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Effects of fertilization on the production of an edible forest fruit: stone pine (*Pinus pinea* L.) nuts in south-west Andalusia

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Abstract

The pine nut from the stone pine (*Pinus pinea* L.) is the most important edible fruit in mediterranean forests. Despite this fact, there is a lack of knowledge regarding the application of agronomy techniques in stands of this species to increase pine nut production. Our study focuses on the effect of mineral fertilization on cone production and size, which in turn, are closely related to nut yield and quality. Cone production and quality was analysed in a randomized experiment installed in south west Spain, comparing the effect of different doses of lime superphosphate, dolomite and potassium. A significant short term increase in cone production and quality exists as a consequence of fertilization, especially in those treatments involving the addition of larger quantity of dolomite. Nevertheless, effect of mineral fertilization on cone yield and quality was lower than expected, so further increments might be achieved through nitrogenous and organic fertilizations to improve soil structure in these sandy soils.

Key words: cone production, dolomite, sandy soils, non-timber forest products.

Resumen

Efecto de la fertilización sobre la producción de un fruto forestal comestible: el piñón de pino piñonero (*Pinus pinea* L.) en Andalucía Occidental

El piñón de *Pinus pinea* L. es el fruto forestal comestible más importante en los bosques mediterráneos. Pese a esto, existe una falta de conocimiento científico acerca de la aplicación de prácticas agronómicas sobre las masas forestales de la especie al objeto de mejorar la producción de piñón. Nuestro trabajo se centra en el análisis del efecto de la fertilización mineral sobre la producción y el tamaño de las piñas, variables muy relacionadas con la cantidad y calidad de piñón. Entre 1993-1999 se realizó el seguimiento de la producción y la calidad de la piña en un experimente aleatorizado instalado en el SO de España, donde se comparaban diferentes dosis de superfosfato de cal, dolomita y cloruro potásico. Se ha detectado una respuesta positiva a la fertilización en la cantidad y calidad de las piñas producidas, especialmente en los tratamientos que planteaban la incorporación de una mayor cantidad de dolomita. El efecto de la fertilización mineral sobre la cantidad y calidad de piña ha sido menor que el esperado, por lo que futuros trabajos deben plantear la necesidad de incorporar fertilizantes nitrogenados y materia orgánica para mejorar la estructura en estos suelos arenosos.

Palabras clave: Pino piñonero, piñón, fertilización, suelos arenosos, productos forestales no maderables.

Introduction

Stone pine (*Pinus pinea* L.) constitutes one of the most important forest tree species producing edible fruits in Mediterranean forests. Natural or afforested stone pine stands occupy more than 600,000 ha in the Mediterranean basin, mainly in the western countries of the area: Spain (computing more than 60% of the total world area for the species), Portugal, France, Morocco, Tunisia and Italy. Management of stone pine stands has generally been multi-objective, encompassing pine nut production, timber, fuelwood and grazing, as well as the important role of these stands for recreation, landscaping and protection against wind erosion on the sandy soils where the species tends to grow.

Pine nut is the main commercial product obtained from Spanish stone pine stands, having been collected

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and consumed by humans since ancient times (López-García, 1980). Total annual amount of cones harvested in Spain, computed using data series from 1980-2000, ranges from 5,000 and 50,000 tons, with an average value of 30,000 tons (MAPA, 2001). These figures represent an annual production of more than 1,200 tons of *white nut* (nut without shell) from Spanish stands.

Due to the economic importance of the pine nut in Spain, an important effort has been made to define the practices and management schedules which favour cone production in stone pine stands. The main areas which have been covered in scientific studies include: silviculture (Montero *et al*., 2004), genetic improvement and clonal selection (Mutke *et al*., 2005a) or grafting propagation (Mutke *et al*., 2003). More recently, other works have focused on modelling cone and pine nut production, taking into account the masting habit of the species (Mutke *et al*., 2005b; Calama and Montero, 2007). In spite of the aforementioned research effort, there is still a lack of scientific and technical knowledge concerning the application of agronomy practices in stone pine stands, such as irrigation, pruning, control and release of herbaceous vegetation or fertilization, to increase cone production and quality.

Fertilization has been a traditional farming practice since ancient times, increasing both the quantity and quality of the crop whether it is fruit, seeds, leaves, roots... However, the importance of fertilization in forestry has been far less prominent, and has generally been associated with enhancing stand growth in intensively managed forests and plantations of timber producing species such as eucalyptus, Douglas fir, Scots pine or Loblolly pine (e.g. Chappell *et al*., 1991; Saarsalmi and Mälkönen, 2001; Zas, 2003a,b; Albaugh *et al*., 2003).

Forest fertilization as a practice for increasing fruit and seed production in forest species has been widely proposed since seminal works by Chandler (1938), Detwiler (1943) and Gemmer (1932). This practice has generally been applied either in seed orchards (Gregory *et al*., 1982; Mikola, 1987; Powell and White, 1994) or in regeneration areas just after the beginning of shelterwood seedling cuttings (Wenger, 1953; McLemore, 1975; Heidmann, 1984). Regarding the field fertilization of forest species with edible production, the only references found in literature are those related to agroforestry systems (Szott and Kass, 1993), specially referred to walnut crops (Gray and Garrett, 1990; Jones *et al*., 1995).

