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One and two-year-old Scots pine (*Pinus sylvestris L*) seedlings from various origins

The effect of various growing conditions on winter hardening

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THESIS ABSTRACT

Faculty: Agriculture and forestry
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Title of thesis: One and two-year-old Scots pine (*Pinus sylvestris L*) seedlings from variable origins
- The effect of variable growing conditions on winter hardening

Supervisors: PhD Niina Stenvall, Metla and MSc, forestry Ossi Vuori, Seamk

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The aim of the thesis was to study the effects of different growing conditions on the winter hardening development of one and two-year-old Scots pine seedlings. This thesis presents the results of survival rate, height growth, time of bud formation, autumn colouration and primary and secondary needle damage. The seed material used in this study was collected from 48 different origins, from Northern Finland to Southern Poland. The seedlings were grown in various conditions, where the growing temperatures and watering treatments were controlled to follow the newest estimations of climate change for years 2100 and 2030. Height growth of the seedlings increased little in the condition 2100. Common freezing testing was used to test the ability of winter hardiness of the seedlings. The highest needle damage was found in 2100 condition with low watering treatment. Foreign origins suffered a lot higher needle damage than the native origins of Finland. It was noted that even if the growing season will lengthen, the benefit of it will be lost, since the day length will stay the same. The results of this study supports the theory that climate warming will increase the risk of frost damage to Scots pine seedlings.

Keywords: climate change, winter hardening, needle damage, freezing testing, *Pinus sylvestris* , Scots pine

SEINÄJOEN AMMATTIKORKEAKOULU

OPINNÄYTETYÖN TIIVISTELMÄ

Koulutusyksikkö: Maa- ja metsätalouden yksikkö
Koulutusohjelma: Metsätalouden koulutusohjelma
Suuntautumisvaihtoehto: Metsätaloustuotanto

Tekijä: Mari Oja

Työn nimi: Eri alkuperää olevien yksi- ja kaksivuotiaiden männyn taimien talveentuminen erilaisissa kasvatusolosuhteissa.

Ohjaajat: FT Niina Stenvall, Metla ja MH Ossi Vuori, Seamk

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Työn tarkoitus oli tutkia erilaisten kasvatusolosuhteiden vaikutusta yksi- ja kaksivuotiaiden männyn taimien pakkasen kestävyyteen. Työssä esitetään tutkimustuloksia olosuhteiden vaikutuksesta taimien kuolleisuuteen, pituuskasvuun, päätesilmun muodostumisen ajankohtaan, syysväritykseen sekä primääri- ja sekundaarineulasten hallavaurioihin. Kokeessa käytetyt siemenet oli kerätty 48 eri paikasta. Siemenalkuperiä oli Pohjois-Suomesta aina eteläisimpään Puolaan. Taimet kasvatettiin erilaisissa kasvatusolosuhteissa, joissa kasvatuslämpötilat ja kastelut oli asetettu mukailemaan uusimpien ilmastonmuutos skenaarioiden malleja vuosille 2100 ja 2030. Taimien pakkaskestävyyttä testattiin yleisesti käytössä olevin pakastustestauksin. Suurimmat neulasvauriot olivat taimilla, jotka kasvoivat olosuhteessa 2100 ja joiden kastelu oli vähäinen. Ulkomaista alkuperää olevat taimet kärsivät huomattavia neulasvaurioita kotimaisiin alkuperiin verrattuna. Työssä ilmenee, että vaikka kasvukausi pidentyisi ilmastonmuutoksen seurauksena, siitä saatu hyöty menetetään hallavaurioiden lisäännyttyä. Työn tulokset tukevat sitä teoriaa, että ilmastonmuutos lisää männyn taimien hallavaurioita.

Avainsanat: Ilmaston muutos, pakkasen kestävyys, hallavauriot, pakastustestaus, *Pinus sylvestris,* Mänty

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1 INTRODUCTION

The global climate is changing rapidly as a result of human activity (IPCC 2007 36-37) According to the most recent estimates the mean temperature in Finland will rise 2-6° degrees Celsius and precipitation may increase 10-40% by the end of the century when compared to the period between 1961-1990. These changes will mainly have an effect on the winter seasons, but there will also be some noticeable changes throughout the year. (Jylhä, et al.,2009 11-12) The changes will appear as increased temperatures and especially rapid changes of temperatures within short time periods. In winter time there will be sudden, noticeably warmer periods in between cold periods (IPCC 2007 30-33). This kind of extreme weather phenomenon will become more common throughout the year (Jylhä, K. et al.,2009 29-65). The frost danger will also become more common; not only during the autumn and spring time, but also in the winter time (Hänninen, H. 2006 895-897) The first studies into this topic were made by Cannel in 1985. An increase in precipitation may also increase the risk of frost damage, especially for seedlings, if the majority of the rain falls as water during the winter, the snow cover that usually protects the seedlings against frost won't exist. Some regional problems may occur due to the lack of rainfall during the growing season, (Jylhä, K. et al 2009 31-33) and these changes will have a definite effect on the forests (Kellomäki, S. Peltola, H. Nuutinen, T, Korhonen, K & Strandman, H. 2007 2345-2350) especially the speed at which the changes take place will have a huge impact on the trees (Kellomäki, S. et al.,2007 2345-2350) and because the trees being planted now will live for a long time, we need to start thinking about the possible consequences of the climate changes well beforehand.

The survival of the tree is based on it's ability to adapt to its environment even under unfavourable conditions (Survival adaptation) and on it's ability to use it's surroundings effectively so that it will not be destroyed by biotic competition (capacity adaptation). As trees cannot move and take cover from snow during the coldest months they have to be cold resistant. (Hänninen, H 1988 3-5). Trees are also liable to other stress factors such as: diseases, pests, grazing, rodents and drought (Mirov, N.T 1967 442-444). Trees can be weakened by these factors and after being damaged, their stress tolerance lowers. These are not necessarily instant threats to a tree's survival but can be fatal in the long run. (Mirov, N.T 1967

442-444) Those trees with the highest stress tolerance will outlive the weak ones and will be able to breed. This means that the annual cycle of the tree must be well abated for the changing weather conditions of its surroundings. The basic aim of survival is not only to stay alive, but to keep on growing and to produce more trees that are able to survive and breed. (Hänninen, H. 1988 3-5)

Scots pine (*Pinus sylvestris L.*) is the most widely distributed tree of the *Pinus*-family (Mirov, N.T 1967. 235-238). It grows in most parts of Europe and Siberia. (Sarvas, R. 1964. 368). It is the only native member of the *Pinus*-family in Finland (Mirov, N.T. 1967. 235-238) Scots pine is the most common tree species in Finland and it is very important for the Finnish forestry industry. Scots pine is mainly used for sawn timber and in pulp production. Scots pine forms either pure pine forests or mixed forests with other tree species. (Valkonen, S. 2008. 134) Scots pine demands a lot of light, but it has very low demands of it's soil nutrients. It usually grows in oligotrophic or mesotrophic soils. It is a pioneer tree species and because of it's thick bark it is known to be very resistant to fire and therefore it will be the first conifer to reproduce in burned areas. (Sarvas, R. 1964. 368-380) It has a reasonably low competitive capacity, but due to its high tolerance to different stress factors it is widely distributed (Mirov, N.T.1967.426-430). Scots pine is a wind pollinating tree, the amount of pollen produced is normally high and spreads widely. The development of the seed is a slow process with Scots pine, after flowering it takes two years before the seed has fully developed and is ready to fall out from its cone. Scots pine needles stay on the tree for approximately three years. (Sarvas, R. 1964. 368-380)
In it's first year of growth it forms only primary needles, unlike the typical needles of the *Pinus*-family, they are one pointed needles with slightly saw-toothed edges (Sarvas, R. 1964. 368-380) These needles appear very shortly after germination (Mirov, N.T. 1967. 370-372). Normally during it's second year of life it begins to form secondary needles, these are in the form of pairs (pair of needles), the type normally seen on the Scots pine. (Sarvas, R. 1964 368-380).
Scots pine has adapted well to the challenging living conditions of the boreal zone, due to its annual cycle. An annual cycle is a chain of differing stages in the physiology of the trees which repeat around the same time each year. (Hänninen, H. 1988.17-32) This annual cycle is controlled by the surrounding temperatures

and by the amount of daylight, so it is important to notice that even though the temperatures change, the amount of daylight during each season remains the same.

The hardening development is considered as a base, where winter hardiness synchronizes with changes in the environmental conditions (Howell, G.S & Weiser, C.J 1970. 390). Winter hardening development takes place in autumn and it is triggered by the shortening of the photoperiod together with lower temperatures (Aronsson, A, 1975. 15-16). The winter hardening process prepares trees for the up coming low temperatures by causing some changes in the plants tissue cell biology. Winter hardiness is a quality of each individual plant and it's governed by genetics or by it's surrounding environment. (Beck, E.H. Heim, R & Hansen, J. 2004. 452-455) The winter hardiness provides seedlings with the ability to survive cold winters (Glerum, C. 1985. 108). Trees of the boreal zone can go into a frost resistant stage called dormancy, to reach this stage, the growth must have stopped completely and the winter hardiness process must have been completed (Hänninen, H. 1988. 13-17). Dormancy is a resting state in which the metabolism has slowed down and the growth has stopped (Lang, A.G. 1987 818-820). The formation of the terminal bud on Scots pine takes place in the autumn and it indicates the end of the growth. The terminal bud remains inactive over the winter and will become active again in the spring. (Mirov, N.T. 1967.404-410) To be able to activate again it demands a colder period in between it's formation and dehardening (Hänninen, H. 1988. 11-17). It has also been thought that the autumn colour of the needles could be an indicator for the cold hardening state of young seedlings. On some one-year-old pine seedlings, the colour of the needles turns to red in late summer or in the autumn time. It is not known for sure why this happens, but there are no studied facts that the change of colour indicates a better cold hardening ability (Toivonen, A .1987. 58-64). In full dormant state, seedlings can cope well without suffering any needle damage, even in temperatures below -70° (Leinonen, I. Repo, T. & Hänninen, H. 1996. 136). When the temperature begins to rise and the amount of daylight begins to increase the dehardening stage of the annual cycle will take place. Dehardening is the stage when the tree begins it's new growth after dormancy. It is known that the most important factor to trigger dehardening is rising temperatures (Aronsson, A, 1975. 5).

