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Upgrading germinability of ponderosa pine seeds from Patagonia, Argentina, by adjusting prechilling periods and applying the IDS technique

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Abstract Ponderosa pine (*Pinus ponderosa* Dogl. ex. Laws.) is the most planted conifer species in the forest-steppe ecotone of Patagonia, Argentina, because of its adaptability and excellent growth rates. In spite of this, and the increasing demand for this species, local commercial seed lots showed low quality, making hazardous seedling production. Aiming to upgrade germinability of a local seed lot, we set an experiment to determine the duration of the prechilling period (0, 10, 20, 30, 40 and 60 days) that promoted the highest seed germination speed (GE) and percentage (GP). Moreover, part of that lot was IDS treated, in an attempt to separate empty and dead filled seeds from viable seeds. Results showed that after 40 days prechilling, GE reached 62%, and GP 70%, both higher than the values obtained under customary conditions. The application of the IDS technique, after 40 days prechilling, 8 h drying at ambient conditions (16 ± 2°C and 50% HR), and 25% seed moisture content (mc), increased GE and GP to 68% and 89%, respectively. Optimal prechilling periods and the application of the IDS technique successfully improved germinability of ponderosa pine seeds from Patagonian stands.

Résumé Le pin á bois lourd (*Pinus ponderosa* Dogl. ex. Laws.) c'est l'éspèce de conifère la plus plantée dans l'écotone forêt-steppe en Patagonie, Argentine, dû à son adaptabilité et ses excellents taux de croissance. Par cette cause, et aussi par la demande croissante pour cette espèce, l'approvisionnement local de semences manque encore de qualité ce qui fait incertaine la production en pépinière. Afin d'améliorer le taux de

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germination des semences locales, nous avons cherché à déterminer la période de stratification froide (0, 10, 20, 30, 40 and 60 jours) donnant la plus grande vitesse de germination (GE) et le plus haut pourcentage (GP). Aussi, une partie des graines ont été traitées par IDS, pour essayer de trier les graines valables de celles mortes ou vides. Les résultats ont montré qu'après 40 jours de stratification froide, GE atteignit 62% et GP 70%, toutes deux plus hautes que les valeurs obtenues dans les conditions initiales. L'emploi de la technique IDS, -avec 40 jours de stratification froide, 8 hs de désechement dans des conditions ambiantes (16 ± 2°C et 50% HR), et 25% de contenu d'humidité de graine- fit augmenter GE et GP jusqu'à 68% et 89%, respectivement. L'ajustement des périodes de stratification froide et l'utilisation de la technique IDS a réussi à améliorer le taux de germination des graines de pin à bois lourd issus de Patagonie.

Keywords Pinus ponderosa · Cold moist stratification · Viable seed separation · Germination improvement

Introduction

Ponderosa pine (*Pinus ponderosa* Dogl. ex. Laws.) is one of the most used conifer species to afforest the ecotone region between the Andean Forests and the steppe in Patagonia, Argentina. This preference is due to the excellent adaptation and growth rates that this species reach in Patagonia, where climate and soil condition are similar to its native region in North America (Gonda 2001). Hence, about 90% of the total production of commercial tree nurseries in Andean Patagonia is dedicated to produce seedlings of this species (Laclau and Leanza 1997). Today, although local ponderosa pine seed production practically replaced importation, the lack of an official quality level for commercialization resulted in low quality and heterogeneous seed lots without assuring an efficient seedling production. In most Patagonian commercial nurseries the only seed treatment applied was cold moist stratification for 21 days according to ISTA rules (ISTA 1993). Higher quality seed lots were required from some nursery producing containerized seedlings. This method implies the use of one, or at most two seeds per container, to produce good quality seedlings in shorter times than the traditional bare-root production (Wenny and Dumroese 1987).

