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RESEARCH ARTICLE

Propagation and Seedling Cultivation of the Endemic Species *Nothofagus alessandrii* Espinosa in Central Chile

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Abstract

Nothofagus alessandrii **is an endemic and endangered species from the Maule Region of Central Chile. The forests it once dominated have been severely degraded and fragmented by human activities, and it is estimated that only 350 ha remain. Yet, available information for propagation and nursery plant cultivation of this species is conspicuously lacking. Future efforts to restore this ecosystem type will rely, in large part, on advancing the capacity to propagate and cultivate this highly threatened species. To this end, we studied the response (germination process and nursery growth) of viable seeds of *N. alessandrii* to different pre-germination treatments, sowing times, and slow-release fertilizer. The highest germination percentages were obtained with cold stratification for periods of 30 and 45 days (84.9 and 82.6%, respectively). Sowing time was also relevant, with seeds obtaining highest germination rates (53%) when sowing during spring season (September) as well as the best growth rates. Both cold stratification and GA_3 treatments can provide adequate germination percentage and similar seedling quality. After one nursery season, no clear effect of slow-release fertilizer on plant growth was observed. The results of this study provide important baseline information for propagation and nursery techniques for restoration programs of *N. alessandrii*.**

Key words: **gibberellic acid (GA_3), ruíl, seeds, slow-release fertilizer, sowing time, stratification.**

Introduction

The mesomorphic region of Chile has been subjected to constant anthropogenic pressure during the last two centuries, resulting in a serious decline in the forests of this region Donoso & Lara 1995. One of the most dramatic examples is that of *Nothofagus alessandrii* Espinosa (Nothofagaceae) (”ruil”), an endemic species of the central region of Chile, which is a species listed in danger of extinction (Benoit 1989). The natural distribution of this species is restricted to shady slopes of the Cordillera de la Costa in the Maule Region of Central Chile (San Martín et al. 1991, 2006). The current range of this species has been drastically reduced to some 350 ha, which are distributed across a highly fragmented landscape (Bustamante & Grez 1995; Bustamante & Castor 1998). Remnant forests of *N. alessandrii* are dispersed within a matrix of *Pinus radiata* D. Don, an exotic species frequently used in forest plantations (Bustamante & Simonetti 2005), which is capable of successfully invading *N. alessandrii* forests (Bustamante & Castor 1998; Bustamante & Simonetti 2005). *N. alessandrii* is not only considered to be Chile’s most threatened tree (Hechenleitner et al. 2005), but, in addition, it has also been estimated that the relative deforestation rate of these forests is one of the highest worldwide (Bustamante & Castor 1998). An urgent study and management of the remaining native vegetation is required in the area in which this species is naturally distributed, an area where 25 conservation biodiversity hotspots have been declared (Myers et al. 2000). In this context, restoration of this highly degraded ecosystem is a priority task. For that reason, it is highly important to determine, among other factors, successful mechanisms of propagation and cultivation of these unique plants in nursery settings to provide materials for restoration projects.

Most of the species of the genus *Nothofagus* possess an endogenous dormancy (Wilcox & Ledgard 1983; León-Lobos & Ellis 2005), although not all of them with the same level (Wardle & Cambell 1976). The treatments most commonly employed to overcome this type of dormancy are cold stratification and soaking seeds in gibberellins, especially in gibberellic acid (GA_3) (Hartmann & Kester 1998; Barceló-Coll et al. 2001). It has been observed that subjecting

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N. alessandrii seeds to cold and moisture treatments increases their germination capacity (GC) (Donoso 1975; Donoso & Cabello 1978). There is also evidence of the advantages of using GA_3 to interrupt the endogenous dormancy status of some species of the genus *Nothofagus* (Rocuant 1984; Espina & Núñez 1996; Espinoza 1997; Subiri 1997). However, the information available on the effect of this hormone on *N. alessandrii* germination is insufficient.

There are few data on the cultivation of *N. alessandrii* plants and results are variable (Farías & Ulloa 1996; San Martín et al. 2006; Santelices & Pérez 2006). Fertilization is one of the most important cultural practices for plant quality, especially for seedlings produced in containers, whose limited volume seriously hinders their growth (Landis 1989). Fertilization has a direct influence on plant growth because it improves rooting capacity when they are transplanted, thus increasing their resistance to water stress, to low temperatures, and to diseases (Malik & Timmer 1998; Floistad & Kohmann 2004; Dumroese et al. 2005). One of the current fertilization methods widely used is "released delivery," which is characterized by a gradual supply of mineral elements over time. This method helps improve nutrient use efficiency and diminish losses from lixiviation, especially in the case of nitrogen (Landis 1989). In the case of *N. alessandrii*, the only reference to fertilizer use in nursery plant cultivation indicates that individuals with an average of 61 cm in height and 5.1 mm root collar can be obtained when using 3 g/L of Osmocote (Santelices & Pérez 2006).

In view of previous results, this work was designed to analyze the germination process of *N. alessandrii* seeds using different pre-germination treatments and to evaluate subsequent seedling development in the nursery. In addition, we studied the response of seedlings to different fertilization treatments of a slow-release fertilizer.

Methods

Plant and Cultivation Material

The *Nothofagus alessandrii* seeds were collected in two successive seasons in 2005 and 2006 during the month of February. In 2005, seeds came from the Lo Ramirez provenance, in Curepto Commune. In 2006, they came from the Quivolgo provenance, in the Commune of Constitucion. These two geographic races are found in the province of Talca, in the Maule Region of Chile (Fig. 1).

The seeds were collected from phenotypically superior trees with regard to diameter, height, straightness, shape, development of the crown, and health. For Lo Ramirez, the number of trees sampled was 11 individuals, and for Quivolgo it was limited to 5, basically due to the seeding restrictions of the species.

