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in

Leone V. (ed.), Lovreglio R. (ed.).
Proceedings of the international workshop MEDPINE 3: conservation, regeneration and restoration of Mediterranean pines and their ecosystems

Bari : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 75

2007
pages 199-205

EFFECTS OF EXPONENTIAL AND CONVENTIONAL FERTILIZATION ON GROWTH NUTRIENT UPTAKE BY FIVE MEDITERRANEAN PINES IN GREENHOUSE

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Abstract

Photoperiod, temperature, humidity and fertilization are the main factors which influence active development in the nursery phase. Fertilizers, which usually contain greater amounts of nitrogen than other nutrients, can be applied in two ways; conventionally, by periodically adding of constant doses of nutrients and exponentially increasing the nutrient doses in accordance with the stage of crop development. Previous experiments have focused on the exponential application of nutrients with the aim of producing a positive effect in pine growth even with relatively low doses.
In the present study, we relate seedlings growth parameters (stem thickness and total weight) with nutrient concentrations in five representative pine species of the Iberian Peninsula (Pinus halepensis, P. pinaster, P. pinea, P. nigra, P. sylvestris), fertilized once a week in two ways, with constant doses of nutrients (conventional fertilization) and with increasing amounts of the nutrients (exponential fertilization). All the pines were grown in a transparent glass greenhouse with controlled temperature, luminosity and humidity (using tensiometers inside the pot). Additionally, temperature and soil humidity were measured daily with ten tensiometers (one per pine-substrate combination). The influence of two different growth substrates was investigated with the conventional fertilization regime: a mixture of perlite-vermiculite (1:1 v/v) and coco peat. The exponential fertilization was conducted only in the perlite-vermiculite mixture as substrate. In both fertilizations, the final quantity of nutrients (i.e., the total quantity of nitrogen) supplied to the different pines was the same (70 mg of nitrogen/plant). The experiment was conducted over 32 weeks. Pines specimens were sampled at weeks 12 and 32 (the end of the experiment).
There was a generally positive response in the pines fertilized conventionally compared with those fertilized exponentially with respect to all 3 studied parameters. Amongst the different pines species, the mountain pines (P. sylvestris and P. nigra) showed lower values following both fertilizations.

INTRODUCTION

Fertilization is one of the most widely cultivation techniques in the nursery, since plant and root development depend on soil fertility and humidity.

The need for quality plants capable of resisting transplant stress, was the driving force behind research into exponential fertilization, an alternative method of fertilization, of which Ingestad and Lund [3] were pioneers in this new fertilization method. Ingestad [2] showed that maximum seedling growth occur when nutrients are applied exponentially increasing the doses until the maximum relative growth of the plant is achieved.

Such exponential fertilization should provide plants with “steady-state nutrition” whereby plants grow with constant internal concentrations and free from nutrient stress [3].

Several authors have studied the influence of the exponential fertilization on conifers and interaction with opportunist nearest vegetation [5]. Such studies have shown the positive effect that such fertilization has on weed control and on the microorgnism population in the rhizosphere, such as mycorrhiza [8], concluding that the combination of exponential fertilization and mycorrhiza results in plants with a greater nutrient content, especially N-P-K.

In the present work, the effect of exponential and conventional fertilization, on the thickness and nutrient accumulation was compared in pines characteristic in the Iberian Peninsula (*P. pinea* L, *P. sylvestris* L, *P. pinaster* Aiton, *P. halepensis* Miller, *P. nigra* Arnold).

MATERIALS AND METHODS

Growth conditions

Seeds for all five pines species came from the Centro Nacional de Mejora Forestal “El Serranillo”. All seeds were surface sterilized with hydrogen peroxide 30% for 30 min and washed tree times with sterile water.The substrate used for both fertilization techniques was Perlite-Vermiculite 1:1 (v:v), while a second substrate coconut coir was studied only for conventional fertilization. The different treatments and the total pine plants can be seen in the table 1.

