Controlling Water Dynamics in Scots Pine (*Pinus sylvestris* L.) Seeds Before and During Seedling Emergence

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Doctoral thesis
Swedish University of Agricultural Sciences
Umeå 2004

Acta Universitatis Agriculturae Sueciae
Sivestria 305

ISSN 1401-6230
ISBN 91-576-6539-7

Tryck: SLU – Grafiska enheten, Umeå 2004

Abstract

Pamuk, G.S. 2004. Controlling water dynamics in Scots pine (Pinus sylvestris L.) seeds before and during seedling emergence. Ph.D. dissertation. ISSN 1401-6230, ISBN 91-576-6539-7

This doctoral thesis examines if water dynamics before and during seedling emergence after autumn and summer direct seeding can be controlled in Scots pine (*Pinus sylvestris* L.) seeds by using hydrophobic polymer coating and hydrophilic (hydrogels) polymer packages. A method was created by using scanning electron microscopy (SEM) to evaluate the quality of the polymer seed coating. Differential scanning calorimetry (DSC) and germination tests indicated that the critical moisture content of Scots pine seeds under freezing conditions was between 15%-20%. After autumn seeding, the coating (Ethocel™ polymer) thickness that resulted in the highest seedling emergence was 2.0% weight gain. On three typical seeding sites this treatment resulted in seedling emergence of 33.7%, 30.0%, and 14.1%, whereas the uncoated seeds had values of only 12.3%, 0.0%, and 1.0%. Summer direct seeding was done with three different scarification techniques - organic removal, huminmix, and mounding. Seedling emergence was compared within each scarification type for different initial seed moistures (dry and invigorated 30% moisture content, not re-dried), soil preparations (2-cm indentation, 2-cm wick and, 6-cm wick), and polymer (hydrogel Ac-Di-Sol®) preparations (powder, gel, and gel/peat). The highest seedling emergence was associated with invigorated seeds prepared with gel/peat package and a 6-cm wick for organic removal (32.5%), huminmix (30.7%) and mounding (18.8%). Invigorated seeds, regardless of treatment, had seedling emergence higher than dry seeds, and control values of dry seeds were significantly lower than for invigorated seeds. The delay of water uptake (probably in combination with sufficient snow cover insulation during winter months) made autumn direct seeding of coated seeds successful. The use of hydrogels, seed invigoration (not re-dried seeds), and soil preparation/wicks improved seedling emergence after summer direct seeding in dry conditions.

Keywords: direct seeding, seed coating, freezing response, seed invigoration, microsite preparation, seedling emergence

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In life, you must try and be the type of person that your dog thinks you are.

Anon

Contents

Appendix

Papers I-IV

This thesis is based on the following papers, which are referred to by their Roman numerals:

I. Pamuk, G.S., Olsson, T., Bergsten, U., and Lindberg, H. 2002. Evaluation of polymer coating on Scots pine (*Pinus sylvestris*) seeds using scanning electron microscopy (SEM). Seed Sci. and Technol. 30: 167-176.

II. Pamuk, G.S., Bergsten, U., and Lingois, P. 2003. Freezing response in Scots pine seeds as assessed by DSC and germination test. Seed Technology 25(2): 92-103.

III. Pamuk, G.S. and Bergsten, U. Autumn direct seeding of Scots pine (*Pinus sylvestris* L.) seeds using Ethyl cellulose coating. (Submitted manuscript).

IV. Pamuk, G.S. Effects of hydrogels, seed invigoration, and soil preparation on seedling emergence of direct seeded Scots pine (*Pinus sylvestris* L.) seeds during a warm and dry summer. (Manuscript).

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Introduction

The boreal forest, which covers approximately 15% of the world's land surface, is the most extensive of all the vegetation regions, extending from a position south of the northern tundra and north of the mid-latitude temperate forests. Environmental conditions are characterized by long, cold winters with short days (typically up to 6 months with average temperatures at or below $0^{o}C$) [Odin 1992]. In the boreal forest, four *genera* of conifers dominate: spruces (*Picea*), pines (*Pinus*), firs (*Abies*), and larch (*Larix*). These four genera usually cover vast areas and can occur as single-species forests (Kaplan 1996). Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies*) dominate the Fennoscandia boreal forest.

In this region, forest regeneration primarily consists of planting, natural regeneration, and direct seeding (planting being the most common method used). In the boreal forests, poor-to-moderately fertile sites are appropriate for direct seeding are (Wennström et al. 1999). In Sweden, about 25% of the total regeneration area is considered suitable for direct seeding (Rasmusson 1978).

A successful direct seeding has many advantages. Direct seeding costs less and produces more seedlings than traditional planting. Direct seeding regeneration costs are roughly half the costs for conventional planting using conventional spacing. Moreover, direct seeding is easier to mechanize than planting, encouraging the development of mechanized regeneration methods as well as further reducing regeneration costs (Wennström et al. 1999). Direct seeding reduces the risk for root and stem deformation and stability problems for seedlings, because it produces seedlings with a more natural and developed root system than planted seedlings produce (Long 1978; Hultén 1982).

The outcome of direct seeding varies greatly and depends on various environmental factors that affect seed germination and seedling survival. Success of direct seeding differs between growing seasons and sites (Tirén 1952; Hagner 1962; de Chantal 2003). Several factors can negatively influence the effectiveness of direct seeding: soil moisture, temperature, frost, evaporation, predation, and competition from ground vegetation (Kinnunen 1992; Winsa 1995; Oleskog and Sahlén 2000; de Chantal 2003). Decreasing the effects of these factors is paramount to a successful direct seeding program.

Seedling emergence and survival

Moisture and evaporation

Since the early days of direct seeding, moisture, and drought have always been considered important factors for germination and seedling emergence during the first growing season (e.g. Vaartaja 1950; Tirén 1952; Winsa 1995; de Chantal

2003). Several studies indicate that desiccation may cause high seedling mortality during their first growing season (Tirén 1934; Arnborg 1943; Tirén 1945; de Chantal 2003). During germination, moisture sources for seeds are most likely precipitation, soil water, and dew. However, rain is an unreliable source of moisture regarding amounts, intervals, and intensities (e.g. Vaartaja 1950; Pratt 1986). It has been standard procedure to remove the organic mat to improve moisture conditions. Winsa (1995) states that seedling emergence is dependent on the availability of capillary soil water. Since precipitation is so unpredictable and young seedling roots have to reach the depth of readily available water, seedling survival may depend on capillary water transport from below the seedbed to the surface (Harper et al. 1961; Schulte and Marshall 1983). In early summer, dew is found in small amounts in the boreal forest region and is not considered an important source of moisture (Bjor 1965).

