Phenological Variation Among Plus-Tree Clones of *Pinus sylvestris* (L.) in Northern Sweden

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Summary

Eighteen plustree clones of *Pinus sylvestris* (L.) from latitudes 67°N, 65°N and 62°N growing in a Swedish coastal clone archive on latitude 63°40'N were tested for cold tolerance on 20 occasions from August 1990 till May 1992 using artificial freeze testing of detached current-year needles. Freezing injury was determined by visual scoring of the proportion of discoloured needle tissue. Clonal variation in needle cold tolerance was significant on all test occasions except for 2 freezing tests in the summer. The southernmost population had a slower autumn cold acclimation than the populations from 65°N and 67°N. In the winter and spring no effect of the latitude of origin on needle cold tolerance was observed. Fluctuations in winter cold hardiness, probably induced by changing weather conditions, were considerable. Winter cold hardiness was uncorrelated with autumn cold hardiness. Rapid deacclimation occurred in April. Early cold acclimation in the autumn was related to poor height growth. The shoot elongation rhythm was uncorrelated with needle cold tolerance at all times of the year. The attachment of current-year needles to the terminal shoot in late summer was positively correlated with needle cold tolerance in early autumn. The needle dry matter proportion in the autumn was positively correlated with needle cold tolerance in early winter. Progeny cold hardiness in early autumn was positively correlated with needle cold tolerance of the mother in early autumn but uncorrelated with needle cold tolerance at other times of the year. Possible implications of selection for phenological characteristics for vegetative propagation and breeding of Scots pine on northern latitudes are discussed.

Key words: cold acclimation, cold tolerance, deacclimation, freeze testing, growth rhythm, needles, Scots pine, temperature.

FDC: 165.53; 165.441; 174.7 *Pinus sylvestris;* (485).

Introduction

Survival at northern latitudes is closely related to the annual rhythm of trees. The rhythm is influenced by photoperiod, temperature and other factors (LEVITT, 1980) and of course, the genotype of the tree. One of the most critical qualities for tree survival in the north is the ability of trees to quickly complete growth in short and often relatively cold summers and initiate autumn cold acclimation under the short summer night conditions still prevailing. The effect of shortening days on cold acclimation of northern conifers and deciduous species has been clearly demonstrated in various freezing experiments (ARONSSON, 1975; CHRISTERSSON, 1978; EKBERG *et al.,* 1979; JONSSON *et al.,* 1981). Later in the autumn and in the winter deeper cold hardiness is mainly obtained in response to low temperatures (WEISER, 1970). Increasing temperatures in the spring cause deacclimation and resumption of physiological activities.

Differences in light and temperature climates of northern latitudes have resulted in a clinal adaptation in the rate of autumn cold acclimation. In Scots pine this relationship has been demonstrated by artificial freeze testing in early autumn of young plants (NILSSON and ERIKSSON, 1986) or detached needles (LINDGREN and NILSSON, 1992; NILSSON and WALFRIDSSON, 1993). It is also obvious from studies of first-year bud-set (MIKOLA, 1982) and dry matter proportion of current-year needles (LANGLET, 1936).

In the summer the timing of shoot elongation (STÅHL, 1984) and flowering (NILSSON, 1981) are clinally related to the latitude of origin. However, the relationships are usually not as strong as for autumn phenology (NILSSON and ÅMAN, 1986). Genetic studies on winter hardiness in forest trees are more often concerned with differences between species (SUTINEN *et al.,* 1992) than within species (DEHAYES, 1990a). In comparison to the wellknown clinal variation in most northern species during cold acclimation, the genetic variation in cold hardiness and other phenological traits during winter and spring is considerably less known. This is partly due to difficulties in finding easily observable phenological criteria in the winter and to uncertainties concerning methodological problems of freeze testing to the low temperatures necessary to induce freezing injury.

The aim of the present study is to (i) provide information on seasonal changes and clonal variation in cold tolerance of Swedish plus-trees of Scots pine, (ii) get better knowledge about how the relationships between cold tolerance and other phenological traits vary over the year under natural outdoor conditions, and (iii) conclude about possible consequences of phenological plustree selection for breeding and vegetative propagation of Scots pine on northern localities.

Material and Method

The phenological study was performed on grafts in a coastal clone archive of Scots pine in north Sweden on latitude 63°40'N. In the clone archive an average of 10 grafts per clone are randomly distributed in 1-tree plots with a spacing of 4x4 meters. The clones were originally selected for good growth and quality at 20 to 21 years of age in north Swedish provenance field trials planted in 1954 (EICHE, 1966). Grafting for the clone archive was made in 1974 and 1975 and planting was carried out 3 years later. For the present study 18 unrelated clones *(Table 1),* 6 from each of latitudes 67°N (northern clone origin), 65°N (central clone origin) and 62°N to 63°N (southern clone origin), were selected in the clone archive. For the northern and southern clones the original stand and the field trial were located close to each other. For the central clones plus-tree selection was made in field trials located one or two latitudes north of the original stand (clone origin).

The 12 northern and central clones is a subset of clones that were studied in various experiments in 1980 to 1985 for clonal variation, based on 6 grafts per clone, in a number of phenological traits during summer and autumn. They were also subjected to a number of artificial early autumn progeny freez-

Table 1. – Origins of the plus-tree clones including the location of the seed source. N = Number of clones.

