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MASTER THESIS

Seed germination of two *Alnus* species

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Declaration:

I hereby declare that I am the sole author of the thesis entitled: “Seed germination of *Alnus incana* and *Alnus glutinosa* in response to different temperature regimes”. I duly marked out all quotations. The used literature and sources are stated in the attached list of references.

In Prague on 30.04.2012

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Valeria Fedorova

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Abstract

Alnus incana and *Alnus glutinosa* species are native and species widely introduced in Europe. It grows naturally in various localities with the different seasonally ranging climate and can adapt to various conditions. The purpose of this study was to analyze the effects of different temperature regimes on the seed germination of *Alnus incana* and *Alnus glutinosa* and to find any significance between species and temperature regimes.

Germination test experiment has been carried out in laboratory to assess an expected effect by using different temperature conditions to seeds germination. After two weeks of cold stratification seeds were incubated under temperature treatments with a light/darkness timer 10/5, 20/5, 25/10 and 25/15. Were calculated the following seed germination parameters: final germination percentage, germination rate index (GRI), germination value and peak value.

Seeds of *Alnus* species had germinated within a time period of about two weeks at all temperatures. Germination was quite rapid, with the peak value being reached on the third or sixth day. Were indicated significant differences on final germination percentage between species only on the third day of the experiment. The same result with temperature regime. Unexpectedly, all variables (excluding the third day) have no major significant differences in the germination behavior and response to alternating temperatures. Effect of temperature regime on germination rate index (GRI) in two species showed significant difference in temperature regimes. Increase in temperature to 25 °C showed a positive effect on germination rate. The temperature regime 20/5 significantly differs with regime 25/15. How it was expected seeds reached final germination percentages (> 50%) over a range of incubation temperatures. We can say that species can germinate well at all temperature regimes in our experiment.

The results from this study confirmed that *Alnus* species can germinate over a wide range of temperatures. The data from this experiment can be used for studying the germination of *Alnus incana* and *Alnus glutinosa species* subjected to understand the temperature requirements for seed germination.

Keywords: *Alnus incana*, *Alnus glutinosa*, seed germination, temperature regime

Abstrakt

Druhy *Alnus incana* a *Alnus glutinosa* jsou v Evropě široce rozšířené. Přirozeně rostou v různých lokalitách se sezónně rozmanitým podnebím a mohou se přizpůsobit různým podmínkám. Cílem této studie bylo analyzovat vliv různých teplotních režimů na klíčení semen *Alnus incana* a *Alnus glutinosa* a případně najít rozdíly mezi danými druhy a teplotními režimy.

Experimentální test klíčení byl proveden v kontrolovaných podmínkách laboratoře za účelem posouzení očekávaného efektu různých teplotních podmínek pro klíčení semen. Po dvou týdnech studené stratifikace byla semena inkubována v různých teplotních a světelných režimech (světlo/tma 10/5, 20/5, 25/10 a 25/15). Následně byly vyhodnoceny parametry klíčení semen: konečné procento klíčivosti, index míry klíčení (GRI), hodnota klíčení a vrcholová hodnota.

Semena druhu *Alnus* vyklíčila v období dvou týdnů při různých teplotách. Klíčení bylo docela rychlé a maximální hodnota klíčení byla dosažena třetí až šestý den. Rozdíly konečného procenta klíčivosti mezi druhy byly významné pouze ve třetím dni experimentu. Stejný výsledek byl pozorován i s různými teplotními režimy. Klíčení (kromě třetího dne) nemělo žádné významné výkyvy v jeho průběhu a reakci na střídání teplot. Vliv teplotního režimu na index míry klíčivosti (GRI) u dvou druhů ukázal významný rozdíl v teplotních režimech. Zvýšení teploty do 25°C ukázalo pozitivní vliv na míru klíčení. Teplotní režim 20/5 se výrazně liší od režimu 25/15. Jak se očekávalo, semena dosáhla konečného procenta klíčení (> 50%) v rozmezí inkubačních teplot. Dle našeho experimentu můžeme říci, že dané druhy mohou dobře klíčit ve všech teplotních režimech.

Výsledky této studie potvrdily, že druhy Alnus mohou klíčit v širokém rozsahu teplot. Údaje z tohoto experimentu lze použít pro studium klíčení druhů *Alnus incana* a *Alnus glutinosa* za účelem pochopení teplotních požadavků pro klíčení semen.

Klíčová slova: *Alnus incana, Alnus glutinosa*, klíčení semen, teplotní režim

Table of Context

1. Introduction

Most seed germination studies can help us to explain the character and response on different factors which influence seed germination of many species in nature. An understanding of seed germination ecology is enhanced by knowledge of the

(1) physiological (germination responses), morphological (development of the embryo), and physical (permeability of coats) states of seeds at the time they matured;

(2) changes in physiological, morphological, and physical states of the seeds that must precede germination;

(3) environmental conditions required for these changes to take place; and

(4) environmental conditions occurring in the habitat between the time of maturation and germination.

Temperature is a major environmental factor which influences on the dormancy states of seeds and seed germination of many different species. Certain temperature conditions, however, require not only the processes of growth and development in general, but also for the individual phases of flowering, fruit ripening, seed germination, seedling emergence, etc.

In this study two Alder species were taken: *Alnus incana* and *Alnus glutinosa*. These species are common in Europe. In Czech Republic it is spread throughout the territory with the exception of the largest mountain ridges and the driest areas. They do not belong to the main economic species, but the specific wet habitats are ecologically important. Alder plantations perform soil protection, reclamation functions to a large extent regulated drains, prevent the formation of avalanches or debris flows and it enriches the soil.

Complete and fresh alder seeds have good germination characteristics. Most other studies argue that freshly seeds of *Alnus* species can germinate without any previous special preparation. Dried seeds require stratification.

These studies are interesting from the point of view of the general biological issues related to seed ecology of *Alnus* species. The results obtained to develop a theoretical understanding the seed germination of this trees.

2. Purpose and aims of the study

The thesis is consisting of two major parts.

The aim of the first part of the thesis is the review of existing literature and studies about seed germination and the ecology of two tree species *Alnus incana* and *Alnus glutinosa*, to analyze the information and data about the seed ecology and germination requirements of Alder species.

The second part is directly related to the investigation about the effects of different temperature regimes on the seed germination of *Alnus incana* and *Alnus glutinosa.* An important aim of this part is to understand and contribute the knowledge about the germination biology of this species. The purpose of the experiment was to:

1) investigate the seeds germination under different temperature conditions,
2) learn the requirements for seed germination,
3) measure seed germination percentage and germination value,
4) find any significance between Alder species and temperature regimes,
5) analyze its interactions.

Our hypothesis was that course of germination for two Alder species will be different, but they will have resembling germination rate. It was also expected, that the germination will be above 50 %.

This was distinguished by testing germination in the laboratory and by analyzing the achieved results.

3. Review of literature

3.1. *Alnus* species

Alder is the common name of a genus of flowering plants (*Alnus*) belonging to the birch family - Familly Betulaceae (MobileReference 2008). The genus *Alnus* includes about 30 species of deciduous trees and shrubs occurring in North America, Europe, and Asia and in the Andes Mountains of Peru and Bolivia (Harrington et al. 2008). In Czech Republic it is spread throughout the territory with the exception of the largest mountain ridges and the driest areas.

3.1.1. *Alnus glutinosa* (L.) Gaertn

The best-known species in Europe is the Common or Black Alder (*Alnus glutinosa*), native to most of Europe and widely introduced elsewhere (MobileReference 2008).

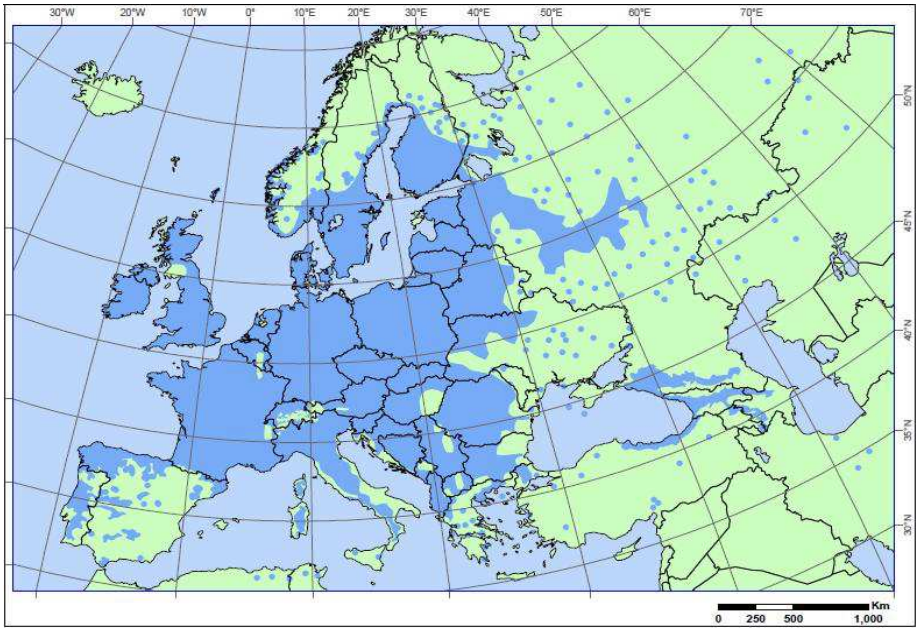


Figure 1. Distribution map of Black alder (*Alnus glutinosa*). (European forest genetic resources programme, www.euforgen.org)

European alder has a broad natural range that includes most of Europe and extends into North Africa, Asia Minor, and western Siberia (Figure 1). Densest distribution is in the lowlands of northern Germany, northern Poland, White Russia, and the northwestern Ukraine. It is naturalized throughout the North America and maritime Canada (Funk 1990). It means that Black Alder is specie able to adapt to very variable climatic conditions (Paule and Gomory 2002). It grows naturally in various temperate localities with the climate ranging from seasonally cold to warm. Under conditions similar to Central Europe, it may be regarded as lowland species as it occurs in the mountains rather sporadically and is replaced at higher altitudes by *Alnus incana* (Forestry Compendium 2012). In Czech Republic it is widely distributed.

Figure 2. *Alnus glutinosa* stand, České Budějovice, Czech Republic

It occupies primarily sites with flowing ground water along streams, but also sites with stagnating ground water like peat bogs (Paule and Gomory 2002). Black alder grows in early successional forest, forests edges, floodplain forest, forest and shrub wetlands, roadsides, yards or gardens. It is adaptable to poor or dry soils (USDA Forest Service

2006). Figure 2 shows the *Alnus glutinosa* forest near České Budějovice, Czech Republic. It is a good example of the wetland forest preference of this specie. *Alnus glutinosa* grows better on flat areas or even in hollows. It is one of the most suited species for planting on the banks of lowland rivers and streams, and along ditches, lakes and ponds (Forestry Compendium 2012).

