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Interactive effects of phosphorus and light availability on early growth of maritime pine seedlings

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Abstract – We examined the response of early growth of maritime pine seedlings to combined levels of light and phosphorus. Seedlings were grown under three levels of phosphorus availability, i.e., two relative addition rates ($RAR = 2$ and 4 g P (100 g⁻¹)P d⁻¹) and a free-access to P, crossed with two light levels (photosynthetic photon flux densities of 150 and 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively). Relative growth rate (RGR) and relative uptake rate of phosphorus (RUR) were computed, as well as the amount of light absorbed per seedling. We found that phosphorus and light acted as limiting factors with a complex interaction. Under low light and at the lowest P level, P and light were co-limiting, i.e., growth was enhanced only when P and light were increased together. Light was the limiting factor for growth under low light conditions at all other levels of P availability. P was the limiting factor at a RAR of 2% under high light. Enhancing P from 4% to free access did not significantly improve growth under high light. RUR was controlled systematically by P availability at 2 and 4% RAR . RGR values were close to RUR values except under free access to P. Therefore, growth-independent accumulation of P was observed under high P conditions. The differences in biomass production among P treatments were explained primarily by the reduced amount of radiation intercepted by the seedlings as a consequence of their reduced leaf area. No effect of P treatments were observed on the calculated radiation-use efficiency (RUE), which was found to be larger under low light. This confirms that pine seedlings adjust to moderate phosphorus deficiency mainly by changing their morphology (leaf area, dry-mass partitioning) while biochemical and photochemical limitations of photosynthesis play only a very secondary role.

phosphorus nutrition / shade / growth / *Pinus pinaster* Ait.

Résumé – Interaction des effets de la disponibilité en phosphore et en lumière sur la croissance de jeunes plants de pin maritime. La croissance de jeunes plants de pin maritime a été suivie en chambre de culture à deux niveaux d'éclairement et trois de disponibilité en phosphore. Deux taux d'addition relatifs en P ($RAR = 2$ et 4 g P (100 g⁻¹)P j⁻¹) et un libre accès au P ont été appliqués en combinaison avec deux niveaux d'éclairement (150 et 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Le taux de croissance relatif (RGR) et le taux d'absorption relatif de P (RUR) ont été calculés. La surface foliaire, la quantité de rayonnement absorbé et l'efficacité d'utilisation du rayonnement (RUE) ont aussi été estimés. Les disponibilités en phosphore et en lumière ont limité la croissance suivant un schéma d'interaction complexe. Au plus faible niveau d'éclairement et d'ajout en phosphore, les deux facteurs étaient co-limitants et la croissance n'était augmentée que lorsque les deux facteurs augmentaient simultanément. La lumière était le facteur limitant pour les traitements sous faible éclairement à tous les autres niveaux de disponibilité en P. Le phosphore était limitant pour des RAR de 2% sous fort éclairement. Le taux de prélèvement relatif en P (RUR) était contrôlé par la disponibilité en P aux taux d'addition relatifs (RAR) de 2 et 4%. Les valeurs de RGR étaient proches des valeurs de RUR sauf au plus fort niveau de disponibilité en P où une accumulation de P indépendante de la croissance a été observée. La réduction de la croissance sous limitation en P est explicable par la réduction de la quantité de rayonnement absorbé par les plants, par suite d'une expansion limitée et plus lente de leur surface foliaire. Il n'y a pas eu d'effet de la disponibilité en P sur le RUE . Le RUE était par contre plus élevé sous faible éclairement. En conclusion, nos résultats confirment que les jeunes plants de pin maritime soumis à une déficience modérée en P ajustent leur croissance (réduction de l'expansion de la surface foliaire) sans effet majeur sur le RUE .

nutrition en phosphore / ombrage / croissance / *Pinus pinaster* Ait.

1. INTRODUCTION

In their natural habitat, forest species are exposed to multiple trophic constraints related to either climatic conditions such as water and light or nutrient availability in soils. Predicting their

response to changes in the availability of a given resource or to combined changes in several resources is of critical importance in the context of global environmental changes. Many studies have been devoted to interactions between light and nitrogen in forest species [1, 7, 9, 16, 29]. These studies

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demonstrated the importance of interactive effects of nitrogen and light on regulating the physiology and growth of seedlings. For example, Elliott and White [16] demonstrated that high nitrogen significantly increased total biomass of red pine seedlings under high irradiance but had no effect in shade.