Technology

Recent research suggests that improving the microsite conditions and using high quality seeds increases the reliability of direct seeding (Winsa 1995; Wennström et al. 1999; Oleskog 2000). Winsa and Sahlén (2001) recommend the use of invigorated seeds and microsite preparation to increase seedling emergence. In recent years, new mechanical seeding techniques and scarification techniques have increased seedling emergence of direct seeded Scots pine (Bergsten 2000). New silviculture techniques and technical advancements need to be developed to reduce the effects of climatic conditions. These advancements may minimize the risk of poor direct seeding results.

Climate and weather

Harsh environmental conditions and a short growing season can negatively influence direct seeding success. The boreal forest has unique environmental factors such as large seasonal variations in air temperature, a seasonal snow cover, and the presence of soil frost during winter (Bonan 1992). The length of the growing season is shorter at higher latitudinal positions, which influences the growing conditions (Von Fircks 1994). Solving the unreliability problem by increasing the number of seeds sown is a practice frequently used when direct seeding. The method cannot become reliable solely through the sowing of a large amount of seeds since many environmental factors affect the results of seeding (Kinnunen 1992; Winsa and Bergsten 1994).

Predation

Seeds are prone to predation from a variety of sources. Forsslund (1944) found more than 20 forest animals in Fennoscandia that eat conifer seeds. Smith and Aldous (1947) found more than 80 forest animals in the United States that consume conifer seeds. Post-dispersal predators are defined as consumers of seeds or seedlings on the ground after dispersal (Janzen 1971). Direct seeded seeds in undisturbed soil have a very low chance of becoming a mature tree mainly because of high predation (Forsslund 1944; Nystrand and Granström 1997). Seed predation can be severe and losses up to 100% have been reported (Crawley 1992). Several authors report that birds may be the most important predator of direct seeded Scots pine seeds (Vaarataja 1950; Heikkilä 1977; Bergsten 1985). Nystrand and Granström (1997) note that birds rely on sight for predation and select forage by seed color. To reduce the effect of predation on seeds of *Pinus sylvestris* and *Picea abies,* they can be covered with a thin layer of substrate (Yli-Vakkuri and Rasanen 1971; Nilsson and Hjältén 2003). Microsite indentations gradually collapse and cover seeds with soil and humus debris. Nilsson and Hjältén (2003) showed that using microsite indentations lowered seed predation and total seed loss.

Seed coating and hydrogel packages

In forestry, coating seeds is a rather new technique, but the concept of coating seeds in general is quite old. Documents referring to seed coating can be traced back to 1868 in files from the U.S. Patent office. The term “coated seed” refers to a single seed coated with an inert material (Burgesser 1950). Taylor and Harman (1990) define the term “seed coating” as an application of a useful material to the seed without changing its general size or shape. Ever since its inception during the 1950s, coated seeds can combat stress conditions found in many areas of New Zealand and Canada (Bailie and Elward 1980). Cotton farmers in Texas use coated seeds to lengthen the growing season since seeds are often subjected to cold soil conditions, which reduce germination and the number of normal seedlings that develop (Struve and Hopper 1995). Coating seeds with liquid-based polymeric adhesives improve performance and physical properties of seeds (Dadlani et al. 1992).

The use of hydrogels is common in arid regions throughout the world but not in the form of packages. Hydrogels are used directly in the soil. Evaporation rates of soils can be reduced considerably when hydrogels are mixed with soils (Johnson 1984; Callahan et al. 1988; Woodhouse 1989). Many hydrogels adhere easily to seeds, which allow high water potentials to be maintained both inside and within the protective polymer layer (Dexter and Miyamoto 1959; Baxter and Waters 1986). Polymer packages can be defined as an application of useful materials to the seed but changes its general size and shape. This differs slightly from traditional seed coating definition since the size and shape of the seed change. These materials may influence moisture close to the seed. Germination rates are influenced by the moisture potential of the seed.soil interface (Hadas 1970).

Uses for forestry

As the goal of direct seeding is to achieve the highest possible number of germinating seeds, the use of coating and polymer packages may improve summer and autumn direct seeding results. The application of polymers to Scots pine seeds creates a more robust seed, a seed that can be successfully direct seeded in the autumn (Figure 1). The autumn direct seeding of Scots pine (*Pinus sylvestris* L.) is presently not a regeneration option in the boreal forest because the seeds readily permit the entry of moisture, which causes seed mortality because the seeds have a low tolerance for freezing temperatures at high moisture contents. If water uptake could be controlled and kept under critical moisture content during the winter months, then seedling emergence the following spring could be achieved. The ability to sow coated pine (*Pinus sylvestris* L.) seeds during the autumn months will enable the seedlings to gain an advantage over ground vegetation the following spring and use the entire growing season. Another advantage to autumn seeding is the longer period to execute seeding. The ability to direct seed Scots pine with less time, climate, and seasonal restraints could increase the demand of autumn direct seeding as a regeneration method in Sweden.

