously spread in a shallow layer on a plastic sheet and exposed to the sun, mixed and turned for even air drying. The soil was sieved through 4 mm mesh plastic sieves to remove rock fragments and other coarse material.

Growth measurements and biomass production

The morphological characteristics of the seedlings (height, root collar diameter, total branches length and terminal buds length) were recorded at the beginning of the experiment (February 2019), before fertilisation, and measured twice a year, repeatedly tree by tree, during the experiment (until November 2021). Increases in height (measured from the soil surface to the top of the terminal bud) and root collar diameter were calculated in relation to the initial values. Height and root collar diameter are the two morphological parameters most commonly studied in forest seedlings. They are easy and quick to measure and give a good estimate of seedling quality and subsequent field performance. The ratio of height to root collar diameter provides information on seedling vigour (HAASE, 2008).

Total branches length was also measured to estimate the vigour and the overall development of the plants throughout the experiment.

Terminal buds are also important indicators of tree health and vigor, as well as growth patterns. By measuring the length of terminal buds, we can monitor changes in growth rates and assess the impact of environmental factors such as temperature, rainfall, and nutrient availability on tree development. Accurately measuring terminal bud length can provide valuable information for tree management decisions, such as pruning or fertilization, to help optimize tree growth and productivity

At the end of the trial (November 2021), after almost three years of fertilisation, plants were cut and weighed, providing data on leaf, stem and root biomass. Plants and soil compositions were also chemically analysed, but these results are not presented.

Statistical analysis

The effect of B (five levels) and N (two levels) fertilisation and their interactions were assessed using two-way analysis of variance (ANOVA) in a randomised complete block design. When the ANOVA results showed a significant effect, Duncan's multiple range test was used for multiple comparisons between treatments (p=0.05). Correlations between aerial and root biomass production and growth parameters such as root collar diameter, height and total branch length were estimated. Statistical analyses were performed using STATISTICA 12 software.

Results and discussion

Growth measurements

A significant effect of the experimental treatments on all the considered growth variables considered in the study was found. However, during the three years of the experiment, B fertilisation and its interaction with N had no significant effect ($p>0.05$) on any of the measured variables, except for B in the height:root collar diameter ratio measured in November 2021 (Table 1). Conversely, the presence or absence of N had a highly significant effect ($p<0.001$) on all the considered morphological characteristics.

Table 1 – p-values derived from ANOVA of the main effects of N fertilisation (N), B fertilisation (B), and their interaction (N x B) on height growth increment (HGI), root collar diameter increment (RCDI), height:root collar diameter ratio (HDR), total branches length (TBL) and terminal buds length (BL)

Source of variation	df	HGI	RCDI	HDR	TBL	BL
N	1	<0.001	<0.001	<0.05	<0.001	<0.001
B	4	0.389	0.093	<0.05	0.865	0.964
N x B	4	0.923	0.348	0.578	0.730	0.976

Height growth increment (HGI)

Shoot height is correlated with the number of needles and therefore provides an estimate of the photosynthetic capacity and transpirational area (HAASE, 2008). The effect of treatments was statistically significant ($p \leq 0.05$) on plant height growth increment (HGI), showing differences in growth with and without N. The average value of HGI of the N-fertilised plants was significantly ($p \leq 0.05$) higher than that of the non-fertilised plants. Figure 1 shows the increments in relation to the initial values of plant height, with (T6 to T10) and without (T1 to T5) N fertilisation.

Root collar diameter increment (RCDI)

Root collar diameter is considered to be the best predictor of seedling survival and growth in the field (DEY and PARKER, 1997). A large stem diameter indicates a larger root system and stem volume (HAASE, 2008). Some authors (DEY and PARKER, 1997; JACOBS *et al.*, 2005; HAASE, 2008) showed that seedlings with a larger initial stem diameter can accelerate early plantation survival and growth.

In this trial, N fertilisation had a positive effect on the increment of this parameter with relation to initial values. The experimental treatments that received N showed a greater development of root collar diameter over time (Figure 2). Conversely, the application of increasing levels of B had no significant effect ($p>0.05$) on this growth parameter.

