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Nursery location and potassium enrichment in Aleppo pine stock 1. Effect on nursery culture, growth, allometry and seedling quality

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Abstract

There is a need for a better understanding of the primary role of macronutrients in Aleppo pine stock quality and for producing larger nutrient-loaded stock, which may be challenging for inland nurseries. The influence of nursery location and fertilization on nursery culture, growth, allometry, and seedling quality of Aleppo pine was studied in seedlings cultivated over the 2006 growing year. Fertilization treatments considered how a K enrichment performed over common programs currently being practiced and divided into three levels of K/N ratio: 0.63-0.89 (normal); 1.81-1.89 (high); and 2.25-2.53 (very high). Results showed that fertilization had a minor effect on seedling growth and allometry in comparison with location, which was the governing factor. However, fertilizing treatments significantly affected final seedling attributes, which has its origin on the early treatment differences that were kept up to the end of culture. Higher nutrient supply treatments produced the highest nutrient concentration in seedlings but they were associated with lower fertilization efficiencies. Fertilizer efficiency was approximately two-fold in the coastal nursery for the three macronutrients, although concentration was higher in the inland nursery due to lower seedling growth. It is concluded that warmer regions are more suitable for producing large stock more efficiently.

Key words: fertilizing efficiency, RGR, nitrogen, phosphorus, degree-day, leachate.

1. Introduction

Seedling quality has a strong influence on field performance and is a prerequisite for reforestation success (Burdett, 1990). Stock quality is determined by specific attributes which are the consequence of the culture growing conditions, giving the nursery a key role in the production of a stress resistant stock, enhancing a better growth and survival response. Aleppo pine (*Pinus halepensis* Mill.) is among the most important species used in forest restoration in the Mediterranean basin because of its resistance to water stress in harsh, xeric, and degraded environments. However, its survival is not always optimal and, under certain site conditions, resistant stock is required (del Campo et al., 2007b). For example, in warm sites with shallow soils, stock quality may make an important difference in field performance, whereas in cooler sites with deep soils this difference disappears. This is practical evidence of the definition of seedling quality as “fitness for purpose” given thirty years ago by Lavender et al. (1980). Stock quality specifications are needed for particularly harsh environments and nurseries must be encouraged to grow these seedlings.

Official standards for Aleppo pine (Council Directive 1999/105/EC, Spanish Royal Decree 289/2003) recommend a minimum root collar diameter of 2 mm and seedling height to be between 8 and 25 cm, although commercial stock is usually below 15 cm due to forester’s preferences. There has been abundant research in the last two decades to address some of the most important issues relating to stock quality of Aleppo pine (Oliet et al., 1997, 2003, 2009; Puértolas et al., 2003, 2005; del Campo et al., 2007a,b). A recent review of this research has concluded that: i) some seedling morphological attributes could be larger than that currently being recommended and produced, with diameter in the 3-4 mm range and height in the 15-30 cm range (Navarro-Cerrillo et al., 2006); ii) nutritional status should maximize seedling nutrient content, with N concentrations around 20 mg g^{-1}, (Oliet et al., 2006); and iii) water status at planting has little impact on field performance of the species (Villar-Salvador et al., 1999). In the case of performance attributes, both frost hardiness and root growth capacity have been shown to affect seedling quality, although to different degrees (Fernández et al., 2003; Pardos et al., 2003; del Campo et al., 2007a).

Although there is much agreement about the need for greater nutrient-loaded seedlings for Aleppo pine, some aspects of seedling quality remain unclear, especially those related to mineral nutrition. For example, knowledge is lacking about the relationship between the species' response in the field and phosphorus, potassium, and nitrogen concentration (Fernandez et al., 2003; Oliet et al., 2006, 2009), which can be manipulated by changing fertilizer nutrient ratios. These studies show that fertilized stock usually have larger biomass, which brings along with it a confounding effect of their single effect in field performance. Thus, there is a need for separating the reciprocal influence of nutritional status on morphology and vice versa, which can be addressed by testing the performance of different-sized seedlings with similar nutrient concentrations and same-sized seedlings with different nutrient concentrations.

However, increasing seedling size to suggested values (Navarro-Cerrillo et al., 2006; Oliet et al., 2009) has important implications for both nursery production and field performance. In the field, integrated testing of large stocktypes, which is the focus of part II of this study (del Campo et al., 2011), is required. In nursery production, fertilization as well as thermal regime have overriding effects on plant growth (Landis et al., 1992). Aleppo pine production in Spain depends primarily on a moderate-sized stocktype grown in 200-250 cm^3 containers, located at the outside or in shade-houses in inland nurseries (500-1000 m.a.s.l.) during a single growing season (del Campo et al., 2007b). Given these conditions, the easiest and most feasible way to increase seedling size is to modify the fertilization program. Warmer coastal regions have traditionally been excluded from forest stock production due to their distance from reforestation areas and forest sites where colder temperatures may disrupt seedling acclimation to planting site (Pardos et al., 2003). Thus, a question that arises when thinking about larger stock production is if inland nurseries would be able to grow large-sized stock based only on fertilization changes, or if additional growing facilities (i.e. greenhouses) structures would be necessary to lengthen the growing season. Another question is whether coastal nurseries, which have more favourable climatic conditions for growing large seedlings, would benefit from this shift in stock specifications.

In practice, modifying fertilization regimes in current nursery production in order to increase growth can be accomplished by increasing the rate of fertilizer applications to the upper range of values that are commonly recommended (Landis et al., 1989). This may result in changes in the growing media solution as an increase in electrical conductivity (EC) (Jacobs and Timmer, 2005), an increase in nutrient leaching, or a decrease in nutrient assimilation efficiency (Edwards, 1985; Broschat, 1995). The combined use of water-soluble fertilizers injected into the irrigation system with controlled-release fertilizers has been proposed as means to enhance growth and improve nutrient efficiency (Eymar et al., 2000). Temperature also affects seedling nutrition (Whitcomb, 1988; Cabrera, 1997). Hence, increasing fertilization would lead to a decrease in efficiency in cooler nurseries. Therefore, the fertilization regime should be adapted to nursery culturing conditions, ensuring adequate concentration of nutrients in the growing media solution (Landis et al., 1989). Most research concerning fertilization practices in forest nurseries has been conducted in a single location, and generally focused on the use of material grown in different nurseries and then subjecting it to experimental treatments (Pardos et al., 2003; Puértolas et al., 2003; del Campo et al., 2007a,b). There is little quantitative information about how a determined fertilization program can modify stock growth and quality by itself when nursery conditions change. The information is even scarcer when considering the effectiveness and effects of different fertilizing nutrient ratios according to nursery location.

Considering these facts, we carried out a study in order to address the following questions: i) What is the comparative ability of inland and coastal forest nurseries to grow large Aleppo pine stock only varying fertilization management? ii) What differences in nursery culture, seedling growth, and seedling quality are associated with particular fertilization programs between coastal and inland nurseries? iii) What are the effects of changing the potassium (and secondary phosphorus) to nitrogen ratio on nursery culture, seedling growth and seedling quality and how does the fertilization system (controlled-release fertilizer, water-soluble fertilizer and K source) influences on it?

2. Materials and methods

Plant material, fertilizer treatments, and nursery culture

Seeds of *Pinus halepensis* Mill. were obtained from the official supplier in Valencia, Banc LLavors Forestals, Generalitat Valenciana, belonging to Spanish provenance region 10 (*Eastern Inland*, 39º03'N, 01º05'W). On April 16, 2006, seeds were sown into a sowing line using 50-alveolus trays (Fores-Pot 200 cm^3) in El Hontanar nursery (40º7’3’’N, 1º21’33’’W, 1200 m.a.s.l). A total of 3500 seedlings (70 trays) were sown in a growing media consisting of a peat-coconut fibre-vermiculite mix (45:45:10 vol.) and kept outside. On June 10, once seedlings had germinated and established, half of the trays were transferred to a nursery at the Universidad Politécnica de Valencia (39º29’12’’N, 0º20’24’’W, 10 m.a.s.l). The seedlings were grown in the open in both nurseries throughout the study, which is a common practice in the region. Both nurseries had contrasting climatic conditions that affect plant culture and growth.

The Hontanar nursery (HO) has a Mediterranean continental climate with an average rainfall and temperature of 572 mm and 10.2ºC (ETP: 603 mm; minimum mean temperature of the coldest month and maximum mean temperature of the warmest month are -2.0 and 28.2ºC respectively). The Universidad Politécnica nursery (UP) has a Mediterranean maritime climate. Average rainfall and temperature are 454 mm and 17.8ºC (ETP: 879 mm; minimum mean temperature of the coldest month and maximum mean temperature of the warmest month are 7.0 and 29.6ºC respectively). Specific data from the 2006 growing year in both nurseries are given in the results and discussion sections.

The main criterion for treatments definition consisted of elevating K:N ratio into currently operating fertilization programs (CRF and water-soluble fertilizer) which was obtained by adding potassium alone or combining potassium with additional increments of nitrogen and phosphorus. Following Edwards (1985) and Landis (2005), potassium sources were chosen according to the prevailing salinity level in irrigation water: potassium sulphate (K_2SO_4, 0-0-52, N-P_2O_5-K_2O) for single potassium enrichment and monopotassium phosphate (KH_2PO_4, 0-51.5-34, N-P_2O_5-K_2O) plus potassium nitrate (KNO_3, 13.5-0-46, N-P_2O_5-K_2O) for potassium, nitrogen and phosphorus enrichment. These fertilizers were applied into currently operating fertilization programs: controlled release fertilizers (CRF) and water-soluble fertilizers injection. In the case of CRF, both a high-end (Osmocote Plus 16-8-12+2MgO 8-9 m) and standard fertilizer commonly used in commercial nurseries in Valencia (Plantacote Pluss 14-8-15 +Mg+TE 8-9 m) were used. Thus, seven treatments were applied with varying dosage and type of fertilizer used (Table 1). The CRF represented control treatments with the standard K:N ratio used in tree nurseries of this species.