Alder is monoecious, so each tree bears both male and female flowers. Male catkins are dark yellow-brown in color, and are up to 5 cm long when they are fully open (Figure 3). At 6 mm. in length, the female flowers are much smaller in size, and are red, erect and cone-like in shape. The flowers appear before the new leaves, in March (or early April), and pollination is by the wind. Pollinated female flowers grow into ovoid fruits about 1.5 cm. in length, which are green in color and grow in clusters of up to 4 at the end of twigs. These ripen and turn woody by October, and release a number of small flat red-brown seeds, each weighing about 0.004 gm (Featherstone 2012).

Figure 3. Male catkins of *Alnus glutinosa,* Chomutov, Czech Republic

The best seeds usually fall first (Funk 1990). The seeds have small 'wings', which are air-filled membranes that enable them to float on water, and dispersal is by both wind

and water. Seeds have been recorded as germinating on the surface of water, and then rooting successfully when they are washed up on land. The empty cones can persist on the tree until the following spring and are a distinctive feature of the alder tree in winter (Featherstone 2012).

Mature alders, at age from 3 to 30 according to the ecotypes and the stand conditions, produce plentiful seed every 3 or 4 years. Germination rates are highly variable, ranging from 10 to 90 per cent according to the crop year and the stand. Mature trees of Black Alder produce near 4000 cones (Claessens et al. 2010).

Alder stands are very important habitats for the survival of many rare plants and animals. One of the reasons for this is that the cones open gradually and release the seed throughout the winter, and are therefore a reliable source of food. Its rapid growth, tolerance of acid soils, and nitrogen-fixing ability, mean that *A. glutinosa* is a desirable species for shelterbelts, mine-spoil rehabilitation, biomass production and landscapes (Forestry Compendium 2012).

3.1.2. *Alnus incana* (L.) Moench

Alnus incana (Grey or Speckled Alder) is a species of alder with a wide range across the cooler parts of the Northern Hemisphere (MobileReference 2008). It is mainly a mountain tree species, but it has been successfully introduced into lowland sites in Europe (Forestry Compendium 2012).

The natural distribution area of Grey Alder stretches approximately from latitude 71°N in Scandinavia to 41°N in the mountains of southern Europe. The species grows spontaneously in northern, central and Eastern Europe, western Asia and in mountain regions of southern and southwestern Europe (Figure 4). In some regions it is difficult to distinguish natural localities from introduced ones, as the species is often planted and easily proliferates from cultivation into the wild (Forestry Compendium 2012).

Figure 4. Distribution map of Grey alder (*Alnus incana*). (http://is.muni.cz/ 2010)

This specie can be finding at different sites: stream banks, temporary watercourses along the roads, moist sites at the foot of upland, grassy bogs, felled and burnt areas, abandoned meadows and plough-lands (Banaev and Bažant 2007). It is a light-demanding, fast-growing tree that is tolerant and grows well on poorer soils. It does not require moist soil (MobileReference 2008). It also grows in sandy loams, grey forest soils, miner tropic peat lands, alluvial soils, and ericaceous bogs, on both poorly drained and well-drained sites (Rook 2004). *Alnus incana* grows naturally in temperate climates similar to *Alnus glutinosa*. However, it stretches far more to the north and grows to higher altitudes in mountain areas. Consequently, Grey Alder is more tolerant of low temperatures than is Black Alder (Forestry Compendium 2012).

It is monoecious tree. Male catkins 7-10 cm long, red-brownish (with yellow anthers), clustered in groups of 2-5 pieces on the twigs (Figure 5). Female catkins are about 3 mm long, red-brown, clustered in groups of 2-5 horizontally (Forestry Compendium 2012). The flowering takes place two weeks earlier than that of Common

alder, that is from March to May depending on the region and altitude; it precedes leaf opening (Suszka et al. 1996).

Figure 5. Grey alder (*Alnus incana*). (Priroda.cz)

Maturation of the seeds is a little earlier than for Common Alder that is from September to November according to the region and altitude. Green to begin with, they gradually turn to a brilliant brown during maturation. Catkins open before winter and the ripe seeds are dispersed from September to December depending on the region and altitude. All winter and sometimes even longer catkins can be seen hanging from the branches (Suszka et al. 1996).

Grey Alder produces abundant seed. The fruits are small, single-seeded nutlet with a narrow wing (Haeussler and Coates 1986). Seeds are transported by the wind for longer distances than those of Common Alder because of their lighter weight and their larger wings (Figure 6). Seeds can be transported by water (Suszka et al. 1996). In general, wet weather following dry weather closes the strobiles, thus terminating a dispersal event (Bonner et al. 2008).

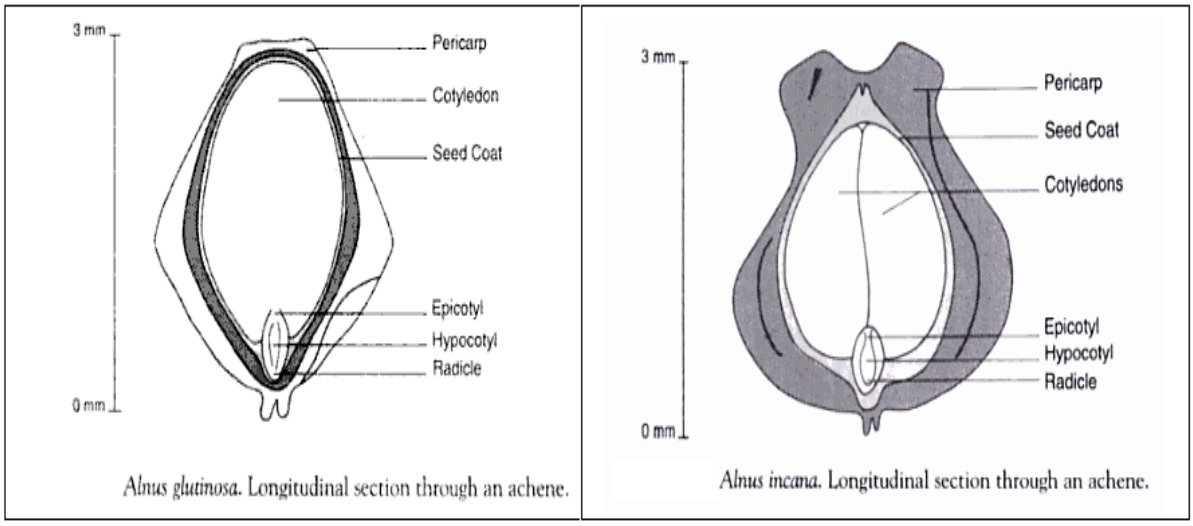


Figure 6. *Alnus glutinosa* and *Alnus incana* longitudinal section through an achene (Suszka et al. 1996)

Alnus incana, as a pioneer nitrogen-fixing species, is utilized in afforestation of bare land. In mountain agricultural areas it often grows on the banks of streams and plays a significant role in erosion control. Grey Alder seems to be potentially suitable for shelterbelts and windbreaks along ditches in sub-mountain regions (Forestry Compendium 2012).

3.2. Seed Dormancy

Plants have evolved many mechanisms and processes that ensure survival. Some species produce prodigious numbers of seeds, so that even if only a tiny proportion germinate and grow, some seedlings will survive. In others, germination at unfavorable times is prevented by a mechanism that is commonly described as dormancy (Bonner et al. 2008).

The regeneration of plant communities from seed depends on seeds being in the right physiological state to germinate in the right place at the right time. In some species, this requirement is satisfied by a regeneration strategy in which seeds germinate as soon as they are shed (Fenner 2000).

Despite the fact that there are enough studies about seed dormancy, it is difficult to find an exact definition of this term. It is a multiple and complex phenomenon (Viémont and Crabbé 2000). Some authors consider that: the frequently used definition of seed dormancy by Harper (1959) is "*the absence of germination of a viable seed under conditions that are favorable to germination*" (Bradford and Nonogaki 2007). But there is some deficiency. In another source that term is defined by Simpson (1990) as: "*the failure of a viable seed to germinate, after a specific length of time, in a particular set of environmental conditions that allow germination after the restrictive state has been terminated by either natural or artificial conditions"* (Nicolás et al. 2003). This term seems more appropriate and profound. Therefore, seed dormancy is caused by a block to the process of germination within the imbibed seed. Dormancy is most easily observed, measured and defined negatively as the failure of a viable seed to germinate, given moisture, air and a suitable constant temperature for radical emergence and seedling growth (Bradford and Nonogaki 2007).

Timing of seed germination can be critical for the survival of natural plant populations, and dormancy mechanisms play a major role in such timing. These mechanisms are pronounced in many ruderals and other species from habitats that are subject to disturbance. Many trees, particularly temperate and tropical species from undisturbed forest, lack pronounced dormancy. The germination of these recalcitrant seeds typically occurs quickly after dispersal (Lambers et al. 2008).

The seeds of some species remain dormant under all conditions, and the seeds of others are only dormant under some conditions. There are a number of natural processes, seasonal stimuli or combinations of the two which overcome dormancy and stimulate germination. And there are a number of artificial substitutes which humans can use to mimic such 'dormancy breakage'. These are called pre-(sowing) treatments or pretreatments for short (Gosling 2007). The stimulatory effects on germination of holding imbibed seeds at low temperatures have been recognized for centuries. Optimum temperatures for the dormancy-breaking effect of chilling are generally close to 5°C (Fenner 2000).

A *dormant* seed (primary or innate dormancy) is one that will not germinate under any combination of normal physical environmental factors that otherwise is favorable for

its germination (Baskin and Baskin 2003). In a dormant seed, the chain of events that leads to germination of the seed is blocked. This block, and hence dormancy itself, can be relieved by a specific factor or combination of factors (e.g., light, temperature regime, and/or specific compounds) (Lambers et al. 2008).

A *non-dormant* seed (secondary or induced dormancy), on the other hand, will germinate over the widest range of normal physical environmental factors possible for the genotype (and considering maternal effects). The non-dormant seed that does not germinate because of the absence of one or more of these physical environmental factors is said to be in a state of quiescence (Baskin and Baskin 2003).

In some cases environmental factors, such as the absence of light, $NO3^-$ and/or a diurnally fluctuating temperature, may keep seeds in a dormant state. It called enforced dormancy (Lambers et al. 2008). The term dormancy is used here because these environmental factors function as an environmental signal that removes a block leading to germination, rather than being involved in metabolism, as is the case for environmental factors such as water, O2, and temperature (Lambers et al. 2008).Enforced dormancy is not always considered as a form of dormancy, but as a mechanism that prevents germination (Lambers et al. 2008).

A number of classification schemes have been proposed to describe seed dormancy. An additional classification is based on the timing of the occurrence of dormancy: *primary dormancy* refers to the type of dormancy that occurs prior to dispersal as part of the seed developmental program, whereas *secondary dormancy* refers to the acquisition of dormancy in a mature seed after imbibition as a result of the lack of proper conditions for germination (Bradford and Nonogaki 2007) Transitions among the various forms of dormancy are illustrated in Figure 7 (Lambers et al. 2008).