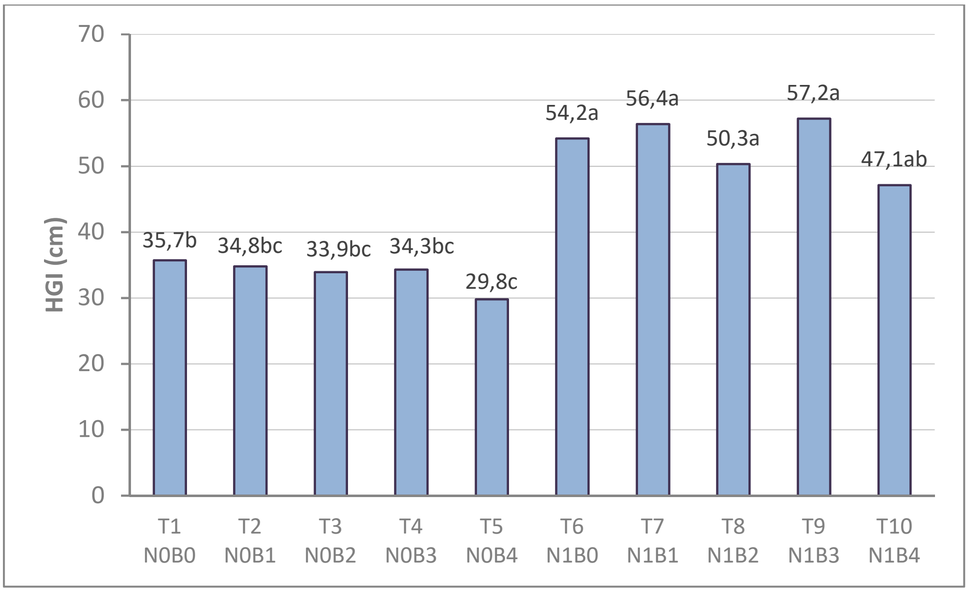

Figure 1 - Response of height growth increment (HGI) to the application of increasing levels of B in the absence (T1 to T5) and in the presence (T6 to T10) of N (different letters indicate significant diferences, p=0.05)

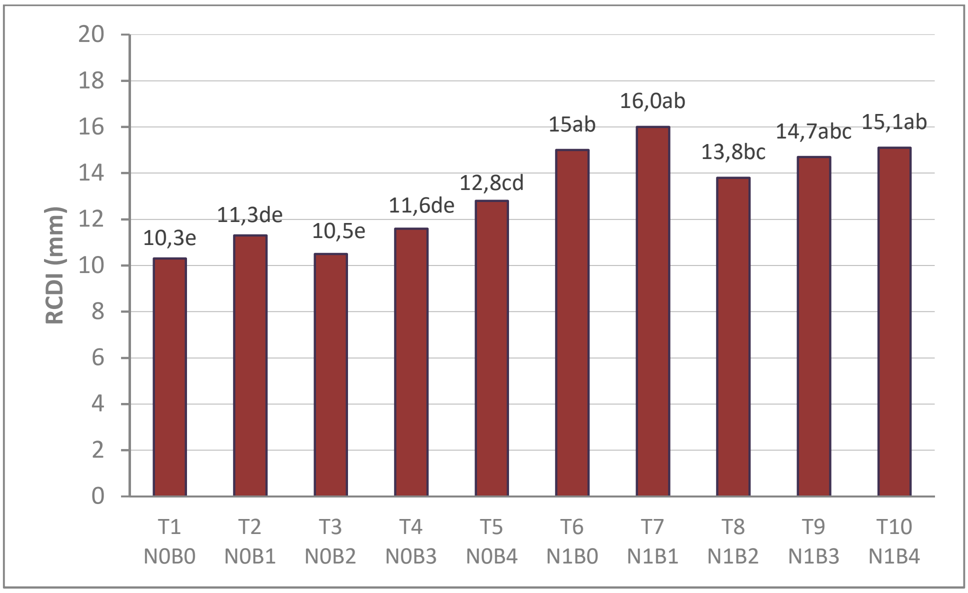

Figure 2 - Response of root collar diameter increment (RCDI) to the application of increasing levels of B in the absence (T1 to T5) and in the presence (T6 to T10) of N (different letters indicate significant diferences, p=0.05)

Height to root collar diameter ratio (HDR)

The ratio of height to root collar diameter provides information about the seedling's sturdiness (HAASE, 2008). Even if height and root collar diameter are on target, the seedling could be unbalanced and it is very important that the shoot is not too tall in relation to the stem diameter. A high ratio indicates a relatively spindly seedling, whereas a lower ratio indicates a stouter seedling. Young plants with high ratios may be susceptible to damage from the wind, drought and frost (HAASE, 2008).