In the case of water-soluble fertilizer injected into the irrigation system (treatments F-K, P-K, P-KNP, O-K and O-KNP, Table 1), treatments were applied twice per week with a 10-L watering can in which the solutions were prepared. Water-soluble fertilization began on June 10th in both nurseries, with the exception of the *Starter* application in treatment F-K, which ran from May 10th to June 9th. In both nurseries, watering, environmental conditions, and measures to prevent fungi and other pests followed standard practices for this species and stocktype. From mid-September to the end of culture, watering was reduced to induce a physiological response in the seedlings to water stress. In each nursery, the 35 trays were divided into three experimental blocks of 14, 14 and 7 trays each (corresponding to 100, 100 and 50 seedlings per fertilization treatment per block). The different measurements were equally applied among these three blocks.

Fertilizer efficiency determinations

To evaluate the effectiveness of the fertilization treatments, leachates from each treatment were collected and analyzed every two weeks. Leachate was collected by sealing a plastic bag to the bottom of each of six individual alveolus selected randomly from the five trays constituting a single treatment. Leachates were left to accumulate in the bags for two weeks and then gathered immediately before the application of the next fertilization, thereby corresponding to four fertilization applications (two per week) and the interim watering. A composite sample of leachates from individual alveolus was taken per treatment, nursery and sampling date, gauged, poured into a plastic recipient and carried to a laboratory (Laboratorio Agroalimentario de Burjassot, Generalitat Valenciana) where the following measurements were made: electrical conductivity (EC, 25ºC mS/cm), pH, and nutrient concentrations (mg/L) of K, NO_3^-, NO_2^-, NH_4^+, Ca, Mg, P and SO_4^{2-}, following standard methods: electrometric method for pH and EC, ion chromatography for N compounds, P and SO_4^{2-}, and optical emission spectrometry for Ca, K, and Mg (APHA, 1998). These data were used to compare: 1) nutrient concentrations and chemical properties in the leachates among fertilization treatments and nursery location, and 2) nutrient (K, N, P) uptake efficiency together with tissue nutrient content, which was calculated monthly as:

$$Ef_i = \frac{Nfc_i - Nfc_{i-1}}{(Nfc_i - Nfc_{i-1}) + Nlc_i} \times 100 \ (\%)$$

where Nfc_i is the foliar nutrient content (mg) at time i (i=15-jul, 15-Aug, 10-sep, 25-oct) and Nlc_i is the leachate nutrient content (mg) accumulated between i-1 and i dates, which corresponds to two or more samples. Leachate nutrient content is preferred to nutrient supply because of the temporal uncertainty of CRF regarding nutrient availability. This formula only calculates the fraction of nutrients recovered, or that found in leachate plus needles. Regarding nitrogen efficiency, the N content of NO_3^-, NO_2^- and NH_4^+ was summed to estimate total nitrogen in the leachate. Final accumulated efficiency was also computed as the foliar nutrient content in October with respect to the total amount of nutrients in the leachate.

Seedling growth and development

From mid July to November, seedling growth and development were determined by measuring morphological and physiological attributes from a randomly selected sample: height (H, cm), stem diameter (D, mm), root and shoot dry biomass at 65ºC (RB and SB, g), root length (m), average root diameter (mm), root fibrosity (percentage of root length with diameter lower than 0.5 mm, %), length of plug pre-existing white-functional roots (m) and nutrient (N, P, K, Ca, Mg and Fe) concentrations (%) in needles. Height and diameter were measured biweekly from a sample of 120 seedlings. The other attributes were measured monthly. Biomass was measured from a sample of 15 seedlings. Root architecture attributes were determined from 5 seedlings using

WinRhizo© v.3.1 software (Regents Instruments Inc.). To determine nutrient concentrations, composite samples of foliar tissue from 15 plants (identical biomass from every seedling) per treatment were oven-dried (70ºC), sieved through a 0.5 mm screen and sent to a laboratory for analysis (Laboratorio Agroalimentario de Burjassot, GV). After preparation of plant tissue by the dry ash method and digesting the samples in concentrated H_2SO_4 with a selenium catalyst, nitrogen was measured by the micro Kjeldahl method with a Kjeltec Auto 1030 Analyser (Tecator, Sweden). Phosphorous was assayed colorimetrically using the phosphomolybdovanadate method (420 nm) in a colorimeter (Technicon Autoanalyzer AAII) and cations were measured using a Varian SpectraAA-10 Atomic Absorption Spectrometer (AOAC, 2000).

In November, the pre-dawn water potential (MPa) of 5 seedlings per treatment was measured using a pressure chamber (Soil Moisture, Santa Barbara, California). Root growth capacity (RGC, g, as dry mass of new white roots) of 15 seedlings per treatment was measured in a growth chamber for 10 days (del Campo et al., 2007a).

Statistical analyses

In general, chemical properties of leachates were not normally distributed according to a Shapiro−Wilk's test. Therefore, a nonparametric Kruskal–Wallis test was used separately for nursery and fertilization factors and Tamhane's T2 test was chosen as the post-hoc test for comparing the means. To compare leachate differences among fertilization treatments, data were normalized for each sampling date due to differences in the volume collected (as watering needs were different according to date and nursery).

Fertilization efficiency and seedling growth were analysed with ANOVA and ANCOVA analyses (Ferrán, 2001), with date as the covariate, following the models:

ANOVA: $y_{ijk} = \mu + \alpha_i + \tau_j + \nu_k + \varphi_{ij} + \varepsilon_{ijk}$

where μ is the true overall mean for y, α_i (i = 1,2), τ_j (j = 1,...,7) and ν_k (k=1,..3) are respectively the deviations due to the nursery location, the fertilization treatment and the block effect fixed factors, φ_{ij} is the interaction between nursery and fertilization treatment and ε_{ijk} is the error term.

ANCOVA: $y_{ijk} = \mu + \alpha_i + \tau_j + \nu_k + \varphi_{ij} + \beta\,(z_{ijk} - z...) + \varepsilon_{ijk}$

where μ is the true overall mean for y, α_i (i = 1,2), τ_j (j = 1,...,7) and ν_k (k=1,..3) are respectively the deviations due to the nursery location, fertilization treatment and the block effect fixed factors after allowance of y to date (z), φ_{ij} is the interaction between nursery and fertilization treatment, β is the true common slope of the regression lines, $z...$ is the overall average of the covariate (date) and ε_{ijk} is the error term.

Seedling growth was analyzed both between consecutive time intervals (in detail) and for the whole growth period (accumulated trend). Regarding the detailed scale, differences between treatments were tested by analyzing the relative growth rates (RGR) of height, diameter, shoot and root dry biomass. RGR was computed for every two consecutive measurements in each variable according to Hoffmann and Poorter (2002). A multivariate ANCOVA or MANCOVA, was performed on the four RGR variables with date as the covariate and fertilizer treatment and nursery as fixed factors. Root architecture variables were also analyzed by means of a MANCOVA analysis.

Differences in the cumulative growth trend along the experiment were analyzed by comparing the slope and intercept of regression lines of each growth variable (height, diameter, shoot and root dry biomass) among treatments and nurseries. Instead of using date of measurement as the x variable, degree-day (calculated over 7.5ºC for the corresponding 2006-date and nursery at each measurement) was chosen because of its better fit than date, as this is the main difference between the nurseries. Growth variables (y) were log-transformed. In addition, seedling allometry between height-diameter and between shoot-root dry biomass was analyzed following the general model $\ln y = a + b \ln x$, where *x=height* or *shoot biomass* and *y=diameter* or *root biomass*. Intercept and slope differences among treatments and nurseries were examined with an F test.

Post-hoc tests on covariate-adjusted factors were conducted by pairwise comparisons of estimated marginal means using Bonferroni adjusted P-levels. In all cases (ANOVAs and ANCOVAs), data were examined to ensure normality and homogeneity of variance (Levene test). When these assumptions were violated, the variables were transformed with power and logarithmic functions to achieve homoscedasticity. In the case of ANCOVAs, regression slopes were tested to be homogeneous and independent of treatments by observing the interaction term between the covariate and the treatment in the ANCOVA output. When the assumption of parallel treatment regression lines was violated, a scatter-plot of the data was examined to decide whether to proceed with ANCOVA or not. A significance level of $P \leq 0.05$ was used for all analyses. Data were analysed with SPSS© 16.0.

