

Fertilization × genotype interaction in *Pinus radiata* open pollinated families planted in three locations in Galicia (NW Spain)

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Abstract. A *Pinus radiata* progeny trial was established in 2003 on three low fertility sites in Galicia to study the relevance of genotype × fertilization interaction. At each site, twenty seven open pollinated families derived from plus trees selected in mature plantations in Galicia (NW Spain) were planted under 9 fertilization treatments following a split-plot design. One year after planting, P and Mg fertilization significantly increased height growth whereas N did not affect or even significantly reduced height growth. Fertilization × site interaction was not significant indicating parallel responses to fertilization in the three sites. Fertilization × genotype interaction was significant in only one site, suggesting some genetic variation in the nutrient use efficiency among the studied genotypes. However, the lack of a significant interaction in the other two sites, and the lack of consistence among the genotypic stability in relation to fertilization across sites, hinder the possibility of breeding in relation to the nutrient efficiency of the genotypes. Indications for further research are given.

Introduction

Pinus radiata D. Don is an important forest tree species in Galicia, NW Spain. High productive plantations occupy around 80,000 ha in this region, with annual yields commonly exceeding $15 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$ (DGCN 2002). The high productivity of radiata pine implies a high water and nutrient demand that Galician forest soils may not satisfy. In fact, nutritional disorders of radiata pine are very common all around its distribution area (Turner and Lambert 1986). In Spain, important nutrient deficiencies have been observed in radiata pine plantations (Mesanza et al. 1993; Romanyà and Vallejo 1996; Merino and Edeso 1999; Zas 2003; Zas and Serrada 2003). Moreover, productivity of radiata pine has been shown to be strongly related to its nutritional status (Sánchez-Rodríguez et al. 2002; Romanyà and Vallejo 2004). Specifically, the main factors limiting growth in Galicia are the P and Mg nutrition, whereas N seems to be well supplied or even in excess. Phosphorus and magnesium fertilization has been thus recommended for *P. radiata* establishment in Galicia (Sánchez-Rodríguez et al. 2002; Zas 2003; Zas and Serrada 2003).

In the early 90s the Forestry Research Center of Lourizán started a breeding program for *P. radiata* in order to procure high genetic quality seed for reforestation in Galicia. This breeding program has included phenotypic mass selection in wild stands and subsequent seed production in clonal seed orchards from the selected material. Tree breeding objectives have been focused mainly on improving growth traits, stem form and branching characteristics. The relevance of nutrition in radiata pine in Galicia suggests that the performance of the improved genetic material may vary in relation to soil fertility. Therefore, it would be highly interesting for breeding purposes to analyze the influence of soil fertility and fertilization practices in the performance of the selected material. Genotype×nutrition interaction has been shown to be quantitatively important in many conifer tree species (see Eriksson et al. 1993). If the differences between genotypes regarding soil fertility conditions are pronounced, separate breeding programs for different soil fertility types might be needed (Jonsson et al. 1992). In a similar way, the best genetic material for intensive (fertilized) silviculture may differ from that for extensive (no fertilized) silviculture.

Significant genotype × nutrition interaction indicates differences in the nutrient efficiency among genotypes, i.e. growth per unit of up taken nutrient. Productivity could thus be increased by breeding for high nutrient use efficiency. Improving nutrient efficiency is a common practice in agricultural crops (e.g. Hammond et al. 2004) and it has received increasing attention in forest trees (Crawford et al. 1991; Li et al. 1991; Jonsson et al. 1992, 1997; Mari et al. 2003a, b). In the case of *P. radiata*, improving nutrient-efficiency could be a first priority objective due its high dependence on soil fertility. The need of fertilization might be minimized by using high nutrient-efficient genotypes.

The aim of the present paper is to analyze the relative importance of genotype × fertilization interaction in a subset of the actual breeding population of *P. radiata* in Galicia, and to explore the possibility to increase productivity through improving the nutrient use efficiency.

Material and methods

Three family×fertilization trials were established in February–March 2003 in the coastal area of Galicia using 1-year-old containerized seedlings (see Table 1). The three sites have typical acidic forest soils upon granite or gneiss with low concentration of nutrients and high organic matter content. The mean values of three topsoil composite samples within each site are shown in Table 1. Each sample was built mixing five 0–20 cm depth sub samples. Climate in the three sites is Atlantic with high annual precipitation, short summer drought and low temperature fluctuation.