The results (Figure 3) showed that although N and B had a statistically significant effect on this ratio (p≤0.05) (Table 1), the results with and without N are very similar and do not show any unbalance in young plants. For the same N level, the application of the higher amount of B seems to cause a decrease of this ratio, as the plants tend to be lower.

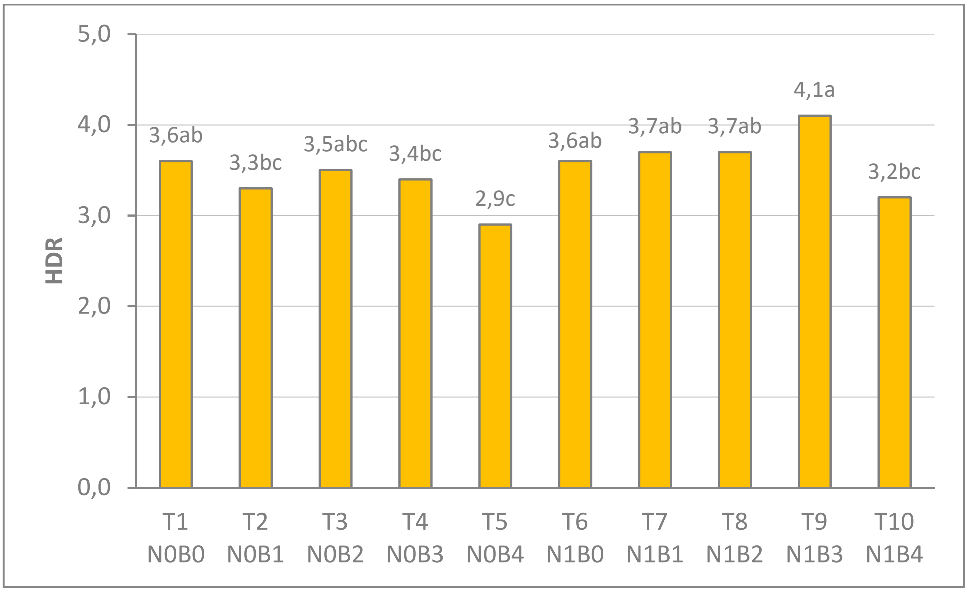

Figure 3 - Response of height to diameter ratio (HDR) to the application of increasing levels of B in the absence (T1 to T5) and in the presence (T6 to T10) of N (different letters indicate significant differences, p=0.05)

Total branches length (TBL)

Nitrogen fertilisation also had a significant (p<0.001) and positive effect on total branch length (Table 1). At the end of the study, the experimental treatments receiving N (T6 to T10) showed, on average, a total branch length (782 cm) twice as high as the treatments without N (370 cm) (T1-T5) (Figure 4).

