Assessing the Hardiness of Aleppo Pine, Maritime Pine, and Holm Oak Seedlings by Electrolyte Leakage and Water Potential Methods

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Cold and drought hardiness of nursery stock were measured just before planting in the region of Valencia (Spain). A number of stock lots of Aleppo pine (Pinus halepensis *Mill.), maritime pine* (Pinus pinaster *Ait.), and holm oak* (Quercus ilex L.) *were assessed between December 1994 and February 1997, during 3 planting seasons. Cold hardiness was evaluated by the electrolyte leakage method and whole-plant freeze testing. Drought avoidance was estimated as the drop in predawn water potential after a period without any watering. Both parameters detected nonhardened seedling lots. The electrolyte leakage method was preferred as it was faster.* Tree Planters' Notes 50(1): 38-43; 2003.

A number of causes are involved in the failure of forest tree plantations. The plants are affected by genetic factors, by conditions of nursery cultivation, and by the environment at the plantation site (Grossnickle and Folk 1993). Sometimes, even when using the same seed source with similar plantation conditions, field performance can be quite different, reflecting differences in factors that are collectively known as seedling quality (Ritchie 1984).

In Spain, the Forest Service of the Community of Valencia, in the eastern Iberian Peninsula, is developing a reforestation program using Aleppo pine *(Pinus halepensis* Mill.), maritime pine *(P. pinaster* Ait.), and holm oak *(Quercus ilex L.).* High variability in the survival of plantations at the same time as substantial changes in nursery cultivation techniques, led to suspicions about the quality of the planting stock.

Seedlings planted in the Mediterranean climate of Spain are grown almost exclusively in containers. In the early 1990s, the polyethylene bag was abandoned in favor of newer systems (rigid containers such as Superleach®, Rootrainers®, Forest pot®, Arnabat®, Styrofoam® block, Ecopot®, and others) to prevent root deformation (Penuelas 1991). The transition was made very quickly, and proper cultivation methods were not always well developed. Additionally, a standard cultivation practice (growing medium, irrigation and fertilization practices, lifting date) was not used. Also occurring during this transition was the change from a plant produced only in state nurseries, usually close to lands to be forested, to the coexistence of private and publicly owned nurseries. In fact, in the region of Valencia, poor performance has been attributed to the stock produced in private nurseries at sea level (the elevation of the majority of the private nurseries) and outplanted in the interior mountains.

In these regions (with a Mediterranean climate), establishing plantations in autumn can be beneficial because the autumn root growth takes place on the site and because the seedling establishment period avoids the possible spring drought. However, plantations established in autumn are in danger of early frost. Thus, it is necessary to know when seedlings are hardened enough to be outplanted.

One-year-old holm oak seedlings (an evergreen species) usually set bud at the end of the growing season and probably enter a deep-rest state. By contrast, the Aleppo and maritime pines never set bud at the end of the 1st growing season and probably remain in a quiescent state, making it difficult to see any morphology changes with hardening. Consequently, this study was conducted to assess the quality of planting stock coming from different nurseries.

Freeze-induced electrolyte leakage, whole-plant freeze testing, and the drop in xylem water potential were chosen to evaluate seedling quality. Electrolyte leakage has been successfully used to assess cold hardiness in other species (Burr and others 1990). It has been operationally tested (Colombo and others 1984) and gives results quickly (Glerum 1985). Xylem water potential is just 1 measure of plant water status Qoly 1985), but tracking it has been useful in reforestation programs (Cleary and Zaerr 1980). The most common application has been monitoring the stress build-up in seedlings during lifting, grading, packing, and storage (Ritchie 1984).

Materials and Methods

Eighty-three stock lots, totaling 10,000 seedlings, from 23 private and public nurseries, were evaluated between December 1994 and February 1997. There were 44 lots of Aleppo pine, 20 of maritime pine, and 19 of holm oak.