The study material consisted of open-pollinated families from 27 maternal plus trees selected for superior growth and form in mature plantations of *P. radiata* in Galicia. The plus trees represent a random subset of a first-generation breeding population of *P. radiata* and are included in a clonal seed

Table 1. Site location and mean soil chemical properties of the 0–20 cm superficial horizon.

	Site A	Site B	Site C
Location	Forgoselo	Rebordelo	Rianxo
Longitude (°W)	8.00°	8.48°	8.77°
Latitude (°N)	43.46°	42.46°	42.60°
Altitude (m)	530	530	90
Parent material	Granite	Gneis	Granite
pH in H ₂ O	4.2	4.0	4.3
C (%) ^a	12.1	10.5	9.8
Total N (g kg ⁻¹) ^a	8.3	7.1	6.3
P Olsen (mg kg ⁻¹) ^b	1.9	4.7	4.2
K ⁺ (mg kg ⁻¹) ^c	119.6	94.9	117.0
Ca ²⁺ (mg kg ⁻¹) ^c	156.7	68.7	52.7
Mg ²⁺ (mg kg ⁻¹) ^c	29.6	25.6	24.4

Values of soil variables are the means of three composite samples of five subsamples each.

^aTotal organic C and total N were analyzed in a LECO CHN 1000 elemental analyzer.

^bAvailable phosphorus following Oslen method.

^cExtracted with NH₄Cl 1 N and analyzed by atomic absorption spectrophotometry.

orchard (Sergude, 42.82°N, 8.45°W) which provides high genetic quality seed for reforestation in Galicia.

The experimental layout at each site was a split-plot design with nine fertilization treatments acting as whole plots and the 27 families as sub plots. One plant of each family was included per sub plot. The experiment was replicated in 10 blocks, resulting in 10 and 90 plants of each family per treatment and site, respectively. The fertilization treatments (control and eight treatments built upon combinations of four commercial fertilizers) were designed to test the effectiveness of different nutrients (Table 2). Fertilization treatments were randomly assigned to the whole plots within each block. Fertilizers were applied immediately after planting, and were distributed around the seedlings in an area of 30 cm in diameter. Doses were defined according to Birk (1994).

Height was measured in all living plants 1 year after plantation. An infestation of *Hylobius abietis* L. in Rianxo (site C) led to stem girdling and, consequently, leader loss in 22.6% of the living seedlings. In this site, only those plants with living leaders were used in the analyses.

Table 2. Codes and composition of the fertilization treatments.

Nutrient	Fertilizer	Dose	Treatment Code							
			control	F0	F1	F2	F3	F4	F5	F6
N	Ammonium nitrate	5 g N plant ⁻¹	–	+	–	+	+	+	–	–
P–Ca	Calcium phosphate	10 g P plant ⁻¹	–	+	+	–	+	+	–	+
K	Potassium sulfate	15 g K plant ⁻¹	–	+	+	+	–	+	+	–
Mg	Magnesium sulfate	5 g Mg plant ⁻¹	–	+	+	+	+	–	+	+

Presence and absence of a given compound in the fertilizer mixture is indicated by (+) and (–), respectively.

Single site analyses were carried out for each site using the MIXED procedure (SAS-Institute 1999) and the following mixed model:

$$Y_{jkl} = \mu + F_j + G_k + B_l + FG_{jk} + GB_{kl} + FB_{jl} + \varepsilon \quad (1)$$

where Y_{jkl} is the height of the seedlings, μ is the overall mean, F_j , G_k and B_l are the effects of fertilization treatment j , genotype (open-pollinated family) k , and block l , respectively, FG_{jk} , GB_{kl} and FB_{jl} are the corresponding interactions, and ε is the experimental random error. To analyze each factor with the appropriate error term, this model was analyzed with the MIXED procedure of the SAS System considering all factors fixed except the FB_{jl} interaction (SAS-Institute 1999).

An analysis across sites was also carried out using the following mixed model:

$$\begin{aligned} Y_{ijkl} = & \mu + S_i + F_j + G_k + B_l(S_i) + SF_{ij} + FG_{jk} + SG_{ik} + GB_{kl}(S_i) \\ & + SFG_{ijk} + FB_{jl}(S_i) + \varepsilon \end{aligned} \quad (2)$$

where S_i is the effect of site i , $B_l(S_i)$ is the effect of block l within site i , and SF_{ij} , SG_{ik} , SFG_{ijk} are the corresponding interactions with the site factor. As for the single-site analysis, this model was analyzed with the MIXED procedure, considering all factors fixed except the $FB_{jl}(S_i)$ interaction.