Table1. Total pine seedlings for each fertilization and substrate treatment

Fertilization	Number of seedlings
Conventional:	
Perlite-Vermiculite	350
Coconut coir	350
Exponential	
Perlite-Vermiculite	300

Substrates were autoclaved (two times in different days, 121 ºC) and placed in growth containers WM (Thermoflan, France) with 450 cm^3 of volume. Ten tensiometers were used to obtain optimal drainage for each fertilization and pine species. Temperature was measured daily in 5 different points of the greenhouse. The pines were maintained with 16 hours light and moved periodically.

Fertilization regimes

For both fertilizations treatments the experiments lasted 32 weeks (fig 1). The total quantity of fertilizer was calculated according to the N content (70 mg) for both the exponential and conventional fertilization. Weekly applications were supplied in the form of a commercial water soluble fertilizer mixture (Peter´s Professional® 20-7-19).

Fertilizer treatments started 3 weeks after germination. The nutrients were added manually with a plastic measuring cap, all seedlings receiving the same volume of solution (30 mL) per application, although fertilizer doses varied with treatments and avoided saline toxicity.

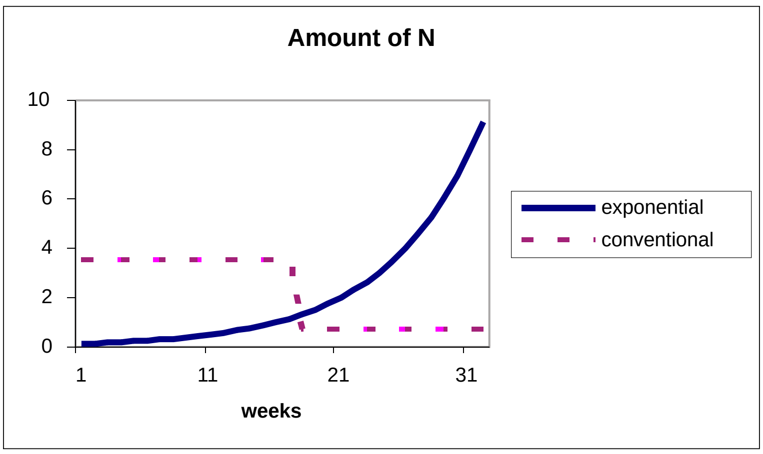

Fig 1. Amount of N provided conventionally and exponentially per seedlings over 32 weeks

For conventional treatment, the fertilization schedule was divided into two steps, providing 3.53 mg N/seedling during the first 17 weeks and 0.666 mg N/seedling during the remaining 15 weeks. The purpose of reducing the N content was the study the interaction between each fertilization method and the ectomycorrhizal fungi, *Lactarius deliciosus* (L. ex Fries) S. F. Gray.

The delivery schedule for exponential fertilization was based on the function described by Ingestad and Lund [4] and Timmer and Armstrong [9]:

$$N_T = N_s\ (e^{rt}\ \ 1)$$

where:
r is the relative addition rate required to increase N_s, the initial level of N, to a final level of $N_T + N_S$.
N_T is the total amount of N applied (70 mg in our study)
N_S is the quantity of N in the seedling at the start of fertilization (0.854 mg for the present study)

After calculating r for the 32 weeks, the amount of N that should be delivered in each fertilization N_t was calculated as follows:

$$N_t = N_s\ (e^{rt}\ \ 1)\ \ N_{t-1}$$

where N_{t-1} is the cumulative amount of N added up to and including the previous application.

Numeric values of weekly released N can be seen in the table 2. The N concentration was also studied in order to avoid salinity problems.