Origin	N	Stand[1] Lat. °N	Stand[1] Elev. m	Field trial[2,3] Lat. °N	Field trial[2,3] Elev. m
North	1	67°09'	330	66°56'	200
	1	"	"	67°14'	470
	4	67°14'	470	"	"
Central	3	65°08'	30	67°14'	470
	3	"	"	66°16'	435
South	3	63°10'	540	63°10'	525
	3	62°03'	385	"	"

[1]) Locality where the open-pollinated seeds for establishing the field trial were collected.
[2]) Growth place of the ortets of the plustree clones.
[3]) The field trials on latitude 66°56' and 67°14' are located on a mild and harsh site, respectively.

ing tests. The results of that study are presented in NILSSON and ÅMAN, (1986). In the present paper clone data from that study are related to the results of a series of artificial freezing tests of detached needles performed in 1990 to 1992 on the same northern and central clones (3 grafts per clone). The 6 southern clones are subjected only to the needle freezing tests and were not included in the phenological study of NILSSON and ÅMAN (1986).

Phenological traits observed in 1980 to 1985

The shoot elongation rhythm was determined from measuring the terminal length (ELONG) on approximately ten occasions per year in 1980 to 1982 and 1985. In the present study the proportion of elongation (RELONG) when the mean elongation had achieved approximately 20% and 70% of the total elongation in October were considered and indicate differences in time for onset and termination of shoot elongation. These occasions typically occurred in late May and mid June.

The receptivity (REC) of female strobili was assessed at midsummer, 1985. Each graft was classified in one of 6 receptivity classes (0=no flower receptive, to 5=receptivity over). The clones are represented by the mean receptivity score of 6 grafts.

Needle attachment (NA) was determined by measuring the power needed to loosen a current-year needle pair from the central part of the terminal shoot. The attachment was measured on three occasions in late July and mid August 1983 and 1984 by pulling a dynamometer attached halfway between needle base and apex in the direction of the needle. Latitudinal variation in the rate of increase in needle attachment during late summer (RUMMOKAINEN, 1982) indicates that needle attachment is a phenological character related to cold acclimation in Scots pine.

The dry matter proportion of current year needles (DRYM) was assessed on 4 occasions between 13 August and 16 October 1985 as the ratio of dry weight after drying at 105° C for 24 h to fresh weight.

Progeny cold acclimation during early autumn was tested in up to 7 artificial whole plant freezing tests at 1 year of age in the early 1980's (cooling rate 3° C h^{-1} with 3 hours at approximately –10° C to –12° C). Freezing injury was assessed by visual scoring of the discolouration of needles approximately ten days after freeze testing, c.f. NILSSON and ERIKSSON (1986).

The above phenological traits were transformed to achieve means and variances of 100 and defined so that high values indicate early phenological rhythms. For traits that were observed for more than 1 year the mean of the transformed values over years were used. For shoot elongation, needle attachment and dry matter proportion the analyses were based on both absolute values and relative the last value in a series of observations (RELONG, RELNA and RELDRYM, respectively).

Needle freeze testing

Current-year needles for artificial freeze testing were collected from 5 2nd order shoots in the upper crown sections of the 4 meters to 7 meters high grafts. From every graft 3 or 4 needle samples, each consisting of 5 needle pairs, were collected and immediately put in small moistened plastic bags that were sealed to decrease needle dessication. After transportation to the laboratory 2 samples per graft were immediately (except for 1 hour of handling the sample bags in room temperature) exposed to artificial freeze testing in 2 programmable Weiss 500/80-180DU freezing cabinets allowing freezing to –80° C. The remaining 2 needle samples were stored in darkness at +6° C till after testing of the first 2 needle samples. All needles were freeze tested within 14 hours from collection. Every graft was tested at 3 or 4 freezing temperatures using different needle samples.

Freeze testing was carried out in darkness by gradual cooling the air in the cabinet during 1.5 h from +5° C to various predetermined minimum temperatures *(Table 2).* The rate of cooling therefore varied between 8° C h^{-1} in the summer to 50° C h^{-1} in some winter tests. After 1 hour at the minimum temperature the needles were thawed by gradual raising of the air temperature to +10° C at the same rate as during freezing. After another 8 h to 10 h in darkness at +10° C the needle samples were moved to a greenhouse with +10° C temperature and 14 h photoperiod (artificial light).

The needles were maintained in the plastic bags from needle collection till visual scoring of the proportion (10% classes) of discoloured tissue of individual needles 1 to 3 weeks after freeze testing (the time from freezing till the injury is clearly visible was longer in late autumn and winter than in summer and early autumn). Based on the observed injury in individual freezing tests, a series of critical temperatures (temperatures inducing a certain level of injury) was obtained for every clone over the whole test period. For this study the critical temperature, $CT_{50/20}$, was defined as the temperature at which half of the frozen needles showed no or slight injury (less than 20% discoloured needle tissue). Estimation of CT-values was made by interpolation between adjacent temperatures. In some winter tests the cold hardiness of individual grafts was better than the minimum test temperature. In these cases critical temperatures were estimated from the the injury levels at the lowest temperature (the lowest critical temperature, –85° C, was given to clones with no visible injury at –75° C.