Studies on interactions between light and phosphorus are still scarce, although phosphorus is a common limiting factor for tree growth in natural environments [52, 57]. Increased growth in response to enhanced P availability was commonly reported for many tree species either in the field or under artificial growth conditions [9, 52, 55, 57]. Chang [9] showed that increasing P supply from 0 to 50 kg P ha⁻¹ increased basal diameter and height increment of two-month-old sweetgum seedlings by 24 and 22%, respectively. Topa and Cheeseman [55] reported that after six weeks of aerobic solution culture, low-P treatment (5 µM P) reduced *Pinus serotina* seedling dry weight, relative growth rate of shoots and roots by 39, 41 and 58%, respectively. Tree growth response to light availability has been studied by many authors. Faster growth was reported under increased light availability for numerous hardwoods and conifers in both natural and greenhouse environments [12, 16, 47, 58]. Elliot and White [16] reported 4 to 5 times more biomass for red pine (*Pinus resinosa* Ait.) seedlings grown under high (> 800 µmol m⁻² s⁻¹) light than under low light (190 µmol m⁻² s⁻¹). On longleaf pine, Jose et al. [29] observed under high light a 48.6 and 56% increase of stem and root biomass, respectively. On four deciduous and pioneer species, Rincon and Huante [47] reported larger relative growth rates (*RGR*) and biomass production under high light (400 µmol m⁻² s⁻¹) as compared with low light (80 µmol m⁻² s⁻¹).

Although separate effects of phosphorus and light availability on tree growth are well documented, there still is a lack of knowledge on how both factors interact. It has been demonstrated that a moderate deficiency in phosphorus first affects shoot growth and leaf development, whereas a pronounced deficiency depresses photosynthesis through a decrease in carboxylation and quantum efficiency [2, 5, 28, 33, 34]. Whereas the effects of phosphorus deficiency on photosynthesis have been investigated extensively at cell and leaf levels, the interaction between phosphorus availability and carbon accumulation at whole plant level is less documented.

The objectives of the present study were to assess how growth rate and biomass of a forest tree species are affected by different levels of light and phosphorus availability and whether some interactive responses to light and phosphorus limitation occur, particularly for phosphorus productivity (P_p) and radiation-use efficiency (*RUE*). The experiment was conducted on maritime pine (*Pinus pinaster* Ait.), a shade-intolerant species grown in southwestern Europe on soils characterised by a low phosphorus availability [50, 51]. Numerous studies have reported a positive effect of P fertilization on maritime pine growth in this context [21, 54, 56]. Indeed, in field conditions, plant nutrient uptake is affected by biotic, e.g., mycorrhization [22] or abiotic factors, such as nutrient availability [18] or soil structure [41], which makes it difficult to study the specific effects of nutrient uptake on growth. In sake of simplicity and for enabling us to monitor the plant uptake rate of phosphorus, our experiment was therefore operated under hydroponic conditions where the nutrient addition rate is controlled accurately.

2. MATERIALS AND METHODS

2.1. Plant material and growth conditions

Seeds of maritime pine (*Pinus pinaster* Ait.) originating from the Landes of Gascogne forest (Southwestern France) were disinfected with a solution of 4% CaCl₂ for 10 min and then germinated in containers filled with moistened vermiculite. The containers were placed in a climate chamber with light supplied by 250 W HQI lamps (16 h day, 8 h night). Photosynthetic photon flux density (PPFD) measured at the plant level was 230 µmol m⁻² s⁻¹. Air temperature and relative humidity were 20 °C day/15 °C night and 70%, respectively. Containers were irrigated daily and treated weekly with a fungicide (cryptonol, 0.3%).

Thirty days after sowing (DAS), 216 seedlings were selected on the basis of their overall uniformity (mean total fresh weight: 0.184 g per seedling) and transplanted into aeroponic growth units (Biotronic, Uppsala, Sweden). The aeroponic growth units were automated to control the composition of the nutrient solution that was continuously recycled and sprayed on the root systems according to the specifications prescribed by Ingestad and Lund [26].

Each growth unit received 36 seedlings. Except for P, the composition of the nutrient solution was adapted from the recommendations given for *Pinus sylvestris* by Ingestad [24] (Tab. I). Details about P supplied to the plants are given later in the text. The pH of the nutrient solution was maintained between 4.0 and 4.5 by regular acid or basic P-free nutrient solution additions.

Six growth units corresponding to six treatments were randomly arranged in a growth chamber with the following climate: 16 h day/8 h night, 20–23 °C day / 15–18 °C night and 70% relative humidity. Light was supplied by 250 W metal-halide lamps (TD 70/150/250, MAZDA, Belgium). The experiment was conducted between July 23 and October 25, 2002.