All the abovementioned fertilization experiences proposed the addition of nitrogenous fertilizer as main nutrient controlling flowering and fruiting process, either as a single element, or as a complete N-P-K fertilization. Though general responses lead to significant increments in fruit and seed productions, a few studies (Wenger, 1953; Powell and White, 1994; Jones *et al*., 1995), have found either slight or null effect of fertilization. Negative or non-significant responses to fertilization may result from a failure to consider such aspects as between-nutrients interaction and blocking, existence of other ecological limiting factors (e.g., water deficiencies), the effect of nutrients stimulating growth of the «wrong» parts of the plant or the season of fertilization. Together with these, due to great between-tree, within-tree and between-year variability in fruit production typical of forest ecosystems, application of statistical analysis disregarding different sources of variability can mislead the truth result from this type of experiences.

Stone pine in West Andalusia usually occupies sandy soils, mainly located close to the coast (<20 km). Stone pine stands in this area are the less fruit producers across the range of the species in Spain. Among the main reasons ruling this small cone yield have been mentioned little nutrient and organic matter soil contents, low water retention capacity, summer rainfall shortage, high rate of pollination failure and loss of female strobili during the first summer after pollination (Montero *et al.,* 2004). Poor soil quality combined with the facility to use mechanized labour (due to the flatness of the territory) pointed to fertilization as an interesting option for increasing cone crops within the area.

Present study analyses and reports the results of an old experience carried out in South West Andalusia to evaluate the effect of different doses of mineral fertilization on cone production (number of mature healthy cones per tree) and quality (defined by visual cone classification). Data from this experience were previously analysed at stand level using classical statistical methods as one-way ANOVA (Montero *et al*., 2004), not showing significant increments in cone production due to fertilization. Main objective of present work is to applying advanced statistical techniques, as mixed modelling, which allows to considering different levels of spatial and temporal correlation in data set, focusing on the identification of those treatments leading to larger and better crops at tree level as well as on definition of the extent of fertilization effect on time. Finally, obtained results were used to evaluate the economical viability of the fertilization.

Material

Experimental design

A fertilization trial was installed in the early 1990's in the *Coto Mazagón* forest, located within the area of influence of the *Doñana National Park* (Huelva province, South-West Andalusia). The trial is located in an even-aged stand, 45-50 years old, covering a flat area 5 kilometres from the coast and 50 meters above sea level. Annual precipitation in the area is 613 mm, with severe drought in summer when June to August precipitation reaches only 22 mm. Average annual temperature is 17.1°C. The site quality of the stand is quite low, with a site index of 11 metres at index age 100 (site index classification by Calama *et al*., 2003).

Within the stand, an 8 hectare zone was selected as the trial area. In this area, eight 1 hectare adjacent square plots (100 m × 100 m) were installed during the spring of 1993. In order to homogenise the initial conditions of the trial, plots were low-thinned to a density of 320 stems/ha (average density reduction was 15%), and a light pruning was applied to the remaining trees.

Five soil analyses were carried out in the trial site and its surrounding area, in order to characterize soil conditions and identify any shortages or imbalance in the chemical elements necessary for female flower stimulation, cone growth and maturation (Table 1). Soil analyses detected several deficiencies in organic matter and in the five macro-nutrients. Textural conditions classified soils as sand, with very low water retention capacity. Together with soil analysis, foliar analyses were carried out during winter 1993 in two trees selected within the trial area in order to evaluate nutritional status and possible deficiencies in mineral contents (Table 2). No optimum values for foliar nutrients have been defined for mature states of the species, so contrast values have been obtained from stone pine stands in Northern Plateau (Santa Regina, 2001), container-seedling conditions (Landis *et al.,* 1989), standard values for conifers as *Pinus radiata* (Zas, 2003b) and proposed nutrient ratios by Ingestad (1979).

Despite low mineral contents on soils, no serious foliar deficiencies were detected, though levels of phosphorous and potassium were under those standards for conifers, and moderate imbalance in N/P (> 12.5) and N/K (> 4) ratios was detected. After analysing soil and foliar nutrient conditions, seven mineral fertilization treatments (Table 3), based on different doses of lime superphospate (18% of P_2O_5), dolomite and potassium chloride (60% of K_2O) were applied in order to evaluate a possible increment in fruit production due to mineral addition. The main reason for selecting these fertilizers was to increase phosphorus and potassium content in soil and leaves to balance nitrogen ratios. Dolomite was proposed to regulate pH, facilitate mineral absorption, increase calcium and magnesium soil contents, and improve microbial activity. Nitrogenous fertilization

Table 1. Soil characteristics of the studied area (average values from five soil analyses)