In the winter injured needles did usually not turn yellow-brown, as they did in the summer and autumn freezing tests, but usually obtained a greyish colour. In the freezing tests from January 1992 till May 1992 the visual scoring of needle discolouration was therefore supplemented by measuring the chlorophyll a fluorescence of the same needle samples using a Plant Stress meter (PSM, Biomonitor AB, Sweden). The measurements, mainly related to PS II, were made after 5 s fluorescence induction at PSM light level 2 following 1 h dark adaptation at room temperature the day after the visual scoring of needle injury. During measurement the needles were placed side by side and the ratio of the variable chlorophyll fluorescence to the maximum fluorescence, F_v/F_m, was determined on a circle area (diameter ca. 10 mm) in the middle of the needle sample. As for the visual scoring of needle injury

critical temperatures, CT_{FL}, were also estimated for the F_v/F_m ratio, as the temperature corresponding to $F_V/F_m=0.50$ on January 9 and February 12, and $F_v/F_m=0.55$ on the following test occasions. The lower value on the first 2 occasions were used because of overall lower F_v/F_m-values at the highest test temperatures with no visible discolouration. Because the chlorophyll a fluorescence measurements were limited to the last third of the test occasions, the F_v/F_m observations are used only for verification of the clonal differences in cold hardiness based on visual injury scoring.

Seasonal changes in cold tolerance were related to temperature observations at the meteorological station at Umeå airport 25 km NE of the clone archive *(Fig. 1)*.

The statistical evaluation of the clonal variation in cold tolerance, and the relationships between needle cold tolerance and other phenological traits were based on either critical temperatures or arcsine transformed proportions of slightly injured needles (<20% discoloured needle tissue) at the test temperature that gave a mean proportion of slightly injured needles closest to 50%. For the statistical analyses the Manova, Pearson corr and Nonpar corr options of the SPSS-X statistical program were utilized.

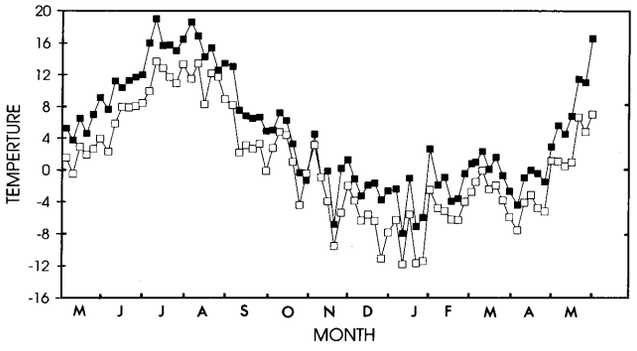

Figure 1. – Mean and minimum temperatures (5 day averages) from May 1991 to June 1992 recorded at the meteorological station at Umeå airport 25 km NE of the clone archive.

Results

Needle cold tolerance

There was a significant effect of clone origin on needle cold tolerance on all test occasions in August and September 1990 and 1991, except for the first test occasion in mid-August 1991

Table 2. – Days of needle freeze testing and utilized freezing temperatures. Results from analyses of variance of the proportion of slightly injured needles (arcsine transformed values) after freeze testing using the model $y_{ijk} = Latitude_i + Clone_{j(i)} + e_{ijk}$. The results are presented for the freezing temperature with mean injury rate closest to 0.5, indicated by bold figures. The F-tests are based on the assumption of fixed latitude (*N*orthern, *C*entral, *S*outhern) and random clone-within latitude effects. Significance levels are shown for linear contrasts comparing northern with central clones (N/C) and southern clones with the mean of northern and central clones (NC/S). Estimated variance components for clone (o^2_{cl}) and error (o^2_e) are presented as percentages of total phenptypic variance.

Day of testing	Significance leves N/C	NC/S	Clone	Variance component s^2_{cl}	s^2_e	Mean injury prop.	Freezing temperature, °C			
1990										
20 Aug	ns	***	**	24	17	**.64**	-7.0	-8.0	**-10**	
3 Sep	ns	***	***	18	3	**.59**	-8.5	**-10.5**	-12	
10 Sep	ns	***	***	8	8	**.48**	-8.5	-10.5	**-13**	-16
17 Sep	ns	**	***	20	21	**.63**	-14.0	**-15.5**	-17	-19
27 Sep	ns	***	***	10	8	**.71**	-17.0	**-20.0**	-23	-27
1991/92										
14 Aug	ns	ns	ns	6	94	**.47**	-6.5	-8.5	**-10**	
3 Sep	ns	*	***	41	17	**.24**	-10	**-12.5**	-14	
24 Sep	ns	**	***	25	10	**.35**	13	-15.5	**-19**	-23
22 Oct	ns	***	***	18	14	**.52**	-18	-21	-26	**-28**
19 Nov	ns	*	*	19	43	**.91**	-25	-31	**-35**	-40
11 Dec	ns	ns	***	52	48	**.63**	-42	-52	**-65**	-75
8 Jan	ns	ns	**	40	51	**.68**	-40	-52	**-63**	-75
12 Feb	ns	ns	***	67	33	**.76**	-40	-52	**-63**	-75
19 Feb	ns	ns	**	35	42	**.80**	-40	-52	**-63**	-75
23 Mar	ns	*	*	22	55	**.10**	**-38**	-52	-63	-75
7 Apr	ns	ns	***	70	30	**.71**	-27	-40	-52	**-63**
22 Apr	ns	ns	***	48	40	**.63**	-40	**-52**	-63	-75
5 May	ns	ns	***	70	30	**.79**	-11	**-20**	-28	-40
11 May	ns	ns	**	39	61	**.65**	-8	-12	-16	**-20**
26 May	ns	ns	ns	15	85	**.19**	-7.5	**-11**	-12	-17

Significance levels: ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

(Table 2). Linear contrasts show that southern clones were significantly less hardy than central and northern clones. The differences between the northern and central clones were nonsignificant. The difference in needle cold tolerance between the southern and northern/central clones gradually increased during the autumn till late September (and till late October 1991).