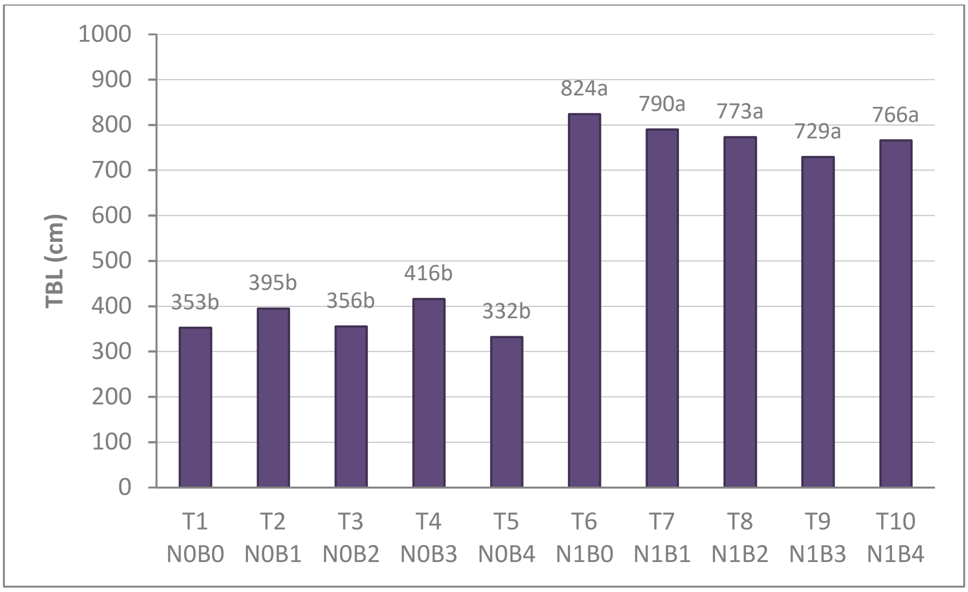

Figure 4 - Response of total branches lenght (TBL) to the application of increasing levels of B in the absence (T1 to T5) and in the presence (T6 to T10) of N (different letters indicate significant differences, p=0.05)

Terminal buds length (BL)

Bud length gives an indication of seedling vigour and shoot potential growth as it is correlated with the number of needle primordia (HAASE, 2008; ROSE and KETCHUM, 2003).

N fertilisation improved terminal bud growth, with significant differences (p=0.05) between treatments without (T1 to T5) and with (T6 to T10) N fertilisation (Figure 5). In March 2021, the average length of terminal buds was 4.1 cm in the plants with N as compared with 1.9 cm in the plants without N.

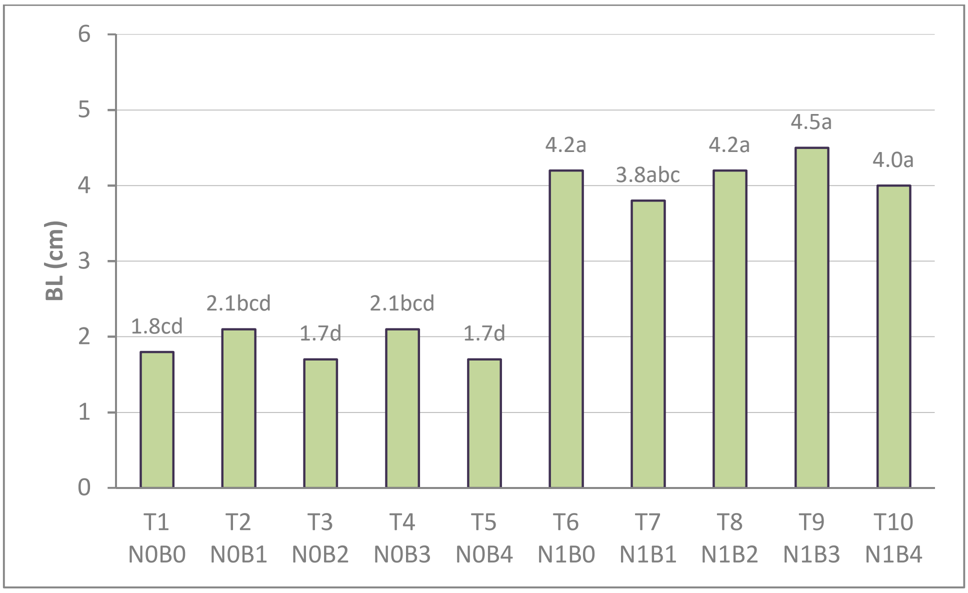

Figure 5 - Response of terminal buds lenght (BL) to the application of increasing levels of B in the absence (T1 to T5) and in the presence (T6 to T10) of N (different letters indicate significant differences, p=0.05)

Biomass production

At the end of the trial, the biomass production was evaluated (Table 2), and a highly significant effect (*p<0.001*) was also observed on the weight of leaves, branches, trunk and roots, due to the presence or absence of N in the fertilisation.

