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# Optimization of seasonality and mother plant nutrition for vegetative propagation of *Pinus pinaster* Ait

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**Abstract** Due to the high economic importance of *Pinus pinaster* Ait., there is considerable interest in developing, improving and extending the use of its families for mass clonal propagation and in breeding programmes. In the current study, we evaluated shoot growth, rooting ability and mini-cuttings production of *P. pinaster* in response to nitrogen fertilization and seasons. We compared eight half-sib families of *P. pinaster* from Asturias and Galicia (Northern Iberian Peninsula), searching for useful parameters and growing conditions to be included in a mass propagation program for clonal family forestry. We fertilized *P. pinaster* seedling mother plants kept in a greenhouse with three levels of nitrogen: high (HN), medium (MN) and low (LN) to evaluate rooting ability of mini-cuttings. In addition, we evaluated the maximal potential production of rooted mini-cuttings considering nine cycles of propagation over 1 year, also using three levels of nitrogen. The HN treatment significantly influenced the rooting process, with length, area and volume of roots all being positively affected. Spring was the most favourable season for mini-cuttings in the HN treatment. This study provides valuable new information to optimize the clonal propagation protocol for *P. pinaster* and shows that the mini-cuttings technique has great potential in mass scale cloning, providing high quality sprout production and well-formed new plants.

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**Keywords** Maritime pine · Nitrogen · Seasonal effect · Rooting · Mini-cuttings

## Introduction

*Pinus pinaster* Ait. is one of the main forest tree species planted in Spain, Portugal and France. Due to its high economic importance and extensive use for afforestation and restoration (Iovieno et al. 2006; Ruano et al. 2009), there is considerable interest in using this species to develop and extend the use of clonal families for mass propagation and in breeding programmes. In some countries, large scale programmes of family forestry-planting, without maintenance of clonal identities is becoming common: seedlings or vegetative multiplied material from full (FS)- or half-sib (HS) families of parents, selected on the basis of breeding values, are planted in single-family blocks. Individual open pollinated (OP) families, FS families and selected clones of different conifers display remarkable stability and predictability of growth performance across sites in the Southern USA, New Zealand and Australia (McKeand et al. 2003; Isik et al. 2004). In order to develop effective protocols for clonal production of *P. pinaster* by stem mini-cuttings, a better understanding of how the nutrition of the stock mother plant influences production, as well as knowledge of maximal annual production capacity and subsequent rooting availability, is essential.

The development of micro-cutting technology for *Eucalyptus* (Assis et al. 1992) highlighted the concept of super-intensive management of the production of vegetative propagules on a commercial scale. The outdoor mini-hedges thus produced were, however, at the mercy of the climate, and problems of adequate maintenance of nutritional status and control of leaf disease, especially during winter, were often encountered. Indoor mini-hedging systems have technical and economic advantages compared with normal outdoor clonal hedging, including higher productivity, lower labor demands, low consumption of chemicals, and also on rooting success (Andersen 1986; Haissig 1986; Read 1987; Hartmann et al. 2002).

