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ESCUELA POLITECNICA SUPERIOR DE GANDIA

Licenciado en Ciencias Ambientales

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**“Effect of climate warming to the dormancy and dehardening of pedunculate oak (*Quercus robur* L.) seedlings in assumed climate conditions of the year 2030 and 2100”**

**TRABAJO FINAL DE CARRERA**

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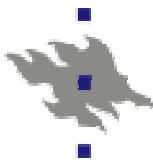
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**Effect of climate warming to the dormancy and dehardening of pedunculate oak (*Quercus robur* L.) seedlings in assumed climate conditions of the year 2030 and 2100**



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## **ABSTRACT**

The climate warming effect on the development of the pedunculate oak (*Quercus robur* L.) during the dormancy state and dehardening development was studied by the simulation of different growth conditions.

The seed material used was obtained from 9 different origins from Poland to South Finland, to ascertain the most appropriate origin to grow in South Finland.

In greenhouses they were simulated two future climatic conditions belonging to the years 2030 and 2100, using the scenario A1B, as well as two conditions of humidity. To find out the state of dormancy and the dehardening ability, we used a freezing test to simulate the injuries caused by the frosts.

In dormancy experiment, the seedlings of the year 2030 presented null injuries and a high rate of survival. On the contrary, by the year 2100 the injuries were 50%. In the dehardening experiment, it was observed that the late frost was a huge threat to the survival, since the mortality increased by 30 % in two weeks. Among all the origins, the one that presents better adaptability was the one from Ruissalo.

The results of this thesis supports the theory that climate warming will increase the risk of frost damage of the one year old seedlings of pedunculate oak in South Finland.

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# **1. INTRODUCTION**

## **1.1 Climate change**

Some observations describing the overall image of a world warming up and other changes in the climate system are listed. The average global temperature at the surface has increased by  $0.6 \pm 0.2$  ° C since the late XIX century. It is very likely that precipitation has increased by 0.5 to 1% per decade in the XX century at most middle and high latitudes of the Northern Hemisphere continents (IPCC 2001). Most of the observed global warming over the last 50 years is likely to have been caused by an increasing concentration of greenhouse gases, but we cannot exclude the natural climate variability in a regional scale as a potential explanation for the trend in Finland (Jylhä et al. 2004).

Climate change in Intergovernmental Panel on Climate Change (IPCC 2001) usage refers to any change in climate over time, whether due to natural variability or as a result of human activity. This usage differs from that in the Framework Convention on Climate Change (UNFCCC 1999), where climate change refers to a change of climate that is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and that is in addition to natural climate variability observed over comparable time periods.

The most remarkable forcing agents that cause climate change have been the increases in atmospheric concentrations of greenhouse gases. The current data sets show the human influence on atmospheric concentrations of these agents, although most of the gases originate from both natural and anthropogenic. The main gases responsible for climate change are carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O), and halocarbons (CFC) (IPCC 2001).

The gas most responsible for climate change is also the greenhouse gas mostly produced by humans, which is the carbon dioxide. The concentration of CO<sub>2</sub> in the atmosphere has increased from 280 ppm in 1750 to 367 ppm in 1999. The present CO<sub>2</sub> concentration has not been surpassed in the last 420,000 years and has not probably occurred, even during the past 20 million years, either (IPCC 2001).

From studies of climate change impacts and adaptations, Jylhä and Laapas (2009) illustrate the estimated temporal evolution of CO<sub>2</sub> emissions and the atmospheric CO<sub>2</sub> concentration in Finland (Figure 1). The tendencies are based in 3 different scenarios, which are documented by Jylhä et al. (cited in Jylhä and Laapas 2009). In our experiment we used the A1B scenario for the simulations of climate change.

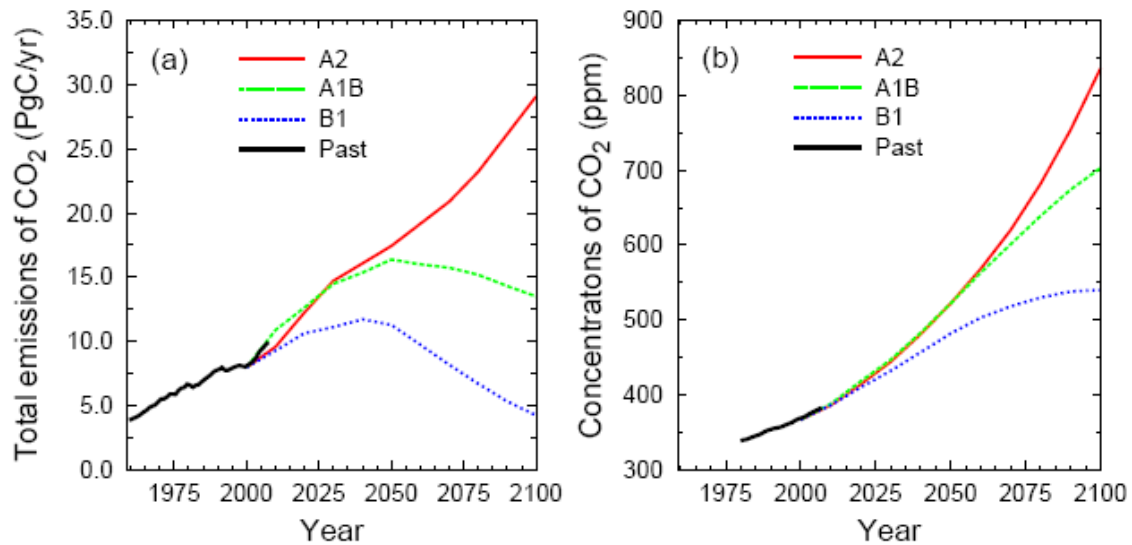


Figure 1. (a): Estimated temporal evolution of the emissions and (b): atmospheric concentration of carbon dioxide under three Special Reports on Emissions Scenarios (SRES) greenhouse gas. Figures for the SRES scenarios are based on IPCC (2001). (Jylhä and Laapas 2009 and Jylhä et al. 2009).

Jylhä et al. (2004) reported that the annual mean temperatures in Finland increased by about 0.7°C from 1901 to 2000. In contrast to temperature, the annual mean fluctuation of precipitation anomalies do not show any statistically significant trend over the 20<sup>th</sup> century. The Finnish Research Programme of Climate Change (SILMU) proposed a climate scenario for the Nordic countries, when the scenario specifies a warming rate of 0.45°C per decade in Finland. Johannesson (1995) suggested that temperature changes in the winter were larger (0.6°C per decade) than in the summer period (0.3°C per decade).

Jylhä, et al. (2009) illustrates on Figure 2 the predictions of mean temperature in Finland using three scenarios. The scenario used in our experiment was the A1B. For the year 2030 the annual average temperature predicted is practically the same in all of the scenarios. However, the simulations done for the year 2100 have differences among themselves, with our scenario in the middle. The annual average is close to 5 positive degrees; for the summer period the temperature is less than in the winter, being the temperature simulated for our experiment of 4 positive degrees.

It is projected that there will be an increase in global average water vapor, evaporation and precipitation. Thus, if it is assumed that the rate of relative precipitation increase is proportional to the rate of warming, it will arrive at a ratio of about 3 to 4% per °C of warming using relatively low values of the ratio of precipitation (Johannesson 1995).

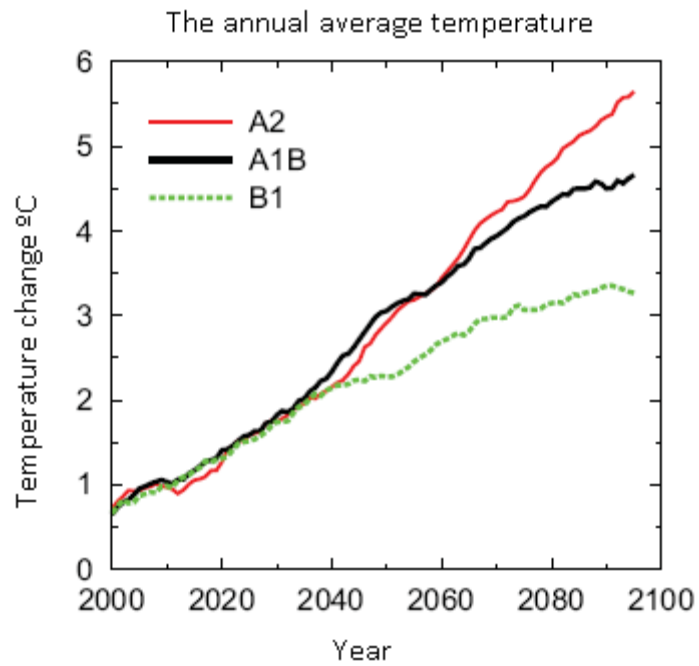


Figure2. Annual mean temperature in Finland modeled and projected employing three SRES scenarios. The figure is based on figures of Jylhä et al. 2009.

Talkkari (1996) predicted that the most drastic changes will take place in northern latitudes, which are mostly covered by boreal forests, and the predicted global climate change is among the major factors affecting future forest development in the boreal zone.



## **1.2 Pedunculate oak (*Quercus robur* L.)**

### **1.2.1 Distribution**

Pedunculate oak have the widest geographic distribution among European white oaks (Figure 3a). It is distributed in Europe from northern Spain to southern Scandinavia and from Ireland to Eastern Europe and reaches the Ural Mountains (Zanetto et al. 1994; Ducouso and Bordacs 2004).

Most of the present South Finland was submerged when the ice receded at the end of the last ice age about 10,000 years ago. This area has been, and still is, subject to rapid land uplift. Colonization of the newly emerged ground in the South and simultaneous decline in the North have made the whole distribution of oak in Finland shift towards South and West (Figure 3). In a way, the process could be seen as the last phase of the post-glacial colonization of Europe by oaks, only now towards south (Vakkari et al. 2005).

Repo et al. (2007) show that the population of pedunculate oak in Finland is strongly fragmented and growing at the northern margin of the species' European distribution and it can only be found in the south of Finland (Figure 3b). It is the only oak species in Finland, even the closely related sessile oak (*Quercus. petraea*) is absent and hence introgression between the species does not affect genetic variability (Mattila et al. 1994; Vakkari et al. 2005).

The cooling climate and expansion of Norway spruce led to a long-lasting decline of oak populations and, starting about 300 years ago, the stands were further fragmented and potentially restricting the flow of genes among local stands owing to increasing human pressure for more agricultural land (Mattila et al. 1994). Oak populations have also been exploited as a source of valuable timber and even destroyed on some occasions. Vakkari et al. (2005) found genetic structural differences in the populations in Finland, because of the fragmentation, and it is affected by population size and age of the population site. Planting of oak is not common in Finland, and the low number of haplotypes and the sharpness of the contact zone support the idea that man-mediated seed transfer has not been very extensive in the past, either.