The influence of latitude on needle cold tolerance gradually increased till late October 1991 ($p<0.001$), and was significant ($p<0.05$) also in mid November. From December, during the whole winter and spring, till the last freezing test on 26 May no effect of latitude was found on cold tolerance, either from discolouration or flourescence observations.

From mid December till mid February the critical temperatures, $CT_{50/20}$, of most clones, irrespective of the origin, were close to or even lower than the capacity (approximately –75° C) of our freezing equipment *(Fig. 2).* On 23 March a drastic decrease in cold tolerance compared to the February tests was observed for all clones. This was probably a consequence of a long and unusually mild period preceding needle collection *(Fig. 1).* The very uniform cold tolerance indicated in *figure 2* was merely caused by too low freezing temperatures in comparison with the actual cold tolerance of the clones. Till 7 April most of the lost cold tolerance was regained (but not for all clones), probably because of a relatively cold first week in April. From 22 April till 5 May the critical temperature rapidly increased from around –50° C to –60° C to approximately –20° C to –25° C for individual clones. After a slightly increased cold tolerance for some clones on 12 May, coinciding with the occurrence of a preceding colder period, the critical temperature on 26 May was back on the same level as in mid-August the previous year *(Fig. 2).*

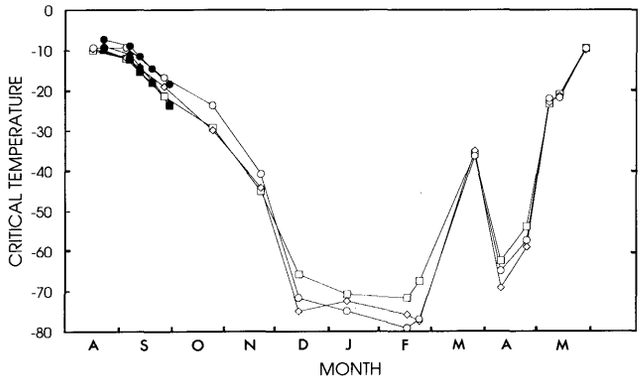

Figure 2. – Average critical temperatures ($CT_{50/20}$) for clones from latitudes 67 °N (□), 65 °N (◇) and 62 °N to 63 °N (○) in the autum of 1990 (filled figures) and from the autum of 1991 to early summer 1992 (open figures).

Clonal variation (within population) was significant ($p<0.001$) on all test occasions but the first one in mid August 1991 and the last one in late May 1992. The ranking of clones for needle cold tolerance was largely the same on the 5 test occasions in August and September 1990 *(Table 3).* In 1991 the correlations between test occasions were significant in September to November but not on as high level as in 1990 *(Table 4).* The critical temperatures in August and September 1990 were positively correlated with critical temperatures in September to November 1991 *(Table 3).* Autumn cold tolerance in 1991 was uncorrelated with cold tolerance on all the following test occasions from December till the last test occasion in late May 1992. Even negative correlations between cold tolerance in the autumn and some winter tests were observed. The critical temperatures estimated from fluorescence measurements (CT_{FL}) in January to May 1992 were significantly ($p<0.01$) correlated with those from needle discolouration, $CT_{50/20}$, and mostly higher than $r=0.70$ *(Table 4).* Although CT_{FL} was generally slightly higher than $CT_{50/20}$, the chlorophyll fluorescence observations verified the nonsignificant influence of latitudinal origin on needle cold tolerance in winter and spring. The CT_{FL}-values also confirm the time-to-time fluctuations in cold hardiness during winter. and spring that were observed by needle discolouration.

Table 3. – SPEARMAN'S rank correlation coefficients (× 100) between critical temperatures, $CT_{50/20}$, on different test occasions in early autumns 1990 and 1991 (n = 18 clones). Bold correlations are significant on level $p < 0.01$.

	1990				
	Aug	September			
	20	3	10	17	27
1990					
3 Sep	**78**				
10 Sep	**88**	**95**			
17 Sep	**89**	**93**	**92**		
27 Sep	**86**	**88**	**90**	**95**	
1991					
14 Aug	08	13	11	14	11
3 Sep	**87**	**90**	**92**	**92**	**92**
24 Sep	**50**	**64**	**66**	**65**	**74**
22 Oct	**68**	**73**	**72**	**68**	**69**
19 Nov	**59**	**72**	**73**	**68**	**65**
11 Dec	18	10	10	12	-05

Correlations between needle cold tolerance and other traits

The relationships between needle cold tolerance and other phenological traits were based on only the 12 northern and central clones.

Shoot growth (ELONG) in the summers of 1981 to 1985 was negatively correlated with needle cold tolerance in early autumn 1990 and 1991 and uncorrelated with cold tolerance at other times of the year. RELONG was uncorrelated with needle cold tolerance during the whole period of hardiness testing from early autumn till early summer *(Fig. 3).*

Early flowering was related to poor needle cold tolerance in some winter tests and to high cold tolerance in early summer. The flowering time was uncorrelated with cold tolerance during autumn and spring *(Fig. 3).*

Needle attachment in late July and early August (both absolute levels and relative values) were positively correlated with needle cold tolerance in late August till late September but uncorrelated with needle cold tolerance at other times of the year *(Fig. 3).*

The needle dry matter proportions in August and early September were uncorrelated with autumn cold tolerance in 1991, whereas positive correlations were obtained with needle cold tolerance in early autumn 1990 *(Fig 3).* High dry matter proportions in late September and, especially, October were related to high needle cold tolerance in early winter. High RELDRYM (early cessation of dry matter increase) was related to high needle cold tolerance in September 1990 but not in 1991.

