Sveriges lantbruksuniversitet
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The Effect of Cold Stratification on Germination in 28 Cultural Relict Plant Species

- With the Purpose of Establishing Germination Protocols

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Effekten av kallstratifiering på groningen hos 28 kulturreliktväxter.
– I syfte att etablera groningsprotokoller

Jonatan Leo

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Abstract

Cultural relict plant species from the Nordic countries have been collected by the Nordic Genetic Resource Center (NordGen) for the purpose of conservation. To ensure high seed vitality in store, regular germination tests need to be conducted. It is important to get a correct viability status, but the knowledge of seed dormancy in the cultural relict plants is often poor. The objective of this study was to investigate how seed dormancy is affected by cold stratification. The study includes 31 accessions from 28 species with the purpose of establishing germination protocols. Furthermore, the study includes three treatments: 0, 2 and 4 weeks cold stratification, followed by germination tests.

The dormancy of 22 of the species was not affected by stratification and 10 of them showed unsatisfying germination percentage (<75 %), probably due to poor seed health or high proportion of immature seeds. Five species benefited of stratification, though the low temperature may be questioned as a dormancy-breaking factor in *Thymus pulegioides* that germinated during the stratification period. Cold stratification reduced seed germination rate in one of the examined species, something which may be due to secondary dormancy or fungal infection.

In addition, the effect of cold stratification in combination with scarification was studied in 4 accessions of non-relict plants: three accessions of *Trifolium pratense* and one accession of *Allium ursinum.* It showed an inter-accessional variation in germination response for *T. pratense* but no response for *A. ursinum*.

Sammanfattning

Kulturreliktväxter från de nordiska länderna har samlats in av Nordiskt Genresurscenter (NordGen) i syfte att bevaras för framtiden i form av frö i en fröbank. För att garantera en god levnadsstatus i lager utförs regelbundna analyser i form av grobarhetstester. Kunskapen om frövilan hos många av reliktväxterna är ofta bristfällig och groningsprotokoller behöver utvecklas. I groningsprotokollerna sammanställs metoder för brytning av frövilan. Det är viktigt för att korrekt kunna analysera frönas vitalitet. Syftet med denna studie är att studera hur frövilan påverkas av en stratifieringsperiod. Studien inkluderar 31 reliktväxtaccessioner från 28 arter. Försöket innehåller tre behandlingar; kallstratifiering 0, 2 samt 4 veckor, följt av groningstest.

Frövilan hos 22 av arterna påverkades inte av en stratifieringsperiod. Tio av arterna visade en låg groningsprocent (<75 %), vilket kan bero på låg kvalitet eller hög andel omogna fröer. Fem av arter gynnades av en kylperiod. Den låga temperaturen kan dock ifrågasättas som en brytningsfaktor för frövila för *Thymus pulegioides* då fröer grodde redan under kylperioden. En art missgynnades av en stratifieringsperiod, vilket kan bero på sekundär frövila eller svampinfektion.

Studien innefattar också en undersökning av effekten av både nötning och stratifiering på frövila hos fyra accessioner icke-kulturreliktväxter: tre accessioner av *Trifolium pratense* och en accession av *Allium ursinum.* Den visade en inter-accessional variation i nötningsrespons på groningen hos *T. pratense* men ingen respons hos *A. ursinum*.

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Contents

Introduction

Cultural relict plants, hereafter called relict plants, are here defined as introduced or native plants that once were cultivated, but now remains naturalised, often as survivors in small populations, bound to the same place or locality where they once were grown (Poulsen *et al*., 2010; Persson *et al*., 2013, unpublished; Solberg *et al*. 2013). Back then, these species were valuable utility crops used for medicine, spices, colorants, fibres etc. Today, we find them in places connected to old settlements like castles, medieval churches and monasteries, old harbours, various kinds of ruins, farms and manors. Even if we do not use them commercially today, or will perhaps never do in the future, they belong to our cultural heritage and should be preserved for future generations. Small populations of relict plants are valuable because of their cultivated history, even though it is a common species. Many of the relict plant populations are threatened by loss of habitats and climate changes. The priority is to protect them *in situ*, but also *ex situ* back-ups are needed. The Nordic Gene Resource Center (NordGen) is responsible for preserving genetic resources *ex situ*, valuable for the horti- and agriculture. Upon request, seeds are distributed for research, plant breeding and cultivation. For this purpose, shared seeds must be viable and in enough quantities. But:

> "To ensure the quality and quantity of the material, germination tests and multiplication must be carried out. The knowledge on how to germinate and how to multiply CRPs [Cultural Relict Plants] is not always present. Germination and regeneration protocols need to be established." (Persson *et al*., 2013, unpublished)

In NordGen, germination tests are conducted every 10th year to study seed viability. A result below 65–75 % is usually considered unsatisfying and, according to NordGen's standards, the accession has to be regenerated to restore high seed vitality. Some seeds need species-specific treatments to germinate. They are said to be dormant. Non-germinating dormant seeds have to be distinguished from dead seeds. It is essential to find out which treatment each species needs to maximise germination percentage, and thus, to get an as accurate viability status as possible.

Objectives

The main objective of this project is to study the effect of cold stratification on seed dormancy in 31 relict plant accessions, in 28 different species, with the purpose to establish germination protocol for germination tests. The accessions were chosen based mainly on poor or no

prior knowledge about seed dormancy and seed availability and quantity in store.

The study also includes a test of seed dormancy in four accessions of non-relict plants, belonging to two different species, and their response to scarification in combination with cold stratification treatment. The purpose is to establish and develop germination protocols and to study inter-accessional variations.

Seed dormancy

All seeds need to absorb oxygen and a species-specific minimum of water to germinate, although, too low or too high water content may inhibit germination (Baskin and Baskin, 1998). Some seeds require additional factors to sprout. Germination is here defined as emergence of radicle from the seed. Germination requirements are factors that need to be present for germination, e.g. a certain temperature range or moisture supply. Seed dormancy can be defined as the failure to germinate under such favourable conditions, although the seed is viable (Bewley, 1997; Baskin and Baskin, 2005). Right germination conditions promote germination in a non-dormant seed. A dormant seed needs a dormancy-breaking treatment to become non-dormant.

Dormancy is of great importance in an evolutionary perspective and in terms of fitness (Baskin and Baskin 1998; Hilhorst 2007). It is essential for a seed to germinate in the exact right time of the year to maximise the probability for survival and growth of the seedling in aspects of competition and environmental conditions. The plant seed needs a cue when to germinate and when it is better to wait for more favourable conditions. Dormancy is also important in aspects of dispersal and as a mechanism for delaying seed germination until it has been spread to new areas (Taiz and Zeiger, 2010).

Seeds may either be *non-dormant*, *conditionally dormant* or *dormant*. Baskin and Baskin (2005) define *non-dormant* seeds as having a high germination rate, with no changes after a dormancy-breaking treatment. If the seeds germinate over broader conditions, for example at a lower temperature, after a dormancy-breaking treatment, the seeds are *conditionally dormant*. *Dormant* seeds need a dormancy-breaking treatment to become *non-dormant* and to germinate. Thus, the fact that a seed needs very specific conditions to germinate does not mean it is dormant.

Different mechanisms of seed dormancy

There are different kinds of mechanisms in the seed that prevent seed germination until after the right environmental cues have occurred. Baskin and Baskin (1998, 2004, 2005) divide seed dormancy into five main groups (classes): *morphological*, *physiological*, *morphophysiological*,

physical and *combinational dormancy*.

In *morphological dormancy*, the embryo is underdeveloped and needs to reach a specific size or development stage to germinate (Baskin and Baskin 1998, 2005). The embryo needs a long period with favourable conditions to grow and then germinate, and not a dormancy-breaking treatment in itself (Baskin and Baskin, 2004).

When the seed experience *physiological dormancy*, the germination is prevented by a physiological mechanism in the embryo, in the seed testa (coat) or in the endosperm (Amen 1968; Baskin and Baskin 1998). The ratio between the two plant hormones Gibberellic Acid (GA) and ABscisic Acid (ABA) is an example of physiological dormancy (Bewley 1997; Baskin and Baskin, 2004). A high GA:ABA ratio promotes germination in many different angiosperm seeds and GA is often added as an external chemical to promote germination. The plant hormone ethylene may also have a dormancy releasing effect (Matilla, 2000; Matilla and Matilla-Vázquez, 2008). Organic or inorganic germination inhibitors from seed tissues, other than the embryo, are also examples of physiological dormancy. Baskin and Baskin (2004) include mechanical dormancy, when the seed testa blocks germination due to low embryo growth force, in the physiological dormancy class. Physiological dormancy are released by environmental factors and can, for example, be overcome by cold or warm stratification, soaking or after-ripening, depending on the species and how deep the dormancy is (Baskin and Baskin, 1998).

The third class is *morphophysiological dormancy*. Seeds in this group need first a dormancy-breaking treatment (warm, cold or both), and then a growth period (warm or cold) to germinate (Baskin and Baskin, 1998).

The fourth class is *physical dormancy*. It is caused by a water-impermeable seed testa that needs some kind of breaking or loosening to initiate germination (Baskin et al. 2000; Baskin and Baskin, 2004). This can be done by dormancy-breaking treatment such as heating or mechanical or chemical scarification.

The final group is the combination of physiological and physical dormancy and is called *combinational dormancy*. The seed has to go through both some kind of stratification treatment to imbibe water and then a physiological dormancy-breaking treatment to germinate (Baskin and Baskin, 2004).

Dormancy-breaking factors

There are many environmental factors that may influence the germination timing and the release of dormancy in seeds, e.g. light, temperature, water, nitrate, hormones, smoke, oxygen and carbon dioxide. Many seeds respond to more than one type of environmental factor. Different dormancy-

breaking treatments can either substitute for each other or be required together or in certain sequences. The factors are either necessary, sufficient or inadequate. The three most influential and important factors in general are temperature, after-ripening and light (Baskin and Baskin, 1998; Taiz and Zeiger, 2010). Dormancy-breaking require, more or less, active biochemical metabolism, therefore low internal water content and freezing reduce eventual dormancy status changes, although low temperature may break physical dormancy.

Temperature is the most important environmental dormancy-breaking factor among herbaceous seed plants from the temperate region. The majority of the species require a cold winter period prior to germination, or to increase their germination rate, in spring (Baskin and Baskin, 1988). *Stratification* is the horticultural term for the treatment (method) using cold or warm temperatures, simulating winter or summer, to break seed dormancy. The temperature may influence the seed testa's water permeability in physically dormant seeds, making them porous and capable of imbibition. In the case of physiological dormancy either low or high temperatures are required for dormancy-breaking, and morphophysiological dormancy needs cold temperatures. Some non-dormant seeds actually enter dormancy, so called secondary dormancy, due to low or high temperature exposition, depending on the species. This trait is common in winter annuals (Baskin and Baskin, 1988).

As a result of water-impermeable seed coats, physically dormant seeds are not capable to imbibe water and germinate. *Scarification* can overcome this constraint, e.g. by softening the seed coat chemically with acid or breaking it mechanically with sandpaper or a scalpel. Physically dormant seeds are known to occur in plant families such as Fabaceae and Malvaceae (Baskin *et al.*, 2000).

The process of *after-ripening* may have an effect on germination requirements or work as a way to escape dormancy, but it may also induce physical dormancy as the drying process makes the seed coat harder and more impermeable (Baskin and Baskin, 1988; Taiz and Zeiger, 2010). A period of dry conditions may affect GA and ABA concentrations and sensitivity and hence act as a dormancy-breaking treatment (Finch-Savage and Leubner-Metzger, 2006). It can increase both the germination rate and speed. However, too dry seeds, about 5 % water content depending on species, inhibit the after-ripening process and thus dormancy-breaking (Finch-Savage and Leubner-Metzger, 2006; Taiz and Zeiger, 2010). Light requirements and seed testa water permeability seem also be related to after-ripening in some species. Dry storage at room temperatures has an after-ripening effect.

Most species require, benefits or are not affected by *light* to germinate (Baskin and Baskin, 1988). Few species require darkness to germinate. It is not clear whether light should be seen as a

dormancy-breaking factor or mere as a required germination condition (Vleeshouwers *et al.*, 1995), although some seeds needs a certain photoperiod to germinate (Taiz and Zeiger, 2010). Light is especially important for seeds which strategy is to dwell in seed-banks and wait for ground disturbance. This trait is very common among plants that have a weedy life strategy. Some seeds sense shade from the red:far-red wavelength ratio, like in the presence of a dense leaf canopy, which inhibit germination (Grime *et al.*, 1981). Phytochrome is responsible for the photochemical reactions that govern seed germination (Shinomura, 1997).

Ecological factors – An indication of seed dormancy patterns

The objective of the present study is mainly to establish germination protocols for laboratory work and not to investigate seed ecology, although seed ecology can provide a hint of the required factors for dormancy-breaking and germination requirements. A study of 274 herbaceous plants from the temperate region showed a relationship between seed dormancy-breaking factors and plant life-cycle types (Baskin and Baskin, 1988). Of the perennial species, 87 % had a germination peak after cold stratification treatment, just 2 % germinated directly after sowing, without a cold treatment, whilst 11 % germinated in autumn when the temperatures decreased. Biennial species showed a peak in germination after a cold treatment. Most of the winter annuals were dormant or conditionally dormant at maturation and germinated in the autumn due to after-ripening or too high summer temperatures, and they did not need cold temperature. About half of the summer annuals were dormant at maturation and one third conditionally dormant. They needed a cold stratification to germinate or to increase germination rate. A meta-analysis of studies on the same matter showed the same pattern (Baskin and Baskin, 1998). Phenology factors, such as time of seed dispersal and germination, and life-cycle strategies are more pervasive on seed dormancy characteristics than evolutionary relationships and type of habitat (Baskin and Baskin, 1988). However, in a study of 403 species in northern England, Grime *et al.* (1981) reported a tendency that woodland herbaceous species have a lower germination rate immediately after dispersal than species in other habitats.

Some generalisations about seed dormancy can be made based on successional stage and survival strategies. Species that often occur on ruderal grounds in an early succession stage and with a weedy behaviour often exhibit similar dormancy characteristics. After-ripening is the most important dormancy-breaking factor for many herbaceous plants with a weedy life-cycle pattern in temperate regions, for examples in weed-like species of Brassicaceae and Caryophyllaceae (Steinbaur and Grigsby, 1957). After-ripening may also affect physiological dormancy by decreasing the cold stratification period needed in perennial, biennial and summer annual weedy plants. Non-dormant seed are more common in weedy species than in non-weedy species in

temperate forest regions (Baskin and Baskin, 1998). Physical and physiological dormancy are the most common dormancy types in weedy species from temperate grass regions.

Intra-species variations in seed dormancy

Dormancy characteristics may vary between different populations of the same species, between individuals of the same population, between different inflorescences on the same individual plant and in the same inflorescence (Baskin and Baskin, 2004). On all these levels, seeds may vary in degrees of dormancy and sensitivity to dormancy-breaking factors, leading to different germination rate (Baskin and Baskin, 1998). Intra-species variations are common in a wide range of plant families. Germination requirements may also vary. For example, plants from a northern distribution germinate, on average, at a higher minimum temperature than those from a more southern distribution (Grime et al., 1981). Other examples of variations in germination requirements are seed sensitivity to soil moisture, light, temperature and soil chemicals (Baskin and Baskin, 1998). A Spanish study of the inter-population variation in *Hypericum perforatum* showed a large discrepancy in dormancy and Pérez-García *et al.* (2006) wrote:

> "...germination from a single population of a species (as has been the case for *Hypericum perforatum* up to now) must be interpreted with caution and that information regarding germination behaviour of a wild species can only be obtained following individual population studies." (Pérez-García *et al.* 2006 pp. 1197)

In contrast to cultivated plant varieties that often have been selected for homogeneous dormancy characteristics and germination requirements, wild populations' dormancy and germination requirements are related and adapted to the local conditions and climate (Baskin and Baskin, 1998).

Baskin and Baskin (2005) write that in most germination tests, "the assumption is that the germination responses obtained at the various test conditions are representative of the population" (Baskin and Baskin, 2005, pp. 164). However, the intra-population variation of germination responses, e.g. due to different hormonal levels, can be large in some wild populations (Finch-Savage and Leubner-Metzger, 2006).

Intra-individual variations also occur and one example is from *Pastinaca sativa.* Hendrix (1984) has discovered germination variations, not only between different individuals, but also among different umbel orders on the same plant. Seeds from a primary umbel are larger and germinate at a higher rate after a winter after-ripening period than smaller seeds from a tertiary umbel. Germination differences in the same individual, and even in the same inflorescence, are

common among flowering plants.