The survival of Scots pine can be threatened by changes in climate (Hänninen, H. 1991.451-454). A warming climate will increase the risk of frost damage in the boreal zone. (Hänninen, H. 1991 451-454;2005 124-127). Higher temperatures in late autumn will delay the winter hardening process of the seedlings. If the development of the winter hardiness process is disturbed, it can have an effect on how deep in to the winter hardening state the seedling will go. (Hänninen, H. 1991. 451-454) If during the dormancy state, temperatures stay above 0° for long enough, dormancy breakdown can occur and the seedling will begin it's growth in mid-winter. (Hänninen, H. 2005. 124-127). Normally these warmer periods are followed by periods with very low temperatures and if the seedling growth has begun, it will be exposed to frost damage (Hänninen, H. 1991.451-454; 2005.124-127). The beginning of new growth will also be disturbed by climate change (Hänninen, H. 2005.124-127). The earlier spring time will cause premature dehardening. This will also expose the seedlings to the threat of frost damage in the spring time (Hänninen, H. Leinonen, I, Repo, T & Kellomäki, S. 1996.233-236). The lengthening of the growing season may increase the growth (Kellomäki, S. & Kolström, M. 1994 212-216), but since the amount of daylight will stay the same there might not be enough light for effective photosynthesis or for effective growth. As a result, the benefit of a longer growing season may be lost due the increasing of frost damage during autumn and spring (Hänninen, H. 2005.124-127).

The Finnish Forest Research Institute is running a project into the effects of climate change during the early years of growth and into the breeding of Finnish forest trees. The research project is called Forests 2050 and began in 2007 and will carry on until 2011. The main idea of the research is to find out if there is enough variations within Finnish forests to adapt to forthcoming climate changes. The aim is also to find the most important factors that support the adaptation of trees, and how to choose the best possible trees for the future with regards to these changes. This research is concentrated on the main tree species: Scots pine (*Pinus sylvestris*), Norway spruce *(Picea abies*), Silver and Pubescent birch (*Betula pendula* and *Betula pubescent*) and the European aspen (*Populus tremula*). Included in the study are some less common tree species: Common oak

(*Quercus robur*) and the Small-leaved lime (*Tilia cordata*). The test material is collected form several different locations from the northernmost part of Finland to the southern Poland. In this project they are also trying to develop suitable methods for pre-testing the seed material before using it for forest breeding. The research is based in Haapastensyrjä´s breeding station in the municipality of Loppi.

Up to 40 000 seedlings are raised each year for the project, the seedlings are raised in controlled conditions inside greenhouses or in natural conditions out in the field. This report is a part of the project and it will concentrate on analyzing the frost damage of one and two year old Scots pine seedlings (Frozen at a temperature of -10°C). These tests were carried out in 2008 and 2009. The freezing test is a commonly used method to test the hardening development of the seedlings. It is known to be a reliable method to test the winter hardiness ability of seed materials (Nilsson, J-E. and Eriksson, G. 1986). The seedlings are grown in controlled light and heat conditions so that the over wintering process proceeds smoothly and under control, this ensures that seedlings with differences in the winter hardening process stand out. The conditions are intended to represent the average conditions of the region the seed material is being tested for. (Aho, M-L. & Pulkkinen, P. 1993. 5-9). This method is commonly used to test the utilization areas of commercial seed material.

The aim of this study is to find out how the growth conditions of Scots pine seedlings affect the winter hardening process. Within the given growing conditions there were different water treatments to find out the effect of watering on the winter hardening process of the seedlings. Another aim of this study is to compare seedlings from different origins, to see how they act in various growing conditions, how the various watering treatments affect them and what effect these factors have on their winter hardening process.

In this study one-year-old and two-year-old seedlings are compared to each other, to find out how they react in the autumn and if it's possible to predict from the one-year-old seedlings how they will react as two-year-old seedlings.

2 MATERIAL AND METHODS

2.1 Material

The Scots pine seedlings used in this study were sown in 2008 at Haapastensyrjä´s breeding station. The seeds for the study were collected from several places; all together there were 48 different origins. 27 of these seed origins were collected from Finland and 14 of them originated from Sweden, Denmark, Estonia, Latvia, Lithuania and Poland (Appendix 1). The seeds were collected from forests, seed orchards and some of the seeds were high breeding quality seeds selected from the Scots pine breeding program (class number 6, Appendix 1).

2.2 Layout of the study

The seeds were sown in trays (40cm by 60cm) filled with peat (N:P:K 14:4:2, Kekkilä Oy, Finland). The sowing took place during April and in the beginning of May. Each tray held 96 seeds in total, consisting of four seeds from each of the 24 different origins. They were laid out in 12 rows, with eight seeds from two origins in each row (Fig 1). The sown seeds were separated into three different growing conditions; one was to stay outside in the natural growing conditions and to be used as a control (Cumulative thermal sum 1244 d.d). The remaining two lots were grown in different departments of a greenhouse. One of the departments was set to follow the current mean temperatures of Loppi (Municipality in the province of Kanta-häme) to imitate the estimated growing conditions in the year 2030 (Cumulative thermal sum 1584 d.d). The other one was set to follow a temperature of +5°C above the current mean temperatures in Loppi to imitate the estimated growing conditions in the year 2100 (Cumulative thermal sum 2530 d.d). The experiment had two watering treatments; one half of the seedlings in the greenhouse conditions were grown with a low amount of watering and the other half with a high amount of watering (Fig 2). The watering conditions were to imitate low and high moisture conditions in the future. The low watering condition was half of the normal precipitation in Jokioinen (Municipality in the province of Kanta-häme) (175 mm/m^2/growing season) and the high condition was double the normal amount of Jokioinen (700 mm/m^2/growing season). The seedlings grown outside had natural precipitation (Fig 2). Due to the shade created by the walls and because of other factors caused by the structure of the greenhouse, the location of the tables was changed to minimize the variation in growth caused by these disadvantages. All seedlings had natural light conditions throughout the whole study. The seedlings stayed in their given conditions throughout the growing season until the freeze testing began. The two-year-olds used in this study were sown in spring 2008, and they too were grown in various conditions just as the one-year-olds had been. They spent their first winter in an over winter storage, typical of tree nurseries and as the spring came they were returned to the same conditions they were in during the previous growing season.

Origin 13	Origin 14	Origin 15	Origin 16	Origin 17	Origin18	Origin 19	Origin 20	Origin 21	Origin 22	Origin 23	Origin 24	Origin 37	Origin 38	Origin 39	Origin 40	Origin 41	Origin 42	Origin 43	Origin 44	Origin 45	Origin 46	Origin 47	Origin 48
8	16	24	32	40	48	56	64	72	80	88	96	8	16	24	32	40	48	56	64	72	80	88	96
7	15	23	31	39	47	55	63	71	79	87	95	7	15	23	31	39	47	55	63	71	79	87	95
6	14	22	30	38	46	54	62	70	78	86	94	6	14	22	30	38	46	54	62	70	78	86	94
5	13	21	29	37	45	53	61	69	77	85	93	5	13	21	29	37	45	53	61	69	77	85	93
4	12	20	28	36	44	52	60	68	76	84	92	4	12	20	28	36	44	52	60	68	76	84	92
3	11	19	27	35	43	51	59	67	75	83	91	3	11	19	27	35	43	51	59	67	75	83	91
2	10	18	26	34	42	50	58	66	74	82	90	2	10	18	26	34	42	50	58	66	74	82	90
1	9	17	25	33	41	49	57	65	73	81	89	1	9	17	25	33	41	49	57	65	73	81	89
Origin 1	Origin 2	Origin 3	Origin 4	Origin 5	Origin 6	Origin 7	Origin 8	Origin 9	Origin 10	Origin 11	Origin 12	Origin 25	Origin 26	Origin 27	Origin 28	Origin 29	Origin 30	Origin 31	Origin 32	Origin 33	Origin 34	Origin 35	Origin 36

Figure 1: The lay out of the origins on trays.