Statistical comparison of treatment means was conducted using the LSMEANS statement of the MIXED procedure. Specific comparisons between different group of treatments were analyzed using the CONTRASTS statement of this procedure (SAS-Institute 1999).

Interclass correlations (r_B) among treatments and sites was estimated to determine the strength of the family by treatment or family by site interaction (Lynch and Walsh 1997). To estimate variance components, the two preceding models were carried out again considering the family effect (G_k) and all the interactions involving this factor (FG_{jk} , SG_{ik} , $GB_{kl}(S_i)$, SFG_{ijk}) as random factors. Interclass correlation was estimated as (Lynch and Walsh 1997):

$$r_B = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{ge}^2} \quad (3)$$

where σ_g^2 is the family variance and σ_{ge}^2 is the family by fertilization or family by site interaction variance.

The family ecovalence (W_i) was estimated to study the stability of each family across the different fertilization treatments within each site (Wricke 1962):

$$W_i = \sum_j (Y_{ij} - Y_{\cdot i} - Y_{\cdot j} - Y_{\cdot \cdot})^2 \quad (4)$$

where W_i is the ecovalence value of the i th family, $Y_{\cdot \cdot}$ is the overall height mean, $Y_{\cdot i}$ and $Y_{\cdot j}$ are the least-square means (LSMEAN) for family i ($i=1-27$) and for treatment j ($j=1-9$), respectively and Y_{ij} is the least-square mean of family i in the treatment j . The relationship between the family ecovalence values estimated at different sites was analyzed by linear regression.

Results

Fertilization had an important effect on height growth in the three sites (Table 3) and in combined sites (Table 4). Despite the differences in site quality among the three sites, the fertilization×site interaction was not significant (Table 4), indicating that the fertilization treatments had similar effects over the three sites. This can also be seen in Figure 1, where parallel responses to the different fertilization treatments can be observed for the three sites. All the eight fertilization treatments significantly increased height growth compared to the control treatment F0 (Figure 1). Among the different treatments, those that included P and Mg fertilization and did not include N (treatments F2 and F7) consistently generated higher growth response than others. Height growth response to the complete fertilization (treatment F1) was also among the highest. Treatments that did not include P (F3 and F6) were consistently the worst in the three sites (Figure 1). These results indicate that P was the principal nutrient affecting height growth of *P. radiata* in the three sites. The application of Mg had also an important positive effect.

The eight fertilization treatments can be grouped into two groups (Table 2): one which include N (F1, F3, F4 and F5), and one without N (F2, F6, F7 and F8). The specific statistical comparison between these two groups indicated

Table 3. Summary of the mixed model for each site analyzed separately.

Effect	DF	Error term	Site A		Site B		Site C	
			F	p < F	F	p < F	F	p < F
Fertilization (F)	8	$F \times B$	10.10	< 0.0001	19.23	< 0.0001	13.37	< 0.0001
Block (B)	9	$F \times B$	1.53	0.1528	3.50	0.0012	1.89	0.0676
Genotype (G)	26	Error	9.17	< 0.0001	14.77	< 0.0001	2.60	< 0.0001
$F \times G$	208	Error	0.95	0.6885	1.26	0.0095	0.98	0.5571
$B \times G$	234	Error	0.85	0.9408	1.26	0.0072	0.94	0.7199

F-ratios and significance levels are presented.

DF = degrees of freedom.

Table 4. Summary of the mixed model analyzing all sites together.

Effect	DF ^a	Error term	F-ratio	p-value
Site (<i>S</i>)	2	<i>F</i> × <i>B(S)</i>	585.53	< 0.0001
Fertilization (<i>F</i>)	8	<i>F</i> × <i>B(S)</i>	36.78	< 0.0001
Genotype (<i>G</i>)	26	Error	15.36	< 0.0001
Block(Site) <i>B(S)</i>	27	<i>F</i> × <i>B(S)</i>	2.13	0.0016
<i>S</i> × <i>F</i>	16	<i>F</i> × <i>B(S)</i>	0.42	0.9768
<i>F</i> × <i>G</i>	208	Error	1.00	0.4747
<i>S</i> × <i>G</i>	52	Error	2.78	< 0.0001
<i>S</i> × <i>F</i> × <i>G</i>	414	Error	1.08	0.1276
<i>G</i> × <i>B(S)</i>	702	Error	1.02	0.3337

^a DF = degrees of freedom.

that the inclusion of N had a negative effect on growth in site B ($F_{1,72}=6.76$, $p=0.011$) and combining all sites ($F_{1,72}=6.25$, $p=0.013$), whereas there were no statistical differences in site A and C ($F_{1,72}=0.87$, $p=0.355$ and $F_{1,72}=2.49$, $p=0.119$, respectively).