The dormancy may also vary over time in one seed. There are differences in germination between mature and immature seeds, depending on species (Baskin and Baskin, 1998). Seeds from some species do not germinate at all if they are collected before maturation while other may not have entered dormancy and hence may germinate to a higher rate than mature seeds. Maturation of seeds on an individual plant may at times differ in degrees of maturity, depending on species. Morphologically dormant seeds fully mature after seed dispersal, although the seeds may need to reach a certain developing stage before leaving the mother plant.

The variation depends mainly on genetics and the environment in which the mother plant grew at seed maturation. Genotype differentiation due to natural selection creates ecotypes that are adapted to the population-specific localities. Baskin and Baskin (1998) mention influential environmental factors such as competition, pests, day length, growing season, light quality, nutrients, moisture and temperature.

Included species and previous germination studies

In most plant families, there are variations in dormancy at both genus and species level. A need for cold stratification to break seed dormancy is very common in Apiaceae which often have a morphological dormancy. Many species of Fabaceae and Malvaceae need scarification to germinate (Grime *et al.*, 1981, Baskin and Baskin 1998). Many of the species included in the present study have more or less weedy life strategies and grow in ruderal places, early in the succession as the first settlers on disturbed grounds. The species and accessions included in the present study are shown in Table 1. For further information about the accessions, see Appendix 1. The scientific names and the Swedish names follow Svensk Kulturväxtdatabas, SKUD (Aldén and Ryman, 2009) and the family names follow the Angiosperm Phylogeny website (Stevens, 01-27-2013).

The Royal Botanical Garden (RBG) in Kew runs the Millennium Seed Bank Partnership with a collection that contains thousands of wild plant species for the purpose of conservation. Germination rate is tested regularly and the results are recorded in the Royal Botanical Garden's Seed Information Database, SID (RBG Kew, 2008). They use an agar substrate, sometimes in combination with GA_3 in the seed tests. The International Seed Testing Association (ISTA) is an authority on seed testing methods and seed science, and their purpose is to develop and standardise seed testing practices (ISTA, 2013). They publish 'ISTA International Rules for Seed Testing'. Most of the species information on germination and dormancy-breaking requirements below is from these two sources.

As for temperature and light conditions, 20/30°C means 20°C at night and 30°C at day and 8/16 h means 8 hours night and 16 h day.

Table 1. List over accessions included in the present study.

Accession Nr	Latin name	Swedish name	Family
Stratification experiment			
NGB21742	*Aethusa cynapium*	Vildpersilja	Apiaceae
NGB20166	*Allium fistulosum*	Piplök	Amaryllidaceae
NGB23673	*Anthemis tinctoria*	Färgkulla	Asteraceae
NGB23598	*Arctium lappa*	Stor kardborre	Asteraceae
NGB21899	*Ballota nigra*	Bosyska	Lamiaceae
NGB23508	*Chenopodium album*	Svinmålla	Amaranthaceae
NGB23477	*Chenopodium album*	Svinmålla	Amaranthaceae
NGB20236	*Cichorium intybus*	Cikoria	Asteraceae
NGB21689	*Cynoglossum officinale*	Hundtunga	Boraginaceae
NGB21874	*Datura stramonium*	Spikklubba	Solanaceae
NGB21774	*Digitalis purpurea*	Fingerborgsblomma	Scrophulariaceae
NGB21740	*Dipsacus fullonum*	Kardvädd	Dipsacaceae
NGB23606	*Dipsacus fullonum*	Kardvädd	Dipsacaceae
NGB21798	*Hyoscyamus niger*	Bolmört	Solanaceae
NGB21968	*Hypericum perforatum*	Äkta johannesört	Hypericaceae
NGB21884	*Leonurus cardiaca*	Hjärtstilla	Lamiaceae
NGB23608	*Lepidium latifolium*	Bitterkrassing	Brassicaceae
NGB23600	*Malva sylvestris*	Rödmalva	Malvaceae
NGB23777	*Melilotus albus*	Vit sötväppling	Fabaceae
NGB24388	*Melissa officinalis*	Citronmeliss	Lamiaceae
NGB21871	*Oenothera biennis*	Nattljus	Onagraceae
NGB21703	*Oenothera glazioviana*	Jättenattljus	Onagraceae
NGB21701	*Pastinaca sativa*	Palsternacka	Apiaceae
NGB21783	*Saponaria officinalis*	Såpnejlika	Caryophyllaceae
NGB21704	*Tanacetum vulgare*	Renfana	Asteraceae
NGB21849	*Tanacetum vulgare*	Renfana	Asteraceae
NGB22478	*Thymus pulegioides*	Stortimjan	Lamiaceae
NGB21684	*Urtica dioica*	Brännässla	Urticaceae
NGB21118	*Verbascum nigrum*	Mörkt kungsljus	Scrophulariaceae
NGB21788	*Verbascum speciosum*	Praktkungsljus	Scrophulariaceae
NGB21962	*Verbascum thapsus*	Kungsljus	Scrophulariaceae
Scarification and stratification experiment			
NGB14193.2	*Trifolium pratense*	Rödklöver	Fabaceae
NGB1143.3	*Trifolium pratense*	Rödklöver	Fabaceae
NGB14440.2	*Trifolium pratense*	Rödklöver	Fabaceae
NGB20014.1	*Allium ursinum*	Ramslök	Amaryllidaceae

Species in the stratification experiment

Aethusa cynapium L. (Apiaceae), NGB21742, Vildpersilja, is a summer or winter annual or biennial, depending on subspecies. It is a poisonous herb that grows on mesic, nutrient-rich soils, in more or less disturbed grounds with high nitrogen content (Flora Nordica, 2010; Mossberg and Stenberg, 2010). Mature seeds of *A. cynapium* are morphophysiologically dormant and need a cold

stratification period and then light to germinate (Roberts and Boddrell, 1985; Baskin and Baskin, 1998). Still, RBG Kew (2008) reported an 89 % germination rate at 9/23°C, 12/12 h germination conditions with GA_3, without stratification.

Allium fistulosum L. (Amaryllidaceae), NGB20166, Piplök, is a perennial geophyte, and originates from East Asia. It is rare in southern Norway where it is found as naturalised from old cultivations (Mossberg and Stenberg, 2010). ISTA (1996) recommends 20°C or 15°C with a cold stratification dormancy-breaking treatment. Specht and Keller (1997) tested germination in 91 wild *Allium* species. An after-ripening treatment for 2 months resulted in an 80 % germination rate at 11°C for *A. fistulosum*.

Anthemis tinctoria L. (Asteraceae), NGB23673, Färgkulla, is a perennial herb that grows on dry, nutrient-rich, calcareous mineral soils, such as along roadsides, embankments and dry meadows (Mossberg and Stenberg, 2010). Studies showed that the seed testa may be an important dormancy factor. Scarification treatments increased germination percentages in two other *Anthemis* species (Ellis and Ilnicki, 1968; Gealy *et* al., 1985), but these species have a winter annual life-cycle. RBG Kew (2008) got 100 % germination at 15 or 15/25 °C, 8/16 h germination conditions for *A. tinctoria*.

Arctium lappa L. (Asteraceae), NGB23598, Stor kardborre, is a biennial that grows on dry to mesic, nutrient-rich, culture-influenced soils (Mossberg and Stenberg, 2010). *Arctium minus* is known to be non-dormant at seed dispersal (Baskin and Baskin, 1988). ISTA (1996) recommends cold stratification and then 20/30 or 20 °C germination conditions for *A. lappa*. RBG Kew (2008) got 100 % germination rate without stratification at 25°C.

Ballota nigra L. (Lamiaceae), NGB21899, Bosyska, is a perennial herb that grows on nitrogenous soils, in ruderal places like ruins and roadsides. It was previously used as a medical plant (Mossberg and Stenberg, 2010). RBG Kew (2008) got 100 % germination at 16°C, 12/12h germination condition.

Chenopodium album L. (Amaranthaceae), NGB23508 and NGB23477, Svinmålla, is a summer annual that grows on ruderal grounds and is often found as a weed in gardens and cultured fields (Mossberg and Stenberg, 2010). A lot of studies have been done about *C. album* germination phenology. Mature seeds are dormant at dispersal and need an after-ripening period to germinate

(Baskin and Baskin, 1977). The seeds have an annual conditionally dormant/non-dormant cycle and germinate primary in spring and early summer (Bouwmeester and Karssen 1993; Baskin and Baskin, 1998). RBG Kew (2008) achieved high germination percentage at a wide range of temperatures.

Cichorium intybus L. (Asteraceae), NGB20236, Cikoria, is a perennial herb that thrives on dry, well drained, nutritious soils, often along roadsides and other ruderal places (Mossberg and Stenberg, 2010). A compilation of studies by Tzortzakis (2009) showed that KNO_3 and GA accelerate germination. ISTA (1996) recommends 20/30°C or constantly 20°C with a KNO_3 treatment.

Fig 1. *Cynoglossum officinale*, NGB21689, Photo: Svein Solberg.

Cynoglossum officinale L. (Boraginaceae), NGB21689, Hundtunga, (Fig. 1) is a biennial herb that grows on ruderal places, like roadsides, and on shore banks in dry, nutrient-rich soil (Mossberg and Stenberg, 2010). Its seeds have a higher germination rate in darkness then in light and Baskin and Baskin (1998) recommend a cold stratification period and low germination temperature. Scarification increases seed germination (Stabell *et al.*, 1996, sec. ref.). The seed testa is water permeable, but makes a mechanically barrier for oxygen uptake. ISTA (1996) recommends cold stratification, KNO_3 treatment and germination conditions at 20/30°C or 20°C in light, for *C. amabile.* RBG Kew (2008) got 100 % germination with a scarification treatment with *C. officinale.*

Datura stramonium L. (Solanaceae), NGB21874, Spikklubba, is a summer annual, poisonous herb, that grows on shore banks and ruderal places, like along roadsides. It originates from Mexico and it has been used as an old medical plant (Mossberg and Stenberg, 2010). It has physiological seed dormancy that seems to be mainly affected by seed coat constrains, embryo hormone levels and

light (Baskin and Baskin, 1998). ISTA (1996) recommends cold stratification and scarification followed by 20/30°C or constantly 20°C germination conditions. RBG Kew (2008) got 83-100 % germination at 30°C without a dormancy-breaking treatment.

Digitalis purpurea L. (Plantaginaceae), NGB21774, Fingerborgsblomma, is a biennial or monocarpic perennial, poisonous, herb that grows on dry to mesic mineral soils, along forest edges, grasslands and timber-felling areas (Mossberg and Stenberg, 2010). The seeds are non-dormant and they germinate the same autumn after dispersal, although light is required (Grime *et al.*, 1981; Baskin and Baskin, 1998). ISTA (1996) recommends cold stratification and 20/30°C or 20°C germination conditions. RBG Kew (2008) got 80-100 % germination at room temperature.

Fig. 2. *Dipsacus fullonum*, NGB21740, Photo: Svein Solberg

Dipsacus fullonum L. (Dipsacaceae), NGB21740 (Fig. 2) and NGB23606, Kardvädd, is a biennial herb that grows in clay-rich soil. It originates from Europe, West Asia and North Africa (Mossberg and Stenberg, 2010). Beaton and Dudley (2007) got 99 % germination from fresh seeds in room temperature and RBG Kew (2008) got 100 % germination with a scarification treatment, but another test without scarification showed equal germination rate.

Hyoscyamus niger L. (Solanaceae), NGB21798, Bolmört, is a summer annual or biennial, depending on when maturation and dispersion occur. Early matured and dispersed seeds germinate the same autumn and the plants live as biennials, whereas late matured and dispersed seeds enter dormancy and germinate in the spring after a cold treatment and live as summer annuals. It is a very poisonous herb that grows on moist, nitrogen-rich soils, on disturbed grounds, gardens, ruins and farms (Mossberg and Stenberg, 2010; CDFA, 2013). *H. niger* seeds are physiologically dormant,

maintained by embryo dormancy and a hard seed testa, and both GA and acidification treatments increase seed germination (Cirak *et al.*, 2004). Baskin and Baskin (1998) recommend cold stratification treatment and RBG Kew (2008) achieved 100 % germination with a 6 weeks 2°C stratification period and 26°C germination conditions.

Hypericum perforatum L. (Hypericaceae),NGB21968, Äkta johannesört, is a perennial herb that grows on nutrient-poor, dry, sandy soils, on dry meadows and rocky grounds (Mossberg and Stenberg, 2010). The seeds are physiologically dormant and it is primary germination inhibitors that control the process. High temperature and darkness prevent germination (Campbell, 1985). A study of *H. perforatum* in Spain showed that there are inter-population variations in germination response, even between accessions from the same type of habitat (Pérez-García *et al.* 2006). RBG Kew (2008) reported 85-100 % germination in temperatures at 15-26°C.

Fig. 3. *Leonurus cardiaca*, NGB21884, Photo: Svein Solberg

Leonurus cardiaca L. (Lamiaceae), NGB21884, Hjärtstilla, (Fig. 3) is a perennial, old medical herb that grows on nutrient-rich soils, on ruderal places like gardens and farms (Mossberg and Stenberg, 2010). ISTA (1996) recommends cold stratification treatment and 20/30 °C germination conditions. RBG Kew (2008) got 97 % and 94 % germination at 12/12 h, 16°C respectively 31°C germination conditions.

Lepidium latifolium L. (Brassicaceae), NGB23608, Bitterkrassing, is a perennial herb that grows on moist soils, on coastal meadows and along roadsides (Mossberg and Stenberg, 2010). Germination is induced by alternating day/night temperatures (Miller *et al.*, 1986). RBG Kew (2008) got 100 % germination at 10/25°C, for *L. latifolium*.

Malva sylvestris L. (Malvaceae), NGB23600, Rödmalva, (Fig. 4) is a biennial or perennial herb that grows on mesic, nitrogen-rich soils, on meadows, farm yards and other ruderal places, in coastal areas (Mossberg and Stenberg, 2010). The seeds are physically dormant and the seed testa needs to become water-permeable to germinate (Van Assche and Vandelook, 2007). ISTA (1996) recommends 20/30°C or 20°C germination conditions.

Fig. 4. NGB23600, *Malva sylvestris*, 2010-09-27, Photo: Svein Solberg.

Melilotus albus Medik. (Fabaceae), NGB23777, Vit sötväppling, is an annual or biennial herb that grows on nutrient-rich coarse sand or clay soils, on embankments, roadsides and disturbed grounds (Mossberg and Stenberg, 2010). Like many other Fabaceae species, *M. albus* has physically dormant seeds. Hamly (1932) states that the dormancy is due to seed coat impermeability but that there is a variation among individual seeds. There are some indications that freezing and thawing may cause the dormancy-breaking in the natural habitat (Baskin and Baskin, 1998). ISTA (1996) recommends a cold stratification before 20°C germination condition. RGB Kew (2008) got high germination percentage with a scarification treatment in a wide range of germination temperatures.

Melissa officinalis L. (Lamiaceae), NGB24388, Citronmeliss, is a perennial herb and originates from southern Europe (Mossberg and Stenberg, 2010). ISTA (1996) recommends cold stratification followed by 20/30°C or 20°C germination conditions. RBG Kew (2008) got high germination rate (>90 %) even without stratification.

Oenothera biennis L. (Onagraceae), NGB21871, Nattljus, (Fig. 5) is a biennial herb that grows on nutrient-poor, coarse sand or clay soils, on embankments, roadsides and disturbed grounds (Mossberg and Stenberg, 2010). According to Baskin and Baskin (1998), the seeds are physiologically dormant and have an annual non-dormant/conditionally dormant cycle in light and a non-dormant/dormant cycle in darkness. They recommend a cold stratification treatment followed by 15/30°C germination conditions and ISTA (1996), in contrast, recommends 20/30°C germination conditions with KNO_3 without stratification. RBG Kew (2008) got a high germination percentage (95-100 %) at a wide range of germination temperatures (21-35°C).

Fig 5. *Oenothera biennis*, NGB21871, Photo: Svein Solberg.

Oenothera glazioviana P. Micheli ex Mart. (Onagraceae), NGB21703, Jättenattljus, is a biennial herb that grows on mineral soils, on roadsides and disturbed grounds. It originates from North America (Mossberg and Stenberg, 2010). Mature seeds are dispersed as non-dormant and germinates primary the same autumn. The seeds are very short lived (Kachi and Hirose, 1985), although, they are orthodox and should manage drying and cold storage. RBG Kew (2008) got 100 % germination at a temperature range of 25-33°C.