At least 100 plants per lot were sampled randomly from the nurseries. Sampling intensity varied from 1 to 10 per 1000 seedlings. During the 1st planting season, measurements were made from December 1994 through February 1995. Over the next 2 y, the measurement periods were from November through February The sampled seedlings were brought to the lab at the same time they were sent to the field for planting. Each lot was measured once. The normal time when these seedlings are extracted and outplanted is late autumn (November 15 to December 15) and late winter (February 1 to March 15); therefore, more than 85% of the lots were analyzed during these periods. The seedlings, all grown in containers (see table 1 for height and diameter of the seedlings, container volumes, and growing densities), were 7 to 11 mo old when brought to the laboratory. Seedlings in their containers were put into perforated cardboard boxes and transported in small closed vans or trucks. Transportation was completed within 3.5 h and was arranged so that the seedlings could start to be tested within 48 hours after leaving the nursery.

Cold hardiness attributes. *A. Index of injury (I;,,). A* temperature of -8 "C (17.6 °F) was chosen to expose tissue samples to in the electrolyte leakage test. According to the methods described by Simpson (1990), the LT_{50} (temperature that kills 50% of the foliage of a seedling) recommended for cold hardiness testing of these species in midwinter is around -10 "C (14 °F). However, at the plantations, the typical minimum midwinter (January) temperature is -8.1 °C (17.4 °F) (infrequently reaching -12 "C, 10.4 "F), and during the usual planting time, the expected temperature is above -6.3 °C (20.7'F). Thus, we believe that -8 "C may be low enough (Royo 1998).

Leaves or needles (two 9-mm-diameter, 0.35-in-diameter, leaf disks, or eight 1-cm-long, 0.39-in-long, needle segments per test tube; 1 tube per seedling; 15 seedlings randomly selected from each lot) were submitted to freezing temperatures following the procedure of McKay and Mason (1991). First, the treatment tubes containing tissue samples (n=11) were placed in a freezer.

The temperature was then dropped from ambient to -8 "C at 5 "C/h (9 "F/h) and maintained for 3 h. After warming to room temperature at 10 "C/h (18 "F/h), the frozen samples, plus another 4 tubes with samples from 4 additional seedlings that were not frozen (control), were submerged in 16 ml (0.54 oz) of deionized water per tube for 24 h. Electrical conductivity of the solution was measured. Samples were then autoclaved at 110 °C (230 °F) for 10 min and electrical conductivity of the solution was measured again after 24 h. Relative electrical conductivity was calculated for each seedling; the 2 kinds of samples were Rs (frozen + autoclave) and Rc (autoclave only). Finally, the index of injury (l_{ip}), expressed as a percentage, was calculated as follows (Glerum 1985):

$$(1) \quad I_{in} = \frac{Rs - Rc}{1 - Rc} \cdot 100$$

B. Whole-plant freeze test (WPFT). To calibrate the electrolyte leakage test results to whole plant response, a browning test was done on another 15 seedlings. Seedlings in their containers, with root systems protected with Styrofoam, were exposed to the same low-temperature treatment as in the previous test. After thawing to room temperature, the seedlings were transferred to a heated greenhouse for 15 d. Each year, greenhouse temperatures were set at 27 "C (80.6 °F) during the day and 17 °C (62.6 "F) at night, and day-length was extended to 12 h (minimum photosynthetic active radiation, PAR, of 150 µmol•m^{-2}.0 provided by 400 W metal halide lamps). Nevertheless, greenhouse temperatures occasionally reached 30 °C (86 °F) during the day and 13 "C (55.4 °F) at night. The damage observed in the shoots was quantitatively estimated according to the proportion of withered leaves or needles:

Level	Foliar damage
1.	<25%
2.	26% to 50%
3.	51% to 75%
4.	75%

Table 1—*Species means and ranges of means among lots within a species for the seedling and container parameters measured for Aleppo pine (*Pinus halepensis *Mill., n = 44 lots), maritime pine (*P. pinaster *Ait., n = 20 lots), and holm oak (*Quercus ilex *L., n = 19 lots)*