The term *secondary dormancy* is used for the type of dormancy that is imposed after seeds have lost primary dormancy. Secondary dormancy may be the result of a prolonged inhibition of germination. The occurrence of secondary dormancy is highly relevant to seed behavior in the soil seed bank as it is central to so-called dormancy cycling. The concept of dormancy cycling has been developed to explain seasonal flushes of seedling emergence of annual temperate species. Dormancy cycling involves repeated

induction and termination of dormancy parallel to seasonal variations in temperature. In this way seeds avoid germination during short favorable spells within the unfavorable season and germinate just prior to the favorable season for plant growth (Bradford and Nonogaki 2007).

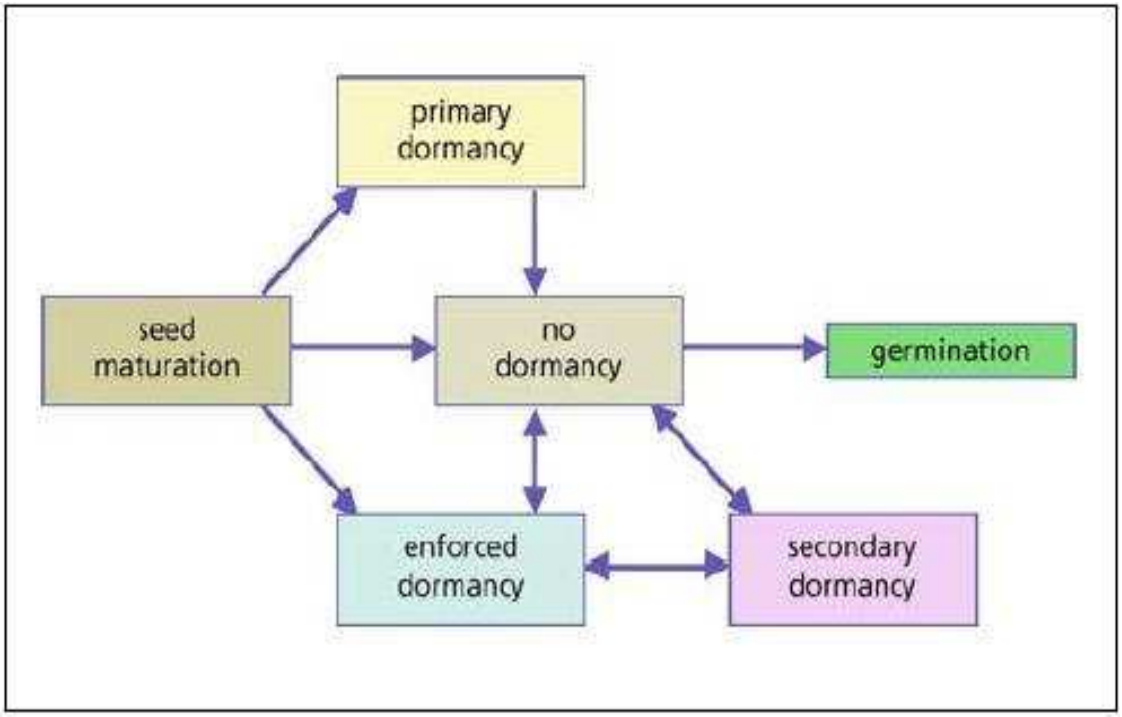


Figure 7. Schematic representation of changes in dormancy after seed maturation (Lambers et al. 2008)

Most classifications in use are derived from the one that was proposed by Nikolaeva (1977) on the basis of seed morphology (Table 1). An additional classification is based on the timing of the occurrence of dormancy: *primary dormancy* refers to the type of dormancy that occurs prior to dispersal as part of the seed developmental program, whereas *secondary dormancy* refers to the acquisition of dormancy in a mature seed after imbibition as a result of the lack of proper conditions for germination (Bradford and Nonogaki 2007). According to Nikolaeva (1969, 1977) there are two types of organic seed dormancy: endogenous and exogenous (Table 1) (Baskin and Baskin 1998).

Type	Cause	Broken by
Endogenous dormancy		
Physiological	Physiological inhibition mechanism (PIM) of germination	Warm and/or cold stratification
Morphological	Underdeveloped embryo	Appropriate conditions for embryo grow/germination
Morphophysiological	PIM of germination and underdeveloped embryo	Warm and/or cold stratification
Exogenous dormancy		
Physical	Seed (fruit) coat impermeable to water	Opening of specialized structure
Chemical	Germination inhibitors	Leaching
Mechanical	Woody structures restrict growth	Warm and/or cold stratification

Table 1. Classification scheme of seed dormancy types (Baskin and Baskin 1998)

Seeds with endogenous dormancy fail to germinate because of factors within the embryo (Geneve 2003). Physiological and morphophysiological are the two major types of endogenous dormancy found in tree species. Morphological dormancy is a third type of endogenous dormancy, but it is most often seen in herbaceous plants (Geneve 1998).

Seeds with physiological dormancy require a period of moist, chilling to satisfy dormancy. A moist, chilling period is called stratification. In nature, physiological dormancy is satisfied by having the seeds in moist soil over the winter. The same conditions can be simulated by keeping the seeds in a plastic bag containing a moist substrate (sand or vermiculite) in the refrigerator for several months. The optimum temperature for stratification is between 1 and 5^0C, which is about the temperature of most refrigerators (Geneve 1998). Endogenous physiological dormancy can be separated into three types based on their "depth" of dormancy. These include nondeep, intermediate, and deep dormancy (Baskin and Baskin 1998). Physiological dormancy at a nondeep level is the most common kind of dormancy in seed banks in temperate climates and occurs in gymnosperms and in all major clades of angiosperms (Lambers et al. 2008). Characteristics of physiological dormancy are shown in the Table 2.

Deep
• Excised embryo produces abnormal seedling. • Gibberellic Acid (GA) does not promote germination. • Seeds require *ca*. 3–4 months of cold stratification to germinate.
Intermediate
• Excised embryo produces normal seedling. • GA promotes germination in some (but not all) species. • Seeds require 2–3 months of cold stratification to break dormancy. • Dry storage can shorten the cold stratification period
Non-deep
• Excised embryo produces normal seedling. • GA promotes germination. • Depending on species, cold (ca. 0–10°C) or warm (≥15°C) stratification breaks dormancy. • Seeds may after-ripen in dry storage. • Scarification may promote germination.

Table 2. Characteristics of dormancy in seeds with deep, intermediate, and non-deep physiological dormancy (Baskin and Baskin 2003).

Morphological dormancy occurs in seeds where the embryo is not fully developed at the time of seed dissemination. Enlargement of the embryo occurs after the seeds have imbibed water and before germination begins. Embryo growth usually occurs by imbibing seeds at warm temperature. Embryo development can take weeks to months to be completed before seedlings finally emerge (Geneve 2003).

The most complex form of endogenous dormancy is displayed by seeds with morphophysiological dormancy (Geneve 2003). This dormancy occurs in seeds with rudimentary or linear embryos, and as the name indicates it is a combination of

morphological and physiological dormancy (Baskin and Baskin 1998). Depending on the species, embryo growth and dormancy break may require (Baskin and Baskin 1998):

(1) warm (≥ 15 °C) stratification only,

(2) cold (0-10 °C) stratification only,

(3) warm followed by cold stratification, or

(4) cold followed by warm followed by cold stratification.

In nature, seeds with morphophysiological dormancy can take several years to germinate because they need to be exposed to summer and winter conditions. To get quicker germination, these seeds can be placed moist in a warm place (about 21 °C) for several months before being moved to the refrigerator for several months more (Geneve 1998).

Exogenous dormancy is imposed upon the seed from factors outside the embryo including the seed coat and/or parts of the fruit. This type of dormancy is commonly referred to as physical dormancy or hard seeds (Geneve 2003).

The major type of exogenous dormancy is called physical dormancy and these are often called hard seeds. Physical dormancy is caused by the outer seed coverings preventing the seed from taking up water. In nature, physical dormancy is most often satisfied by exposing the seed to high temperature conditions. Since this can take many years, gardeners treat seeds with physical dormancy by scarification. The three most common ways to scarify seeds include hot water, acid, or scratching the seed surface (Geneve 1998). In nature, exposure to high temperature or extreme fluctuating temperatures is the most likely cause of dormancy release (Geneve 2003).

Chemical dormancy as the name implies is caused by germination inhibitors which accumulate in the fruit and seed covering during development (Arteca 1996). This type of dormancy is broken by removal of the pericarp or leaching of the fruits (Baskin and Baskin 1998).

And the last one is the mechanical dormancy. This dormancy means that seed coverings, mostly woody fruit walls, prevent embryo penetration. Different types of

dormancy frequently occur together (e.g. morphophysiological dormancy and physical plus physiological dormancy) (Willan 1987).

Dormancy is an adaptive trait which seeds acquire during their development and maturation, and can be defined as the repressive state which temporarily blocks germination. Dormancy loss can be affected by a number of environmental factors like light, temperature, nutrients, drought, after-ripening and fire. Plants have evolved different strategies to utilize dormancy to their advantage. Some plant species produce seeds which are non-dormant, while others produce seeds which are weakly or deeply dormant (Pua and Davey 2010). Other plants can produce seeds with different degrees of dormancy, depending on environmental stimuli, and the dormancy can be maintained even if the external conditions are suitable for germination. Seed dormancy probably evolved as a mechanism for permitting germination when the environmental conditions become more conducive to growth and reproductive success (Pua and Davey 2010).

3.3. Seed germination

Seed germination is the emergence of a new plant from seed. A quiescent embryo contained in a dry seed becomes active after hydration (imbibition), although dormant seeds still do not germinate after full hydration under optimal conditions. In a narrow scientific definition, seed germination is complete when the radicle penetrates through the covering tissues such as the testa (seed coat) and endosperm (Pua and Davey 2010). It occurs when the growth potential of the embryo can overcome the constraints imposed by the covering structures (Bradford and Nonogaki 2007).

It is the event that marks the transition between two developmental stages of a plant: seed and seedling. The seed has a package of food reserves that makes it largely independent of environmental resources for its survival (Lambers et al. 2008). It includes numerous events, e.g. protein hydration, subcellular structural changes, respiration, macromolecular synthesis, and cell elongation, none of which is itself unique to germination (Bewley and Black 1994). The visible sign that germination is complete is usually the penetration of the structures surrounding the embryo by the radicle; the result is often called visible germination (Bewley 1997).

Germination consists of three overlapping processes (Willan 1987):

(1) Absorption of water mainly by imbibition, causing a swelling of the seed and eventual splitting of the seed coat,

(2) Enzymatic activity and increased respiration and assimilation rates which signal the use of stored food and translocation to growing regions,

(3) Cell enlargement and divisions resulting in emergence of radicle and plumule.

Components of the germination process, however, may occur in a seed that does not achieve radical emergence. Even when conditions are apparently favorable for germination, so that inhibition, respiration, synthesis of nucleic acids and proteins, and a host of other metabolic events all proceed, culmination in cell elongation does not occur, for reasons that are still poorly understood; such a seed expresses dormancy (Bewley and Black 1994).