Pastinaca sativa L. (Apiaceae), NGB21701, Palsternacka, is a biennial or monocarpic perennial herb that grows on nutrient-rich, sand or clay soils, along roadsides, embankments, slopes or ruderal places (Mossberg and Stenberg, 2010). It is a mid-successional species. The seeds have morphological dormancy and ecological studies of *P. sativa* by Baskin and Baskin (1979) showed that only a few mature seeds germinate at high summer temperatures (35/20°C). This prevents seeds to germinate during the autumn. A winter after-ripening treatment lowers the temperature required for germination so that the seeds may germinate in early spring. In *P. sativa*, germination is partly

affected by furanocoumarins that work as growth inhibitors (Hendrix, 1984). ISTA (1996) only recommends 20/30°C germination conditions. RBG Kew (2008) got a 93 % and 83 % germination percentage with pre-sowing 6°C stratification treatment in 12 respectively 8 weeks and then 9/23°C, 12/12 h germination conditions with GA_3.

Saponaria officinalis L. (Caryophyllaceae), NGB21783, Såpnejlika,(Fig. 6) is a perennial herb that grows on nutrient-rich moist mineral soils, on beaches, meadows and in ruderal places (Mossberg and Stenberg, 2010). The seeds need a cold stratification period to germinate (Steinbaur and Grigsby, 1957). ISTA (1996) recommends cold stratification and germination conditions at 10/15°C with light. RBG Kew (2008) got high germination rate both with and without stratification.

Fig. 6. *Saponaria officinalis*, NGB21783, Photo: Svein Solberg

Tanacetum vulgare L. (Asteraceae), NGB21704 and NGB21849, Renfana, (Fig. 7) is a perennial herb that grows on dry, sandy, humus-rich soils, on beaches, roadsides, embankments and disturbed grounds. It is an old medical and spice plant (Mossberg and Stenberg, 2010). Hogenbrik and Wein (1992) showed that the germination percentage 10-foldet when daily germination temperature cycles increased from 20/10°C to 30/15°C. For most of the *Tanacetum sp*., ISTA (1996) recommends cold stratification and 20/30°C or constantly 20°C germination conditions with light. RBG Kew (2008) got high germination rate in a wide range of temperatures (11-33°C) for *T. vulgare*.

Fig. 7. *Tanacetum vulgare*, NGB21704, Photo: Svein Solberg.

Thymus pulegioides L. (Lamiaceae), NGB22478, Stortimjan, (Fig. 8) is a perennial herb that grows on dry to mesic, nutrient-rich moraine soils, in grasslands, roadsides and embankments (Mossberg and Stenberg, 2010). ISTA (1996) recommends 20/30°C or constantly 20°C germination conditions for *T. vulgaris.* RBG Kew got 100 % germination at 20°C, 8/16 h germination conditions for *T. pulegioides*.

Fig 8. *Thymus pulegioides*, NGB22478, Photo: Svein Solberg.

Urtica dioica L. (Urticaceae), NGB21684, Brännässla, is a perennial herb that grows on mesic, humus- and nitrogen-rich soils, in manure-piles, ditches, shrubberies and woods (Mossberg and Stenberg, 2010). The seeds are dependent on light to germinate and cold stratification does not improve germination rate (Tylor, 2009). RBG Kew (2008) has only records on germination tests without germination pre-treatments, but got 100 % with 8 weeks cold stratification at 6°C followed by 19/33°C germination conditions.

Verbascum nigrum L. (Scrophulariaceae), NGB21118, Mörkt kungsljus, is a biennial herb that grows on dry mineral soils, along roadsides, embankments and disturbed grounds (Mossberg and Stenberg, 2010). RBG Kew (2008) got 100 % germination with cold stratification at 2°C for 6 weeks and 26°C germination conditions.

Verbascum speciosum Schrad. (Scrophulariaceae), NGB21788, Praktkungsljus, is a biennial herb that grows on dry mineral soils. It is naturalised along roadsides, on embankments and disturbed grounds and originates from Southeast Europe, Turkey and Northern Iran (Mossberg and Stenberg, 2010). RBG Kew (2008) got 100% germination at 25°C.

Verbascum thapsus L. (Scrophulariaceae), NGB21962, Kungsljus, is a biennial herb that grows on dry, warm, mineral soils, on slopes, roadsides, embankments and disturbed grounds (Mossberg and Stenberg, 2010). The species has physiologically dormant seeds and an annual conditionally dormant/non-dormant cycle in light and dormant/non-dormant cycle in darkness (Vanlerberghe and Van Assche, 1986; Baskin and Baskin, 1998). The primary germination peak occurs in spring. Baskin and Baskin (1998) recommend a cold stratification followed by 20/35°C germination conditions. RGB Kew (2008) got high germination rate both with and without stratification.

Species in the scarification and stratification experiment

Allium ursinum L (Amaryllidaceae), NGB20014, Ramslök, is a perennial geophyte that grows on nutrient-rich moist humus-rich soils, in woodlands and gorges (Mossberg and Stenberg, 2010). The seeds have morphophysiological dormancy at maturation (Baskin and Baskin, 1998). Studies by Ernst (1979) shows that *A. ursinum* requires both a long warm period at 15-20°C and a subsequent 4 month cold period at 5°C to germinate.

Trifolium pratense L. (Fabaceae), NGB14193.2, NGB1143.3 and NGB14440.2, Rödklöver, is a perennial herb that grows on nutrient-rich soils, on grasslands, ley fields, roadsides and disturbed grounds (Mossberg and Stenberg, 2010). Seeds from *T. pratense* are conditionally dormant and have a physical or combinational dormancy (Baskin and Baskin, 2004; 1998). Some seeds will germinate after an after-ripening period but the germination percentage increases after scarification. RBG Kew (2008) reported 100 % germination rate with scarification treatment in a wide range of germination temperatures.

Materials and Methods

The study on the 31 cultural relict plant accessions and four non-relict accessions (Table 1) was conducted at the seed laboratory at Nordic Gene Resource Center (NordGen), Alnarp, Sweden, from January to March 2013.

Pre-experimental treatments

The seeds have been collected at different locations at different times in Denmark, Finland and Sweden. For information on the specific collection date, location, habitat and population sample size, see Appendix 1. The seeds were collected from plants in the same population and stored at room temperatures before drying to an internal water content of 3-7 % (depending on oil content in the seed), packed in hermetic bags and then frozen to -18°C in ordinary freezers. Exceptions are accessions of *Thymus pulegioides* – NGB22478, *Melissa officinalis* – NGB24388, *Cynoglossum officinalis* – NGB21689 that were newly collected and only dried, but not frozen. No seed germination tests were performed before storage

Experiments

Two experiments were carried out:

1) Stratification
2) Scarification and stratification

Stratification experiment

Germination tests, with cold stratification as the only factor, were performed on 31 different accessions from 28 different species. The study included three treatments, null, two and four weeks cold stratification, with three replications for each treatment, of approximately 25 seed per replicate. Baskin and Baskin (1998) recommend 50 seed with three replications. Due to limited seed supply, only 25 seeds per replicate were used.

About 25 seeds per sample were counted in a seed counter (Contador, Pfeuffer), (Fig. 9). The seeds were spread out evenly, primary to prevent spread of fungus and allelopatic interference, on filter paper (Munktell Filter AB, Falun, Sweden), wetted with tap-water, in sterile Petri-dishes. In

most cases, the kind of germination substrate does not influence germination. It is just a matter of water retention capacities and that the seeds do not get drenched (Baskin and Baskin, 1998). The two stratification treatments were made in a cold room at +4-5°C, for two and four weeks, respectively.

The germination tests were conducted in an incubator (MIR-254, Panasonic), (Fig. 9), at 20/30 °C, with 8/16 hours diurnal cycles. According to Baskin and Baskin (1998), studies show that an alternating diurnal temperature rhythm is, in most cases, better for germination. The temperature regime was according to ISTA (1996) recommendations to maximise germination for most of the studied species. The germination status was evaluated and recorded once a week for three weeks. A seed was defined as germinated when 2 mm of the radicle had emerged (Bewley, 1997). After germination, the seeds were removed and discarded.

Fig. 9. The seed counter (left), Contador, Pfeuffer and the incubator (right) MIR-254, Panasonic. Photo: Jonatan Leo

Scarification and stratification experiment

Germination tests, with cold stratification and scarification treatment, were performed on four accessions from two different species (*Trifolium pratense* and *Allium ursinum*), with three replicates of approximately 25 seed each. The samples from the three *T. pratense* accessions were treated with three different cold stratification periods (0, 2 and 4 weeks) for scarified and non-scarified seeds, which made six treatments in total. Due to shortage of seeds, *A. ursinum* accessions were treated with two different cold stratification periods (0 and 3 weeks) for scarified and non-scarified seeds, i.e. four treatments in total. The seeds were scarified with sandpaper, grade 120. The seed counting was done manually. The stratification and germination tests were conducted in the same way as in the stratification experiment, as explained above.

Tetrazolium test

Tetrazolium test is a method used for seed viability analyses. Living tissue stains red, while dead tissue remains uncoloured. The tetrazolium test was performed according to the instructions in the Annex to chapter 6 in International Rules for Seed Testing (ISTA, 1996, pp. 203-204). Only species that did not germinate in present study were analysed.

The tetrazolium tests were conducted on 20 seeds each of *Allium ursinum*, *Aethusa cynapium* and *Cynoglossum officinale*, plus 20 *Avena sativa* seeds, with the lemmas removed, as a control. The seeds were soaked in tap water in petri dishes for 20 hours. For the *Allium ursinum* seeds, a longitudinal cut was made with a scalpel in the endosperm, but not trough the embryo. For the *Avena sativa*, *Aethusa cynapium* and *C. officinale* seeds, the cut was made at three quarters of the length of the endosperm and trough the embryo. Five controls for each species were killed in a microwave oven at 750 W, 15 sec. The seeds were put in 1 % tetrazolium for 18 hours in an incubator at + 30°C. At the evaluation, the seeds were cut in half, under a stereo microscope, and the viability were assessed by studying the colour of the embryo and endosperm, according to ISTA worksheets (ISTA, 2011).

Statistical analyses

The cold stratification (scarification) effect on the seed germinability after 21 days in the seed incubator was analysed statistically. The methods used were logit binomial confidence intervals and Two Proportions Tests, with 95 % significance. These methods are useful when analysing the probability of an event with two possible outcomes (in this case germination or no germination), where n is the total number of seeds in one accession and x is the sum of germinated seeds in that accession (Olsson *et al.*, 2010). The statistical computer programs used were R version 2.15.2 (R Development Core Team, 2008) and Minitab 16 (Minitab 16 Statistical Software, 2010).

Results and Discussion

Stratification experiment

The germination percentage and significant difference after 21 days, for the stratification experiment, are shown in Table 2. The accessions are divided into three groups, depending on their response to cold stratification treatment:

A) No response
B) Positive response
C) Negative response

For all raw data, see Appendix 2. The results for the accessions with significant results are shown in diagrams.

(A) Stratification treatments do not affect germination rate

Twenty-three species did not respond to cold stratification treatment and exhibited no significant difference between the treatments, see Appendix 2. This group is here divided into three subgroups with similar germination patterns. Depending on their germination percentage, different conclusions are made about dormancy status and further investigations. The subgroups are:

(A1) High germination rate
(A2) Suboptimal germination rate
(A3) No germination or very low germination rate

(A1) *Allium fistulosum, Arctium lappa, Datura stramonium, Digitalis purpurea, Dipsacus fullonum, Leonurus cardiac, Lepidium latifolium, Melissa officinalis, Urtica dioica, Verbascum nigrum, V. speciosum* and *V. thapsus* have a high satisfying germination rate (75-100 %) with no significant difference between the treatments. The cumulative germination percentages are shown in Fig. 10 for *V. nigrum.* The other accessions in this subgroup show similar patterns. The conclusion is that they were non-dormant at the beginning of the experiment. Providing favourable germination requirements, these species have no problem germinating, although after-ripening and/or high incubation temperatures may have had a positive impact on the seed germinability. These factors

may have overridden other dormancy-breaking requirements. It is an essential notation in case of new collections or future germination tests. Both after-ripening and cold stratification may be sufficient treatments to change dormancy status in these species.

Table 2. Germination percentage and significant differences between cold stratification treatments after 21 days. A1 - no response with high germination, A2 - no response with low germination, A3 - no response with no or very low germination, B - positive response and C - negative response, * - significant at $p \leq 0.05$, - - no significance.

Accession number	**Latin name**	**Mean germination %, after 21 days**			**Significant difference between treatments**			**Group**
		0 weeks	2 Weeks	4 weeks	0 and 2 weeks	0 and 4 weeks	2 and 4 weeks	
NGB21742	*Aethusa cynapium*	0	1	0	-	-	-	A3
NGB20166	*Allium fistulosum*	85	92	97	-	-	-	A1
NGB23673	*Anthemis tinctoria*	62	66	68	-	-	-	A2
NGB23598	*Arctium lappa*	82	90	88	-	-	-	A1
NGB21899	*Ballota nigra*	47	25	5	-	*	-	C
NGB23508	*Chenopodium album*	29	32	25	-	-	-	A2
NGB23477	*Chenopodium album*	50	37	44	-	-	-	A2
NGB20236	*Cichorium intybus*	46	46	37	-	-	-	A2
NGB21689	*Cynoglossum officinale*	4	1	2	-	-	-	A3
NGB21874	*Datura stramonium*	100	99	99	-	-	-	A1
NGB21774	*Digitalis purpurea*	92	100	92	-	-	-	A1
NGB21740	*Dipsacus fullonum*	89	97	93	-	-	-	A1
NGB23606	*Dipsacus fullonum*	100	97	100	-	-	-	A1
NGB21798	*Hyoscyamus niger*	0	26	15	*	*	-	B
NGB21968	*Hypericum perforatum*	28	60	78	*	*	-	B
NGB21884	*Leonurus cardiaca*	99	100	99	-	-	-	A1
NGB23608	*Lepidium latifolium*	100	99	100	-	-	-	A1
NGB23600	*Malva sylvestris*	27	25	27	-	-	-	A2
NGB23777	*Melilotus albus*	14	18	17	-	-	-	A2
NGB24388	*Melissa officinalis*	96	81	94	-	-	-	A1
NGB21871	*Oenothera biennis*	15	7	8	-	-	-	A2
NGB21703	*Oenothera glazioviana*	17	16	13	-	-	-	A2
NGB21701	*Pastinaca sativa*	23	45	59	-	*	-	B
NGB21783	*Saponaria officinalis*	29	83	92	*	*	-	B
NGB21704	*Tanacetum vulgare*	61	60	42	-	-	-	A2
NGB21849	*Tanacetum vulgare*	83	86	81	-	-	-	A2
NGB22478	*Thymus pulegioides*	47	80	68	*	-	-	B
NGB21684	*Urtica dioica*	88	94	92	-	-	-	A1
NGB21118	*Verbascum nigrum*	97	99	97	-	-	-	A1
NGB21788	*Verbascum speciosum*	99	99	96	-	-	-	A1
NGB21962	*Verbascum thapsus*	98	98	100	-	-	-	A1

The germination results from the present study for *Arctium lappa*, *M. officinalis*, *Datura stramonium*, *V. thapsus*, *V. speciosum*, *Leonurus cardiaca* and *Lepidium latifolium* are in line with RBG Kew's results (2008). RBG Kew got a high germination percentage for *V. nigrum* with cold stratification, Baskin and Baskin (1998) recommended cold stratification for *V. thapsus*, and ISTA recommended stratification and scarification for *D. stramonium* and stratification for *A. lappa*, *Allium fistulosum*, *M. officinalis*, *Digitalis purpurea*, *Leonurus cardiaca* and *Lepidium latifolium*, but neither of these treatments where required in present study.

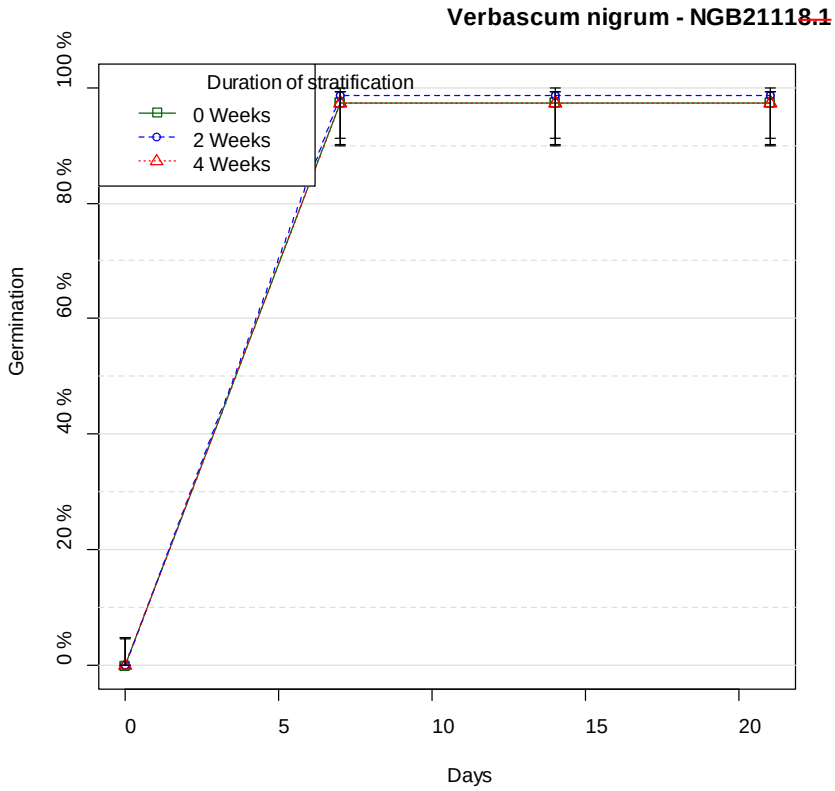

Fig. 10. The cumulative germination percentage for *Verbascum nigrum*, 0, 2 and 4 weeks cold stratification treatment, 0, 7, 14 and 21 days after start of germination tests. Error bars marks the binomial confidence interval with 95 % significance. *V. nigrum* shows no differ between treatments.