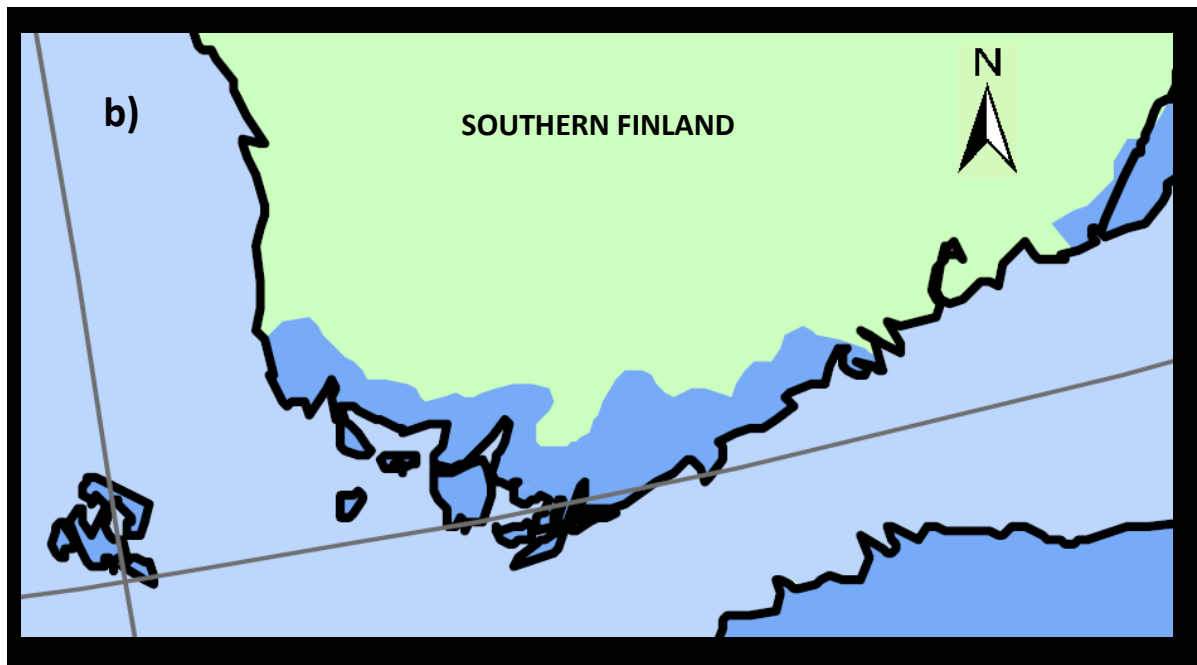
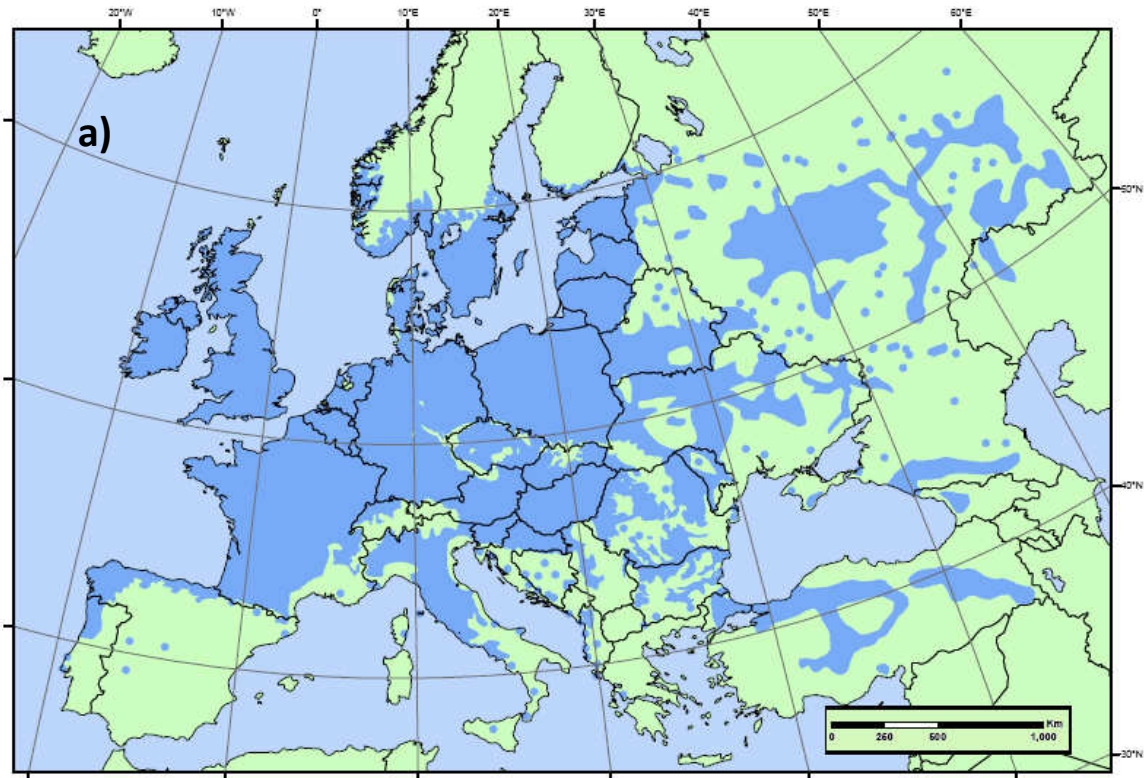


Figure3. a) Natural distribution area of *Quercus robur* in Europe. b) Natural distribution area of *Quercus robur* in Finland, where its distributions is limited to the south. Compiled by members of the EUFORGEN Network. (Ducousso and Bordacs 2004).

### **1.2.2 Description, morphology and habitat**

*Quercus robur* L. is a deciduous tree which belongs to the *Fagaceae* family and is also synonymous of *Quercus pedunculata* Ehrh. Pedunculate oak is a large tree that reaches 30–40 m in height and it is a long-lived tree that can live even longer than 800 years. The morphology of the top is broad and rounded or irregular and has a trunk straight, short and thick in individual specimens, with thick and tortuous branches with bark greyish or whitish, very cracked and brownish tonality in old trees (Ducousso and Bordacs 2004).

The adult leaves are large, glabrous, shortly stalked, with 5 - 7 lobes and auriculate at the base, in alternating arrangement with elongated stipules early falling and they are dark green and shiny in the front and lighter beneath. It is monoecious and allogamous, anemophilous and predominantly outcrossing. The flowers are unisexual; the male flowers grouped in catkins pendulums, while the females are solitary and axillaries. Trees usually reach seedbearing age at between 40 and 100 years old. The fruit are an acorn, 2-2.5 cm long, which are long peduncle (having a peduncle of 3-7 cm long). Acorns fall from cups and sprout next spring, and the oak seedlings keep their seed-leaves within the husk, and send up sturdy shoots bearing first scale-leaves, then typical leaves (Edlin 1978, Galán 1998 and White 2005).

Pedunculate oak can grow on most types of soil, but preferentially grows on rich, humid soils from sea level to 1800m. It needs a humid cold climate, where there are few signs of summer drought (Galán 1998 and Ducousso and Bordacs 2004).

Natural hybridization in oaks has been reported in many studies. In European white oaks hybridization is asymmetric: *Quercus petraea* preferentially pollinates *Quercus robur*, but the closely related *Quercus petraea* is absent in Finland and long-distance pollen flow is (probably) not as efficient so in more central areas (Vakkari et al. 2005).

### **1.2.3 Role in biodiversity and utilization**

Pedunculate oak have a huge role in biodiversity, since the tree supports a wealth of organisms which benefit from the food, support and shelter it supplies. Its acorns are rich in starch and provide food for birds and small mammals, and the leaves are eaten by insects. Gall wasps lay their eggs inside the leaves, where their larvae then secrete a chemical causing the leaf to mutate and form a gall (oak apple). In years with a high population of caterpillar or moth larvae almost all the leaves on a tree can be eaten, yet the English oak is able to survive this. In autumn the soft leaves break down easily

to form a rich leaf mould beneath the tree, supporting a wealth of invertebrates and a range of fungi (Kew Royal Botanic Gardens 2011).

At around 200 years old branches start to die back and fall when hit by severe storms. This dead wood is a vital habitat for invertebrates (White 2005). Even after its death the English oak continues to support biodiversity. The decaying wood can host a vast array of fungi and its hollow trunks provide roosting sites for owls and hibernation sites for bats (Kew Royal Botanic Gardens 2011).

They have been planted and managed for timber and barrel staves, for tanning leather, for hundreds of years. Notably used for furniture, ship and house building until the start of the twentieth century when iron became more successful material of choice (Galán 1998 and White 2005). One of the reasons why they were used so widespread was that the oak is able to withstand changes in humidity. Their acorns are also suitable for cattle and pig feed (Galán 1998).

The use of pedunculate oak is also widely applied for ornamental use and for planting in parks, gardens and botanical gardens in the temperate regions of most continents for its aesthetic value (White 2005). Especially in Finland, this species is relevant for the ornamental use since it is the only species of oak that has a natural distribution.

### **1.3 Plant adaptation to northern areas**

Plants are subjected, and must adapt to many different abiotic stresses (water, temperature...), low temperature may be the most critical determinant of plant distributional limits (Salisbury & Ross, cited in Lennartsson 2003). In northern areas, low temperature is the major environmental factor limiting the growth, development and the geographical distribution of plants (Lindén 2002).

During the period of 1971-2000 in the south of Finland (Helsinki Kaisaniemi), the annual variation of the absolute temperature shows a wide variation of 65°C between summer temperature with 31°C and winter with -34°C (Finnish Meteorological Institute). To withstand these variations of temperature and prevent the death, the trees have to synchronize the annual temperature cycle closely with their internal annual cycle of cold hardening and dehardening (Sakai and Larcher cited in Lennartsson 2003).

#### **1.3.1. Cold acclimation**

Cold acclimation is the ability to withstand the subsequent freezing temperatures in response to a period of low but non-freezing temperatures, in order to survive frosts by avoiding freezing through protecting their vital organs. (Xin and Browse 2000). Several factors may affect the level of cold hardiness attained (Sakai and Larcher, cited in Lennartsson 2003), e.g. nutritional status, the occurrence of pest, diseases, and drought, but the two most important factors are light and temperature. Gordon (1970) concludes that their results indicate that acclimation is induced by short days and frost or low temperature. Aversely, Anisko (cited in Lennartsson 2003) found in a recent study, by the modeling of cold hardening for several taxa of deciduous woody plants, that the initiation of the cold hardening process induced by growth cessation and dormancy is internally regulated and independent of temperature.