Table 2 – p-values derived from ANOVA of the main effects N fertilisation (N), B fertilisation (B), and their interaction effect (N x B) on leaves, branches, trunk and root dry matter biomass

Source	df	leaves	branches	trunk	roots
N	1	<0.001	<0.001	<0.001	<0.001
B	4	0.019	0.968	0.233	0.074
N x B	4	0.040	0.498	0.539	0.355

The results showed a high effect of N fertilisation; in fact, the total biomass production due to N fertilisation was up to twice higher than the non-N supplied plants (Figure 6).

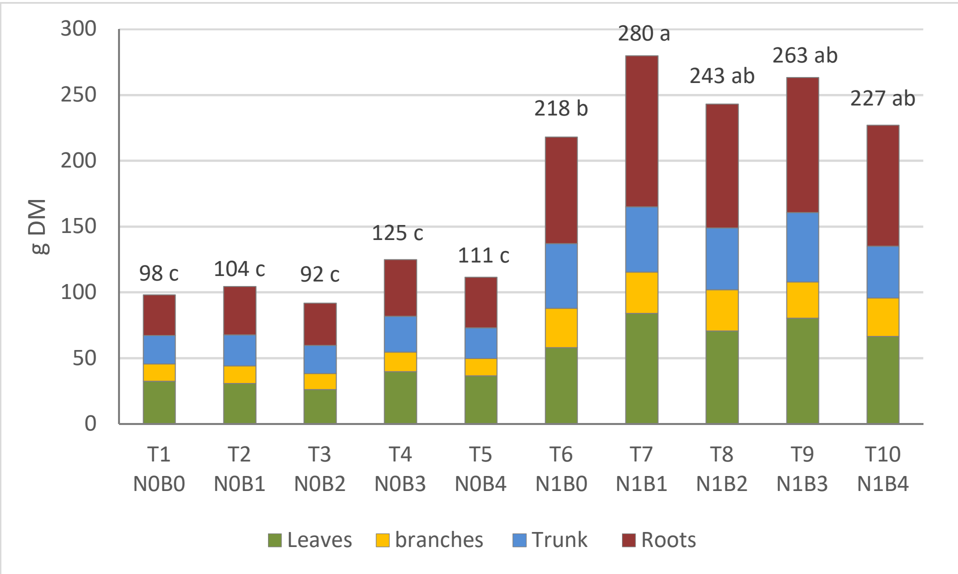

Figure 6 - Response of total produced dry matter (DM) biomass (leaves, branches, trunk and roots) to the application of increasing levels of B in the absence (T1 to T5) and in the presence (T6 to T10) of N (different letters indicate significant differences p=0.05)

The lack of response to B can be explained by the fact that, during the study period, the young *Pinus pinea* plants were efficient in using this micronutrient, even if it was available in small quantities in the soil (according to the analytical method used for its evaluation) or can also be due to the low levels of B tested.

Root biomass contributed with 34 % to 39 % of the total biomass of these young pine trees.

Highly significant and positive correlations (p≤0.001) were found between aerial and root biomass production and root collar diameter (RCD), height (HG) and sum of branch length (SBL). The highest correlation coefficient between biomass production and growth measurements was found for total biomass (TBio) with the sum of branch length (SBL) (r=0.902***), suggesting that this parameter can gives a good estimate of the expected total biomass production (Table 3).

Table 3 – Correlation matrix showing interrelations between biomass production and growth measurements (r values)

Parameters	LB	BB	TB	RB	TBio	RCD	HG
LB							
BB	0.893***						
TB	0.890***	0.867***					
RB	0.909***	0.847***	0.871***				
TBio	0.969***	0.919***	0.937***	0.974***			
RCD	0.801***	0.872***	0.839***	0.779***	0.840***		
HG	0.779***	0.769***	0.937***	0.825***	0.860***	0.748***	
SBL	0.875***	0.976***	0.875***	0.826***	0.902***	0.867***	0.796***

LB–leaves biomass, BB-branches biomass, TB-trunk biomass, RB – roots biomass, TBio-total biomass, RCD-root colar diameter, HG-height growth, SBL-Sum of branch length; r- correlation coefficient