2.2. P and light treatments

Three rates of P supply (high P, intermediate P and low P), combined with two levels of irradiance (high light: PPFD of 400–500 µmol m⁻² s⁻¹ and low light: PPFD of 120–180 µmol m⁻² s⁻¹) were applied between seedling transplantation (30 DAS) and the end of the experiment (105–110 DAS). In the high P treatment (HP, or free-access), P was supplied as KH₂PO₄ at a growth-saturating concentration of 516 mM P. For intermediate (IP) and low (LP) treatments, P was supplied at a daily relative addition rate (*RAR*) of 4 and 2 g P (100 g⁻¹)P d⁻¹, respectively [26]. Assuming the relative growth rate, *RGR*, was close to the relative addition rate, *RAR*, the amount of P added at day *t* (P_A) into the nutrient solution was calculated as:

$$P_A = P_s \left[\exp \left(\frac{RGR}{100} \right) - 1 \right] \quad (1)$$

where P_s is the amount of P in seedlings on day *t*, and *RGR* the relative growth rate of the seedlings in each growth unit. In the LP and IP treatments, nutrient solution conductivity was adjusted to 80–150 µS cm⁻¹ by addition of P free nutrient solution. In the free-access treatment (HP), nutrient solution conductivity was adjusted to 300–350 µS cm⁻¹ by the addition of nutrient solution containing P. A high conductivity value was chosen to maintain a high P concentration in the nutrient solution of the free-access treatment (HP).

The low irradiance level was obtained by shading plants with four layers of white cloth, whereas seedlings subject to high light treatment were exposed to unobstructed light. Homogeneity of irradiance within each growth unit was checked with a PPFD light sensor (Li-190 SB, Licor Ltd, Lincoln USA). The resulting photosynthetic photon flux

Table I. Composition of the stock nutrient solutions (mM).

Compound	Basic solution		Acid solution	
	P-free	With P	P-free	With P
NH ₄	5593	5714	0	0
NO ₃	1550	1429	7143	7143
P	0	516	0	516
K	1151	1151	1151	1151
Ca	150	150	150	150
Mg	247	247	247	247
S	250	250	250	250
Fe	0.7	0.7	0.7	0.7
Mn	4	4	4	4
Cu	0.03	0.03	0.03	0.03
Zn	0.06	0.06	0.06	0.06
B	0.2	0.2	0.2	0.2
Mo	0.007	0.007	0.007	0.007
Na	0.22	0.22	0.22	0.22
Cl	0.033	0.033	0.033	0.033

density (PPFD) at the top of the seedlings was 120–180 and 400–500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the LL and HL treatments, respectively.

2.3. Plant measurements and chemical analysis

Three plants per treatment were sampled each week for a non-destructive measurement of the total fresh weight per seedling and calculation of the relative growth rate (*RGR*). Each plant was carefully removed from the growth unit, and hanged on a wooden frame. The root system was blotted dry between absorbing papers before the seedling was weighed. Plants were subsequently replaced in their growth unit. Additionally, three plants per treatment were randomly sampled every 15 d for measuring dry weight at 65 °C of shoot and root, as well as the total P content of the seedling. Five plants per treatment were harvested at 68, 88 and 105–110 DAS for morphologic measurements. Length, width and dry weight of three euphylls (the “primary needles” for maritime pine seedlings, see [31, 40]) of the main stem and auxiblasts (the axillary shoots which have the same structure than the main shoot) were measured. The area and area-to-mass ratio of individual euphylls were subsequently calculated. The total euphyll area was estimated from the area-to-mass ratio of individual euphylls and the total euphyll dry weight. The same procedure was used to estimate the pseudophyll (two “secondary needles” which compose the brachyblasts, see [31, 40]) area. The total foliage area per plant was calculated by summing euphyll and pseudophyll areas. The remaining shoot and root system were dried at 65 °C, weighed and N and P contents measured colorimetrically with a Technicon Autoanalyser II [43].

2.4. Control of environmental conditions

The photosynthetic photon flux density (PPFD) was measured continuously for each growth unit at the level of seedlings using amorphous silicon cells (Solems, France) as proposed by Chartier et al. [11]. Air and nutrient solution temperatures of each growth unit were monitored using Cu-Cr thermocouples recorded with a datalogger (CR23X, Campbell Scientific France, Paris). Relative humidity in the chamber was measured with a relative humidity probe (HMP35AC, Campbell Scientific). Air and nutrient temperatures, as well as humidity meas-

Table II. Mean and standard deviation of diurnal air and nutrient solution temperatures (°C) and incident PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$) for each treatment during the experimental period ($n = 74\text{--}94$ days).

Treatments	Air (°C)	Nutrient solution (°C)	PPFD $\mu\text{mol m}^{-2} \text{s}^{-1}$
LL-LP	22.3 ± 1.3	21.0 ± 1.5	184 ± 17
LL-IP	21.5 ± 1.2	23.0 ± 1.3	157 ± 11
LL-HP	21.0 ± 1.3	24.1 ± 1.5	122 ± 26
HL-LP	22.3 ± 1.4	21.8 ± 1.3	410 ± 23
HL-IP	24.0 ± 1.2	22.3 ± 1.0	501 ± 26
HL-HP	22.0 ± 1.4	22.8 ± 1.8	424 ± 46

urements, were performed every 10 min and hourly and average values computed. The average daily values of climatic conditions corresponding to each treatment are shown in Table II. As expected, PPFD differed between low (120 to 180 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and high light (410 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Although air in the growth chambers was stirred, average air temperature differed slightly among growth units and treatments. To account for these differences, growth kinetics were expressed on a thermal time basis (*TT*, °C days) calculated on a daily basis as follows:

$$TT = \sum \left[\frac{(T_X + T_N)}{2} - T_b \right] \quad (2)$$

where T_X is the maximum daily air temperature (°C), T_N the minimum daily temperature (°C) and T_b the base temperature. Since no reference exists in the literature about the base temperature for maritime pine, an arbitrary value of $T_b = 10$ °C was used for calculations. All measured temperatures were above this base value so that this arbitrary choice may alter the absolute values of calculated thermal time but not the relative values between treatments.