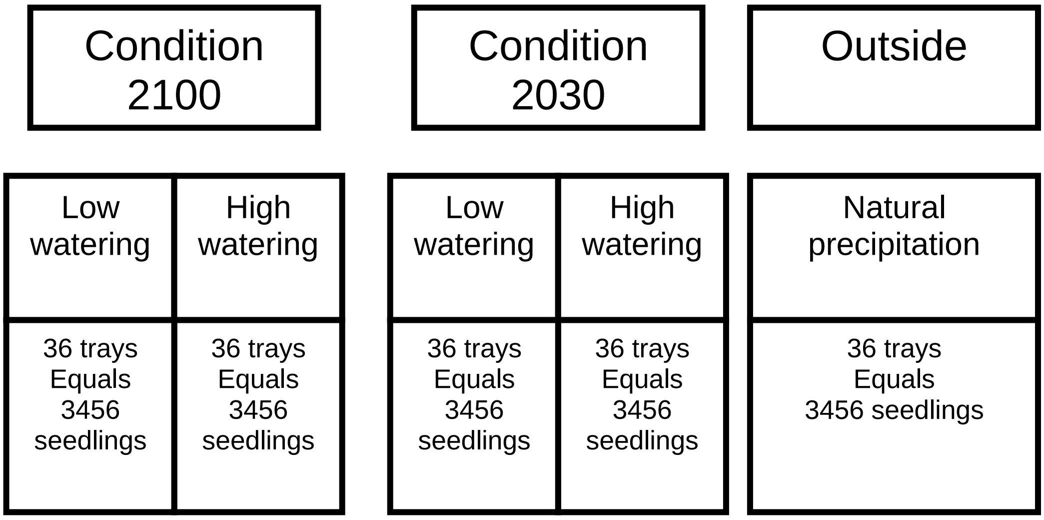

Figure 2: The study layout

2.3 Measurements and observations

The height of all of the seedlings was measured using a ruler from the base of the plant to the very top. The number of dead or non existing seedlings was also noted. The formation of the terminal bud on the one-year-old seedlings was observed weekly after week 37. The formation of the terminal bud was observed on all of the seedlings as one-year-old. The colour of the seedlings was visually classified in to three classes. Class number one indicates a red colour, class number two is for seedlings that have started to turn red, but still have some green needles and class number three is for the seedlings that were completely green.

2.4 Freezing tests

The freezing testing began in September and ended in of October 2009. The freezing tests (-10°C) were done for one replication from each condition per week. There were five different replications from the one-year-olds and five replications from the two-year-olds. Replication was taken in the freezing chamber during working hours (Fig. 3). The temperature of the chamber was set at +5°C. At 9.00 pm the temperatures began to drop at a rate of 3°C/h. By 2.00 am it reached the temperature of -10°C. The temperature remained at -10°C for two hours. From 4.00 am the temperatures began to rise again at 3°C/h and it reached +5°C again at 9.00 am. After spending the night in the chamber, the replication was moved back to its growing conditions. This was repeated for all of the replications

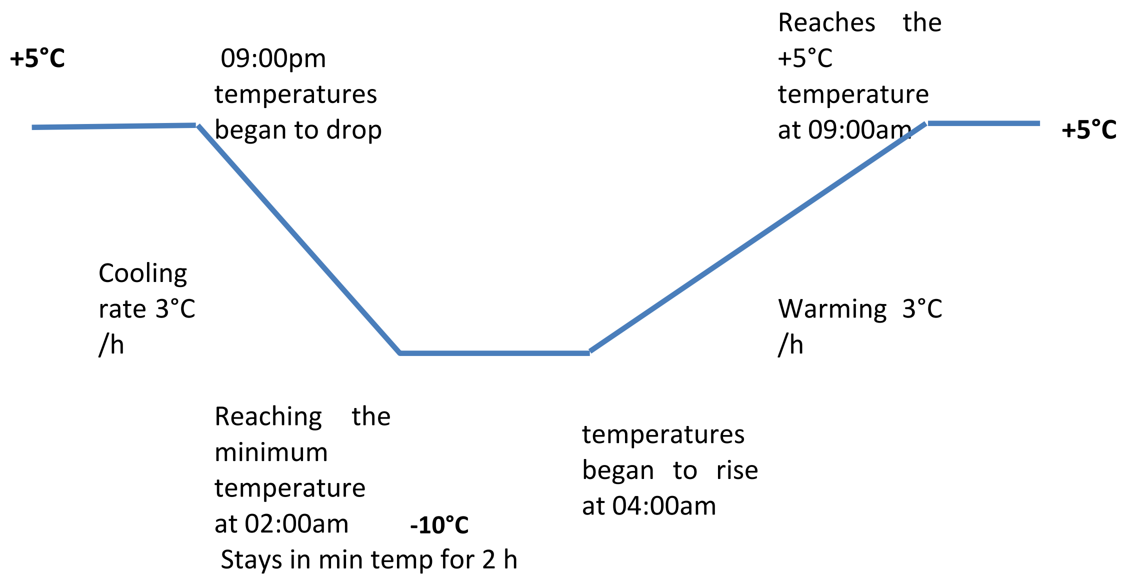

Figure 3: The cycle of freezing test

2.5 Classification of needle damage

The classification of the needle damage was done to each replication approximately two weeks after the freezing test. The damage was evaluated visually. The damage was observed for primary and secondary needles. A primary needle is a type of needle that a seedling forms during the first months of it's life, the difference to a normal needle is that it is only a one pointed needle. Pine trees form secondary needles (pair of needles) as they grow older, usually during their second growing season. Observations from both needle categories were classified in six classes: 1) 0%, 2) 1-19% 3) 20-39% 4) 40-59% 5) 60-79% 6) 80-100%.

2.6 Statistical analysis

The data was analysed using the SPAW Statistics package (version 17, SPSS Inc. 2009). Differences in height and needle damage of the one- and two-year old seedlings between origin class, growth conditions, time of the freezing tests and the water treatments were analysed with ANOVA (GLM) followed by Tukey´s post hoc test at a 5% risk level. Observations of height and needle damage were normally distributed. The following linear model explained the analysis of variance concerning height and needle damage of the seedlings: $X_{ijkl} = \mu + C_i + G_j + T_k + W_l + C_iG_j + C_iT_k + C_iW_l + G_jT_k + G_jW_l + T_kWl + C_iG_jT_k + e_{ijkl}$

Where μ is the overall mean, C_i is the effect of ${}_i$th class of origin, G_j is the effect of ${}_j$th growing condition, T_k is the effect of ${}_k$th time of freezing testing, W_l is the effect of ${}_l$th watering treatment, C_iG_j is the interaction of ${}_i$th class of origin and ${}_j$th growing condition, C_iT_k is the interaction of ${}_i$th class of origin and ${}_k$th time of freezing testing, C_iW_l is the interaction of ${}_i$th class of origin and ${}_l$th watering treatment, G_jW_l is the interaction of ${}_j$th growing condition and ${}_l$th watering treatment, T_kW_l is the interaction of ${}_k$th time of freezing testing and ${}_l$th watering treatment, $C_iG_jT_k$ is the interaction of ${}_i$th class of origin, ${}_j$th growing condition and ${}_k$th time of freezing testing and e_{ijkl} denotes the random error. The effect of water treatment and some of the interactions could not be determined with respect to the condition outside because there was only natural precipitation and no water treatments.

3 RESULTS

The number of seedlings in this study was 9600. Each of the growing conditions and watering treatments had 960 seedlings, for both one and two year-olds (Table 1). The amount of dead seedlings was quite even in all growth conditions. The highest death rate was with one-year-old seedlings grown outside (9.0%) and the lowest death rate was in the 2100 condition with high a watering treatment. (in one-year-old 2.1% and on two-year-old 2.3% seedlings) (Table1). With the two-year-olds some of the seedlings died during their second year of life. The highest death rate was in the 2100 condition with low watering treatment (1.6%) and the lowest death rate was in the 2030 condition with high watering treatment (0.3%). The total number of seedlings left for the freezing tests was 9078, according to the table 5.4% of the seedlings died at some point during the growing seasons.

Table 1: The death rate of the seedlings.

Age of the seedling (in years)	Growth condition	Water treatment	Original number of seedlings	Number of dead seedlings as a one year old	Dead seedlings as a one year old in %	Number of dead seedlings as a two year old	Dead seedlings as a two-year-old in %	Number of seedlings in freezing testing
1	Condition 2100	High	960	20	2.1			940
		Low	960	39	4.1			921
		Total	1920	59	3.1			1861
	Condition 2030	High	960	32	3.3			928
		Low	960	53	5.5			907
		Total	1920	85	4.4			1835
	Control	Natural	960	86	9.0			874
	Total		4800	230	4.8			4570
2	Condition 2100	High	960	22	2.3	12	1.3	926
		Low	960	38	4.0	15	1.6	907
		Total	1920	60	3.1	27	1.5	1833
	Condition 2030	High	960	56	5.8	3	0.3	901
		Low	960	64	6.7	13	1.5	883
		Total	1920	120	6.3	16	0.9	1784
	Control	Natural	960	55	5.7	14	1.5	891
	Total		4800	235	4.9	57	1.2	4508
Grand total			9600	465	4.8	57	0.6	9078

3.1 The height of the seedlings

The growth condition had a clear effect (F=1182.98; p<0.001) (Table 2a) on the height of the one-year-old seedlings (Fig. 4). The watering treatments also had a significant effect on the growth of the seedlings (F=618.15; p<0.001)(Table 2a). The seedlings grown in the 2100 condition with a high amount of watering were the tallest and the seedlings grown in natural conditions and with natural watering were the shortest. All together the seedlings grown in the 2100 condition were the tallest ones, even with the low watering treatment. The significant difference in height of the seedlings between the growth conditions (F=588.512; p<0.001) and watering treatments (F=89.851; p<0.001) (Table 2b) was visible after two years of growth (Fig. 5). The two-year-old seedlings grown in the condition 2100 and with high watering treatment were the tallest, as they had been as one-year-olds. Two-year-old seedlings grown outside were significantly shorter (the average height 70.4mm) than the ones grown in the warmer conditions (condition 2100 the average height 250.5mm and condition 2030 the average height 181.9mm). In low watering treatment the height difference between conditions 2100 and 2030 evened out for the two-year-old seedlings (Fig 5) compared to the one-year-olds (Fig 4).