Seeds were collected from trees spaced approximately 50 m apart in an attempt to capture the maximum genetic variation possible. The fruits were collected directly from the branches and transported to the Catholic University of Maule's nursery on the same day. There, the seeds were cleaned and stored in paper bags at 4°C (Hartmann & Kester 1998) for 5 months to be grown the following spring. Once the seeds were cleaned, purity and weight analyses were conducted according to ISTA (2006). In addition, using a selection of five samples of 400 seeds each in a flotation test, their viability was determined (Table 1).

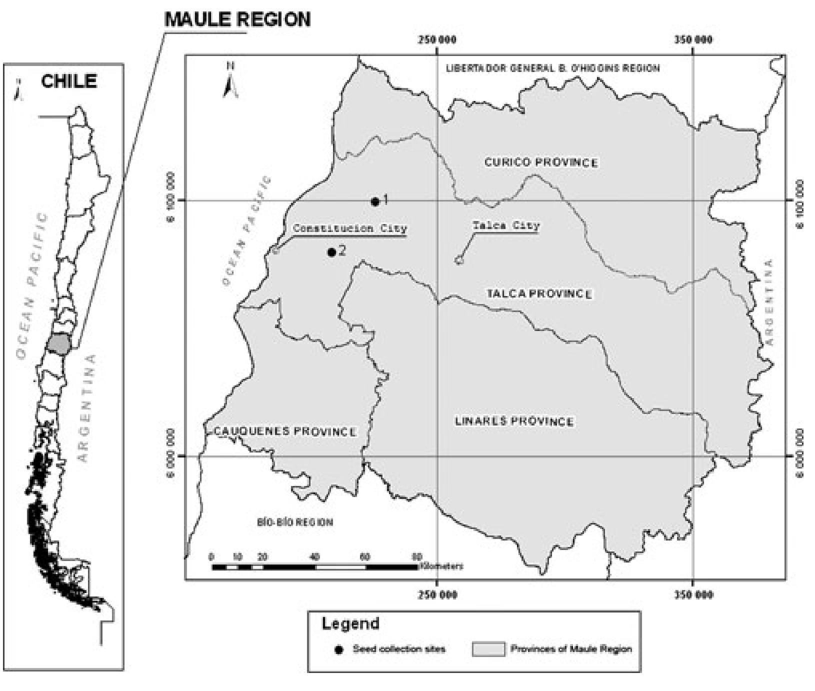

Figure 1. Location of seed collection sites (1 = Lo Ramirez and 2 = Quivolgo).

Table 1. Parameters of the seeds used in the germination and fertilization trails of *Nothofagus alessandrii*.

	Provenance	
Parameter	*Lo Ramirez*	*Quivolgo*
Seed Purity (%)	72	85
Viability* (%)	17.9	5.5
Weight of 1000 seeds (g)	8.3	7.8

*Viability was calculated by the floating test.

Three independent trials were carried out, two of them in the Campus San Miguel of Catholic University of Maule nursery, located in Talca city, Maule Region. The third was performed in Quivolgo nursery near the city of Constitucion, also in the Maule Region of Chile. In all the cases, the seed used was selected based on its morphological characteristics, rejecting any seed that showed any external anomaly. Only viable seed was employed; by means of a flotation test in distilled water for 24 hours the viable seeds were separated from the infertile ones.

The trials were done under nursery conditions, and the seeds sown in rigid plastic containers with a 140-cm^3 cavity (Termomatrices). The substrate consisted of a mixture of composted bark of *Pinus radiata*, perlite, and vermiculite (6:2:2 v), which was combined with the slow-release fertilizer Osmocote 18-6-12 (9.7% ammonium; 8.3% nitrate; 6% available phosphorus [P_2O_5]; and 12% of soluble potassium [K_2O]) for a period of 9 months. Slow-release fertilizer was applied at a rate of 3 g/L of substrate for all treatments except fertilization, in which its dosage was varied to test the effect of fertilizer application rate (see below). During their cultivation, the plants were protected by a 50% plastic sunshade mesh. In the germination process, they were watered daily with micro-sprinklers so that the seed was always kept moist. Later, once the seedlings emerged, the substrate was maintained at a field capacity.

In the stratification treatments, the seed was mixed with damp sand, previously disinfected with 5% sodium hypochlorite for 20 minutes, and then placed in polyethylene bags in a refrigerator at a temperature of 4°C (±1°C) for periods of 30, 45, and 60 days. In the treatments with GA_3, the seeds were soaked for 24 hours in concentrations of 25, 50, 100, 200, and 400 ppm. For the control plants, the seeds were only soaked in distilled water for 24 hours. All the treatments were carried out sequentially in order for the germination process to be initiated on the same day and thus maintain the same conditions in all the treatments. Sowing was done on 23 September 2005, and the trial was conducted in the Catholic University of Maule nursery.

To evaluate the effect of the fertilization on the development of the plants grown during a nursery season, seeds from the Quivolgo provenance were used. Internal dormancy was first broken by soaking seeds for 24 hours in a gibberellic acid solution (GA_3) at 200 ppm, diluted in distilled water. Seeds were then sown on seedbeds in mid-October 2006, and the seedlings were replanted in the containers in the last week of December 2006. The fertilizer application rates assayed were 2, 4, 6, 8, and 10 g/L of substrate. The plants were cultivated in the Forestal Celco company nursery.

Experiment Design and Measurements

With an experiment assay designed as completely randomized fixed-effect blocks, three replications per treatment, and 44 seeds per unit, a first stage in the germination process was evaluated. Next, the development of the plants in the nursery after a single-season growth period, because of the treatments on the seeds, was assessed. Sowing was done on 23 September 2005.

The evaluation of the sowing time effect was also made by an assay with an experiment design in completely randomized fixed-effect blocks, with three replications per treatment, and 44 seeds per experiment unit. Sowing was carried out in mid-July, August, and September 2005 (each time on the 23rd of the month).