The temperature has a modulator effect and is externally regulated and dependent on the accumulated chill or heat. Winter and spring changes of cold hardiness are mainly dependent on ambient air temperature. Temperature has a direct effect on short-term changes in hardiness: the level of hardiness increases as temperature drops and decreases as it rises (Lindén 2002).

Induction of dormancy involves the cessation of growth, the build-up of cryoprotective substances (e.g. sugars and proteins), the down-regulation of photosynthesis and respiration, the shedding of leaves, and the development of dormant buds. These

active physiological changes in a tree are fundamentally important for the subsequent phase of physical cold hardening (Lambers et al., cited in Lennartsson 2003). Effective hardening in winter can be achieved by a two-step acclimation of 1 – 2 weeks, starting at a temperature of 0° to -3°C and then, dropping from -5° to -10°C (Sakai and Larcher, cited in Lennartsson 2003). Dormancy gradually develops through different stages, starting with the predormancy state and ending with the true dormant state, in which plants can no longer be activated by temporary warming, a state that is attained in November- December (Larcher , cited in Lennartsson 2003).

The ultimate survival of woody plants is dependent, on not only the maximal capacity of cold hardening, but also on the timing and rate of both cold acclimation and loss of cold hardening, the stability of cold hardiness, and the ability to reacclimate after unseasonably warm periods (Larcher, cited in Lindén 2002).The correct timing of cold hardening and dehardening is important for the growth and survival of oak (Jensen and Deans, cited in Repo et al. 2007). At the onset of winter, woody plants are more responsive to hardening stimuli than to dehardening temperatures. During deep dormancy, hardiness variation is reduced in both directions. However, in late winter, after dormancy release, plants are more easily dehardened and less easily rehardened (Heide, cited in Lindén 2002). After the breaking of endodormancy, only the xylem had the capacity to rearden (Warmund et al., cited in Palonen and Lindén 1999). For the winter survival of raspberry, the retention of cold hardiness once attained and the capacity to rearden after a warm period are possibly the most crucial factors (Palonen and Lindén 1999).

Breaking of dormancy and the commencement of growth can only occur after extensive periods of chilling, with temperatures between -3°C and 12°C and an optimum of 3 and 5°C. The induction of dehardening is triggered by the reduction in the critical day length and temperature is important because it governs the speed at which terminal buds are formed, being both factors are the most essential (Lennartsson 2003). So, even if there is an extended thaw in early winter, the trees will not start growing (Lambers et al., cited in Lennartsson 2003).

For woody plants the most hazardous times of the year, with respect to frost injuries, is are the autumn (when cold hardening has not been fully completed) and the spring (when dehardening may have started). For both of these times, frosts can be severe and frequent. (Sakai and Larcher, cited in Lennartsson 2003). Repo et al. (2007) suggested that in the case of pedunculate oak, injuries to the phloem, cambium and cortex, would be detrimental in the early stages of cold hardening. Thus therein lies the importance of proper acclimatization synchronized with the annual climatic cycle (Lennartsson, 2003). If the plant fails to give adequate acclimatization will be given a process of freezing.

### **1.3.2. Frost damage and risk of frost damage**

Extracellular freezing subjects cell membranes to various types of stress, involving a physical effect of the low temperature *per se*, freeze-induced reduction in the surface area and solute concentration effects, freeze dehydration of the protoplasm, (Sakai and Larcher, cited in Lindén 2002). The water potential of ice is lower than that of liquid water. Consequently, extracellular ice crystals grow by drawing water from cells until the water potential of ice and cell are equal, thus dehydrating the cell contents. The water potential of ice falls as temperature falls, hence cellular dehydration becomes progressively greater as temperature falls (Gusta et al., cited in Pearce 2001) Freeze may occur for other reasons, for example, cells that deep supercool will die if their capacity for supercooling is exceeded.

Oaks belong to a species with deep-supercooling in their xylem ray parenchyma cells, whereas cells of other tissues without such a property may avoid freezing by means of dehydration (George et al., 1982, cited in Repo et al. 2007). Other factors include: large ice masses affecting tissue or organ structure; embolisms in xylem vessels; disease, which may enter through lesions or exacerbate damage (Pearce 2001). These stresses may cause irreversible alterations in the structure and functioning of cell membranes and thus, bring about cell death. (Lindén 2002).

Signs of freezing injury can be detected by visual examination of the plants, externalizing, usually, develop a brown or yellowish color on injured tissues (Howell and Weiser, cited in Lindén 2002).

Climate change may play an important role in development and survival trees. Johannesson (1995) reported that the annual mean temperatures and precipitation in Finland could increase. Thus, the risk of snow damage could increase compared to the present day. This is because, the frequency of snowfalls at temperatures of around zero could increase. Consequently, the snow damage risk may increase. (Nykänen et al. 1997). Also the variability in minimum temperature may increase in a warmer climate. This could lead to an increased probability of frost damage even in the absence of warming if the state of frost hardiness of the plant cannot track the increased variability in temperature (Kramer and Hänninen 2009).

The distribution of tree species in northern latitudes is conditioned primarily by the capacity of acclimation to low temperatures. Weather conditions during the preceding summer and fall are relevant for successful overwintering at high latitudes, due to their impact on the annual cycle of vegetative growth (Lindén 2002). Pedunculate oak (*Quercus robur* L.) grows on the border of its northernmost distribution range in

southern Finland. Repo et al. (2007) hypothesized that short growing seasons and insufficient cold hardiness during autumn, and the consequent frost damage, are key factors that restrict the northward growth of oak.

#### **1.4. How to test cold acclimation?**

Due to the importance of cold acclimation for winter survival of northern plants, it was necessary to establish a method to determine levels of plant cold hardiness, dormancy and dehardening and thereby to obtain data of injury and survival levels (Lindén 2002).

To standardize the freezing test protocol, Levitt (cited in Lindén 2002) proposed the following steps as basic requirements:

1. The plants must be inoculated to ensure freezing.
2. Cooling must occur at a standard rate.
3. A single freeze must be used for a standard length of time.
4. Thawing must occur at a standard rate of warming.
5. Post-thawing conditions must be standardized.

Using the methodology of cooling simulation, the temperature is lowered at a rate of 1 to 2°C/h to imitate natural frost events. A common approach is to use a fixed rate of 2 to 6°C/h (Lindén 2002), but in nature the rate is usually less (Levitt 1972). As shown by Harrison et al. (cited in Lindén 2002), the rate of cooling is critical in the temperature range where the amount of unfrozen water is sufficient to cause injury. Lennartsson (2003) exposed that the most crucial aspects of freeze tests are the control of initiate ice formation at -2 °C to avoid supercooling, to apply slow cooling (3 °C/h) to ensure equilibrium freezing, and finally to apply slow thawing. Freezing tests can be evaluated by assessing the plant's capacity for regrowth after freezing and the extent of visible injuries and the mortality of seedlings (Lennartsson 2003).

Lindén (2002) in her doctoral thesis, *Measuring cold hardiness in woody plants*, lump together different alternative methods with in regard to the common controlled freezing tests. The content of dry matter or soluble carbohydrates, as well as carbohydrate composition, is often correlated with plant or tissue cold hardiness. Others investigations have show the relationship between the quality or quantity of amino acids, proteins and lipids and the level of cold hardiness (e.g. Yoshida, Khanizadeh et al., Arora and Wisniewski , Arora et al., cited in Lindén 2002). Additional



methods used to determine cold hardiness without freezing include electrical impedance analysis (Coleman, Repo et al., Repo et al., Väinölä and Repo, cited in Lindén 2002) and measurement of the ability to withstand plasmolysis (Siminovitch and Briggs, Levitt, cited in Lindén 2002). The indirect hardiness indicators often work well for some plants or tissues, or under certain conditions, yet none of them can be trusted as a general measure of cold hardiness in all plants (Lindén 2002).

### **1.5 Aim of study**

The main aim of this master thesis was to assess the effects of climate warming in the process of cold acclimation of one year old seedling of pedunculate oak (*Quercus robur* L.). The target of this study was to deal with the state of deep dormancy and the process of dehardening.

In the experiment we tried to find out the difference of behavior among all origins and between Finland and more Southern seedlings. And get the most adequate origin for growth in South Finland in future conditions.

We intended to find out how frost damage affects on the seedlings, analyzing the injuries and survival. To do that, the seedlings were subjected to different previous summer conditions of temperature and humidity, for the years of 2030 and 2100 simulated. All this was replicated with the aim of knowing the cold acclimation rate too.

With all this, we tried to ascertain the viability of the natural oak stands in South Finland, as well as their use as ornamental trees in future years.

## **2. MATERIAL AND METHODS**

### **2.1. Plant material**

The oak acorns (*Quercus robur* L.) were collected from 9 different areas (Figure 4), using seeds from the forest, orchard and urban parks (Table 1). The most seeds origin came from the southern Finland, but there was also from other countries of north of Europe, so we made two big useful groups to analyze. The first group appointed "Finland" with seeds only from the south of Finland, and the other appointed "more Southern" with seeds from southern Sweden, Latvia and north Poland.

There were two different types of stands; one a natural stands origin and the other planted. In Malmi origin seed is probably source from Estonia, and the Annalapuisto seeds came from Finland (*Pulkkinen pers. comm.*).

Thus entries has been divided into three categories, depending upon the typology of stand in which acorns were extract

- Park: The stand is definitely planted and the genetic origin of the trees is undefined.
- Forest stand: In all probability its origin is natural, but due to the uncertainty of its origin cannot be excluded that have been artificially planted.
- Seed orchard: The trees were planted by man with the purpose to produce acorns for commercial exploitation.

The material was collected during the summer of 2008 except the seeds from Poland that had been collected during the summer of 2007.