The requirement for alternating temperatures in *L. latifolium* seems to be fulfilled (Miller *et al.*, 1986). *D. purpurea* is non-dormant at seed dispersal (Grime *et al.*, 1981; Baskin and Baskin, 1998) and the result from the present study confirms this. Both accessions of *Dipsacus fullonum* and *U. dioica* in the present study showed the same germination patterns as earlier studies (Beaton and Dudley 2007; RBG Kew 2008; Tylor 2009). There are differences in germination between the *D. fullonum* accessions. TRANEKÆR CASTLE GP06 has a significantly higher germination percentage than MOLS SS0501 (Table 3), though both satisfying. This may be due to inter-population variations, differences in seed health or proportion of immature seeds.

In the present study, *A. fistulosum* germinated under the two and four week stratification temperature at 4-5°C and Specht and Keller (1997) report that an after-ripening treatment for two months resulted in 80 % germination at 11°C. Together, this confirms that it is after-ripening and not temperature that affects dormancy in *A. fistulosum*.

(A2) *Anthemis tinctoria, Chenopodium album, Cichorium intybus, Malva sylvestris, Melilotus albus, Oenothera glazioviana, O. biennis* and *Tanacetum vulgare* are not affected by cold stratification with no significant differences between the treatments and show a low, unsatisfying germination rate (<75 %). The result may be caused by pre-experimental treatments, unfavourable germination conditions, low seed health, impure seed sample, immature seeds or intra-population variations.

Mature seeds of *Chenopodium album* are dormant at dispersal and need an after-ripening period to germinate (Baskin and Baskin, 1977). The germination percentages in the present study are low in both accessions. Perhaps the after-ripening was insufficient. The seeds in the present study germinated during the cold period, which indicate that low temperatures do not affect dormancy status. The fact that RBG Kew (2008) got high germination rate at a wide range of temperatures, and that a lot of mould was recorded in the present study, indicate a low seed health in both accessions. There is a significant difference between the two accessions (Table 3). HØEGHOLM BL071023 has a higher germination rate than KOLLERUM BL0710230104. The variance was not caused by the amount of after-ripening as the two accessions were collected at the same date.

For *Cichorium intybus*, Tzortzakis (2009) showed that KNO_3 speed up germination and ISTA (1996) also recommend it. Possibly nitrate is a necessary requirement for *C. intybus* to germinate.

Seeds of *O. glazioviana* are non-dormant, but very short-lived according to Kachi and Hirose (1985). The after-ripening process might have had a negative impact on seed vitality in the present study. Although, RBG Kew (2008) got high germination percentages at 25–33°C for both *O. glazioviana* and *O. biennis*.

Both accessions of *T. vulgare* showed a low germination rate and were unaffected by a cold stratification. Interestingly, RBG Kew (2008) got high germination rate in a wide range of temperatures (11-33°C). This indicates a low seed quality (low seed health and/or a large proportion of immature seeds) in the tested accessions in the present study. It was hard to see whether the sample was clean and to distinguish seeds from other dry flower parts. There was a significant difference between the accessions in the present study. GUDHJEM SS1007 (86 %) had a higher

germination percentage than AGERSØ SS0703 (61 %) after 21 days with no stratification treatment (Table 3). This might be due to inter-population variations in germination requirements or differences in seed quality.

Table 3. Two Proportions Tests between the two accessions of *Tanacetum vulgare, Dipsacus fullonum, Chenopodium album* after 21 days, without stratification treatment. All comparisons show a significant difference.

Species	**Accessions**	**Fishers exact test: p-value**
Tanacetum vulgare	GUDHJEM SS1007 > AGERSØ SS0703	0.003
Dipsacus fullonum	TRANEKÆR CASTLE GP06 > MOLS SS0501	0.002
Chenopodium album	HØEGHOLM BL071023 > KOLLERUM BL0710230104	0.013

According Van Assche and Vandelook (2007), *Malva sylvestris* seeds are physically dormant, like many other species in Malvaceae, and need scarification to imbibe water and germinate. The absence of scarification is a probable explanation to the low germination performance in the present study, although ISTA (1996) do not mention scarification in their recommendations.

Like in Malvaceae, many species in Fabaceae, including *Melilotus albus,* have physically dormant seeds and need scarification to germinate. Results from RGB Kew (2008) confirm this. The absence of scarification may explain the results in the present study. According to Hamly (1932), dormancy is variable among individual seeds, and this might explain the partial germination in the present study. Some seeds of both *M. albus* and *Malva sylvestris* germinated during the cold period, which suggests that stratification does not affect seed dormancy.

The unsatisfying germination rate in *A. tinctoria* may also be caused by the absence of scarification. The seed coat is shown to be an important dormancy factor for the winter annual *Anthemis* species (Ellis and Ilnicki, 1968; Gealy *et* al., 1985), but no data is available for the perennial *A. tinctoria*. RBG Kew (2008) got 100 % germination at 15 or 15/25 °C, for *A. tinctoria* and the seeds germinated during the cold period in the present study, which indicate that germination may benefit from lower germination temperatures.

The after-ripening process may have had a negative impact on the germination of the accessions in Group A2. If this is the case, new collections and avoidance of drying is recommended. Suboptimal germination condition is another explanation. The germination may, for example, have been inhibited by the high incubation temperature. The result might be an expression of intra-population variations where some seeds need additional dormancy-breaking treatments, for example two cold periods. Poor seed health or impure seed samples are also a plausible explanation.

Depending on species, the low germination rate may reflect the proportion of collected immature seeds. However, the results are unsatisfying and further investigations, and perhaps dormancy studies and seed health tests, need to be carried out.

Larger accession batches in store may be needed to guarantee enough regeneration mother material. In this case, the decline of germinability is more important than the actual germination percentage.

(A3) *Cynoglossum officinale* and *Aethusa cynapium* showed no germination results, or only a few seeds germinated. The tetrazolium test showed that 20 of 20 seeds are alive in both accessions. The conclusion is that the seeds are still dormant. The result may have been caused by unsatisfying germination requirements, or, like in A2, that the after-ripening process may have had a negative impact on the germination. However, further investigations need to be done in order to find out which dormancy-breaking factor that is needed for germination.

The large seeds of *C. officinale* have a hard seed coat and Stabell *et al.* (1996, sec. Ref.) claim that scarification increases the germination rate, by enhancing oxygen uptake, and the results by RBG Kew (2008) confirm this. The absence of scarification may explain the results in the present study. A contributing factor may be the presence of light during germination, as Baskin and Baskin (1998) states that *C. officinale* has a higher germination rate in darkness than in light.

The low germination of *A. cynapium* may be due to the high incubation temperature. In all, 16 seeds germinated one week after final reading in room temperature with constant temperature of approximately 21°C. It is unclear whether it was the lower temperature *per se*, or the temperature change, that enhanced germination. RBG Kew (2008) got a high germination percentage (89 %) in 9/23°C, but they also used GA_3. As *A. cynapium* experience morphophysiological dormancy (Roberts and Boddrell, 1985), the GA_3 might be a crucial factor. Further investigations are needed to resolve these ambiguities.

(B) Stratification treatment increase the germination rate

Five species benefited from cold stratification: *Hyoscyamus niger, Hypericum perforatum, Pastinaca sativa, Saponaria officinalis* and *Thymus pulegioides* (Table 2). For *Hyoscyamus niger, Hypericum perforatum,* and *S. officinalis,* two weeks and four weeks of stratification were significantly better than no stratification, but four weeks were not significantly better than two weeks. For *P. sativa* and *T. pulegioides*, four weeks stratification was significantly better than no stratification, but two weeks were not significantly better than no stratification and four weeks were not significant better than two weeks. The results were not depending on differences in imbibation

time. The germination percentages were significantly higher in the four weeks of stratification treatments than in the no stratification treatments, after 21 days imbibation time on moist filter paper, in all accessions of group B.

For *Hypericum perforatum*, RBG Kew (2008) got 85–100 % germination in temperatures at 15–26°C. In contrast, the present study showed that *H. perforatum* benefits by a cold stratification, but does not reach high germination rate after four weeks of stratification (Fig. 11). According to Campbell (1985), it is primary germination inhibitors that control the process and hence soaking the seeds in water may increase germination. He also states that lower germination temperatures may increase germination, which should be considered in future tests. An inter-population variation in germination response (Pérez-García *et al.* 2006) should also be considered in other collection germination studies.

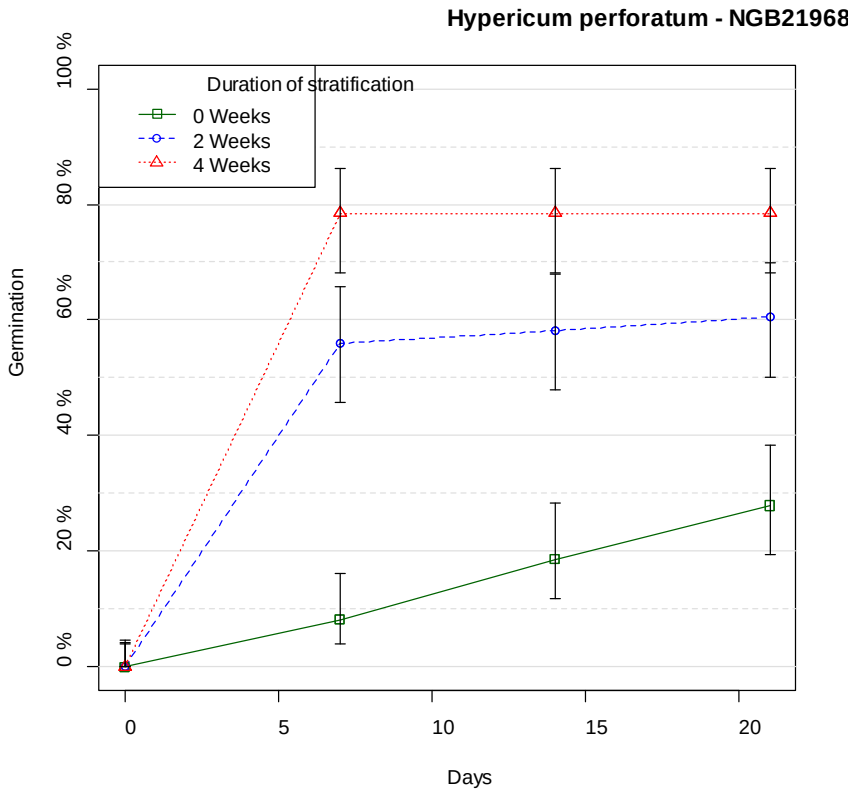

Fig. 11. The cumulative germination percentage for *Hypericum perforatum*, 0, 2 and 4 weeks cold stratification treatment, 0, 7, 14 and 21 days after start of germination tests. Error bars marks the binomial confidence interval with 95 % significance. *H. perforatum* show significantly higher germination percentage for 2 and 4 weeks than 0 weeks stratification, after 21 days.

The results of *Hyoscyamus niger* in the present study confirm earlier studies and recommendations (Baskin and Baskin 1998; RBG Kew 2008). Cold stratification benefited germination, but the percentage was still low after four weeks, and there were no significant differences between two

and four weeks of stratification (Fig. 12). A longer cold stratification period, GA and/or scarification might be needed to increase germination and to release the physiological dormancy that is maintained by embryo and the hard seed coat (Cirak *et al.*, 2004).

The results from *S. officinalis* in the present study are coherent with earlier studies and recommendations (Steinbaur and Grigsby, 1957; ISTA 1996; RBG Kew 2008), although RBG Kew also got high germination rate without stratification, which is in conflict with the results from the present study that showed an increase in germination after a cold stratification compared to no stratification (Fig. 13).

RBG Kew (2008) reported 100 % germination at 20°C of *T. pulegioides*, but the present study showed that a cold period benefits germination (Fig. 14). Still, many seeds germinated during the cold treatment, which might indicate that a low temperature stimulate germination rather than releases the dormancy and that 30/20°C are suboptimal germination conditions.

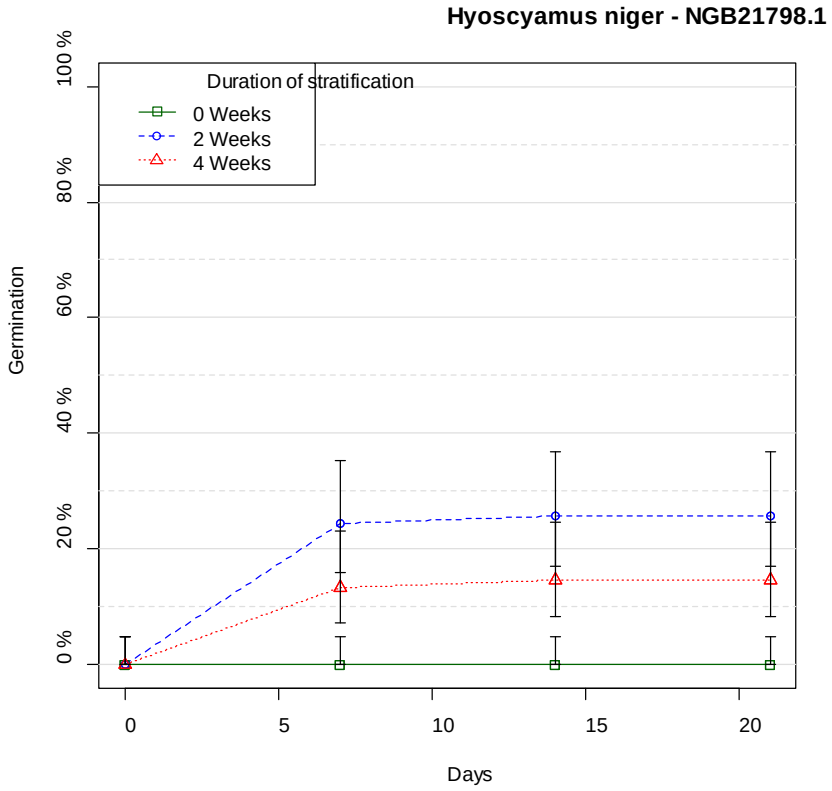

Fig. 12. The cumulative germination percentage for *Hyoscyamus niger*, 0, 2 and 4 weeks cold stratification treatment, 0, 7, 14 and 21 days after start of germination tests. Error bars marks the binomial confidence interval with 95 % significance. *H. niger* shows significantly higher germination percentage for 2 and 4 weeks than 0 weeks stratification, but no difference between 2 and 4 weeks.