Visual symptoms of nutritional inbalances

Typical symptoms of N deficiency were visualised in this study. These observations were consistent with those reported by several authors (MALAVOLTA *et al.*, 1997; MENGEL and KIRKBY, 2001; BRYSON *et al.*, 2014). Nitrogen deficiency disrupts the plant's ability to produce chlorophyll, eventually resulting in yellow or pale green leaves. As N is a mobile nutrient, the plant can easily transport the nutrient between tissues. Therefore, when a plant is deficient in N, it is translocated from older and lower leaves to younger and upper leaves of the plant. This is why N deficiency will first cause the lower leaves to become yellow. Later, the upper leaves will turn light green, then yellow, and older leaves will often fall prematurely. A plant that cannot produce chlorophyll through photosynthesis will also have difficulties of producing energy. This leads to slow growth, thin stems and weak branches (BRYSON *et al.*, 2014; VARENNES, 2003).

The characteristic symptoms of B deficiency described in the literature for various pine species (SCHLATER and GERDING, 1984; STONE, 1990; MENGEL and KIRKBY, 2001; GUPTA and SOLANKI, 2013), such as abundant resin flow, various disturbances in apical dominance, delayed development and, in some cases, twisted apices and side branches, were not detected in this study.

Opposing to what was reported by SCHLATTER and GERDING (1984), any symptoms of B deficiency were found in this study and no significant effects of B fertilisation were found on height growth.

However, data not presented in this paper allowed the identification of a highly significant effect (p≤0.001) of the experimental treatments on needle B content, showing a very clear effect of B application on foliar B content. Soil B composition was also significantly affected by the addition of increasing levels of this micronutrient, showing a highly significant increase (p≤0.001) with rising amounts applied.

Conclusions

The obtained results point to the benefit of establishing N fertilised plantations to increase initial plant growth. The application of this nutrient at a juvenile stage of stone pine stands will allow a better vegetative development of the plants and may constitute an important reserve of this nutrient to be used at later stages of their development, especially in soils of low fertility.

Regarding B, further research is needed to clarify the importance of this nutrient in the early growth stages of stone pine. It is also essential to carry out calibration experiments in order to obtain more accurate information on the availability of B in the soil, particularly in forest stands.

This study should be complemented by field trials under different edaphic conditions.

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References

BRYSON, G.M., MILLS, H.A., SASSEVILLE, D.N., JONES, J.B., BARKER, A.V., 2014. *Plant Analysis Handbook IV. A guide to sampling, preparation, analysis and interpretation for agronomic and horticultural crops*. Micro-Macro Publishing. Inc.Athens, Georgia. ISBN 978-1-878148-03-2. 609 pp.

CAÑELLAS, I., FINAT, L., BACHILLER, A., MONTERO, G., 1999. Comportamiento de planta de *Pinus pinea* en vivero y campo: ensayo de técnicas de cultivo de planta, fertilización y aplicación de herbicidas. *Investigación Agraria: Sistemas y Recursos Forestales* **8**(2): 335-360.

CARDOSO, J.C., BESSA, M.T., MARADO, M.B., 1973. Carta de solos de Portugal (1:1 000 000). *Separata da Agronomia Lusitana*, vol. **33**, tomos I-IV, Versão do Atlas do Ambiente, Lisboa: Comissão Nacional do Ambiente.

CERASOLI, S., MAILLARD, P., SCARTAZZA, A., BRUGNOLI, E., CHAVES, M.M., PEREIRA, J.S., 2004. Carbon and nitrogen winter storage and remobilisation during seasonal flush growth in two-year-old cork oak (*Quercus suber* L.) saplings. *Annals of Forest Science* **61**: 721-729.

CORREIA, A.C.P., 2013. *Balanço de carbono em ecossistemas mediterrânicos*. Tese apresentada para obtenção do grau de doutor em Engenharia Florestal e dos Recursos Naturais, ISA, Lisboa. 89 pp.

DEY, D.C., PARKER, W.C., 1997. Morphological indicators of stock quality and field performance of red oak (*Quercus rubra* L.) seedlings underplanted in a central Ontario shelterwood. *New Forests* **14**: 145-156.

FINCK, A., 1982. *Fertilizers and fertilisation. Introduction and practical guide to crop* fertilisation. Verlag chemie. Weinheim. ISBN: 0895730529. 438 pp.