Table 2. Fertilization schedule for both fertilizations. Amounts based on N (mg)

Week	Exponential	Conventional	ppm Exponential	ppm Conventional
1	0.334	3.530	11.142	117.667
2	0.332	3.530	11.055	117.667
3	0.334	3.530	11.133	117.667
4	0.341	3.530	11.382	117.667
5	0.354	3.530	11.812	117.667
6	0.373	3.530	12.434	117.667
7	0.398	3.530	13.263	117.667
8	0.430	3.530	14.318	117.667
9	0.381	3.530	12.705	117.667
10	0.438	3.530	14.585	117.667
11	0.502	3.530	16.744	117.667
12	0.577	3.530	19.221	117.667
13	0.662	3.530	22.066	117.667
14	0.760	3.530	25.331	117.667
15	0.872	3.530	29.079	117.667
16	1.001	3.530	33.382	117.667
17	1.150	3.530	38.322	117.667
18	1.320	0.666	43.993	22.200
19	1.515	0.666	50.503	22.200
20	1.739	0.666	57.976	22.200
21	1.997	0.666	66.555	22.200
22	2.292	0.666	76.403	22.200
23	2.631	0.666	87.709	22.200
24	3.021	0.666	100.688	22.200
25	3.468	0.666	115.587	22.200
26	3.981	0.666	132.691	22.200
27	4.570	0.666	152.326	22.200
28	5.246	0.666	174.867	22.200
29	6.022	0.666	200.743	22.200
30	6.913	0.666	230.448	22.200
31	7.936	0.666	264.549	22.200
32	7.936	0.666	264.549	22.200
	69.827mg N	**70 mg N**		

Seedlings harvesting and statistical analysis

Seedlings root thickness was measured at week 17 and 32 (the end of the experiment). Three randomized samples were taken for each pine-fertilization-substrate combination. The samples were dried in an oven 70 ºC for 72 hours. Dried pines were ground in a grinder for chemical analysis of the nutrient content (N-P-K).

The statistical analysis of the nutrient concentration of the different pine species was carried out with the univariant model of the SPSS statistical program (SPSS for windows 12.0; SPSS Inc., Chicago, IL, USA), with the Games-Howell post-hoc comparison because there was no homogeneity in the varience. Seedlings root thickness was also measured with SPSS but, in this case, with ANOVA of repeated measures.

RESULTS

Seedling root thickness.

The conventional fertilizer treatment resulted in statistically significant increase in root thickness for all the pine species (fig 2). Pines fertilized conventionally resulted in an increment in growth development at week 17 and 32 (fig 3).

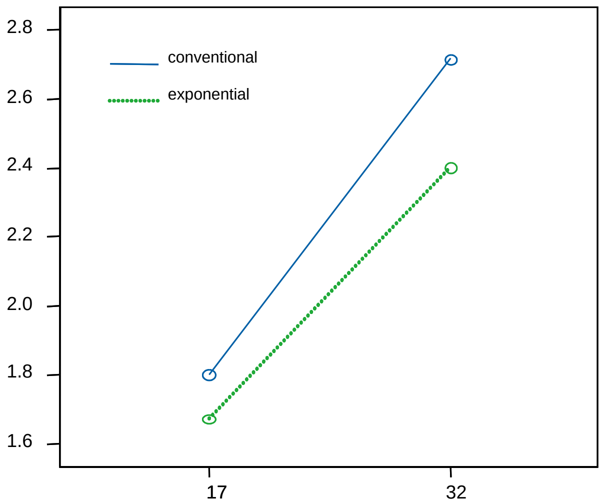

Fig. 2. Root thickness (cm) differences between both fertilization treatments for all the 5 pine species during the experiment

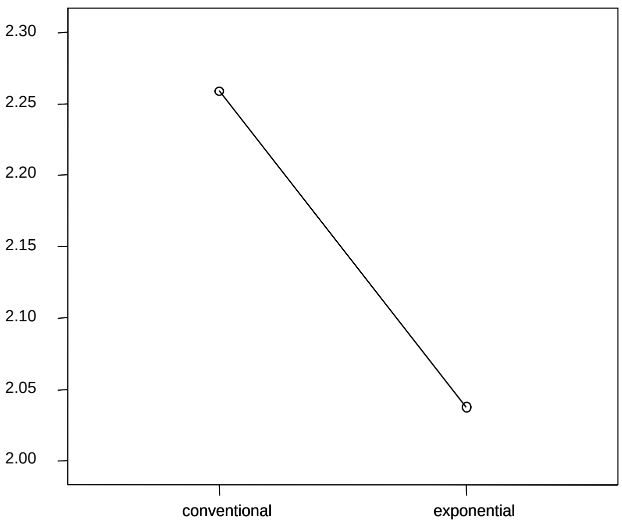

Fig. 3. Root thickness (cm) evolution for both fertilizations in weeks 17th and 32th