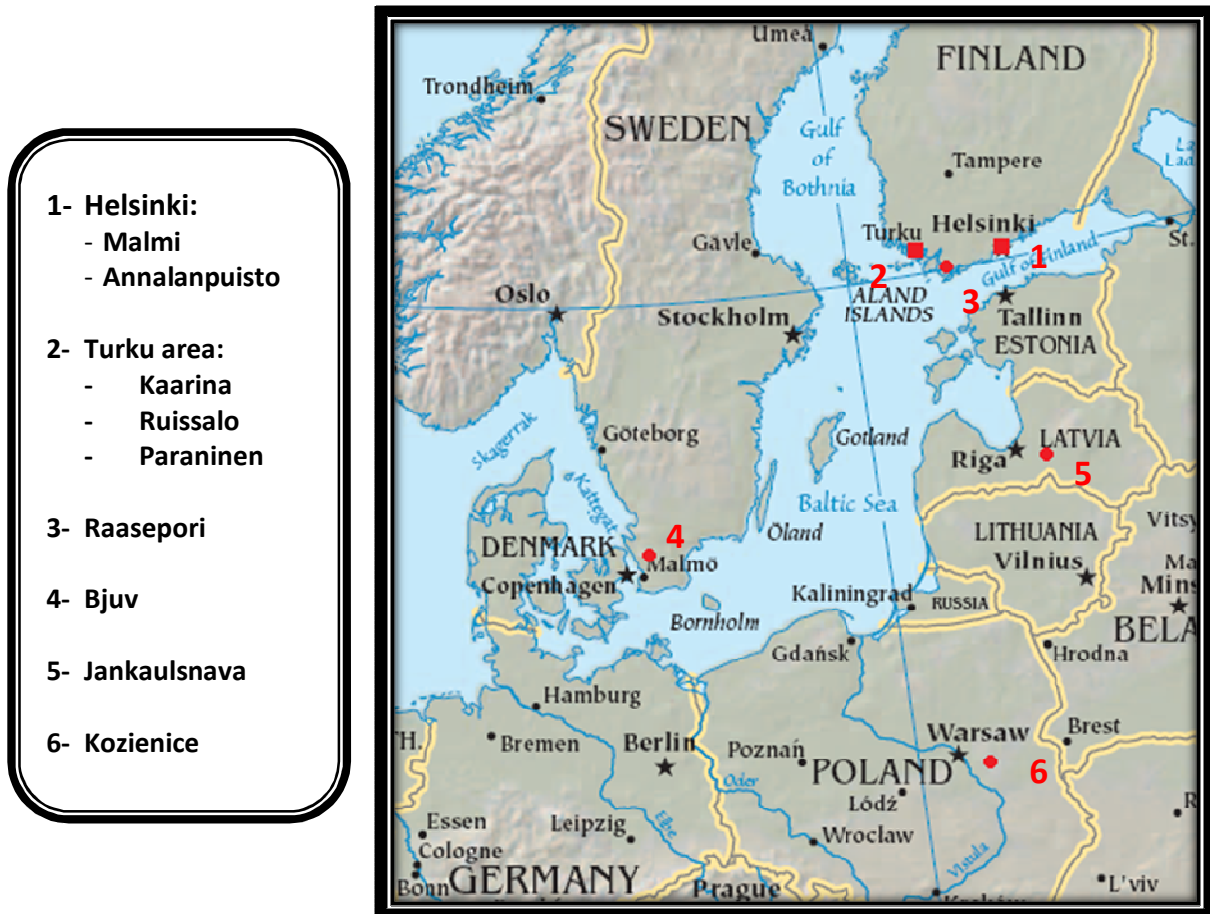


Figure 4. Location of the different origins of pedunculate oak acorns in Europe and Finland. Helsinki and Turku area have more than one sample used for the experiment.

Table 1. Origin and characteristics of the oak seeds used.

Group	Country	Origin	Area	Type of place	Type	Latitude (N)	Longitude(E)	Day degrees
Finland	Finland	Helsinki	Malmi	Park	Planted	60°14'195"	25°1'647"	1250
	Finland	Helsinki	Annalanpuisto	Park	Planted	60°12'745"	24°58'459"	1250
	Finland	Kaarina	Katariinanlaakso	Forest stand	Natural	60°24'713"	22°16'344"	1250
	Finland	Turku	Ruissalo	Forest stand	Natural	60°25'525"	22°7'512"	1250
	Finland	Raasepori	Tenhola	Forest stand	Natural	60°0' 472"	23°5'729"	1250
	Finland	Parainen	Parainen	Forest stand	Natural	60°14'844"	22°13'276"	1250
More Southern	Sweden	Söderåsen	Söderåsen	Forest stand	Natural	56° 5' 037"	13° 14' 574"	1550
	Latvia	Jankaulsnava	Jankaulsnava	Seed orchard	Planted	56°41'187"	25°58'129"	1650
	Poland	Kozienice	Kozienice	Forest stand	Natural	51°58'553"	21°55'117"	1900

## **2.2. Conditions**

### **2.2.1 Previous summer condition**

The present study was carried out in Finnish Forest Research Institute's Haapastensyrjää Unit (60° 37' 04", 24° 25' 4", 125 meters a. s. l.) There were two dates of sowing; the first acorns were sowed the 8.04.2009 for the condition of year 2100 and the second date was the 29.04.2009 for the condition of year 2030. The seeds were sowed in a box of 40 spaces and the boxes were collocated randomly in tables of 36 boxes. The growth substrate used was 100% of peat the type of Kekkilä White 420 W with ph of 5.5 and 2.0 mS/cm of conductivity. The fertilizing used was NPK 16-4-17 with the nutrient proportion of 9.0% for NO<sub>3</sub>-N and 6.5% for NH<sub>4</sub>-N.

For the same experiment (for both, dormancy to dehardening) have simulated different growing conditions in greenhouses. On the one hand we have two types of controlled temperatures that simulate the future growth condition of the Year 2100 and Year 2030.

The temperature used to simulate the thermal conditions provided for the years 2030 and 2100 were obtained by adding day and night +1°C to year 2030 and +4°C to year 2100 to the outside temperature of Haapastensyrjä breeding station in the year 2009. The temperature inside of the greenhouse never fell below of 0°C and the light hours of the material was not manipulated. Temperature sum of the Year 2100 had 2355 days degrees and for Year 2030 had 1615 dd, and the original temperature sum in the Year 2009 was 1270 dd.

In the greenhouse the moisture was also controlled to obtained different growth condition and simulates the effects of this variable in the experiment. They were controlled by two different water treatments; the wet moisture condition with 700 mm/m<sup>2</sup>/year and the dry moisture condition with 260 mm/m<sup>2</sup>/year. The quantity of water per week was 7.0 l/box for wet treatment and 1.75 l/box for dry treatment. These values correspond to double amount of water for the wet moisture and 50% less to the dry moisture compared to the long term local average rainfall measured in Jokioinen (research station). The irrigation process was manually using a hosepipe in both treatments. Wet moisture material was irrigated twice per week and dry material was only once per week. The situations of boxes were changed randomly three times to minimize the location effects.

For each moisture and temperature conditions, the same replications were done with the 9 different seeds origins for dormancy and 8 for dehardening experiment. For

each origin were done 4 and 5 replications, for dehardening and dormancy experiment respectively, according to the number of freezing tests necessary for the experiment. And finally, each freezing test had 4 seedlings (Table 2 and 3).

Table 2. Number of oak material in dormancy experiment.

Dormancy				
Temperature	Moisture	Origin	Freezing tests	Total seedlings
Year 2100	Wet	9	5	180
	Dry	9	5	180
Year 2030	Wet	9	5	180
	Dry	9	5	180
Total				720

Table 3. Number of oak material in dehardening experiment.

Dehardening				
Temperature	Moisture	Origin	Freezing tests	Total seedlings
Year 2100	Wet	8	4	128
	Dry	8	4	128
Year 2030	Wet	8	4	128
	Dry	8	4	128
Total				512

For each condition used, i.e. for each combination of temperature, moisture, origin and finally the different freezing test dates, were sowed four seedlings replicates.

### 2.2.2 Growth conditions during dormancy experiment

After the summer growth and hardening testing all the material was transferred to outside on the day 16.11.2009. The place was partly covered with plastic, but had open ceilings. The material in these conditions did not have any temperature control and could suffer a lot of minus temperatures. But the seedlings should acclimate correctly and be in deep dormancy.

When the dormancy testing started, the boxes from the outside cold storages were taken into the same greenhouse of previous summer growth. In the greenhouses the temperature never was less than +2°C. To evaluate the effect of frost in relation with

the length of high temperature treatment, the material was subject to one freeze testing per week.

### **2.2.3 Growth conditions during dehardening experiment**

In dehardening experiment all the material was in outside condition for the purpose of getting a natural timing of dehardening as the length of high temperatures and night length. Like in dormancy experiment, the material was subject to one freeze testing per week, corresponding the first material tested with a night length of 8 hours and 25 minutes and the last one a night length of 6 hours and 25 minutes.

### **2.3. Freeze testing**

The freezing tests were made to simulate the frost occurring in the hibernation periods in order to ascertain the effects produced on the seedlings when they are in the state of dormancy and dehardening. The freezing test was an operation that affects the variables analyzed such as injuries, survival and height growth.

All material used was submitted to the freezing test and were made different replications with the same conditions (seed origins, temperature and humidity) in order to ascertain the importance of the frost effect on seedlings depending on the day on which frost occurs. The freezing test experiment period started the last week of 2009 and ended the 8th week of 2010 for the dormancy experiment and for the dehardening experiment started the 17th week of 2010 and ended the 20th week of 2010.

Freezing test process was consisting to transfer the material to be tested in a cold chamber with an automatically controlled temperature. The original temperature of the chamber was 5°C and from 21:00 the temperature cooled down constantly 3 °C per hour to reaching a minimum temperature of -10 °C at 2:00. During next two hours the temperature remained constant and then begins to warm up 3°C/h for 5 hours until 9:00 when it reached the initial temperature (Fig 5). After the freezing test the boxes were returned to their original condition in the greenhouse.

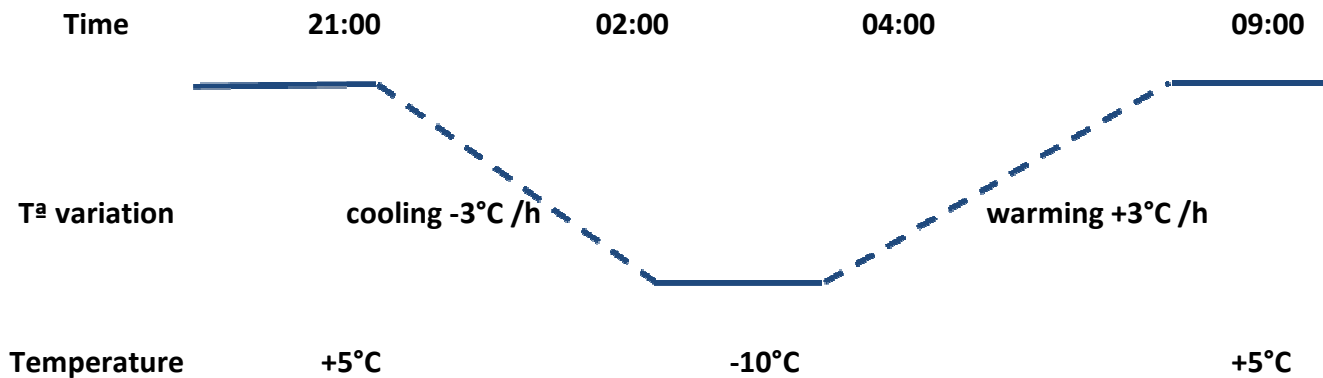


Figure 5. Freezing test process; temperature variation in the time.