3. Results

Leachate analysis, fertilizing efficiency and seedling nutrient status during the culture

Chemical properties of leachates (EC, NO_3^-, P and K) differed significantly with both, nursery location and fertilization factors (Figure 1A. Kruskal-Wallis analyses not shown). Comparing location, EC and sulphate concentration showed a parallel trend, being significantly (P<0.001) higher in the UP nursery (EC average of 3.87 mS/cm) compared to the HO nursery (EC average of 1.84 mS/cm) throughout the culture. By contrast, leachate macronutrient concentrations were significantly lower in the UP nursery, averaging 145, 282 and 12.6 mg l^{-1} of K, NO_3^- and P respectively, compared to 216, 530 and 31.7 mg l^{-1} (K, NO_3^-, P; P-values: 0.006, 0.002 and 0.000 respectively) in the HO nursery. Regarding the fertilization treatments, Kruskal-Wallis test revealed significant differences in all variables ($P < 0.001$) except NO_2 and NH_4. Treatments F-K (regular water-soluble fertilizer plus K_2SO_4) and P-KNP (Plantacote plus KH_2PO_4 plus KNO_3) were markedly different from the other five treatments, producing higher nutrient concentrations, higher EC and lower pH in the leachates (EC: 3.21 and 4.85 mS/cm; pH: 6.23 and 6.68; K: 281 and 305 mg l^{-1}; NO_3: 669 and 638 mg l^{-1}; P: 32 and 48 mg l^{-1}, for F-K and P-KNP treatments respectively). In contrast, O-KNP (Osmocote plus KH_2PO_4 plus KNO_3) and O-K (Osmocote plus K_2SO_4) treatments presented a contrary pattern (EC: 1.85 and 2.14; pH: 7.27 and 7.03; K: 113 and 146; NO_3: 274 and 273; P: 17 and 7, for O-KNP and O-K treatments respectively). The remaining treatments presented an intermediate pattern, with O (Oscomote) and P (Plantacote) presenting lower EC, potassium and phosphorus than the average, and P-K (Plantacote plus K_2SO_4) presenting lower pH and nitrates and higher K concentration in the leachate than the average.

This pattern had a direct and significant influence on fertilization efficiency, which differed among the fertilization treatments and between both nurseries (Table 2). However, ANCOVA results showed a minor effect of fertilization treatment on efficiency relative to nursery and date (see F values in Table 2). Overall efficiency for the three macronutrients was twice as high in the UP nursery as the HO nursery (Table 2). Efficiency tended to increase from July to October in all treatments in the UP coastal nursery, peaking at values around 50% for nitrogen and phosphorus and 21% for potassium (total treatments average). In the inland nursery (HO), efficiency increased until September (19, 17 and 10% for N, P and K respectively) but then decreased by approximately one half in October.

Potassium efficiency was the lowest among the three macronutrients and was higher in the P, O and O-KNP treatments compared to the F-K and P-KNP treatments (Table 2). However, a Tukey's test showed only marginal differences ($p \leq 0.1$) between O and F-K treatments. Nitrogen efficiency was also lower in the P-KNP and especially the F-K treatments (significant differences only between P and F-K). Finally, phosphorus efficiency was slightly lower than that of nitrogen (Table 2) and was significantly higher for the O and O-K treatments with respect to the F-K treatment as well as for the O-K compared to the P-KNP treatment.

Final accumulated efficiency (Figure 1B) reveals the temporal influence of efficiency values presented above. It is notable that there are i) higher values in the UP nursery, ii) higher values in N and P with respect to K and iii) lower values in F-K and P-KNP treatments.

Seedling nutrient concentrations along the experiment differed significantly between nurseries for N, P and K and between fertilization treatments for P and K (Table 3). Differences in seedling N concentration were not consistent among fertilization treatments during the study period, which likely explains why no significant effect was detected. Macronutrient concentrations were higher in the HO nursery and for treatments F-K and P-KNP throughout the experiment (Figure 2). P, P-K and O-KNP treatments showed higher fluctuations in seedling nutrient concentration from date to date (ANCOVA considered values from five different dates). No interactions were found between nursery and treatments, indicating that the pattern of the seven treatments with different macronutrient concentrations was constant in both nurseries (Figure 2).

Seedling growth: RGR, cumulative trend and allometry

Multivariate tests of MANCOVA (*Pillai's Trace, Wilks' Lambda, Hotelling's Trace and Roy's Largest Latent Root*) revealed no significant fertilization effect on RGRs from July to November. Main effects confirmed this fact for the individual RGR variables in height, diameter, shoot and root biomass (Table 3). In contrast, nursery location and especially measurement date, significantly affected RGRs, which were higher in the first weeks and in the coastal nursery (UP) (Figure 3). Mean values for RGR_H in the UP nursery were about twice those in the HO nursery, whereas the remaining variables were closer between the nurseries, although significantly lower in HO (Table 3). Fertilization treatments were not significant, but some intra-nursery differences highlight: in the HO nursery, RGR (all variables) was significantly higher in F-K than in O and O-K. In the UP nursery, RGR in biomass and diameter were higher in O-K than in P and P-KNP (not shown).

MANCOVA multivariate tests on root architecture indicated a significant effect of nursery location and fertilization treatment. However, main effects confirmed this fact in all root variables set only in the nursery factor (values for HO and UP were respectively: 10.2 and 15.3 m for root length; 0.34 and 0.38 mm for average root diameter; 81 and 77% for fibrosity; and 3.1 and 4.6 m for white-functional roots length). Regarding the fertilization factor, only root length was lower in F-K with respect to the remaining treatments (except O-K) and the length of white roots was higher in P-K compared to F-K, O-K and O-KNP.

The cumulated growth trend of seedlings (height, diameter, shoot and root biomass) along the culturing period presented an excellent fit with the degree-day variable (Figure 4 A to D) and both slope and intercept terms were significantly different between nurseries (Table 4). On the other hand, cumulative seedling growth was affected by fertilization treatments to a much lower degree, as height was the only variable showing significant differences in slope (Table 4). This was due to the F-K treatment, which had a significantly higher slope coefficient than the P and P-KNP treatments (data not shown). Despite this, only one regression line was fitted for simplicity (Figure 4A). Regression intercepts were significantly different for height and diameter growth (Table 4), indicating, in the latter, that differences among treatments are due only to initial differences at the beginning of the measuring period (July 2006). In contrast, no effect of fertilization on biomass growth was detected.

Regarding seedling allometry, results again revealed a significant effect of nursery location on both height-diameter and shoot-root allometric relationships but not of fertilization (Table 4). Thus, only one regression line per nursery has been plotted for each pair of variables, independent of treatments (Figure 4 E-F). In particular, above ground biomass (height or shoot biomass) was larger than below ground biomass (diameter or root biomass) in the UP coastal nursery, as indicated by its lower allometric coefficient (slope). This pattern was more pronounced for height-diameter than for shoot-root biomass (slope values are closer).

Final seedling quality

In contrast with the above results, final seedling attributes differed substantially among fertilization treatments and nurseries, although the latter had greater weight in the differences with a minor contribution of fertilization treatment and fertilization x nursery interaction (F values, Table 5). Exceptions were [N], which differed only among fertilization treatments, and water potential and root growth capacity, which differed only between nurseries. In general, plants from the inland nursery (HO) had lower biomass, height and diameter, and higher nutrient (P and K) concentrations, water potential (-0.12±0.05 MPa in HO and -0.18±0.08 MPa in UP) and root growth capacity (0.125±0.105 g in HO and 0.049±0.027 g in UP).

Regarding the fertilization treatments, due to the significant interactions with location, a nursery separated post-hoc test was performed (Table 5). In the HO nursery, all potassium fertilization treatments showed significant higher concentrations in this nutrient than the P and O treatments (control). The F-K treatment showed the highest values in both nurseries (1.51% and 1.03% for HO and UP respectively). Final K concentration in the UP nursery was very similar among treatments except for F-K. Fertilization with KH_2PO_4 and KNO_3 led to higher macronutrient concentrations (N, P and K) than K_2SO_4, although differences were not always significant. Most treatments followed a similar pattern when considering either nutrient concentration or content (not shown), although the F-K treatment showed lower nutrient contents (N and K), especially in the UP nursery. In general, the F-K treatment was associated with lower seedling development, higher SB/RB ratio and higher nutrient concentrations. Osmocote treatments (O, O-K and O-KNP) presented a higher N concentration than Plantacote treatments; phosphorus concentration was in agreement with its supply, being higher in P-KNP and O-KNP treatments. Treatments O and P-KNP presented higher morphology values independent of the nursery; O-KNP showed lower height and root biomass in the HO nursery but the opposite was true in the UP nursery.

4. Discussion

The results have identified differences in fertilization efficiency, seedling growth, allometry and final quality attributes.

Regarding fertilization efficiency and seedling nutrient status, leachate and efficiency results demonstrated important differences between nurseries and a considerable gap between the F-K and P-KNP treatments with respect to the other fertilization treatments. Differences between nurseries can mainly be attributed to their contrasting climates, (i.e., continental and coastal) which are known to affect seedling quality and field performance in Aleppo pine (Pardos et al., 2003; Puértolas et al., 2005). Temperature is one of the main factors influencing plant nutrition and root function of this species, with optimal values in the 18-29ºC range (Whitcomb, 1988). During the study period, the weekly average temperatures in the continental (HO) nursery ranged from 13.2 to 24.4ºC, whereas in the coastal nursery (UP) weekly average temperatures were between 19.0 and 27.8ºC. These differences had a pronounced effect on most of the variables measured in this work.