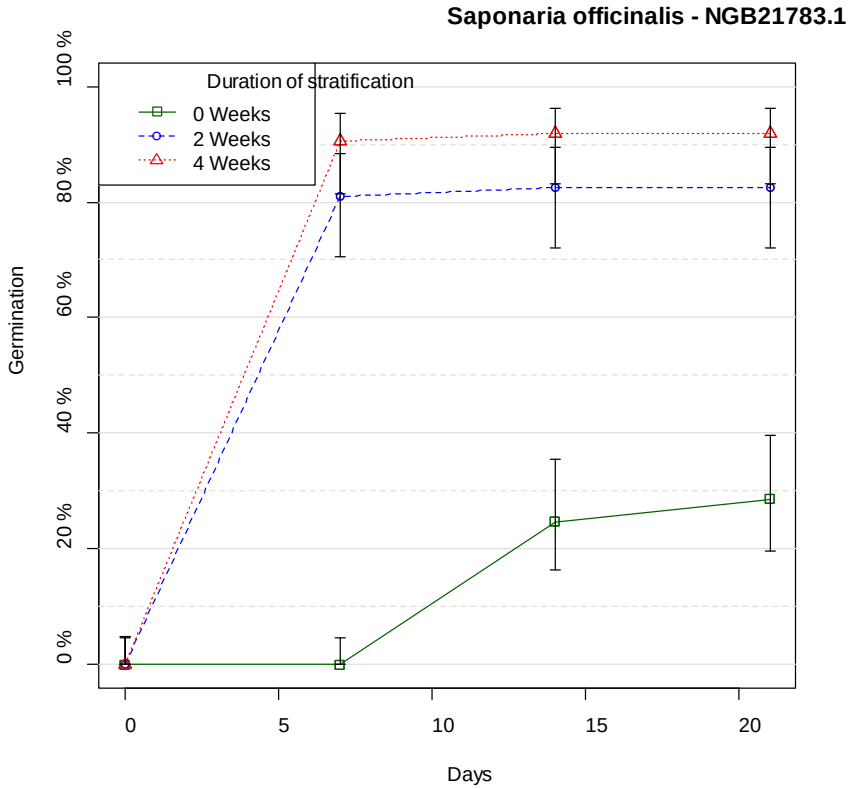

Fig. 13. Cumulative germination percentage for *Saponaria officinalis*, 0, 2 and 4 weeks cold stratification treatment, 0, 7, 14 and 21 days after start of germination tests. Error bars marks the binomial confidence interval with 95 % significance. *S. officinalis* show significant higher germination percentage for 2 and 4 weeks than 0 weeks stratification, but no difference between 2 and 4 weeks.

The germination of *P. sativa* increased by cold stratification in the present study (Fig. 15), but did not reach a satisfying germination rate. Results from RBG Kew (2008) suggest that four weeks are too short. RBG Kew got 93 % and 83 % germination rate with a 6°C stratification treatment of twelve and eight weeks, respectively. According to Baskin and Baskin (1979), only a few seeds germinate at high temperatures without a cold dormancy-breaking period, and in the present study, about 24 % germinated without stratification. The germination process may be accelerated by soaking the seeds, due to leaching of the furanocoumarins growth inhibitors (Hendrix, 1984). The intra-individual variation may lead to a variance in required stratification length.

Four weeks of cold stratification were adequate for *S. officinalis* (92 %) and *Hypericum perforatum* (78 %) but a longer cold period would perhaps increase germination even further (Table 2). The germination results of *P. sativa* and *Hyoscyamus niger* were not satisfying. *P. sativa* showed 59 % after 21 days, four weeks stratification and *H. niger* showed 26 % after 21 days, two weeks of stratification and 15 % after 21 days four weeks of stratification. The statistical analyses indicate that *P. sativa* would benefit by a longer stratification treatment.

Factors like fungal infections and a high incubation temperature may have inhibited germination in all species in this group. The amount of immature seeds may be another explanation.

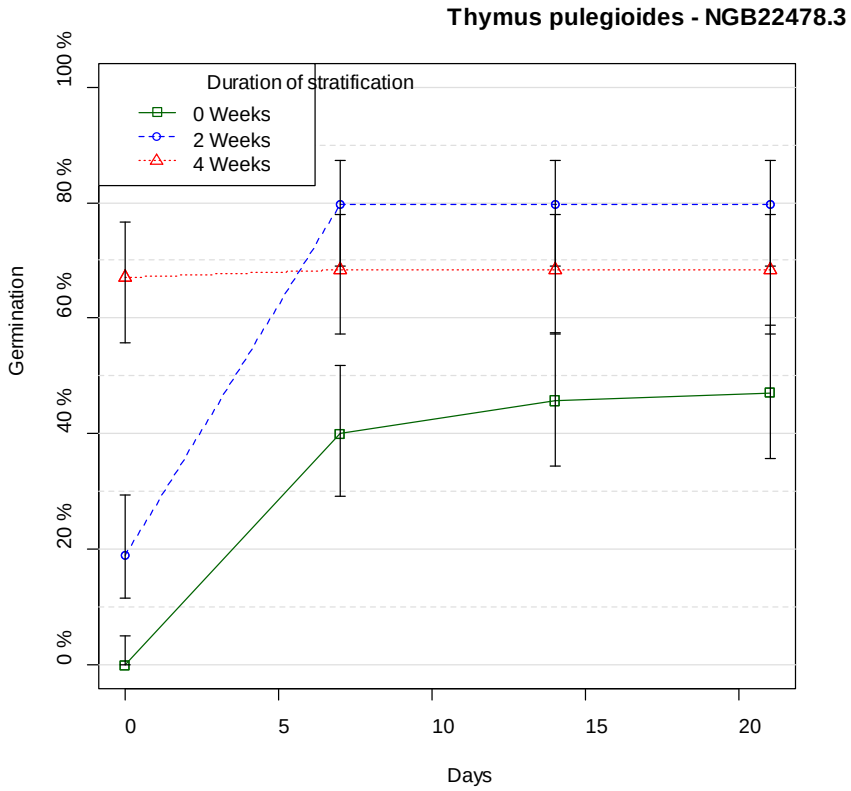

Fig. 14. Cumulative germination percentage for *Thymus pulegioides*, 0, 2 and 4 weeks cold stratification treatment, 0, 7, 14 and 21 days after start of germination tests. Error bars marks the binomial confidence interval with 95 % significance. 4 weeks stratification got significant higher germination percentage than 0 weeks in *T. pulegioides*.

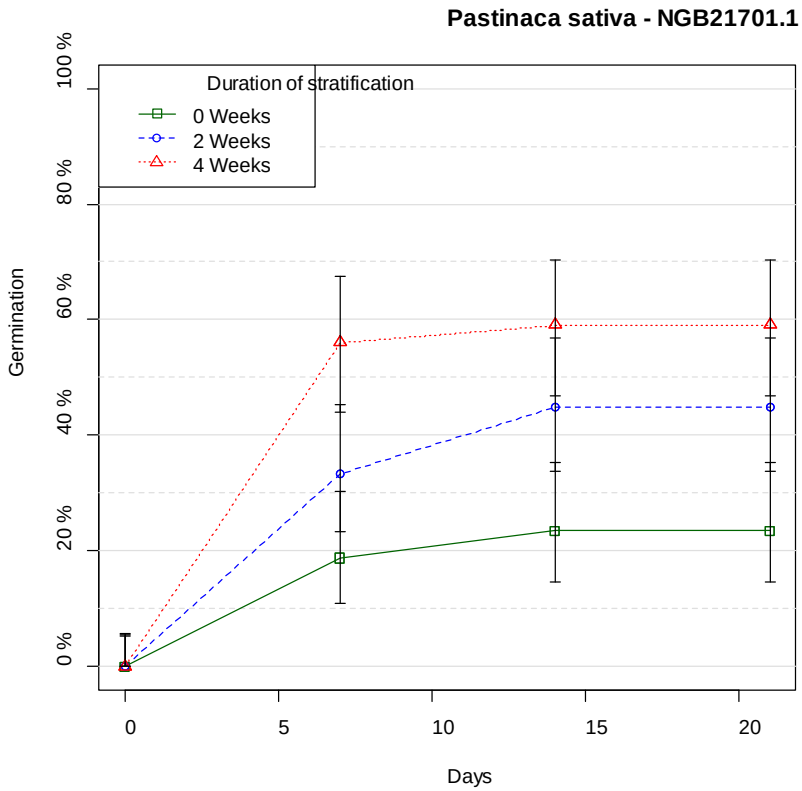

Fig. 15. The cumulative germination percentage for *Pastinaca sativa*, 0, 2 and 4 weeks cold stratification treatment, 0, 7, 14 and 21 days after start of germination tests. Error bars marks the binomial confidence interval with 95 % significance. Four weeks stratification got significantly higher germination percentage than 0 weeks in *P. sativa*.

(C) Stratification treatment decrease the germination rate

Cold stratification had a negative impact on *Ballota nigra* seed germination. The germination percentage decreased after cold stratification (Fig. 16). Four weeks were significantly lower than no stratification and two weeks of stratification (Table 2). Two weeks were not significantly lower than 0 weeks. It is not likely that cold temperatures impair seed vitality, but the seeds may have entered secondary dormancy. A plausible hypothesis is that the result is caused by fungal infection and that the stratification treatment resulted in a longer time for the seeds to become infected. As stated above, mould may be caused by low seed vitality and thus fungicide treatment may possibly not have helped. RBG Kew (2008) showed germination test results on 100 % germination at 16°C. Compared to RBG Kew's germination temperature, 20/30°C may have inhibited germination and benefited the fungus in the present study. None of the treatments showed a satisfying germination rate. Even without a stratification treatment, germination only reached 48 %. There might be other factors that affect dormancy status.

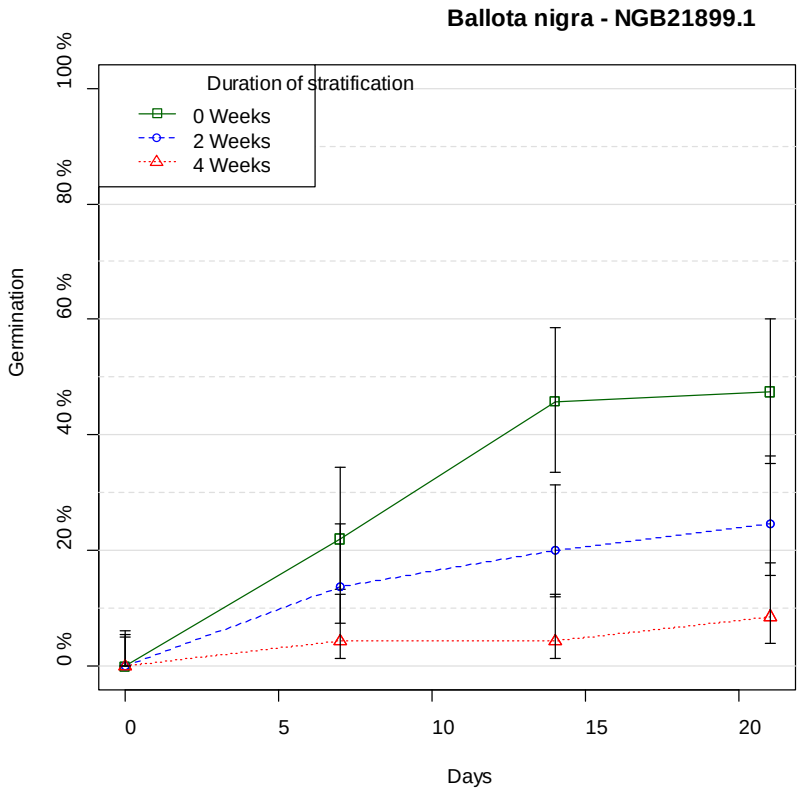

Fig 16. The cumulative germination percentage for *Ballota nigra*, 0, 2 and 4 weeks cold stratification treatment, 0, 7, 14 and 21 days after start of germination tests. Error bars marks the binomial confidence interval with 95 % significance. Four weeks stratification got significant lower germination percentage than 0 weeks in *B. nigra*.

Scarification and stratification experiment

The raw data from the scarification and stratification experiment is shown in Appendix 3.

No *Allium ursinum* seeds germinated during the experiment, even though the tetrazolium test showed 100% vitality. Neither stratification nor scarification or a combination of the two broke the dormancy. Other measurements have to be taken into consideration. Studies show that the seeds are morphophysiologically dormant at maturation and need a moist warm period followed by a cold period to germinate (Baskin and Baskin, 1998; Ernst, 1979). The absence of a moist warm period before cold stratification may explain why no seeds germinated in the present study.

Trifolium pratense seeds are conditionally dormant and have a physical or combinational dormancy. Some seeds will germinate after an after-ripening period but the germination percentage increases after scarification (Baskin and Baskin, 1998; 2004). Results from RBG Kew (2008)

confirm this.

The present study showed that there are inter-population differences in scarification responses of seed dormancy in *T. pratense* (Table 4). Two proportion tests showed that scarification promote germination in STENSJÖN IB0104 (Fig. 19) and KOTILA HM0101 (Fig. 17), but have no effect in REPOLANKYLÄ ME0202 (Fig. 18). This might be due to variations in seed coat thickness and therefore water-permeability. The variation may be caused by genetic or environmental factors. The amount of hard coated seeds in a batch might be caused by temperature and amount of precipitation during mother plant growth and seed maturation (Öhlund, unpublished). The same mechanical effect of scarification is hard to obtain and it is therefore difficult to guarantee the same treatment for all seeds.

The present study showed that cold stratification had no effect on dormancy-breaking in *T. pratense*. Many seeds germinated during the cold period, which indicate a wide required germination temperature. The after-ripening has probably affected the required germination temperature.

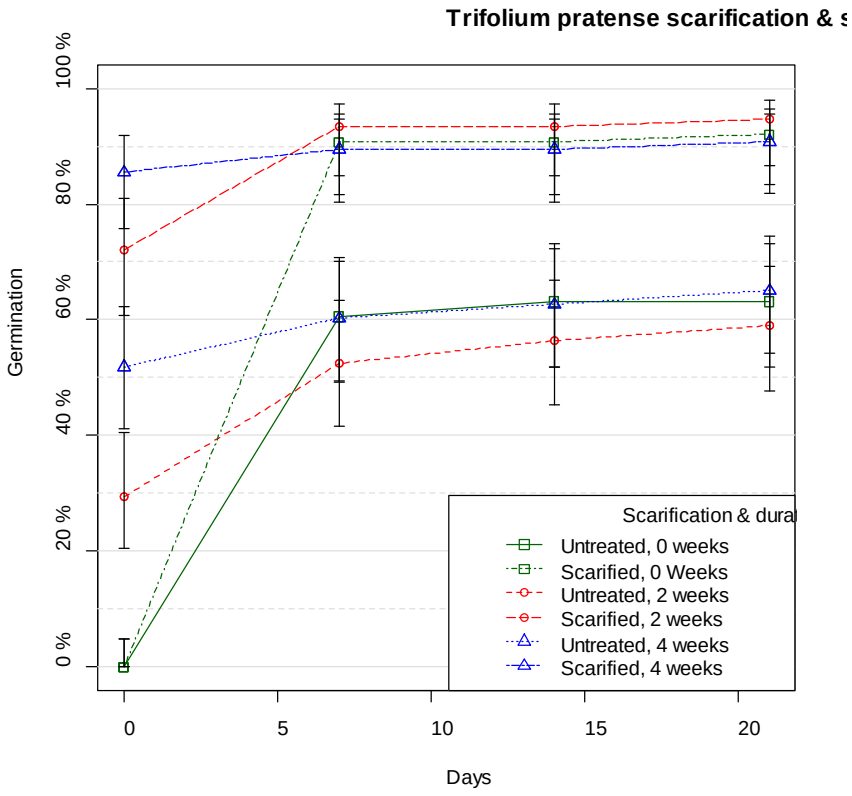

Fig. 17. The cumulative germination percentage for the six treatments in *Trifolium pratense*- NGB14440.2. Error bars marks the binomial confidence interval with 95 % significance.

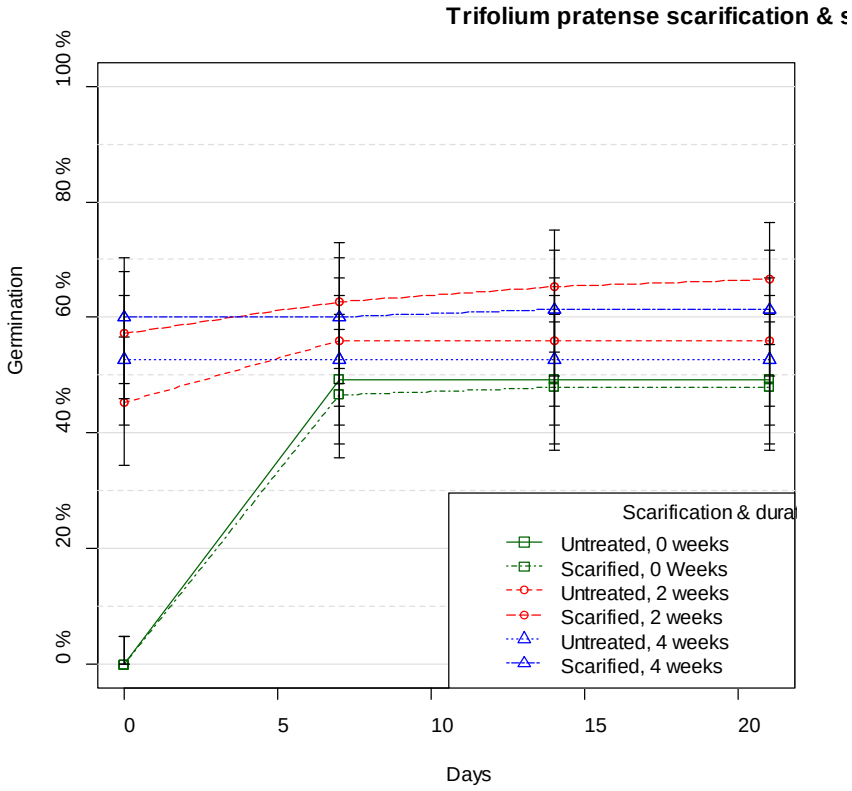

Fig. 18. The cumulative germination percentage for the six treatments in *Trifolium pratense*- NGB1143.3. Error bars marks the binomial confidence interval with 95 % significance.