3.3.1. Temperature and germination

Seed germination is a complex process involving many individual reactions and phases, each of which is affected by temperature (Copeland and McDonald 2001). The influence of temperature on foxtail seed dormancy changes with the time and development (Inderjit 2004.). The response to temperature depend on a number factors, including the species, variety, growing region, quality of the seed, and duration of time from harvest (Copeland and McDonald 2001).

Roberts (1988) recognized three separate physiological processes in seeds affected by temperature: firstly, temperature, together with moisture content, determines the rate of deterioration in all seeds; secondly, temperature affects the rate of dormancy loss in dry seeds and the pattern of dormancy change in moist seeds; and, thirdly, in non-dormant seeds temperature determines the rate of germination (Fenner 2000).

Often the same temperatures can have opposite effects on seeds depending on their dormancy state (Inderjit 2004.). As a general rule, temperate-region seeds require lower temperatures than do tropical-region seeds. High-quality seeds are able to germinate

under wider temperature ranges than low-quality seeds (Copeland and McDonald 2001). The optimum temperature for most seeds is between 15 and 30 °C (Copeland and McDonald 2001). Temperatures of 20-25 °C can rapidly promote germination of non-dormant and near complete after-ripened seeds, but dormant seeds at hose temperatures after-ripen very slowly or not at all (Inderjit 2004).

In seasonal climates, temperature is of course a good indicator of the time of year and is therefore implicated strongly in determining the timing of germination (Fenner and Thompson 2005). Two main types of responses are discerned in climates with seasonally changing temperatures: summer and winter annuals (Lambers et al. 2008).

At summer annuals species produce seeds in autumn and germinate in the spring (Lambers et al. 2008). When seeds Are dispersed in the autumn, they are either truly dormant or in a state of relative dormancy. If in a state of relative dormancy germination is possible only over a restricted range of high temperatures. Since prevailing temperatures are below these values, germination is effectively prevented. During the winter, dormancy is released as a result of chilling (Fenner 2000). A long exposure (1—4 months) of imbibed seeds to low temperature (approximately 4°C; stratification or chilling) relieves dormancy by gradually decreasing the minimum temperature for germination. This seasonal change in dormancy restricts germination to spring, the beginning of the most suitable season for growth in temperate climates (Lambers et al. 2008). During early spring as soil temperatures begin to rise, the temperature range for germination and prevailing temperatures overlap and germination occurs, providing that the requirement for other factors, such as light (Fenner 2000).

Winter annuals set seed in spring and early summer; they generally germinate in autumn (Lambers et al. 2008). When seeds are dispersed in late spring/early summer, they are either truly dormant or in a state of relative dormancy. If in a state of relative dormancy, germination is possible only over a restricted range of low temperatures. Since prevailing temperatures at this time of the year are above these values, germination is prevented. During the summer, exposure to high temperatures results in dormancy release via the processes of dry after-ripening and warming (Fenner 2000). Exposure to relatively high summer temperatures gradually relieves the dormancy by increasing the maximum temperature that allows germination. This occurs even without imbibitions (Lambers et al.

2008). This seasonal dormancy pattern causes the seeds to germinate in autumn (Lambers et al. 2008). During the autumn, as soil temperatures begin to fall, the temperature range for germination and prevailing temperatures overlap and germination occurs (Fenner 2000). It is the most suitable season for many species from Mediterranean climates (Lambers et al. 2008).

In a series of studies on geographical variation in germination temperature in Europe some scientists concluded that both minimum and maximum temperatures for germination varied consistently along a north--south gradient; both were lower in Mediterranean species compared with those from northern Europe. Indeed, some workers have identified a typical 'Mediterranean' germination syndrome, a key feature of which is a rather low optimal temperature (typically 5- 15 °C) for germination. At the opposite extreme, Arctic species need higher temperatures for germination. In northern Europe, the priority is to avoid germinating during or immediately before the severe winter, which often seems to be best arranged by needing relatively high temperatures for germination (Fenner and Thompson 2005).

Seeds of temperate woody plants can germinate over a wide range of temperatures, from a minimum of 2 or 3 °C, to a maximum of about 45 °C. Natural seedbeds do not remain at constant temperatures, but experience diurnal fluctuations from lows at night to highs in the daytime. Most temperate woody plants have adapted to these conditions and germinate most rapidly at alternating temperatures of approximately 20 °C at night and 30 °C in the daytime. Other species germinate faster at lower temperatures regimes, for example, 15 to 25 °C or 10 to 20 °C, or at constant temperatures of 5 to 22 °C (Bonner et al. 2008).

Despite the dominant role of temperature in the control of seasonal patterns of dormancy in seeds, it is important to note that temperature does not act alone. Several studies have clearly demonstrated that other factors – for example, light, nitrate and desiccation – can all influence the expression of dormancy in seeds (Fenner 2000).

3.3.2. Light and germination

Light is one of the environmental factors that can influence germination of the seeds. The ability to detect different aspects of the light environment enables the seed to have at least some control over where and when germination takes place (Fenner and Thompson 2005). The ultimate effect of light on seeds depends on genotype and on environmental factors during ripening of the seeds, during dormancy and during germination itself. These environmental factors may be light or factors other than light (Fenner 2000).

The light climate under natural conditions has many components, some of which are used by seeds for regulation of dormancy. Three major types of light responses can be distinguished: light requirement, light intensity and duration of exposure and last one spectral composition of daylight (Lambers et al. 2008).

A light requirement prevents germination of seeds that are buried too deeply in soil. Such seeds germinate only when exposed to light, and thus do not germinate below a soil depth where no light penetrates (Lambers et al. 2008). The most obvious significance of a light requirement for dormancy breaking is avoidance of germination too deep in the soil for the seedlings to reach the surface on the available nutrient reserves (Fenner 2000). Most light-requiring seeds are small (Fenner 2000). Germination occur only when the soil is turned over or the seeds otherwise reach the soil surface where they are exposed to light (Lambers et al. 2008).

In studying the light requirement for germination, it is important to test seeds at a daily photoperiod and in continuous darkness at each of the daily alternating temperature regimes. Seeds need to be tested in light and darkness when they are freshly matured and at regular intervals during the dormancy-breaking period because their light requirement may change as they come out of dormancy (Baskin and Baskin 1998).

Light intensity and duration of exposure (photon dose, integrated over a period of time) determine whether dormancy enforced by darkness is broken. A steep light gradient exists near the soil surface (Lambers et al. 2008). Higher light intensity or temperature may stimulate germination, but to a greater or lesser degree (or have no effect), depending

on the species (Fenner 2000). The influence of light intensity on different species varies greatly (35).

The spectral composition of daylight as modified by a leaf canopy also influences the timing of germination after disturbance of vegetation (Lambers et al. 2008). The greatest promotion of germination occurs in the red area (660-700 nm) with a peak at 660 nm, followed by inhibition zone in the far-red area above 700 nm (Copeland and McDonald 2001). The seeds may subsequently get mixed into the soil, where a light requirement further enforces dormancy and where the risks of predation are smaller than at the soil surface (Lambers et al. 2008).

Day length and other environmental factors may contribute to the phenotypic plasticity and diversity of seed germination in many plant species. The germinability of seeds of many species is affected by day length during seed development and maturation. In some plant species, short days result in higher germinability (Fenner 2000). In some cases, day length plays a part in determining the timing of germination (Fenner and Thompson 2005). Day-length detection is often highly dependent on the temperature regime, especially chilling. Photoperiod sensitivity is likely to increase in importance with latitude because of the large seasonal variation in day length. A means of detecting day length would enable seeds to limit germination to favorable seasons (Fenner and Thompson 2005). In all these situations, the ability to detect the intensity, quality or periodicity of the light provides the seed with information it requires about its environment (Fenner and Thompson 2005).

Temperature itself has dual effects. It affects germination and the dormancy status of the seed. A seed may be light-requiring at one temperature but not at another one; e.g. lettuce seeds do not require light at low temperatures, but do at higher ones (Fenner 2000).

3.4. Seed storage behavior

Storage may be defined as the preservation of viable seeds from the time of collection until they are required for sowing (Willan 1987). Seed storage is the

preservation of viable seed until their sowing/requirement (Ahlawt and Bisht 1999). Seeds are generally categorized into the following types (Berjak and Pammenter 2003):

• **Orthodox s**eeds that can be dried, without damage, to low moisture contents, usually much lower than those they would normally achieve in nature. Their longevity increases with reductions in both moisture content and temperature over a wide range of storage environments.

These seeds can be stored for relatively long periods at subfreezing temperatures—if their moisture contents are reduced to about 5 to 10% (wet weight basis). Most species of the economically valuable tree genera of the Northern Temperate Zone are classified as having true orthodox seeds: fir (*Abies* P. Mill.), alder (*Alnus* P. Mill.), birch (*Betula* L.), ash (*Fraxinu*s L.), larch (*Larix* P. Mill.), spruce (*Picea* A. Dietr.), pine (*Pinu*s L.) etc (Bonner et al. 2008).

• **Recalcitrant s**eeds that do not survive drying to any large degree, and are thus not amenable to long term storage.

Seeds cannot be desiccated but can be stored at or slightly below freezing Genera with temperate-recalcitrant seeds include buckeye (*Aesculus* L.), chestnut (*Castanea* P. Mill.), oak (*Quercus* L.), and redbay (*Persea* P. Mill.) (Bonner et al. 2008).

• Intermediate seeds that are more tolerant of desiccation than recalcitrant's, though that tolerance is much more limited than is the case with orthodox seeds, and they generally lose viability more rapidly at low temperature.

These seeds can be dried to moisture levels almost low enough to meet orthodox conditions (12 to 15%) but are sensitive to the low temperatures typically employed for storage of orthodox seeds. Viability is retained usually only for a few years (Bonner et al. 2008).

Storage environment is obviously very important in extending the life of seeds. The general objective is to reduce the metabolism of the seeds as much as possible without damaging them and to prevent attack by microorganisms (Bonner et al. 2008).

3.5. Seed dormancy and germination of *Alnus* spp.

The female catkins of alder are woody cones; the cones contain oval nutlets that do not break up at maturity (Carole 1997). Color is also a good indicator of maturity: immature cones are green whereas mature cones are mottled shades of green, yellow, gray, or brown. Strobiles should be collected as soon as they are ripe, for the largest seeds with the best germinability are usually released first. Thus, both seed quality and seed yield are higher if collections are made in the fall rather than in the winter or spring (Harrington et al. 2008).

Both species seeds are orthodox (Table 3) - seeds that can be dried without harm, and once dried can be frozen, stored for years with little deterioration and relatively easily revived (Forest Research 2012). It is shallowly dormant species. They have the potential to be dried and stored, if required (Gosling 2007).