FOYER, C.H., NOCTOR, G., HODGES, M., 2011. Respiration and nitrogen assimilation: targeting associated metabolism as a means to enhance nitrogen use efficiency. *J. Exp. Bot*. **62**: 1467–1482.

FREIRE, J.A., RODRIGUES, G.C. TOMÉ, M., 2019. Climate change impacts on *Pinus pinea* L. silvicultural system for cone production and ways to contour those impacts: a review complemented with data from permanent plots. *Forests* **10**: 169. DOI:10.3390/f10020169

GUPTA, U.C., 1979. Boron nutrition of crops. *Adv. Agron*. **31**: 273-307. ISBN 0-12-000731-2.

GUPTA, U., SOLANKI, H., 2013. Impact of boron deficiency on plant growth. *International Journal of Bioassays* **2**(07): 1048-1050. ISSN:2278-778X.

HAASE, D.L., 2008. Understanding forest seedling quality: Measurements and interpretation. *Tree Planters´Notes* **52**(2): 24-30.

ICNF, 2019. *6º Inventário florestal nacional - IFN6.2015*. Relatório final. Instituto da Conservação da Natureza e das Florestas, Lisboa. 284 pp. http://www2.icnf.pt/portal/florestas/ifn/resource/doc/ifn/ifn6/IFN6_Relatorio_completo-2019-11-28.pdf.

JACOBS, D.F., SALIFU, K.F., SEIFERT, J.R., 2005. Relative contribution of initial root and shoot morphology in predicting field performance of hardwood seedlings. *New Forests* **30**: 235–251.

LI, G., WANG, J., OLIET, J.A., JACOBS, D.F., 2015. Combined pre-hardening and fall fertilization facilitates N storage and field performance of *Pinus tabulaeformis* seedlings. *iForest* 9: 483-489. DOI: 10.3832/ifor1708-008

LOEWE, M.V., BALZARINI, M., ÁLVAREZ, A., DELARD, C., NAVARRO-CERRILLO, R.M., 2016. Fruit productivity of stone pine (*Pinus pinea* L.) along a climatic gradient in Chile. *Agric For Meteorol* **223**: 203–216.

LUIS, V.C., PUÉRTOLAS, J., CLIMENT, J., PETERS, J., GONZÁLEZ-RODRÍGUEZ, A.M., MORALES, D., JIMÉNEZ, M.S., 2009. Nursery fertilization enhances survival and physiological status in Canary Island pine (*Pinus canariensis*) seedlings planted in a semiarid environment. *Eur J Forest Res*. **128**: 221–229. DOI: 10.1007/s10342-009-0257-7

MALAVOLTA, E., VITTI, G.C., OLIVEIRA, S.A., 1997. *Avaliação do estado nutricional das plantas. Princípios e aplicações*. 2ª ed. Piracicaba: POTAFOS. 319 pp.

MARCELO, M.E., JORDAO, P., FONTES, L., TOMÉ, M., CALOURO, F., 2017. Effect of fertilization on the mineral composition of stone pine needles**.** In: Carrasquinho I. (ed.), Correia A.C. (ed.), Mutke S. (ed.), *Mediterranean pine nuts from forests and plantations*. Zaragoza: CIHEAM, 2017. Pp. 13-16. (Options Méditerranéennes: Série A. Séminaires Méditerranéens; n. 122). ISBN: 978-2-85352-574-9. http://om.ciheam.org/om/pdf/a122/00007237.pdf.

MARCELO, M.E., CORREIA, A., PARTIDÁRIO, A.C., GONÇALVES, A.C., ALEXANDRE, C., SILVA, C.S., SEMPITERNO, C., CALOURO, F., CARRASQUINHO, I., SILVESTRE, J., TOMÉ, M., JORDÃO, P., MANO, R., FERNANDES, R., SOARES-DAVID, T., VALDIVIESSO, T., 2022. *A Fertilização do Pinheiro-manso - Recomendações para uma gestão florestal sustentável* (Fertilization of Stone Pine - Recommendations for a sustainable forest management). GO FERTIPINEA, Edição da UNAC - União da Floresta Mediterrânica. Lisboa. ISBN 978-989-33-3560-4. 20 pp. https://www.unac.pt/images/Manual_FERTIPINEA.pdf.