2.5. Calculations and statistics

To account for the temperature difference observed between growth units, the individual relative growth rate of each seedling (*RGR* in $\text{g g}^{-1} (\text{°C days})^{-1}$) was expressed on a thermal time basis as follows:

$$RGR = \frac{(\ln(W_{TT_2}) - \ln(W_{TT_1}))}{TT_2 - TT_1} \quad (3)$$

where *W* is the total plant fresh weight (g) and *TT* the thermal time (°C days). For consistency, the *RGR* were also recalculated on a thermal time basis. Similarly, relative uptake rate of P (*RUR* in $\text{g P g}^{-1} (\text{°C days})^{-1}$) was calculated from P content as follows:

$$RUR = \frac{(\ln(P_{TT_2}) - \ln(P_{TT_1}))}{TT_2 - TT_1} \quad (4)$$

where P_{TT_1} and P_{TT_2} (g P) are amounts of P in the plant at thermal times TT_1 and TT_2 (°C days), respectively.

Assuming growth was exponential and according to Ingstad's theory [25–27], a plant which growth is limited by P availability is considered to be at a steady-state when the plant P concentration remains constant over time:

$$\frac{d(P/W)}{dt} = 0. \quad (5)$$

Under these conditions, it follows that:

$$RUR = \frac{1}{P} \frac{dP}{dt} = \frac{1}{W} \frac{dW}{dt} = RGR. \quad (6)$$

Phosphorus productivity P_P (growth rate per unit of phosphorus in the plant) was defined as the slope of the linear relationship between *RGR* and plant P concentration ($C_P = P/W$) [24, 26]:

$$\frac{dW}{dt} \times \frac{1}{W} = P_P \times C_P. \quad (7)$$

Moreover, when P availability limits P uptake, the steady state is obtained when *RUR* is controlled by a numerically equal and constant relative addition rate (*RAR*). Therefore, under steady-state conditions (constant P concentration in the plant), *RGR* of a plant whose growth is limited by P availability equals *RUR*, and *RAR* [25, 26]:

$$RAR = RUR = RGR. \quad (8)$$

During the first days after seedling transplantation into the growth units, *RGR* changed rapidly and reached a steady value. For each treatment, a period of constant *RGR* was identified statistically, and non steady-state *RGR* values were discarded. Light interception by seedlings was calculated according to Forseth and Norman [19]. Specifically, incident PPFD was partitioned into a direct and a diffuse component, respectively Q_{diff} and Q_{dir} . Q_{dir} was defined as the vertical downward photosynthetic photon flux density whereas Q_{diff} was estimated as the average PPFD received from five angular sectors corresponding to four horizontal sectors and upward reflection. On average, measured Q_{dir} and Q_{diff} were 70% and 30% of the total incident PPFD, respectively.

The amount of direct light intercepted by sunlit foliage ($Q_{i,\text{sun}}$) was calculated as:

$$Q_{i,\text{sun}} = K \cdot Q_{\text{dir}} \cdot F_{\text{sun}} \quad (9)$$

where *K* was the foliar absorption coefficient calculated according to Campbell [6] with a mean inclination angle of the foliage plane from the horizontal, γ , assumed to be 45 degrees ($K = 0.644$), and F_{sun} being the sunlit foliage area index calculated as:

$$F_{\text{sun}} = \frac{1 - \exp\left(\frac{-KF}{\cos(\theta)}\right)}{K \cdot \cos(\theta)} \quad (10)$$

where *F* was the foliage area index of each seedling ($\text{cm}^2 \text{cm}^{-2}$) and θ was the zenith angle of the light source (assumed to be zero in our experiment). *F* was given by equation (11), where *L* was the total foliage area per seedling ($\text{cm}^2 \text{seedling}^{-1}$) and *A* the projected seedling foliage area on an horizontal surface ($\text{cm}^2 \text{seedling}^{-1}$) estimated assuming the seedling foliage was entirely contained in a vertical cylinder with a radius *r* given as $r = l \cos(\gamma)$ with *l* being the average needle length so that:

$$F = \frac{L}{A}. \quad (11)$$

Total foliage area per seedling was interpolated between measurements using the ratio of foliage area to stem height as estimated from destructive measurements and stem height values which were measured weekly during the experiment. The diffuse PPFD intercepted by both faces of foliage ($Q_{i,\text{shade}}$) was calculated as:

$$Q_{i,\text{shade}} = 2 \cdot F \cdot Q_{\text{diff}} \exp(-0.5F^{0.7}) \quad (12)$$

where 0.5 and 0.7 were coefficients depending on foliar orientation which account for the radiation extinction in the foliage [42].