For the two assays made, the germination process was monitored daily during a span of 86 days, and it was considered successful when the plumule reached a length that doubled the seed's diameter (i.e., around 1 cm in length). After that time, the following calculations were made: the GC, as the proportion of seeds germinated with respect to the total seeds sown (value expressed as a percentage); Czabator's maximum value as the maximum quotient of cumulative germination percent at day x, divided by x (Czabator 1962); and the germination energy, expressed as the cumulative germination percent occurring at the time of the maximum value. After a period in the nursery (8 months), namely, at the beginning of May 2006, the morphological attributes: stem length (L), root collar diameter (D), aboveground biomass (AB), root biomass (RB), and total biomass (TB) were measured. To calculate the biomass, nine plants per treatment were selected randomly. With this information, several indices were calculated: the slenderness index (SI), the root shoot index (RSI) (Iverson 1984), and the Dickson index (DI) (Dickson et al. 1960), according to the following formulas:

$$\mathrm{SI} = \frac{L}{D}$$

$$\mathrm{RSI} = \frac{\mathrm{AB}}{\mathrm{RB}}$$

$$\mathrm{DI} = \frac{\mathrm{TB}}{(L/D) + (\mathrm{AB}/\mathrm{RB})}$$

In addition, at the beginning of April 2006, before the vegetative recess period began, the nutritional status of the plants was evaluated by the content of the macronutrients N, P, K, Ca, and Mg present in their leaves. The foliar analysis was made in the Technology Center for Soils and Crops at Talca University, following the methodology of Sadzawka et al. (2007) by collecting three leaves per plant from each experiment unit.

The effect of the fertilization was evaluated from a random block design with three replications and 99 plants per treatment. At the beginning of May, namely, at the end of the vegetative growth period of the plants, the same attributes and indices indicated for the previous assays were measured. The macronutrient level was also assessed in mid-March and mid-April of the same year, following the methodology previously described.

The carbohydrate content of the aboveground part and of the roots was also quantified. The total content of carbohydrates was calculated by adding together the reducing sugar and starch values (Stolz 1966). The procedure followed to determine the reducing sugars was the one described by Southgate (1969) and Dubois et al. (1956), with respect to what referred to extraction and quantification, respectively. To calculate the starch content, the methodology proposed by Munson and Walker, described by Schmidt-Hebbel (1973), was followed.

Data Analysis

The analyses of variance (ANOVAs) and the comparison of means were made using the general linear model (GLM) procedure from the statistics program SPSS for Windows V. 15. In order to normalize those variables expressed as percentages, before carrying out the ANOVAs, they were transformed into angular values with the formula $y' =$ arcsine $\sqrt{p}$ (Ostle 1992). In those cases in which the variables presented a great deal of dispersion, the data were transformed logarithmically (Martínez-González et al. 2001). The mean values showing significant differences were compared with the Tukey test at a 5% level.

Results

Evaluation of the Effect of Stratification and of Gibberellic Acid on the Germination and Attributes of the Nursery-Grown Plants

Significant differences in the GC and maximum value because of the germination treatment applied to the seeds were observed (Table 2). The highest capacity was obtained when stratifying the seeds for 30 days at 4°C, whereas the lowest corresponded to those which did not receive any treatment. On soaking the seeds in gibberellic acid, rates comparable to those of the stratification treatments were obtained. The development of the germination with the best stratification and GA_3 immersion treatments, compared with the control, is shown in Figure 2. However, no statistical differences were recorded as a consequence of the pre-germination treatments in most of the morphological attributes of the plants, although they were indeed seen in the RB (Table 3; Fig. 4). Nor were any statistical differences observed in the SI or DI, only in the root shoot (Table 3).

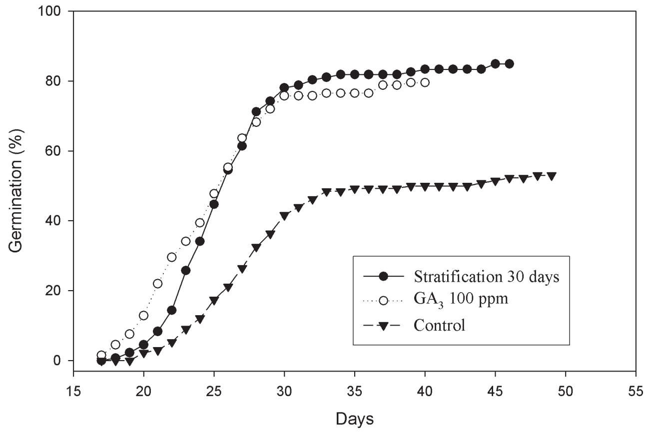

Figure 2. Germination percentage to the best stratification and GA_3 soaking treatments, in comparison to the control.

Evaluation of the Sowing Time Effect on the Germination and Attributes of the Nursery-Grown Plants

With respect to the seeds sown in September, significant differences were observed in most of their variables in the germination process compared with the other treatments (Table 2). Significant differences were recorded in most of the

Table 2. Effect of different pre-germination treatments and sowing season on *Nothofagus alessandrii* germination from Lo Ramirez provenance (mean values with the same letter are not significantly different, $p < 0.05$).

Treatment	*Germination Capacity (%)*	*Maximum Value (% per day)*	*Germination Energy (%)*	*Energy Period (days)*
Pre-germination treatment				
Control	53.03 b	1.51 b	47.73	32
Stratification 30 days	84.85 a	2.63 a	78.79	30
Stratification 45 days	82.58 a	2.57 a	76.52	28
Stratification 60 days	75.00 ab	2.61 a	64.39	25
$GA_3$25 ppm	75.76 ab	2.37 ab	68.18	29
$GA_3$50 ppm	65.91 ab	2.28 ab	62.88	28
$GA_3$100 ppm	79.55 ab	2.54 ab	73.49	29
$GA_3$200 ppm	75.00 ab	2.40 ab	66.67	28
$GA_3$400 ppm	62.88 ab	2.24 ab	59.09	26
Sowing season				
July	4.55 b	0.06 b	4.55	51
August	12.12 b	0.18 b	9.09	55
September	53.03 a	1.51 a	47.73	32

Table 3. Effect of different pre-germination treatments and sowing season on morphological attributes and quality index of *Nothofagus alessandrii* seedling from Lo Ramírez provenance (mean values with the same letter are not significantly different, $p < 0.05$).