#### **2.4. Data processing**

First, the entries that had not germinated or were dead before the freeze test were removed.

- Height before the freeze test: We measured the height of the seedlings before they were subjected to the freeze test.
- The material was subjected to the freeze test according to the protocol described above.
- The percentage of seedlings survival after the freezing test was calculated.
- The before and after freezing test high data of seedlings that were used to calculate the percentage of seedlings injuries produced after the freezing test.

#### **2.5. Statistical analysis**

Once the experimental data was obtained, the data was processed to determine the source effects; temperature, moisture, origin and freeze test day. Those sources have direct influence on the plants growth and development, the survival and the injuries in the dormancy and dehardening process.

The statistical software used to perform every analysis was the SYSTAT 9 for Windows. With this program it was obtained all necessary statistical information to get the results, as well as to do the relevant tables, graphs and figures. The Analysis of Variance (ANOVA), was used to find out the results of the various models raised. In the



## ***Material and Methods***

analysis, on one hand, the independent variables were: temperature condition, moisture condition, seeds origin and time of freeze testing. On the other hand, the dependent ones were: the seedling height, percentage of survival, percentage of injuries and the time of bud formation.

From this original data, there were summarized the seedling height average and as well as the survival and injuries percentage. It was reported that measures of variability using the standard deviation with the mean (mean  $\pm$  SE). Also, all bar graph figures had a standard error of 0.6825 as an error bar.

Correlations analysis between the height before freeze testing and injuries level were done using the type Pearson and corrected by the probabilities of Bonferroni.

For best results it was used for the following data transformations:

The parameters box edge and table edge representing the edge effect of the boxes and tables were created respectively, in the different variables to analyze. This effect is due to a competition between the seedlings depending on the location in the box, and also that irrigation could reach differently depending on the location of the box at the tables. This might affect the values of variables that are analyzed.

The freezing test did not work properly in all sessions, so we had to remove some material with the objective of improve the analysis. Due to the lack of entries required to create the survival ANOVA model, a new variable that grouped the seedlings entries into two largest groups; Finland and South was created in both experiments.

- **Dormancy experiment:**

The time of freeze testing became in treat time because the first week of treatment was the number 51 and therefore, it would have been an error in the testing data freeze, so this was named treat time 0.

In survival model, the origin North Poland was deleted because they had insufficient inputs to make a quality analysis.

- **Dehardening experiment:**

In some models, few origins were eliminated because they had insufficient inputs to make a quality analysis.

- Injuries: The origin North Poland in dehardening experiment was deleted.
- Survival model: The origin North Poland in dormancy experiment and North Poland and South Swedish in dehardening experiment were deleted.

### 3. RESULTS

#### 3.1. Survival after growth period

The seedlings survival after growth period was not uniform in all origins ( $p < 0.05$ ). Lowest survival corresponds to Polish origin (Figure 6) with 48% ( $\pm 7.3\%$ ) and the origin with high seedling survival percentage corresponds to Parainen (Finland) with 91.4% ( $\pm 6\%$ ). Only the origin and its interaction with temperature were significant (Table 4).

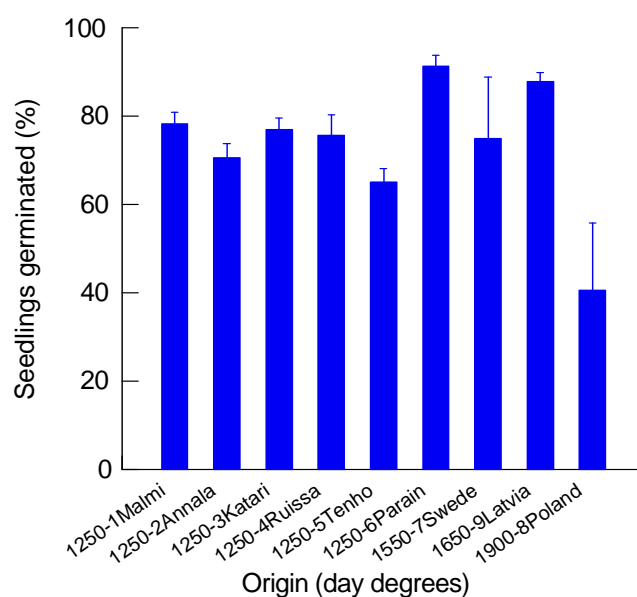


Figure 6. The mean percentage of oak seeds survival according of the origin (day degrees). SE = 0.6825 included as an error bar, in all bar figures of the results.

Table4. Effect of previous summer temperature, origin and moisture and their interaction into survival of oaks during the Year 2030 and Year 2100 growth conditions.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Origin	8247.227	8	1030.903	3.595	0.004
Temperature	556.475	1	556.475	1.941	0.173
Moisture	119.357	1	119.357	0.416	0.523
O * T	7648.342	8	956.043	3.334	0.006
O * M	115.207	8	14.401	0.050	1.000
T * M	0.075	1	0.075	0.000	0.987
T * M * O	335.980	8	41.997	0.146	0.996
Error	9749.396	34	286.747		

### 3.2. Height before freezing test

The mean height was 21.4 mm bigger for the condition Year 2030 with 166.2mm ( $\pm 2.7$ mm) than for the Year 2100 with 144.8mm ( $\pm 2.6$ mm). For the moisture, the wet condition was 13.3 mm bigger than the dry condition, with 162.1mm ( $\pm 2.6$ mm) and 148.8mm ( $\pm 2.7$ mm) respectively.

There are some differences among the seedling origin ( $p < 0.001$ ) being the origin variable the most relevant (Table 5). The highest oak origin corresponds to the North Poland with a 229.3mm ( $\pm 7.2$ mm) tall average and the lowest height average were the seedlings from Parainen in Finland with 111mm ( $\pm 5.4$ mm) (Figure 7).

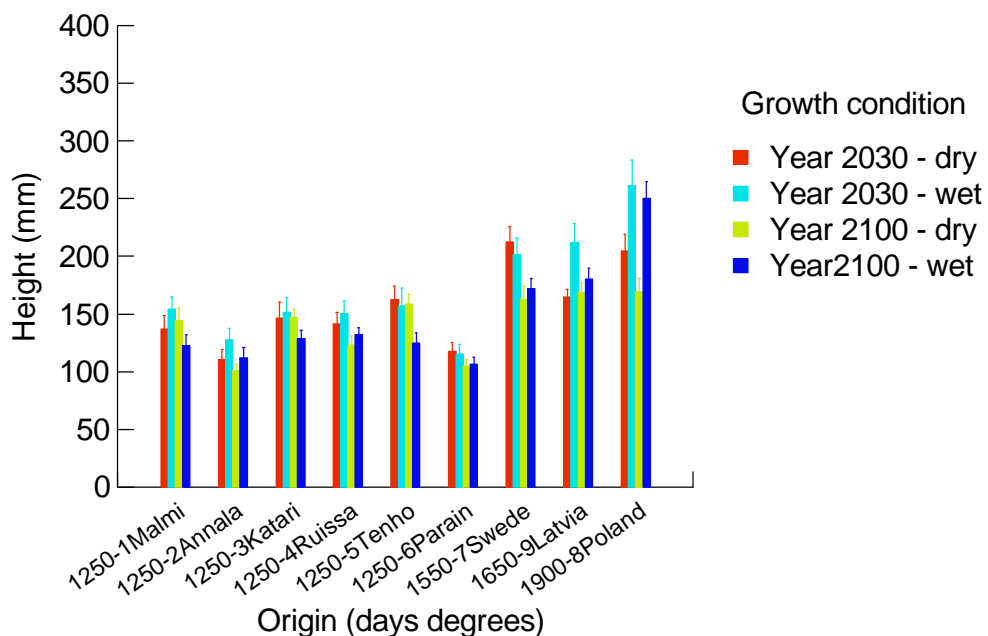


Figure 7. The mean height of oak seedlings according of the origin (day degrees) and clustered by growth condition (Year 2030 and Year 2100).

In addition, among seedlings origins, there were also significant differences between the seedling from Finland and more Southern, being the Southern 65 mm taller, with 197mm ( $\pm 3.5$ mm), than the Finland seedlings with 132mm ( $\pm 2.4$ mm).

Table5. Effect of previous summer temperature and moisture condition together with origins of oak on the height growth of oaks seedlings during the Year 2030 and Year 2100 growth condition.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Origin	970225.604	8	121278.2	42.619	0.000
Temperature	92588.656	1	92588.656	32.537	0.000
Moisture	35854.1	1	35854.1	12.6	0.000
O * T	33572.361	8	4196.545	1.475	0.162
O * M	122248.967	8	15281.121	5.37	0.000
T * M	13411.682	1	13411.682	4.713	0.030
T * M * O	22764.563	8	2845.570	1	0.434
Box edge	3793.685	1	3793.685	1.333	0.249
Table edge	21169.711	1	21169.711	7.439	0.007
Error	2307834.07	811	2845.665		

The height model shows that origin, temperature and moisture were significant ( $p < 0.001$ ). Also the interactions of origin with moisture and the temperature with the moisture were significant (Table5).

The effect of the random moisture in the box into the table was significant ( $p < 0.05$ ), but not the same into the box for the seedlings.

### 3.3. Dormancy

#### 3.3.1. Injuries

In the seedling growth during previous summer condition of Year 2030 the injuries level was almost null in both water treatments, 2.7% ( $\pm 2.3\%$ ) for the dry condition and 0.7% ( $\pm 2.2\%$ ) for the wet condition. On the contrary for the condition Year 2100 the injury was much more elevated than the condition Year 2030. The high injuries levels were 56.7% ( $\pm 2.3\%$ ) corresponding to the wet condition and 48.2% ( $\pm 2.3\%$ ) to the dry condition (Figure 8).