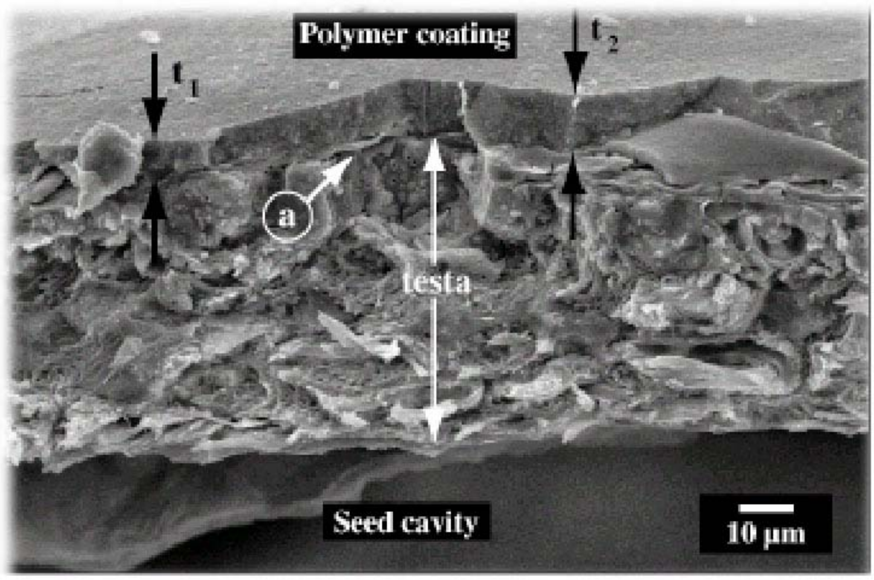

Figure 1. Scanning electron microscope (SEM) photo of polymer (approximately 2% weight gain) coated Scots pine seed. a = seed coat. t1 and t2 = polymer coating thickness.

Summer drought and evaporation are two factors that negatively affect summer direct seeding. The possible use of polymer packages for summer seeding of Scots pine is the application of super absorbent (hydrophilic) materials to improve moisture conditions at the microsite, which reduces evaporation in seeds. Hydrogels can be used in forestry by either being put into the soil or molded around the seed (**IV**). When hydrogels are put into the soil, they can serve as a holding tank for water. Hydrogels can absorb water to the microsite and/or prevented from percolating into the soil. Kochekzadh et al. (2000) and Specht and Harvey-Jones (2000) state that when soil dries, the water is slowly released back into the soil from the polymeric water reservoir enabling plants to thrive even during droughts. Hydrogel packages (mixture of hydrogel, water, and peat) could serve as water reservoir maintaining a high water supply around germinating seeds.

Objectives

This doctoral thesis examines if water dynamics can be controlled in Scots pine (*Pinus sylvestris* L.) seeds using polymer coating or polymer packages. The main hypothesis is that the use of polymer coating/packages could increase seedling emergence and survival in both autumn and spring direct seeding. The objectives of the different studies are listed below:

- To establish a method for measuring the thickness and surface uniformity of polymer coating coverage using scanning electron microscopy (SEM) (current methods do not sufficiently detail the surface uniformity or thickness of the coating). A method for rating seed coating quality to delay water uptake was created and evaluated in laboratory tests (**I**).

- To learn more about freezing effects in Scots pine seeds in early germination stages at various moisture contents by measuring responses to freezing temperatures using differential scanning calorimetry (DSC) technique. This was performed to identify critical moisture content (**II**).

- To find a coating material and a coating procedure/thickness that could keep the moisture content of Scots pine seeds below a critical level during the winter months, allowing for seed germination the following spring (**III**).

- To evaluate whether seedling emergence after summer direct seeding can be improved by using hydrophilic polymers, invigorated (not re-dried) seeds, and soil preparation (**IV**).

Material and methods

Polymers

Polymers have physical properties that allow for the creation of numerous products and functions such as reduce water uptake or improve water-holding capacity. The polymers should possess qualities that make them unique and economically advantageous. Polymers have physical properties that include materials that respond to temperature changes or are time aging dependant. Polymers can change their permeability when heated or cooled by just a few degrees or by an extended duration of time. These changes involve a physical (not a chemical) change (Figure 2).

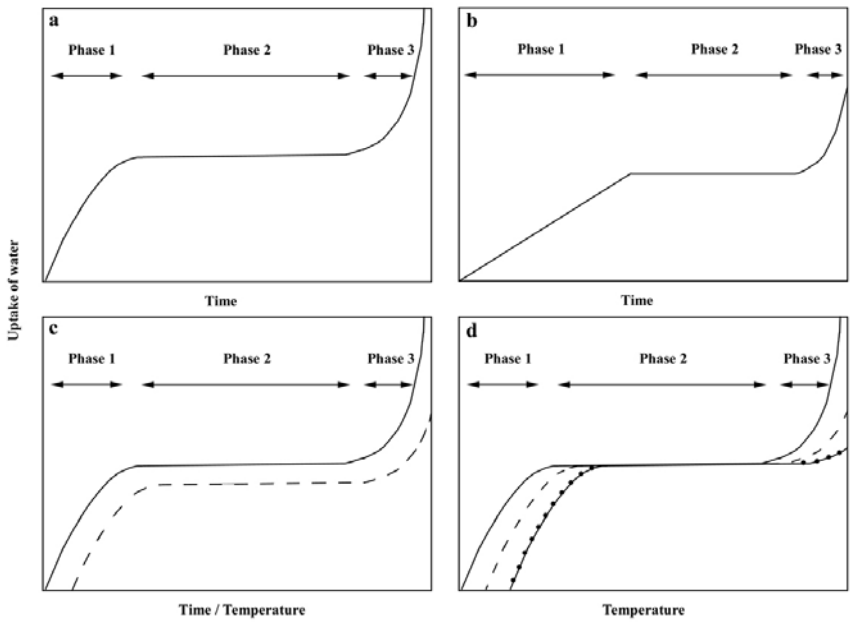

Figure 2. a) Water uptake regimes of normal seed (cf. Bewley and Black 1994); b) Ethocel™ coated Scots pine seeds results from (III); c) invigoration effect (--) compared to water uptake of normal seed (—); d) temperature release coating responses to different temperatures (--) and (··) compared to water uptake of normal seed (—).

Properties of the polymer materials have to be analyzed to find the right polymer for the application. Mechanical and thermal tests are used to describe specific characterizations of polymers. Typical tests for mechanical and thermal characterization include density, tensile strength, crystalline melting point, and glass-temperature. When considering the use of polymer material to delay water uptake, Bergsten and Lindberg (1997) note that glass-temperature, melting temperature, and moisture diffusion should be considered. Glass-temperature refers to an increase in molecular movement and decreases stiffness of materials.