Scientific name	**Common name**	**Storage/dormancy characteristics**	**Storage moisture content and temp-res**	**Pretreatment weeks warm (15°C)**	**Pretreatment weeks (4 ° C)**
Alnus cordata	Alder (Italian)	Orthodox/Shallow	8-10% / <4°C	0	6 (3-9)
Alnus glutinosa	Alder (black or common)	Orthodox/Shallow	8-10% / <4°C	0	6 (3-9)
Alnus incana	Alder (grey)	Orthodox/Shallow	8-10% / <4°C	0	6 (3-9)
Alnus rubra	Alder (red)	Orthodox/Shallow	8-10% / <4°C	0	6 (3-9)
Alnus virdis	Alder (green)	Orthodox/Shallow	8-10% / <4°C	0	6 (3-9)

Table 3. Storage and pretreatment and characteristic of *Alnus* species (Gosling 2007)

It is important to note, that some untreated seeds will germinate, but pretreatment will usually stimulate quicker germination of more seeds over a wider range of conditions (Gosling 2007). Alders shallow dormancy characteristics can make them a little more challenging to germinate, especially to achieve the maximum germination possible (Gosling 2007).

In natural conditions the seeds of Common Alder germinate easily, but the germination depends upon the presence and quality of the light. In the dark these seeds do not germinate or only poorly. The germination is enhanced by daylight (Suszka et al. 1996). Although European Alder seeds can germinate immediately after they are shed, stratification and cold treatment enhance their germination capacity (Funk 1990). The optimum conditions for *A. glutinosa* seed germination occur at pH 4 and 25 °C in either light or dark (Klein 2007).

Specie	**Cold Storage** (1-5 °C) days	**Germination test condition**			**Germination rate**		**Germination**
		Temperature °C		Days	Amount	Days	Avg %
		Day	Night		%		
A. glutinosa							
fresh seeds	0	25	25	21	21	5	28
dried seeds	0	25	25	21	9	5	13
dried seeds	180	25	25	21	27	5	35
A. incana							
fresh seeds	0	25	25	21	21	5	29
dried seeds	0	25	25	21	12	5	16
dried seeds	180	25	25	21	25	5	34

Table 4. Stratification and germination *Alnus incana* and *Alnus glutinosa* species from Finland (Bonner et al. 2008)

Germination of Grey Alder usually requires exposed mineral soil, which may need to be saturated (Rook 2004). The seed requires no treatment or chilling to break dormancy and can be expected to germinate immediately following dispersal if conditions are favorable (Haeussler and Coates 1986). Fresh seeds of European and Grey Alders also germinated promptly without stratification; but dried seeds, at moisture of content of 8 to 9%, were dormant (Table 4). Germination capacity of the dried seeds, after stratification for 180 days at 5 °C was higher than that of fresh seeds (Bonner et al. 2008).

All these seeds (whether freshly collected or dry-stored) are slow to germinate and/or only capable of germinating over a narrow range of conditions until pretreated. Pretreatment usually consists of incubating seeds for a relatively short period of time (3 to 12 weeks) under moist conditions at c. 4°C (a so-called prechill) (Forest Research 2012).

4. Seed germination analysis of two *Alnus* species (*Alnus incana* and *A. glutinosa*).

4.1. Materials and methods

4.1.1. Study area

The seeds of *Alnus incana* and *Alnus glutinosa* were collected in 7 different localities of Czech Republic, which were divided into 5 groups (Table 5). In Czech Republic it is spread throughout the territory with the exception of the largest mountain ridges and the driest areas. Typical habitats for alders are wet and muddy soils in alluvial forests, moist sites, along streams, temporary watercourses along the roads, ponds and lakes. It can be found in floodplain forests around the large flows in the lowest positions. For example, in the Řevničov locality *Alnus* species were found in the lowest and wet positions of forest between Upper and Lower ponds Kracle. Location Špindlerův Mlýn is characterized by the highest point above sea level (706 m.n.m.).

Selected locations were mapped using the Flora Database of the Czech Republic (Figure 8). This base contains data of plant species and it is helpful to complete the map of plants species distribution of the country.

№		Lokalition	GPS	Quadrant
1	I,G	Horažďovice, district Klatovy	49°18'34.48"N, 13°43'39.48"E	6648
2	I,G	Hutě, district Tabor	49.305789 N, 14.513767 E	6653
3	I,G	Řevničov žst., district Rakovník	50°8'38.869"N, 13°50'25.889"E	5848
4a	G	Klokočka, district Mladá Boleslav	50°29'53.484"N, 14°54'35.956"E	5455
4b	I	Špindlerův Mlýn, district Trutnov	50°43'26.097"N, 15°35'24.045"E	5259
5a	I	Hradsko, distrct Mělník	50°25'42.189"N, 14°35'58.188"E	5553
5b	G	Olšany (Studená), district Jindřichův Hradec	49°08'24.0" N, 15°14'56.0"E	6857

Table 5. Localities *Alnus incana* (I) and *A. glutinosa* (G) collections

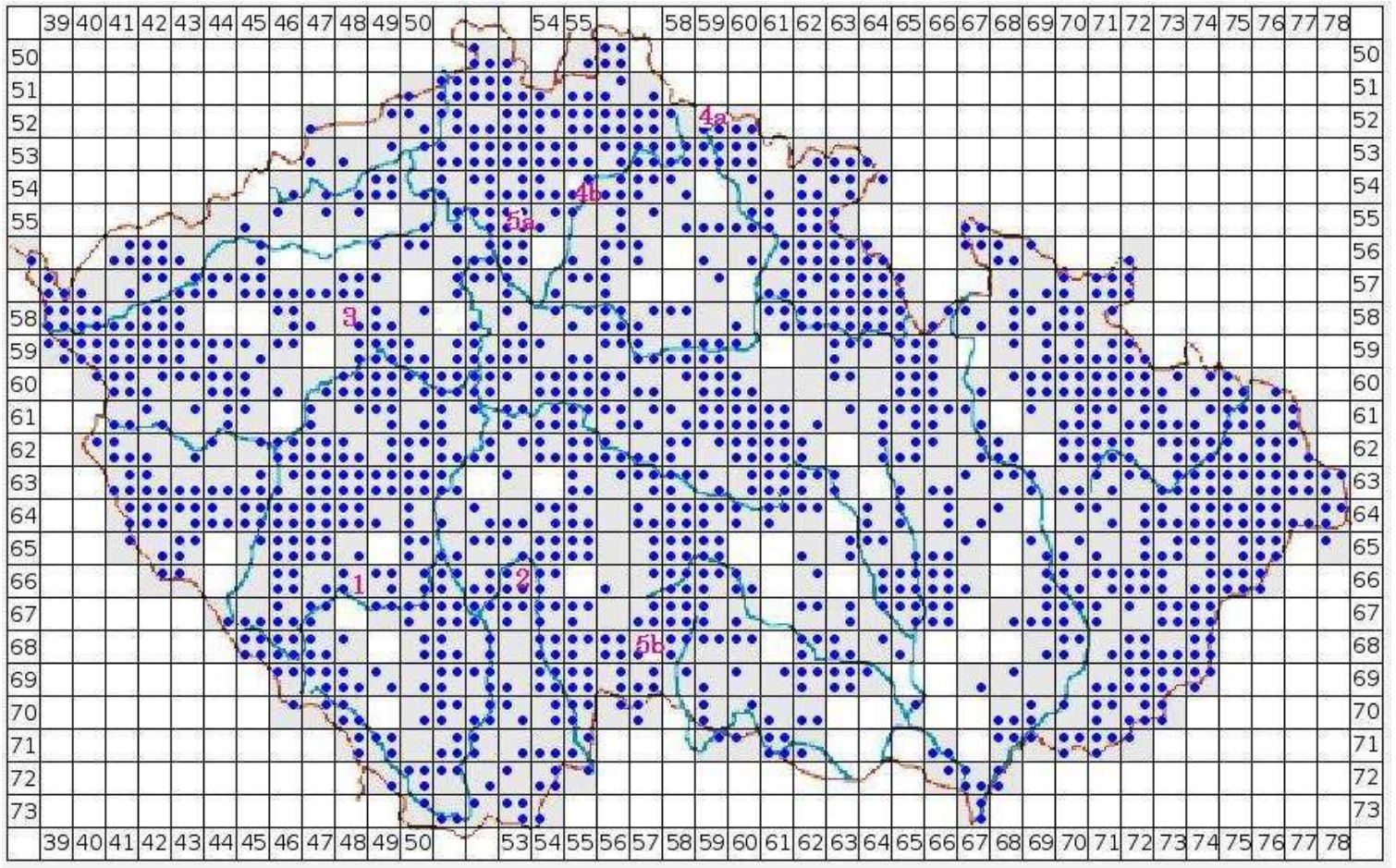


Figure 8. Species distribution map of *Alnus glutionosa* and i*ncana* in Czech Republic. Numbers from 1 to 5 correspond to the numbers in Table 3. Locations: 1. Horažďovice, 2. Hutě, 3. Řevničov žst., 4a. Klokočka, 4b. Špindlerův Mlýn, 5a. Hradsko, 5b. Olšany (Studená), (Flora Database of Czech Republic. http://florabase.cz)

4.1.2. Seed collection

Cones should be collected only when seeds are sufficiently mature (Bonner et al. 2008). Strobiles should be collected as soon as they are ripe, for the largest seeds with the best germinability are usually released first. Thus, both seed quality and seed yield are higher if collections are made in the fall rather than in the winter or spring (Bonner et al. 2008).

It is important to note that there is a problem of rapid dispersal or of seed pests exists and provided the earliest time for safe collection of immature fruits can be established (Willan 1987). In this case it is better to start collect since the Alder catkins do not disintegrate at maturity. They may be collected from standing or recently felled trees as soon as the bracts (scales) start to separate on the earliest-ripening strobiles (Carole et al. 1997).

The cones of the study species were gathered from different individual stands in autumn during September-November 2011. It was the time when they reached their maturity. It was better to collect cones from the tree, not on the ground, because of seed quality characteristics. The seeds were collected by hand from 5 different individual trees.

4.1.3. Preparation of seeds

Cones need to be cleaned of twigs, bark, foliage and other impurities before they go for extraction, cleaning, storage or sowing (Willan 1987). Alder strobiles will open after being exposed in drying racks in a well-ventilated room for several weeks at ambient air temperature. They can be opened in a shorter time by drying them in a kiln at 27–38°C. Most seeds will fall out of the strobiles during the drying process; however, the remaining seeds may be extracted by shaking or tumbling if necessary (Carole et al. 1997).

Following this, cones were spread out to the paper sheets for drying at room temperature for few days. After drying cones were opened and seeds fall out easily as a result of manual stirring (Figure 9). For each collection, the seeds were calculated and stored in paper bags at room temperature during 3 months before the experiment.