One important aspect of stock plant management is N fertility because N concentrations affect carbohydrate levels, shoot production and adventitious rooting (Rowe et al. 2002a). Nutrition is a key factor affecting rooting predisposition because of its involvement in determining the morphogenic response of plant parts. Nitrogen is of particular importance because it is essential in high amounts and because changes in its availability induce large variations in seedling performance (Villar-Salvador et al. 2004; Villar-Salvador et al. 2005). Nitrogen concentration was found to be positively correlated with rooting ability of chrysanthemum cuttings under natural radiation in a greenhouse during spring and summer, even when sugar concentrations were low (Druege et al. 2000). However, high nitrogen supply to stock plants, meeting or even surpassing the levels necessary for maximum growth, often decreases subsequent rooting of cuttings (Roeber and Reuther 1982; Haissig 1986; Henry et al. 1992). Such negative effects have been attributed to decreased carbohydrate reserves and an altered C:N ratio, since increasing nitrogen supply lead to decrease starch concentration (Reddy et al. 1996). The response of sugars to nitrogen level has often been found to be less pronounced than that of starch (Rufty et al. 1988; Guidi et al. 1998). Fertilization enhances new root growth capacity and the out-planting survival and growth of conifer seedlings from mesic environments (Timmer et al. 1991; Van den Driessche 1992; Landis et al. 1995). Some studies with *P. halepensis* and *Pseudotsuga* indicate that seedling mortality in the field is lower when leaf N concentration is high (Van den Driessche 1988; Oliet et al. 2005). Many studies have reported the effect of fertilization on conifer seedlings (Van den Driessche 1991, 1992; Rowe et al. 2002b; Villar-Salvador et al. 2004; Oliet et al. 2005; Hunt et al. 2011; Lazcano et al. 2010), however the mini-cuttings cultivation conditions of *Pinus* species have received less attention and no information on nutritional aspects is available for maritime pine (Martins et al. 2009). Seasonal variation in nutrient levels in stock plant must also be considered when attempting to form roots on cuttings. For most conifers, reducing soluble carbohydrates are usually high during winter and low in summer, whereas starch is high in spring and autumn and low throughout the remainder of the year (Kozlowki and Keller 1966; Rowe et al. 2002a).

The main aim of the present study was to optimize the clonal propagation protocol of Majada et al. (2011) by evaluating the effects of fertilization and season, searching for the best growing conditions for each time of the year to maximize production and increase the performance of the cuttings useful parameters and growing conditions. We addressed the following specific questions: (1) How do shoot growth, rooting ability and mini-cuttings production of *P. pinaster* respond to N fertilization and season? (2) Is there any relationship between N fertilization rate and season in eight half-sib clonal families of *P. pinaster*? (3) Are there differences in propagation ability of selected half-sib families from a large base breeding population across the genetic diversity of *P. pinaster*?

## Materials and methods

### Mother plant material and culture conditions

Seeds of five half-sib families selected from plus trees were collected from Atlantic metapopulations located in Northern Spain (Alto la Llama 4, Alto la Llama 8, Armayán 5, Cadavedo 4, Castropol 9) and three more half-sib families (first generation) were collected in the Sergude seed orchard (Sergude 11, Sergude 13 and Sergude 19) located in Galicia (Table 1). The experiments were carried out at the SERIDA experimental station, "La Mata", in Grado, Asturias, Spain, located at 43° 23′N 6° 4′W at 60 m a.s.l. The seeds were sown in 250 mL containers (Cetap 54-Universal), in a 4:1 (v:v) mixture of peat (PINSTRUD) and vermiculite (VERLITE) and, following germination, seedlings were grown-on in a greenhouse for 9 months. At the end of January, the leader shoot of all seedlings was

**Table 1** Geographic and climatic information of different families employed in experiments

| Family code | Population | Province | Latitude and longitude | Mean altitude range (m) | Annual rainfall (mm) | Mean annual temperature (°C) |
|---|---|---|---|---|---|---|
| Alto 4 | Alto la LLama | Asturias | 43° 28′ N 6° 49′ W | 503 | 1,155 | 11.5 |
| Alto 8 | | | | | | |
| Arma 5 | Armayán | Asturias | 43° 18′ N 6° 29′ W | 532 | 1,160 | 11.4 |
| Cada 4 | Cadavedo | Asturias | 43° 32′ N 6° 25′ W | 180 | 1,316 | 13.2 |
| Cast 9 | Castropol | Asturias | 43° 50′ N 6° 98′ W | 391 | 1,179 | 12.6 |
| Lamu 2 | Lamuño | Asturias | 43° 33′ N 6° 13′ W | 85 | 1,282 | 13.4 |
| Segu 11 | Sergude | Galicia | | 275 | 1,741 | 13.1 |
| Segu 13 | | | | | | |
| Segu 19 | | | | | | |

pruned at least 10 cm from the tap root and five mother plants from each family were selected.