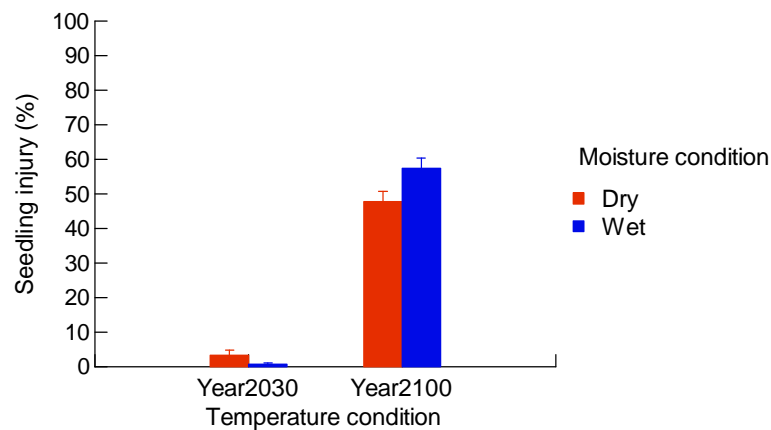


Figure 8. The mean percentage of oak seedlings injuries according of the temperature condition (Year 2030 and Year 2100) and clustered by moisture condition (wet and dry).

Most of the injuries level was explained for the previous summer temperature conditions (Table 6), there was large differences between the injuries caused to the seedlings depending on the temperature of treatment ( $p < 0.001$ ). The injuries depending of the large origin variable were similar among the Finland and south seedlings being of 1 % of difference. Also the interactions of temperature\*moisture, freeze testing week\*moisture, large origin\* freeze testing week\* moisture and temperature\* freeze testing week\*moisture were significant (Table 6)

## Results

Neither boxes position in the table nor the seedling positions in the boxes were significant (Table 6).

Table 6. Effect of previous summer temperature and moisture conditions together with oak origins and the time of testing into the injuries of seedlings during dormancy period.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Large Origin	119.735	1	119.735	0.192	0.622
Temperature	285716.873	1	285716.873	457.756	0.000
Freeze testing week	1031.119	4	257.780	0.413	0.799
Moisture	1104.360	1	1104.360	1.769	0.184
O * T	2.325	1	2.325	0.004	0.951
O * F	1444.895	4	361.224	0.579	0.678
O * M	350.118	1	350.118	0.561	0.454
T * F	1478.422	4	369.605	0.592	0.688
T * M	2981.788	1	2981.788	4.777	0.029
F * M	8835.572	4	2208.893	3.539	0.007
O * T * F	625.398	4	156.349	0.250	0.909
O * T * M	64.161	1	64.161	0.103	0.749
O * F * M	8745.417	4	2186.354	3.503	0.008
T * F * M	8726.891	4	2181.723	3.495	0.008
O * T * F * M	5546.734	4	1386.684	2.222	0.066
Box edge	891.438	1	891.438	1.428	0,233
Table edge	1438.730	1	1438.730	2.305	0,130
Error	305842.356	490	624.168		

### 3.3.2. Survival

The mean survival of seedlings in the previous summer condition Year 2030 was 99.3% ( $\pm 1.9\%$ ), having practically null mortality. In the growth condition Year 2100 the survival was less, with 80.5% ( $\pm 1.9\%$ ) (Figure 9). During the dormancy experiment, this difference is statistically significant ( $p < 0.001$ ) (Table 7)

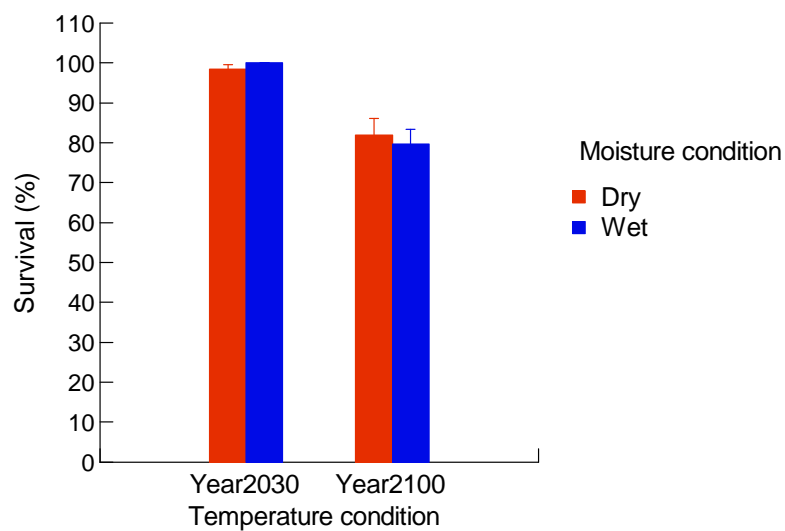


Figure 9. The mean percentage of survival in oak seedlings according of the temperature condition and clustered by moisture condition (wet and dry).

The other relevant source that explain the survival of the seedlings was their origin ( $p < 0.05$ ). The minimum percentage of survival seedlings per origin was 75% ( $\pm 3.7\%$ ) from Malmi origin, and the maximum with 97 ( $\pm 3.7\%$ ) were Latvia origin (Figure 10).

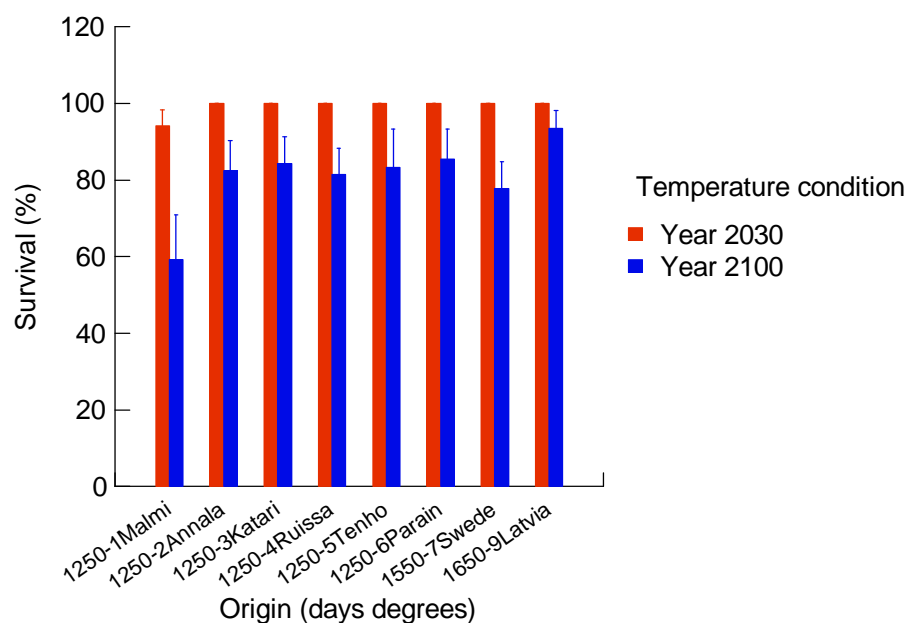


Figure 10. The mean percentage of survival in oak seedlings according of the origin (day degrees) and clustered by growth condition (Year 2030 and Year 2100).

The factors temperature condition and seedling origin was significant, but on the contrary any interactions were significant (Table 7).

Table7. Effect of previous summer temperature and moisture conditions together with oak origins and the time of testing into the survival of seedlings during dormancy period.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Origin	5289.248	7	755.607	3.032	0.008
Temperature	12228.261	1	12228.261	49.073	0.000
Freeze testing week	1092.205	4	273.051	1.096	0.366
O * T	2687.679	7	383.954	1.541	0.169
O * F	7659.023	28	273.537	1.098	0.368
T * F	1030.966	4	257.742	1.034	0.396
T * F * O	7120.331	28	254.298	1.021	0.457
Error	16944.444	68	249.183		



### 3.4. Dehardening

#### 3.4.1. Injuries

The seedlings injury augmented in all growth condition from 5.2% ( $\pm 4.4\%$ ) of average in the first freeze testing in the week 17 to the week 19 with a 59.1% ( $\pm 4.6\%$ ) of average. After this trend, the injury direction decreased in the last freeze testing week, the average was 52.6% ( $\pm 5.0\%$ ), except the growth condition Year 2030 dry where injuries still increase (Figure 11).

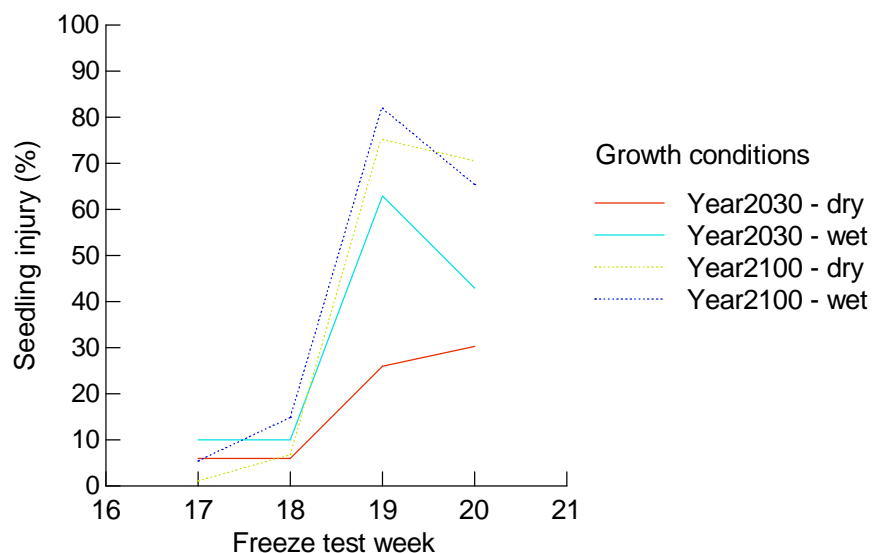


Figure 11. The mean percentage of injuries in oak seedlings according of the freeze testing (weeks) and clustered by growth condition (Year 2030 and Year 2100).

Temperature of treatment ( $p < 0.05$ ), the moisture condition ( $p < 0.05$ ) and their interaction ( $p < 0.05$ ) explained significantly injuries variation (Table 8)

Seedlings grown in Year 2030 condition during previous summer had significantly lower injuries level than seedlings grown in more warm Year 2100 conditions. There were less injuries in dry moisture condition than in the wet condition. The minimum injuries were 13.4% ( $\pm 4.7\%$ ) for Year 2030 dry and 35.2% ( $\pm 4.3\%$ ) for the wet condition. Otherwise, the maximum injuries were 38.8% ( $\pm 4.9\%$ ) for the Year 2100 dry and 41.5% ( $\pm 4.3\%$ ) for the wet condition (Figure 12).