Parameter		Aleppo pine $\bar{x} \pm s_{\bar{x}}$	Aleppo pine Range	Maritime pine $\bar{x} \pm s_{\bar{x}}$	Maritime pine Range	Holm oak $\bar{x} \pm s_{\bar{x}}$	Holm oak Range
Shoot height	(cm)	10.4 ± 0.4	19.9 - 6.3	10.1 ± 0.7	19.4 - 5.0	11.8 ± 0.6	22.7 - 6.3
	(in)	4.1 ± 0.7	7.8 - 2.5	4.0 ± 0.3	7.6 - 2.0	4.7 ± 0.2	8.9 - 2.5
Stem diameter	(mm)	2.1 ± 0.1	3.1 - 1.2	2.2 ± 0.1	3.5 - 1.4	3.8 ± 0.1	5.5 - 2.6
	(in)	0.08 ± 0.01	0.12 - 0.05	0.09 ± 0.01	0.14 - 0.06	0.15 ± 0.01	0.22 - 0.10
Cell volume	(cm^3/cell)	211 ± 6	300 - 150	209 ± 6	300 - 150	317 ± 28	810 - 200
	(in^3/cell)	12.9 ± 0.4	18.3 - 9.2	12.8 ± 0.4	18.3 - 9.2	19.3 ± 1.7	49.4 - 12.2
Cell density	(plants/m^2)	368 ± 12	600 - 200	353 ± 16	600 - 250	298 ± 12	400 - 194
	(plants/ft^2)	34.2 ± 1.1	55.7 - 18.6	32.8 ± 1.5	55.7 - 23.2	27.7 ± 1.1	37.2 - 18.0

Plant water status assessed by the drop in water potential ($\Delta\Psi$). Plants in their original containers were watered to field capacity in the afternoon upon arrival at the laboratory. The following day, predawn water potential (Ψ_1) was measured with a pressure chamber. The seedlings were then kept in a growth chamber for 15 d without watering. During the day, the chamber conditions were 22 °C (71.6 °F) air temperature, 65% relative humidity, and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR at plant level during a 16-h photoperiod. At night the temperature was 16 °C (60.8 °F), with 80% relative humidity. Predawn water potential was measured again at the end of the 15 d (Ψ_{15}). Visual damage (VD) was also quantified as in the browning test. The drop in water potential was calculated as:

(2) $\Delta\Psi = \Psi_1 - \Psi_{15}$

Results

The only significant difference among species for all hardiness tests (table 2) was for initial needle water potential (Ψ_1, $P < 0.001$). The oak Ψ_1 averaged 2 to 3 times more negative than the Ψ_1 of the pines. However, after 15 d without water, the Ψ_{15} for the 3 species did not differ.

Water stress avoidance ($\Delta\Psi$) followed a bimodal frequency distribution in Aleppo pine and, to a lesser extent, in maritime pine (figure 1, left). There were 2 types of responses: a decrease in water potential less than 1.5 MPa, identifying high-avoidant lots, and a decrease greater than 2.5 MPa in less-avoidant lots, with few lots in between. There were no significant correlations among $\Delta\Psi$, container volume, root dry mass, and seedling shoot-to-root ratio (r^2 L 0.12, $P > 0.05$, data not shown). The ratio of root dry mass to container volume was not similar for all lots: it ranged from 1.3 to 3.6 g/L (0.17 to 0.48 oz/gal) for pines and 5.0 to 9.2 g/L (0.67 to 1.23 oz/gal) for holm oak.