## Experimental design

### *Effect of nitrogen on rooting ability*

The mother plants were then fertilized weekly with one of three levels of nitrogen in a total volume of 120 mL/plant: High Nitrogen (HN): 543.58 mg $L^{-1}$/; Medium Nitrogen (MN): 386.92 mg $L^{-1}$; or Low Nitrogen (LN): 309.69 mg $L^{-1}$, in a base solution of $P_2O_5$ (249.05 mg $L^{-1}$), $K_2O$ (436.97 mg $L^{-1}$), MgO (2.24 mg $L^{-1}$), Mo (0.35 mg $L^{-1}$), Fe (3.71 mg $L^{-1}$), Mn (2.45 mg $L^{-1}$), Zn (0.35 mg $L^{-1}$), B (0.35 mg $L^{-1}$) and Cu (1.89 mg $L^{-1}$). The mother plants were placed in an air-conditioned glasshouse maintained at 70 % relative humidity (RH) and at 25 ± 2 °C during the day with a night-time minimum temperature of 10 °C.

Commencing after 3 months, the hedged mother plants were examined monthly, apical stem cuttings (orthotropic shoots resulting from hedging) were collected for rooting evaluation when the number of suitable cutting were at least one per plant. At each hedging, the number of cuttings 3–5 cm in length suitable for use as stem mini-cuttings was recorded for each mother plant. We selected 480 mother plants (5 mother plants per treatment × 8 families × 4 replications per treatment × 3 $N_2$ treatment) to evaluate the effect of nutrition on rooting ability. Cuttings were soaked in a solution of 3 mL $L^{-1}$ fungicide (Iprodiona 25 %) for 10 min, and dipped in a 1 g $L^{-1}$ IBA solution for 10 s before planting into pots filled with a 1:1 (v:v) mixture of perlite and peat (77 pots of 60 $cm^3$ per tray, 1,023 pots $m^{-2}$). Selected mini-cuttings were treatment with as described above (glass-house conditioned and were fertilized weekly with N 543.58 mg $L^{-}$ and the same base solutions). We also determined the nutrient content (N, C and S) of the cuttings' needles using a 2 g dry sample. All determinations were made in triplicate. Nutrient content (N, C and S) was measured following standard procedures at the Research Support Service of the University of Oviedo using a PerkinElmer 2400 Series II CHNS/O Elemental Analyzer, based on the classical Pregl-Dumas method: dry and powered samples were combusted in a pure oxygen environment, with the resultant combustion gases measured in an automated fashion. The trays were then placed in an air-conditioned glasshouse on an ultra-fog bench and maintained during the day at 25 ± 2 °C and 90 % RH following the *P. pinaster* protocol of Majada et al. (2011). Plants were allocated to treatments using a completely randomized nested design, and the rooting of the cuttings was assessed after 60 days (Majada et al. 2011).

### *Effect of season*

The mother plants were cultured in 250 mL containers, as described above, to evaluate the effect of season on potential production of mini-cuttings and the effect of nutrition across a whole year. After apical pruning, mother plants were fertilized weekly with the same three levels of nitrogen solution, as above, for 1 year. Cuttings were usually taken monthly. In total, nine cutting times were evaluated: 60, 90, 120, 150, 180, 210, 300, 330 and 360 days after leader shoot pruning. We were not able to take any mini-cuttings for two cycles during the winter season (cycles 240 and 270: December and January respectively) due to reduced vegetative growth. A random sample of cuttings from all mother plants were selected at each cycle-assay and submitted to the same fungicide, hormone and culture conditions as in the previous experiment. A total of 600 cuttings (5 mother plants × 8 families × 3 repetitions × 5 cuttings) were selected. The trays were placed in similar conditions to those described above but the nursery was heated to maintain a minimum of 14 °C at night during autumn and winter.

Data collection and analysis

We evaluated the number of mini-cuttings, length of mini-cuttings, percentage of cuttings that formed roots, shoot diameter, shoot dry biomass, root dry biomass, number of principal roots, root length, root surface area and root volume. Morphological evaluation of roots was made with the interactive scanner-based image analysis programme WinRhizo 2002 software (®Regent Instruments, Canada) version WinPro. Analyses of variance (ANOVA) were performed on the number of mini-cuttings produced per plant, length of mini-cuttings, shoot diameter, number of roots, root length, root surface area, root volume, shoot and root dry biomass. Tukey's multiple range test was performed when significant treatment differences were detected ($\alpha = 0.05$). The percentages of cuttings that formed roots were transformed with the arcsin function before the analysis of variance (LSD test, $\alpha = 0.05$), but we showed the original data. All statistical analyses were performed using SPSS Inc.®, Win TM, version 12.