Element	Content	Qualification (following Cobertera, 1993)
Sand (%)	90.0-95.01	Sand (USDA)
Lime (%)	5.1-7.5	
Clay (%)	1.4-3.3	
Organic matter (%)	0.10-0.39	Very low
N (Kjendahl %)	0.01-0.08	Low
K (ppm)	20-40	Very low
P (Bray ppm)	2-7	Very low
Ca (meq/100 g)	< 0.5	Very low
Mg (meq/100 g)	< 0.25	Very low
Water retention (mm)	104-122	Very low
pH	5.76- 6.53	Moderately acid

Table 2. Results from foliar analysis and contrast values

Foliar analyses			Contrast value			
Nutrient	Content	Nutrient ratio	Santa Regina (2001)	Landis *et al.* (1989)	Zas (2003b)	Ingestad* (1979)
N (%)	1.02-1.05	1.00	0.98	1.40-2.20	1.30-2.20	1.00
P (%)	0.06-0.08	0.07	0.13	0.20-0.40	0.10-0.30	0.20
K (%)	0.22-0.31	0.26	0.25	0.40-1.50	0.50-0.90	0.55
Ca (%)	0.11-0.16	0.13	0.36	0.20-0.40	0.12-0.70	0.20
Mg (%)	0.24-0.26	0.24	0.22	0.10-0.30	0.10-0.18	0.10

* Ingestad control values are referred to nutrient ratios.

Table 3. Proposed fertilizer doses

	Fertilization treatments (kg/ha)							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**Control**
Lime superphosphate (18%)	600	450	300	600	600	300	120	0
Dolomite	800	800	800	400	800	400	160	0
Potasium chloride (60%)	250	250	250	250	125	125	125	0

was not considered since foliar contents on this element were considered acceptable.

Each plot was randomly assigned to one of these seven fertilization treatments, leaving a control plot without fertilization. Fertilizations were carried out in May 1993. Treatments 6 and 7 were applied once more in May 1995. Initially the experiment was to be replicated in another nearby stand in 1994, but after installing the plot this second trial was abandoned.

Data collection

Between 1993 and 1995, cones were collected each autumn from 30 selected trees per plot. The cones were cropped by climbers, counted separately for each tree, and classified into four categories according to size and presence of abnormalities. This classification is similar to that carried out by the pine nut industry when fixing prices. These categories were assigned an ordinal level: A (big cones, without defects); B (medium cones without defects); C (small cones without defects or cones of all sizes with defects); D (very small cones and/or with important defects). Cones were also collected and counted each autumn between 1996 and 1999 but only from 10 selected trees per plot, and cones were not classified according to its quality.

Methods

Basic assumptions

To achieve main work objectives some basic suppositions were assumed. First we considered that as fertilization took place in May 1993, the first expected increase in number of cones would be that corresponding to 1996 (since female flowers emerge in Andalusia during April year t, while mature cones cannot be collected until November, year t+2), so analyses testing differences on *number of cones per tree* due to fertilization were carried out using only data series 1996-1999.

Second assumption is related with possible pseudoreplication (Hurlbert, 1984) caused by not being able to separate the effect of fertilization from other inherent effects specific to each plot (including thinning effect). We have used 1993-1995 cone production data series in order to detect possible inherent *a priori* differences among plots. If we do not detect systematic differences we assumed that differences for the series 1996-1999 (*a posteriori* differences) were most likely related to fertilization.

Finally, with respect to cone quality we assume that the effect of fertilization can be noticeable even in the cones cropped during the autumn following fertilization, since the greatest increase in weight of two-year old cones is attained during the spring and summer prior to maturation (Montero *et al.,* 2004), so analyses were then carried using the only available cone quality series (1993-1995).

Analysis of variable: number of cones per tree

Number of cones per tree is a typically heterocedastic and non-normally distributed variable. Several works focusing on this variable (Mutke *et al.,* 2005a,b; Calama and Montero, 2007) have proposed a logarithmic transformation to attain basic assumptions for statistical inference and define a multiplicative effect of analysed factors (treatment, year, and tree). To deal with meaningless transformed values associated with zero crops, a term +1 is added to the original number of cones per tree.

Repeated measures analysis of variance: mixed model approach

The available data consists of repeated observations of a variable (number of mature cones) taken from the same trees during different years. These trees are located within different plots, each corresponding to a different fertilization treatment. The main aim of the study is to detect possible differences in the number of mature healthy cones per tree for different fertilization treatments through a series of years. In this kind of analysis independence between observations coming from the same tree or the same year is a basic assumption which is not allowable. Additional source of dependence is given since two measurements from the same tree corresponding to consecutive years might be more correlated than two measurements separated a larger number of years.