MARSCHNER, P., 2012. M*ineral nutrition of higher plants*. 3th Ed. Elsevier Ltd. Academic Press. ISBN: 978-0-12-384905-2. 650 pp.

MENGEL, K., KIRKBY. E.A., 2001. *Principles of plant nutrition*. 5th Ed. Dordrecht: Kluwer Academic Publishers. 849 pp.

MUMCU KÜÇÜKER, D., BASKENT, E.Z., 2017. Spatial and temporal changes of stone pine forest in Turkey: a case study in Ayvalik forest planning unit. In: Carrasquinho I. (ed.), Correia A.C. (ed.), Mutke S. (ed.), *Mediterranean pine nuts from forests and plantations*. Zaragoza: CIHEAM, 2017. Pp. 7-12. (Options Méditerranéennes: Série A. Séminaires Méditerranéens; n. 122). ISBN: 978-2-85352-574-9. http://om.ciheam.org/om/pdf/a122/00007236.pdf.

MUÑOZ, V.F., 2016. *Caracterización de la variabilidad, crecimiento y producción del pino piñonero* (Pinus pinea *L.*) *en Chile en función del clima y de algunas prácticas silviculturales.* Doctoral thesis. UCOPress, Córdoba. 353 pp.

OLIET, J.A., TEJADA, M., SALIFU, K.F., COLLAZOS, A., JACOBS, D.F., 2009. Performance and nutrient dynamics of holm oak (*Quercus ilex* L.) seedlings in relation to nursery nutrient loading and post-transplant fertility. *European Journal of Forest Research* **128**: 253-263. DOI: 10.1007/s10342-009-0261-y

ROSE, R., KETCHUM, J.S., 2003. Interaction of initial seedling diameter, fertilization. and weed control on Douglas-fir growth over the first four years after planting. *Annals of Forest Science* **60** (7): 625-635.

SALIFU, K.F., TIMMER, V.R., 2003. Nitrogen retranslocation response of young *Picea mariana* to nitrogen-15 supply. *Soil Science Society of America Journal* **67**: 309-317. DOI: 10.2136/sssaj2003.3090

SCHLATTER, J.E., GERDING, V., 1984. Deficiencia de Boro en Plantaciones de *Pinus radiata* D. Don en Chile II. Principales causas y corrección. *Bosque* **6** (1): 32–43.

SILVA, A.M.S., 1982. *Carta litológica de Portugal na escala 1:1.000.000*. Atlas do Ambiente. Lisboa: Comissão Nacional do Ambiente. (https://dados.gov.pt/pt/datasets/atlas-do-atlas-do-ambiente-cna-carta-litologica/)

STONE, E.L., 1990. Boron deficiency and excess in forest trees: A review. *Forest Ecology and Management* **37**: 49-75.

TEASDALE, R.D., RICHARDS, D.K., 1990. Boron deficiency in cultured Pine cells. *Plant Physiol.* **93**: 1071-1077.

VALE, R., COUTINHO, J., BENTO, J., 1999. Boron deficiency in a young *Pinus pinea* plantation. In: Bech J. (ed.), *Extended abstracts of the 6th Intenational Meeting on Soils with Mediterranean Type Climate*. 4-9 July 1999, Barcelona, 99. Pp. 363-364.

VARENNES, A., 2003. *Produtividade dos solos e ambiente*. Escolar Editora. ISBN 972-592-156-9. 490 pp.

VILLAR-SALVADOR, P., PUERTOLAS, J., CUESTA, B., PENUELAS, J.L., USCOLA, M., HEREDIA-GUERRERO, N., REY BENAYAS, J.M., 2012. Increase in size and nitrogen concentration enhances seedling survival in Mediterranean plantations. Insights from an ecophysiological conceptual model of plant survival. *New Forests* **43**: 755-770. DOI: 10.1007/s11056-012-9328-6

ZHOU, G., PENG, S., LIU, Y., WEI, Q., HAN, J., ISLAM, M., 2014. The physiological and nutritional responses of seven different citrus rootstock seedlings to boron deficiency. *Trees: Structure and function* **28** (1): 295–307.