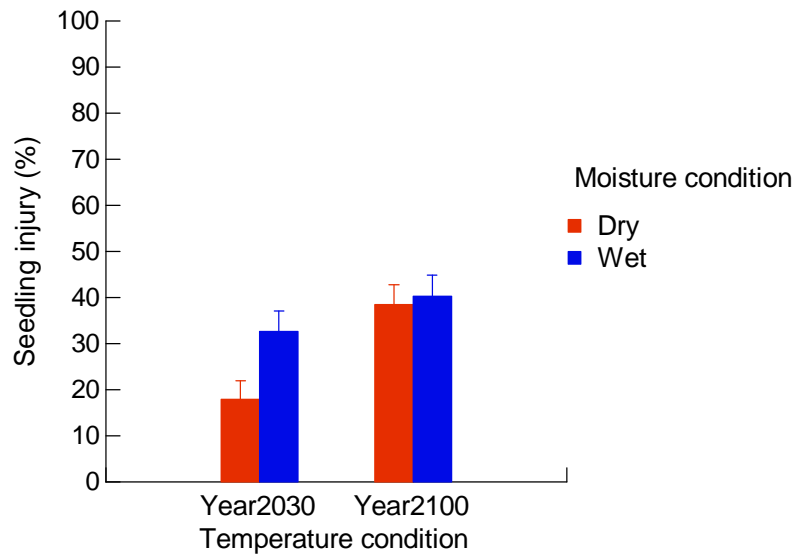


Figure 12. The mean percentage of oak seedlings injuries according of the temperature condition (Year 2030 and Year 2100) and clustered by moisture condition (wet and dry).

Variations of injuries were explained by previous summer temperature, freeze testing week and moisture (Table 8). The variable large origin was not significant ( $p > 0.05$ ) as the differences were less than 0.5 %, thus there were not differences between the Finnish and more Southern seedlings.

There were also some significant interactions to explain the variability; temperature\* freeze testing week, temperature\*moisture, freeze testing week\*moisture, large origin\*temperature\* freeze testing week (Table 8).

The effect of the position of the box in the table was significant ( $p < 0.05$ ), but not the seedling position in the boxes.

Table 8. Effect of previous summer temperature and moisture conditions together with oak origins and the time of testing into the injuries of seedlings during dehardening period.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Large origin	4.065	1	4.065	0.004	0.947
Temperature	10783.164	1	10783.164	11.931	0.001
Freeze testing week	95580.102	3	95580.102	0.004	0.000
Moisture	6544.409	1	6544.409	7.241	0.008
O * T	3.657	1	3.657	0.004	0.949
O * F	503.597	3	167.866	0.186	0.906
O * M	891.104	1	891.104	0.986	0.321
T * F	22986.459	3	7662.153	8.478	0.000
T * M	4027.482	1	4027.482	4.456	0.036
F * M	8394.943	3	2798.314	3.096	0.027
O * T * F	9993.465	3	3331.155	3.686	0.012
O * T * M	938.541	1	938.541	1.038	0.309
O * F * M	4508.667	3	1502.889	1.663	0.175
T * F * M	6962.153	3	2320.718	2.568	0.054
O * T * F * M	2208.521	3	736.174	0.815	0.487
Box edge	1187.051	1	1187.051	1.313	0.253
Table edge	10015.377	1	10015.377	11.081	0.001
Error	283793.275	314	903.800		

### 3.4.2. Survival

The average percentage of survival of the oak seedlings was 83.8% ( $\pm 2.7\%$ ) during dehardening experiment. Previous summer temperature condition did not explain ( $p>0.05$ ) the survival variation (Table 9). The moisture was significant ( $p<0.05$ ) with 88.2% ( $\pm 3.8\%$ ) survival for the dry condition and 79.2% ( $\pm 3.8\%$ ) for the wet condition.

In the first freeze testing week the survival was around 100% not depending much of previous summer growth condition. Then the survival decreased until the freeze testing week 19 with 70% ( $\pm 4.2\%$ ) of survival. In the last freeze testing week the survival increased up to 79.5% ( $\pm 4.2\%$ ) (Figure 13).

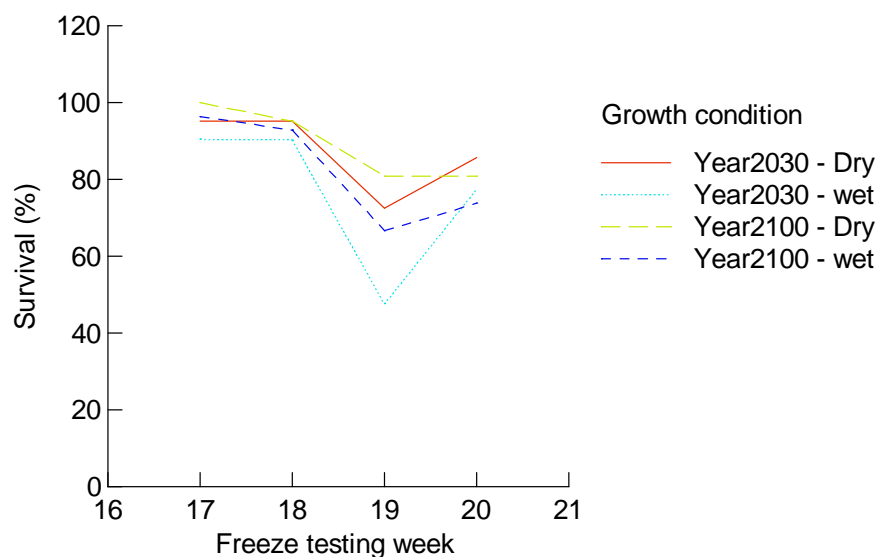


Figure 13. The mean percentage of survival in oak seedlings according of the freeze testing (week) and clustered by growth condition (Year 2030 and Year 2100).

The lowest survival seedling was the origin from Tenhola (Finland) with 68.8% ( $\pm 5.5\%$ ) of average and the highest average was from Ruissalo (Finland) with 95.8% ( $\pm 5.1\%$ ) of survival (Figure 14).

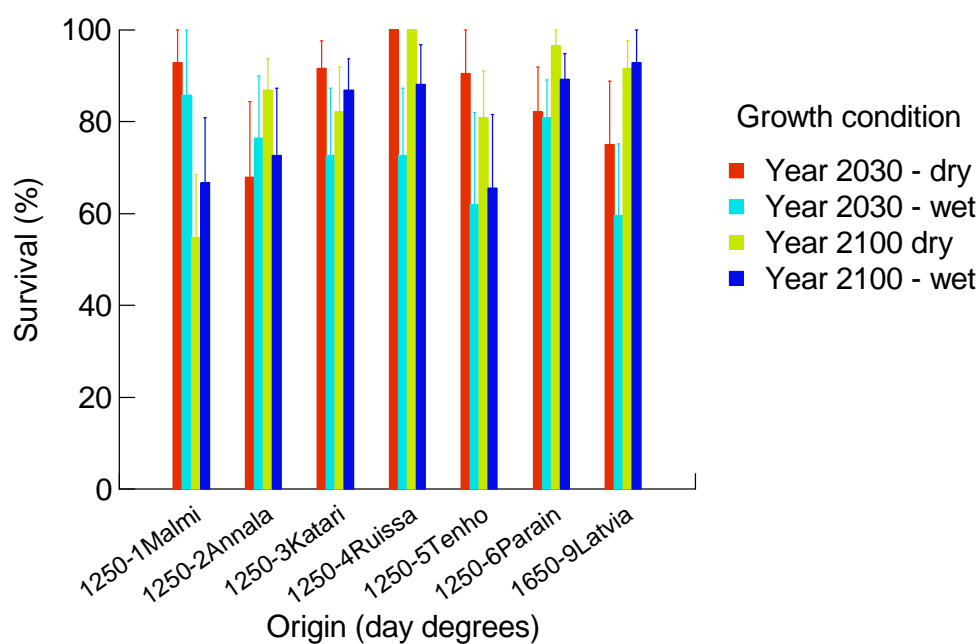


Figure 14. The mean percentage of survival of oak seedlings according of the origin (day degrees) and clustered by growth condition (Year 2030 and Year 2100).

Survival variation was explained significantly by origin and freeze testing week and also their interaction was significant (Table 9). The temperature condition was not significant ( $P > 0.05$ ).

Table 9. Effect of previous summer temperature and moisture conditions together with oak origins and the time of testing into the survival of seedlings during dormancy period.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Origin	9165.267	6	1527.544	3.134	0.010
Temperature	411.793	1	411.793	0.845	0.362
Freeze testing week	14906.174	3	4968.725	10.195	0.000
O * T	5709.005	6	951.501	1.952	0.089
O * F	18407.503	18	1022.639	2.098	0.018
T * F	1212.672	3	404.224	0.829	0.483
T * F * O	10135.403	18	563.078	1.155	0.329
Error	26805.556	55	487.374		

### **3.5. The effect of seedlings size on the injuries**

There were in dormancy experiment a negative correlation,  $r = -0.167$  ( $p = 0.000$ ,  $N = 849$ ) between the seedlings size before the freeze testing and the results of the injuries. In origin level only Malmi,  $r = -0.203$  ( $p = 0.041$ ,  $N = 101$ ) and Sweden,  $r = -0.326$  ( $p = 0.001$ ,  $N = 110$ ) had significant correlations.

There were also negative correlation  $r = -0.102$  ( $p = 0.012$ ,  $N = 615$ ) between the seedlings height and injuries in dehardening experiment. In origin level only Malmi,  $r = -0.215$  ( $p = 0.049$ ,  $N = 84$ ) and Parainen,  $r = -0.208$  ( $p = 0.001$ ,  $N = 110$ ) had significant correlations.

## **4. DISCUSSION**

### **4.1 Experimental process**

Greenhouses create problems because of difficulties in randomize tested material to obtain similar kind of growth conditions. Even though the tables were randomized three times during growing period the difficulties still persisted. One of the main disadvantages was due to the use of automated irrigation in greenhouses. This creates effect on the edge of the tables and boxes. This effect consists in unequal contribution of water by the irrigation system to the seedlings, depending on the location of the boxes in the tables as well as their own seedlings in each box. In the ANOVA model we could include these sources as covariates to determine their effects on the seedlings.

The results of the table location, indicate that the error that provides the unequal amounts of water supplied by the irrigation system together with the competition of plants, influences in the height of the seedling. This also has an influence in the injuries level in dehardening experiment. This results show an error in the process of changing the boxes among the tables or in the methodology used. On contrary, in any case the boxes not were affected fort the water irrigation, so we can assume that seedlings position in the box have no effects.