## Results

Fertilization significant affected mini-cuttings production (Table 2). An increase in N fertilisation, especially high N fertilisation, enhanced the number of mini-cuttings produced annually (Fig. 1). Furthermore, time of collection also influenced mini-cuttings production with a significant interaction between season and N fertilization for this parameter (Table 2) and the highest mini-cuttings production was observed in spring and the lowest in winter (Fig. 1).

HN treatment significantly increased rooting ability of cuttings that increased from 66.7 %, with LN, to 75.2 %, with HN (data no showed). The differences were significant between the extreme levels ($\alpha = 0.05$). However, we also observed a significant influence of time of collection on the percentage of cuttings that formed roots (Table 2). The percentage of rooted cuttings increased with HN during summer, autumn and winter but this effect was not observed during spring when, in fact, the highest percentages of rooting were observed with LN (Table 3). Furthermore, a significant interaction between N treatment and season on the percentage of cuttings that formed roots was found. The HN cuttings developed more roots than those fertilized with LN, although again this was not observed in spring, when mini-cuttings developed more roots than in any other period. The other root variables (length, surface area and volume) were significantly influenced by the time of collection and, once more, the highest values were observed in spring time. Morphological variables were also significantly influenced by N fertilization, although this effect was not observed during the spring and autumn seasons (Fig. 2).

The effect of fertilization on rooting response varied greatly among the families studied (Table 3). A significant interaction was observed between N fertilization and families in relation to percentage of rooted cuttings, indicating that families differed significantly in response to fertilization (Table 2). However, this interaction was not found in relation to the root morphological variables, length, surface area and volume ($P = 0.128$, $P = 0.092$ and $P = 0.085$, respectively).

**Table 2** ANOVA *P* value for number of mini-cuttings, percentage of rooted cutting, number of roots and different variables of biomass allocation for mini-cuttings of *P. pinaster* from mother plants allocated to different N fertilization treatments grown in the greenhouse for one year

| | Number of mini-cuttings 3–5 cm | % Rooted cutting | Number of roots | Shoot length (mm) | Shoot diameter (mm) | Shoot dry mass (g) | Root dry mass (g) | Root length | Root surface area | Root volume |
|---|---|---|---|---|---|---|---|---|---|---|
| Family | **<0.0001** | **<0.0001** | **<0.0001** | 0.095 | **<0.0001** | **0.005** | **0.001** | 0.308 | 0.106 | 0.139 |
| N | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | 0.0026 | **<0.0001** | **<0.0001** | 0.013 | **<0.0001** | **<0.0001** |
| Cycles | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** |
| Fam × N | **<0.0001** | **<0.0001** | 0.189 | **<0.0001** | 0.184 | 0.204 | 0.078 | 0.128 | 0.092 | 0.085 |
| N × Cycles | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | 0.160 | **0.012** | **<0.0001** |
| Fam × N × Cycles | 0.912 | 0.064 | 0.547 | **<0.0001** | **0.021** | **0.023** | 0.283 | 0.985 | 0.948 | 0.877 |

Values in bold are significant ($P < 0.05$)