Early progeny cold acclimation in the autumn was related to high needle cold tolerance of the mother clone in early autumn but not with needle cold tolerance at other times of the year *(Fig. 3).*

Table 4. – SPEARMAN'S rank correlation coefficients (× 100) between critical temperatures ($CT_{50/20}$) on the 15 freezing occasions from August 1991 to May 1992 (n = 18 clones). The right column shows correlations between $CT_{50/20}$ and CT_{FL} (critical temperature based on chlorophyll fluorescence) on the freezing occasion indicated in the left column. Bold correlations are significant on level $p < 0.01$ (one-tailed tests). Low correlations in the range $-0.30 < r < 0.30$ are indicated by "<".

	$CT_{50/20}$														
	Aug	Sep	Sep	Oct	Nov	Dec	Jan	Feb	Feb	Mar	Apr	Apr	May	May	
Day	14	3	24	22	19	11	8	12	19	23	7	22	5	11	CT_{FL}
3 Sep	<														
24 Sep	<	**59**													
22 Oct	<	**83**	40												
19 Nov	<	**62**	40	**56**											
11 Dec	<	<	<	<	<										
8 Jan	-46	<	<	<	<	43									**69**
12 Feb	-35	**-55**	-50	-34	<	**55**	45								**57**
19 Feb	-42	-32	-52	<	<	52	49	**82**							**74**
23 Mar	<	<	<	<	-33	<	<	<	<						30
7 Apr	<	<	<	<	<	68	<	38	47	<					**82**
22 Apr	-40	<	<	<	<	40	43	42	**59**	<	**56**				**77**
5 May	<	<	<	38	<	-36	<	<	<	<	<	<			**77**
11 May	<	<	<	<	<	<	<	<	<	<	<	<	**74**		15
26 May	<	<	<	<	<	<	<	<	31	<	<	<	44	48	**73**

Discussion

Test conditions

The cooling and thawing rates were as high as 50° C h^{-1} in some winter freezing tests. Certainly even faster temperature fluctuation can occur in nature, but not with as large amplitudes as in our winter tests. The combination of very large temperature fluctuations and high rates of cooling and thawing in the winter tests is extreme and has probably negatively affected the estimated critical temperatures compared to slower freezing rates (SAKAI, 1979; GLERUM, 1985). However, the freezing arrangement allowed all needles to be tested for an equally long period of time. Furthermore, the rapid cooling during hardening means that hardening during freezing was probably avoided. On the other hand intracellular ice formation, which is usually lethal in nature, may occur with rapid cooling. Because many needles were uninjured even at the highest cooling rates, injury from intra-cellular freezing was not a major problem in the freezing tests.

In our study the time from needle collection till freeze testing was usually less than 14 h. Except for one hour at room temperature the needles were stored in darkness at +6° C during this time. DEHAYES *et al.* (1990b) found no dehardening of excised shoots of red spruce stored at +4° C for 72 hours during winter, and we did not find that the winter cold hardiness of detached Scots pine needles was affected by up to one week of storage at +5° C compared to exposure of intact needles to outdoor temperatures varying between of 0° C and –5° C (unpublished study). This indicates that the management of needles from collection till freeze testing did not severely affect the critical temperatures in our study.

The environmental conditions after exposure to harmful temperatures may influence the level of freezing injury (LUNDMARK and HÄLLGREN, 1987), and consequently the levels of critical temperatures. The conclusion about clonal variation in cold tolerance presented here are based on the assumption that clone by post-freezing environment interactions are negligible.

The estimated critical temperatures are representative only of experiments performed as in our study and must not be considered the temperature at which plants and trees are killed in nature. Furthermore, cold tolerance varies between different types of tissue like stem, needle and bud (GLERUM, 1973; LARCHER, 1973; BURR *et al.*, 1990), between similar tissue located on different parts of a plant (TIMMIS and WORRAL, 1974), between needles of different age (DEHAYES *et al.*, 1990a), and between detached tissue and intact trees (GLERUM, 1973, 1976). As in our study, the clinal variation in injury after artificial needle freeze testing of Scots pine and lodgepole pine provenances in late summer and autumn (LINDGREN and NILSSON, 1992; NILSSON and WALFRIDSSON, 1993) is very similar to the clinal covariation of field mortality with latitude of origin observed in Swedish provenance field experiments (EICHE, 1966; REMRÖD, 1976; ERIKSSON *et al.*, 1980; PERSSON and STÅHL, 1990; PERSSON, 1994). Differences in needle cold tolerance in late summer and early autumn indicate differences in the phenological rhythm during the shortening photoperiod in autumn that are of importance for the survival of Scots pine in north Sweden. Although different from our tests in rate of cooling, time at the minimum temperature, tested tissue (shoots), method to assess freezing injury and other factors, results on Scots pine in Finland (REPO, 1992) agree well with ours as concerns the timing of seasonal changes in autumn and spring (winter hardiness was not studied by REPO). The method of freeze testing detached tissue to study genetic variation in cold tolerance during cold acclimation and deacclimation, therefore appears quite robust.