A material changes from glass behavior to rubber behavior within a small temperature range. Since moisture diffusion drastically increases when temperatures exceed the glass-temperature, materials with low glass-temperatures are desirable for delaying water uptake in seeds. Therefore, the permeability of a material is temperature dependent (Flodberg 2002).

Materials are liquids when temperatures are above the melting temperature. Therefore, a high melting temperature is desired if the function of the material is to delay water uptake (Bergsten and Lindberg 1997). Mechanical and physical properties of materials are important (Flodberg 2002). Polymers used in seed coating must not be harmful or toxic to the seed. A material has to withstand collisions (between seeds and against the coating chamber) that occur during the coating process (**I**). During the coating process materials (with relatively low molecular weight) and seeds have the tendency to adhere to one another (Bergsten Lindberg 1997). Many polymers such as ethyl cellulose have advantageous application properties and provide adequate tensile strength and a low water absorption rate (Anon 1998).

Two classes of hydrogels commonly used are generally classified as natural polymers or synthetic polymers (Mikkelsen 1994). Hydrophilic polymers can be chemically manipulated to produce different characteristics within each general class. Semi-synthetic polymers are initially derived from cellulose and then combined with forms of petrochemicals, which are usually altered cationically or anionically (Mikkelsen 1994). Synthetic polymers are used over natural polymers since they tend to last longer in the soil and have stored water available for plant absorption (Johnson and Veltkamp 1985). Hydrogels used to improve water-holding capacities must have rapid swelling and good water uptake properties possibly by having high capillary action. Forming capabilities, long-term stability, and low evaporation rate are advantageous especially when using the polymers in direct seeding. Packages (mix hydrogels, water, and peat) formed around seeds must not hinder germination or have negative effects towards seeds. Furthermore, polymer materials have to be affordable, acceptable and environmentally safe.

The polymers used in this thesis are divided into two major groups: hydrophobic and hydrophilic.

Hydrophobic

A popular use of hydrophobic polymer-coated seeds is to delay water uptake. Since the first stage of germination is the physical process of imbibition, germination may be delayed or prevented if the water supply is restricted (Shephard and Naylor 1996). Several studies have examined seeds coated with a hydrophobic polymer to examine the polymer’s ability to reduce or delay water uptake. These studies used various species. Many hydrophobic polymers have been used with beneficial results such as polyvinylidene chloride (PVdC) (West et al. 1985), polyurethane (Bauman 1967), a combination of two polymers (shellac and PVdC) (Klein and Sachs 1992), and using the natural polymer Lanolin (Priestley and Leopold 1986).

Improving direct seeding results under sub-optimal germination conditions may be accomplished using hydrophobic polymer coated seeds. There are several reasons for seeding under sub-optimal germination conditions including decreasing the time for a crop to mature, lengthening the growing season, improving the ability to compete with other vegetation, etc. Studies have shown the detrimental effects of low temperature on imbibing and emerging seeds. Treating seeds with chemical additives improves emergence and protects seeds from environmental factors (Dadlani et al 1992). Struve and Hopper (1995) concluded that when cotton is planted early under sub-optimal conditions, only 40-60% of the total seeds establish productive plants. Imbibition injuries are attributed to rapid water uptake by low moisture content seeds sown in cold, wet soils (Herner 1986). Taylor (1987) observed a slight improvement in germination from seeds coated with hydrophobic polymer compared to the control seeds when exposed to cold and wet conditions. Priestley and Leopold (1986) in agreement with earlier studies, achieved beneficial results by slowing the imbibition rate of soybean seeds. Schrieber and LaCroix (1967) delayed the emergence of fall-sown wheat until spring with a complex seed coat, including beeswax, polystyrenes and ethylene cellulose. The coated seeds remained intact and remained dry and viable during the winter months.

This study uses the hydrophobic polymer Ethocel™ developed by Dow Chemical (**III**). Ethocel™ polymer is made of primarily ethyl cellulose, which is insoluble in water and a versatile and thermoplastic polymer. Ethyl cellulose is a product of the reaction of ethyl chloride with alkali cellulose. Furthermore, it is odorless, colorless, and film-forming. In addition, it meets the requirements of numerous FDA regulations for food applications. Ethocel™, which is used in protective coatings as binders and flavor fixatives, is safe for use in food contact applications (Anon 1998). Ethyl cellulose coated soybean seeds absorb water at a reduced rate compared to uncoated seeds, and there is no negative effect of ethyl cellulose on the seeds' ability to germinate (Hwang and Sung 1991). Ethocel™ is considered a typical hydrophobic polymer, a polymer that delays water uptake.

Hydrophilic

Hydrophilic polymers (hydrogels) are used as water super-absorbers with the capability of absorbing 400 g to 1500 g of water per dry gram of hydrogel (Bowman and Evans 1991; Johnson 1984; Woodhouse and Johnson 1991). Hundreds of types of hydrogels exist; they are used in oil recovery, medical grafting separations, food processing, personnel care products, and in laboratory supplies (Bouranis et al. 1999). Hydrogels have been used for many years (over forty years in the diaper industry) (Orzolek 1993). The hydrophilic polymer used in this thesis was Ac-Di-Sol® croscarmellose sodium (**IV**). Ac-Di-Sol® is a polymer developed by FMC Corporation that is an internally cross-linked form of sodium carboxymethylcellulose (NaCMC). Cross-linking makes it an insoluble, hydrophilic, highly absorbent material, resulting in good swelling properties and its fibrous nature gives it water wicking capabilities (Anon 1998). Ac-Di-Sol® is considered a typical material within hydrogels regarding swelling properties and water holding capabilities.

Scanning electron microscopy (SEM)

The scanning electron microscope (SEM) is used in medical science and such fields as materials development, metallic materials, ceramics, and semiconductors. The SEM furnishes an image of surfaces and is capable of producing both high magnification and good depth of field, so that even at low magnifications it is often more useful than an optical light microscope. SEM provides a three-dimensional high-resolution image of cells and tissues.

SEMs are patterned after reflecting light microscopes and yield similar information such as topography, morphology, and composition. The topography refers to surface features. Morphology is the shape, size, and arrangement of the particles making up the object lying on the surface of the sample (e.g., seed coating). The elements and compounds of the sample are its composition and are composed of their relative ratios in areas (~ 1 micrometer in diameter).