The highest response to fertilization occurred in the poorest growth site (Site A). The relative response to the best treatments (F2 and F7) was about 165% in this site, while it was around 140% in the other two sites.

Differences among families were also highly significant in all cases (Tables 3 and 4). Analyzing each site independently, the genotype × fertilization

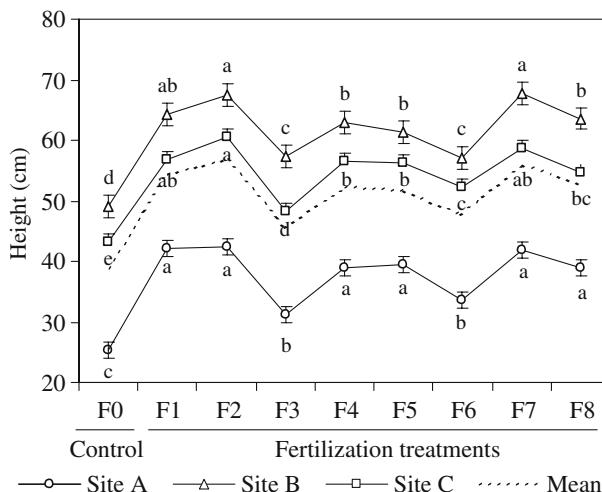


Figure 1. Height (least square means ± standard errors) of 1-year-old radiata pines under no fertilization (Treatment F0) and under 8 different fertilization treatments (F1–F8, see Table 2 for treatment descriptions) at site A (circles), site B (triangles) and site C (squares). Overall mean of the three sites (dotted line) is also shown. Different letters within the same site indicated significant differences among treatment means ($p < 0.05$).

interaction was significant only in one out of the three sites (Site B, Table 3). However the relative importance of this interaction in site B was low as indicated by a high interclass correlation among treatments ($r_B=0.86$). Some differences among genotypes in the response to fertilization may occur but these differences seem to be not very important. Joint analysis across sites showed that genotype \times fertilization interaction was not significant (Table 4). On the contrary genotype \times site interaction was significant (Table 4) suggesting that other factors rather than nutrition are the responsible of the genotype \times environment interaction. Nevertheless, the interclass correlation among sites was again relatively high ($r_B=0.78$), indicating that the strength of the genotype \times environment interaction was scarce.

Table 5 shows the ecovalence values for each family across the different fertilization treatments in the three sites. High ecovalence values indicate a high sensibility to fertilization whereas low ecovalence values indicate similar family

Table 5. Family ecovalence (Wricke 1962) across the different fertilization treatments in the three test sites.

Family code	Ecovalence (%)		
	Site A	Site B	Site C
00	7.2	2.0	1.8
01	1.3	2.0	3.4
08	2.6	4.7	4.2
11	4.8	5.7	2.7
22	1.2	2.9	5.3
30	2.6	3.7	0.8
32	5.2	3.4	2.5
42	7.7	3.4	2.1
45	1.5	5.3	4.5
46	4.1	1.2	3.7
49	7.7	1.5	4.6
51	2.4	3.6	3.3
55	8.7	4.5	2.6
68	1.7	11.1	7.2
69	5.4	1.1	4.4
72	2.8	2.1	2.0
73	2.4	3.8	2.8
74	0.5	3.4	3.2
76	2.3	4.6	3.3
77	2.2	5.0	5.0
78	4.0	3.6	1.7
79	7.7	4.5	2.7
80	4.6	5.4	4.1
83	2.9	3.2	12.3
84	1.4	3.5	5.8
86	3.4	3.1	1.7
89	1.7	1.6	2.3

performance irrespective of fertilization. Despite considerable variation in the ecovalence can be observed within each site, there were no families that can be identified as consistently stable or sensible in the three sites. In fact, the ecovalence values were not related from site to site ($r^2=0.03-0.07$, $p>0.05$).

Discussion

The response to the fertilization treatments agreed with the nutritional disorders frequently found in *P. radiata* stands in northern Spain. Phosphorus and Mg are the most common nutrient deficiencies in young (Merino and Edeso 1999; Zas 2003) and mature *P. radiata* plantations (Mesanza et al. 1993; Romanyà and Vallejo 1996; Sánchez-Rodríguez et al. 2002) in northern Spain. On the contrary, N is usually well supplied or even in excess (Sánchez-Rodríguez et al. 2002; Zas 2003; Zas and Serrada 2003). Furthermore, site index of *P. radiata* has been found to be positively related with available soil P and foliar P, and negatively with total soil and foliar N (Sánchez-Rodríguez et al. 2002; Romanyà and Vallejo 2004). Thus, the positive effect of the P and Mg fertilization and the negative effect of the N addition was expected.