In our study large residual variances were found on the first freezing occasion in mid August 1991 and the last test occasion in late May 1992 (94% and 85%, respectively, of the phenotypic variance), compared to 3% to 2% in the autumn and usually 40% to 50% of the phenotypic variance in the winter and spring. Because so many factors vary between test occasions (e.g. weather conditions, test temperatures, latitudinal effects on cold tolerance) we can not conclude whether the large sum-

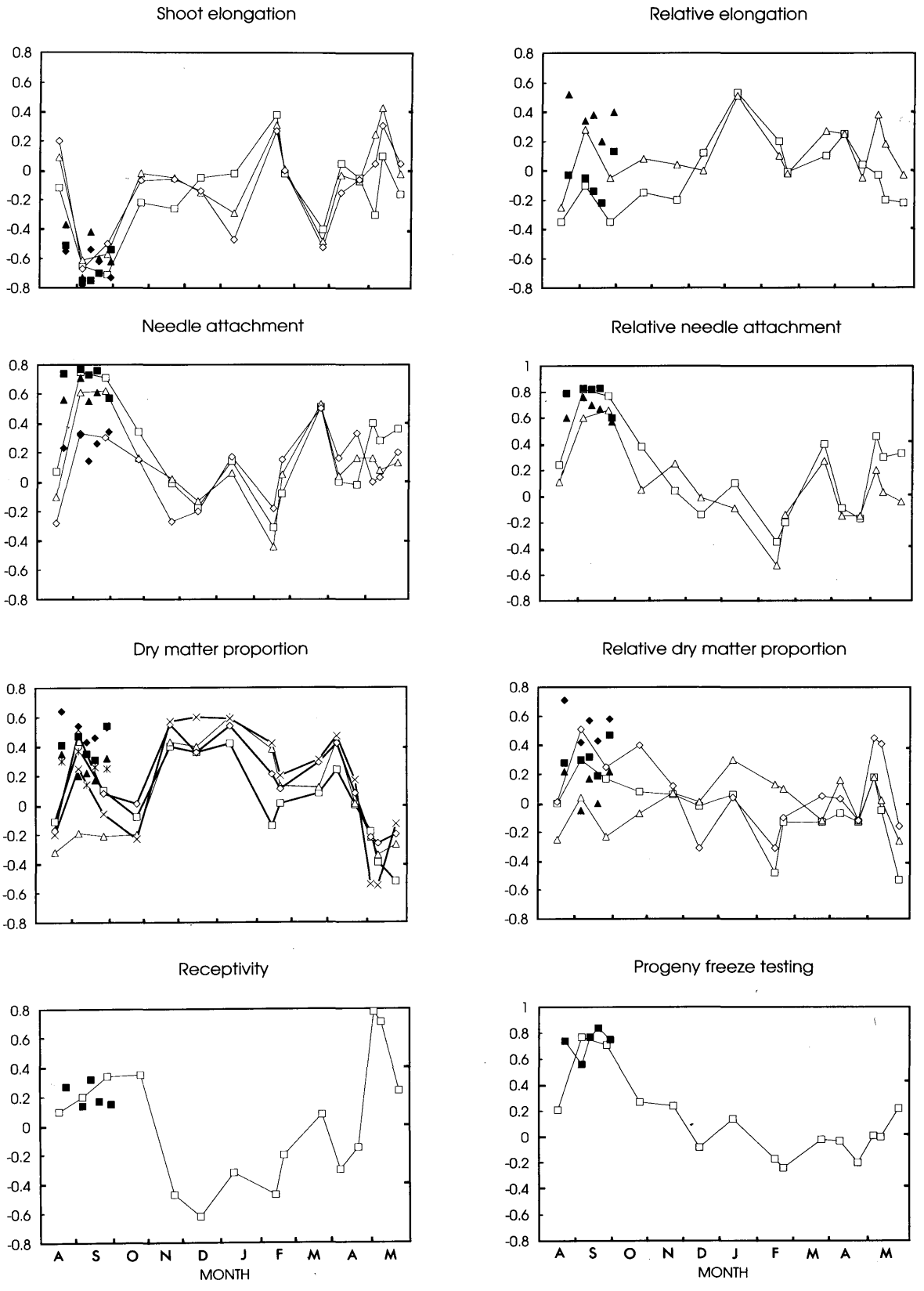

Figure 3. – PEARSON correlations between needle cold tolerance (proportion of slightly injured needles at the test temperature indicated in *Table 2*) and other phenological traits and progeny cold hardiness in early autum as assessed from artifical whole-plant freeze testing based on the n = 12 northern and central clones. Positive correlations indicate that high needle cold tolerance is related to an early rhythm of the compared phenological trait. The following labels show correlations calculated for different test occasions. Shoot elongation: □ early June, △ late June, ◇ September; Needle attachment: □ late July, △ early August, ◇ mid August; Dry matter proportion: □ mid August, △ early September, ◇ late September, × mid October. Filled and open labels indicate needle freezing tests in 1990 and 1991 to 1992, respectively.

mer residuals indicate less reliability of the frezing tests in the summer, or show real variation between grafts indicating that the cold tolerance of Scots pine is more sensitive to microenvironmental variation in the summer than at other times of the year.