						Quality Index		
Treatment	*Stem Length (cm)*	*Root Collar Diameter (mm)*	*Aboveground Biomass (g)*	*Root Biomass (g)*	*Total Biomass (g)*	*Slenderness*	*Root Shoot*	*Dickson*
Pre-germination treatment:								
Control	53.1 a	4.9 a	3.73 a	1.93 ab	5.66 a	10.9 a	3.1 ab	0.43 a
Stratification 30 days	65.4 a	4.7 a	4.12 a	1.30 ab	5.42 a	13.8 a	4.3 a	0.33 a
Stratification 45 days	59.2 a	4.3 a	4.52 a	1.69 ab	6.21 a	13.8 a	3.8 ab	0.37 a
Stratification 60 days	61.3 a	4.8 a	4.06 a	1.36 ab	5.42 a	12.6 a	4.0 ab	0.37 a
$GA_3$25 ppm	58.9 a	4.9 a	3.86 a	2.31 a	6.17 a	12.1 a	2.7 b	0.43 a
$GA_3$50 ppm	59.7 a	5.0 a	3.58 a	1.50 ab	5.08 a	12.0 a	3.4 ab	0.37 a
$GA_3$100 ppm	69.0 a	5.0 a	3.99 a	1.24 b	5.23 a	13.9 a	4.3 a	0.33 a
$GA_3$200 ppm	68.0 a	5.1 a	3.98 a	1.41 ab	5.39 a	13.3 a	3.8 ab	0.30 a
$GA_3$400 ppm	61.0 a	5.0 a	3.48 a	1.63 ab	5.11 a	12.7 a	3.3 ab	0.37 a
Sowing season								
July	22.52 b	3.1 b	1.17 b	1.62 a	2.78 b	5.7 b	1.7 a	0.43 a
August	34.04 b	4.0 b	1.85 b	2.46 a	4.31 ab	8.4 ab	1.8 a	0.47 a
September	53.10 a	4.9 a	3.73 a	1.93 a	5.66 a	10.9 a	3.1 a	0.43 a

morphological attributes of the nursery-grown plants during a cultivation period (Table 3; Fig. 5). Although the germination began later with the September sowing, the convenience of this treatment is clearly observed in Figure 3. The seeds of those plants sown in the spring showed better development than those sown in the winter. However, when analyzing the effect of the sowing time on the quality indices of the plants, statistical differences were observed only in the SI (Table 3). The seedling foliar macronutrients levels were 1.06 mg/g for N, 0.10 mg/g for P, 0.36 mg/g for K, 0.68 mg/g for Ca, and 0.63 mg/g for Mg (Figs. 4 & 5).

Evaluation of the Fertilization Effect on the Attributes of the Nursery-Grown Plants

As a result of the application of different slow-release fertilizer doses, statistical differences were only observed in the root collar diameter of the *Nothofagus alessandrii* plants (Table 4). However, no significant differences were recorded when analyzing the quality indices of the plants (Table 4). Generally, as the fertilizer concentration increased, so did the macronutrient content in the plants. Finally, the carbohydrate content in the stem was, on average, 1.5 ppm and in the roots 1.3 ppm, and no great differences were observed between the treatments.

Discussion

In those species whose seeds present an endogenous dormancy, it is necessary to develop some effective strategies that permit to ensure the highest germination percentages, and this can be done by means of cold stratification and gibberellic acid application treatments (Barceló-Coll et al. 2001; Zhou et al. 2003; Yang et al. 2007). In this study, only stratifications of 30 and 45 days differed significantly from the control with regard to GC, and stratifications of 30–45–60 days differed

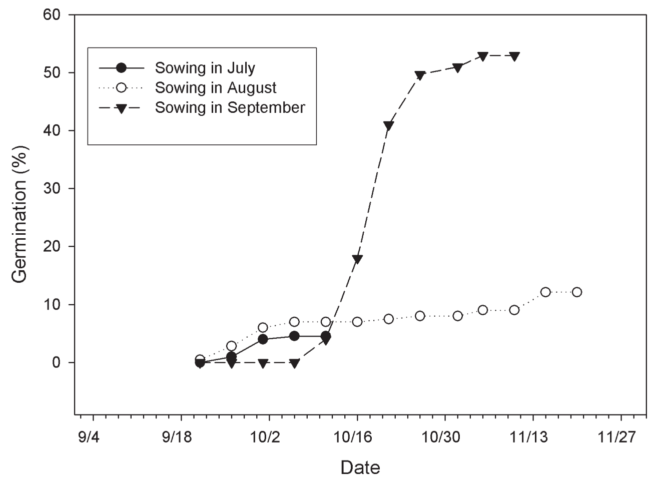

Figure 3. Effect of the sowing season on *Nothofagus alessandrii* germination.

significantly from the control with regard to maximum value. None of the GA_3 treatments differed significantly from the control.