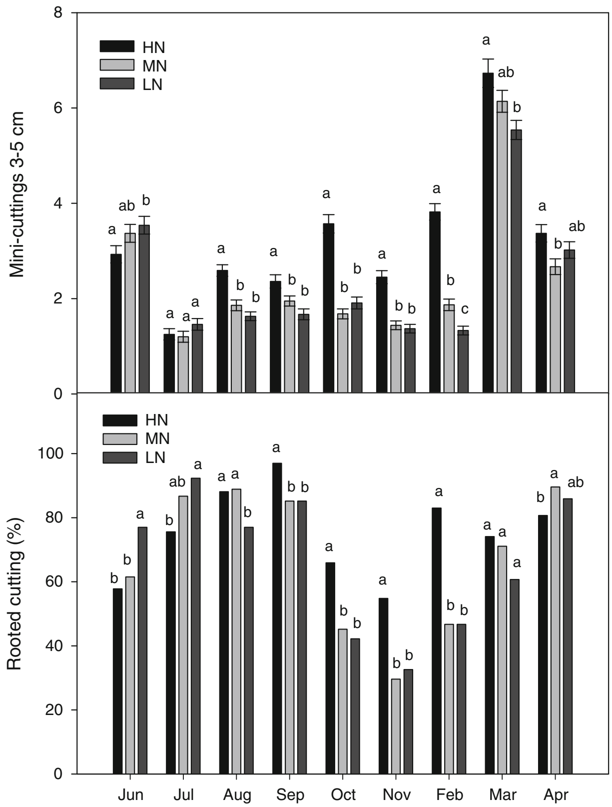


**Fig. 1** Mini-cuttings of 3–5 cm in length during 1 year (mean ± SE) and percentage of rooted cutting with three levels of nitrogen (*HN* high nitrogen, *MN* medium nitrogen and *LN* low nitrogen). Mean values followed by *same letter* are not significantly different at 5 % level

All parameters evaluated, except shoot diameter and root length, showed a positive effect of nitrogen fertilization. The family effect was significant in all cases, except for the shoot length, root length, root surface area and root volume. In addition, there was a significant interaction between fertilization and seasonality for biomass (root and shoot) (Table 2).

The N and C concentrations of the cutting needles were significantly correlated with the percentages of cutting that formed roots, but this relationship was not observed for S concentration (Table 4).

## Discussion

Fertilization affected almost all the morphological plant features measured and also enhanced both the rooting capacity and the mini-cuttings production. This response has also been observed in other species such as *P. taeda* (Rowe et al. 2002a, b), *Betula glandulosa* (Nams et al. 1992), *P radiata* (Zas et al. 2006) and *Eucalyptus* sp. (Higashi et al. 2000).

**Table 3** Effects of nitrogen level on the percentage of rooted cuttings during spring, summer, autumn and winter seasons in the different families studied

| | Spring | | | Summer | | | Autumn | | | Winter | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HN | MN | LN | HN | MN | LN | HN | MN | LN | HN | MN | LN |
| Alto 4 | 46.7 b | 73.3 ab | 86.6 a | 100 a | 93.3 a | 86.6 a | 73.3 a | 46.7 a | 73.3 a | 100 a | 53.3 b | 20 b |
| Alto 8 | 60 a | 73.3 ab | 100 b | 93.3 a | 93.3 a | 80 a | 60 a | 46.6 a | 40 a | 86.7 a | 53.3 ab | 40 b |
| Arma 5 | 80 ab | 66.6 b | 100 a | 93.3 a | 80 a | 86.6 a | 73.3 a | 13.3 b | 26.6 b | 66.6 a | 46.6 a | 53.3 a |
| Cada 4 | 53.3 b | 73.3 ab | 93.3 a | 100 a | 93.3 a | 80 a | 60 a | 13.3 b | 40 ab | 86.6 a | 33.3 b | 73.3 a |
| Cast 9 | 66.7 a | 46.7 a | 53.3 a | 93.3 a | 86.6 a | 66.7 a | 46.7 a | 40 a | 33.3 a | 80 a | 46.6 b | 60 b |
| Segu 11 | 40 a | 53.3 a | 66.7 a | 93.3 a | 100 a | 80 a | 46.6 a | 40 a | 6.6 b | 80 a | 40 b | 66.6 a |
| Segu 13 | 46.7 a | 60 a | 40 a | 100 a | 100 a | 93.3 a | 53.3 a | 40 a | 20 a | 86.6 a | 80 a | 33.3 b |
| Segu 19 | 93.3 a | 53.3 b | 86.6 ab | 66.6 a | 100 a | 86.7 a | 40 a | 13.3 a | 33.3 a | 86.7 a | 40 b | 40 b |

Letter codes indicate significant differences between treatments (HN: High Nitrogen, MN: Medium Nitrogen and LN: Low Nitrogen) (LSD test, $P < 0.05$)