High correlations between visible needle injury and chlorophyll a fluorescence in the autumn (LINDGREN and HÄLLGREN, 1993) and between visual needle injury and whole plant freezing test using either visual scoring or specific impedance data (PULKKINEN and AHO, 1992) indicate high reliability of the utilized method for study of provenance variation in cold tolerance of Scots pine during autumn cold acclimation. However, in the winter discolouration of injured needle tissue was usually not as clearly visible and the time till assessment of injury considerbly longer than in the autumn, late spring and early summer. Furthermore, freeze testing was restricted to temperatures above −75° C, which was not low enough to cause discolouration of the most hardy clones in some winter tests. These factors may have affected the magnitude of the observed short-term fluctuations in winter hardiness of individual clones. However, although the chlorophyll fluorescence was measured for only a minor part of the needle tissue compared to all tissue for the discolouration assessments, high correlations ($r>0.70$) were obtained between $CT_{50/20}$ and CT_{FL} on most test occasions in winter and spring 1992. Only when we failed to select a proper range of test temperatures (on 23 March and 11 May) were the correlations nonsignificant. This indicates that artificial needle freeze testing, using either visual scoring or clorophyll flrorescence, is useful in studies of clonal variation in needle cold tolerance during winter, spring and early summer as well as in the autumn. To what extent differences in needle cold tolerance indicate differences in susceptibility of Scots pine to weather damage during winter and spring should, however, be more carefully studied.

Seasonal changes

The freeze test results indicate that recessions in the cold acclimation process of Scots pine populations do normally not occur in the autumn. This is supported by an unpublished study where we found that high temperatures (+15° C) for three weeks from late September until the middle of October slowed down but did not prevent further cold acclimation. During deacclimation REPO (1991) found that artificially grown Scots pine seedlings can regain cold hardiness in low temperature conditions. His observation was supported by a slight rehardening (<3° C) of the most dehardened clones in our study, following a short period of colder weather in early May.

In mid winter the critical temperatures were, except for 2 clones, always better than −60° C. However, on 23 March a considerable dehardening ($CT_{50/20}$ higher than −40° C) was observed for all clones. It can, therefore, not be excluded that naturally occuring mild winter periods may cause a dehardening that can induce needle frost injury, repairable or irrepairable, to Scots pine in Sweden.

The timing of rapid deacclimation in spring, which occurred when the daily mean temperature was approximately +5° C, agrees with observations on Scots pine in Finland (KOSKI, 1985; REPO, 1992). However, the deacclimation rate (° C $week^{-1}$) was higher in our study, possibly because of different test methods.

Different adaptive strategies for cold acclimation, winter cold hardiness and deacclimation were demonstrated by two of the northernmost clones. One clone was the least cold tolerant of all clones studied in the autumn and early summer but among the most cold tolerant in the winter. The other clone was the opposite with the poorest winter cold tolerance but best cold tolerance of all clones in the autumn and early summer. The early cold acclimating clone was selected on a very harsh locailty with a survial rate of only 5% after 20 years as compared to 80% survival where the clone with late cold acclimation was selected. It is possible that the clone differences are indications on different adaptive strategies on harsh and mild localities.

One of the few survivors in the harshest field trial showed a cold acclimation rate from August till late September that was similar to that for the most southern clones. The slow cold acclimation was confirmed in the progeny freezing tests indicating that late cold acclimation in the autumn needs not be critical on the individual level. However, it can not be excluded that a genotype with late cold acclimation on a mild locality (the phenological observations were made in mild climate near the coast) can have an early cold acclimation rhythm, relative other genotypes, on a harsher locality (the northern field trial) although NILSSON *et al.* (1991) did not find any family by site interaction for the rate of first-year cold acclimation of Scots pine.

Correlations between phenological traits

The relationships between needle cold tolerance and other phenological traits were based on clones from only the northern and central populations, between which the differences in needle cold tolerance were nonsignificant in all freezing tests. Furthermore, partial correlations between pairs of variables, controlling for the latitude of origin, were of the same magnitude as the Pearson correlations. This indicates that relationships between needle cold tolerance and other traits were unaffected by the difference in latitude of origin between the 2 plustree populations.

Highly significant influence of latitudinal origin on needle cold tolerance in late summer and autumn (based on all 3 populations), and nonsignificant influence of latitude from winter to early summer suggest that the natural selection in the field trials was more directed towards early cold acclimation in the autumn than towards cold hardiness at other times of the year. However, changes in needle cold tolerance in early summer were measured for needles produced the previous summer, and may not be the best indication on genetic variation in phenological rhythm during early summer. A more reliable measure of early summer phenology is probably the shoot elongation rhythm itself, which in our study was uncorrelated with the cold tolerance of last-year's needles.

Nonsignificant correlations between winter cold tolerance and autumn cold tolerance indicate that the genetic control of cold hardiness varies over the year. Significant correlations between needle cold tolerance and flowering phenology in June, needle attachment in late summer and dry matter proportion in the autumn suggest that different phenological traits in the autumn are regulated by partly the same genes.

Implications for selection and breeding

Some conclusions concerning selection strategy for vegetative propagation and breeding of Scots pine for phenological adaptation on northern latitudes are suggested. Phenotypic plustree selection on northern localities indirectly increases the rate of autumn cold acclimation as compared to plustree selection on more southern localities, but has no effect on winter cold hardiness and rate of deacclimation in spring. Significant clone effects on the rate of autumn cold acclimation *(Table 2)* indicate that phenotypic within-population selection based on artificial freeze testing of detached needles in the autumn can further improve the rate of cold acclimation.

Positive correlations between needle cold tolerance and progeny cold tolerance in artificial freezing tests during early autumn found in this study, and between rate of cold acclimation in whole plant freezing tests and survival in field experiments of the same families (NILSSON and ANDERSSON, 1987; NILSSON *et al.,* 1991) indicate that phenotypic selection based on needle freeze testing in early autumn also has positive effects on field survival on northern latitudes, not only for vegetatively propagated trees but also for plustree progenies of Scots pine. In breeding programs for Scots pine selection for high needle cold tolerance in the autumn should be especially useful when sparse flowering is an obstacle for progeny testing, which is often the case on northern localities and in young populations. Needle freeze testing must, however, not replace progeny testing in breeding programs aimed at high survival, but rather be used to screen for the most promising candidates.