On the other hand, observed differences between treatments were more or less congruent with the nutrient concentrations in the fertilizers (F-K and P-KNP treatments supplied higher nutrient amount). It is known that increasing nutrient dosage usually leads to an increase in nutrient uptake (Oliet et al., 1997, 2003, 2004) but also leaching (Oliet et al. 1999), thus inducing a lower efficiency. The method of continuous leachate collecting used here revealed considerable variation in the leaching volume and nutrient concentration during the study (Broschat, 1995; Stowe et al., 2010). This makes fully reliable comparisons with other studies difficult, although mean EC in the F-K and P-KNP treatments in the UP nursery were high, very high or in the danger area (Oliet et al., 2004; Jacobs and Timmer 2005) regardless of the extraction method. This is likely to be a direct consequence of the water gypsum content and its contribution to EC (Papadopoulos, 1986), as sulphate leaching in treatments without potassium sulphate averaged 1046mg L^{-1} in the UP nursery compared to 177 mg L^{-1} in the HO leachates. Efficiency values for N, P and K were below the usual range found in the literature (Cabrera, 1997; Huett, 1997b; Oliet et al., 2004), especially those observed in the HO nursery. This may be due to the different ways of reporting efficiency among authors, whether or not the amount of nutrient supplied is considered, the remaining amount in prills in the case of CRF, the nutrient content of the substrate solution, the leaching fraction, or loss of nutrients such as nitrogen due to volatilization and denitrification (Niemiera and Leda, 1993). Moreover, it should be noted that the efficiencies measured here correspond only to the nutrient content of needles, the biomass of which was between 39 and 57% of total seedling biomass depending on the treatment and date (overall average was 47%). This means that the efficiencies reported here could be increased by a factor of about 1.7-2.5, considering the variation in nutrient concentration in different tissues reported for this species (Oliet et al., 1999; 2004; Royo et al., 2001). This would increase UP efficiency values to be within the ranges found by the studies cited above for N and P (46-90% in N; 60-94% in P) but not potassium (41-88% in K), which is likely due to the higher amounts supplied in our case which have whittle it down. Efficiencies were also low in the HO nursery (in spite of the above mentioned arguments), which is in agreement with the higher nutrient concentration found in the leachates from this nursery and is a likely consequence of lower nutrient uptake rates due to lower crop development and root plug colonization (Oliet et al., 2004).

EC values above 4 mS cm^{-1} (saturated media extract) may result in root injury and reduce seedling growth (Huett, 1997a; Jacobs and Timmer 2005). However, seedlings from the UP nursery did not show apparent deficiencies in root development, but rather had higher values in root architecture than those from the HO nursery (e.g. treatment P-KNP in UP). Working with Aleppo pine, Oliet et al. (2004) registered EC values above 5 mS cm^{-1} in the saturation extract and reported a good seedling quality in terms of growth and nutritional status. In this study, the F-K treatment (which presented high EC) showed lower morphological values in some of the above and below ground variables considered. However, growth rates in this treatment over the July-October period did not differ from the other treatments nor its biomass increment through time.

Then, the differences should be produced before, between May 10, when the application of *Starter* fertilizer began, and July 14, when the first measurement took place. In this period, the only known factor that could have contributed to the impediment of growth in the F-K treatment is high salinity from the *Starter* fertilizer, which was applied in concentrations of 70, 174 and 141 mg L^{-1} of N, P and K respectively, when recommended values are 50, 100 and 100 mg L^{-1} for N, P and K respectively (Landis et al., 1989). This would explain the significant differences in the intercept of height and diameter growth for this treatment (see Figure 4). Germination and establishment phases in seedling growth are especially sensitive to salinity build-up in the growing media solution (Jacobs and Timmer 2005), although in our case these differences in growth were lower than those attributed to the nursery factor.

Nursery location has been found to have a determinant effect on seedling growth and its nutritional status during culturing. Thus, results indicate that the fertilization treatment had a minor influence on seedling development relative to temperature-related factors such as nursery location and sampling date. This is corroborated by the goodness of fit obtained between cumulative seedling growth and temperature of the growing season (degree-day), which is commonly found to have the largest effect on growth and biomass accumulation (Whitcomb, 1988; Nedlo et al., 2009). However, Aleppo pine is also able to respond significantly to fertilization treatments in terms of morphological and physiological attributes (Oliet et al., 1999, 2003). In fact, our results indicate that there were differences in the final values of quality attributes due to fertilization. Then, the absence of differences in growth rates according to fertilization treatment is likely due to the much higher importance of nursery location over fertilization treatment when they are considered together, thus masking the former the effect of the latter. Previous studies on Aleppo pine observed this same general pattern, with seedlings in the range

9-16 cm height for inland nursery stock and above 20 cm height for coastal or greenhouse produced stock (Oliet et al., 1999, 2003, 2009; Puértolas et al., 2003; del Campo et al., 2007a,b). Another argument in explaining the absence of treatment differences in growth rates but not in final quality attributes has been stated previously and is related with the very early stage of this experiment, before any growth measurement took place (from germination to mid July). Figure 4 indicates that some differences among treatments were produced in that phase, maintained over the culturing period and finally translated into final quality attributes, thus giving a special importance to the early stage of nursery culture in this species. RGR is used to avoid the additive effect of seedling size on growth (which follows the compound interest law), so initial differences between treatments were not detected in the growth analysis carried out in this work. Thus, beyond a threshold of nutrient supply, Aleppo pine appears to be not very sensitive to fertilization, and temperature is the main factor governing seedling growth and development.

However, growth-temperature slopes were different for each nursery indicating that some other factor might have played a role in growth rates between nurseries. Water stress conditioning in the hardening phase was carried out by reducing watering from late September onwards. However, this technique was difficult in the cooler nursery (HO) because of higher humidity and lower evaporation characteristic of the region and season. In addition, smaller seedlings did not transpire fast enough to dry out the plugs (Villar-Salvador et al., 2004). Thus water stress conditioning could only be carried out effectively in the coastal nursery, where higher seedling size and evaporation rate induced moderate to strong water stress during the final growth phase. This can be demonstrated by the pre-dawn water potential and root growth capacity, which only showed differences between nurseries and are generally lower for Aleppo pine under water stress conditioning (Villar-Salvador et al., 1999; Royo et al., 2001). The influence of this cultural treatment on seedling growth can be observed in Figure 4, where UP values beyond 3500 degree-day showed lower dependence on temperature. In fact, deducing these values from the plot, slope and intercept differences between both regression lines would smooth, indicating a very similar temperature influence on seedling growth independently of the nursery.

Allometric analysis indicates that the aboveground portion of seedlings was the preferred sink for photosynthesis gains in the UP nursery. This result is in agreement with results for RGR, in which the difference between height (and shoot biomass) and diameter (and root biomass) growth rates were higher in the UP nursery than in the HO nursery. This pattern could be a consequence of the lower temperatures in the continental nursery. In some pine species, lower temperatures have been found to stimulate calliper and/or root growth while higher temperatures stimulate needle growth (Gowin et al., 1980; Hellmers and Rook, 1973 cit. in Landis et al., 1992). However, allometric differences in our case are more likely a consequence of the high growing density (390 plants m^{-2}) and limited container volume (200 cm^3). For a given seedling size, higher growing density is associated with higher shoot length (Landis et al., 1990). In addition, it is known that root restriction imposed by container volume may hinder allometry expression (Climent et al., 2008) and compel plants to allocate greater resources to shoots. Root biomass of large Aleppo pine seedlings grown in 200-230 cm^3volumes usually falls within the range found here, i.e. 1.40-1.70 g, independently of shoot size (Oliet et al., 1997, 2004, 2009). These values would be the upper limits of root biomass in this container volume. Once the limits are reached, seedlings allocate more biomass to their shoot.

Despite differences among fertilizer treatments in the final quality assessment, again nursery appears to have been the main governing factor differentiating the stock. All treatments in the UP nursery agree with morphological values recently proposed for this species (Navarro-Cerrillo et al., 2006). On the other hand, HO seedling values are similar to those previously reported for this species and nursery (del Campo et al., 2007a,b), which exhibited medium to high survival. Regarding physiological attributes, nutrient concentrations were in the upper range or higher than those observed in similar studies (Oliet et al., 2004, 2009; Fernández et al., 2003; del Campo et al., 2007a, b). However, it has been proposed that K/N and P/N ratios are more important than the content of each nutrient separately (Landis et al., 1989) and that 0.45-0.55 and 0.14-0.20 would be the adequate range for K/N and P/N, respectively. In this study, UP seedlings fell within these ranges but HO seedlings surpassed them, suggesting acclimation to low temperatures as described by Fernández et al. (2003). These authors also observed higher root growth capacity for cold hardened seedlings, but Pardos et al. (2003) did not found any relation between nursery location and RGC. In fact, the lower RGC of UP seedlings was likely related to the hardening irrigation practised in that nursery, as explained before. Several studies have demonstrated lower values in RGC test when water stress is mediated (Tinus, 1996; Villar-Salvador et al., 1999; Vallas-Cuesta et al., 1999). Thus, this cultural practice seems easier to treat in warmer nurseries.

5. Conclusions

The results found in this study indicate that inland nurseries relying only on fertilization changes may be limited in their capacity to grow large stock-types of Aleppo pine. Temperature appeared to have an overriding effect on seedling growth and high doses of fertilizer were associated with low efficiencies. Two-year-old stock-type or greenhouse infrastructure represent an alternative. Warmer regions seem to be more suitable for producing large stock more efficiently (more cost-effective, better crop control with watering, higher fertilizer efficiencies, etc.). Fertilization regimes had little effect on seedling growth relative to nursery location, although their relative effect was fairly consistent within each nursery (no interaction occurred). However, fertilization did affect final seedling attributes, which was likely due to the presence of early differences between treatments that were maintained until the end of culture. Most differences among the fertilizer programs can be explained by the total amount of nutrients supplied (N, P and K). K/N or P/N ratios have not performed similarly in the different variables considered in this study. Thus, K/N or P/N did not seem to play an important role in seedling nutrition.