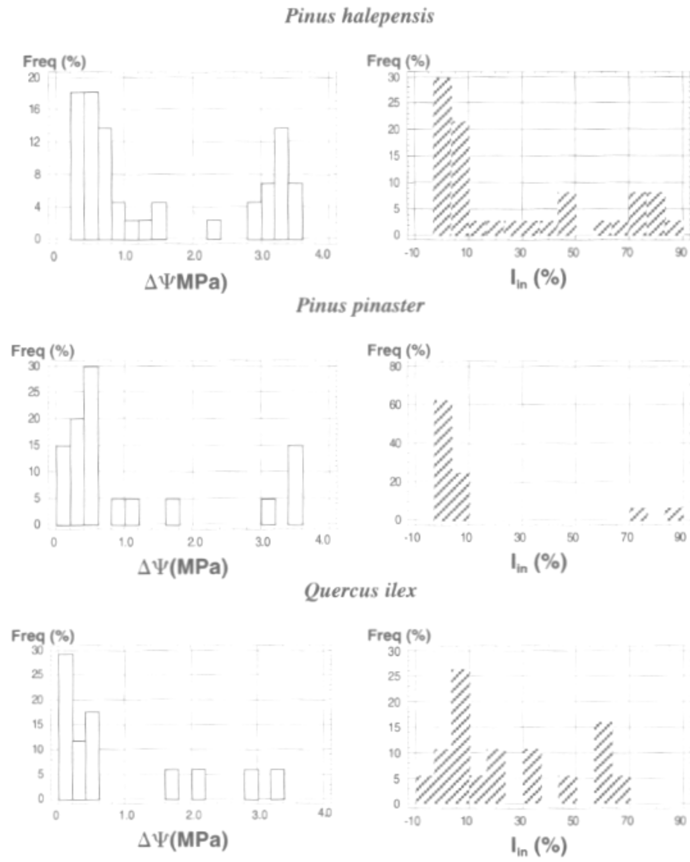


*Figure 1—Frequency distribution of the water potential drop ($\Delta\Psi$ after 15 d without watering) (left) and index of injury (I_{in},-8 °C, 17.6 °F) (right) for individual stock lots of Aleppo pine (*Pinus halepensis *Mill., n = 44 lots), maritime pine (*P. pinaster *Ait., n = 20 lots), and holm oak (*Quercus ilex *L., n = 19 lots). Each lot (15 seedlings per lot) was measured once, when shipped to the field from a nursery.*

A bimodal response was also observed for I_{in} in the 3 species (figure 1, right), although with a less visible 2nd peak than for $\Delta\Psi$. This response contrasted with an unimodal distribution for other morphological and physio-

Table 2—*Species means and ranges of means among lots within a species for the hardiness parameters over all test dates for Aleppo pine (*Pinus halepensis *Mill., n = 44 lots), maritime pine (*P. pinaster *Ait., n = 20 lots), and holm oak (*Quercus ilex *L., n = 19 lots)* [a]

	Aleppo pine		Maritime pine		Holm oak	
Parameter	$\bar{x} \pm s_{\bar{x}}$	Range	$\bar{x} \pm s_{\bar{x}}$	Range	$\bar{x} \pm s_{\bar{x}}$	Range
I_{in} (%)	29.8 ± 5.4 a	96.5 - 0.0	12.1 ± 6.8 a	86.8 - 0.0	22.7 ± 6.0 a	68.4 - 0.0
WPFT rating[b]	2.2 ± 0.2 a	4.0 - 0.5	1.7 ± 0.3 a	4.0 - 0.5	1.7 ± 0.2 a	3.6 - 0.5
$-\Psi_1$ (MPa)	0.53 ± 0.03 b	0.23 - 1.06	0.38 ± 0.04 a	0.23 - 0.80	1.05 ± 0.07 c	0.68 - 2.06
$-\Psi_{15}$ (MPa)	2.04 ± 0.21 a	0.56 - 4.00	1.51 ± 0.31 a	0.43 - 4.00	1.58 ± 0.28 a	0.67 - 4.00
$\Delta\Psi$ (MPa)	1.60 ± 0.02 a	4.01 - 0.27	1.13 ± 0.28 a	3.57 - 0.17	0.72 ± 0.26 a	3.30 - 0.00
Visible damage rating[b]	1.5 ± 0.2 a	4.0 - 0.5	1.1 ± 0.2 a	4.0 - 0.0	1.0 ± 0.3 a	3.0 - 0.0

[a] For each row, means followed by the same letter are not statistically different (Bonferroni, 95%).
[b] WPFT (whole-plant freeze test) and visible damage rating scale, based on the proportion of withered foliage 15 d after shoot exposure to –8 °C (17.6 °F) and 15 d without watering, respectively: 1. < 25%, 2. 26% to 50%, 3. 51% to 75%, 4. > 75%.

logical parameters measured, such as shoot height and nutrient content (data not shown).

The following results were derived from the correlation analyses between the hardiness parameters (tables 3, 4 and 5):

1. Good correlations were found between the WPFT and l_{in} (figure 2, left), and between VD and AT. The determination coefficients (r^2) were higher than 0.65 for the 3 species, and greater than 0.85 for the 2 pines.

2. h_n was highly correlated with AP in the 3 species (figure 2, right). The correlation was higher for the 2 pine species *($r >$* 0.94, $P < 0.0001$) than for the holm oak ($r = 0.78, P < 0.001$).