The use of SEM and proper tilt angles made it possible to determine the polymer layer thickness and enabled the study of fracture surface (depth) of coated seeds. Analysis was conducted to characterize the coating thickness variation in seeds. A thickness profile plot was obtained by measuring the coating surface uniformity and thickness. Measurements were taken equally throughout the circumference of the seed. The surface morphology and uniformity were analyzed to detect damages to the coating coverage. Therefore, systematically performed microscopy was used to measure the damages and categorize them. By using SEM technology, we have been able to identify the need for better quality coating (**I, III**).

Differential scanning calorimetry (DSC)

DSC simultaneously measures a sample and reference material as a function of temperature while both the sample material and the reference are subjected to a controlled temperature program. DSC measures the difference between the energy inputs to the sample and the reference. The modes of measurement in DSC are a power compensation method and a heat-flux method. In the former case, the compensatory power flow is measured, or the energy required to maintain a temperature (T) null-balance between the sample and the reference by compensating for heat exchange is measured. DSC employs two separate heat sources for the sample and reference as well as two Pt sensors for the temperature measurement (Wunderlich 1990).

The output of a DSC instrument is a thermogram, which plots the differential heat flow at Q/dT (heat transfer/temperature change) against temperature. A change in the dependent variable dQ/dT (heat transfer change/temperature change) represents in fact an absorption or evolution of heat by the sample. The temperature at the peak, Tmax, is also of interest as a "transition temperature" but it does depend on the rate at which the temperature is being changed.

Precise results and sharp transition peaks are observed by DSC thermograms. The rigid endothermic peak for hydrated test materials is characterized as the gel-to-liquid-crystalline phase transition (Ladbroke and Chapman 1969). As a result of hydrophobic interactions, a rather sharp DSC peak indicates the high degree of cooperative activity present in such systems, a peak that accompanies the transition. The changes in temperature caused by a reaction (combined with the values of the specific heat and the mass of the reacting system) make it possible to determine the heat of reaction.

Calorimetry can determine the amount of heat flow that occurs in a process. Thermodynamics divide the universe into two parts: the system and the surroundings. A calorimeter is a well-insulated device that surrounds the system. Since it is insulated, heat does not flow from the calorimeter to the outside environment. Thus, any heat that flows out of the reaction will be absorbed by the calorimeter and can be measured by the change in temperature of the calorimeter. In the same way, if the reaction is endothermic, the heat flow into the system will come from the calorimeter, thus cooling the calorimeter (Wunderlich 1990).

The base line and the peak of the process or endotherm characterize the analysis of a typical melting transition curve. Characteristic temperatures include: the beginning of melting, (Tb), the onset of melting (Tm), the peak temperature (Tp), and the point where the base line is finally recovered or the end of melting (Te).

The DSC technique has given insight to the freezing responses in seeds of different moisture contents (**II**). The results have led to the possible identification of the critical moisture content of seeds during freezing (**II**). DSC complements germination tests in conjunction with a freezing regime, producing compatible results. A better understanding of correlation of moisture content and freezing temperatures is needed.

Results and Discussion

Polymer coating thickness and morphology

To achieve a successful coating to delay water uptake, it is necessary to have a uniform coverage and damage-free coat over the entire seed surface (**I**; Taylor 1987). The lack of uniformity results in uneven hydration through the seed tissue (Taylor 1987). Methods to evaluate the coating surface quality and uniformity, includes visual inspection, West et al. (1985) measure cross-sections of the coat. A dial thickness gauge is one way to determine quality of coating surface (Hwang and Sung 1991). A coating index that measures the weight of materials (seed, coating material, and adhesive) added during the coating process and the total weight of material coming out has been recently developed (Scott et al. 1997). However, if the coating is applied to control water uptake, these methods do not sufficiently detail the surface uniformity or thickness of the coating. A method for measuring the thickness and surface uniformity of polymer coating coverage by using scanning electron microscopy (SEM) may allow for better results for water uptake studies on coated seeds in the future (**I**).

The evaluation of coated pine seeds showed two types of damage (**I**). One damage type displayed an absence of coating material on relatively large areas of the seed and the presence of pores and cracks on the seed coat. The absence of coating material always occurred together with cracks. The reason for this damage seems to be outside impact. The absence of coating material is believed to be associated with the handling or shipping of the coated and dried seed.

Imprint-like damages were classified as areas totally without coating but smaller in comparison to the first identified damage class. This “imprint-type” of damage also contained cracks which were fewer in number than the “absence-type” of damage. The coat seems to be thinner over the damaged area; therefore, the imprints most likely occurred because of seed collision against the walls of the coating chamber or between seeds during the coating process.

The success of seed coating to delay or control water uptake may increase if a method to evaluate the uniformity and thickness of seed coating can be applied as follows:
(i) Surface mapping for large damages with light microscopy using a small randomly selected batch of seeds.
(ii) If (i) shows no damage, surface exploration for cracks with SEM.
(iii) If necessary, coating surface examination for small pores (under 0.01 mm^2) through the film using SEM.
(iv) A final examination for coating thickness variations using SEM.

It is unlikely that an encapsulation treatment can totally inhibit moisture uptake (McGee et al. 1988). A thicker coating surface may reduce the amount of cracks, but sufficient moisture still infiltrates the coating surface. This causes cracks to increase in size, allowing for more water uptake. Higher application rates of coating material improve the moisture barrier possibly by reducing the number of cracks (McGee et al. 1988; Klein and Sachs 1992).

The need for a damage-free coat was evident since cracks and pores in the seed coat allow moisture to enter the seed. The coating material should be elastic and easy to apply. The application of more layers of coating seems to reduce water uptake. However, cracks and pores are still present, but moisture has a more difficult time finding its way into the seed through more layers of coating. Coatings were thinner at the radicle and cotyledon ends but did not contain areas lacking coating (**III**). Despite the slow entries of moisture into the seed, beneficial results were obtained (**III**). No ill effects from the coating material were observed in seedling emergence. Methods to test the quality of the seed coat have to be invoked when the goal of the coating is to delay or control water uptake.