Several alternatives have been proposed in forest literature (e.g. Moser *et al.*, 1990; Gumpertz and Brownie, 1993; Zhao *et al.,* 2005) to deal with this lack of independence, including separate analysis of variance (ANOVA) at each time period, univariate and multivariate repeated measurements ANOVA and mixed modelling, which is the approach proposed in this work. Mixed modelling show several advantages, as the capacity for considering multiple sources of heterogeneity and correlation, inclusion of time effect as a random component, flexibility to assign non-spherical structures for the within-subject variance-covariance matrix and possibility of handling unbalanced incomplete data. Proposed mixed model for analysing fertilization effect over single tree cone crop is given by:

$$y_{ijk} = \mu + w_i + h_j + wh_{ij} + \gamma_{ik} + \varepsilon_{ijk} \qquad [1]$$

where y_{ijl} represents the logarithm of the number of cones (plus 1) cropped from tree k (fertilized with treatment i) during year j; μ represents the intercept of the model; w_i is the fixed treatment (fertilization) effect; h_j is the time effect, considered as random; wh_{ij} represents the random time × treatment interaction; γ_{ik} is a random tree effect, specific to sampling unit (tree) k; h_j, wh_{ij} and γ_{ik} are assumed to be normally distributed with mean zero and variances σ^2_h, σ^2_v and σ^2_γ respectively. Finally, ε_{ijk} is a random error term defining within-subject pattern of variability. Evaluated structures for within-subject covariance matrix were (Verbeke and Molenberghs, 2000):

— Compound symmetry (CS):

$$\begin{bmatrix} \sigma^2 & & \\ & \sigma^2 & \\ & & \sigma^2 \end{bmatrix}$$

— Autoregressive order 1 (AR1):

$$\begin{bmatrix} \sigma^2 & \rho\sigma^2 & \rho^2\sigma^2 \\ & \sigma^2 & \rho\sigma^2 \\ & & \sigma^2 \end{bmatrix}$$

— Huyhn-Feldt (H-F):

$$\begin{bmatrix} \sigma^2_1 & \frac{\sigma^2_1+\sigma^2_2}{2}-\lambda & \frac{\sigma^2_1+\sigma^2_3}{2}-\lambda \\ & \sigma^2_2 & \frac{\sigma^2_2+\sigma^2_3}{2}-\lambda \\ & & \sigma^2_3 \end{bmatrix}$$

— Unstructured (UN):

$$\begin{bmatrix} \sigma^2_1 & \sigma_{12} & \sigma_{13} \\ & \sigma^2_2 & \sigma_{23} \\ & & \sigma^2_3 \end{bmatrix}$$

Variance-covariance structures for within-subject observations were evaluated on the basis of log-likelihood ratio test (LRT) and Akaike's Information Criterion (AIC). Level of significance for fertilization treatments (fixed effect w) was tested using Type III F-tests. Level of significance for random components year and treatment × year was evaluated through Wald test and LRT. Analyses were carried out using SAS proc MIXED, separately for the data series not *influenced by fertilization* (1993-1995) and that *influenced by fertilization* (from 1996).

Testing fertilization effect

As extent of fertilization effect is assumed not to last permanently when analysing the series 1996-1999 first one-way ANOVA's were fitted separately for each year in order to detecting the year when fertilization effect becomes negligible. In a second step, global differences between fertilization treatments were evaluated using mixed modelling approach over the series including those years with significant treatment effect. Contrasts between treatments were carried out using multiple pairwise comparisons among adjusted least square means of the treatments. Other *a priori* orthogonal interesting partial contrasts were also evaluated in order to identify individual effect of each added nutrient.

Analysis of variable: cone quality

Analyses for cone quality were done for the available 1993-1995 series, using a chi-square contrast test over a three way contingency table, testing the existence of dependence patterns between treatment, cone quality and year. Analyses were also done separately for each year, in order to detect the duration of the fertilization effect. Analyses were performed using the SAS procedure CATMOD.

Cost-benefit analysis

Despite fertilization effects over cone production are delayed at least three years with respect to application, given the shortage of data series economic analysis is only based on contrasting the cost of fertilization against the expected additional returns derived from increased cone crops, without considering more complex financial studies involving net present value of delayed incomes.

Results

Analysis of variable: number of cones per tree

The mean value and standard deviation (per year and treatment) of the variable number of cones per tree are shown in Table 4 and Figure 1. Together with a clear pattern of synchrony among treatments pointing to a climatic dependence on masting habit, it must be indicated the great increment in individual cone production shown by treatments 1, 2 and 5 between 1995 and 1996, reaching values over 12-15 cones per tree. These treatments are also the larger producers in 1997 (6-8 cones per tree) and 1998 (2 cones per tree). Contrasting with this, control plot shows the smallest production in 1997 and 1998, with values of production per tree close to zero, coinciding with two very bad crops detected in the rest of province (average provincial production per tree of 0.6 and 0.9).

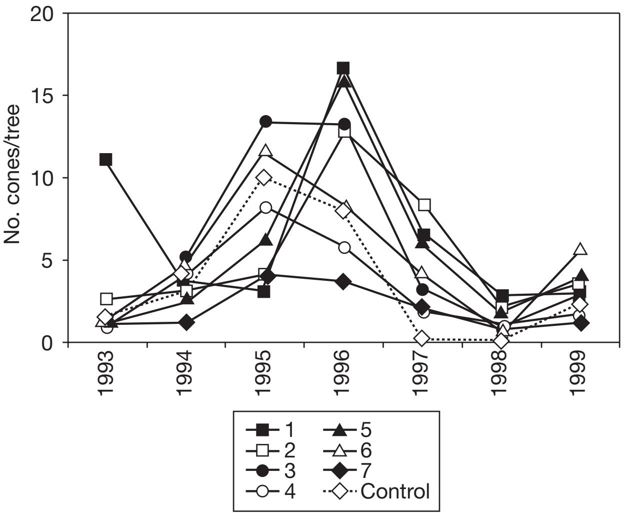

Figure 1. Average number of cones/tree per treatment and year.