The highest germination percentages were obtained when stratifying the seeds for 30 and 45 days, when they gave significantly higher values than those obtained with nontreated seeds. However, they did not differ statistically from those generated when soaking the seeds in gibberellic acid. When stratifying the seeds for one month, the highest rates were also reached for the maximum value and the germination energy. The germination speed was similar for all the stratification treatments and was not differentiated from that observed with soaking the seed in GA_3 at 100 ppm. For most of the treatments tested, the start of germination began around day 17, including the control seed. The seeds treated with gibberellic acid began to germinate before those stratified for

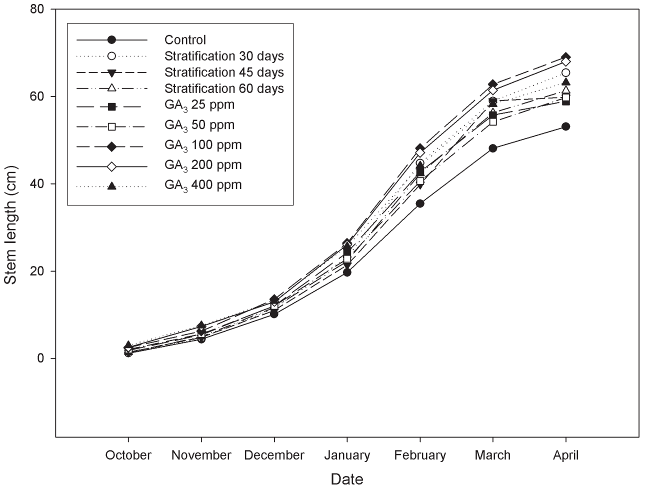

Figure 4. Effect of germination treatment on the stem length growth of *Nothofagus alessandrii*.

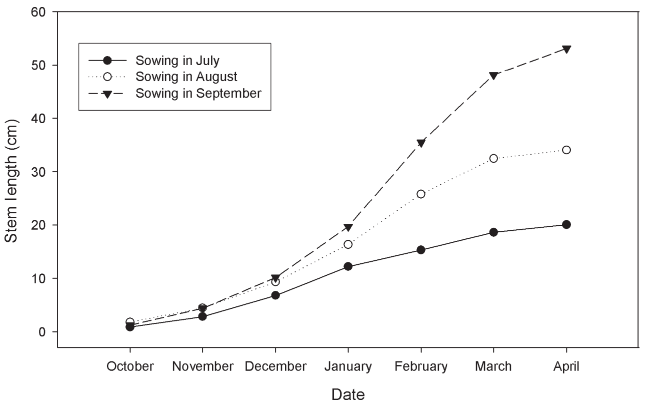

Figure 5. Effect of the sowing season on the stem length growth of *Nothofagus alessandrii*.

30 days. However, later, the germination rates equaled each other (around day 26) and remained very similar for the rest of the assay.

The average GC reached when stratifying the seed for 30 days was significantly higher than those (47%) obtained by Donoso (1975) with the same treatment. This difference may be due to environmental factors at the time when the seed was harvested, or it could be an artifact of population-level genetic differences. That author worked with seeds with a 20% viability, which were collected from the area of Empedrado, a more southern location than that of the seeds used in our study. When comparing the effect of gibberellic acid on seeds of the same geographic origin, the results obtained in this study were similar to those presented by Espina and Núñez (1996), who indicated an 85% GC when soaking the seeds for 24 hours in a GA_3 solution at 25 ppm. However, the results of Espina and Núñez (1996) are somewhat inconsistent—there was an ample GC for nontreated seeds in all stratification treatments (from 15 to 60 days)—but when the treated seeds were stratified for 4 weeks, their germination did not exceed 5%. On contrasting our results with those reported by Rocuant (1984), who worked with a more southern provenance and managed to obtain a germination percentage of almost 15% by treating the seeds with 25 ppm of GA_3, a significant difference was again observed in *Nothofagus alessandrii* germination. This author did not carry out a flotation test to eliminate the infertile seeds but only homogenized the material, removing perforated or small seeds, which could partly account for the differences observed between studies. He also indicated that stratification for 30 days at 4°C did not result in any germination, further demonstrating that *N. alessandrii* germination is somewhat erratic.

In this study, the best results in the *N. alessandrii* germination process were obtained when stratifying the seeds for 30 or 45 days at 4°C, and they were comparable to those with GA_3 immersion at 100 ppm for 24 hours. However, considering the differences observed with the results presented by other authors, it would seem necessary to investigate the germination process in more depth. It would be of interest, for instance, to compare the effect of stratification with other temperature ranges and the gibberellic acid immersion in varying exposure times, for different provenances, and in different seasons since this species is characterized by having irregular cycles of seed production (Cabello 2004).

The use of gibberellic acid as a germination promoter of seeds with endogenous dormancy is widespread (Barceló-Coll et al. 2001; Zhou et al. 2003; Cerabolini et al. 2004; Sivakumar et al. 2006). Nevertheless, in spite of improving the germination percentage, there is a risk of producing plants with undesirable traits (Rascio et al. 1998; Cabello 2004). On comparing the morphological attributes, the control did not differ significantly from any of the seeds' pre-treatment. Differences only occurred between two GA_3 treatments. On analyzing

Table 4. Effect of the fertilization treatment (Osmocote) on the morphological attributes and quality index of *Nothofagus alessandrii* seedling from Quivolgo provenance (mean values with the same letter are not significantly different, $p < 0.05$).

Treatment	*Stem Length (cm)*	*Root Collar Diameter (mm)*	*Aboveground Biomass (g)*	*Root Biomass (g)*	*Total Biomass (g)*	*Quality Index*		
						Slenderness	*Root Shoot*	*Dickson*
2	13.2 a	3.0 ab	0.40 a	0.31 a	0.71 a	4.5 a	2.3 a	0.12 a
4	18.1 a	3.2 a	0.48 a	0.40 a	0.88 a	5.8 a	2.2 a	0.13 a
6	14.8 a	2.9 bc	0.51 a	0.37 a	0.88 a	5.2 a	2.4 a	0.13 a
8	13.5 a	2.7 c	0.73 a	0.34 a	1.07 a	5.0 a	3.2 a	0.15 a
10	14.3 a	2.8 bc	0.59 a	0.39 a	0.98 a	5.1 a	2.5 a	0.15 a

seedling quality, the control did not differ significantly from any of the pre-germination treatments. Statistical differences were only observed in the RSI.

The growth rate reached by the plants during a cultivation period, nearly 60 cm in height and 5 mm root collar diameter, can be considered as being adequate. Although there is no official norm for this species, on comparing it to the one for *N. nervosa* (Phil.) Dim. et Mil. (INN 2005*a*), it is possible to affirm that the plants produced not only would fulfill the minimum growth demands but also they would greatly exceed them.