Response of Scots pine seeds at different moisture contents and freezing temperatures

Critical moisture content for pine seeds during early germination stages may have been detected using differential scanning calorimetry (DSC) [**III**]. Seeds with higher moisture content (20%-30%) responded to melting temperatures significantly higher than seeds with lower moisture content [dry (7%)-15%]. Seeds with higher moisture contents (20%, 25%, and 30%) had a wider endothermic peak (-30.51 J/g, -40.36 J/g, and –47.02 J/g). The presence of two exothermal peaks seems to indicate that seeds with moisture content in excess of 30% could have two different types of water. Water in seeds is classified as bound or free; it could be theorized that these different types of water could respond differently to freezing temperatures. Dry seeds responded to melting temperatures as low as –40 °C and do not readily give off much energy. Seeds with high moisture content (20%-30%) responded to melting temperatures (5.0 °C, 4.6 °C, and 2.8 °C) higher than that of pure water (2.7 °C). This leads us to believe that the seed coat has an insulation character and a chemical solution in the seed that lowers the freezing point of water.

In a complementary experiment, seeds of different initial moisture contents were incubated for 2 or 5 days and subsequently exposed to –5 °C or –20 °C during 2 days or 5 days. Then a germination test was performed (21 days, 20 °C). Germination capacity range was from 97% (control) to 58% (30% moisture content, 5 days of incubation, 5 days of freezing at –20 °C). Germination capacity after freezing was significantly higher (81%-83%) for seeds with moisture contents from dry to 20% compared to seeds with moisture contents between 25%-30% (68%-72%). Seeds exposed to –20 °C for 2 and 5 days had significantly lower germination capacity (72%) than seeds exposed to –5 °C (80%-83%).

Scots pine (*Pinus sylvestris* L.) seeds are unsuitable for autumn direct seeding because they readily permit the entry of moisture that causes seed mortality due to a low tolerance for freezing temperatures at high moisture contents. A critical moisture content (15-20%) of survival/mortality from freezing temperatures has been noticed for Scots pine seeds (**II**). For autumn direct seeding of Scots pine, the application of polymer coating to seeds to delay water uptake may keep the moisture content of seeds under the critical level during the winter months with low freezing temperatures, allowing for seed germination the following spring.

Autumn direct seeding of Scots pine (*Pinus sylvestris* L.) seeds using ethyl cellulose coating

Coated seeds had significantly lower moisture content before snowfall than that of uncoated seeds for sites and seedbed preparations (experiment established 2000)

(**III**). An increase in coating thickness decreases moisture content before snowfall. On a moist site with no seedbed treatment, the maximum moisture content of 47% before snowfall was measured for uncoated seeds and the minimum of 29% for the thickest coated seed (20.0% weight gain). Significant differences in moisture content were found between the sample plots using microsite preparation and those without microsite preparation. On average, experiments conducted 2000 had lower moisture content (28.5%) when no seedbed treatment was done compared to moisture content (35.6%) of seedbed-treated plots. Differences in seed moisture content between sites indicated a positive reaction to increasing soil moistures. Of the three sites suitable for direct seeding, the drier sites had lower moisture content (21.3%) before snowfall compared to the two mesic sites (39.9%) [**III**].

On all sites, the maximum number of seedlings was found on sample plots of coated seeds. Autumn direct seeding resulted in significantly lower seedling emergence for uncoated seeds with or without seedbed preparation (3.9% and 1.1%) compared to 2.0% (weight gain) coated seeds (19.8% and 8.9%). Significantly, higher seedling emergence was found in plots with seedbed treatment (13.9%) compared to no seedbed treatment (6.5%) with coated seeds. On average, the coating thickness with the best seedling emergence (19.8%) for all sites after autumn seeding was 2.0% weight gain. On the three typical seeding sites, this coating thickness entailed a seeding emergence of 33.7%, 30.0%, and 14.1% after autumn seeding, and the control values were only 12.3%, 0.0%, and 1.0%, respectively (**III**). Thus, maintaining moisture contents in autumn direct seeded pine seeds under a critical level during winter months will enable seeds to survive freezing temperatures and germinate the following spring.

Seed moisture contents before snowfall exceeded the critical values, but seedling emergence could still be high in the spring. In northern Sweden, snow covers the ground for more than 6 months a year. Because of snow's insulating capacity, snow cover could reduce the risk of seeds being exposed to extreme cold temperatures. The presence of an insulating snow cover decreases the soil heat loss during winter; the thickness of the snow determines the insulation value (Gray and Male 1981; Bergsten et al. 2001). The temperature under snow was not measured but it could be theorized that the temperature under the snow was not as harsh as the air temperature above the snow (Bergsten et al. (2001). Mellander et al. (in press) observed no soil temperatures under –10 °C at 10-cm below the soil surface and a minimum daily average air temperature of –34.1 °C in January in northern Sweden. Seedling emergence is significantly higher (even at high moisture contents) if Scots pine seeds are exposed to only a few degrees below zero than if the temperature is as low as -20 °C. The duration of exposure is also important (**II**). Another possibility is that the moisture content of seeds decreased under the snow cover. A freeze-drying effect (decreased moisture contents) was noticed when incubated Scots pine seeds were subjected to freezing temperatures of –5 °C and –20 °C for 2 days and 5 days (Pamuk et al. loc cit.).

Polymer seed coating and autumn direct seeding are two well-known applications to seeds; however, their combination in the boreal forest in Sweden has never been practiced. A number of experiments have been conducted within seed coating and direct seeding which makes it easy to conceive theories of applying polymer coating to Scots pine seeds to achieve successful autumn direct seeding. Using polymer-coated Scots pine seeds made it possible to delay water uptake in the field tests until snowfall, at the sites with dry or mesic conditions-typical sites for direct seeding. Ethyl cellulose-coated Scots pine seeds delayed water uptake compared to untreated seeds. It seems that seed coating could provide the necessary reduction of moisture content within the seed for autumn direct seeded Scots pine seeds to survive the winter months, and germinate the following spring.