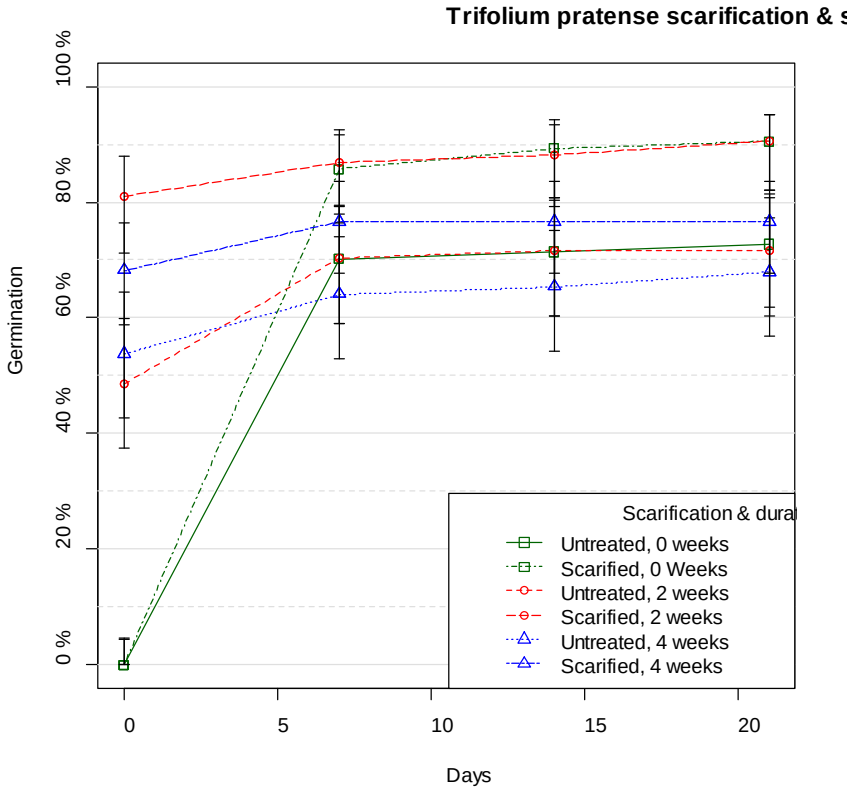

Fig. 19. The cumulative germination percentage for the six treatments in *Trifolium pratense*- NGB14193.2. Error bars marks the binomial confidence interval with 95 % significance.

Table 4. Two Proportions tests between scarified and non-scarified seeds in the three *Trifolium pratense* accessions without stratification treatment. Scarification promotes germination in accession NGB14193.2 and NGB14440.2, but has no effect in accession NGB1143.3.

Accession	Accession name	Fisher's exact test, p-value
NGB14193.2	STENSJÖN IB0104	0.004
NGB1143.3	REPOLANKYLÄ ME0202	1.000
NGB14440.2	KOTILA HM0101	0.000

Remarks

After-ripening and cold stratification are the most common dormancy-breaking factors and are appropriate for initial dormancy-breaking studies and dormancy screenings. More comprising studies are needed to maximise germination and to confirm the type of dormancy and germination requirements for each species, especially in an ecological perspective without optimal or overriding germination conditions or dormancy-breaking factors. Conclusions about conditionally dormancy cannot be made in the present study due to the high incubation temperature. It is possible that a cold stratification may lower the required germination temperature in the studied species. The seeds of *Thymus pulegioides* – NGB22478, *Melissa officinalis* – NGB24388, *Cynoglossum officinalis* – NGB21689 had not been frozen, and the result might differ after freezing.

In nature, wild plant seeds that mature at the same year, may germinate over many years (Baskin and Baskin, 1998). The mechanism that controls this might be due to intra-population variations in germination requirements or dormancy. With optimal germination requirements in laboratory germination test, the intra-population germination requirements can be reduced as a source of error, but dormancy variations call for additional treatments to get the maximum germination and a correct viability status.

The results in the present study show the germination response of the 31 accessions. Some samples originate from small size populations and sometimes from just a few individual plants, like the seeds from *Aethusa cynapium* (five plants), *Digitalis purpurea* (one plant), *Oenothera glazioviana* (three plants), *Urtica dioica* (four plants) and *Verbascum speciosum* (two plants). To draw conclusions about a species, many different populations have to be analysed. However, these results can give an indication of the species germination characteristics. The knowledge of intra-

species germination variations is often scarce, but in a conservation context, specific population characteristics are more interesting than average species traits. In the perspective of germination protocol establishment, the results from the present study give an indication of where to start. Variance may exist, but it is more important in an ecological view and may be more or less neglected in most cases in the laboratory, where dormancy-breaking treatments and germination requirements are optimised.

The seed quality (health and viability) is often unknown in wild collected seeds. Factors like infections, pests and disease organisms, mother plant growth conditions and nutrient status may affect the germination rate, and pre-experimental tests should be performed to confirm the seed quality. Fungal infection was a problem in many samples and might be a sign of poor seed health, reduced vitality or immature seeds. It is hard to know whether they would have germinated even in absence of fungus. A fungicide would perhaps give a higher germination rate in accessions with large problems.

The time of collection might be crucial in some species due to intra-population or intra-individual maturity status variations. A seed has a more or less optimal collection time that often occur at seed dispersal. Only mature seeds should be collected, although maturity status is often difficult to evaluate in the field. Repetitive collection times might be necessary.

One limitation was that only one incubator with one temperature regime was used. As mentioned above, high temperature inhibits seed germination in some species, and some may have performed better if a lower germination temperature had been used. The high temperature may also have benefited the fungus. High temperatures may override other dormancy-breaking requirements like cold stratification, and should be considered if germination studies are done with lower germination temperatures.

Summary

The majority, 22 out of 28 species, showed no effect of cold stratification. Twelve species had a high, satisfying germination percentage (75–100 %): *Allium fistulosum, Arctium lappa, Datura stramonium, Digitalis purpurea, Dipsacus fullonum, Leonurus cardiaca, Lepidium latifolium, Melissa officinalis, Urtica dioica, Verbascum nigrum, V. speciosum* and *V. thapsus.* Eight species showed a low, unsatisfying germination percentage (<75 %): *Anthemis tinctoria, Chenopodium album, Cichorium intybus, Malva sylvestris, Melilotus albus, Oenothera glazioviana, O. biennis* and *Tanacetum vulgare.* These accessions need further investigations in germination requirements, dormancy-breaking factors, and/or seed quality. The poor results may also have been caused by a high proportion of immature seeds. Two species, *Aethusa cynapium* and *Cynoglossum officinale,* showed no germination at all or only a few seeds germinated, even though Tetrazolium tests show 100 % vitality. They might require another dormancy-breaking treatment to germinate. Five accessions benefited from cold stratification: *Hyoscyamus niger, Hypericum perforatum, Pastinaca sativa, Saponaria officinalis* and *Thymus pulegioides.* However, cold stratification as a dormancy-breaking effect can be questioned in *T. pulegioides* because of germination during the cold stratification period. Cold stratification had a negative impact on *Ballota nigra* seed germination, either as consequence of secondary dormancy or fungal infections.

Trifolium pratense showed an inter-accessional variation in germination response to scarification treatment. Two accessions benefited by scarification and one accession was unaffected. The variation may have been caused by genetic factors or mother plant growth environment. *Allium ursinum* did not germinate at all, even though Tetrazolium test show 100 % vitality. Additional dormancy-breaking treatments are probably needed.

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Appendix 1.

Included accessions with accession number, accession name, taxon, collection date, origin country, origin place, source population size.

Accession Nr	Accession name	Latin name	Collection date	Origin Country	Place name	Source population	Remarks
NGB21742	EBELTOFT SS0601	*Aethusa cynapium*	20090927	Denmark	Ebeltoft by	5 plants	At construction area in city center
NGB20166	AUEN	*Allium fistulosum*		Norway			
NGB23673	KALØ BL200710230501	*Anthemis tinctoria*	20071023	Denmark	Kalø		
NGB23598	HESSELAGER GÅRD GP0802	*Arctium lappa*	20101029	Denmark	Hesselager gård	20 plants	At a castle built year 1500-1600
NGB21899	ØRBY SS1401	*Ballota nigra*	20090930	Denmark	Ørby		Old village
NGB23508	KOLLERUM BL0710230104	*Chenopodium album*	20071023	Denmark			
NGB23477	HØEGHOLM BL071023	*Chenopodium album*	20071023	Denmark	Høegholm	15 plants	From an old garden
NGB20236	LOMMA SS0103	*Cichorium intybus*	20080804	Sweden	Lomma	50 plants	Roadside
NGB21689	BORREBY SS0105	*Cynoglossum officinale*	20090921	Denmark	Borreby	10 plants	Old garden, at a castle from 1556
NGB21874	EBELTOFT SS1102	*Datura stramonium*	20090929	Denmark	Ebeltoft		Harbour area
NGB21774	HOLSTEINBORG SS0212	*Digitalis purpurea*	20090921	Denmark	Holsteinborg	1 plant	Old garden, at a castle
NGB21740	MOLS SS0501	*Dipsacus fullonum*	20090927	Denmark	Mols bjerge	10 plants	Stony area with grazing
NGB23606	TRANEKÆR CASTLE GP06	*Dipsacus fullonum*	20100928	Denmark	Tranekær borg		
NGB21798	HAMMERSHUS SS0113	*Hyoscyamus niger*	20091008	Denmark	Hammershus (Bornholm)		Inside ruin fortress wall
NGB21968	KOLLERUP GODS BL0103	*Hypericum perforatum*	20071022	Denmark	Kollerup gods	25 plants	At a castle
NGB21884	HAMMERSHUS SS0117	*Leonurus cardiaca*	20091006	Denmark	Hammershus (Bornholm)	50/100 plants	
NGB23608	VALDEMAR SLOT GP0501	*Lepidium latifolium*	20100928	Denmark	Valdemar slot	100 plants	On a sea shore, at a castle
NGB23600	KALEKO MØLLLE GP0301	*Malva sylvestris*	20101027	Denmark	Kaleko mølle	10 plants	At an old water mill
NGB23777	SJÆLLANDS ODDE LA1109270602	*Melilotus albus*		Denmark			
NGB24388	BRUNDBY SS20120926	*Melissa officinalis*	20120925	Denmark	Brunby, Samsø	30 plants	
NGB21871	STRANDS SS0201	*Oenothera biennis*	20090928	Denmark	Strands	8 plants	
NGB21703	AGERSØ SS0702	*Oenothera glazioviana*	20090922	Denmark	Agersø	3 plants	Sea shore on an island
NGB21701	STIGSNES SS0601	*Pastinaca sativa*	20090922	Denmark	Stigsnes	20/1000 plants	Sea shore
NGB21783	ESBY SS1201	*Saponaria officinalis*	20090920	Denmark	Esby		
NGB21704	AGERSØ SS0703	*Tanacetum vulgare*	20090922	Denmark	Agersø	2 plants	
NGB21849	GUDHJEM SS1007	*Tanacetum vulgare*	20091008	Denmark	Gudshjem (Bornholm)	40/1000 plants	
NGB22478	TOMMERUP GB2002	*Thymus pulegioides*	20020730	Denmark	Tommerup		
NGB21684	BORREBY GP0102	*Urtica dioica*	20090921	Denmark	Borreby, Skjælsør	4/4 plants	At a castle built 1556
NGB21118	FALSLEV MARIAGER	*Verbascum nigrum*	20081001	Denmark	Falslev Mariager		
NGB21788	ØRBY SS1301	*Verbascum speciosum*	20090929	Denmark	Ørby, Mols bjerge	2 plants	Field margin at a farm
NGB21962	KOLLERUP GODS BL0102	*Verbascum thapsus*	20071022	Denmark	Kollerup gods	6 plants	

Accession Nr	Accession name	Latin name	Collection date	Origin Country	Place name	Source population	Remarks
NGB14193.2	STENSJÖN IB0104	*Trifolium pratense*	20000816	Sweden	Karlsberg, Stensjön		
NGB1143.3	REPOLANKYLÄ ME0202	*Trifolium pratense*	19810917	Finland	Repolankylä, Rautalampi		
NGB14440.2	KOTILA HM0101	*Trifolium pratense*	19980924	Finland	Kotila, Piippola	10 plants	
NGB20014.1	Alnarp	*Allium ursinum*	2008	Sweden	Alnarpsparken, Lomma		

Appendix 2.

Raw data for species included in the stratification experiment with accession number, germination day, stratification treatment, n = total amount of seeds in one accession, x = total amount of germinated seeds in one accession and logit confidence interval with germination mean, upper and lower values.

Accession	Treatment	Days	n	x	mean	lower	upper
NGB24388.1	0 Weeks	0	77	0	0,000	0,000	0,047
NGB23777.1	0 Weeks	0	80	0	0,000	0,000	0,045
NGB23673.1	0 Weeks	0	86	0	0,000	0,000	0,042
NGB23608.1	0 Weeks	0	75	0	0,000	0,000	0,048
NGB23606.1	0 Weeks	0	82	0	0,000	0,000	0,044
NGB23600.1	0 Weeks	0	104	0	0,000	0,000	0,035
NGB23598.1	0 Weeks	0	74	0	0,000	0,000	0,049
NGB23508.1	0 Weeks	0	76	0	0,000	0,000	0,047
NGB23477.1	0 Weeks	0	76	0	0,000	0,000	0,047
NGB22478.3	0 Weeks	0	70	0	0,000	0,000	0,051
NGB21968.1	0 Weeks	0	86	0	0,000	0,000	0,042
NGB21962.1	0 Weeks	0	77	0	0,000	0,000	0,047
NGB21899.1	0 Weeks	0	59	0	0,000	0,000	0,061
NGB21884.1	0 Weeks	0	74	0	0,000	0,000	0,049
NGB21874.2	0 Weeks	0	77	0	0,000	0,000	0,047
NGB21871.1	0 Weeks	0	76	0	0,000	0,000	0,047
NGB21849.1	0 Weeks	0	78	0	0,000	0,000	0,046
NGB21798.1	0 Weeks	0	74	0	0,000	0,000	0,049
NGB21788.1	0 Weeks	0	82	0	0,000	0,000	0,044
NGB21783.1	0 Weeks	0	77	0	0,000	0,000	0,047
NGB21774.1	0 Weeks	0	76	0	0,000	0,000	0,047
NGB21742.1	0 Weeks	0	77	0	0,000	0,000	0,047
NGB21740.1	0 Weeks	0	62	0	0,000	0,000	0,058
NGB21704.1	0 Weeks	0	82	0	0,000	0,000	0,044
NGB21703.1	0 Weeks	0	63	0	0,000	0,000	0,057
NGB21701.1	0 Weeks	0	64	0	0,000	0,000	0,056
NGB21689.2	0 Weeks	0	78	0	0,000	0,000	0,046
NGB21684.1	0 Weeks	0	77	0	0,000	0,000	0,047
NGB21118.1	0 Weeks	0	77	0	0,000	0,000	0,047
NGB20236.1	0 Weeks	0	68	0	0,000	0,000	0,053
NGB20166.2	0 Weeks	0	68	0	0,000	0,000	0,053
NGB24388.1	0 Weeks	7	77	47	0,610	0,498	0,712
NGB23777.1	0 Weeks	7	80	11	0,138	0,078	0,231
NGB23673.1	0 Weeks	7	86	49	0,570	0,464	0,670
NGB23608.1	0 Weeks	7	75	74	0,987	0,911	0,998
NGB23606.1	0 Weeks	7	82	82	1,000	0,956	1,000
NGB23600.1	0 Weeks	7	104	10	0,096	0,053	0,170
NGB23598.1	0 Weeks	7	74	59	0,797	0,691	0,874
NGB23508.1	0 Weeks	7	76	21	0,276	0,188	0,387
NGB23477.1	0 Weeks	7	76	34	0,447	0,340	0,560
NGB22478.3	0 Weeks	7	70	28	0,400	0,292	0,518
NGB21968.1	0 Weeks	7	86	7	0,081	0,039	0,161
NGB21962.1	0 Weeks	7	77	75	0,974	0,902	0,993