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References

AOAC 2000 Official methods of analysis. Association of Official Analytical Chemists, 17th ed., Washington, DC.

APHA 1998 Standard methods for the examination of water and waste water. 20th ed. American Public Health Association, Washington, DC.

Broschat, T.K. 1995 Nitrate, phosphate and potassium leaching from container grown plants fertilized by several methods. *HortScience* **30(1)**, 74-77.

Burdett, A.N. 1990 Physiological processes in plantation establishment and the development of specifications for forest planting stock. *Can. J. For. Res*. **20**, 415-427.

Cabrera, R.I. 1997 Comparative evaluation of nitrogen release patterns from controlled release fertilizers by nitrogen leaching analysis. *HortScience* **32(4)**, 669-673.

Climent, J., Alonso, J., Gil, L. 2008 Short Note: Root restriction hindered early allometric differentiation between seedlings of two provenances of Canary Island pine. *Silvae Genet*. **57**, 4–5.

Del Campo, A.D., Navarro-Cerrillo, R.M., Hermoso, J., Ibáñez, A.J. 2007a Relationships between site and stock quality in *Pinus halepensis* Mill. reforestations on semiarid landscapes in eastern Spain. *Ann. For. Sci.* **64**, 719-731.

Del Campo, A.D., Navarro-Cerrillo, R.M., Hermoso, J., Ibáñez, A.J. 2007b Relationships between root growth potential and field performance in Aleppo pine. *Ann. For. Sci*. **64**, 541-548.

Del Campo, A.D., Hermoso, J., Flors, J., Lidón, A., Navarro-Cerrillo, R.M. 2011 Nursery location and potassium enrichment in Aleppo pine stock 2. Performance under real and hydrogel-mediated drought conditions. *Forestry*, In revision.

Edwards, I.K. 1985 How to maximize efficiency of fertilizers in a forest tree nursery. Proc. of the Intermountain Nurserymen Association Meeting, Fort Collins, CO. 99-103.

Eymar, E., Cadahia, C., Sánchez, A., López-Vela, A. 2000 Combined effect of slow release fertilizer and fertigation on nutrient use of *Cupressus glabra* grown in nursery conditions. *Agrochimica* **XLIV(1-2)**, 39-48.

Fernández, M., Royo, A., Gil, L., Pardos, J.A. 2003 Effects of temperature on growth and stress hardening development of phytotron-grown seedlings of Aleppo pine (*Pinus halepensis* Mill.). *Ann. For. Sci*. **60**, 277–284.

Ferran Aranaz, M. 2001 SPSS para Windows. Análisis estadístico. McGraw-Hill/Interamericana de España. 421 p.

Gowin, T., Lourtioux, A., Mousseau, M. 1980 Influence of constant growth temperature upon the productivity and gas exchange of seedlings of Scots pine and European larch. *Forest Sci.* **26(2),** 301-309

Hoffmann, W.A., Poorter, H. 2002 Avoiding bias in calculations of relative growth rate. *Ann. Bot*. **80**, 37–42.

Huett, D.O. 1997a Fertiliser use efficiency by containerised nursery plants. 1. Plant growth and nutrient uptake. *Aust. J. Agric. Res.* **48**, 251-258.

Huett, D.O., 1997b. Fertiliser use efficiency by containerised nursery plants. 2. Nutrient leaching. *Aust. J. Agric. Res.* **48**, 259-265.

Jacobs, D.F., Timmer, V.R. 2005 Fertilizer-induced changes in rhizosphere electrical conductivity: relation to forest tree seedling root system growth and function. *New For.* **30**, 147–166.

Landis, T.D. 2005 Macronutrients, Potassium. *Forest Nursery Notes*, **Winter 05**, 5-11.

Landis, T.D., Tinus, R.W., McDonald, S.E., Barnett, J.P. 1989 Seedling nutrition and irrigation, Vol. 4, The Container Tree Nursery Manual. Agric. Handbk. 674. Washington, DC: U.S.D.A., Forest Service. 119 p.

Landis, T.D., Tinus, R.W., McDonald, S.E., Barnett, J.P. 1990 Containers and growing media, Vol. 2, The Container Tree Nursery Manual. Agric. Handbk. 674. Washington, DC: U.S. U.S.D.A., Forest Service. 88 p.

Landis, T.D., Tinus, R.W., McDonald, S.E., Barnett, J.P. 1992 Atmospheric Enviroment, Vol. 3, The Container Tree Nursery Manual. Agric. Handbk. 674. Washington, DC: U.S.D.A. Forest Service. 145 p.

Lavender, D., Tinus, R., Sutton R., Poole, B. 1980 Evaluation of Planting Stock Quality. *N. Z. J. Forest Sci.* **10**, 293–300.

Navarro-Cerrillo, R.M., Villar-Salvador, P., del Campo, A. 2006 Morfología y establecimiento de los plantones, in: Calidad de planta forestal para la restauración en ambientes Mediterráneos. Estado actual de conocimientos. Cortina, J. et al. (eds.), Organismo Autónomo de Parques Naturales, MMARM, Madrid, pp, 67-88.

Nedlo, J.E., Martin, T.A., Vose, J.M., Teskey, R.O. 2009 Growing season temperatures limit growth of loblolly pine (*Pinus taeda* L.) seedlings across a wide geographic transect. *Trees* **23,** 751–759.

Niemiera, A.X., Leda, C.E. 1993 Nitrogen leaching from Osmocote fertilized pine bark at leching fractions of 0 to 0.4. *J. Environ. Hortic*. **11(2)**, 75-77.

Oliet, J., Planelles, R., Lopez, M., Artero, F. 1997 Efecto de la fertilización en vivero sobre la supervivencia en plantación de *Pinus halepensis*, *Cuad. Soc. Esp. Cien. For.* **4** 69-79.

Oliet, J., Segura, M.L., Martín, F., Blanco, E., Serrada, R., López, M., Artero, F. 1999 Los fertilizantes de liberación controlada lenta aplicados a la producción de planta forestal de vivero. Efecto de dosis y formulaciones sobre la calidad de *Pinus halepensis* Mill. *Inv. Agrar.-Sist. Recursos Fore.* **8(1),** 207-228

Oliet, J., Planelles, R., Artero, F., Martínez, E., Alvarez Linarejos L., Alejano, R., Lopez, M. 2003 El potencial de crecimiento radical en planta de vivero de *Pinus halepensis* Mill. Influencia de la fertilización. *Inv. Agrar.-Sist. Recursos Fore.* **12(1),** 51-60.

Oliet, J., Planelles, R., Segura, M.L., Artero, F., Jacobs. D.F. 2004 Mineral nutrition and growth of containerized *Pinus halepensis* seedlings under controlled-release fertilizer. *Sci. Hortic.* **103(1),** 113-129

Oliet, J.A., Valdecantos, A., Puértolas, J., Trubat, R. 2006 Influencia del estado nutricional y el contenido en carbohidratos en el establecimiento de las plantaciones, in: Calidad de planta forestal para la restauración en ambientes Mediterráneos. Estado actual de conocimientos. Cortina, J. et al. (eds.), Organismo Autónomo de Parques Naturales, MMARM, Madrid, pp 89-117.

Oliet, J., Planelles, R., Artero, F., Valverde, R., Jacobs, D.F., Segura, M.L. 2009 Field performance of *Pinus halepensis* planted in Mediterranean arid conditions: relative influence of seedling morphology and mineral nutrition. *New For.* **37**, 313–331.

Papadopoulos, I., 1986. Effect of high sulphate irrigation waters on soil salinity and yields. *Agron. J.* **78**, 429-432

Pardos, M., Royo A., Gil L., Pardos J. 2003 Effect of nursery location and outplanting date on field performance of *Pinus halepensis* and *Quercus ilex* seedlings. *Forestry* **76(1)**, 67-81.

Puertolas, J., Gil, L., Pardos, J.A. 2003 Effects of nutritional status and seedling size on field performance of *Pinus halepensis* planted on former arable land in the Mediterranean basin. *Forestry* **76(2)**, 159-168.

Puertolas J., Gil L., Pardos J.A. 2005 Effects of nitrogen fertilization and temperature on frost hardiness of Aleppo pine (*Pinus halepensis* Mill.) seedlings assessed by chlorophyll fluorescence. *Forestry* **78(5)**, 501-511.

Royo, A., Gil, L., Pardos, J. 2001 Effect of water stress conditioning on morphology, physiology and field performance of *Pinus halepensis* Mill. seedlings. *New For.* **21**, 127-140.

SPSS Inc. 2003 SPSS for Windows, release 12.0, SPSS. Inc., Chicago.

Stowe, D.C., Lamhamedi, M.S., Carles, S., Fecteau, B., Margolis, H.A., Renaud, M., Bernier, P.Y. 2010 Managing irrigation to reduce nutrient leaching in containerized white spruce seedling production. *New For.* DOI 10.1007/s11056-010-9193-0

Tinus, R.W. 1996 Root growth potential as an indicator of drought stress history. *Tree physiol.* **16**, 795-799.

Vallas-Cuesta, J., Villar-Salvador, P., Peñuelas, J., Herrero, N., Domínguez, S., Nicolas, J. 1999 Efecto del aviveramiento prolongado sin riego en la calidad funcional de brinzales de *Pinus halepensis* Mill y desarrollo en campo. *Montes* **58**, 51–58.