3. There was a difference between the 2 pines and the holm oak. 'P_1 was correlated with the WPFT and I_{in} in the 2 pines. However, there were no significant correlations for 'P_1 in the oak.

Discussion

The studied parameters followed different patterns: a unimodal distribution for height and a bimodal distribution for cold hardiness and drought avoidance. The bimodal pattern allows the separation of 2 different groups of plants: hardy and nonhardy.

Apart from outplanting, the only procedure for unequivocally evaluating damage after freezing tests is to hold the seedlings in a greenhouse or growth chamber for several weeks and then visually inspect them (Ritchie 1991). In our study, results from the whole-plans freeze test were highly correlated ($r > 0.69$) with those from the electrolyte leakage test. Therefore, results from the latter method can be used alone, as they are related to the true cold hardiness of the seedlings. A similar relationship occurred with AT and VD, with even higher correlation coefficients ($r > 0.88$). Several variables (container volume, available water, growing medium composition, phenological stage of the seedlings, seedling size, vapor pressure deficit, for example) can alter the rate of seedling dry-down and the subsequent predawn 1P reading. This may explain the lack of relationship between X'P and some of those variables.

The general stress resistance that plants show when acquiring cold hardiness (Lavender 1985; Burr 1990) would explain the high correlation found between the index of injury and X P in the 3 species. This correlation is largely affected by environmental changes throughout the annual growth cycle of seedlings (Levitt 1980; Burr 1990). Additionally, good correlations have usually been found between cold and drought hardiness and field performance (Mattsson 1997). These correlations reduce the number of tests needed for seedling quality evaluation. The measurement of cold hardiness using the electrolyte leakage method permits the estimation of hardi-

Table 3—*Linear correlations (coefficient and significance level) between measured parameters for Aleppo pine* (Pinus halepensis *Mill.). All coefficients are significant*

Parameter	I_{in}	WPFT	Ψ_1	Ψ_{15}	$\Delta\Psi$	VD
I_{in}	1					
WPFT	**0.91**	1				
	0.0000					
Ψ_1	**-0.63**	**-0.63**	1			
	0.0000	**0.0000**				
Ψ_{15}	**-0.85**	**-0.70**	**0.62**	1		
	0.0000	**0.0000**	**0.0000**			
$\Delta\Psi$	**0.94**	**0.72**	**-0.53**	**-0.92**	1	
	0.0000	**0.0000**	**0.0002**	**0.0000**		
VD	**0.78**	**0.59**	**-0.57**	**-0.93**	**0.85**	1
	0.0000	**0.0000**	**0.0001**	**0.0000**	**0.0000**	

Table 4—*Linear correlations (coefficient and significance level) between measured parameters for maritime pine* (Pinus pinaster *Ait.). All coefficients are significant*

Parameter	I_{in}	WPFT	Ψ_1	Ψ_{15}	$\Delta\Psi$	VD
I_{in}	1					
WPFT	**0.99**	1				
	0.0000					
Ψ_1	**-0.78**	**-0.78**	1			
	0.0001	**0.0001**				
Ψ_{15}	**-0.96**	**-0.85**	**0.69**	1		
	0.0000	**0.0000**	**0.0007**			
$\Delta\Psi$	**0.94**	**0.82**	**-0.62**	**-0.99**	1	
	0.0000	**0.0000**	**0.0037**	**0.0000**		
VD	**0.73**	**0.73**	**-0.44**	**-0.91**	**0.93**	1
	0.0004	**0.0004**	**0.0490**	**0.0000**	**0.0000**	