For example, the norm contemplates a minimum value of 3 mm for the root collar diameter and this is one of the most important attributes for predicting the subsequent development of the plants in the field (Mexal & Landis 1990; Mexal et al. 2002). The degree of plant development can also be seen by observing the slenderness coefficient, which indicates that with all the treatments, including the control, the material generated was stable.

When analyzing the nutrient concentrations in all the plants produced, it can be verified that the principal macronutrients (N, P, and K) are below the limits recommended for different forestry species (Landis 1989; INN 2005*b*). *N. alessandrii* is considered as being a species capable of efficiently using its nutrients (San Martín et al. 2006), so that lower values for this physiological index could probably be assumed. Generally, no effect of the pre-germination treatments was observed on the quality of plants during a cultivation period, and it is possible to affirm, mainly in the light of their morphological attributes, that their quality was acceptable for their establishment in the plantation. However, the quality of a nursery-produced plant should be tested in the field, where the survival ability and initial growth of the saplings could be evaluated.

In some species of the genus *Nothofagus*, as an alternative to breaking the internal dormancy, it has been proposed to sow seeds in the winter, in such a way that the seed is submitted to low temperature and high moisture conditions and, thus, with the arrival of the spring a natural stratification process has been produced (Espinoza & Cabello 1993; Espinoza 1997). The results of this study do not coincide with what was previously indicated since the seeds sown in the spring gave a better response in all the variables analyzed compared with those sown in the winter, although for those sown in August no significant difference for root and TB was observed.

Although the germination began later with the September sowing, this treatment is clearly superior to the others. Not only was a higher germination rate obtained at the end of the period, but it was also faster. In this same sense, the maximum value was reached in 32 days, and, by contrast, in the other months it was reached in over 50. With regard to the seeds sown in July, the germination began 2 months after the treatment was established, that is in September, but the germination subsequently came to a halt, in spite of the improvement in the temperature conditions in that period.

The advantages of sowing the seeds with no treatment in September with respect to doing so in July or August are obvious. However, as mentioned, by stratifying the seed for 30 or 45 days and sowing it at that time its GC increased by over 29%. The seeds probably require a longer time of “natural stratification,” and it would be of interest to analyze the germination performance of seeds sown as from April or May. The high percentage obtained in September could indicate that *N. alessandrii* seeds have no deep dormancy.

The benefits from a September sowing were also manifested in some morphological traits of plants during a cultivation period grown in the nursery, especially in stem length and root collar diameter. However, the seeds of those plants sown in July and August had a higher coefficient of slenderness than those sown in September. On analyzing the growth of the latter, it could be seen that they grew higher to the detriment of the root collar diameter.

At present, nursery fertilization is an important cultural treatment, especially in container-grown plants, because of its effect on plant quality, with plants that are larger and have a better resistance to drought stress, low temperatures, and diseases being obtained (Malik & Timmer 1998; Floistad & Kohmann 2004).

In this study, no great differences were observed in the growth of the plants during a cultivation period of *N. alessandrii* because of applying different doses of Osmocote to them. As the slow-release fertilizer concentration increased, a slight increase in the diameter growth was recorded, with this variable subsequently remaining at relatively equal values. Thus, the toxicity level described by various authors was probably not reached (Salifu & Timmer 2003; Salifu & Jacobs 2006), and the plants were kept within an optimal interval or with an excessive consumption of nutrients. *N. alessandrii* probably reaches an optimal growth level with less nutritional requirements than other species due to its efficiency in using nutrients (San Martín et al. 2006). The dynamics of the plants’ height growth was similar to that observed in the diameter, although no statistic differences were noted in that variable.

With all the treatments applied, plants with a SI going from 4.5 to 5.8 were generated, which indicated that the plants’ height and diameter growth were well balanced. The highest values recorded in the morphological attributes analyzed indicated that the optimum growth level could be found at around 4 g/L of Osmocote, which should be monitored in the plantation. However, the plants’ size did not reach the minimum demanded by the norm established for *N. nervosa* (INN 2005*a*), and it was clearly inferior to that achieved with plants subjected to the different pre-germination treatments mentioned above. The plants did not manage to show their potential, with the production of plant material being necessary, in this case, of two cultivation periods, probably due to the sowing being done in this case later (October).

Total sugar content in the roots was generally homogeneous, and no great differences were noticed between the treatments. On the contrary, their contents in the stem had a greater variation and the plants fertilized with 8 g/L gave a significantly lower content than the rest.

Root collar diameter obtained (3.3 mm), and the quality indices and nutrient and total sugar contents, permitted the assumption that they would be apt for establishment in the field. However, and as mentioned before, the quality of the plants should be assessed in the field by taking into account their capacity to survive, and, then to develop roots and grow.

Conclusions

Evidence has shown that the *Nothofagus alessandrii* seed possesses an endogenous dormancy, an obstacle that can be overcome with cold stratification or gibberellic acid immersion treatments, sowing the seed in the spring (September). The highest GC has been achieved when stratifying the seed for 30 days (85%), with a daily germination rate of 2.6%. The best response to GA_3 treatments was observed with a concentration of 100 ppm (79%) and a daily 2.52% rate. In addition, soaking the seed in gibberellic acid at 100 ppm initially increased the speed of germination, although it was later equaled with stratification for 30 days. Finally, the sowing time was fundamental in the germination process, and with winter sowings (in July and August) the germination rate obtained was too low to be sufficient for totally breaking seed dormancy.

The pre-germination treatments assayed did not have any effect on the development of the plants grown in the nursery during a cultivation period. Similar quality plants were obtained based on both morphological traits and quality indices. Thus, sowing in September and under the specific treatments outlined here (especially applying fertilization with Osmocote in a concentration equal to 3 g/L of substrate), it is possible to produce plants with a stem length of over 60 cm and a root collar diameter of nearly 5 mm.