Seed quality implies a series of attributes that include purity, vigour, or germinability of a seed lot (Bonner et al. 1994). In seed lots collected in Patagonia, the percentages of dead and empty seeds could reach values varying from 30 to 60% (N. M. Pasquini, personal communication). Physiological dormancy can be removed through cold-moist stratification (prechilling), as already demonstrated for several conifer species (Bonner et al. 1989; Wang and Pitel 1991; Willan 1991; Bradbeer 1992). This technique consists in incubating imbibed seed within a moist medium at 1 to 5°C for a few weeks up to several months. The duration of this treatment could vary according to different species, varieties, and ecotypes, and should be determined for each particular case (Bonner et al. 1994). Previous references about ponderosa pine seeds were not consistent; ISTA rules (ISTA 1993) recommended 0 or 21 days prechilling. In other editions (ISTA 1996, 1999, 2003) chilling requirements were not mentioned. On the other hand, the Association of Official Seed Analysts (AOSA 1981, 1984), suggested 28 days stratification. Weber (1988) showed indirect evidences that prechilling requirements varied geographically for ponderosa pine seeds coming from populations grown in central Oregon, and that these differences could be related to the severity of the summer drought of the area each population came from. Seeds coming from

mesic locations appear to have greater prechilling requirements than those coming from areas with shorter and rain-limited summers (xeric locations). In another study on 149 ponderosa pine populations from the State of Oregon, Weber and Sorensen (1990), recommend that the stratification period for those populations should be at least 60 days, and that this period should be increased in nurseries located in areas with low spring temperatures or for breeding zones which present more variable rains and mesic environmental conditions. They finally concluded that chilling period should be adjusted according to the differences in population geographic location. On the other hand, none of the prechilling rules specify moisture levels that seeds should reach after moistening before applying the cold treatment (see ISTA 1993, 1996, 1999, 2003; AOSA 1981, 1984). Evidences suggested, however, that for conifer species, seed moisture content (mc) should be around 30% to optimize the efficacy of prechilling (Tanaka and Edwards 1986; Gosling and Rigg 1990; Downie and Bergsten 1991; Tillman Sutela 1996).

Physical quality of ponderosa pine seeds in Patagonia is partially improved by the use of air screen cleaner equipment (locally called MAZ), which eliminates inert material and empty seeds, obtaining lots showing from 98 to 99% of filled seeds (Basil and Leanza 2001). However, some seeds within the filled fraction showed an incomplete, soft, uncoloured content and they were not able to germinate and to produce seedling at the end of the assay (ISTA 1999). MAZ could not separate them due to similarities in weight. To remove those seeds, the use of the Incubation-Desiccation-Separation technique (IDS, Simak 1984) appeared an alternative way for separating filled and viable seeds from those filled but non-viable. This technique is based on the principle that when moistened seeds are subjected to uniform drying conditions, viable seeds release the bond-stored water at a slower rate than non-viable seeds (Simak 1984). Differences in density allow then the separation of the two fractions through immersion in water (sunk = viable, floating = non viable), (Simak 1984).

In the case of dormant seeds, such as those corresponding to ponderosa pine (Krugman et al. 1989; Weber and Sorensen 1990), the incubation step could be substituted by a period of stratification (Simak 1984). This technique was successfully applied to improve seed quality (germinability and vigour) in lodgepole pine (*Pinus contorta var. latifolia* Engelm.) (Simak 1984; Downie and Wang 1992), Jack pine (*Pinus banksiana* Lamb.) (Downie and Wang 1992), and caribian pine (*Pinus caribaea*, Poulsen 1995). However, results were not successful when this technique was applied to white spruce (*Picea glauca* (Moench) Voss) (Downie and Wang 1992). At present, there are no references that the IDS technique was applied to ponderosa pine seeds whose origins are from stands grown in the temperate zone of southern South America (the Patagonian region of Argentina and Chile).

The objectives of the present study were twofold: (1) to improve the quality of commercial ponderosa pine seed lots commonly used in Patagonian determining the optimal prechilling period, and (2) separating viable from non-viable seeds by applying the IDS technique.