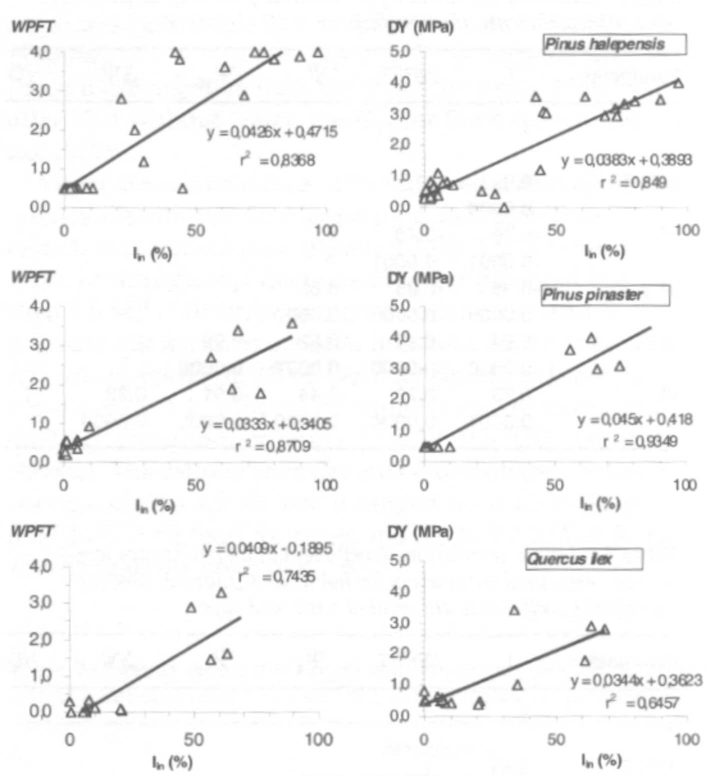
Table 5—*Linear correlations (coefficient and significance level) between measured parameters for holm oak* (Quercus ilex *L.). Significant coefficients are printed with bold type*

Parameter	I_{in}	WPFT	Ψ_1	Ψ_{15}	$\Delta\Psi$	VD
I_{in}	1					
WPFT	**0.69**	1				
	0.0023					
Ψ_1	0.15	-0.37	1			
	0.5526	0.0825				
Ψ_{15}	**-0.77**	**-0.72**	0.44	1		
	0.0014	**0.0024**	0.0768			
$\Delta\Psi$	**0.78**	**0.69**	-0.30	**-0.99**	1	
	0.0010	**0.0042**	0.2340	**0.0000**		
VD	**0.69**	**0.55**	-0.34	**-0.88**	**0.88**	1
	0.0061	**0.0337**	0.1883	**0.0000**	**0.0000**	

ness in an easier and quicker way than the whole-plant test. As long as the index of injury does not surpass 30% after freezing to -8 °C (17.6 °F) as described (figure 2), it can be assumed that plants have good drought avoidance and are ready to be planted. If water potential is used as the parameter to assess stress resistance, the DY value, following the correlation with $_{in}$, must be under 1.5 MPa.

Autumn plantations are an alternative to early spring plantations if drought periods are frequent in spring, as long as seedlings are hardened at the nursery before lifting. The relationships among seedling quality (physiological attributes, morphological parameters) and cultural and climatic conditions during the year need to be established for each nursery-species (or nursery-genotype) combination, so that lifting and outplanting schedules will be successful. The cold-hardiness test (-8 "C, 30% h_n) can be used to compare production regimes for these species to eliminate the unsuccessful regimes, especially at coastal nurseries where the chilling requirements of seedlings are not always completed.

Figure 2-Relationship between the whole-plant freeze test (WPFT) rating (based on the proportion of withered foliage 15



d after shoot exposure to -8 'C (17.6 °F): 1. < 25%, 2. = 26% to 50%, 3. 51 to 75%, 4. > 75%) and index of injury (1~,, -8 'C, 17.6 °F) (left), and between the water potential drop (DY after 15 days without watering) and index of injury (h_{am}, *-8 'C, 17.6 'F) (right) for individual stock lots of Aleppo pine* (Pinus halepensis *Mill.,* n=44 *lots), maritime pine (P* pinaster *Ait., n = 20 lots) and holm oak* (Quercus ilex L., n = *19 lots). Each point represents the mean value of a stock lot (15 seedlings per lot). Each lot was measured once when shipped to the field from a nursery.*

Conclusion

Electrolyte leakage and water potential tests proved to be very useful in assessing the hardiness level of seedlings against cold and drought, respectively. The relationship between cold hardiness and drought avoidance permits inferring the status of one from the other. Electrolyte leakage was preferred as it was faster. Nevertheless, further research concerning correlation of the seedling quality test results with field performance should be carried out.