Testing initial homogeneity: data series 1993-1995

The best structure for within-subject covariance was complete unstructured UN (Table 5). This type of structure indicates the existence of a common pattern associated with tree effect, but no clear trends of correlation

Table 4. Mean value and standard deviation (in parenthesis) in number of cones per tree

Treatment	1993*	1994*	1995*	1996**	1997**	1998**	1999**	Average 93-95	Average 96-99
1	11.0 (27.6)	3.7 (7.7)	2.9 (4.7)	16.6 (17.1)	6.4 (7.1)	2.7 (2.1)	3.3 (3.2)	5.9	7.3
2	2.7 (3.0)	3.3 (3.5)	4.2 (6.4)	12.9 (7.1)	8.3 (4.3)	2.2 (1.7)	3.7 (3.6)	3.4	6.8
3	1.4 (2.4)	5.2 (8.2)	13.4 (21.5)	13.3 (14.9)	3.2 (2.5)	0.8 (0.9)	2.6 (2.5)	6.7	5.0
4	0.9 (1.6)	4.0 (6.6)	8.2 (12.1)	5.8 (6.4)	1.9 (1.8)	0.8 (1.2)	1.6 (1.3)	4.4	2.5
5	1.3 (1.8)	2.7 (4.5)	6.2 (4.3)	15.8 (32.7)	6.1 (10.9)	1.5 (2.1)	3.8 (7.3)	3.4	6.8
6	0.8 (1.4)	4.6 (8.9)	11.7 (19.3)	8.1 (6.0)	4.2 (3.6)	0.6 (0.8)	5.6 (6.2)	5.7	4.6
7	1.2 (1.4)	1.2 (1.8)	4.0 (5.5)	3.7 (3.3)	2.2 (3.4)	1.0 (1.6)	1.3 (1.8)	2.1	2.1
Control	1.0 (2.1)	4.2 (3.8)	10.1 (15.7)	8.0 (10.6)	0.2 (0.6)	0.1 (0.3)	2.3 (5.3)	5.1	2.7
Average	2.5 (10.3)	3.6 (6.1)	7.5 (13.2)	10.5 (15.1)	4.1 (5.6)	1.2 (1.6)	3.0 (4.4)	4.6	4.7

* Data from 30 trees per plot. ** Data from 10 trees per plot.

Table 5. Within-subject variance-covariance structure

Structure	N	-2RLL	p > LRT	AIC
Data series 1993-1995				
CS	3	1,854.5	< 0.0001	1,860.5
AR1	4	1,810.6	< 0.0001	1,818.6
HF	6	1,817.6	< 0.0001	1,829.6
UN	**8**	**1,772.3**	**—**	**1,788.3**
Data series 1996-1998				
CS	3	533.0	< 0.0001	536.0
AR1	4	528.3	< 0.0001	536.3
HF	**6**	**505.7**	**0 .3499**	**517.7**
UN	8	503.6	—	519.6

n: number of variance components to estimate. -2RLL: -two times restricted log-likelihood. p > LRT p-value for likelihood ratio test (evaluated against most UN structure). AIC: Akaike's Information Criterion. Bold indicates selected structure.

among tree observations. Table 6 shows results for fitting mixed model [1] to 1993-1995 data series considering this structure. No significant global plot effect was identified ($p = 0.9322$), so null hypothesis of plot equality cannot be rejected. This would indicate that, *a priori,* there are no global inherent differences among the analysed plots with respect to cone production. Wald test indicated a nonsignificant year effect, although large level of explained variability by this component points to between year differences in cone production, confirming species masting habit. There is also a significant plot × year interaction, indicating non-parallel production trends between plots. Figure 2A shows plot behaviour for 1993-1995 period, defined by the sum of the best linear unbiased estimator (BLUE) for plot effect and the best linear unbiased predictor (BLUP) for random plot × year effect. As it can be seen no systematic common pattern of arrangement due to cone production can be detected. Under these conditions, any possible differences detected in cone production for the 1996-1999 series are assumed to be related, at least in part, to fertilization.

Extent of fertilization effect

The data series 1996-1999 include those observations of cone production possibly influenced by fertilization. A one-way ANOVA for each year within 1996-1999 series revealed significant differences between treatments for years 1996 (p-value = 0.0390); 1997 ($p = 0.0002$) and 1998 ($p = 0.0032$), whereas no significant between-

Table 6. Mixed model fitting statistics

	1993-1995 (UN structure type)			1996-1998 (HF structure type)		
	Effect	**p-value**	**Variance component**	**Effect**	**p-value**	**Variance component**
Fixed effects	Treatment (w)	0.9322	—	Treatment (w)	0.0501	—
Random components	Year (σ^2_h)	0.1909	0.1293	Year (σ^2_h)	0.1626	0.4701
	Treatment × year (σ^2_{wh})	0.0199	0.1244	Treatment × year (σ^2_{wh})	0.0749	0.03404
Error	σ^2_1	< 0.0001	0.5929	σ^2_1	< 0.0001	0.8604
	σ^2_2	< 0.0001	0.8125	σ^2_2	< 0.0001	0.8134
	σ^2_3	< 0.0001	1.1908	σ^2_3	< 0.0001	0.3537
	σ_{12}	< 0.0001	0.2880	λ	< 0.0001	0.2789
	σ^2_{13}	< 0.0001	0.2102			
	σ^2_{23}	< 0.0001	0.6534			

Note: In H-F and UN structures tree variance component σ2γ is assumed to be included in the within-subject structure, so is not independently estimated.