High correlations with needle cold tolerance *(Fig. 3)* indicate that positive effects on survival can also be obtained from selecting plustrees with high needle attachment in late summer or high dry matter proportions in needles in the autumn. Negative correlations between height growth and needle cold tolerance in early autumn indicate that early cold acclimation and increased survival are not easily combined with maximum growth. In programs for breeding and vegetative propagation aiming at high volume production on northern latitudes artificial needle freeze testing to increase survival must, therefore, be accomplished by other tests to avoid undesired negative selection for growth.

Naturally occurring low temperatures are usually considered no problem for winter hardy Scots pine. Our results, however, indicate that mild winter periods might decrease the cold tolerance to levels where naturally occurring fluctuations in air temperature may be crucial. Because there were no differences in winter cold tolerance between clones from various latitudes, and all clones studied showed a drastic loss in cold tolerance in response to high winter temperatures, neither provenance transfer nor breeding or vegetative propagation seem to be profitable ways to eliminate the risk of considerable dehardening of Scots pine plustrees during warm winter periods.

It is likely that the positive effect on autumn cold hardiness obtained from the natural selection taking place in the field trials during the first 20 years before our phenotypic plustree selection was more effective in the northern and central populations than in the southern one. This probably implied an indirect negative effect on growth that was stronger on the northern than on the southern localities. However, the effects of the phenotypic plustree selection for growth and tree quality on e.g. hardiness traits are uncertain. The results and conclusions presented here should, therefore, be limited to young plustree populations that have been exposed to natural selection for a number of years. More general conclusions can not be made until similar phenological studies have been made on populations exposed to other selection pressures and other climate and weather conditions than here.

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Multipurpose Gene Conservation in *Quercus suber* – a Portuguese Example

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Summary

Quercus suber, the native forest tree of highest economic value to Portugal is continuously declining. Creating good conditions for future evolution is the most important gene conservation objective for *Quercus suber.* A simple breeding programme to improve the production of good cork is also included in the gene conservation objective. Gene conservation of species accompanying *Quercus suber,* or even dependent on *Quercus suber,* is a third objective. The suggested sampling of gene resource populations is mainly based on climatic, soil and management conditions of *Quercus suber* populations. Active selection of trees with good cork in small multiple populations growing over a broad array of site conditions is suggested to match the joint evolutionary and breeding objective in gene conservation. Management of large natural populations to create maximum habitat diversity is suggested to take care of the gene conservation of accompanying species. Knowledge of the genetic structure of *Quercus suber* and of gene flow is urgently needed and ought to be given priority in genetic research.

Key words: Quercus suber, cork oak, gene conservation objectives and methods, research needs.

FDC: 165.3; 165.4; 176.1 *Quercus suber;* (469).

Introduction

Quercus suber is the native forest tree of highest economic value to Portugal. The value of the export of cork amounts to approximately 200 million US $ per year (SANTOS and MARTINS, 1993).

The actual range of the species is restricted to the western part of the Mediterranean area, in which the effect of summer drought is ameliorated by the wet Atlantic winds. The northernmost populations are found in southern France. The key climatic factors for the geographical limits of the species are essentially the annual precipitation and the winter temperatures whereas the species is not demanding with respect to soil conditions. The range of the annual precipitation of the areas in which *Quercus suber* is growing varies between 400 mm and 2,500 mm although always with dry summers.

According to NATIVIDADE (1950) and TEIXEIRA and PAIS (1976) palaeobotanical data are insufficient to get a reliable picture of *Quercus suber* distribution during the Cenozoic or even during the beginning of the Quaternary when the Mediterranean region acquired its present orographic profile. Together with other species in the same genus it dominated the native forests in Portugal (NATIVIDADE, 1950; TEIXEIRA and PAIS, 1976). The prominent role of the *Quercus* genus is reflected in the ecological zonation of Portugal (ALBUQUERQUE, 1954).

Even if Portugal was outside the zone of ice cover during the glaciations, the climate turned cooler and became harsh to *Quercus suber* and other evergreen oak species. The compensatory effect of water and thermic balance on southern slopes with calcareous soil provided the microclimatic conditions under which *Quercus suber* could survive. From such refuges the species migrated and formed larger populations. During the xerothermic interglacial periods northern slopes housed the *Quercus suber* populations. As the climate turned cooler new migrations took place again. Today isolated populations are still to be found in spite of strong impact from anthropogenic factors. These vestiges of passed epochs reveal that the species is well able to cope with changed climatic conditions. Present observations lend further support for this. Whenever the human pressure is relaxed *Quercus suber* colonizes the freed areas within a few decades. This must be attributed to large genetic variation in adaptive traits and to its reproductive ability. This is certainly of great value for enabling the species to cope with the rapid change of the environmental conditions taking place today. However, it should be emphasized that the speed of the change now is higher than ever before (DAVIS and ZABINSKI, 1992).

In spite of a stable area of approximately 660 000 hectares (ANONYMOUS, 1993) there has been a decline in *Quercus suber* owing to lower stocking accompanied by reduced production of high quality cork (CABRAL and SARDINHA, 1993). Climatic factors, pests and diseases as well as mismanagement were among the factors of greatest importance for the decline of *Quercus suber* during the recent decades. CABRAL and SARDINHA (1993) noted a relationship between extended droughts and

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