Materials and methods

Seed collection and preliminary tests

Seeds used in this study came from a commercial lot collected in March 2000, at the end of the summer season, from different ponderosa pine stands grown near the city of Trevelin, Chubut, Argentina (43°04′ S, 71°27′ W). All stands were located at altitudes ranging from

350 to 450 m.a.s.l., with a precipitation regime varying from 670 to 750 mm per year. Mean an annual temperature of the collection area was 9.6°C, with winter (July) mean temperature of 3.2°C, and summer (January) mean of 16.2°C. After collection, seeds were cleaned by an air screen cleaner equipment (MAZ) to remove inert matter and other small debris. This procedure allowed to obtain a seed lot purity of 99.9%. After this time period, seeds were stored in plastic containers at room temperature for 3 months to mimic the short-term storage conditions regularly used by most Patagonian nurseries. Seeds were then taken to the lab, and a random sample was used for preliminary tests to determine seed lot quality, as shown in Table 1. The rest of the seed lot was placed in a plastic container and remained stored at temperatures ranging from 16 ± 2°C, and Relative Humidity (RH) between 40 and 50% during summertime, and 7 ± 2°C and RH between 50 and 60% during wintertime, for 3 years. After that, seeds were used for the analyses reported in this study.

Imbibition duration and moisture content before prechilling

Before to prechilling, seeds were water-soaked during 9, 16, 24, 32, 48 and 56 h at 18°C to determine the time to attain a moisture content of around 30%, according to the recommendations given for other conifers (Tanaka and Edwards 1986; Gosling and Rigg 1990; Downie and Bergsten 1991; Tillman Sutela 1996). Seed moisture content was determined on a fresh weight basis by oven-drying two seed samples of 5 g each at 103°C for a period of 17 ± 1 h (ISTA 1999).

Prechilling treatment

To determine the optimal prechilling period, seeds were water-soaked for 24 h (which was the time at which seeds reached 30% moisture content), then they were placed into polyethylene bags, and stratified at 3–5°C for 0 (control), 10, 20, 30, 40, and 60 days. After each prechilling periods, four 50-seeds replication were placed to germinate in plastic boxes containing sterilized sand (moistened with distilled water) at 20/25°C. The boxes were exposed to alternate daily light and dark periods (8 h of fluorescent light, coincident at the highest temperature, and 16 h of darkness, coincident with the lowest temperature). The germination temperatures used were chosen based on previous studies, which suggest alternate temperatures of 20/30°C (ISTA 1999), 15/25°C (Krugman et al. 1989), and 20/25°C (Weber and Sorensen 1990). We used sterilized sand as a substrate instead of the paper is suggested by ISTA (1999), because it maintains better the humidity content, allowing at the same time lower fungi contamination.

Seed were considered as germinated when hypocotyl protruded the sand surface and had a normal appearance. Germination energy (GE) and germination percentage (GP) were

Table 1 Preliminary analyses of ponderosa pine seeds used in this study†

Testing date	Purity (%)	Weight per 1,000 seeds (g)	No. of seeds per kg	Moisture content (mc) (%)	Germination Energy (GE)* (%)	Germination Percentage (GP)*
July/00	99.9	49.5	20.202 ± 820	7	39.0	66.0

* Prechilling: 3 weeks (3°–5°C)

† According to ISTA rules (ISTA 1993)

then evaluated as the percentage of seeds germinated at 7 (GE) and 21 (PG) days after sowing, respectively (ISTA 1999). Non-germinated seeds were submitted to cutting test at the end of germination test and classified as fresh, dead and empty (ISTA 1999). The percentage of dead and empty seeds indicated the unproductive seeds of the lot studied. The prechilling treatment that gave the best results in terms of GE and GP was then used as the control in the subsequent application of the IDS technique.

Adjustment of the IDS technique

(a) Incubation (prechilling)

The incubation step in the IDS technique was replaced by 3 weeks of cold-stratification (prechilling) as suggested by Simak (1984). A seed sample was placed in water over a period of 24 h at 18 ± 2°C, to attain a moisture content of about 30%, and then incubated as mentioned before at 3/5°C for 40 days. This period was selected because it was the one that gave the best GE and GP in the previous test. After this period, a sample of these seeds was placed to germinate and used as control in the subsequent application of the IDS technique.

(b) Drying

The remaining sample was divided into four fractions, spread out on a net-bag to allow drying at ambient conditions (16/18°C and 50% RH) for 0, 6, 8 and 10 h. These were then the treatments against which the control was compared by applying the IDS technique. Then, a small fraction of seeds of each treatment were used to determine the seed moisture content, according to ISTA rules (ISTA 1999).