The PPFD absorbed by a seedling (Q_a mol seedling $^{-1}$ day $^{-1}$) is given by:

$$Q_a = a \cdot A(Q_{i,\text{sun}} + Q_{i,\text{shade}}) \quad (13)$$

where *a* (= 0.9) is needle absorbance in visible light [4].

Radiation-use efficiency (*RUE* in g DW mol^{-1}) was estimated as the slope of the linear regression between total dry biomass accumulated after seedling transplantation and photosynthetically active radiation absorbed (cQ_a) cumulated over the same period. Daily absorbed PPFD was calculated for five individual seedlings per treatment.

The six combinations of light and P levels could not be replicated. We therefore assumed the lack of a significant growth-unit effect. We took care to minimize the effects of the difference in temperature or incident light between growth units, and assumed the possible residual effects linked to a particular growth unit was unlikely to corrupt the large differences in the responses. Therefore, we considered each seedling as a replicate, though this is not in accordance with the strict statistical sense of a replicate. For most of the measured variables, two-way analysis of variance with interaction and linear regression based respectively on values measured on individual seedlings and averaged values per growth unit were performed using the ANOVA and GLM procedures of SYSTAT 10 for Windows (SPSS Inc. Chicago, USA). Significant differences between means were separated using the LSD procedure. The first order risk was fixed at $\alpha = 0.05$.

3. RESULTS

3.1. Relative Growth Rate (*RGR*) in response to phosphorus and light

A two-way analysis of variance showed that *RGR* was significantly affected by P and light treatments and that a significant interaction existed between both factors (data not shown). At low light (LL), *RGR* was unresponsive to P availability (Fig. 1). Conversely, at high light (HL), *RGR* increased significantly with P availability between low (LP) and intermediate (IP) P levels, but not between IP and free access to P (HP) (Fig. 1). Under LP, *RGR* did not differ significantly between light levels ($\alpha > 0.05$, tests not shown). Conversely, at IP and HP, *RGR* increased significantly with increasing light level ($\alpha < 0.05$, tests not shown).

Table III. Mean values of the relative uptake rates (RUR in $\text{mg P (mg P)}^{-1} (\text{°C day})^{-1}$) obtained during the steady state period for each light and P treatment. Mean values per treatment were obtained by averaging 3–4 individual values. For each light treatment, values annotated by the same letter are not significantly different (LSD test, $\alpha = 0.05$).

	RUR	
	HL	LL
HP	0.00730 ^a \pm 0.00154	0.00420 ^a \pm 0.00057
IP	0.00335 ^b \pm 0.00038	0.00272 ^a \pm 0.00039
LP	0.00101 ^c \pm 0.00064	0.00161 ^b \pm 0.00043

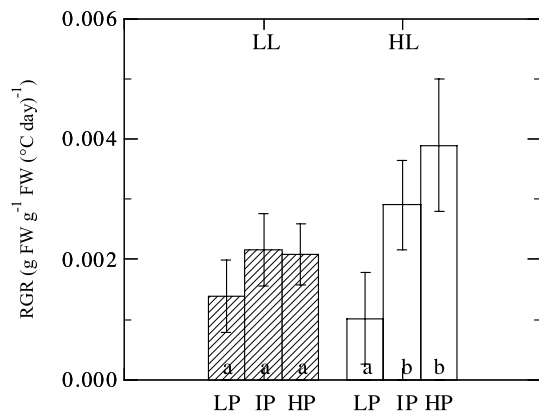


Figure 1. Relative growth rate (RGR) of *Pinus pinaster* seedlings grown under three P regimes combined to two levels of irradiance: LP (RAR of $2 \text{ g P (100 g)}^{-1} \text{ P d}^{-1}$), IP (RAR of $4 \text{ g P (100 g)}^{-1} \text{ P d}^{-1}$) and HP (free access to P); HL ($400\text{--}500 \mu\text{mol m}^{-2} \text{ s}^{-1}$) and LL ($120\text{--}180 \mu\text{mol m}^{-2} \text{ s}^{-1}$). RGR was calculated on a thermal time basis. Mean values per treatment were obtained by averaging individual values of RGR observed during the steady state period ($n = 8\text{--}9$ individual values per treatment). For each light treatment, bars annotated with the same letter are not significantly different (LSD test, $\alpha = 0.05$). Each vertical bar indicates the standard error of the mean.