Table 2a and b: Analysis of variance of effects of growing condition, origin class of the seedlings and the watering treatments and their interactions on the height of the seedlings at one-year-old (a) and at two-year-old (b)

(a)

Source	d.f	F	p
Growing condition	1	1182.984	0.000
Class of origin	5	94.245	0.000
Water treatment	1	618.151	0.000
G.condition*C. of origin	5	12.714	0.000
G.condition* W.treatment	1	2.958	0.086
C. of origin*W.treatment	5	3.820	0.002
G.condition*C.of origin*W.treatment	5	2.689	0.020

(b)

Source	d.f	F	p
Growing condition	1	588.512	0.000
Class of origin	5	21.256	0.000
Water treatment	1	89.851	0.000
G.condition*C. of origin	5	3.055	0.009
G.condition* W.treatment	1	454.564	0.000
C. of origin*W.treatment	5	2.194	0.052
G.condition*C.of origin*W.treatment	5	6.957	0.000

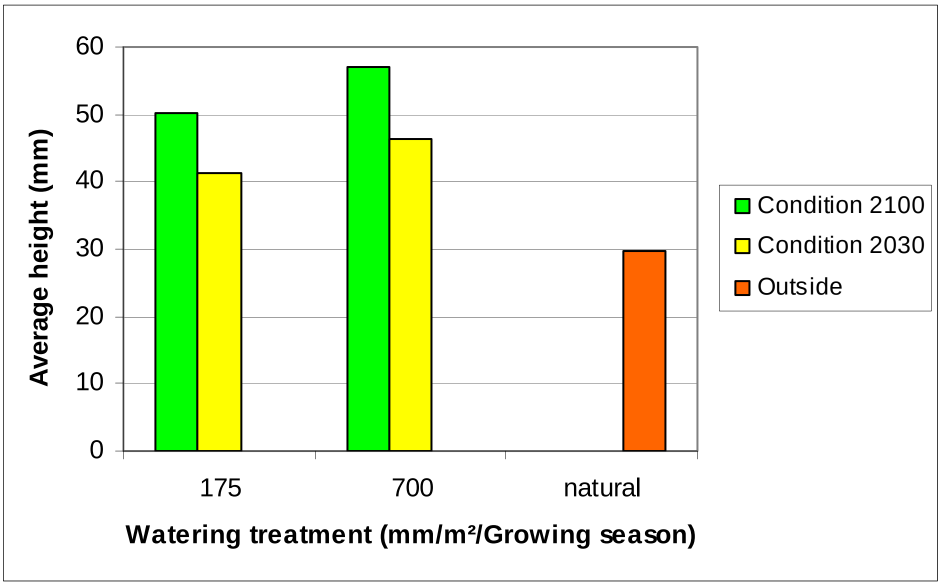

Figure 4: The effect of different watering treatments on the height of the seedlings in different growth conditions (Measured on week 27 in 2008 as one-year-old).

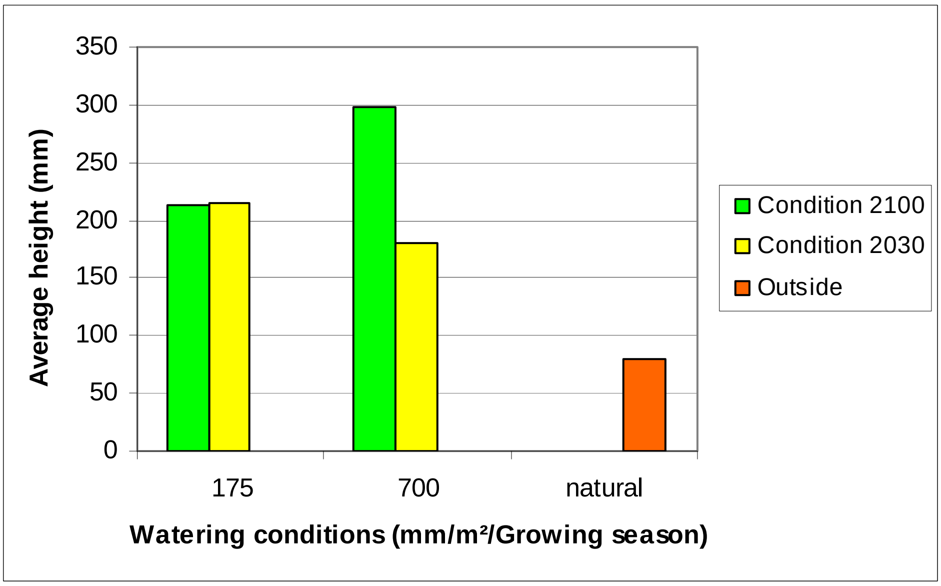

Figure 5: The effect of different watering treatments on the height of the seedlings in different growth conditions (measured before freezing in 2009 as two-year-olds).

There were differences in height ($F=94.245$, $p<0.001$) between origin classes of the one-year-old and two-year-old seedlings ($F=21.256$, $p<0.001$). In the one and two-year-old seedlings, the class 1 seedlings differed in height from all other classes significantly. In one-year-old seedlings, all origin classes, except class 2 and 3, differed significantly in height between each other. In two-year-old seedlings, the class 4 had a significant difference in height between class 3 and 6. The high quality breeding material (class 6) was the tallest in all growth conditions in both one- and two-year-olds (Fig. 6 and 7). The northernmost originated seedlings (class 1) were the shortest, except in one-year-old grown outside, where the seedlings from the classes 4 and 5 were the shortest (Fig. 3). In the two-year-olds the northernmost origins were significantly shorter than the other origins.

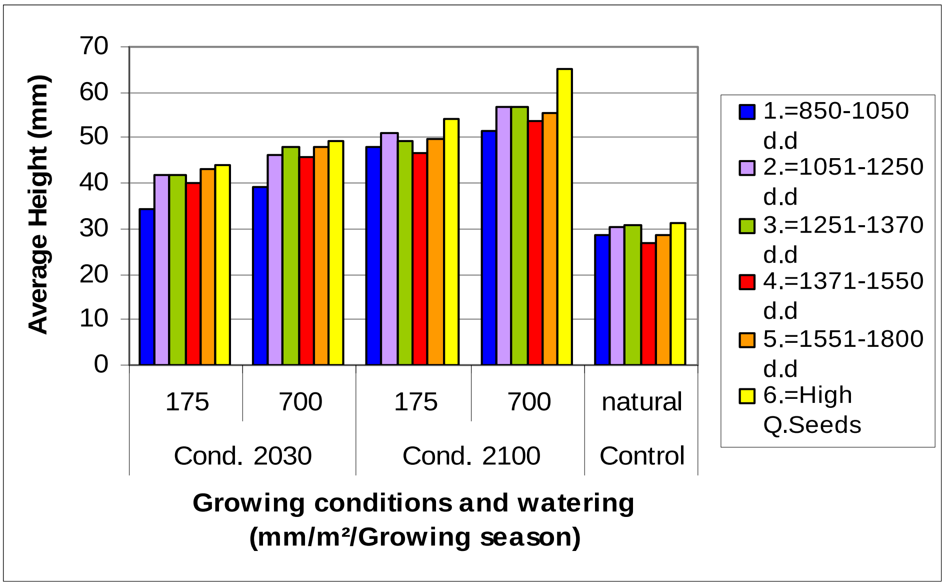

Figure 6: The effect of different growth conditions and watering treatments on the height of the seedlings within different origins (seedlings measured as one-year-olds in 2008).

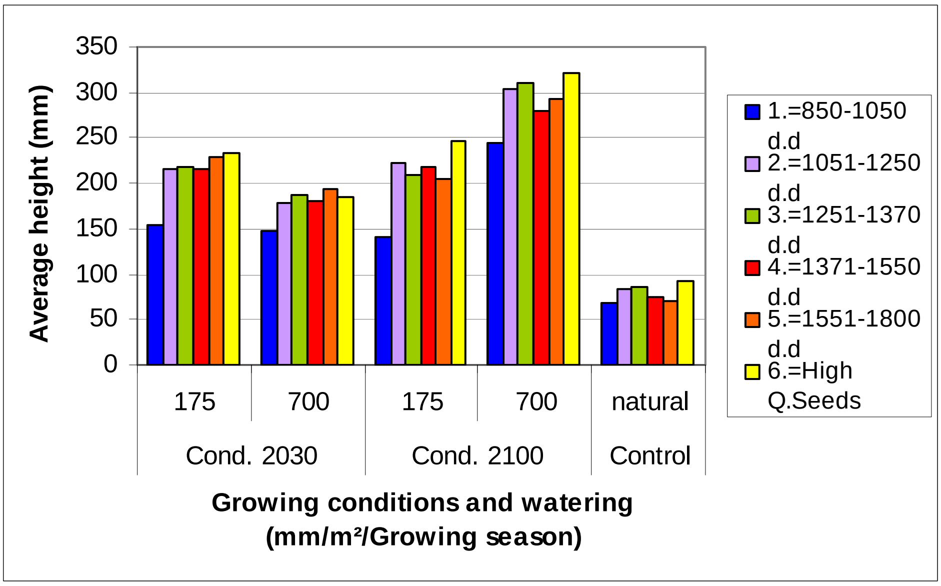

Figure 7: The effect of different growth conditions and watering treatments on the height of the seedlings within different origins (seedlings measured as two-year-olds in 2009).