(c) Separation

For each drying treatment (excluding the Control) were then soaked in water, stirred for about 1 min and allowed to separate in sunken and floating fractions. Each fraction was collected separately, air dried for about 30 min to take away surface water, weighed and put to germinate separately following the conditions previously reported to determine their GE and GP, and fresh, empty and dead seeds (see prechilling treatment).

Experimental design and statistical analyses

Five treatments and two levels each were then set as follows: (1) Control (original sample), (2) 0 h drying and floating (IDS 0, F) and 0 h drying and sunk (IDS 0, S), (3) 6 h drying and floating (IDS 6, F) and 6 h drying and sunk (IDS 6, S), (4) 8 h drying and floating (IDS 8, F) and 8 h drying and sunk (IDS 8, S), and 5) 10 h drying and floating (IDS 10, F) and 10 h drying and sunk (IDS 10, S). A completely randomized design was used, with four replicates per treatment (5) at two levels (F and S). Every treatment-level consisted in 200 seeds. Germination Energy (GE) and Germination Percentage (PG) data were arcsine transformed to homogenize variances, and analysed through ANOVA techniques. To determine significant differences among mean values of the treatments, we used the Duncan Test ($P \leq 0.05$) with the InfoStat Statistical Package (InfoStat 2002).

Results and discussion

Seed imbibition

Our results showed that ponderosa pine seeds collected in Patagonia increased from 7 to 35% mc after 56 h imbibition at ambient temperature (18 ± 2°C), but needed only 16–24 h to reach 30% mc (Fig. 1). Many authors reported that optimal moisture content to promote germination in many conifer seeds was 30–35% (Downie and Bergsten 1991; Tilman Sutela 1996). However imbibition time varied among different species (Tilman Sutela 1996; Gosling and Rigg 1990). In our case, longer periods of imbibition (24 h) increased seed mc by only 3%. This suggests that longer imbibitions periods may not be necessary (Patagonian nurseries use customarily 48 h or more). Results were consistent with Weber and Sorensen (1990) study on ponderosa pine in central Oregon. In contrast in Idaho, Wenny and Dumroese (1987) adopted an imbibition time of 48 h. Although the seeds mc, and the time period to achieved it may be critical, only a few papers has focused on this aspect (Gosling and Rigg 1990; Downie and Bergsten 1991; Tillman-Sutela 1996).

Effect of stratification on germinative energy (GE) and germination percentage (GP)

Results showed that cold-moist stratification favour both GE and GP, and that an increase in the duration of this pre-treatment improved these germination parameters (Table 2). The GP increased significantly from 0 to 10, and from 10 to 20-day prechilling ($P \leq 0.05$) and did not show any significant variation when stratification time exceeded 20 days (Table 2). In this table comparisons between GP values and percentage of fresh seeds showed that after 20-day stratification the majority of fresh seeds had germinated producing normal seedlings. However, chilling periods longer than 20 days had a beneficial effect resulting in a significant increase of GE ($P \leq 0.05$) up to 40-day stratification. Germination energy was an indicator of germination speed in a specific period of time (in this case 7 days after

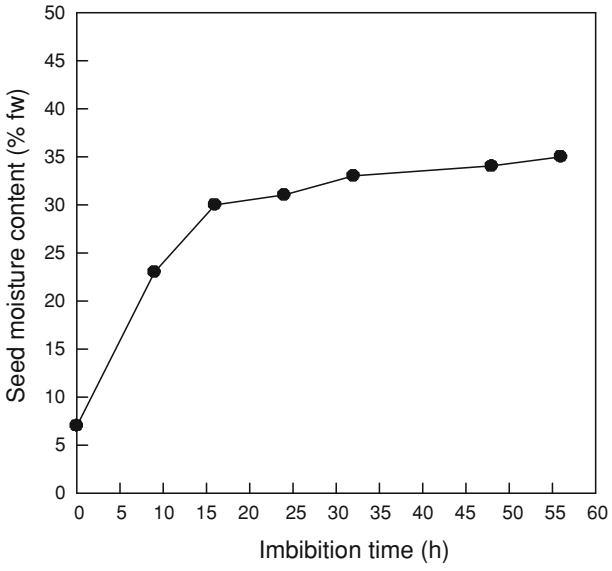

Fig. 1 Moisture content achieved by ponderosa pine seeds soaked in distilled water for different time periods