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References

Burr KE. 1990. The target seedling concepts: bud dormancy and cold-hardiness. In: Rose R, Campbell SJ, Landis TD, editors. Target seedling symposium: proceedings, combined meeting of the Western Forest Nursery Associations; 1990 Aug 13-17; Roseburg, OR. Fort Collins (CO): USDA Forest Service, Rocky Mountain Forest and Range Experiment Station. General Technical Report RM-200. p 79-90.

Burr KE, Tinus RW, Wallner SJ, King RM. 1990. Comparison of three cold hardiness tests for conifer seedlings. Tree Physiology 6: 351-369.

Cleary BD, Zaerr JB. 1980. Pressure chamber techniques for monitoring and evaluating seedling water status. New Zealand Journal of Forest Science 10(1): 133-141.

Colombo SJ, Webb DP, Glerum C. 1984. Frost hardiness testing: An operational manual for use with Extended Greenhouse Culture. Ontario Ministry of Natural Resources. Forest Research Report No. 110. 14 p.

Glerum C. 1985. Frost hardiness of coniferous seedlings: principles and applications. In: Duryea ML, editor. Proceedings: Evaluating seedling quality: principles, procedures, and predictive abilities of major tests; 1984 Oct 16-18; Corvallis, OR. Corvallis (OR): Forest Research Laboratory, Oregon State University. p 107-126.

Grossnickle SC, Folk RS. 1993. Stock quality assessment: forecasting survival or performance on a reforestation site. Tree Planters' Notes 44(3): 113-121.

Jolt' RJ. 1985. Techniques for determining seedling water status and their effectiveness in assessing stress. In: Duryea ML, editor. Proceedings: Evaluating seedling quality: principles, procedures, and predictive abilities of major tests;

1984 Oct 16-18; Corvallis, OR. Corvallis (OR): Forest Research Laboratory, Oregon State University. p 17-28.
Lavender DP. 1985. Bud dormancy. In: Duryea ML, editor. Proceedings: Evaluating seedling quality: principles, procedures, and predictive abilities of major tests; 1984 Oct 16-18; Corvallis, OR. Corvallis (OR): Forest Research Laboratory, Oregon State University. p 7-16.
Levitt J. 1980. Responses of plants to environmental stresses. Vol. 1. Chilling, freezing, and high temperature stresses. New York (NY): Academic Press. 497 p.
Mattsson A. 1997. Predicting field performance using seedling quality assessment. New Forests 13: 227-252.
McKay HM, Mason WL. 1991. Physiological indicators of tolerance to cold storage in Sitka spruce and Douglas-fir seedlings. Canadian Journal of Forest Research 21: 890-901.
Penuelas JL. 1991. La calidad de la planta forestal [The quality of forest nursery stock]. In: Proceedings, Jornadas sobre situation actual y tecnicas modernas para la produccion de plantas forestales [Present situation and modern techniques in forest nursery stock production]. Madrid (Spain): Universidad Politecnica de Madrid y Colegio de Ingenieros de Montes [Polytechnic University of Madrid and Forest Engineering Association]. p 1-5.
Ritchie GA. 1984. Evaluating seedling quality. In: Duryea ML, Landis TD, editors. Forest nursery manual: production of bareroot seedlings. The Hague (The Netherlands): Martinus Nijhoff/Dr. W. Junk Publishers. p 243-257.
Ritchie GA. 1991. Measuring cold hardiness. In: Lassoie JP, Hinckley TM, editors. Techniques and approaches in forest tree ecophysiology. Boca Ration (FL): CRC Press. p 557-584.
Royo A. 1998. La calidad de la planta forestal y su relacion con el comportamiento en campo [The quality of forest nursery stock and its relation to field performance]. Aplicacion a Pinus *halepensis Mill., Pinus pinaster* Ait. *y Quercus ilex L.* [dissertation]. Madrid (Spain): ETS1 de Montes, Universidad Politecnica de Madrid [School of Forestry, Polytechnic University of Madrid]. 150 p.
Simpson DG. 1990. Frost hardiness, root growth capacity, and field performance relationships in interior spruce, lodgepole pine, Douglas-fir, and western hemlock seedlings. Canadian Journal of Forest Research 20: 566-572.