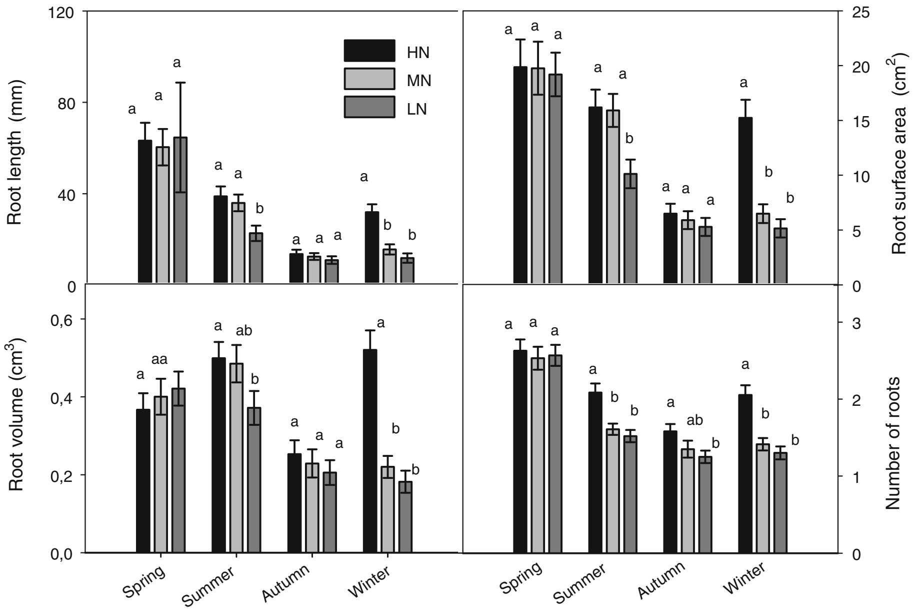


**Fig. 2** Effect of nitrogen level *HN* high nitrogen, *MN* medium nitrogen and *LN* low nitrogen) and seasonality on the number of roots, root length, root volume and root surface area (mean ± SE). Mean values followed by the *same letter* are not significantly different at 5 % level

**Table 4** Pearson correlation matrix of nutrient content in needles mini-cutting and percentage of rooted cutting

| | N | C | N/C | S | Rooted cutting (%) |
|---|---|---|---|---|---|
| N | 1 | 0.124 | **0.960**** | 0.218* | **0.340**** |
| C | | 1 | 0.159 | 0.210* | **0.199*** |
| N/C | | | 1 | 0.159 | **0.284**** |
| S | | | | 1 | 0.018 |
| Rooted cutting (%) | | | | | 1 |

Values in bold are significant, ** correlation at $P < 0.01$; * correlation at $P < 0.05$

The length of the maintenance period in the greenhouse for cuttings depends on the species, the climate conditions and the rooting process. For example, 60, 70 and 28 days are recommended for *Sapium glandulatum*, *Liquidambar* and *Eucalyptus* cuttings, respectively (Rieckermann et al. 1999; Ferreira et al. 2001; Alfenas et al. 2004). Information about how many cycles of hedging can be made per year for pines are scarce (but see Rowe et al. 2002a; Sharma and Verma 2011). In the Rowe et al. (2002a) study, three cutting cycles per year were made using loblolly pine, and they found that the highest number of 9 cm orthotropic shoots was produced in the spring, followed by summer, and then winter. However the authors recommend taking the cuttings earlier, before they have reached the 9 cm length described in their study. In this way the number of shoots available for propagation is greatly increased. In the current study, taking 3–5 cm cuttings allowed the number of cutting cycles per year, in *P. pinaster*, to be increased to 9, albeit with a similar gradient of production: higher in spring, followed by summer, autumn and winter.