Of the different pine species, *P. pinea* developed the greatest root thickness (fig 4), although the difference between fertilizations was small at the end of the experiment. *P. halepensis* and *P. pinaster* showed the largest differences (statistically significant), between treatments, in favour of the conventional treatment.

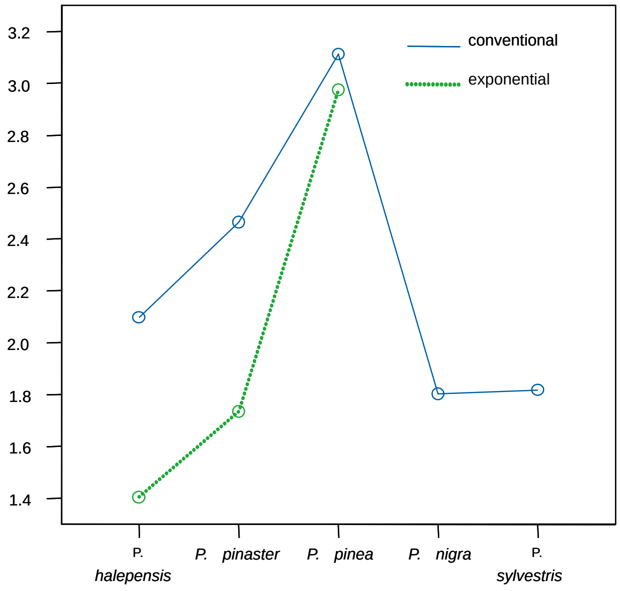

Fig 4. Root thickness differences (cm) for each pine species and fertilization method

Almost all the populations of *P. nigra* as well as *P. sylvestris* died at the outset of the experiment so the values are not shown.

The effect of coconut coir substrate in conventional treatment produced an increase in root thickness diameter in all the pine species with the exception of *P. halepensis*, the increase being maximum in *P. pinaster* and *P. pinea* (data not shown).

Nutrient content

The nutrient concentration in terms of N-P-K differed between fertilization treatments (fig 5). Conventional fertilization led to more N and K being accumulated in the leaves than in the exponential treatment, although P concentration was highest after exponential fertilization. None of these differences was statistically significant.

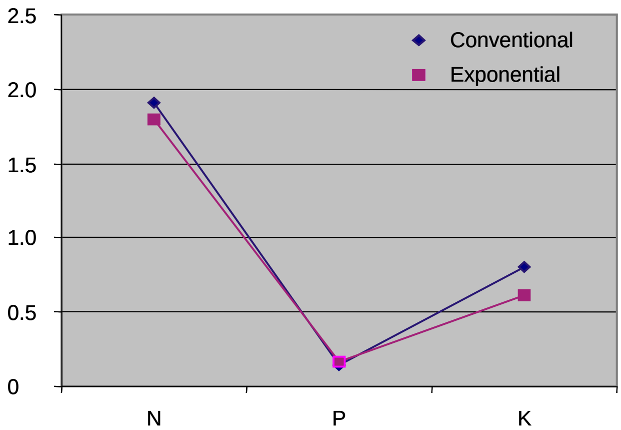

Fig. 5. N-P-K concentration (mg) in leaves for each fertilization treatment

N accumulation in pine leaves differed between species and fertilization method while *P. pinaster* and *P. halepensis* showed the highest values after conventional fertilization, the N concentration in *P. sylvestris* and *P. pinea* was highest in the case of exponential fertilization (fig. 6). For conventional fertilization, the type of substrate gave rise to significant differences, N accumulation being greater when Perlite-Vermiculite was used rather than coconut coir.