There is a relationship between the height of seedlings and the percentage of injuries that occur after the freeze testing, having a negative correlation. If the height of seedling decreased, the percentage of injuries inflicted by freeze testing tends to increase.

The seedlings' height depends on their origin. Thus, when analyzing the different parameters (injuries and survival) related to previous summer temperature, moisture and origin, they will be influenced by the height that also depends on their origins. Therefore, the origin is being evaluated twice when using this method. But this has little relevance since the correlation is not strong because it is close to 0.

Experimental measurement of cold hardiness provides a useful means for examination of different hardiness characteristics in plants, and for defining the impact of environmental factors on the level of hardiness, being possible to test conditions not yet available in nature. For testing the cold hardiness level we used the freeze testing, being a good system to simulate the frost situation, but not the possible effects of the snow cover.

## ***Discussion***

The fact of having used greenhouses to simulate the predicted different thermal, as well as the use of freeze testing, provides us a methodology easy to use and replicate. But this only limits to a laboratory level, and it can be different from the results obtained in field experimentation. All in all, Lindén (2002) points out that the results from laboratory tests can be used to supplement and accelerate field experimentation.

### **4.2 Effect of growth condition on oak seedling development**

#### **4.2.1 Survival after growth period**

The average of survival before freeze testing was about 74%, with differences of survival among the origins in excess of 25% units of the average. This is the case of Polish origins with only 48% survival seedlings (figure 6). Probably this was because they were collected in 2008, one year before than the rest of the acorns. García-Fayos (2001) asserts that acorns of *Fagaceae* family, from Mediterranean species, lose their viability quickly under ambient conditions, this fact could explain the low survival of Polish seedlings before the freeze testing. Excluding Poland origin, there were not significant differences among the origins from Finland and the other origins.

#### **4.2.2 Height before freezing test**

The tallest seedlings were from the previous summer temperature condition of year 2030 with 13% higher than seedlings of year 2100 (Figure 7). In lower temperature summer, the oak growth taller (in south Finland) than in high temperature condition. This contrasts with Matala (2005) that show that in warming conditions produced by climate change the trees should growth taller than in lower summer temperature.

It was seen that the seedlings from the wet growth condition was 8.2 % taller than dry condition. For the pedunculate oak seedlings, the lack of water in the dry conditions has a negative effect on their growth.

Origins had a big influence to the height of the seedlings. The seeds from Finland, with a 1250 day degrees in nature growth conditions, had similar growth being the smallest. More Southern origins, was about 33% taller than Finland origins. The tallest origin was from Poland, which also has the highest day degrees, taking the double of height than Parainen. Significantly, there was a relationship between the growth of seedling and the day degree of the seed origin, taking taller seedlings in major temperature sum.



### **4.3 Dormancy state**

The survival of the seedlings to the winter depends on both the ability to reach the maximum capacity of cold hardening, and the timing and rate of cold acclimation, the level of cold tolerance during dormancy and the loss of hardening (Lennartsson 2003). When the oak seedlings is in deep dormancy then can tolerance temperatures about -40°C (Repo et al. 2008). In this experiment we took seedlings from deep dormancy and exposed them to warm conditions and noticed that they cannot tolerance even -10°C temperatures without injuries.

The previous summer growth temperatures seems also have an significant impact on the break-down of dormancy. With higher summer temperature, higher injuries during dormancy break down experiment. Moisture, by itself, had no significant effect.

By the year 2030, the seedlings are in a complete state of cold hardiness, in which they are capable of bearing the simulated frosts, having null injuries and a survival of almost 100%. On the contrary, by year 2100 it can be observed that the frosts will have huge consequences in the deep dormancy, since there is a 50% rate of injuries and a 20% rate of mortality (Figure 8). Thus, comparing the results of both years, we conclude that the highest temperatures, during the period of growth, directly affect the risk of frost damage and survival during the dormancy state.

There are variations in the survival among the different origins, but not in injuries, that as it is observed in the figure 10, largely owe to the low percentage of survival of Malmi and the high ones of Latvia, with a difference of 22% units. Nevertheless, the origin of Malmi is a park type, so it is possible that this fact could slant the results and finally the origin is not significant due to its weak value.

#### **4.4 Dehardening development**

The most critical periods for the survival of the plants are, rather, the spring and autumn periods when cold hardiness levels are low, but frosts are frequent (Lennartsson 2003). In our experiment, we found the injuries that are caused by the frosts, depending on the time of the annual cycle of the trees, through the freezing tests in different dates. According to Kramer and Hänninen (2009), the evaluation of the impact of climate change on tree growth requires the synchronization of the entire annual cycle.

The process of dehardening is crucial for the evaluation of the injuries and the survival, but unlike in dormancy, the temperature of growth is just significant for the injuries. In any case, for the injuries it is relevant and follows the same pattern as in dormancy experiment, having also influence the effect of previous summer temperatures. Being year 2100 the most vulnerable growth condition to the frosts, with a rate of 15% units more injuries than in year 2030.

There is a great dependence of the survival and mortality with regard to the timing of frost (Table 8 and 9). It can be observed that the dehardening process in the figures 11 and 13 in relation to the injuries and survival. Until the week 18, the seedlings are in deep dormancy and are not almost susceptible to the frosts, having injury percentages lower than 10% and a survival average of 90%. The next week (19) is the most crucial since it begins the real loss of hardening and the seedlings are more sensitive to the frosts because they start to growth, presenting percentages of 80% of injury in the seedlings in year 2100 and of 50% in year 2030. And having a remarkable decrease of the percentage of survival, though the previous summer temperature is not significant for the survival (Table 9). From this week onwards, the frosts have a larger effect since the resistance to the frosts has been lost. Finally, in the last week analyzed, we observe large percentages of injuries and survival, but they are lower and higher, respectively, than in the previous week. This fact does not have explication, theoretically it should follow the tendency and have more level of injuries and less of survival. We do not know the reasons of this fact; it might be due to experimental failures or to the need of more experimentation.

As have been observed, the timing of cold hardening is crucial for the survival of seedlings of oak (Jensen and Deans, cited Repo et al. 2007). So the main effect of a milder winters temperatures, due to climate change, can bring forward the lost of frost resistance in spring and therefore, it can increase the risks of frost damage, especially in high latitude ecotypes (Myking and Heide 1995)

Unlike in dormancy, the moisture does explain some of the variability. We observe that the more wet moisture is, in both simulated thermal conditions, the higher the injuries

## ***Discussion***

are, and therefore, the least survival with almost 10 % less than in dry conditions. Thus, the risk in injuries caused by frosts increases with climate change, since this means an increase in moisture.

Likewise in dormancy, there are variations in survival among the different origins, but there are not in injuries. There is no relation between day degrees and origins; the maximum percentage corresponds to Ruissalo and the minimum one to Teenhola, both being Finnish origins. On the contrary to what Kriebel and Wang have exposed in a publication of Beuker (1993) about the bud burst among Northern and Southern origins related to days degree; the Northern origins flush earlier than the Southern origins, which should affect the injuries caused by the frosts in the Northern origins, fact that does not occur in our experiment.

## **5. CONCLUSIONS**

The results show that there are differences of height between Finland origins with more Southern origins. However, these differences are not shown sufficiently in injuries and survival levels to assert, that there are significant differences in oak seedlings frost damage into both groups. In any case, taking into account the percentages of injury and survival of dormancy and dehardening experiments, we can assert that the most adaptable origin to climate change will correspond to the one from Ruissalo, having a natural origin from a forest stand. On the other hand, the least adaptable origins are those from Tenhola and Malmi, having the first one a forest stand origin and the last one an origin from a park. Thus, for managing future plantations of ornamental oak and avoiding the frost damage, the most appropriate origin should be the one from Ruissalo. But, for managing natural stands it should be used the suitable seeds to keep the genetic structure (Vakkari et al. 2006)

Davis and Kabinski (cited in Kramer and Hänninen 2009) have expressed that trees may not have adequate genetic diversity to adapt to the changing environmental conditions, and in fragment landscapes, rapid climate change has the potential to overwhelm the capacity for adaptation in many plant populations and a reduction in their ability to resist from environmental perturbations such extreme climatic events (Jump and Penuelas 2005). On the contrary, (Hamrick and Godt, cited in Kramer and Hänninen 2009) trees have high phenotypic plasticity that allows them to withstand large environmental fluctuations during their lifetime.

Adaptive responses of trees are particularly important at the edges of the geographic distribution of trees because demographic processes at the leading edge differ from those at the rear edge of a species area (Hampe cited in Kramer and Hänninen 2009). At the northern of the distribution of tree species the phenology, particularly the timing of the onset of the growing season, is an important adaptive trait, as it balances the effect of frost. For Scots pine (Leinonen et al. 1997) the onset of active growth and the simultaneous loss of hardening competence caused by high temperatures seem to be the essential factors for dehardening of Scots pine, thus higher winter temperatures in a changing climate may result in an earlier loss of frost hardiness in winter and spring, and increase the risk of damage caused by frosts.

It is shown in our study that on the one hand, the oak seedlings depend heavily on the timing of dehardening. On the other hand, warming summers produced in the year 2100 increase the risk of frost in dormancy state and during dormancy break-down. So, we can conclude that the climate warming will increase the risk of frost damage to pedunculate oak in one year old seedlings

## **Conclusions**

Also, taking into account the vulnerability of the Finland oak stand due to fragmentation (Repo et al. 2007 and Kramer and Hänninen 2009) and the negative effects of frosts in the year 2100, we can deduce that the viability of the stands will be less than the one presented in year 2030.

The oak is a very attractive species for the ornamental use in the Southern Finland. But when predicting the aptitude of these trees for the future climatic conditions, it should be taken into account the results obtained. The thermal conditions that currently present cities like Helsinki, due to urban heat island effect, correspond to those simulated in the year 2030. The monthly average temperature is around 1.2°C higher in the city center than in Helsinki-Vantaa airport (Hongisto 2005). Damage caused in oak seedlings by frost in current years, are lower than those simulated for the thermal conditions of the year 2100. Therefore, the aptitude of oak plantations as an ornamental tree in parks will decline in future years.

Thus, with the results obtained, it is required the necessity of following investigating the effects of climate change on the growth of first year old seedlings of the oak. We do not know whether the effects of climate warming vary considerably in the oaks that are older than one year or in adults. Neither do we know the effects that could have on the natural stands nor did its threats, (already fragmented and threatened), since it has only been used a small-scale simulation and with a greenhouse and not in natural conditions.

Another point to develop deeply would be the study of climate change effects on the oak particularly, and boreal forest in general, in the dormancy state. Since there are hardly any studies previously realized and its evaluation requires more study.

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