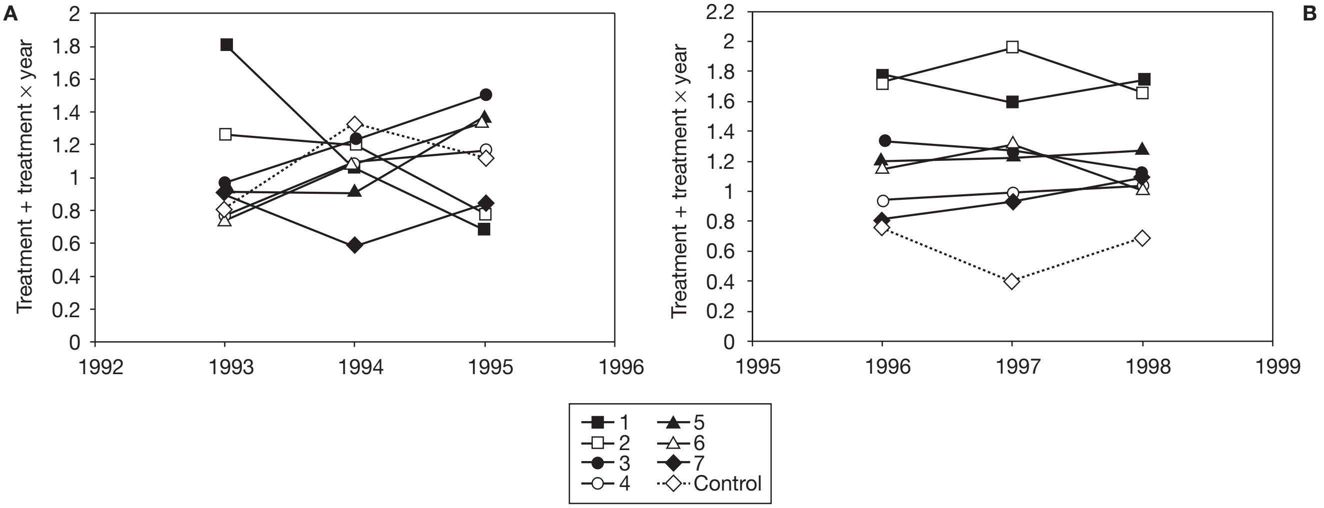

Figure 2. Time trends for treatment BLUE + treatment × year BLUP. A) Data-series 1993-1995; B) Data series 1996-1998.

treatment differences were detected in 1999 (p = 0.2430). Table 7 shows Tukey's range test for years 1996-1999. It can be seen that for 1997-1998 doses 1 and 2 are significantly deviated with respect to the control plot. In 1996 the ranking is similar, but the treatment with the poorest results is not the control plot, but rather, dose 7. In 1999, though the differences were non-significant, an increase in production was detected for dose 6 (probably associated with the repetition of fertilization treatment in late spring 1995). One way ANOVA results point to a three-year duration of fertilization effect, so mixed model analysis for detecting global differences were then carried out for the 1996-1998 series.

Testing fertilization effect: data series 1996-1998

Best within-subject variance structure is H-F one (Table 5), pointing again to the existence of common tree effect and the no-existence of a pattern of similarity in consecutive years. Table 6 show results after fitting model [1] considering prior variance structure to 1996-1998 data series. In this case, the treatment effect is slightly significant (p-value = 0.0501), indicating that there exist significant differences in cone production for 1996-1998 data series due to fertilization. Again main part of unexplained variability is associated with year effect, pointing again to a year dependence on cone production, likely related with climatic factors. Finally, treatment × year significant effect indicates a pattern of year response in cone production specific for each plot, as was also shown for the 1993-1995 series. Graphical analysis plotting the sum of BLUE of treatment plus BLUP of treatment × year (figure 2B) against year show in this case a clear pattern of arrangement between treatment series, with largest productions attained all the years in treatments 1 and 2, and lowest values associated with control.