3.2 Development of terminal buds

The growth condition (F=1380.266, p<0.001) (Table 3), class of origin (F=78.589. p<0.001) and watering treatment (F=104.918, p<0.001) had a significant effect on the formation of the terminal bud. In autumn, before the freezing tests 72.3% of the one-year-old seedlings in the condition 2100 a developed terminal bud. In condition 2030 62.6% of the seedlings and outside 21.1% developed terminal buds (Table 4a). In the condition 2100 and 2030 the northernmost origins developed terminal buds more often than the other origins (Table 4b). The difference in terminal bud formation between origins was more clear cut for the outside condition: 60.4% of the seedlings from the northern origins and only 1.2-4.9% of the foreign origins (class 4 and 5) developed terminal buds (Table 4b).

Table 3: Analysis of variance of effects of growing condition, origin class of the seedlings and the watering treatments and their interactions on the formation of the terminal bud of all one-year-old seedlings.

Source	d.f	F	p
Growing condition	1	1380.266	0.000
Class of origin	5	78.589	0.000
Water treatment	1	104.918	0.000
G.condition*C. of origin	5	131.722	0.000
G.condition* W.treatment	1	0.024	0.876
C. of origin*W.treatment	5	9.577	0.000
G.condition*C.of origin*W.treatment	5	4.34	0.001

Table 4a and b. The effect of different growing conditions and watering treatments on the formation of the terminal bud (a), the effect of growing condition and origin class on the formation of the terminal bud (b).

(a)

Growing condition	Water treatment	Development of terminal bud %
2100	Low	70.2
	High	74.3
	Average	72.3
2030	Low	65.4
	High	59.9
	Average	62.6
Outside	Natural	21.1
	Average	21.1

(b)

Growth condition	Origin class	Development of terminal bud %
2100	1	80.2
	2	75.5
	3	74.1
	4	65
	5	60.2
	6	78.3
2030	1	84.7
	2	66.6
	3	63.7
	4	51.1
	5	50.8
	6	62.9
Outside	1	60.4
	2	26.3
	3	25.4
	4	4.9
	5	1.2
	6	17.7

3.3 The autumn colour of the seedlings

The class of origin had significant effect on the autumn colouring of the seedlings ($F=54.473$, $p<0.001$) (Table 5). Only the seedlings grown outside had a clear autumn colour (Table 6a). The watering treatments did not affect the colouring of the seedlings. However, there were differences between origins. Over 50% of the seedlings from the northernmost origins and only 1.6-7.4% of the seedlings from foreign origins had red autumn colouring (Table 6b).

Table 5: Analysis of variance of effects of growing condition, origin class of the seedlings and the watering treatments and their interactions on the autumn colouring of the seedlings

Source	d.f	F	p
Growing condition	1	0.024	0.878
Class of origin	5	54.473	0.000
Water treatment	1	0.024	0.878
G.condition*C. of origin	5	0.008	1.000
G.condition* W.treatment	1	0.024	0.878
C. of origin*W.treatment	5	0.008	1.000
G.condition*C.of origin*W.treatment	5	0.008	1.000

Table 6a and b: The different autumn colourings of the seedlings between growing conditions (a) and the difference in the autumn colouring of the seedlings grown outside between different origin classes (b). The key to the colour classes: 1 indicates completely red needles, 2 indicates needles that have partly turned red but also have some green needles amongst them and 3 is for completely green needles.

(a)

Growing condition	Colour class	Percentage of plans
2100	1	0
	2	0
	3	100
2030	1	0
	2	0.1
	3	99.9
Outside	1	22.9
	2	39.9
	3	37.2

(b)

Class of origin	Colour class	Percentage of plans
1	1	52.9
	2	32.9
	3	14.3
2	1	29.9
	2	38.1
	3	32
3	1	26.5
	2	43
	3	30.5
4	1	7.4
	2	43.8
	3	48.8
5	1	1.6
	2	34.9
	3	63.6
6	1	23.4
	2	46
	3	30.6

3.4 The effect of growth conditions on the winter hardening of pine seedlings

It was clearly noticeable that the amount of needle damage decreased the further the autumn got (Fig. 8 and 9). The normal phenomenon is clear in all conditions in both one- and two-year-old seedlings. However, there were large differences between the hardening rate of the seedlings grown in different conditions (Table 7a and b). The one-year-old seedlings grown in the 2100 condition had the largest damage through the autumn. The seedlings grown in this condition had a high amount of needle damage even in the second half of September (Fig 8). For the one-year-old seedlings the lowest needle damage occurred, depending on the date of the freezing test, either in condition 2030 or with the seedlings grown outside (Fig 8). In the beginning of September the two-year-old seedlings grown in 2100 condition had the highest amount of needle damage. However, with the next three freezing tests the seedlings from the outside condition had the most damage. For some reason all of the conditions had lower damage on the third week of testing than on the forth week. During the fifth week of testing there was hardly any damage in any of the conditions (Fig 9). The two-year-old seedlings grown in the condition 2030 had the lowest amount of needle damage though out the freezing tests compared to the other conditions (Fig 9).

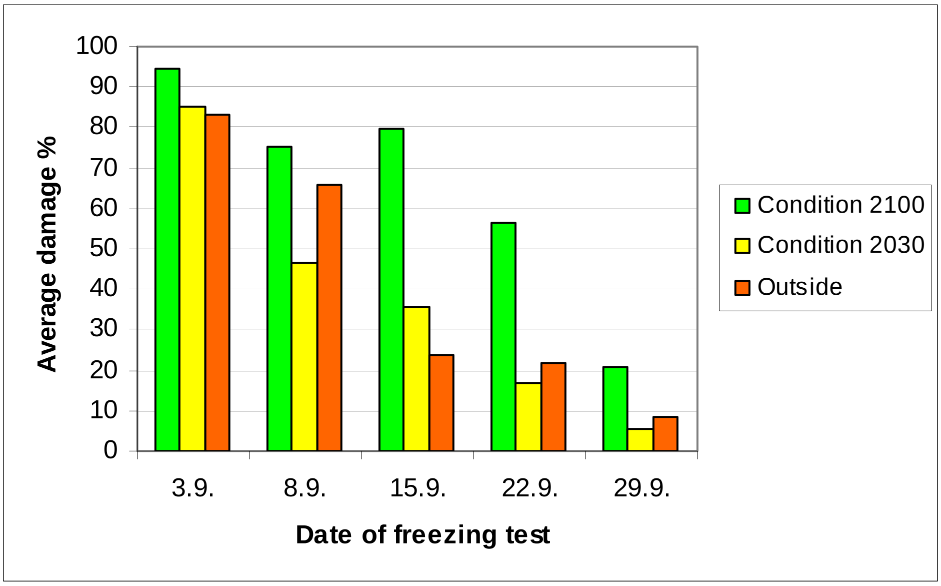

Figure 8: The average frost damage on different growing conditions in 2008 (seedling tested as one-year-olds)

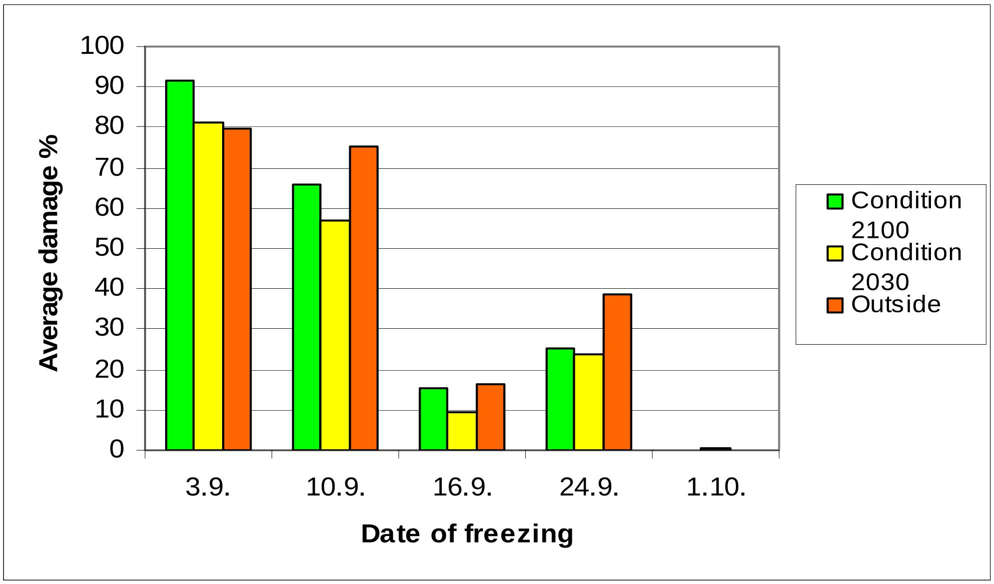

Figure 9: The average frost damage on different growing conditions in 2009 (seedlings tested as two-year-olds)

Table 7a and b: Analysis of variance the effects of growing condition, origin class of the seedlings and the time of the freezing test and their interactions on the primary needle damage of the one-year-old seedlings (a) and on the secondary needle damages of the two-year-old seedlings (b).

a.)

Source	d.f	F	p
Growing condition	2	456.856	0.000
Class of origin	5	415.502	0.000
Replication	4	879.404	0.000
G.condition * C. of origin	10	16.985	0.000
C.of origin * Replication	20	22.565	0.000
G.condition * Replication	8	42.732	0.000
G.condition* C.of origin* Replication	40	7.123	0.000

b.)