Villar-Salvador, P., Ocaña, L., Peñuelas, J. and Carrasco, I., 1999. Effect of water stress conditioning on the water relations, root growth capacity, and the nitrogen and non-structural carbohydrate concentration of *Pinus halepensis* Mill. (Aleppo pine) seedlings. *Ann. For. Sci.* **56(6)**, 459-465.

Villar-Salvador, P., Peñuelas Rubira, J.L., Vallas-Cuesta, J. 2004 Dessication patterns of *Pinus halepensis* seedlings grown in different types of containers. *Cuad. Soc. Esp. Cien. For.* **17**, 93-99.

Whitcomb, C.E. 1988 Plant production in containers. Stillwater, OK: Lacebark Publications. 633 p.

Treatment code	**Fertilizer description**	**Date of application** (2006)	**Fertilizer Dosage**(2) g l^{-1}	**Total nutrient supply,** mg $plant^{-1}$			**K/N**	**P/ N**
				K	**N**	**P**		
P	Plantacote (CRF(1))	Sowing to lifting	4†	100	112	28	0.89	0.25
O	Osmocote (CRF)	Sowing to lifting	4†	80	128	28	0.63	0.22
F-K	Starter(7-40-17)	Starter: 10.5-10.6	1*	439	243	136	1.81	0.56
	Grower (18-11-18)	Grower:11.6-18.9	1*					
	Finisher (4-19-35)	Finisher:19.9-19.10	0.6*					
	K_2SO_4	10.6-19.10	0.241*					
P-K	Plantacote (CRF)	Sowing to lifting	4†	252	112	28	2.25	0.25
	K_2SO_4	10.6-19.10	0.241*					
P-KNP	Plantacote (CRF)	Sowing to lifting	4†	252	139	88	1.81	0.63
	KH_2PO_4	10.6-19.10	0.172*					
	KNO_3		0.131*					
O-K	Osmocote (CRF)	Sowing to lifting	2.5†	202	80	18	2.53	0.23
	K_2SO_4	10.6-19.10	0.241*					
O-KNP	Osmocote (CRF)	Sowing to lifting	2.5†	202	107	78	1.89	0.73
	KH_2PO_4	10.6-19.10	0.172*					
	KNO_3		0.131*					

Table 1. Seven fertilization treatments applied to *Pinus halepensis* stock grown in two nurseries with contrasting climate (coastal: UP and continental: HO). (1) Controlled-Release Fertilizer. (2) Dosage in water* (twice weekly) or in substrate† (at time of sowing).

<table>
<tr><td></td><td colspan="2">F(13,42); MSE=119.8</td><td colspan="2">F(13,42); MSE=186.7</td><td colspan="2">F(13,42); MSE=184.5</td></tr>
<tr><td>Source of variation (fixed)</td><td colspan="2">Potassium</td><td colspan="2">Nitrogen</td><td colspan="2">Phosphorus</td></tr>
<tr><td>Date (covariate)</td><td colspan="2">11.98**</td><td colspan="2">27.23**</td><td colspan="2">28.03**</td></tr>
<tr><td>Nursery</td><td colspan="2">25.79**</td><td colspan="2">79.78**</td><td colspan="2">80.14**</td></tr>
<tr><td>Fertilization Treatment</td><td colspan="2">2.94*</td><td colspan="2">2.82*</td><td colspan="2">4.51**</td></tr>
<tr><td>Nursery x Fert. Treat.</td><td colspan="2">0.57</td><td colspan="2">0.78</td><td colspan="2">0.91</td></tr>
<tr><td colspan="7">Efficiency (%)</td></tr>
<tr><td>P</td><td colspan="2">12.6(8.3)</td><td colspan="2">29.1(19.6)</td><td colspan="2">24.1(23.8)</td></tr>
<tr><td>O</td><td colspan="2">14.2(10.9)</td><td colspan="2">21.0(17.6)</td><td colspan="2">25.8(20.8)</td></tr>
<tr><td>F-K</td><td colspan="2">4.0(2.9)</td><td colspan="2">11.0(9.1)</td><td colspan="2">11.0(7.5)</td></tr>
<tr><td>P-K</td><td colspan="2">8.1(7.1)</td><td colspan="2">28.1(18.5)</td><td colspan="2">22.7(21.6)</td></tr>
<tr><td>P-KNP</td><td colspan="2">5.7(4.3)</td><td colspan="2">18.5(17.7)</td><td colspan="2">11.9(11.5)</td></tr>
<tr><td>O-K</td><td colspan="2">7.0(4.5)</td><td colspan="2">21.9(17.2)</td><td colspan="2">31.8(23.7)</td></tr>
<tr><td>O-KNP</td><td colspan="2">11.2(10.1)</td><td colspan="2">25.3(23)</td><td colspan="2">22.8(22.3)</td></tr>
<tr><td>Nursery average</td><td>HO</td><td>UP</td><td>HO</td><td>UP</td><td>HO</td><td>UP</td></tr>
<tr><td></td><td>5.8(5.3)</td><td>12.2(8.8)</td><td>10.8(8.8)</td><td>33.4(17.7)</td><td>9.6(6.7)</td><td>33.2(21.6)</td></tr>
</table>

Table 2. Summary of the ANCOVAs (F value, degrees of freedom, Mean Square Error and significance: $^*P \leq 0.05$, $^{**}P \leq 0.01$) performed on macronutrient efficiency and average (plus standard deviation) of macronutrient efficiency (from four different dates) according to nursery location (HO: Hontanar; UP: Polytechnic University) and fertilization treatment. Post-hoc groups are described in text.

Source	N	K	P	Relative Growth Rate (RGR)			
				Height	Diameter	Shoot Biomass	Root Biomass
Power transf.	2.525	0.854	1.525	-	-	Variance heterog.	-
Degrees freedom	13, 105	13, 105	13, 105	6, 146	6, 146	6, 146	6, 146
MSE	1.563	0.019	0.0036	0.0014	0.0014	0.0042	0.0036
F: Date (Cov.)	319.1**	81.26**	15.58**	175.2**	141.293**	282.77**	295.88**
F: Nursery Locat.	12.32**	65.17**	55.25**	57.3**	65.347**	21.768**	16.64**
F: Fertiliz. treat.	1.6	10.44**	12.43**	1.33	0.603	0.973	0.576
F: Nurs. x Fertiliz.	0.15	0.80	0.48	0.39	0.31	0.59	0.58
Average HO	2.13%(0.29)	1.25%(0.22)	0.36%(0.09)	0.037cm cm^{-1}week^{-1}	0.055mm mm^{-1}week^{-1}	0.130g g^{-1}week^{-1}	0.145g g^{-1}week^{-1}
Average UP	1.96%(0.32)	1.00%(0.25)	0.28%(0.07)	0.081cm cm^{-1}week^{-1}	0.060mm mm^{-1}week^{-1}	0.157g g^{-1}week^{-1}	0.153g g^{-1}week^{-1}

Table 3. Summary of the ANCOVAs (variable transformation, Mean Square Error, F value, degrees of freedom and significance: *$P \leq 0.05$, **$P \leq 0.01$) performed on macronutrient (N, K, P) concentration in needles and on RGR (Height, Diameter, Shoot and Root Biomass) of Aleppo pine seedlings according to nursery location and fertilization treatment. The last two rows show the means (plus standard deviation) of each nursery location from different culture dates. Post-hoc groups of fertilization treatments in nutrient concentrations are represented in Figure 2.

		Growth trend analysis								**Allometric analysis**			
	y-value	ln_Height		ln_Diametr.		ln_Shoot B.		ln_Root B.		ln_Diamtr.		ln_Root B.	
	x-value	Degree-day (^{o}C)								ln_Height		ln_Shoot B.	
	Source	*Itercp.*	*Slope*	*Itercp.*	*Slope*	*Itercp.*	*Slope*	*Itercp.*	*Slope*	*Itercp.*	*Slope*	*Itercp.*	*Slope*
Nursery Location	F-value	30.50**	10.77*	209**	48**	74.9**	11.9**	170**	31.9**	160.8**	180.7**	120.2**	18.9**
Fertilization Treatments	F-value	9.01**	2.28*	3.91*	0.3	1.88	0.22	1.29	0.05	0.24	0.34	0.33	0.61

Table 4. Results of the comparison among regression lines (representing growth trend of Aleppo pine with degree-day and allometric models) according to nursery location and fertilization treatments: F-value and significance of ANOVAs performed on intercept and slope variables in the models fitted (*$P \le 0.05$; **$P \le 0.01$).