Corrected least squares means (Table 8) reveals large productions associated with treatments 2 and 1,

Table 7. Single year one-way ANOVA and Tukey's range test for data series 1996-1999

Treatment	1996	1997	1998	1999
1	2.5016^{a}	1.4202^{ab}	1.1144^{a}	1.1485^{a}
2	2.4978^{a}	2.1063^{a}	1.0045^{a}	1.2338^{a}
3	2.2607^{a}	1.2579^{abc}	0.4682^{ab}	1.0204^{a}
4	1.5729^{a}	0.8659^{bc}	0.4159^{ab}	0.8253^{a}
5	1.8626^{a}	1.1258^{abc}	0.6356^{ab}	0.7621^{a}
6	1.9749^{a}	1.4330^{ab}	0.3584^{ab}	1.5010^{a}
7	1.2794^{a}	0.7340^{bc}	0.4682^{ab}	0.6174^{a}
8	1.5857^{a}	0.1099^{c}	0.0693^{b}	0.5663^{a}

Values with the same letter indicate non-significative differences ($p < 0.05$).

Table 8. Corrected global least squares means and significant least squares differences. Data series 1996-1998

Treatments	Least square mean	Group
	Data 1996-1998	
1	1.6990	A
2	1.7880	A
3	1.2373	AB
4	0.9923	BC
5	1.2339	AB
6	1.1584	BC
7	0.9399	BC
8	0.6210	C

Groups with same letter indicate non significant ($p > 0.10$) differences.

similar smaller values for doses 5, 6 and 3, while lowest productions associated with control treatment, followed by treatments 7 and 4. By arranging multiple comparison between each pair of treatments, significant differences ($p < 0.05$) were identified between treatment 1 and treatments 4, 7 and control, as well as between treatment 2 and treatments 4, 7 and control. Significant differences ($p < 0.10$) were detected between treatments 2 and 6, and between treatments 3 and 5 and control. Largest least square difference (1.1688, p-value = 0.0032) was detected between treatment 2 and the control plot, indicating that on average (random year and treatment × year components are zeroes), treatment 2 leads to cone crops approximately 3.2 times ($\exp^{1.1668}$) larger than without fertilization.

Several a priori partial contrasts were evaluated (Table 9) to detect the influence of each separate nutrient. Significant differences were detected between doses adding 800 kg/ha of dolomite with respect to those adding smaller quantities ($p < 0.05$) and between doses adding 250 kg/ha of potassium chloride against those adding 125 kg/ha ($p < 0.10$). Confirming this result, Figure 3 shows the highly significant linear relation between the total amount of dolomite added and the average difference in number of cones per tree with respect to control attained for the 1996-1998 series.

Table 9. Partial *a priori* contrasts

Contrast	t	p-value
Superphosfate 600 kg/ha *vs* superphosfate lower doses	0.16	0.8789
Dolommite 800 kg/ha *vs* dolomite lower doses	2.59	0.0215
Potasium 250 kg/ha *vs* potasium lower doses	1.79	0.0945

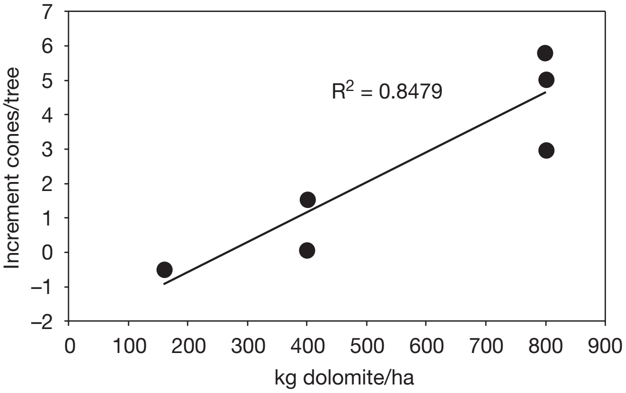

Figure 3. Relation between amount of dolomite added and average increment (1996-1998 series) in number of cones per tree with respect to control plot.

Analysis of variable: cone quality

The three-way contingency table indicated global independence between treatment and cone quality, as well as global dependence between year and cone quality ($p = 0.0527$ in the likelihood ratio test when compared with the complete dependence model). This result points to a between-year cone quality variability, probably related to climatic effects, but little effect of fertilization on cone quality. Two-way contingency tables, developed separately for each year, indicated a significant relationship between cone quality and treatment in 1993 ($\chi^{-2} = 49.9739$, $p = 0.0003$), whereas it indicated independence for 1994 ($\chi^{-2} = 2.4021$, $p = 0.3766$) and 1995 ($\chi^{-2} = 31.8188$, $p = 0.0610$). The dependence detected in the 1993 result was caused by a larger than expected number of A class cones (bigger cones) in treatments 1 and 2.

Cost-benefit analysis

Average cone production in control plot during the 1993-1999 series is close to 200 kg/ha.year. Assuming that treatment 2 lead to cone crops 3.2 times larger than untreated plots, an average expected cone crop under doses 2 could reach values of 640 kg/ha.year. If the extent of fertilization effect is 3 years and actual value of cones on tree is 0.20 €/kg, fertilization could lead to a increment in production of 1,380 kg/ha (income increment 276 €/ha) in three years with respect to control plots.

Actual prices for dolomite, superphosphate and potassium chloride are 10, 20 and 19 €/100 kg (Anonymous, 2005), respectively, while cost for spreading nutrients is about 30 €/ha. Considering these figures the cost for fertilization of one hectare applying dose 2 is approximately 250 €/ha, so expected benefit associated with fertilization with this dose, after a three year cycle, is only about 25 €/ha. In view of this expected benefit, and taking into account associated risks (as pest, fires, drought…) fertilization using the most favourable treatment (dose 2) does not seem to be, by the moment, a profitable practice.