Source	d.f	F	p
Growing condition	2	36.270	0.000
Class of origin	5	293.560	0.000
Replication	4	1408.377	0.000
G.condition* C. of origin	10	3.518	0.000
C. of origin* Replication	8	11.799	0.000
G.condition * Replication	20	40.034	0.000
G.condition* C.of origin* Replication	40	3.654	0.000

3.5 The effect of various watering treatments on winter hardening

There were significant differences (F= 36.360, p<0.001) between watering treatments in different growing conditions for the one-year-old seedlings (Fig.10). For two-year-old seedlings, statistically the effect of different watering treatments was significant (F=7.379, p<0.007), but in practice the difference was hardly noticeable (Fig. 11). Amongst all of the growing conditions for both one and two-year-old, the needle damage was slightly higher for the low watering treatment. The outside control seedlings had only natural watering and as such cannot be compared to the watering treatments.

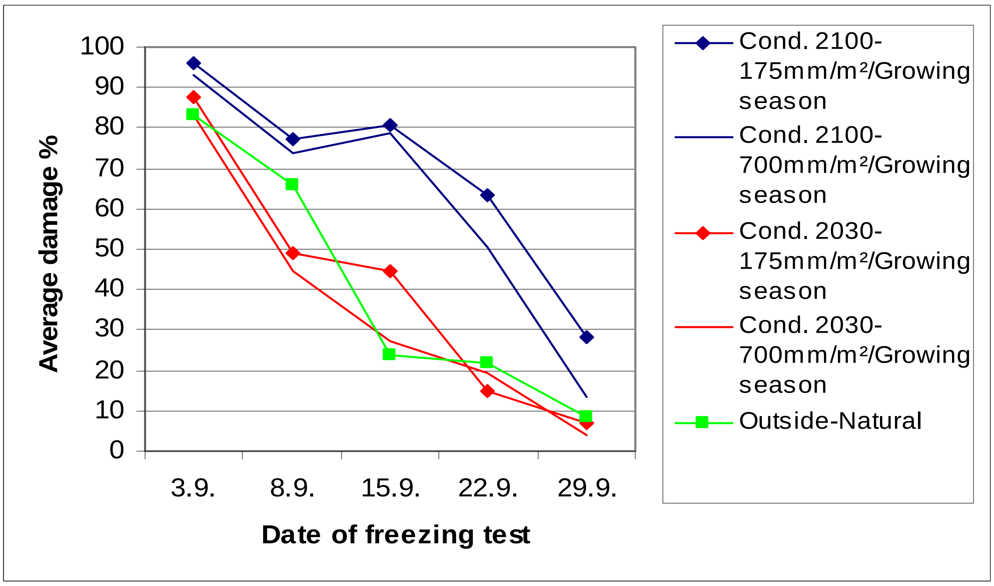

Figure 10: The effect of different watering treatments on the winter hardening of seedlings in autumn 2008 (seedlings tested as one-year-old).

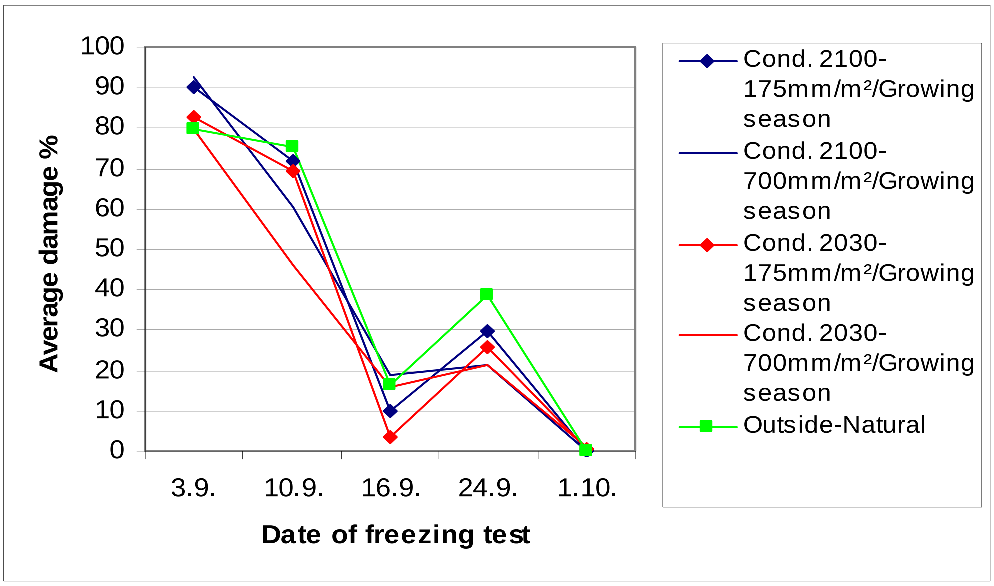

Figure 11: The effect of different watering treatments on the winter hardening of seedlings in autumn 2009(seedlings tested as two-year-old).

3.6 The effect of winter hardening on different origins

There were differences ($F = 415.502$; $p < 0.000$) in the amount of needle damage within all of the origins. The highest amount of damage was for the southern most origins (class 5): for one-year-olds the average damage was 83.2% and for two-year-old 65.1%. The winter hardening rate of the seedlings from the northernmost origin (Class 1) developed fast and the seedlings began to withstand the freezing test well after two weeks. Seedlings from class 1 had the lowest needle damage for both one and two-year-olds in all of the conditions (Fig. 12a and 13a). The average needle damage in one-year-old seedlings from origin class 1 was 16.4% and for two-year-old 14.7%. For one-year-old seedlings classes 2 and 6 (average needle damage 41.5-45.6%), and also classes 6 and 3 (average needle damage 45.1-47.5%) did not significantly differ from each other. Also for the two-year-old seedlings the classes 2 and 6 (average needle damage 30.6-34.2%) and 6 and 3 (average needle damage 34.2-35.9%) did not significantly differ from each other. One-year-old seedlings in all origin classes had the highest amount of damage in the 2100 conditions (Fig. 12a). In 2030 condition and outside condition the damage was at quite the same level for all origin classes (Fig. 12b and c). Seedlings tested as two-year-olds acted very similarly to the one-year-olds. The amount of damage decreased evenly as expected, although in all of the conditions the replication tested on the 16th of September seemed to tolerate the frost better than in the replication tested a week later on the 24th of September (Fig. 13 a, b and c).

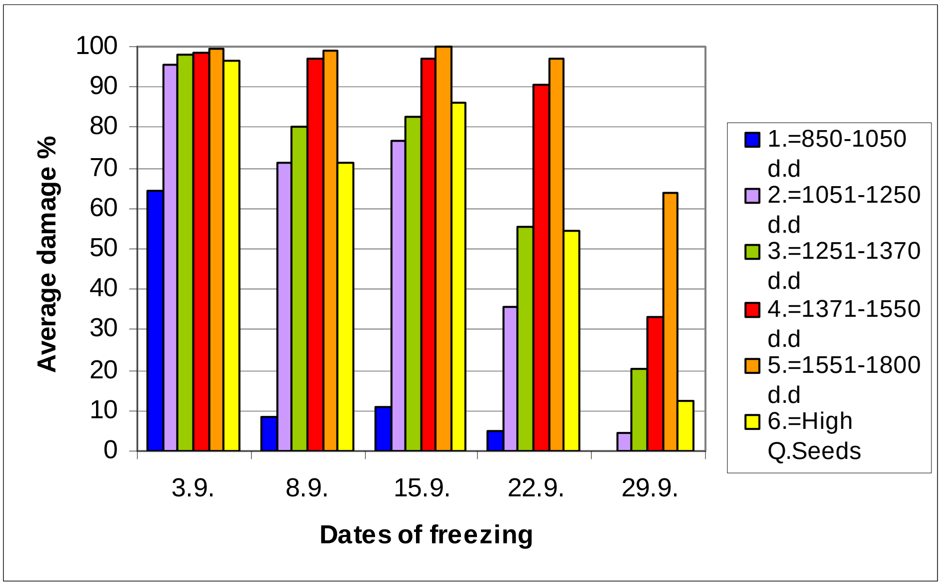

Figure 12a: The winter hardening of seedlings from different origins, grown in 2100 condition (Seedlings tested as one-year-old).

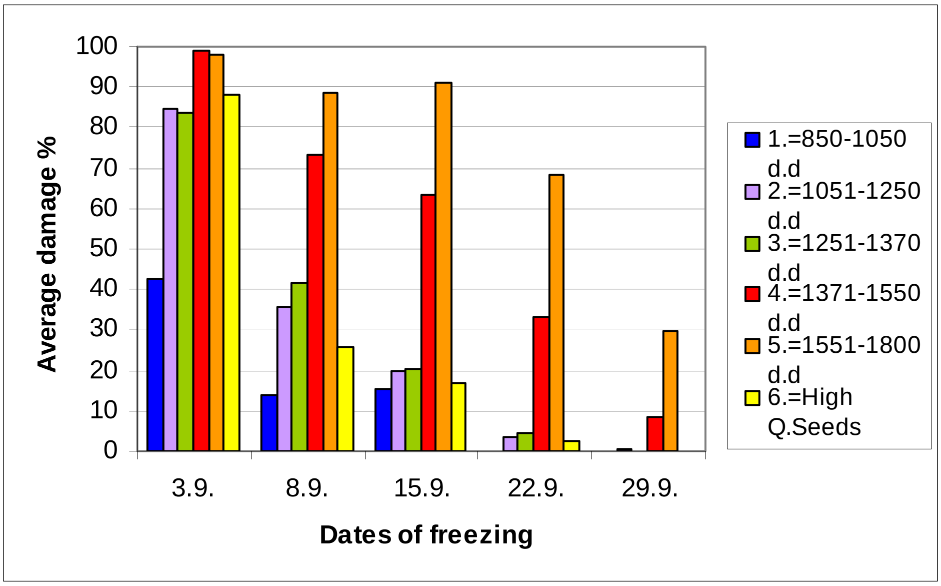

Figure 12b: The winter hardening of seedlings from different origins, grown in 2030 condition (Seedlings tested as one-year-old).