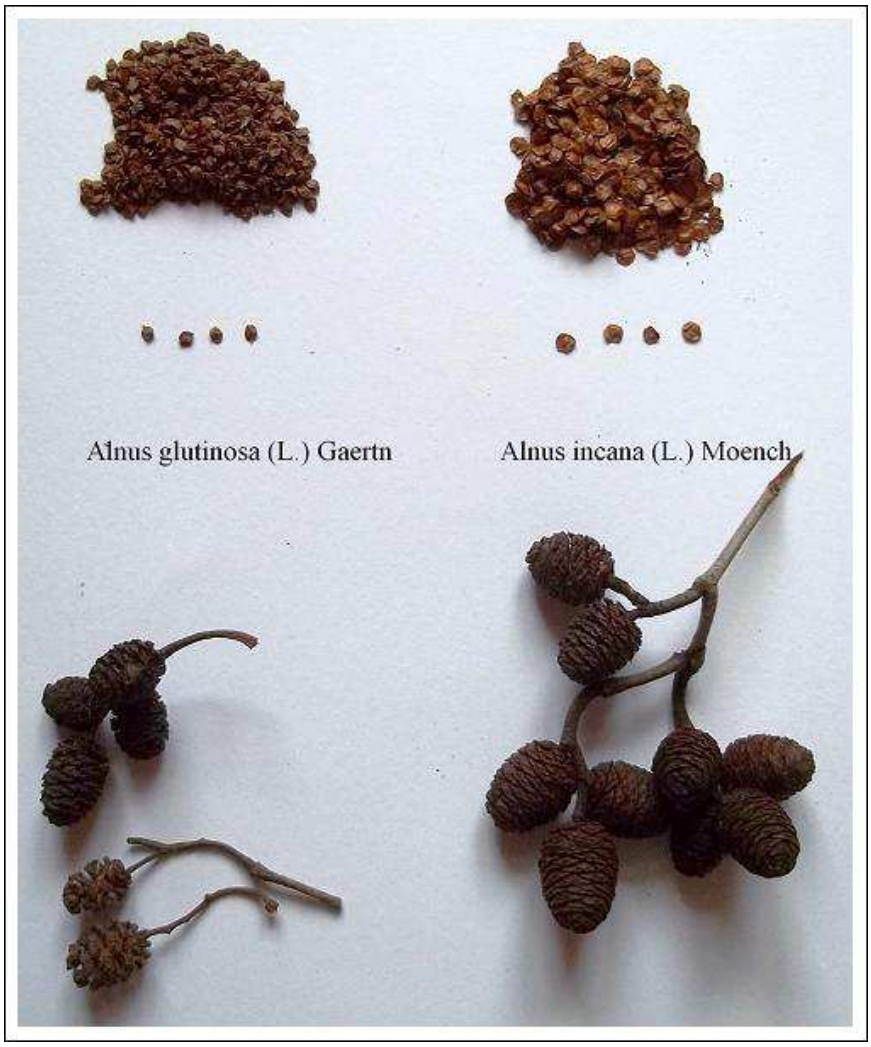


Figure 9. The seeds and cones of study species

4.1.4. Germination test

Before preparation small and damaged seeds were excluded. The seeds were randomly sampled by hand. Appropriate seeds were placed in 10 cm diameter Petri dishes on a single layer of filter paper, wetted by distilled water. One Petri dish contains 5 single rounds of filter paper with fifty seeds from different locality (Figure 10). Generally, there were four dishes with 250 seeds for each species and each treatment.

Dormancy is the condition of a seed that prevents it from germinating when it is placed in conditions that are favorable for germination. Dormancy must be overcome in order to conduct the germination test, just as when trying to grow seedlings (Bonner et al. 2008). Pre-germination chilling (commonly called “prechilling” and traditionally called “stratification”) is the procedure most used for breaking dormancy in forest seeds (Bonner et al. 2008) Because of that dishes were placed in a temperature controlled incubator without illumination for two weeks at temperature equal 5 °C.

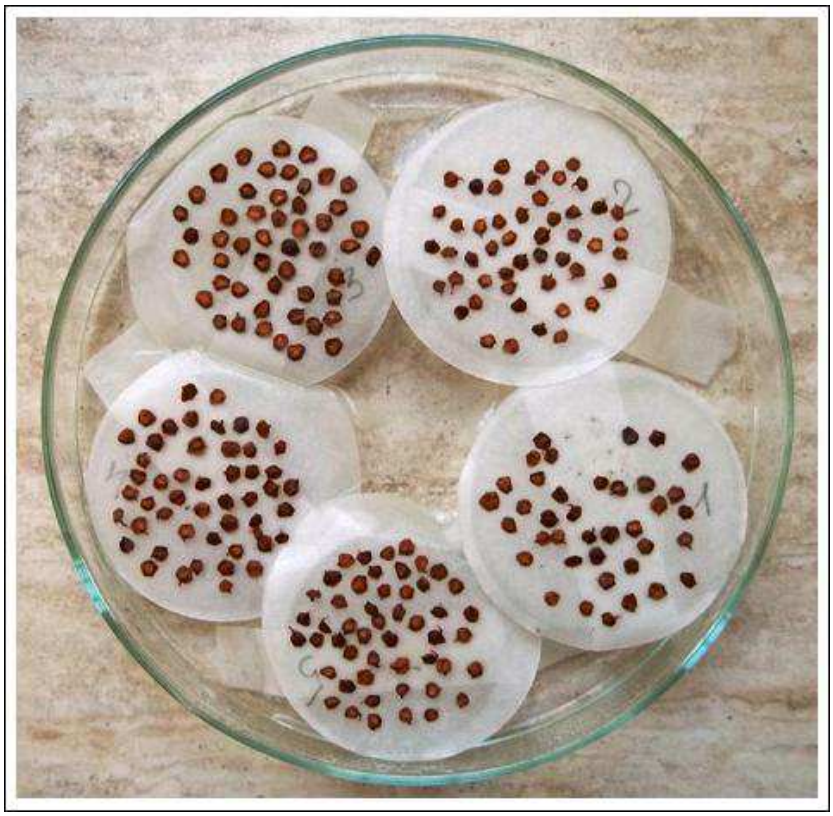


Figure 10. Prepared Petri dish with *Alnus incana* seeds

The main aim of a laboratory germination test is to estimate the maximum number of seeds which can germinate in optimum conditions (Willan 1987). For determining the effect of temperatures, dishes were allocated into environmental test chambers (Figure 11) with four different temperature treatments with a light/darkness timer 10/5, 20/5, 25/10 and 25/15 (Figure 12).

Figure 11. Environmental test chamber

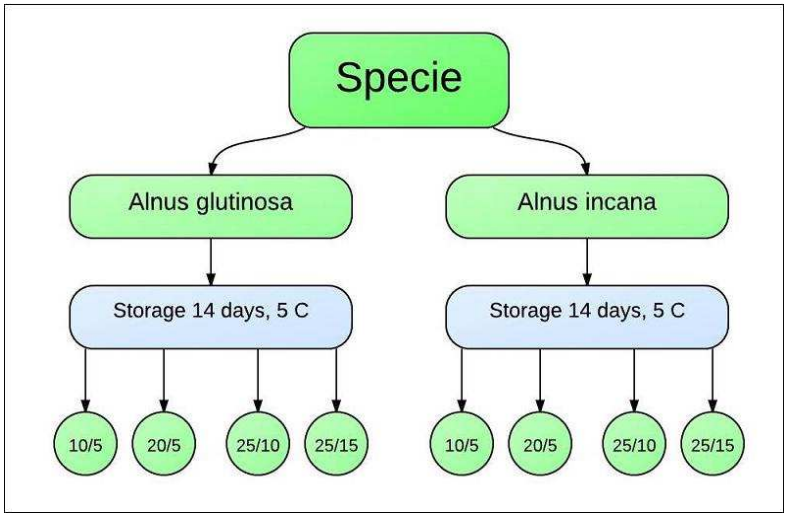


Figure 12. Schematic experimental design treatments preparation. Two species: *Alnus incana* and *Alnus glutinosa*. Four germination temperatures with 5 replications. Each replicate (1-5) include 50 seeds from chosen locality.

A seed is considered to have germinated after the emergence and development from the seed embryo of those essential structures which are indicative of the seed's capacity to produce a normal seedling under favourable conditions (Willan 1987). An example of germination is displayed in Figure 13.

Figure 13. Germinated seeds of *Alnus glutinosa*

4.1.5. Germination measurements

Three characteristics of germination were determined: final germination percentage, mean time to germinate (MTG) and germination rate index (GRI).

Germination percentage (GP) or total germination is the most common expression of seed germination. It is calculated by this formula (Carole et al. 1997):

$$GP = \frac{\text{number of germinated seeds}}{\text{number of filled seeds}} \text{ x } 100\ \%$$

Germination Value aims to combine in a single figure an expression of total germination at the end of the test period with an expression of germination energy or speed of germination (Willan 1987). Germination value (GV) is a product of two additional variables: mean daily germination and peak value (Kolotelo et al. 2001):

$$GV = MDG * PV, \quad \text{where:}$$

MDG (mean daily germination) - is simply the germination capacity (or germination percentage) divided by the number of days in test,

PV (peak value) - is the point at which the cumulative germination percent divided by the number of days is maximum. The PV describes germination rate.

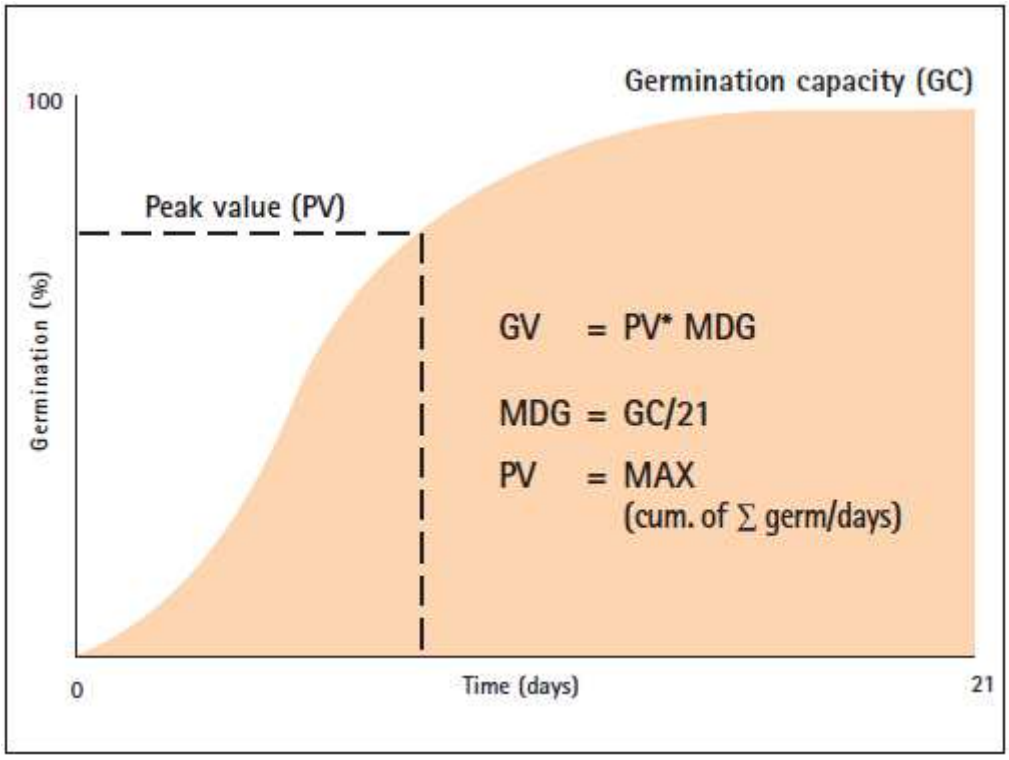


Figure 14. A graphical representation of germination capacity (GC), peak value (PV), and germination value (GV), also termed vigour (Kolotelo et al. 2001)

The absolute magnitude of GV depends upon the species, but values usually range from 10 to 60. Germination values have no units, and have not been widely accepted by those who prefer separate reporting of germination rates and total germination (Carole et al. 1997).