3.2. Relationships between relative growth rate (RGR), relative uptake rate (RUR) and relative addition rate (RAR)

A two-way analysis of variance showed that RUR values were significantly affected by P but not by light treatments (data not shown). Under high light (HL), RUR significantly increased from LP to IP and to HP (Tab. III). Under low light, RUR significantly increased between LP and IP but not between IP and HP (Tab. III).

Figure 2 shows the relationships between RGR , RUR and RAR for all P and light treatments. RAR could not be calculated for HP. RAR was two fold larger at IP than under LP, as expected from the P addition treatments used. RUR increased with RAR under both high and low light treatments. Moreover, RUR was close to RAR for both LP and IP (Fig. 2). This demonstrates that at these P levels, P uptake was limited by P availability whatever the light availability. RGR was close to RUR

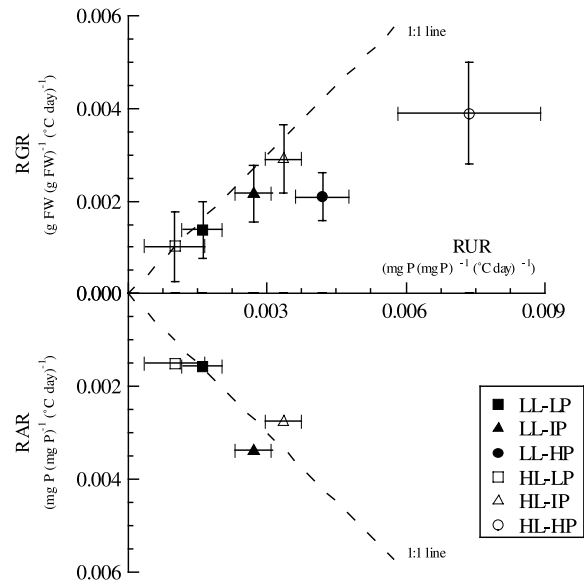


Figure 2. Relationships between relative growth rates (RGR), relative uptake rates of P (RUR) and relative addition rates of P (RAR) for *Pinus pinaster* seedlings grown under two light levels (low light (LL) and high light (HL)) and three P regimes (low P (LP), intermediate P (IP) and high P (HP)). Relative addition rates (RAR) could not be calculated for the high P (free access) regime. RGR , RUR and RAR values were calculated on a thermal time basis. Mean values of RGR ($n = 8\text{--}9$ individual values per treatment) and individual values of RUR ($n = 2\text{--}3$ individual values per treatment), respectively. Only individual values of RGR and RUR obtained during the steady state period were considered. Each vertical and horizontal bar indicates the standard error of the mean.

for LP and IP. However, RUR and RGR observed at HP deviated severely from the 1:1 relationship, suggesting that P uptake was no longer related to the increment in dry biomass. Indeed, RUR for HP was larger than RGR which demonstrates that growth was not controlled by P at this level and seedlings accumulated P independently of growth. The incident light intensity was certainly below saturation at this stage and was likely the main limiting factor at HP.

Figure 3 shows the relationship between RGR and plant P concentration, with the slope indicating the phosphorus productivity (P_p). Under high light, RGR increased sharply with increasing plant P concentration at LP and IP, and the slope of the linear relationship between plant P concentration and RGR , i.e., phosphorus productivity, was highest (Fig. 3). It levelled off at higher P concentrations where phosphorus productivity dropped. RGR reached its maximum value ($0.0038 \text{ g FW (g FW)}^{-1} (\text{°C day})^{-1}$) at an optimum P concentration between 0.002 and $0.004 \text{ g P (g DW)}^{-1}$, close to the value found by Ericsson and Ingestad [17] in birch seedlings. Conversely, under low light, RGR was independent of plant P and no relationship was found between P accumulated by seedlings and growth.

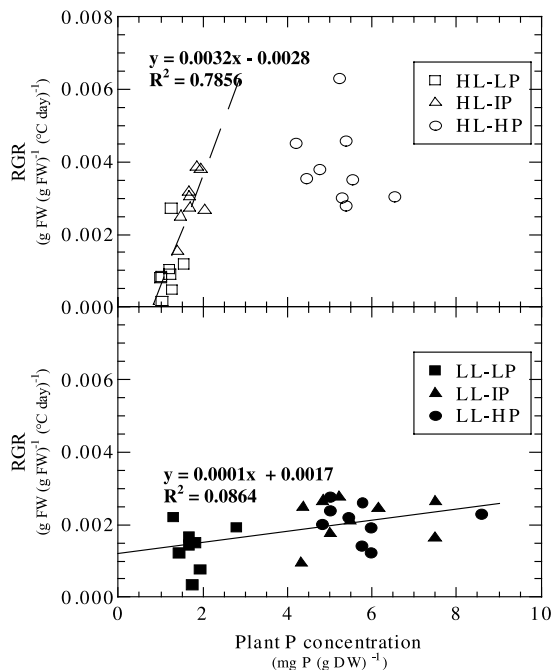


Figure 3. Relationships between relative growth rates (RGR) and plant P concentration for *Pinus pinaster* seedlings grown under two light levels (high light (HL) and low light (LL)) and three P regimes (low P (LP), intermediate P (IP) and high P (HP)). Each symbol corresponds to one individual seedling.