Table 2 Germination energy (GE) and Percentage (GP), empty (E), fresh (F) and dead (D) ponderosa pine seeds used in this study, subjected to six different prechilling periods

Prechilling (days)	GE	GP	E	F	D
	%				
0	0.0a	31.0a	3.0	41.0a	25.0
10	7.0a	51.0b	4.0	19.0b	26.0
20	34.0b	66.0c	4.0	6.0c	24.0
30	50.0c	71.0c	3.0	1.0c	25.0
40	62.0d	70.0c	5.0	1.0c	24.0
60	59.0d	73.0c	3.0	0.0c	24.0

Note: Lower case letters indicate significant differences at ($P \leq 0.05$)

sowing) and it expressed seed vigour in trees and shrubs seeds (Bonner 1989; Bonner et al. 1994). In relation to GP, our results coincide with those suggested by ISTA (1993) and AOSA (1984), i.e. 21 and 28 days of stratification, respectively. In contrast and related to germination speed, our results indicated that the stratification time should be at least 40 days to obtain the highest GE percentages. This was consistent with results obtained by Weber and Sorensen (1990), who stated that for this species, germination speed and uniformity increased after longer stratification (up to 60 days) and higher incubation temperature.

On the other hand, the percentage of empty seeds in this lot varied from 3 to 5% (according to the sample), indicating that the cleaning operation did not attain the desired goal of eliminating all empty seeds. Also, the proportion of dead seeds varied from 24 to 26%, which added to 3–5% of empty seeds, gave a total of 27–31% of unproductive seeds in the seed lot studied. It is important to note that these numbers are within these values recorded for many other analyses performed for ponderosa pine seeds lots from Patagonian nurseries (N. M. Pasquini, unpublished report). This high percentage of unproductive seeds limited the use of this poor quality seed lot for commercial purposes, and was the reason why we essayed the IDS Technique.

Application of the IDS technique

Incubation, drying and separation

As previously stated, the 40-day stratification, at around 30% mc was selected as a control because it was the treatment that gave the bests results in the preliminary test. The values obtained with the Control treatment for GP and GE were 70 and 62%, respectively (Table 3, row 1). The same table shows the effects of the different periods of drying and separation over the effectiveness of the IDS technique. The application of this technique, separating sunken (viable) and floating (non-viable) seeds, significantly improved ($P \leq 0.05$) GP and GE of the sunken fractions compared to the control at all drying periods tested (Table 3, rows 2–5). GP values of the sunken fraction increased from 13% (IDS 1S) to 19% (IDS 3S) whereas GE improved from 2% (IDS 4S) to 6% (IDS 2S and IDS 3S) as compared to the Control. Longer drying periods (decreasing seed mc) produced a higher separation of the floating and sunken fractions, although they did not improve GP and/or GE of the sunken fraction (see Table 3.). Under ambient conditions prevailing during the test (16 ± 2°C, and 50% RH), the highest GP and GE of the sunken fraction were obtained

Table 3 Drying time, moisture content, sample weight (percentage), GP and GE of IDS separated fraction of ponderosa pine seeds (S = sunken; F = floating). Drying conditions: 16 ± 2°C, 50% RH. Seeds used as Control were those prechilled for 40 days but neither dried nor separated with water

Treatment	Drying time (h)	Moisture content (mc) %	% of sample weight	GP %	GE %
S					
Control	0	32	100	70.0a	62.0a
IDS 1 S	0	32	91	83.0b	65.0a
IDS 2 S	6	27	82	86.0b	68.0a
IDS 3 S	8	25	81	89.0b	68.0a
IDS 4 S	10	24	74	83.0b	64.0a
F					
IDS 1 F	0	32	9	11.0a	2.0a
IDS 2 F	6	27	18	20.0b	12.0b
IDS 3 F	8	25	19	23.0b	12.0b
IDS 4 F	10	24	26	26.0b	12.0b