No clear effect on the development of seedlings was observed when applying different doses of slow-release fertilizer Osmocote. The quality indices and root collar diameter indicated that all of these plants were appropriate for afforestation. However, the morphological attributes obtained were comparatively lower than the results for plants sown in September one year previously, which were treated with 3 g/L of the same fertilizer.

Implications for Practice

- Endogenous dormancy of the regional endemic *Nothofagus alessandrii* can be successfully overcome in a nursery sowing during springtime, applying gibberellic acid or by cold stratification. Without utilizing these practices both the percentage of germination and the seedling quality are very poor.
- The application of slow-release fertilizer Osmocote, following manufacturer-suggested doses (3 g/L), produces *N. alessandrii* seedlings with acceptable quality indicators.
- Improvements in the capacity to produce viable seedlings in the nursery of the endemic tree *N. alessandrii* are important for restoration efforts in this unique, but highly degraded ecosystem.

LITERATURE CITED

Barceló-Coll, J., G. Nicolás-Rodrigo, B. Sabater-García, and R. Sanchéz-Tamés. 2001. Fisiología Vegetal. Ediciones Pirámide, Madrid.

Benoit, I. editor. 1989. Libro rojo de la flora terrestre de Chile (Primera parte). Corporación Nacional Forestal, Santiago, Chile.

Bustamante, R., and A. Grez. 1995. Consecuencias ecológicas de la fragmentación de los bosques nativos. Ambiente y Desarrollo (Chile) **11:**58–63.

Bustamante, R., and C. Castor. 1998. The decline of an endangered ecosystem: the ruil (*Nothofagus alessandrii*) forest in Central Chile. Biodiversity and Conservation **7:**1607–1626.

Bustamante, R., and J. Simonetti. 2005. Is *Pinus radiata* invading the native vegetation in Central Chile? Demographic responses in a fragmented forest. Biological Invasions **7:**243–249.

Cabello, A. 2004. *Nothofagus macrocarpa* "roble de Santiago" y las dificultades de propagación de las especies del género *Nothofagus*. Revista del Jardín Botánico Chagual (Chile) **2:**37–41.

Cerabolini, B., R. De Andreis, R. M. Ceriani, S. Pierce, and B. Raimondi. 2004. Seed germination and conservation of endangered species from the Italian Alps: *Physoplexis comosa* and *Primula glaucescens*. Biological Conservation **117:**351–356.

Czabator, F. J. 1962. Germination value: an index combining speed and completeness of pine seed germination. Forest Science **8:**386–396.

Dickson, A., A. L. Leaf, and I. E. Hosner. 1960. Quality appraisal of white spruce and white pine seedlings stock in nurseries. The Forestry Chronicle **36:**10–13.

Donoso, C. 1975. Aspectos de la fenología y germinación de las especies de *Nothofagus* de la zona mesomórfica, Boletín Técnico No. 34. Facultad de Ciencias Forestales, Universidad de Chile, Santiago.

Donoso, C., and A. Cabello. 1978. Antecedentes fenológicos y de germinación de especies leñosas chilenas. Ciencias Forestales (Chile) **1:**31–40.

Donoso, C., and A. Lara. 1995. Utilización de los bosques nativos en Chile: pasado, presente y futuro. Pages 363–387 in J. J. Armesto, C. Villagrán, and M. K. Arroyo, editors. Ecología de los bosques nativos de Chile. Editorial Universitaria, Santiago, Chile.

Dubois, M., K. A. Gilles, J. A. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry **28:**350–356.

Dumroese, R. K., D. S. Page-Dumroese, K. F. Salifu, and D. F. Jacobs. 2005. Exponential fertilization of *Pinus monticola* seedlings: nutrient uptake efficiency, leaching fractions, and early outplanting performance. Canadian Journal of Forest Research **35:**2961–2967.

Espina, J., and L. Núñez. 1996. Características germinativas del ruíl (*Nothofagus alessandrii* Espinosa) para la zona de Curepto. Taller de Habilitación Profesional. Universidad Católica del Maule, Talca, Chile.

Espinoza, N. 1997. Técnicas de propagación por semillas de hualo (*Nothofagus glauca* (Phil.) Krasser). Tesis Ingeniería Forestal. Universidad de Chile, Santiago.

Espinoza, N., and A. Cabello. 1993. Propagación de hualo (*Nothofagus glauca*) por semillas. Page 13 in Avances en Silvicultura, Publicaciones Misceláneas Forestales No. 1. Departamento de Silvicultura, Universidad de Chile, Santiago.

Farías, R., and R. Ulloa. 1996. Características germinativas del ruíl (*Nothofagus alessandrii* Espinosa) para la zona de Curepto. Informe Taller de Habilitación. Universidad Católica del Maule, Talca, Chile.

Floistad, I. S., and K. Kohmann. 2004. Influence of nutrient supply on spring frost hardiness and time of bud break in Norway spruce (*Picea abies* (L.) Karst.) seedlings. New Forests **27:**1–11.

Hartmann, H., and D. Kester. 1998. Propagación de plantas, principios y prácticas. Editorial Continental, México.

Hechenleitner, P., M. Gardner, P. Thomas, C. Echeverría, B. Escobar, P. Brownless, and C. Martínez. 2005. Plantas amenazadas del centro-sur de Chile. Distribución, conservación y propagación. Primera Edición. Universidad Austral de Chile y Real Jardín Botánico de Edimburgo, Valdivia, Chile.

INN (Instituto Nacional de Normalización). 2005*a*. NCH2957/5 Madera. Material de propagación de uso forestal. Parte 5: requisitos generales para raulí. Instituto Nacional de Normalización (INN), Chile.

INN (Instituto Nacional de Normalización). 2005*b*. NCH2957/0 Madera. Material de propagación de uso forestal. Parte 0: Producción y comercialización. Instituto Nacional de Normalización (INN), Chile.