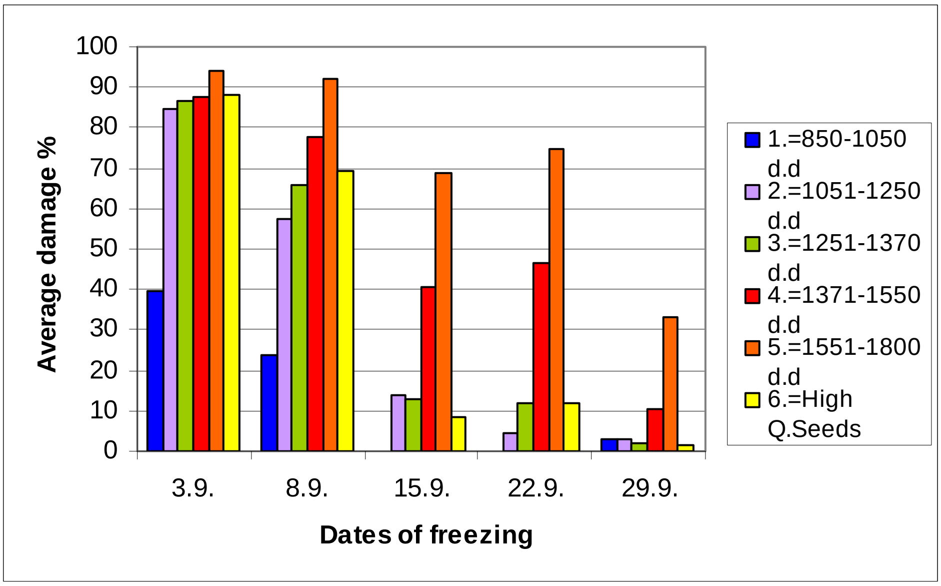

Figure 12c: The winter hardening of seedlings from different origins, grown outside in natural conditions (Seedlings tested as one-year-old).

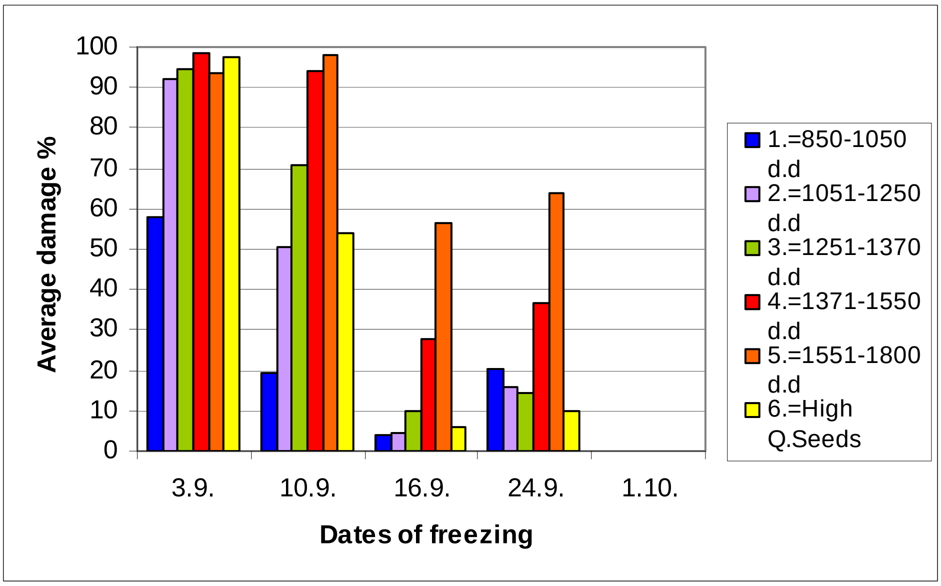

Figure 13a: The winter hardening of seedlings from different origins, grown in 2100 condition (Seedlings tested as two-year-old).

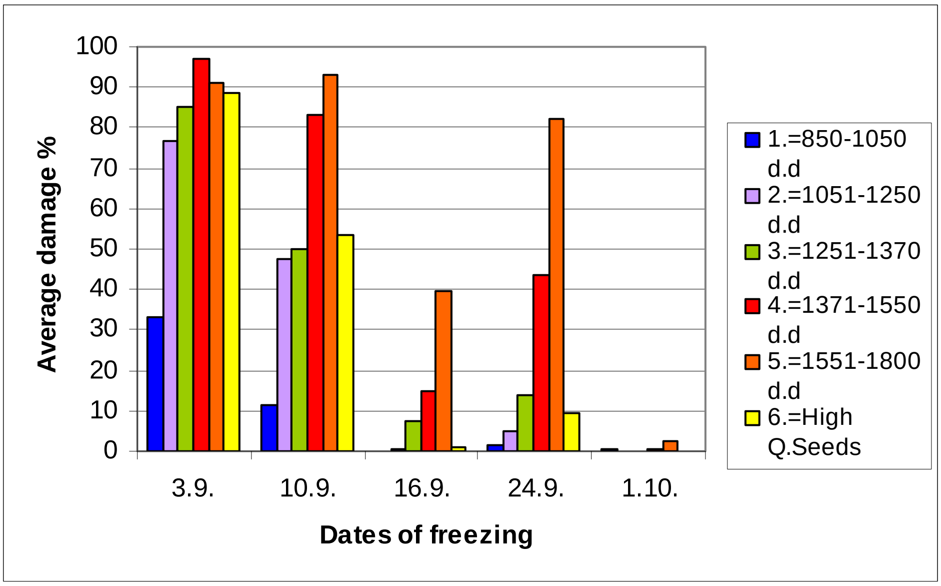

Figure 13b: The winter hardening of seedlings from different origins, grown in 2030 conditions (Seedlings tested as two-year-old).

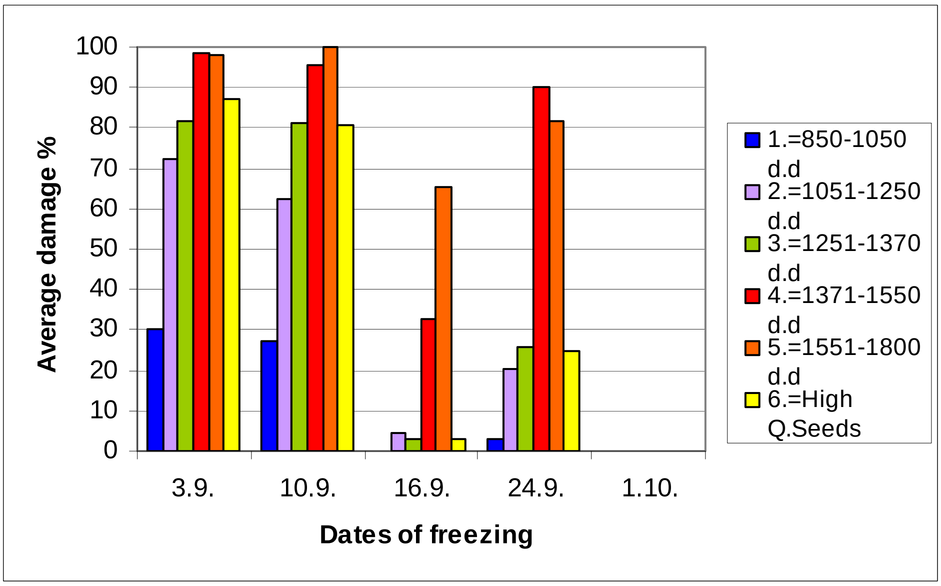

Figure 13c: The winter hardening of seedlings from different origins, grown outside in natural conditions (Seedlings tested as two-year-old).

4 CONCLUSIONS

The study was carried out successfully. The germination and death rate of the seedlings was quite similar in all the conditions. On average the death rate for two-year-old seedlings was lower than for one-year-old. It seems that the seedlings are stronger and stress tolerant during their second growing season than they are during their first year of growth. The death rate in greenhouse conditions seemed to be a little bit higher on the seedlings with low watering treatment. In the warmer conditions the risk for drought stress is greater and the importance of watering increases. The slightly higher death rate on seedlings grown outside than grown in greenhouse conditions may be partly due to the problems with birds eating the seeds despite the protective covers.

In the 2100 condition and with high water treatment the seedlings grew best. Furthermore, the seedlings grown in the greenhouse with low watering treatment were taller than the seedlings grown outside. With two-year-old seedlings the height difference between different conditions grew even larger and was clearly noticeable. With the low watering treatment the height difference seemed to even out between the conditions during the second year of growth. With the high watering treatment the height difference between the growing conditions got even larger. If the precipitation is low the benefit in height growth during the first growing season will be lost during the second year of growth, but if the precipitation will be at a high level the height growth will clearly benefit from the change in climate.

The seedlings from different origins reacted very individually to the different growing conditions and watering treatments. The high quality seed material seedlings were the highest independent to the growing conditions or watering treatment. The northernmost seedlings were almost always the shortest ones, except in the outside condition. The seedlings that originated from central and southern Finland were also tall, compared to the foreign and northern origins, in all conditions. In condition 2030 the origins height difference between the watering treatments was not as clear as it was in condition 2100. The foreign originated seedlings did not seem to benefit from the more suited watering and heat conditions of the greenhouse as much as the Finnish seedlings. The foreign seedlings clearly suffered from the different light conditions in Finland. According to this the benefit in height growth of favourable water and heat conditions will be lost if the light conditions are not favourable. The seedlings from different origins acted very similarly in different growing conditions during their first and second year of growth.