3.3. Leaf area development, PPFD absorbed and radiation use efficiency (RUE)

Figure 4 shows the total leaf area per seedling calculated at the three sampling dates versus thermal time after transplantation. P availability affected leaf area development only under high light ($\alpha < 0.05$) (Fig. 4). At low light availability, P did not affect leaf area ($\alpha > 0.05$) (Fig. 4). Light availability affected leaf area development only at the highest P level ($\alpha < 0.05$). These results are consistent with those observed for RGR (Fig. 1). At the end of the experiment, seedlings under HL and HP had the highest total leaf area ($132.8 \pm 33.9 \text{ cm}^2$), whereas seedlings in the HL-LP treatment had the lowest ($32 \pm 6.5 \text{ cm}^2$).

The relationships between total biomass (estimated at 105–110 DAS) and absorbed irradiance cumulated per seedling for all light and P treatments (Fig. 5) revealed an effect of the light level on the slope of the dry biomass - absorbed irradiance relationship, i.e., radiation use efficiency (RUE). This indicates that seedlings grown under low light had higher RUE . Conversely, P treatments did not affect RUE . Indeed, under high light a unique linear relationship was observed for all P treatments between total biomass produced per seedling and cumulated absorbed irradiance. This demonstrates that under high light, growth was modulated by P nutrition via leaf area expansion rather than via a change in RUE .

The linear relationship between total biomass and absorbed irradiance had a steeper slope under low than under high light. In that case, the calculated absorbed irradiance was not very different among P treatments, since they did not affect leaf area,

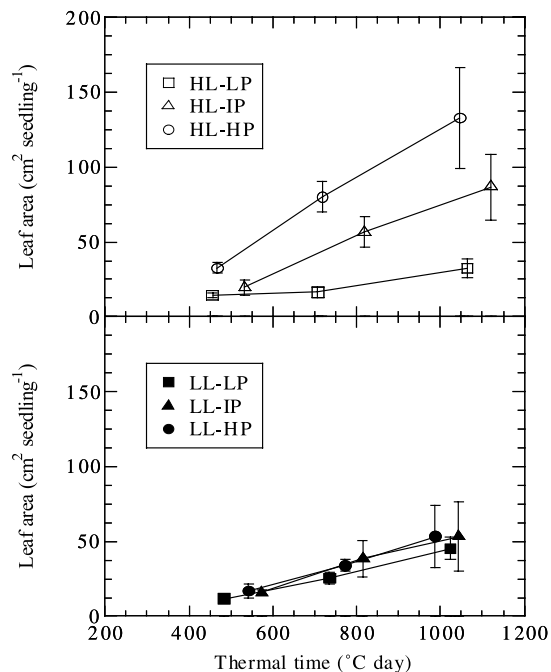


Figure 4. Total leaf area per plant of *Pinus pinaster* seedlings grown under two light levels (high light (HL) and low light (LL)) and three P regimes (low P (LP), intermediate P (IP) and high P (HP)). Mean values per sampling date and treatment were calculated by averaging 5–10 individual values. Each vertical bar indicates the standard error of the mean.

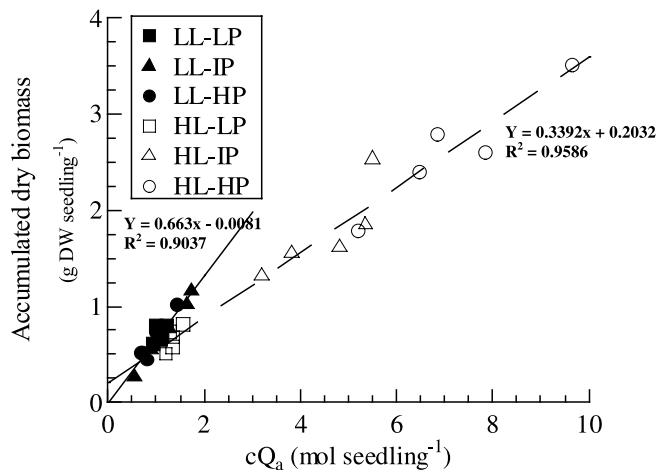


Figure 5. Relationship between the dry weight produced per seedling and the amount of absorbed irradiance (cQ_a) for *Pinus pinaster* seedlings grown under two light levels (high light (HL) and low light (LL)) and three P regimes (low P (LP), intermediate P (IP) and high P (HP)). Each symbol corresponds to one individual seedling harvested after 105–110 days.

resulting in the range of cumulated absorbed irradiance being mainly created by the within-treatment variability between seedlings. As for high light treatments, the relationship between cumulated absorbed irradiance and seedling biomass was the same for all P treatments.