Discussion

In the present study, we have found that mineral fertilization of sandy soils to improve cone production results in slight significant global differences in the number of cones per tree. Except doses 4, 6 and 7, the rest of proposed treatments showed significant ($p < 0.10$) larger crops than control treatment considering the three years period 1996-1998. These results contrast with those previously obtained with the same data set (Montero *et al*., 2004), who did not identify significant differences between treatments after applying ANOVA over stand level values. In this sense, consideration of different sources of spatial and temporal correlation, as well as the analysis of response at tree-level, is helpful for detecting significant differences in such type of highly correlated data.

Best cone crops are attained with treatments adding larger amounts of phosphorus, potassium chloride and dolomite (doses 1 and 2), leading to average crops per tree over 3 times larger than control. Application of doses 3 and 5 lead to average cone crops 1.75 times larger than control. These treatments are characterized by adding the largest amount of dolomite but smaller levels of potassium chloride and superphosphate. Treatment 4 adds the largest quantities of potassium chloride (250 kg/ha) and superphosphate (600 kg/ha) but only incorporates 400 kg/ha of dolomite, leading to similar nonsignificant increases (with respect to control) as treatment 7 (which incorporates the small amount of the three analysed nutrients).

These results, together with those obtained from the proposed *a priori* contrasts (Table 8) seems to indicate that dolomite is, among the three mineral elements evaluated, the most important in controlling cone production. Liming with dolomite has been used in forest fertilization to increase soil content on Ca and Mg, since low solubility of dolomite makes these elements less prone to leaching, which is the most common mode of nutrient loss in sandy soils (Mupangwa and Tagwira, 2005). Together with this nutritional effect, liming favours availability for absorption of other essential nutrients, as phosphorous; improves structural conditions of soil and activates soil microbian activity (Bara, 1990; Fuentes, 1994). With respect to fruit and seed production, dolomitic liming resulted in significant medium and long-term crop increments (e.g. Long *et al.,* 1997; Mupangwa and Tagwira, 2005) on sandy and acid soils.

A three-year (1996-1998) duration of fertilization effect has been identified, with largest significant differences in 1997 and 1998 and nonsignificant differences for 1999. Highest significant response in fourth and fifth-year after fertilization (second and third-year of expected response) points to an indirect response in cone production to fertilization through increased foliage growth and carbohydrate reserves which leads to a delayed flowering response – rather than a direct nutritional one which will result in largest crop differences for first expected year (1996). Similar delayed indirect response in fruit production has been identified in *Pinus palustris* (McLemore, 1975) and *Picea mariana* (Smith, 1987) as well as in different deciduous species (Chandler, 1938; Long *et al.,* 1997).

Regarding cone quality, a significant dependence between cone classification and fertilization treatment was only identified for the first crop after fertilization (1993), suggesting a positive relation between treatments 1 and 2 (those in which a large amount of fertilizer was added to the soil) and the best cone quality (class A). A slight quality increase in the short term was also reported for both forest and agricultural species (as shown by, e.g., Gray and Garrett, 1999), indicating a direct nutritional response in fruit size and contents. Nevertheless, more accurate results could be obtained by testing a cone level continuous covariate, as weight, rather than a categorical visual classification.

The results obtained demonstrate the existence of a positive response to fertilization in cone production and, to a lesser extent, in cone quality. However, this response would seem somewhat lower than expected after a fertilization program, and, from an economical point of view, fertilization using proposed doses for increasing cone production cannot be considered by the moment a profitable practice. Two main reasons could explain these poor results: 1) inappropriate selection of nutrients 2) ignoring other limiting factors in cone production, as water deficiency. In South West Andalusia, soils typically show a large proportion of sand, low levels of mineral nutrients and organic matter, and low water holding capacity. These characteristics lead to large nutrient losses through leaching and to water shortage. The fertilization treatments proposed in this study mainly deal with chemical conditions (mineral deficiencies and pH correction) rather than with physical (soil structure). In that sense, future fertilization experiences should consider improvement of soil structure by addition of organic amendments and/or nitrogenous fertilization, together with the repeated application of nutrients in different cycles.

Conclusions

The stone pine has great potential as an alternative crop for areas with poor sandy soils throughout the Mediterranean area, bearing in mind the minimal attention required by stone pine forest stands or plantations (compared with traditional farming), the increasing demand for pine nuts and the compatibility among nut production and other timber and non-timber products of Mediterranean forests, such as fuelwood, mushrooms, hunting or grazing. Present work has revealed the potential for increasing cone production through mineral fertilization. New studies focusing on the application of both organic manure and nitrogenous fertilization, as well as on the interaction between fertilization and other agronomy techniques such as pruning, clone selection, grafting or irrigation should complement the initial results derived from this study, allowing farm holders, landowners and forest managers to improve nut production and increase the incomes derived from these marginal sandy areas.

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