Germination rate (GR) is often expressed as $R_{50,}$ or the number of days it takes 50% of the sown seeds to germinate (Arteca 1996). In the study were used the **germination rate index** (GRI), which is measured as (Ahmadizadeh 2011):

$$GRI = G_1/1 + G_2/2 + \ldots + G_x/x, \text{ where}$$

G1 - germination percent in first day,

G2 - germination percent in second day to final experiment.

4.1.6. Statistical analysis

For continuous data such as percent germination, analysis of variance is suitable analysis method (Carole et al. 1997). In this germination experiment the significance of mean was tested by Factorial analyses (ANOVA). Final germination percentages were transformed using Arcsine square root transformation technique. Data were analyzed using Statistica™ software (StatSoft 2007), version 8.0. Means were separated using Tukey's Honestly Significant Difference (HSD). The figures were drawn by using software EXCEL.

4.2. Results

In the case of both species the first seeds from all sites germinated rapidly in distilled water during two days after being allocated into environmental test chambers. Germination was quite rapid, with the peak being reached on the third or sixth day, when more than 50 % of all the seed had germinated (Figure 15). In general, seed germination of *A. glutinosa* has the highest results in total germination percentage.

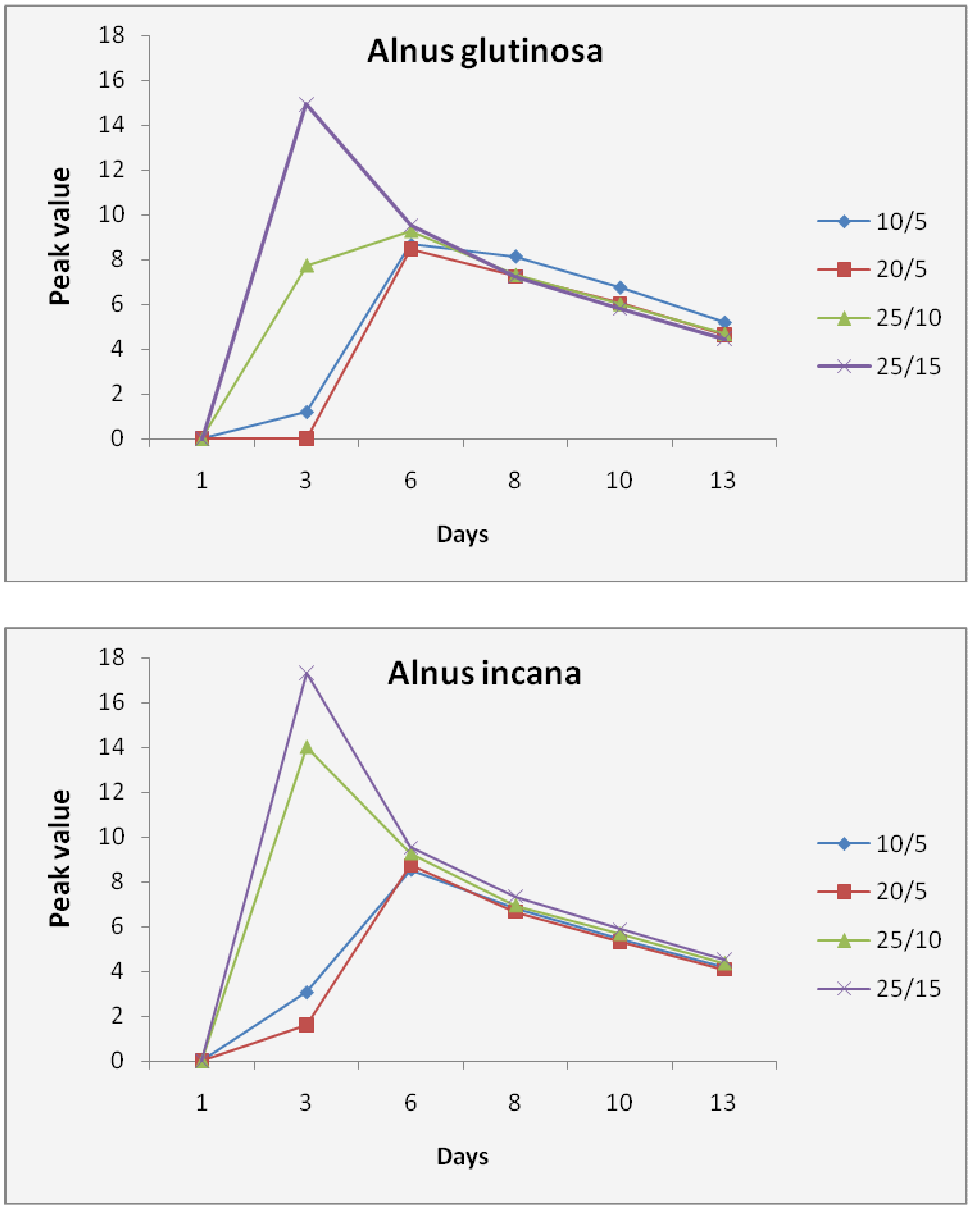


Figure 15. The peak value of germination of *A. glutinosa* and *A. incana.*

Source *of* variation	3 day			6 day			8 day			10 day			13 day		
	df	F -ratio	P level	df	F -ratio	P level	df	F -ratio	P level	df	F -ratio	P level	df	F -ratio	P level
Specie	1	19.914	***	1	0.002	0.960	1	0.846	0.364	1	1,405	0.244	1	1.492	0.230
T regime	3	65.020	***	3	0.377	0.770	3	0.160	0.922	3	0,160	0.922	3	0.155	0.925
Specie x T regime	3	0.758	0.525	3	0.013	0.997	3	0.284	0.836	3	0,425	0.736	3	0.406	0.749

Table 6. Two-way analysis of variance showing the effect of temperature regimes on final germination (performed on pooled data, n=50). Data were transformed (arcsin $\sqrt{}$ %) to achieve normality. *** $P < 0.001$

The effect of temperatures regimes on *A. incana* and *A. glutinosa* and interactions between it are shown in Table 6. Two-way ANOVA tests of germination indicated significant differences on final germination percentage between species only on the third day of the experiment ($P < 0.001$). The same result with temperature regime ($P < 0.001$). The effect of temperature on both species is not significant for the rest days. The two-way interaction between temperature regimes and specie was not significant ($P > 0.05$). Unexpectedly, all variables (excluding the third day) have no major significant differences in the germination behavior and response to temperature regimes. Figure 16 shows the observed signifficant difference between two species. Seeds produced by *A. incana* germinated better at this day.

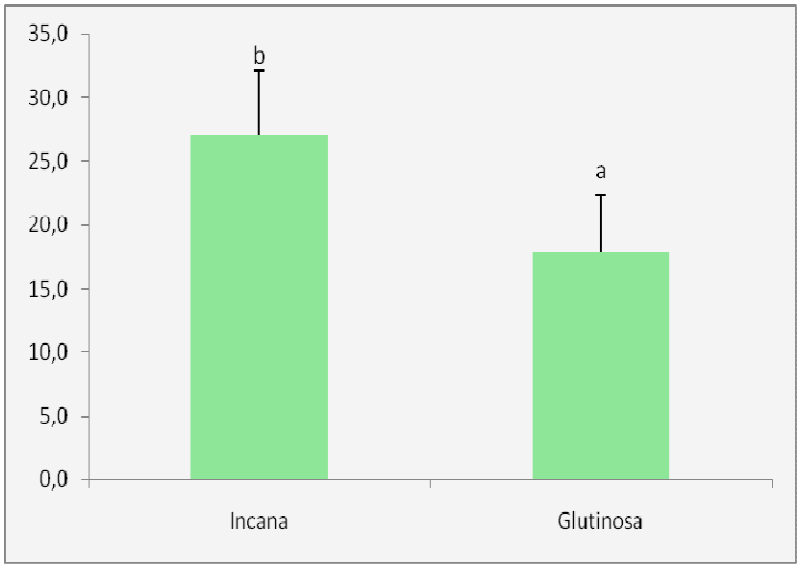
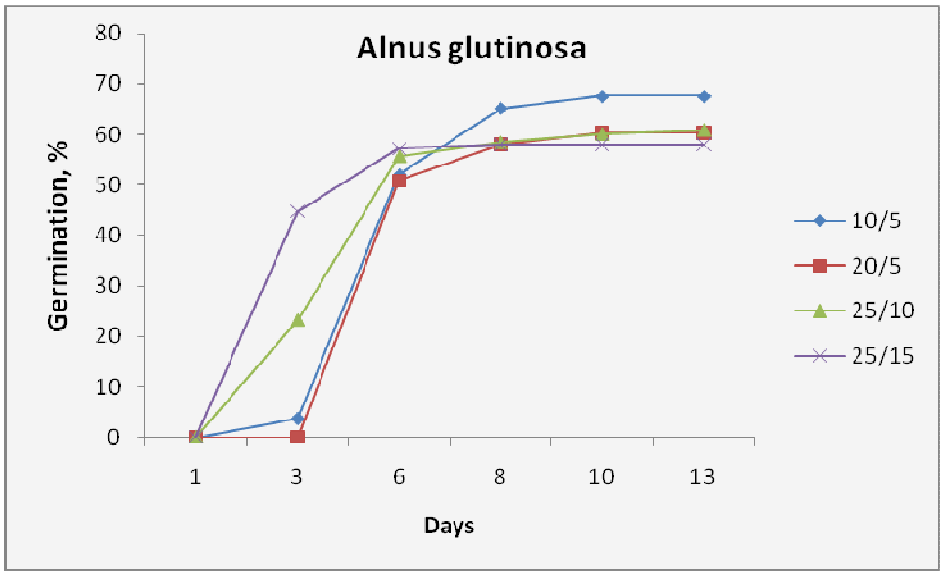


Figure 16. Final percentage of germination seeds (with SE) of *Alnus incana* and *Alnus glutinosa* on the third day of experiment. Bars with the different letters were significantly different in multiple range comparison (Tukey's test) a $P < 0.05$

The germination responses of *A. incana* and *A. glutinosa* seeds to the temperature are shown in Figure 17. Plots *a* and *c* shows the course of germination at the four different temperature regimes. This shows that it is very important to pay attention to the start and peak of germination, because of some differences or similarity between two species ant

temperatures. Looking forward these graphs we can conclude that the process of germination looks the same way. The beginning in germination at highest temperatures (25/10 and 25/15) started one day earlier than two another (10/5 and 20/5). Seeds were able to germinate at all chosen temperatures between 10 °C and 25 °C and the optimal temperature corresponds to 25 °C. According the end of germination *A. glutinosa* reached the highest result a 10/5 C. For *A. incana* the best result in germination was in 25/15 C.