4. DISCUSSION

Our results reveal the occurrence of a complex interaction between phosphorus and light. At the lowest level of P and light (LP-LL treatment), relative growth rate (*RGR*) was enhanced only when both P and light were increased together, which means that the two factors were co-limiting. At higher levels of P (IP and HP), *RGR* increased with light, which means that light was the limiting factor. This result is consistent with the available knowledge on the photosynthetic requirements of *Pinus pinaster* [2, 3, 34]. Indeed, incident PPFD was 120 to 180 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which is far below the saturating PPFD for maritime pine [34].

Under the highest light level, P was limiting only under low P and intermediate P, but not from IP to high P (HP). This lack of response to increasing P availability between IP and HP may be explained by the fact that even under high light in our experiment (400–500 $\mu\text{mol m}^{-2} \text{s}^{-1}$), PPFD was not saturating and growth remained probably limited by light.

The differences in *RGR* induced in our experiment were caused by the differential amount of intercepted radiation due to lower plant leaf area expansion in relationship with either P [48, 49] or light limitations rather than a reduced carbon assimilation rate (e.g., photosynthesis per unit of leaf area). In maritime pine, Ben Brahim et al. [2] have reported that under moderate P deficiency (between 0.0013 and 0.0017 g P per g dry matter in plants), the lower plant growth was paralleled by a slower development of the foliage. Under more severe P deficiencies (between 0.0004 and 0.0013 g P per g dry biomass) a tight negative correlation was observed between phosphorus leaf concentration and photosynthetic capacity [15, 34]. Our experiment was conducted in the intermediate range of P deficiencies, so that the absence of effect of P treatments on *RUE* is consistent with these results. Pine seedlings adjust to moderate phosphorus limitation mainly by changing their morphology (leaf area, dry-mass partitioning) while biochemical and photochemical limitations of photosynthesis would play a secondary role, if any. We conclude that leaf area and consequently the amount of light absorbed by seedlings was the main process limiting the carbon gain and dry matter increment for seedlings grown under mild P deficiency. Similar conclusions were produced for other plant species where low P was found to have a larger impact on leaf area expansion than on the rate of photosynthesis per unit leaf area [14, 20, 45]. Thus, a moderate P deficiency affects the morphological component, while severe deficiencies mainly affect the physiological component. In a meta-analysis of literature based on 75 observations, it was reported that on average the morphological component of *RGR* was more important than the “physiological” component in explaining the effects of nutrient limitation on growth [44].

Resource use efficiency was always highest at the lowest P availability and decreased as the resource concentration increased. In the case of phosphorus, the decrease in phosphorus productivity at high level of P availability may be attributed to a growth independent accumulation, which may be interpreted as P storage. In our experiment, growth independent P consumption was observed in the P free-access treatments. This can be attributed to the accumulation of inorganic phosphorus (Pi) in the vacuole [32, 46] and to a higher concentration of P incorporated in organic compounds in the cell as polyphosphate

or phytate [13]. This ability may be ecologically important in enabling plants to face seasonal variations in phosphorus availability observed in the field [10]. Luxury consumption and large vacuolar storage are interpreted as potentially contributing to future productivity by several authors [35, 39]. P remains in its oxidised form and a relatively large part is incorporated in structural cell components, such as phospholipids and nucleic acids. A smaller fraction of P is used as a component of the machinery of the plant's energy metabolism, where it is incorporated into glycolysis and the Calvin cycle [36, 37]. This growth independent accumulation of P, i.e. lower phosphorus productivity, observed under low light conditions was also interpreted as a potential storage of P for red pine seedlings grown under 190 $\mu\text{mol m}^{-2} \text{s}^{-1}$ [17]. The lack of growth response to nutrient under low light conditions has also been reported for beech seedlings [38] and other trees species [7].

The decrease of radiation use efficiency with increasing PPFD may be primarily explained by a decrease in quantum use efficiency, a well-documented characteristic of photosynthesis in C3 plants, an enhancement in respiration due to higher plant temperature may also have played a secondary role. It may be noted that *RUE* was calculated to account for intercepted and not incident light, so that increased self shading cannot be invoked as an explanation of the decrease in *RUE* with increasing plant size.

The calculated *RUE* obtained in our experiment (0.3–0.4 (HL) to 0.6–0.7 (LL) g DW mol⁻¹ = 2.8–3.8 to 5.7–6.6 g DW MJ⁻¹) were higher than values found by numerous authors for several pine species (1.3–1.9 g MJ⁻¹) [8, 23, 30, 53]. Indeed, in the literature, studies on seedlings and mature trees predominantly calculated *RUE* on the basis of the above ground dry matter production, whereas we expressed *RUE* as the total dry matter production per MJ. Consequently, our *RUE* values were higher than those reported in the literature, since the seedling root dry mass accounted for between 20 and 40% of total dry mass (for high P treatments and low P treatments, respectively).

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