Accession	Treatment	Days	n	x	mean	lower	upper
NGB21899.1	0 Weeks	7	59	13	0,220	0,132	0,343
NGB21884.1	0 Weeks	7	74	73	0,986	0,910	0,998
NGB21874.2	0 Weeks	7	77	77	1,000	0,953	1,000
NGB21871.1	0 Weeks	7	76	4	0,053	0,020	0,132
NGB21849.1	0 Weeks	7	78	65	0,833	0,734	0,901
NGB21798.1	0 Weeks	7	74	0	0,000	0,000	0,049
NGB21788.1	0 Weeks	7	82	81	0,988	0,919	0,998
NGB21783.1	0 Weeks	7	77	0	0,000	0,000	0,047
NGB21774.1	0 Weeks	7	76	69	0,908	0,819	0,955
NGB21742.1	0 Weeks	7	77	0	0,000	0,000	0,047
NGB21740.1	0 Weeks	7	62	28	0,452	0,333	0,576
NGB21704.1	0 Weeks	7	82	49	0,598	0,488	0,698
NGB21703.1	0 Weeks	7	63	9	0,143	0,076	0,252
NGB21701.1	0 Weeks	7	64	12	0,188	0,110	0,302
NGB21689.2	0 Weeks	7	78	0	0,000	0,000	0,046
NGB21684.1	0 Weeks	7	77	59	0,766	0,659	0,847
NGB21118.1	0 Weeks	7	77	75	0,974	0,902	0,993
NGB20236.1	0 Weeks	7	68	25	0,368	0,262	0,488
NGB20166.2	0 Weeks	7	68	53	0,779	0,666	0,862
NGB24388.1	0 Weeks	14	77	69	0,896	0,806	0,947
NGB23777.1	0 Weeks	14	80	11	0,138	0,078	0,231
NGB23673.1	0 Weeks	14	86	52	0,605	0,498	0,702
NGB23608.1	0 Weeks	14	75	74	0,987	0,911	0,998
NGB23606.1	0 Weeks	14	82	82	1,000	0,956	1,000
NGB23600.1	0 Weeks	14	104	22	0,212	0,144	0,300
NGB23598.1	0 Weeks	14	74	61	0,824	0,721	0,895
NGB23508.1	0 Weeks	14	76	21	0,276	0,188	0,387
NGB23477.1	0 Weeks	14	76	37	0,487	0,377	0,598
NGB22478.3	0 Weeks	14	70	32	0,457	0,345	0,574
NGB21968.1	0 Weeks	14	86	16	0,186	0,117	0,282
NGB21962.1	0 Weeks	14	77	75	0,974	0,902	0,993
NGB21899.1	0 Weeks	14	59	27	0,458	0,336	0,585
NGB21884.1	0 Weeks	14	74	73	0,986	0,910	0,998
NGB21874.2	0 Weeks	14	77	77	1,000	0,953	1,000
NGB21871.1	0 Weeks	14	76	11	0,145	0,082	0,243
NGB21849.1	0 Weeks	14	78	65	0,833	0,734	0,901
NGB21798.1	0 Weeks	14	74	0	0,000	0,000	0,049
NGB21788.1	0 Weeks	14	82	81	0,988	0,919	0,998
NGB21783.1	0 Weeks	14	77	19	0,247	0,163	0,355
NGB21774.1	0 Weeks	14	76	70	0,921	0,835	0,964
NGB21742.1	0 Weeks	14	77	0	0,000	0,000	0,047
NGB21740.1	0 Weeks	14	62	52	0,839	0,725	0,911
NGB21704.1	0 Weeks	14	82	50	0,610	0,501	0,709
NGB21703.1	0 Weeks	14	63	10	0,159	0,088	0,271
NGB21701.1	0 Weeks	14	64	15	0,234	0,147	0,353
NGB21689.2	0 Weeks	14	78	2	0,026	0,006	0,097
NGB21684.1	0 Weeks	14	77	66	0,857	0,760	0,919
NGB21118.1	0 Weeks	14	77	75	0,974	0,902	0,993

Accession	Treatment	Days	n	x	mean	lower	upper
NGB20236.1	0 Weeks	14	68	28	0,412	0,302	0,532
NGB20166.2	0 Weeks	14	68	57	0,838	0,731	0,908
NGB24388.1	0 Weeks	21	77	74	0,961	0,886	0,987
NGB23777.1	0 Weeks	21	80	11	0,138	0,078	0,231
NGB23673.1	0 Weeks	21	86	54	0,628	0,521	0,723
NGB23608.1	0 Weeks	21	75	75	1,000	0,952	1,000
NGB23606.1	0 Weeks	21	82	82	1,000	0,956	1,000
NGB23600.1	0 Weeks	21	104	28	0,269	0,193	0,362
NGB23598.1	0 Weeks	21	74	61	0,824	0,721	0,895
NGB23508.1	0 Weeks	21	76	22	0,289	0,199	0,401
NGB23477.1	0 Weeks	21	76	38	0,500	0,389	0,611
NGB22478.3	0 Weeks	21	70	33	0,471	0,358	0,588
NGB21968.1	0 Weeks	21	86	24	0,279	0,195	0,383
NGB21962.1	0 Weeks	21	77	75	0,974	0,902	0,993
NGB21899.1	0 Weeks	21	59	28	0,475	0,351	0,601
NGB21884.1	0 Weeks	21	74	73	0,986	0,910	0,998
NGB21874.2	0 Weeks	21	77	77	1,000	0,953	1,000
NGB21871.1	0 Weeks	21	76	11	0,145	0,082	0,243
NGB21849.1	0 Weeks	21	78	65	0,833	0,734	0,901
NGB21798.1	0 Weeks	21	74	0	0,000	0,000	0,049
NGB21788.1	0 Weeks	21	82	81	0,988	0,919	0,998
NGB21783.1	0 Weeks	21	77	22	0,286	0,196	0,396
NGB21774.1	0 Weeks	21	76	70	0,921	0,835	0,964
NGB21742.1	0 Weeks	21	77	0	0,000	0,000	0,047
NGB21740.1	0 Weeks	21	62	55	0,887	0,782	0,945
NGB21704.1	0 Weeks	21	82	50	0,610	0,501	0,709
NGB21703.1	0 Weeks	21	63	11	0,175	0,099	0,288
NGB21701.1	0 Weeks	21	64	15	0,234	0,147	0,353
NGB21689.2	0 Weeks	21	78	3	0,038	0,012	0,113
NGB21684.1	0 Weeks	21	77	68	0,883	0,790	0,938
NGB21118.1	0 Weeks	21	77	75	0,974	0,902	0,993
NGB20236.1	0 Weeks	21	68	28	0,412	0,302	0,532
NGB20166.2	0 Weeks	21	68	58	0,853	0,748	0,919
NGB24388.1	2 Weeks	0	75	0	0,000	0,000	0,048
NGB23777.1	2 Weeks	0	105	17	0,162	0,103	0,245
NGB23673.1	2 Weeks	0	74	17	0,230	0,148	0,339
NGB23608.1	2 Weeks	0	76	0	0,000	0,000	0,047
NGB23606.1	2 Weeks	0	81	0	0,000	0,000	0,045
NGB23600.1	2 Weeks	0	90	9	0,100	0,053	0,181
NGB23598.1	2 Weeks	0	73	0	0,000	0,000	0,049
NGB23508.1	2 Weeks	0	76	20	0,263	0,177	0,373
NGB23477.1	2 Weeks	0	73	0	0,000	0,000	0,049
NGB22478.3	2 Weeks	0	74	14	0,189	0,115	0,295
NGB21968.1	2 Weeks	0	91	0	0,000	0,000	0,040
NGB21962.1	2 Weeks	0	82	0	0,000	0,000	0,044
NGB21899.1	2 Weeks	0	65	0	0,000	0,000	0,055
NGB21884.1	2 Weeks	0	75	0	0,000	0,000	0,048
NGB21874.2	2 Weeks	0	77	0	0,000	0,000	0,047

Accession	Treatment	Days	n	x	mean	lower	upper
NGB21871.1	2 Weeks	0	76	0	0,000	0,000	0,047
NGB21849.1	2 Weeks	0	78	0	0,000	0,000	0,046
NGB21798.1	2 Weeks	0	74	0	0,000	0,000	0,049
NGB21788.1	2 Weeks	0	75	0	0,000	0,000	0,048
NGB21783.1	2 Weeks	0	74	0	0,000	0,000	0,049
NGB21774.1	2 Weeks	0	78	0	0,000	0,000	0,046
NGB21742.1	2 Weeks	0	76	0	0,000	0,000	0,047
NGB21740.1	2 Weeks	0	68	0	0,000	0,000	0,053
NGB21704.1	2 Weeks	0	84	0	0,000	0,000	0,043
NGB21703.1	2 Weeks	0	76	0	0,000	0,000	0,047
NGB21701.1	2 Weeks	0	69	0	0,000	0,000	0,052
NGB21689.2	2 Weeks	0	73	0	0,000	0,000	0,049
NGB21684.1	2 Weeks	0	78	0	0,000	0,000	0,046
NGB21118.1	2 Weeks	0	75	0	0,000	0,000	0,048
NGB20236.1	2 Weeks	0	64	0	0,000	0,000	0,056
NGB20166.2	2 Weeks	0	72	0	0,000	0,000	0,050
NGB24388.1	2 Weeks	7	75	55	0,733	0,622	0,821
NGB23777.1	2 Weeks	7	105	18	0,171	0,111	0,256
NGB23673.1	2 Weeks	7	74	48	0,649	0,534	0,748
NGB23608.1	2 Weeks	7	76	75	0,987	0,912	0,998
NGB23606.1	2 Weeks	7	81	79	0,975	0,907	0,994
NGB23600.1	2 Weeks	7	90	13	0,144	0,086	0,233
NGB23598.1	2 Weeks	7	73	66	0,904	0,812	0,954
NGB23508.1	2 Weeks	7	76	23	0,303	0,210	0,414
NGB23477.1	2 Weeks	7	73	25	0,342	0,243	0,458
NGB22478.3	2 Weeks	7	74	59	0,797	0,691	0,874
NGB21968.1	2 Weeks	7	91	51	0,560	0,457	0,659
NGB21962.1	2 Weeks	7	82	80	0,976	0,908	0,994
NGB21899.1	2 Weeks	7	65	9	0,138	0,074	0,245
NGB21884.1	2 Weeks	7	75	75	1,000	0,952	1,000
NGB21874.2	2 Weeks	7	77	76	0,987	0,914	0,998
NGB21871.1	2 Weeks	7	76	5	0,066	0,028	0,149
NGB21849.1	2 Weeks	7	78	67	0,859	0,763	0,920
NGB21798.1	2 Weeks	7	74	18	0,243	0,159	0,353
NGB21788.1	2 Weeks	7	75	74	0,987	0,911	0,998
NGB21783.1	2 Weeks	7	74	60	0,811	0,705	0,885
NGB21774.1	2 Weeks	7	78	78	1,000	0,954	1,000
NGB21742.1	2 Weeks	7	76	0	0,000	0,000	0,047
NGB21740.1	2 Weeks	7	68	66	0,971	0,890	0,993
NGB21704.1	2 Weeks	7	84	51	0,607	0,499	0,705
NGB21703.1	2 Weeks	7	76	11	0,145	0,082	0,243
NGB21701.1	2 Weeks	7	69	23	0,333	0,233	0,452
NGB21689.2	2 Weeks	7	73	1	0,014	0,002	0,091
NGB21684.1	2 Weeks	7	78	70	0,897	0,808	0,948
NGB21118.1	2 Weeks	7	75	74	0,987	0,911	0,998
NGB20236.1	2 Weeks	7	64	30	0,469	0,351	0,590
NGB20166.2	2 Weeks	7	72	66	0,917	0,827	0,962
NGB24388.1	2 Weeks	14	75	57	0,760	0,651	0,843

Accession	Treatment	Days	n	x	mean	lower	upper
NGB23777.1	2 Weeks	14	105	19	0,181	0,118	0,266
NGB23673.1	2 Weeks	14	74	49	0,662	0,548	0,760
NGB23608.1	2 Weeks	14	76	75	0,987	0,912	0,998
NGB23606.1	2 Weeks	14	81	79	0,975	0,907	0,994
NGB23600.1	2 Weeks	14	90	18	0,200	0,130	0,295
NGB23598.1	2 Weeks	14	73	66	0,904	0,812	0,954
NGB23508.1	2 Weeks	14	76	24	0,316	0,222	0,428
NGB23477.1	2 Weeks	14	73	27	0,370	0,267	0,486
NGB22478.3	2 Weeks	14	74	59	0,797	0,691	0,874
NGB21968.1	2 Weeks	14	91	53	0,582	0,479	0,679
NGB21962.1	2 Weeks	14	82	80	0,976	0,908	0,994
NGB21899.1	2 Weeks	14	65	13	0,200	0,120	0,315
NGB21884.1	2 Weeks	14	75	75	1,000	0,952	1,000
NGB21874.2	2 Weeks	14	77	76	0,987	0,914	0,998
NGB21871.1	2 Weeks	14	76	5	0,066	0,028	0,149
NGB21849.1	2 Weeks	14	78	67	0,859	0,763	0,920
NGB21798.1	2 Weeks	14	74	19	0,257	0,170	0,368
NGB21788.1	2 Weeks	14	75	74	0,987	0,911	0,998
NGB21783.1	2 Weeks	14	74	61	0,824	0,721	0,895
NGB21774.1	2 Weeks	14	78	78	1,000	0,954	1,000
NGB21742.1	2 Weeks	14	76	0	0,000	0,000	0,047
NGB21740.1	2 Weeks	14	68	66	0,971	0,890	0,993
NGB21704.1	2 Weeks	14	84	51	0,607	0,499	0,705
NGB21703.1	2 Weeks	14	76	12	0,158	0,092	0,258
NGB21701.1	2 Weeks	14	69	31	0,449	0,337	0,567
NGB21689.2	2 Weeks	14	73	1	0,014	0,002	0,091
NGB21684.1	2 Weeks	14	78	72	0,923	0,839	0,965
NGB21118.1	2 Weeks	14	75	74	0,987	0,911	0,998
NGB20236.1	2 Weeks	14	64	30	0,469	0,351	0,590
NGB20166.2	2 Weeks	14	72	66	0,917	0,827	0,962
NGB24388.1	2 Weeks	21	75	61	0,813	0,709	0,886
NGB23777.1	2 Weeks	21	105	19	0,181	0,118	0,266
NGB23673.1	2 Weeks	21	74	49	0,662	0,548	0,760
NGB23608.1	2 Weeks	21	76	75	0,987	0,912	0,998
NGB23606.1	2 Weeks	21	81	79	0,975	0,907	0,994
NGB23600.1	2 Weeks	21	90	22	0,244	0,167	0,344
NGB23598.1	2 Weeks	21	73	66	0,904	0,812	0,954
NGB23508.1	2 Weeks	21	76	24	0,316	0,222	0,428
NGB23477.1	2 Weeks	21	73	27	0,370	0,267	0,486
NGB22478.3	2 Weeks	21	74	59	0,797	0,691	0,874
NGB21968.1	2 Weeks	21	91	55	0,604	0,501	0,699
NGB21962.1	2 Weeks	21	82	80	0,976	0,908	0,994
NGB21899.1	2 Weeks	21	65	16	0,246	0,157	0,365
NGB21884.1	2 Weeks	21	75	75	1,000	0,952	1,000
NGB21874.2	2 Weeks	21	77	76	0,987	0,914	0,998
NGB21871.1	2 Weeks	21	76	5	0,066	0,028	0,149
NGB21849.1	2 Weeks	21	78	67	0,859	0,763	0,920
NGB21798.1	2 Weeks	21	74	19	0,257	0,170	0,368