Source	**Height, cm**		**Diam., mm**		**Shoot B, g**		**Root B, g**		**N, %**		**P, %**		**K,%**	
Nursery	7630**		2184**		539**		127.1**		.184		1031**		661**	
Fertiliz.	19.2**		36.5**		3.3*		10.8**		64.0**		115**		105**	
Nur x Fert	9.0**		11.0**		4.3**		3.8**		7.77**		32.3**		19.9**	
Nurs. Fert.	HO	UP	HO	UP	HO	UP	HO	UP	HO	UP	HO	UP	HO	UP
P	8.8^{a}	20.3^{b}	2.69^{cd}	3.70^{b}	1.08^{a}	2.60^{ab}	1.03^{bc}	1.47^{b}	1.66^{a}	1.72^{ab}	0.39^{b}	0.21^{a}	0.96^{b}	0.79^{ab}
O	9.4^{b}	22.1^{c}	2.67^{bcd}	4.17^{d}	1.16^{a}	3.10^{b}	0.91^{abc}	1.60^{b}	2.02^{c}	1.89^{cd}	0.26^{a}	0.18^{a}	0.79^{a}	0.71^{a}
F-K	8.8^{a}	18.2^{a}	2.5^{ab}	3.22^{a}	1.16^{a}	1.88^{a}	0.80^{ab}	0.88^{a}	1.88^{b}	2.00^{d}	0.39^{b}	0.29^{c}	1.51^{e}	1.03^{c}
P-K	9.5^{b}	20.9^{bc}	2.68^{cd}	3.95^{c}	1.19^{a}	2.95^{b}	1.04^{c}	1.56^{b}	1.69^{a}	1.66^{a}	0.39^{b}	0.19^{a}	1.18^{d}	0.74^{ab}
P-KNP	9.6^{b}	21.6^{bc}	2.82^{d}	4.04^{cd}	1.21^{a}	2.98^{b}	1.03^{bc}	1.52^{b}	1.75^{a}	1.67^{a}	0.52^{c}	0.30^{c}	1.26^{d}	0.84^{ab}
O-K	8.6^{a}	18.8^{a}	2.55^{abc}	3.69^{b}	1.02^{a}	2.62^{b}	0.92^{abc}	1.33^{b}	1.72^{a}	1.71^{ab}	0.28^{a}	0.18^{a}	1.06^{c}	0.75^{ab}
O-KNP	8.4^{a}	21.9^{c}	2.47^{a}	3.70^{b}	0.94^{a}	2.89^{b}	0.74^{a}	1.46^{b}	1.76^{a}	1.80^{bc}	0.58^{d}	0.25^{b}	1.22^{d}	0.86^{b}
Average	9.0	20.5	2.6	3.8	1.11	2.72	0.92	1.4	1.78	1.78	0.39	0.23	1.13	.81

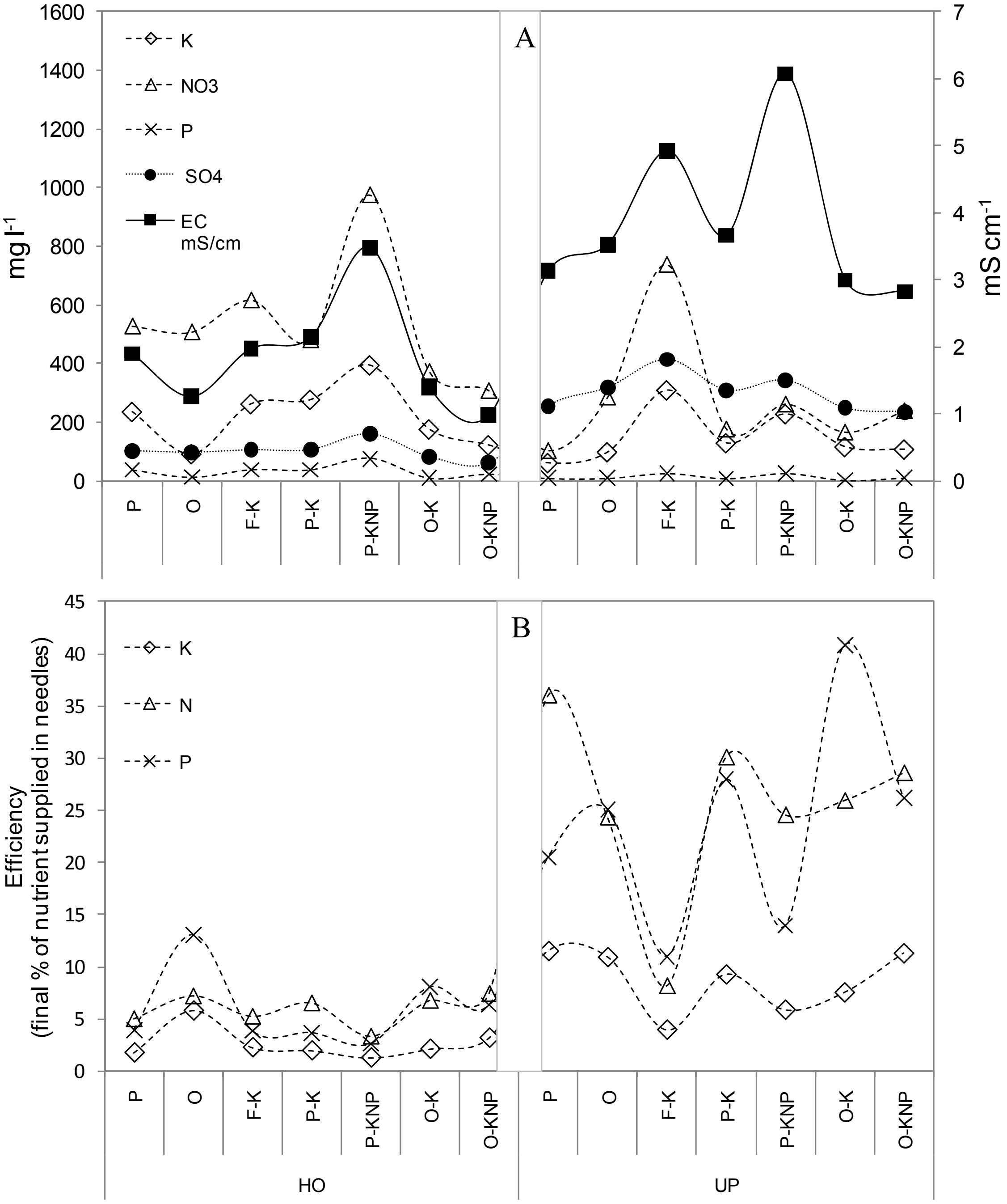
Table 5. Summary of ANOVAs (F-value, significance: *$P\leq0.05$; **$P\leq0.01$, treatments and nursery means and post-hoc groups) performed on final values of morphological and physiological attributes of *Pinus halepensis* grown under seven fertilization enrichment treatments in two nursery locations (HO: inland; UP: coastal). In a column, different letters among treatments indicate significant differences according to Tukey test ($P\leq0.05$).

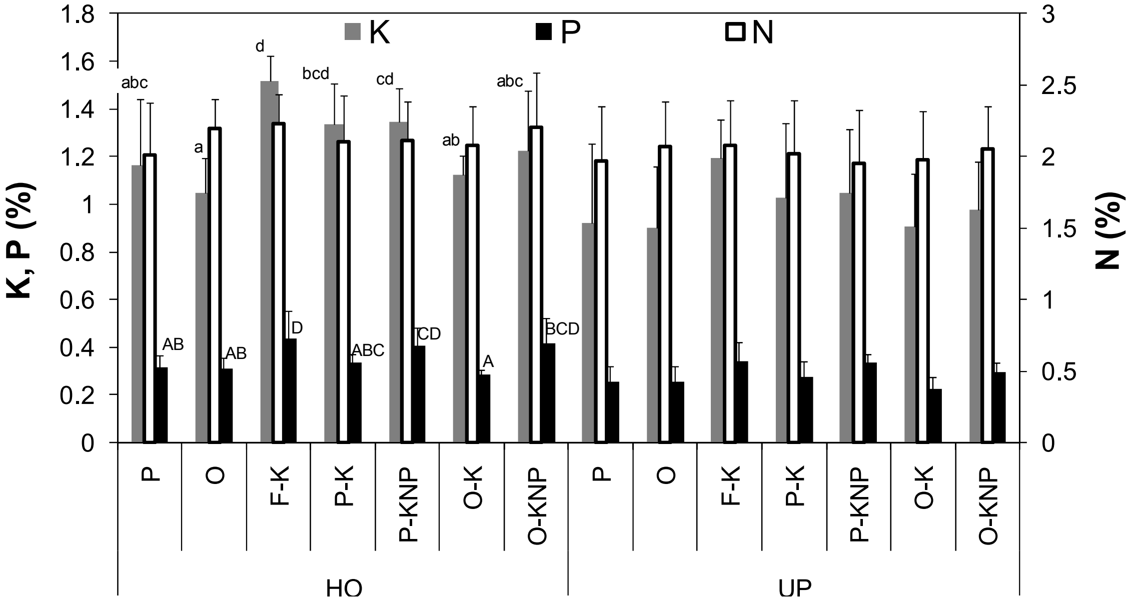
Figure 1. Leachate macronutrient concentrations and EC (A) and final macronutrient fertilizing efficiency (B), calculated as the total amount of nutrient leached from June to October and the final nutrient content in the seedling, of Aleppo pine seedlings grown under seven fertilization treatments in two nursery locations with contrasting climates (HO: inland; UP: coastal).

Figure 2. Mean macronutrient concentrations in Aleppo pine needles during culture (four sampling dates) in seven fertilization treatments grown in two forest nursery locations (HO: inland; UP: coastal). For K and P, different letters indicate statistical differences in the ANCOVA post-hoc test at $P \leq 0.05$.These groupings refer to fertilization treatment independent of the nursery (are the same in UP location). Mean values and standard error are reported.