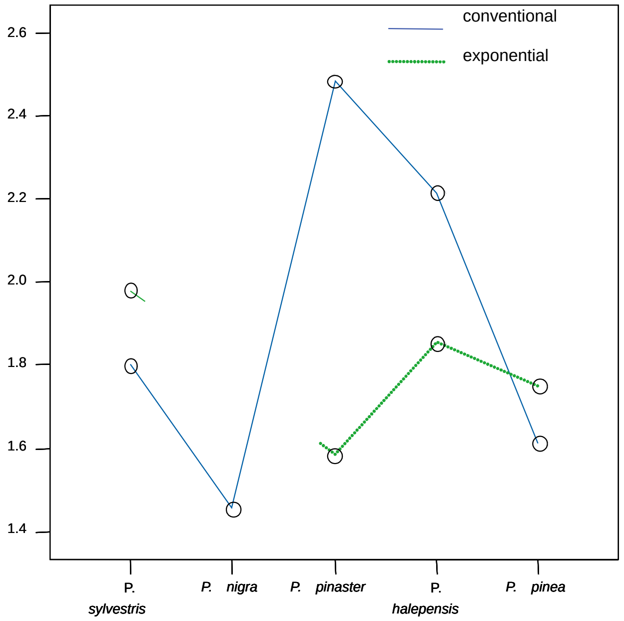

Fig. 6. N concentration (mg) in leaves of each pine and fertilization method

P accumulation in *P. pinea* and *P. halepensis* was highest after exponential fertilization while the rest of the pine species showed similar values for both fertilization methods (data not shown). The K concentration in pine leaves was higher with conventional fertilization for all species, particularly in the case of *P. pinaster* (data not shown).

DISCUSSION

Exponential fertilization of pines in the greenhouse resulted in lower root thickness in all the Iberian pines studied, although the amount of N-P-K in leaves was greater in some varieties, as observed by Hawkins et al. [1]. The rates of exponentially fertilized pines would possibly have been greater if larger amounts of nutrients had been added.

Similarly the length of the research might not have favoured exponential fertilization. 70 mg of N per seedling was supplied during the 32 weeks the experiment lasted. The curve of the exponential nutrient treatment reveals the low dose of nutrients released during the first weeks, which presumably caused serious injures even death during the first stages of growth, especially for *P. nigra* and *P. sylvestris*. During the first 17 weeks, conventional fertilization released 3.53 mg of N per seedling and week, which was probably excessive because, although no cases of injures was observed in the seedlings, no evidence of mycorrhization in pine roots was seen after the addition of mycorrhizal fungi (17 week). Parladé et al [6] on the other hand, obtained good results for mycorrhization in *P. pinaster* and *P. sylvestris* after using 3.6 mg of N per seedling every 14 days.

The size of the container probably played a relevant role and 450 cm^3 containers used could have led to diffusion of the nutrients rendering them unavailable to seedlings. Containers in previous works were no bigger than 110 cm^3 with the exception of those used by Qu et al. [7] working with *Larix* (287 cm^3) all quite different from the size used in the present work. The diffusion of nutrients could be especially important in early stage of pine development, when small roots are unable to colonize a great volume in the container. This lack of nutrients could have had a more drastic effect in the pines fertilized with the exponential method.

The findings, then, demonstrated the great care that should be taken with the nutrient supply during low-dose exponential fertilization regimes if initial plant stunting or death is to be avoided.

Further research is needed to modify or adjust the exponential curve to provide an appropriate release of nutrients during the growing season.

Acknowledgements

The authors wish to thank Mr J. Peñuelas and Mr P. Villar Salvador from Centro Nacional de Mejora Forestal "El serranillo" for valuable comments about the exponential fertilization, as well as Mr J. A Sánchez Saorín from Servicio Experimentación Agrícola Forestal (SACE, Universidad de Murcia) for providing technical support and profitable suggestions in pine fertilization management

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