The formation of the terminal bud was clearly affected by the surrounding growing conditions. The condition 2100 had the highest rate of bud formation and the outside condition had the lowest. Amongst different origins the northernmost seedlings formed terminal buds clearly more often than any other origin. It is clear that the northern seedlings are more used to the light conditions in Finland and will begin their terminal bud development even when the temperatures are higher than normal. This shows that the development process of the terminal bud is triggered by the amount of light. The foreign originated seedlings had the lowest rate in bud formation in all conditions. The biggest difference between origins was in the outside condition, where the northern seedlings formed buds very often and the foreign seedlings formed hardly any buds at all. The high quality seed material seedlings did not form terminal buds any better than the average seedlings from central Finland. Especially the northern originated seedlings began to form terminal buds approximately at the same time as they would in normal conditions. According to this, the formation of terminal buds on our native seed material should not be disturbed too much even if the temperatures began to rise.

The autumn colouration of the seedlings was the strongest in the outside condition. In the condition 2100 none of the seedlings turned red, as the autumn progressed. This shows that the autumn colouration is dependent on the surrounding temperature. The warmer it is, the less autumn colouration will occur. The strongest autumn colouration was on the northernmost seedlings and the lowest rate of autumn colouration was on the foreign seedlings. The more southern the origin, the weaker is the autumn colouration. According to this the autumn colouration is a quality of more northern trees and the warming of the climate can weaken the rate of autumn colouration.

The growth conditions did have an effect on the winter hardening process of the seedlings. The needle damage decreases the further autumn gets in all of the conditions, some faster than others. It is clearly noticeable that the winter hardening process is slower than usually on the seedlings grown in condition 2100. There is still a high percentage of needle damage even on the fifth week of the freezing tests on one-year-old seedlings. The lowest needle damages on both one and two-year-old seedlings were either on the condition 2030 or on the outside condition, depending on the date of the freezing test. On the two-year-olds all of the seedlings seemed to begin their winter hardening process faster than the one-year-olds. There was hardly any needle damage on the fifth week of the freezing tests in any of the conditions. On two-year-olds also the highest percentage of needle damage occurred in the 2100 condition. On the two-year-olds the amount of needle damage in all of the conditions was for some reason lower on the third week of the freezing tests than it was on the forth week of the freezing tests. The reason to this can not be explained. According to the results of this study, a small rise in growing temperature is not necessarily yet a threat to the winter hardening process of the pine seedlings, but if the mean temperatures rise by +5°C or more it will have a slowing effect on the development of the winter hardening process on Scots pine seedlings and therefore the climate change will increase the risk of frost damage to Scots pine seedlings.

The different water treatments also had an effect on the winter hardening process of the seedlings. Clearly the seedlings with low watering treatment had a higher percentage of needle damage. This shows that the stress caused by drought has a negative effect on the development of winter hardening. So if the dry seasons become more common, especially if the dry season takes place in the late summer or during autumn, this will also increase the risk of frost damage to the young seedlings.

The difference of origin had a clear effect on the winter hardening of the seedlings in all of the growing conditions. In all conditions the northernmost origins became winter hardy a lot faster than any other origin. The Central Finland and Southern-Finland originated seedlings also became winter hardy reasonably fast. After the third week of the freezing tests in all of the conditions the amount of needle damage decreased noticeably. Also the high quality seed material seedlings acted similarly to the Central and Southern-Finland origin class, and became winter hardy after the third week of the freezing tests. This shows that the high quality seed material doesn’t seem to act any better or worse against frost than any other seed material collected from the same regions. The highest percentage of needle damage in all conditions in both one and two-year-old seedlings was on the southern most origins. Both of the foreign origin classes suffered a high amount of needle damage even on the fifth week of freezing testing as one-year-old and became winter hardy hardly at all. The ability to winter hardening seems to depend strongly on the origin of the seedling. Clearly the southern originated seedlings had a lower ability to become winter hardy and therefore the seed material from more southern countries will not live successfully in Finland, even if the on coming climate changes will make the growing conditions of Finland more favourable for them. As two-year-olds all origins acted very similarly as they did as one-year-olds, under the different conditions, although all of them became winter hardy a week earlier as two-year-olds than they did as one-year-old. This proves that the seedlings ability to winter hardening as a two-year-old can be predicted from the winter hardiness development of one-year-old. So even if the height growth of Scots pine seedlings will increase due to higher temperatures and longer growing seasons, the benefit of those factors will be lost due the increasing risk of frost damage.

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APPENDIX 1: **List of seed origins**

Origin num.	Origin	seeds collected from	Lat	Long	altitude	Average d.d	origins d.d class	Y of ripening	Seed ID
1	Rovaniemi	Forest	66º55'	25º35'		900	1	1988	M29880744
2	Kuhmo	Forest	63°42'-64°29'	28°44'-30°33'	0-200	1000	1	2003	T03030269
3	Rautavaara (surroundings)	Forest	63°12'-63°45'	28°02'-28°46'	105-315	1050	1	2002	T03021201
4	Multia (surroundings)	Forest	62°01'-62°40'	24°01'-25°15'	105-265	1100	2	2002	T03021204
5	Keuruu (surroundings)	Forest	61°38'-62°55'	23°00'-25°42'	65-265	1150	2	2003	T03030250
6	Parikkala	Forest	61°12'-61°46'	28°15'-29°52'	45-174	1200	2	2003	T03030227
7	Heinävesi (surroundings)	Forest	61°16'-62°39'	24°26'-29°46'	60-200	1150	2	2002	T03021203
8	Pori (surroundings)	Forest	60°52'-61°45'	21°23'-23°52'	22-169	1200	2	2003	T03030249
9	Rauma (surroundings)	Forest	60°37'-61°20'	21°08'-23°23'	65-144	1250	2	2002	T03020295
10	Sweden 1	Forest	57°22'	15°38'		1450	4	1977	FO-H-20
11	Sweden 2	Forest	57°55'	15°38'		1450	4	1983	FO-H-27
12	Denmark	Forest	58°50'	9°30'		1700	5	2004	DK/A3423
13	Estonia 1	Forest	58°52'	23°34'		1400	4		S1-06-004
14	Estonia2	Forest	58°50'	23°48'		1400	4		S1-06-006
15	Latvia 1	Forest	57°20'	25°50'		1550	4		
16	Latvia 2	Forest	56°40'	23°50'		1550	4		
17	Lithuenia 1	Forest	55°10'	25°00'		1650	5	2006	18PM2
18	Lithuenia 2	Forest	55°40'	22°25'		1650	5	2002	58PM2
19	Poland 1	Forest	53°30'-53°40'	17°30'-17°35'		1800	5		184/05
20	Poland 2	Forest	53°30'-53°40'	17°30'-17°35'		1800	5		185/05
21	Poland 3	Forest	53°30'-53°40'	17°30'-17°35'		1800	5		186/05
22	Poland 4	Forest	53°30'-53°40'	17°30'-17°35'		1800	5		187/05
23	Männikkö	282	60°45'	26°40'	40	1300	3	2004	M29040104
24	Vitikkala	323	61°29'	26°57'	130	1216	2	2004	M29040057
25	Nummela	358	60°30'	24°43'	90	1262	3	2004	M29040083
26	Nurmijärvi	372	60°29'	24°41'	95	1263	3	2004	M29040059
27	Seppälä	406	62°13'	27°44'	88	1167	2	2004	M29040074
28	Latvia: Talsi	so	57°15'	22°30'		1550	4	2000	
29	Laakerinmaa	83	61°54'	24°53'	170	1154	2	2007	M29070025
30	Männikkö	282	60°45'	26°40'	40	1300	3	2007	M29070007
31	Kalloila	201	60°47'	26°22'	60	1280	3	2007	M29070008
32	Nurmijärvi	372	60°29'	24°41'	95	1263	3	2007	M29070006
33	Nummela	358	60°30'	24°43'	90	1262	3	2007	M29070011
34	Vitikkala	323	61°29'	26°57'	130	1216	2	2007	M29070009
35	Ruoksu	155	60°59'	25°14'	135	1218	2	2007	M29070017
36	Kanteleenniemi	152	61°35'	26°17'	115	1217	2	2007	M29070031

37	Alkärr	400	60°03'	23°52'	35	1337	3	2007	M29070022
38	Suhola 2	404	62°14'	27°41'	88	1167	2	2007	M29070013
39	Peräsuo	405	62°13'	27°42'	85	1167	2	2007	M29070014
40	Seppälä	406	62°13'	27°44'	88	1167	2	2007	M29070015
41	Ruunamäki	409	xx°xx'	xx°xx'		1005	1	2007	M290070016
42	E39xE618C	so	xx°xx'	xx°xx'			6	1999	G04990072
43	E468DxE620C	so	xx°xx'	xx°xx'			6	1992	R01920513
44	E468DxE1591	so	xx°xx'	xx°xx'			6	1992	R01920515
45	E604xE1029	so	xx°xx'	xx°xx'			6	1996	R01960381
46	E615AxE1592	so	xx°xx'	xx°xx'			6	1996	R01960383
47	E2257xE79	so	xx°xx'	xx°xx'			6	1985	R01850836
48	E2872xE1686	so	xx°xx'	xx°xx'			6	1995	R01950565