The best fertilization treatments produced a height growth increment ranging between 1.4 and 1.7 folds over the unfertilized control. The benefits of such an important response may be particularly important in these radiata pine plantations through a forwardness of the canopy closure and, consequently, a reduction of weed competition. However, establishment fertilization should only be recommended if combined with effective control of any competing vegetation until canopy closure. Otherwise, the application of fertilizers can stimulate weed growth more than tree growth (Will 1985). Further evaluations are needed to analyze the perseverance of the fertilization effects in mature ages.

Most of the studies regarding genetic variation in nutrient-related traits have been carried out under controlled conditions in greenhouse or growth chambers. Genetic differences in nutrient use efficiency (NUE, i.e. growth per unit of nutrient) and other equivalent measures and/or in the response to fertilization are evident in many tree species (Mullin 1985; Crawford et al. 1991; Li et al. 1991; Dewald et al. 1992; Jonsson et al. 1997; Mari et al. 2003a; Xiao et al. 2003) and also in *P. radiata* (Fife and Nambiar 1995). However there are only few studies where genotype \times nutrient interaction is analyzed in real field conditions. For example, Crawford et al. (1991) found important rank changes in *Pinus taeda* families after fertilization in field, whereas Fife and Nambiar (1995) found markedly different responses to N addition among *P. radiata* families in a sandy soil in South Australia. These field studies were usually carried out in one site only (Crawford et al. 1991; Fife and Nambiar 1995), thus, repeatability of the genetic variation in the response to fertilization across sites was not analyzed. Xiao et al. (2003) analyzed the intra-specific variation

in growth and foliar nutrient response to fertilization in families of *Pinus eliottii* and *P. taeda* planted in two locations in north Florida, but they did not study the relationships across sites. In our study, the results were not consistent across sites. Genotype × fertilization interaction was significant in one site, indicating different genetic response to fertilization in this site, i.e. genetic variation in nutrient use efficiency among the studied families. However, genotype × fertilization interaction was no significant in the other two sites suggesting that, in these sites, all the open-pollinated families had similar responses to the different fertilization treatments. Moreover, the genotypic sensibility to fertilization within each site, estimated as the family ecovalence value, was not related across sites. Some stable families in one site (i.e. equivalent relative performance irrespective of the fertilization treatment) may show a high sensibility to fertilization in other sites. Other factors, such as initial soil nutrient levels, pH, texture, or mycorrhizae may be interacting. For example, response to fertilization should be higher when nutrient availability before fertilization is low and becomes smaller as the original nutrient levels increase. This general form of non-linear response varies among genotypes (Namkoong et al. 1992) and, thus, the specific response of a given genotype may vary (in relation to other genotypes) from site to site due to different soil start conditions. Besides, the lack of consistence among the genotypic stability across the three sites could be due to no reliable estimations of genotypic stability in those sites where the interaction was not significant.

The results of the present work confirmed P nutrition to be critical in *P. radiata* in Galicia. Superphosphate applications at establishment can be recommended as a routine practice. Improving the P-use-efficiency (PUE) by breeding should also be highly desirable. The use of P-use-efficient genotypes may substitute the need of P fertilization. This may be particularly interesting because P fertilizers are a non-renewable resource and cheap sources of P will be exhausted within the next 60–90 years (Runge-Metzger 1995). Furthermore, a widespread use of P fertilizers can pollute local watercourses leading to eutrophication. In fact, improving PUE in agricultural crops is becoming an issue of major importance (Hammond et al. 2004). The results presented here indicated that genotypes may respond differently to fertilization but this variable response could differ across sites. Our results suggest that some families of the breeding program are more nutrient-efficient than others, and improving the nutrient efficiency appeared to be possible, but at this point further research is needed. Fertilization treatments were designed upon combinations of several fertilizers complicating the interpretation of the results regarding one single nutrient. New experiments with just P fertilizers applied in different doses on soils with different P availabilities should be carried out to further investigate the possibility to improve PUE. Furthermore, our results are limited because of the young age of the experiment and post-planting stress may be affecting too much the results. Therefore, new analysis at older ages are necessary before any practical conclusion is given.

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