Note: Different lower case letters in each column indicate significant differences ($P \leq 0.05$) within each fraction (S) or (F)

after a drying period of 8 h, when the sample had a 25% mc. In this case, the separation of dead seeds allowed to increase GP of that fraction from 70 to 89%. For the same treatment, the floating fraction, which represented 19% of the sample weight, showed a GP of 23%. By recalculating this numbers, and by applying this technique, the floating fraction represented only 4% of the total amount of germinable seeds in the original seed lot sample. Our results showed then, that the application of IDS technique was useful for upgrading germination vigour and percentage of ponderosa pine seeds from stands grown in Patagonia, and were in close agreement with the findings of other authors that applied a similar technique to other conifers (Simak 1984; Downie and Wang 1992; Poulsen 1995; Tilman Sutela 1996; Demelash et al. 2002).

The values of GE, GP, empty, fresh, and dead seeds obtained from the different fractions (Sunken and Floating) by the application of the IDS technique are presented in Table 4. The apparently high proportion of empty seeds found in the floating fraction that appeared after the germination test could be the result of two different causes. The first one is that, as previously stated, the MAZ did no eliminate all empty seeds (5% in the control). The second cause could be that during the IDS germination test, many of these seeds often decay, appearing as empty at the end of it. As a result of this, the true proportion of empty seeds could often be overestimated (Simak 1991).

The results presented here also suggested that the application of the IDS technique may have an additional invigorating effect (GE) on ponderosa pine seeds that exceeded the simple separation in viable and non-viable seed fractions. This technique also increased the GP as related to the control treatment (whose seeds only received prechilling for 40 days at 3–5°). The average germination percentage (GP) of the two fractions (S and F) was higher (76, 74 and 76%) than the control (70%). It is probable that these drying and repeated moistening processes could be, in part, the responsible for these germination differences. Under natural conditions, the process of seed wetting and drying occurs often, and this has proven to improve germination percentages (Henckel 1961). Seed re-imbibition during the IDS process could have acted, then, as a sort of priming, as re-hydration could allow some

Table 4 Germination Energy (GE) and Percentage (GP), empty (E), fresh (F) and dead (D) ponderosa pine seeds IDS treated (S = sunken; F = floating). Drying conditions: 16 ± 2°C, 50% RH. Seeds used as control were those prechilled for 40 days but neither dried nor separated with water

Treatment	Drying time (h)	GE	GP	E	F	D
S						
Control	0	62b	70a	5	1	24a
IDS 1 S	0	65b	83b	0	0	17b
IDS 2 S	6	68b	86b	0	0	14b
IDS 3 S	8	68b	89b	0	0	11b
IDS 4 S	10	64b	83b	0	0	17ab
F						
IDS 1 F	0	2a	11a	13a	0	76a
IDS 2 F	6	12b	20b	17a	0	63b
IDS 3 F	8	12b	23b	13a	0	64b
IDS 4 F	10	12b	26b	14a	0	60b

Note: Different lower case letters in each column indicate significant differences ($P \leq 0.05$) within each fraction (S) or (F)

seed reparative process (Downie and Bergsten 1991). This priming effect was recorded in several horticultural seeds (Parera and Canliffe 1994; Mac Donald 1999). To be conclusive, however, more specific investigations would be necessary to corroborate our explanation about these germination differences.

Conclusions

According to the results obtained in this study, it is possible to improve germinability (GP) and germination energy (GE) of ponderosa pine seeds from stands grown in Patagonia by applying the IDS technique with a slight modification. This modification consisted in replacing the step of incubation with a period of prechilling, which should last at least for 40 days. The best separation between sunken = viable, and floating = nonviable seed fractions was obtained when seeds were dried to a mc of 25%. By following this simple procedure, Patagonian nurseries could improve the quality of local ponderosa pine seed lots up to about 20% GP (from 70 to 89%) and 100% (from 34 to 68%) GE compared to typical procedure used in those nurseries, where the only treatment adopted is 21 days prechilling.

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