a)



b)

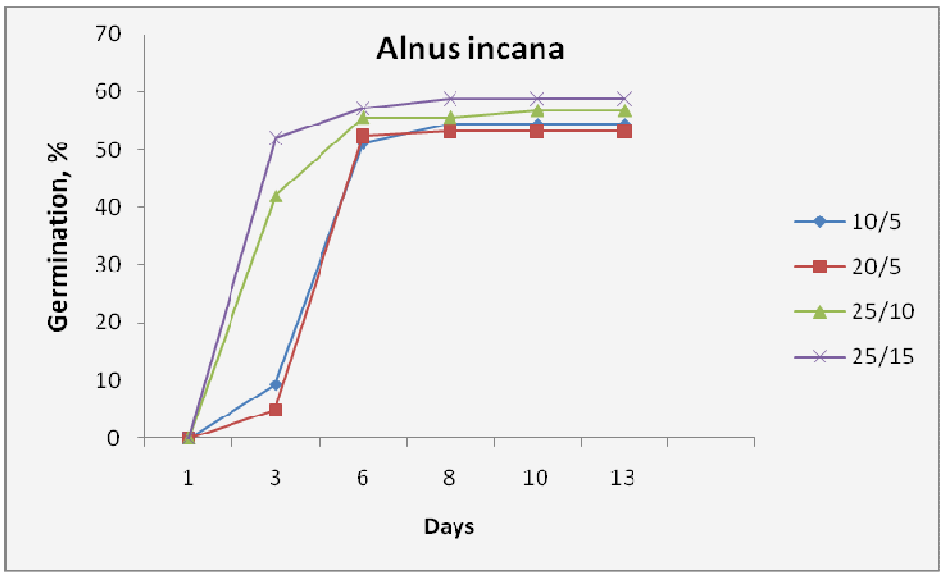


Figure 17. Germination course of *Alnus glutinosa* (a) and *Alnus incana* (b)

Results of a two-way ANOVA showed effect of temperature regime on germination rate index (GRI) in two species (Table 7). There was found significant difference in temperature regimes ($P < 0.001$). Figure 18 showed it significant difference. GRI seems different in its response to chosen conditions. Increase in temperature to 25 °C showed a positive effect on germination rate. The temperature regime 20/5 significantly differs with regime 25/15.

Source of variation	d.f.	F-ratio	P level
Specie	1	0.301	0.586
T regime	3	10.045	***
Specie x Tregime	3	0.389	0.761

Table 7. Two-way analysis of variance showing the effect of temperature regimes on Germination rate index (GRI) both species. Data were transformed (arcsin $\sqrt{}$%) to achieve normality. *** $P < 0.001$

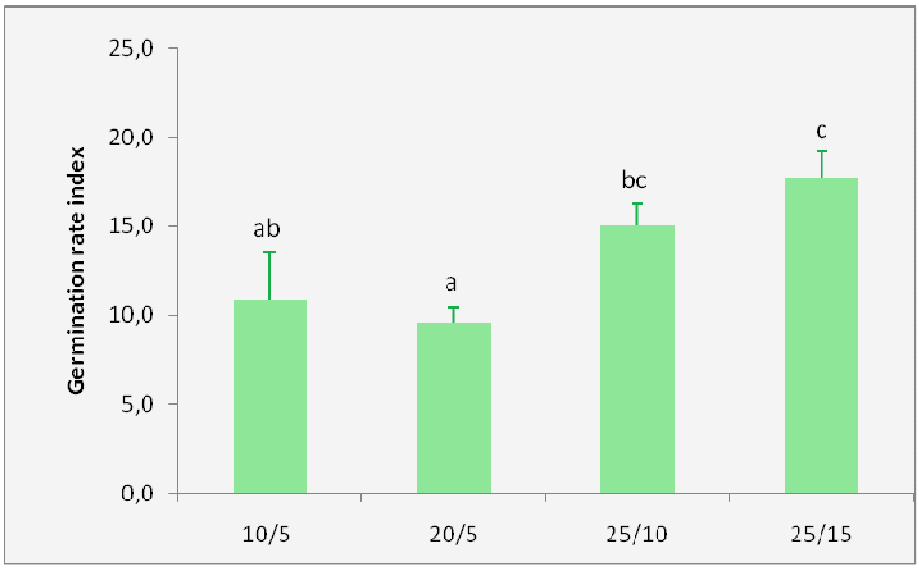


Figure 18. Germination rate index (with SE) of *Alnus incana* and *Alnus glutinosa*. Bars with the different letters were significantly different in multiple range comparison (Tukey's test) a $P < 0.05$

4.3. Discussion

Seeds of temperate woody plants can germinate over a wide range of temperatures, from a minimum of 2 or 3 °C, to a maximum of about 45 °C. Natural seedbeds do not remain at constant temperatures, but experience diurnal fluctuations from lows at night to highs in the daytime. Most temperate woody plants have adapted to these conditions and germinate most rapidly at alternating temperatures of approximately 20 °C at night and 30 °C in the daytime. Other species germinate faster at lower temperatures regimes, for example, 15 to 25 °C or 10 to 20 °C, or at constant temperatures of 5 to 22 °C (Bonner et al. 2008).

In a high proportion of species from temperate regions, particularly those adapted to spring emergence, exposure to cold temperatures under moist conditions releases seed dormancy (Bradford and Nonogaki 2007). The usual procedure for stratification is to refrigerate fully imbibed seeds at 1 to 5 °C for 1 to 6 months. This procedure simulates the natural winter conditions of temperate seeds that are lying on the forest floor (Bonner et al. 2008). It is important to note, that some untreated seeds will germinate, but pretreatment will usually stimulate quicker germination of more seeds over a wider range of conditions (Gosling 2007).

Alders shallow dormancy characteristics can make them a little more challenging to germinate, especially to achieve the maximum germination possible (Gosling 2007). According to Baskin and Baskin (1998) *Alnus incana* and *Alnus glutinosa* belong to species that have nondeep physiological dormancy. In germination study of Schalin (1967) it was found that dry storage and stratification resulted a decrease in germination. So we can say that presence of these dormancy characteristics in seeds means that they become nondormant during cold stratification. Complete and fresh Alder seeds have good germination characteristics. Most other studies argue that freshly seeds of *Alnus* species can germinate without any previous special preparation. Dried seeds require stratification. Several studies supported this conclusion for *Alnus* species and showed the differences and interactions between storage and germination character (Schalin 1967, Baskin and Baskin 1998, Gosling 2009, Harrington et al. 2008).

In our study cold-stratified seeds of *Alnus* species had germinated within a time period of about two weeks at all temperatures (10/5, 20/5, 25/10 and 25/15) in the laboratory experiment. In the case of both species the first seeds from all sites germinated rapidly in distilled water during two days after being allocated into environmental test chambers. It is importatnt to pay attention on the differences between species in the start of germination. *Alnus incana* begin to germinate more rapidly than *Alnus glutinosa* (Figure 15). The same result had Schalin study experiment: the highest results occurred during the first five days and the rest during the following three days. No late germination was recorded during the three-week duration of the experiments (Shalin 1967).

The analysis of variance (ANOVA) for final germination percentage showed that there was no significance for differences between temperature conditions (Table 6). It was found that, only on the third day there was a significant difference between species ($P < 0.0001$). The same result with temperature regime ($P < 0.0001$). Unexpectedly, in the results all variables (excluding the third day) have no major significant differences in the germination behavior and response to temperature regimes. However, it is not clear what caused this nonsignificant result. We considered to observe differences in the range of temperatures.

A. incana and *A. glutinosa* seeds reached final germination percentages (> 50%) over a range of incubation temperatures between 10°C and 25°C under at light and between 5 ° C and 15 ° C dark period. Germination was quite rapid, with the peak being reached on the third or sixth day, when more than 50 % of all the seed had germinated. Figure 16 shows the observed signifficant difference in germination starting between two species. Seeds produced by *A. incana* germinated better at the third day. It means that this species is faster in the beginning of germination.

Results of a two-way ANOVA showed effect of temperature regime on germination rate index (GRI) in two species (Table 7). There was found significant difference in temperature regimes ($P < 0.001$). Figure 18 showed this significant difference. GRI looks different in its response to chosen conditions. Increase in temperature to 25 °C showed a positive effect on germination rate. The temperature regime 20/5 significantly differs with regime 25/15.

For both populations, the germination tests showed that the optimal peak value of germination was occurred at 25 °C (Figure 15). This confirms data of Gosling (2009) study research: warmer temperatures (25 or 30EC) and alternating temperatures (20/30EC) promote even faster and more complete emergence.

However, the highest germination percentage was differing for two species. For *A. glutinosa* it was 10/5 temperature regime, for *A. incana* 25/15 (Figure 17). According Baskin and Baskin (1998) cold stratification decrease the minimum temperature for germination of *A. glutinosa* seeds from 18 to 7 ° C. Gosling (2009) states that prechill widens the range of temperatures over which a significant proportion of live seeds can germinate to include 10, 15 and even 35 °C. Clearly, prechilling has several beneficial effects to the germination which are only manifest the following spring (Gosling 2009). So, in our experiment cold stratification could influence on the high result of germination in the 10/5 temperature conditions.

The germination with final percentage more than 50 % occurred at all regimes from 10/5 to 25/15 °C in both species, while all temperature treatments had no effect on them. The final germination of *Alnus incana* varies from 53 to 59 %, for *Alnus glutinosa* from 58 to 68 % in four chosen regimes. *Alnus incana* collections have lowest germination at 20/5 °C and high germination result at 25/15 (59 %). This species does not show large variation in final germination percentage. We can say that species can germinate well at all temperature regimes in our experiment.

5. Conclusion

This study highlighted the importance of temperature in regulating the dormancy and germination of *Alnus incana* and *Alnus glutinosa.* Alders have a wide range of temperature in which they germinate. Our study indicates that species can germinate well at all temperature regimes in our experiment. How it was expected seeds reached final germination percentages (> 50%) over a range of incubation temperatures between 10°C and 25°C under at light and between 5 ° C and 15 ° C dark period. It is important to pay attention on the beginning and peak value of germination, because of sensitiveness of seeds to different temperature conditions.

Despite the dominant role of temperature in the control of seasonal patterns of dormancy in seeds, it is important to note that temperature does not act alone. Many studies have clearly demonstrated that other factors – for example, light, nitrate and desiccation – can all influence the expression of dormancy in seeds (Fenner 2000). Several studies consider that, seeds of European alder germinated as well in continuous darkness as under normal day length, recent work indicates that seed germination of many alder species is markedly affected by light regime (Bonner et al. 2008).

It may be suggested for the future germination studies of Alnus species to take into account light pattern. It may be better to chose more wide range of temperatures regimes with lowest and highest values or to include to the study field experiment. This will give more data, which can help to do more clear results. It also may be suggested to do control treatment without stratification for better understanding the interaction between seed dormancy of specie and temperature regimes.

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