Accession	Treatment	Days	n	x	mean	lower	upper
NGB21788.1	2 Weeks	21	75	74	0,987	0,911	0,998
NGB21783.1	2 Weeks	21	74	61	0,824	0,721	0,895
NGB21774.1	2 Weeks	21	78	78	1,000	0,954	1,000
NGB21742.1	2 Weeks	21	76	1	0,013	0,002	0,088
NGB21740.1	2 Weeks	21	68	66	0,971	0,890	0,993
NGB21704.1	2 Weeks	21	84	51	0,607	0,499	0,705
NGB21703.1	2 Weeks	21	76	12	0,158	0,092	0,258
NGB21701.1	2 Weeks	21	69	31	0,449	0,337	0,567
NGB21689.2	2 Weeks	21	73	1	0,014	0,002	0,091
NGB21684.1	2 Weeks	21	78	73	0,936	0,855	0,973
NGB21118.1	2 Weeks	21	75	74	0,987	0,911	0,998
NGB20236.1	2 Weeks	21	64	30	0,469	0,351	0,590
NGB20166.2	2 Weeks	21	72	66	0,917	0,827	0,962
NGB24388.1	4 Weeks	0	76	0	0,000	0,000	0,047
NGB23777.1	4 Weeks	0	76	12	0,158	0,092	0,258
NGB23673.1	4 Weeks	0	83	32	0,386	0,287	0,494
NGB23608.1	4 Weeks	0	77	0	0,000	0,000	0,047
NGB23606.1	4 Weeks	0	87	0	0,000	0,000	0,042
NGB23600.1	4 Weeks	0	87	6	0,069	0,031	0,145
NGB23598.1	4 Weeks	0	74	0	0,000	0,000	0,049
NGB23508.1	4 Weeks	0	73	15	0,205	0,128	0,313
NGB23477.1	4 Weeks	0	71	2	0,028	0,007	0,106
NGB22478.3	4 Weeks	0	76	51	0,671	0,558	0,767
NGB21968.1	4 Weeks	0	79	0	0,000	0,000	0,046
NGB21962.1	4 Weeks	0	77	0	0,000	0,000	0,047
NGB21899.1	4 Weeks	0	70	0	0,000	0,000	0,051
NGB21884.1	4 Weeks	0	78	0	0,000	0,000	0,046
NGB21874.2	4 Weeks	0	78	0	0,000	0,000	0,046
NGB21871.1	4 Weeks	0	76	0	0,000	0,000	0,047
NGB21849.1	4 Weeks	0	92	0	0,000	0,000	0,039
NGB21798.1	4 Weeks	0	75	0	0,000	0,000	0,048
NGB21788.1	4 Weeks	0	79	0	0,000	0,000	0,046
NGB21783.1	4 Weeks	0	74	0	0,000	0,000	0,049
NGB21774.1	4 Weeks	0	79	0	0,000	0,000	0,046
NGB21742.1	4 Weeks	0	68	0	0,000	0,000	0,053
NGB21740.1	4 Weeks	0	74	0	0,000	0,000	0,049
NGB21704.1	4 Weeks	0	83	0	0,000	0,000	0,043
NGB21703.1	4 Weeks	0	78	0	0,000	0,000	0,046
NGB21701.1	4 Weeks	0	66	0	0,000	0,000	0,054
NGB21689.2	4 Weeks	0	79	0	0,000	0,000	0,046
NGB21684.1	4 Weeks	0	79	0	0,000	0,000	0,046
NGB21118.1	4 Weeks	0	74	0	0,000	0,000	0,049
NGB20236.1	4 Weeks	0	71	0	0,000	0,000	0,051
NGB20166.2	4 Weeks	0	68	40	0,588	0,468	0,698
NGB24388.1	4 Weeks	7	76	62	0,816	0,713	0,888
NGB23777.1	4 Weeks	7	76	13	0,171	0,102	0,273
NGB23673.1	4 Weeks	7	83	56	0,675	0,567	0,767
NGB23608.1	4 Weeks	7	77	77	1,000	0,953	1,000

Accession	Treatment	Days	n	x	mean	lower	upper
NGB23606.1	4 Weeks	7	87	87	1,000	0,958	1,000
NGB23600.1	4 Weeks	7	87	21	0,241	0,163	0,342
NGB23598.1	4 Weeks	7	74	65	0,878	0,782	0,935
NGB23508.1	4 Weeks	7	73	18	0,247	0,161	0,358
NGB23477.1	4 Weeks	7	71	30	0,423	0,314	0,540
NGB22478.3	4 Weeks	7	76	52	0,684	0,572	0,778
NGB21968.1	4 Weeks	7	79	62	0,785	0,681	0,862
NGB21962.1	4 Weeks	7	77	77	1,000	0,953	1,000
NGB21899.1	4 Weeks	7	70	3	0,043	0,014	0,125
NGB21884.1	4 Weeks	7	78	75	0,962	0,887	0,988
NGB21874.2	4 Weeks	7	78	77	0,987	0,915	0,998
NGB21871.1	4 Weeks	7	76	6	0,079	0,036	0,165
NGB21849.1	4 Weeks	7	92	75	0,815	0,723	0,882
NGB21798.1	4 Weeks	7	75	10	0,133	0,073	0,230
NGB21788.1	4 Weeks	7	79	76	0,962	0,889	0,988
NGB21783.1	4 Weeks	7	74	67	0,905	0,815	0,954
NGB21774.1	4 Weeks	7	79	73	0,924	0,841	0,965
NGB21742.1	4 Weeks	7	68	0	0,000	0,000	0,053
NGB21740.1	4 Weeks	7	74	68	0,919	0,831	0,963
NGB21704.1	4 Weeks	7	83	36	0,434	0,332	0,542
NGB21703.1	4 Weeks	7	78	10	0,128	0,070	0,222
NGB21701.1	4 Weeks	7	66	37	0,561	0,440	0,675
NGB21689.2	4 Weeks	7	79	1	0,013	0,002	0,084
NGB21684.1	4 Weeks	7	79	71	0,899	0,810	0,949
NGB21118.1	4 Weeks	7	74	72	0,973	0,898	0,993
NGB20236.1	4 Weeks	7	71	25	0,352	0,250	0,469
NGB20166.2	4 Weeks	7	68	66	0,971	0,890	0,993
NGB24388.1	4 Weeks	14	76	71	0,934	0,851	0,972
NGB23777.1	4 Weeks	14	76	13	0,171	0,102	0,273
NGB23673.1	4 Weeks	14	83	56	0,675	0,567	0,767
NGB23608.1	4 Weeks	14	77	77	1,000	0,953	1,000
NGB23606.1	4 Weeks	14	87	87	1,000	0,958	1,000
NGB23600.1	4 Weeks	14	87	24	0,276	0,192	0,379
NGB23598.1	4 Weeks	14	74	65	0,878	0,782	0,935
NGB23508.1	4 Weeks	14	73	18	0,247	0,161	0,358
NGB23477.1	4 Weeks	14	71	31	0,437	0,327	0,553
NGB22478.3	4 Weeks	14	76	52	0,684	0,572	0,778
NGB21968.1	4 Weeks	14	79	62	0,785	0,681	0,862
NGB21962.1	4 Weeks	14	77	77	1,000	0,953	1,000
NGB21899.1	4 Weeks	14	70	3	0,043	0,014	0,125
NGB21884.1	4 Weeks	14	78	77	0,987	0,915	0,998
NGB21874.2	4 Weeks	14	78	77	0,987	0,915	0,998
NGB21871.1	4 Weeks	14	76	6	0,079	0,036	0,165
NGB21849.1	4 Weeks	14	92	75	0,815	0,723	0,882
NGB21798.1	4 Weeks	14	75	11	0,147	0,083	0,246
NGB21788.1	4 Weeks	14	79	76	0,962	0,889	0,988
NGB21783.1	4 Weeks	14	74	68	0,919	0,831	0,963
NGB21774.1	4 Weeks	14	79	73	0,924	0,841	0,965

Accession	Treatment	Days	n	x	mean	lower	upper
NGB21742.1	4 Weeks	14	68	0	0,000	0,000	0,053
NGB21740.1	4 Weeks	14	74	69	0,932	0,848	0,972
NGB21704.1	4 Weeks	14	83	36	0,434	0,332	0,542
NGB21703.1	4 Weeks	14	78	10	0,128	0,070	0,222
NGB21701.1	4 Weeks	14	66	39	0,591	0,469	0,702
NGB21689.2	4 Weeks	14	79	2	0,025	0,006	0,096
NGB21684.1	4 Weeks	14	79	73	0,924	0,841	0,965
NGB21118.1	4 Weeks	14	74	72	0,973	0,898	0,993
NGB20236.1	4 Weeks	14	71	26	0,366	0,263	0,484
NGB20166.2	4 Weeks	14	68	66	0,971	0,890	0,993
NGB24388.1	4 Weeks	21	76	72	0,947	0,868	0,980
NGB23777.1	4 Weeks	21	76	13	0,171	0,102	0,273
NGB23673.1	4 Weeks	21	83	57	0,687	0,580	0,777
NGB23608.1	4 Weeks	21	77	77	1,000	0,953	1,000
NGB23606.1	4 Weeks	21	87	87	1,000	0,958	1,000
NGB23600.1	4 Weeks	21	87	28	0,322	0,232	0,427
NGB23598.1	4 Weeks	21	74	65	0,878	0,782	0,935
NGB23508.1	4 Weeks	21	73	19	0,260	0,173	0,372
NGB23477.1	4 Weeks	21	71	31	0,437	0,327	0,553
NGB22478.3	4 Weeks	21	76	52	0,684	0,572	0,778
NGB21968.1	4 Weeks	21	79	62	0,785	0,681	0,862
NGB21962.1	4 Weeks	21	77	77	1,000	0,953	1,000
NGB21899.1	4 Weeks	21	70	6	0,086	0,039	0,178
NGB21884.1	4 Weeks	21	78	77	0,987	0,915	0,998
NGB21874.2	4 Weeks	21	78	77	0,987	0,915	0,998
NGB21871.1	4 Weeks	21	76	6	0,079	0,036	0,165
NGB21849.1	4 Weeks	21	92	75	0,815	0,723	0,882
NGB21798.1	4 Weeks	21	75	11	0,147	0,083	0,246
NGB21788.1	4 Weeks	21	79	76	0,962	0,889	0,988
NGB21783.1	4 Weeks	21	74	68	0,919	0,831	0,963
NGB21774.1	4 Weeks	21	79	73	0,924	0,841	0,965
NGB21742.1	4 Weeks	21	68	0	0,000	0,000	0,053
NGB21740.1	4 Weeks	21	74	69	0,932	0,848	0,972
NGB21704.1	4 Weeks	21	83	36	0,434	0,332	0,542
NGB21703.1	4 Weeks	21	78	10	0,128	0,070	0,222
NGB21701.1	4 Weeks	21	66	39	0,591	0,469	0,702
NGB21689.2	4 Weeks	21	79	2	0,025	0,006	0,096
NGB21684.1	4 Weeks	21	79	77	0,975	0,904	0,994
NGB21118.1	4 Weeks	21	74	72	0,973	0,898	0,993
NGB20236.1	4 Weeks	21	71	26	0,366	0,263	0,484
NGB20166.2	4 Weeks	21	68	66	0,971	0,890	0,993

Appendix 3.

Raw data for *Trifolium pratense* with accession number, germination day, stratification treatment, scarification treatment where s = sandpaper treatment and o = no sandpaper treatment, n = total amount of seeds in one accession, x = total amount of germinated seeds in one accession, mean germination and logit confidence interval with upper and lower value.

Accession number	Days	Stratification	Scarification	n	x	mean	lower	upper
NGB14440.2	0	0 Weeks	o	76	0	0,000	0,000	0,047
NGB14193.2	0	0 Weeks	o	77	0	0,000	0,000	0,047
NGB1143.3	0	0 Weeks	o	73	0	0,000	0,000	0,049
NGB14440.2	0	0 Weeks	s	75	0	0,000	0,000	0,048
NGB14193.2	0	0 Weeks	s	84	0	0,000	0,000	0,043
NGB1143.3	0	0 Weeks	s	75	0	0,000	0,000	0,048
NGB14440.2	0	2 Weeks	o	78	23	0,295	0,204	0,405
NGB14193.2	0	2 Weeks	o	74	36	0,486	0,375	0,599
NGB1143.3	0	2 Weeks	o	75	34	0,453	0,345	0,566
NGB14440.2	0	2 Weeks	s	75	54	0,720	0,608	0,810
NGB14193.2	0	2 Weeks	s	84	68	0,810	0,711	0,880
NGB1143.3	0	2 Weeks	s	75	43	0,573	0,460	0,680
NGB14440.2	0	4 Weeks	o	83	43	0,518	0,411	0,623
NGB14193.2	0	4 Weeks	o	78	42	0,538	0,428	0,645
NGB1143.3	0	4 Weeks	o	74	39	0,527	0,414	0,638
NGB14440.2	0	4 Weeks	s	76	65	0,855	0,757	0,918
NGB14193.2	0	4 Weeks	s	107	73	0,682	0,588	0,763
NGB1143.3	0	4 Weeks	s	75	45	0,600	0,486	0,704
NGB14440.2	7	0 Weeks	o	76	46	0,605	0,492	0,708
NGB14193.2	7	0 Weeks	o	77	54	0,701	0,590	0,793
NGB1143.3	7	0 Weeks	o	73	36	0,493	0,381	0,606
NGB14440.2	7	0 Weeks	s	75	68	0,907	0,817	0,955
NGB14193.2	7	0 Weeks	s	84	72	0,857	0,765	0,917
NGB1143.3	7	0 Weeks	s	75	35	0,467	0,357	0,579
NGB14440.2	7	2 Weeks	o	78	41	0,526	0,415	0,633
NGB14193.2	7	2 Weeks	o	74	52	0,703	0,589	0,796
NGB1143.3	7	2 Weeks	o	75	42	0,560	0,447	0,668
NGB14440.2	7	2 Weeks	s	75	70	0,933	0,850	0,972
NGB14193.2	7	2 Weeks	s	84	73	0,869	0,779	0,926
NGB1143.3	7	2 Weeks	s	75	47	0,627	0,513	0,728
NGB14440.2	7	4 Weeks	o	83	50	0,602	0,494	0,702
NGB14193.2	7	4 Weeks	o	78	50	0,641	0,529	0,739
NGB1143.3	7	4 Weeks	o	74	39	0,527	0,414	0,638
NGB14440.2	7	4 Weeks	s	76	68	0,895	0,803	0,946
NGB14193.2	7	4 Weeks	s	107	82	0,766	0,677	0,837
NGB1143.3	7	4 Weeks	s	75	45	0,600	0,486	0,704
NGB14440.2	14	0 Weeks	o	76	48	0,632	0,518	0,732
NGB14193.2	14	0 Weeks	o	77	55	0,714	0,604	0,804
NGB1143.3	14	0 Weeks	o	73	36	0,493	0,381	0,606

Accession number	Days	Stratification	Scarification	n	x	mean	lower	upper
NGB14440.2	14	0 Weeks	s	75	68	0,907	0,817	0,955
NGB14193.2	14	0 Weeks	s	84	75	0,893	0,807	0,943
NGB1143.3	14	0 Weeks	s	75	36	0,480	0,370	0,592
NGB14440.2	14	2 Weeks	o	78	44	0,564	0,453	0,669
NGB14193.2	14	2 Weeks	o	74	53	0,716	0,604	0,807
NGB1143.3	14	2 Weeks	o	75	42	0,560	0,447	0,668
NGB14440.2	14	2 Weeks	s	75	70	0,933	0,850	0,972
NGB14193.2	14	2 Weeks	s	84	74	0,881	0,793	0,935
NGB1143.3	14	2 Weeks	s	75	49	0,653	0,539	0,752
NGB14440.2	14	4 Weeks	o	83	52	0,627	0,518	0,724
NGB14193.2	14	4 Weeks	o	78	51	0,654	0,542	0,751
NGB1143.3	14	4 Weeks	o	74	39	0,527	0,414	0,638
NGB14440.2	14	4 Weeks	s	76	68	0,895	0,803	0,946
NGB14193.2	14	4 Weeks	s	107	82	0,766	0,677	0,837
NGB1143.3	14	4 Weeks	s	75	46	0,613	0,499	0,716
NGB14440.2	21	0 Weeks	o	76	48	0,632	0,518	0,732
NGB14193.2	21	0 Weeks	o	77	56	0,727	0,618	0,815
NGB1143.3	21	0 Weeks	o	73	36	0,493	0,381	0,606
NGB14440.2	21	0 Weeks	s	75	69	0,920	0,833	0,964
NGB14193.2	21	0 Weeks	s	84	76	0,905	0,821	0,952
NGB1143.3	21	0 Weeks	s	75	36	0,480	0,370	0,592
NGB14440.2	21	2 Weeks	o	78	46	0,590	0,478	0,693
NGB14193.2	21	2 Weeks	o	74	53	0,716	0,604	0,807
NGB1143.3	21	2 Weeks	o	75	42	0,560	0,447	0,668
NGB14440.2	21	2 Weeks	s	75	71	0,947	0,866	0,980
NGB14193.2	21	2 Weeks	s	84	76	0,905	0,821	0,952
NGB1143.3	21	2 Weeks	s	75	50	0,667	0,553	0,764
NGB14440.2	21	4 Weeks	o	83	54	0,651	0,543	0,745
NGB14193.2	21	4 Weeks	o	78	53	0,679	0,569	0,773
NGB1143.3	21	4 Weeks	o	74	39	0,527	0,414	0,638
NGB14440.2	21	4 Weeks	s	76	69	0,908	0,819	0,955
NGB14193.2	21	4 Weeks	s	107	82	0,766	0,677	0,837
NGB1143.3	21	4 Weeks	s	75	46	0,613	0,499	0,716