ISTA (International Seed Testing Association). 2006. International rules for seed testing. 2006 edition. International Seed Testing Association (ISTA), Bassersdorf, Switzerland.

Iverson, R. D. 1984. Planting stock selection: meeting biological needs and operational realities. Pages 261–266 in M. L. Duryea, and T. D. Landis, editors. Forest nursery manual. Oregon State University, Corvallis.

Landis, T. D. 1989. Mineral nutrients and fertilization. Pages 1–67 in T. D. Landis, R. W. Tinus, S. E. McDonald, and J. P. Barnett, editors. The container tree nursery manual. Volume 4. Agricultural handbook 674. Department of Agriculture, Forest Service, Washington, D.C.

León-Lobos, P., and R. H. Ellis. 2005. Seed survival in Chilean *Nothofagus* in response to desiccation and storage. Seed Science Research **15:**113–123.

Malik, V., and V. R. Timmer. 1998. Biomass partitioning and nitrogen retranslocation in black spruce seedlings on competitive mixed wood sites: a bioassay study. Canadian Journal of Forest Research **28:**206–215.

Martínez-González, M., J. De Irala-Estevez, and F. J. Faulín-Fajardo. 2001. Bioestadística amigable. Ediciones Díaz de Santos, Madrid.

Mexal, J. G., and T. D. Landis. 1990. Target seedling concepts: height and diameter. Pages 13–17 in R. Rose, S. J. Campbell, and T. D. Landis, editors. Proceedings, Western Forest Nursery Association 13–17 August 1990, Roseburg, Oregon. General Technical Report RM-200. U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado.

Mexal, J. G., R. A. Cuevas Rangel, P. Negreros-Castillo, and C. Paraguirre Lezama. 2002. Nursery production practices affect survival and growth of tropical hardwoods in Quintana Roo, Mexico. Forest Ecology and Management **168:**125–133.

Myers, N., Mittermeier, M. A., Mittermeier, C. G., Da Fonseca, G., and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature **403:**853–858.

Ostle, B. 1992. Estadística aplicada. Décimosegunda reimpresión, Limusa, México.

Rascio, N., P. Mariano, F. Dalla Vecchia, N. La Rocca, P. Profumo, and P. Gastaldo. 1998. Effects of seed chilling or GA_3 supply on dormancy breaking and plantlet growth in *Cercis siliquastrum* L. Plant Growth Regulation **25:**53–61.

Rocuant, L. 1984. Efecto de giberelina y de tiourea en la germinación de semillas: especies del género *Nothofagus*. Bosque (Chile) **5:**53–58.

Sadzawka, A., M. A. Carrasco, R. Grez, M. L. Mora, H. Flores, and A. Reaman. 2007. Métodos de análisis de tejidos vegetales. Revisión 2007, Serie Actas INIA No. 40. Instituto de Investigaciones Agropecuarias, Santiago, Chile.

Salifu, K. F., and V. R. Timmer. 2003. Optimizing nitrogen loading in *Picea mariana* seedlings during nursery culture. Canadian Journal of Forest Research **33:**1287–1294.

Salifu, K. F., and D. F. Jacobs. 2006. Characterizing fertility targets and multi-element interactions in nursery culture of *Quercus rubra* seedlings. Annals of Forest Science **63:**231–237.

San Martín, J., V. Mourgues, A. Villa, and C. Carreño. 1991. Catastro actualizado de la distribución y estado de conservación de los bosques de ruíl en la VII Región. Informe Proyecto Investigación y Desarrollo Forestal CHI/89/003. Corporación Nacional Forestal, Talca, Chile.

San Martín, J., R. Santelices, and R. Henríquez. 2006. *Nothofagus alessandrii* Espinosa, Ruíl. Familia: *Fagaceae*. Pages 390–400 in C. Donoso, editor. Las especies arbóreas de los bosques templados de Chile y Argentina, autoecología. Marisa Cuneo Ediciones, Valdivia, Chile.

Santelices, R., and F. Pérez. 2006. Caracterización del material forestal de reproducción de cinco procedencias de *Nothofagus alessandrii* Espinosa. Page 204 in Actas Tercer Congreso Chileno de Ciencias Forestales. Universidad de Concepción, Chile.

Schmidt-Hebbel, H. 1973. Ciencia y tecnología de los alimentos. Editorial Universitaria, Santiago, Chile.

Sivakumar, V., R. Anandalakshmi, R. R. Warrier, M. Tigabu, P. C. Oden, S. N. Vijayachandran, S. Geetha, and B. G. Singh. 2006. Effects of presowing treatments, desiccation and storage conditions on germination of *Strychnos nux-vomica* seeds, a valuable medicinal plant. New Forests **32:**121–131.

Southgate, D. A. T. 1969. Determination of carbohydrates in foods. Journal of the Science of Food and Agriculture **20:**331–335.

Stolz, L. P. 1966. Factors influencing root initiation in an easy and difficult to root chrysanthemum. Proceedings of the American Society for Horticultural Science **92:**622–626.

Subiri, M. 1997. Técnicas de propagación por semillas y de producción de plantas en vivero de *Nothofagus obliqua* de tres procedencias. Memoria Ingeniería Forestal. Universidad de Chile, Santiago, Chile.

Wardle, P., and A. D. Campbell. 1976. Winter dormancy in seedlings of mountain beech (*Nothofagus solandri* var. *cliffortioides*) near timber line. New Zealand Journal of Botany **74:**183–186.

Wilcox, M. D., and N. J. Ledgard. 1983. Provenance variation in the New Zealand species of *Nothofagus*. New Zealand Journal of Ecology **6:**19–31.

Yang, Q., W. Ye, and X. Yin. 2007. Dormancy and germination of *Areca triandra* seeds. Scientia Horticulturae **113:**107–111.

Zhou, L., J. Wu, and S. Wang. 2003. Low-temperature stratification strategies and growth regulators for rapid induction of *Paris polyphylla* var. *yunnanensis* seed germination. Plant Growth Regulation **41:**179–183.