Effects of using hydrogels, seed invigoration, and soil preparation on seedling emergence of direct seeded Scots pine (*Pinus sylvestris* L.) seeds during a warm and dry summer

Effects of hydrogels, seed invigoration, and soil preparation on seedling emergence of direct seeded Scots pine (*Pinus sylvestris* L.) were studied for three types of scarification (organic removal, huminmix (mixed humus layer and mineral soil), and mounding). Seedling emergence was compared within each scarification type for different initial seed moisture content (dry and invigorated 30% moisture content not re-dried), soil preparations (2-cm indentation, 2-cm wick and, 6-cm wick), and polymer preparations (powder, gel, and gel/peat). The experiment was established in late June to early July-after the time that normally is used for direct seeding in the region. The summer was characterized as warmer and drier than normal. The highest seedling emergence (32.5%) was noticed when organic removal scarification was done and invigorated seeds were prepared with gel/peat package and a 6-cm wick. The same treatment also resulted in the best seedling emergence for huminmix and mounding 30.7% and 18.8%, respectively. Invigorated seeds (17.5%), regardless of treatment, had higher seedling emergence than dry seeds (10.7%). A higher seedling emergence was noticed in the majority of treatments using the hydrogel than that of the control.

Differences were observed between scarification methods for all treatment combinations; seedling emergence was higher when scarified by organic removal (18.7%) compared to huminmix (14.5%) and mounding (9.2%). Seedling emergence was 18.8% in sample plots using mounding, 30% MC seeds, 6-cm wicks, and gel/peat package.

On all sample plots and treatments, invigorated seeds (not re-dried 30% MC) had higher seedling emergence except organic removal and powder package. On the average for all scarifications and treatments, 30% MC seeds had a seedling emergence of 17.5% compared to dry seeds with an emergence of 10.7%. Control values for seedling emergence of dry seeds were 2.3% (organic removal), 3.9% (huminmix) and 4.6% (mounding) whereas the lowest average seedling emergence for dry seeds using treatments was 15.3% (organic removal), 7.5% (huminmix), and 4.7% (mounding). However, invigorated control seeds had seedling emergence values of 4.7% (organic removal), 14.1% (huminmix), and 14.7% (mounding) whereas the highest average seedling emergence for 30% MC seeds regardless of treatment was 32.5% (organic removal), 30.7% (huminmix), and 18.8% (mounding).

Packages positively affected seedling emergence with higher values ranging 13.2% to 32.5% than the control (2.3% and 4.7%) when organic removal was used. Huminmix and mounding control values were with dry seeds (3.9%) and invigorated seeds (14.1% and 4.6% and 14.7%, respectively). Seedling emergence for packages ranged between 4.6% to 30.7% and 4.7% to 18.8%, respectively for these scarification methods. The gel/peat package had the highest seedling emergence (16.1%) regardless of seed moisture and scarification, compared to powder (11.3%) and gel (15.0%).

Significant differences were noticed for wick effects and the 6-cm preparation had the highest seedling emergence on the average for all treatments and scarifications (16.0%) compared to surface (12.4%) and 2-cm wick (14.0%). The 6-cm wick had the highest seedling emergence when used with organic removal regardless of treatment (21.8%) compared to the 2-cm wick, which had a seedling emergence of 18.1% and on the surface 16.2% emergence.

The interaction of the treatments gave several two-factor effects, which were significantly different. Package/seed and package/wick effects were significant according to the variance analysis when using organic removal and huminmix but not with mounding. Mounding resulted in near significant differences with two-factor effects, package/wick. The treatment that had the highest seedling emergence on all scarification methods (organic removal, huminmix, and mounding) was the invigorated seed, 6-cm wick, and gel/peat package, 32.5%, 30.7%, and 18.8%, respectively.

The best results found in this experiment (**IV**) were with wicks at 6-cm and a gel/peat mixture. The 6-cm wick would have the ability to absorb and thus release more water than the other treatments. The polymer/seed packages could serve as water holding tanks. Applying seeds with hydrogels is a technique for maintaining a high water potential around germinating seeds, ensuring that soil water content does not fall below critical levels before germination. Using wicks (2-cm and 6-cm) increased seedling emergence possibly by reaching capillary water. Seedling emergence in mounding was lower than the other two scarification treatments (organic removal and huminmix), but the results were more favorable than expected. This may be because the hydrogels increase moisture levels at the microsite or reached capillary water.

In many cases, seeds invigorated to 30% moisture content and not re-dried had higher seedling emergence than the dry seeds. Seedling emergence varied with regards to microsite preparation and seed invigoration (seeds were re-dried) when direct seeding at different dates (Winsa and Sahlén 2001). Winsa and Sahlén (2001) suggest that the reduced seedling emergence may be caused by evaporation. If the combination of invigorated seeds and microsite preparation are used in direct seeding, then improved and less varying regeneration results can be expected.

By using hydrogels as a package to seeds, evaporation of seed moisture can be reduced. The structure of most hydrogels may account for their ability to resist evaporation (Johnson and Veltkamp 1985). The gel adheres easily to the seed and the package allows high water potentials to be maintained both inside the seed and within the protective layer (Dexter and Miyamoto 1959; Baxter and Waters 1986). Evaporation rates of soils can also be reduced considerably when polymers are mixed with soils, especially soils with coarse texture and high permeability (Johnson 1984; Callahan et al. 1988; Woodhouse 1989). Hydrogels have improved moisture retention of sandy loam and loamy sand soils, possibly enhancing the soil's ability to store plant-available water (Hemyari and Nofziger 1981). Water uptake by seeds and subsequent germination rates are strongly influenced by moisture potential at the seed-soil interface (Hadas 1970).

Conclusions

- A method using SEM for evaluating the thickness and uniformity of polymer coating was established.
  - The absence of coating material seems to be associated with the handling or shipping of the coated and dried seed.
  - Coatings are thinner at the radicle and cotyledon ends of the seed where hydration begins.

- The variation of coating thickness and damage could be attributed to the coating process. A high quality coating requires a high quality coating process.
  - Imprint-type damages were fewer in number and not as deep as cracks. These damages occurred as a result of seed collision against the walls of the coating chamber or between seeds during the coating process.
  - Damages (pores) were associated with the drying process. This means that materials with greater elasticity should be used to avoid cracking during the drying process.