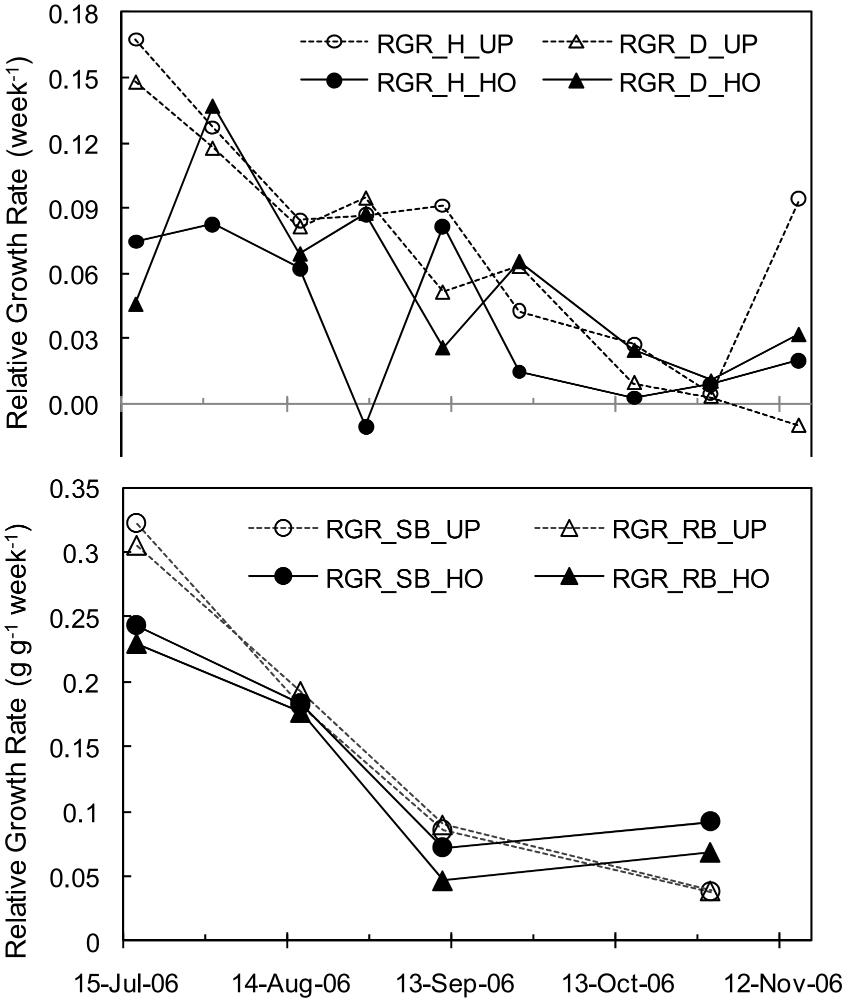
Figure 3. Weekly relative growth rates in height (H, cm $cm^{-1}week^{-1}$), diameter (D, mm mm^{-1} $week^{-1}$), shoot biomass (SB, g g^{-1} $week^{-1}$) and root biomass (RB, g g^{-1} $week^{-1}$) of Aleppo pine seedlings grown in two forest nursery locations (—HO: inland and ---UP: coastal) (fertilization factor did not exert a significant effect on RGR).

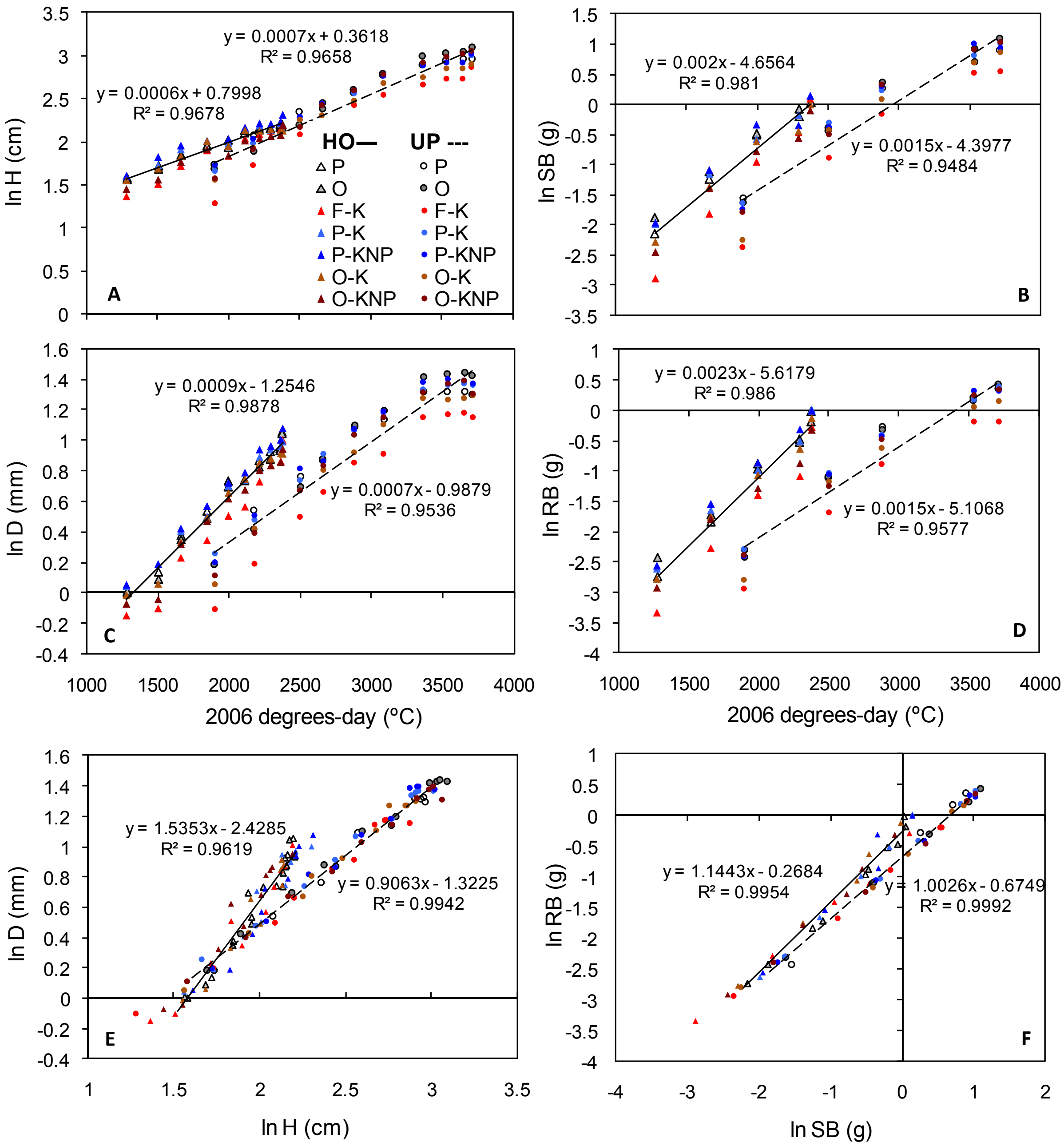
Figure 4. Regression models between growth variables and degree-day (A to D) and allometric models for Diameter-Height and Root Biomass-Shoot Biomass (E and F) of Aleppo pine according to nursery location (—HO: continental and ---UP: coastal) (fertilization factor originated almost no differences). All models are significant at $P \leq 0.01$.


A
B
mg l-1
mS cm-1
Efficiency (final % of nutrient supplied in needles)
K
NO3
P
SO4
EC mS/cm
N
0
200
400
600
800
1000
1200
1400
1600
1
2
3
4
5
6
7
5
10
15
20
25
30
35
40
45
P
O
F-K
P-K
P-KNP
O-K
O-KNP
HO
UP



K
P
N
K, P (%)
N (%)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
3
2.5
2
1.5
1
0.5
0
abc
AB
a
AB
d
D
bcd
ABC
cd
CD
ab
A
abc
BCD
P
O
F-K
P-K
P-KNP
O-K
O-KNP
P
O
F-K
P-K
P-KNP
O-K
O-KNP
HO
UP


RGR_H_UP
RGR_D_UP
RGR_H_HO
RGR_D_HO
Relative Growth Rate (week^{-1})
0.18
0.15
0.12
0.09
0.06
0.03
0.00
RGR_SB_UP
RGR_RB_UP
RGR_SB_HO
RGR_RB_HO
Relative Growth Rate (g g^{-1} week^{-1})
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
15-Jul-06
14-Aug-06
13-Sep-06
13-Oct-06
12-Nov-06


A
ln H (cm)
y = 0.0007x + 0.3618
R² = 0.9658
y = 0.0006x + 0.7998
R² = 0.9678
HO—
UP ---
△ P
△ O
▲ F-K
▲ P-K
▲ P-KNP
▲ O-K
▲ O-KNP
○ P
● O
● F-K
● P-K
● P-KNP
● O-K
● O-KNP
B
ln SB (g)
y = 0.002x - 4.6564
R² = 0.981
y = 0.0015x - 4.3977
R² = 0.9484
C
ln D (mm)
y = 0.0009x - 1.2546
R² = 0.9878
y = 0.0007x - 0.9879
R² = 0.9536
2006 degrees-day (°C)
D
ln RB (g)
y = 0.0023x - 5.6179
R² = 0.986
y = 0.0015x - 5.1068
R² = 0.9577
2006 degrees-day (°C)
E
ln D (mm)
y = 1.5353x - 2.4285
R² = 0.9619
y = 0.9063x - 1.3225
R² = 0.9942
ln H (cm)
F
ln RB (g)
y = 1.1443x - 0.2684
R² = 0.9954
y = 1.0026x - 0.6749
R² = 0.9992
ln SB (g)
1000
1500
2000
2500
3000
3500
4000