The average mini-cuttings production rate per mother plant found in this study (3.28 per 30 days) was less than those presented by *Eucalyptus* (9.7 per 30 days) and *Ilex paraguariensis* (3.4 per 30 days) (Assis et al. 2004; Titon et al. 2006; Wendling et al. 2007, 2010). However, our procedure resulted in higher cutting yields than in other studies with other species: *I. paraguariensis* cultivated in plastic bags (cuttings of 15, 10 and 9 cm in length) (Wendling et al. 2007), and *Cedrela fissilis* cultivated in 200 $cm^3$ plastic tubes (Xavier et al. 2003), which resulted in production rates of 1.9 per 30 days and 1.3 per 30 days, respectively. Given the productivity of each ortet throughout the year (with all cycles and families assessed), we estimate gives a total productivity of 5,932 *P. pinaster* mini-cuttings per square metre under the best conditions tested (HN).

The number of shoots produced per hedge also increased with increasing N rates, and a positive correlation between high N and high rooting percentage was found. However, in others works, increased N rates have not necessarily resulted in high rooting percentages (Rowe et al. 2002b). Our results showed a positive effect of increased N during the year except in spring. This may be the result of the fast growth of cuttings in this period diluting the effect of the N application.

Family plasticity in relation to nutrient availability is under genetic control (Martins et al. 2009). The genetic effect on the response of mother plants and rooting capacity of mini-cuttings has been widely discussed by many authors (Rowe et al. 2002a, b; Chung and Lee 1994). Although it is well known that there is abundant genetic variation for plastic responses (Pigliucci 2005), there is very little empirical evidence allowing the quantification of this variation and the estimation of heritability of plastic responses, especially for forest trees (Chambel et al. 2005; Martins et al. 2009). Obviously between-family and between-clone genetic variation resulting in families with greater or lesser rooting ability is of vital importance to large scale clonal forestry propagation systems. Although studies on genetic variability in relation to rooting in *Pinus* are scarce, Baltunis et al. (2005), working with 2,200 clones of *P. taeda*, found moderate to high family and clonal mean heritabilities. More recently, family genetic variability of the rooting characteristics of white spruce (*Picea glauca* (Moench) Voss) cuttings was evaluated for 75 half-sib families (Gravel-Grenier et al. 2011). The results obtained show that the root initiation phase (B + 0) and the root development phases (B + 1 and B + 2) are under strong genetic control. This suggests that an indirect and efficient selection procedure for white spruce families based on measurement of above-ground morphological characteristics could be developed. The significant family × fertilization interaction found in this study suggests that differences exist in nutrient uptake or assimilation. Similar results were found by Rowe et al. (2002b), where tissue N concentrations increased for all families tested with increasing applied N level. This strong genetic control of morphological characteristics indicates that the selection of superior genotypes is possible for intensive forest clonal propagation programmes (Gravel-Grenier et al. 2011; Shepherd et al. 2005).

Time of collection was also a significant factor affecting rooting capacity. Although specific fertilization for each season has not been used in this study, the significant interaction between season and N fertilization ($P < 0.001$) for mini-cutting production suggests that a specific fertilization regime for each season could be used in this kind of propagation system, as proposed by Oliet et al. (2011).

Given the difficulties associated with vegetative propagation using cuttings from adult trees (Alvarez et al. 2009; Rieckermann et al. 1999), and that a system capable of producing rooted family clonal cuttings is in great demand (Gocke 2006; Amri et al. 2010), the present study provides important new information. It shows that the mini-cuttings technique has great potential for mass scale clone production of *P. pinaster*, not only producing high quality sprouts from mini-clonal hedges and well formed new plants but also greatly increasing productivity rates. Previous research with Fraser fir (*Abies fraseri*) (Wise et al. 1985), loblolly pine (*P. taeda*) (Cooney 1999), radiata pine (*P. radiata*) (Bolstad and Libby 1982; Libby et al. 1972), Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) (Black 1972) and Norway spruce (*Picea abies* L.) (Bentzer 1993) has demonstrated that continuous hedging of a stock plant provides a way to increase macro-cutting production and maintain juvenility. Considering hedging's important role as a stock plant management technique that maintains juvenility (Hartmann et al. 2002), we would recommend that the application of mini-cuttings in pines be increased in propagation programs. Field trials are already under way to compare the growth vigour of mini-cuttings and seed-derived plants. However, long-term studies need to be conducted to further evaluate the number of collections that can be made from the mini-clonal hedge without significant loss of productivity and the performance of family-genotypes selected according to their genetic merit.

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