- The critical moisture content of Scots pine seeds during freezing conditions seems to be 15%-20%.
  - Freeze damage increased with the combination of high seed moisture, decreased temperatures, and increased freezing duration.
  - Seeds with moisture content in excess of 30% have two exothermal peaks in DSC experiments, indicating the presence of bound and free water.

- A thickness of 2.0% weight gain resulted in the highest seedling emergence the following spring after autumn direct seeding of Scots pine.
    - Increasing the coating thickness lowered the moisture content of seeds before snowfall.
    - Microsite preparation increased moisture content of seeds before snowfall.
    - Microsite preparation increased seedling emergence.

- The combination of hydrogels (Ac-Di-Sol polymer) packages, invigorated seeds (not re-dried), and soil preparation/wicks may increase seedling emergence under dry conditions after summer seeding.
    - The gel/peat package, invigorated seeds, and 6-cm wick resulted in the highest seedling emergence.

Applications and future research

This method to evaluate coating coverage may allow for better results using coated seeds for future autumn direct seeding (**I**). The method provides valuable information regarding the quality of the coating. A high quality coat in regards to uniformity and thickness is necessary for achieving beneficial results of autumn direct seeding. The method can be used in other fields of study where the goal is to delay or reduce water uptake such as seeding two grains simultaneously with the coated seed germinating later.

It is possible, at typical sites for direct seeding, to achieve a sufficient control of water uptake by using Ethocel™ (ethyl cellulose) polymer-coated Scots pine seeds (**III**). Seed coating could provide the necessary reduction of moisture content within the seed for autumn direct seeded seeds to survive the winter months and germinate the following spring. Seeds germinated early in the growing season will have seedlings that are more developed than seeds germinated later in the season. Another advantage to autumn seeding is that it allows for a longer period to execute seeding and reduces the time-constraint pressure. Coated seeds may be more robust (extra coat) thus more tolerant to injury. The seeds may even be easier to disperse mechanically since they are heavier and can be coated to the same size and shape.

According to the results of summer direct seeding during a warm and dry summer, seedling emergence was higher than expected because of the detrimental conditions (**IV**). The use of hydrogels, wicks, and invigorated seeds may extend the period for direct seeding during the summer months. Summer-sown seeds are more susceptible to warm and dry environmental conditions, which increase the risk of drought and evaporation.

In this study, microsite preparation was used in the field experiments (**III**, **IV**). In accordance with earlier studies, microsite preparation had a positive effect on seedling emergence.

Further investigations could help determine other polymer materials that are more effective in the boreal forest. The polymer material has to meet some general criteria to be considered for use in autumn direct seeding of pine; that is, the coating material must be easy to apply and spread evenly. Another function of the coating material is to delay water uptake in the seed until a critical temperature reached or until the coat allows moisture to enter the seed.

As mentioned earlier, there are several factors, which cause inconsistencies when using direct seeding such as predation and competition from ground vegetation. A polymer coat can repel predators or camouflage the seed. Disguising seeds with various color schemes may reduce seed predation since many predators use their keen eyesight to detect seeds. The seeds could be colored with lacquer or dye thus serving to camouflage seeds to avoid detection from birds and rodents. However, many predators detect seeds using their olfactory senses. Creating odorless seeds by applying color or clear lacquer may decrease predation from predators with a sensitive sense of smell.

Seed coating can be used in spring/summer seeding to enhance growth and germination. It can be designed to create a nutritious environment in the immediate vicinity of the germinating seed. This provides a “boost” for the seedling in its critical early stages of development (Baile and Elward 1980). Beneficial results from slow release fertilizers have been achieved in seedbeds of *Picea abies* and *Abies grandis*. The fertilizers improved height growth in seedlings but had little effect on seedling numbers. Reforestation activities in New Zealand use slow-release fertilizers in nutrient-deficient areas to lengthen the duration of response (Hunter and Smith 1995). Slow-release fertilizers are not rapidly lost through leaching and can be combined with hydrophilic polymers. Another advantage of using hydrophilic polymers along with slow-release fertilizers is they do not rapidly disperse providing additional benefits to the seedlings later in the growing cycle (Baile and Elward 1980).

Further studies involving hydrogels, invigorated seeds, and soil preparation are needed. The factors in combination with each other have led to improvements in seedling emergence. Testing soil moisture levels of the microsite and seed evaporation are required to better understand the relation of hydrogels, invigorated seeds, and soil preparation on seedling emergence during summer seeding.

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Acknowledgements

It seems a along time ago, but as a Master's student at SLU I never dreamed of pursuing a doctoral degree. While finishing up my Master's thesis I read a description of a doctoral project in seed coating. The subject sounded extremely interesting and now at the end of the project I was right. Seed coating and regeneration is an exciting and fascinating field within silviculture.

I would like to give my greatest and deepest of thanks to my two supervisors Urban Bergsten and Henrik Lindberg for their interest in my project and me. The advice and encouragement has been tremendous and this I truly appreciate. The knowledge I have gained is a direct reflection on Urban and Henrik.

I would also like to thank my co-authors Tomas Olsson and Philippe Lingois for their expertise in analyzing and using scanning electron microscopy and differential scanning calorimetry. The hours spent analyzing with you was time well spent!

I am also grateful to have been employed at Vindelns Experimental Forests where I have an office at Svartberget. There have been so many fun moments during fika, work related parties, even statistics class. All of my co-workers in Vindeln have touched my personal and professional life and that is something I hold dear to me.

Rune Johansson and Margareta Söderström who have offered helpful advice and guided my efforts in the laboratory. Sören Holm has helped me a great deal with my statistics, thank you. I would like to thank my language proofreaders at Accent Språkservice for their speedy and thorough assistance. I would also like to extend my gratitude to the Kempe foundation for their economic contribution to my doctoral studies. I would also like to thank Skogsvårdsstyrelsen in Norrbotten for allowing me the extra time to finish my thesis. Last but certainly not the least, I would like to thank all my colleagues, friends, and family who have supported me throughout my time spent working with my studies, which has culminated in this thesis. A special thanks to my two Schillerstövare (Woody and Glimma) who have listened to me when I complained and have given me a great outlet for excess energy by hunting hare and fox.