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Interdependence between seed age and aerated steam treatment intensities

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Samband mellan ålder på utsäde och värmebehandlingsintensitet

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Abstract

Aerated steam treatment is a modern and environmental friendly method for seed borne fungus sanitation, mainly used on cereals. The method involves exposure to heat and high humidity, factors which are known to induce a fast ageing of seeds, thus resulting in reduced storability. This thesis clarifies the interdependence between seed age and the aerated steam treatment intensities.

Two studies were carried out in this thesis. In the first study the storage longevity of seeds treated with aerated steam was examined. The second study investigated the shelf-life of the pre-tests used to determine the aerated steam treatment tolerance of a seed lot, by testing the treatment tolerance of seeds aged prior to the treatment. Both studies were carried out on winter wheat seed (*Triticum aestivum* L.) with different moisture contents.

The storage longevity study showed that seeds treated with high aerated steam treatment intensities had more reduced storability than seeds treated with lower aerated steam treatment intensities. Seeds from all aerated steam treatments maintained 85% viability or above after artificial ageing comparable to 0.5 years of storage, and dry seeds treated at low intensities maintained 85% viability or above after artificial ageing comparable to 1.6 years. To determine this, the seeds were treated with a range of aerated steam treatment intensities prior to artificial ageing of the seeds. The ageing was performed by rapid ageing designed to induce changes comparable to the effects of storage for 0.5, 1.6 and 2.5 years in 10 °C.

In the shelf-life of the pre-tests study it was found that the $LD_{0.1}$ aerated steam treatment tolerance decreases linearly with age with 0.62-0.85 kJ/m^3 during a year, which should be considered when the aerated steam intensity for a seed lot is chosen. To investigate this, the seeds were aged by rapid ageing corresponding to 0.5, 1.6 and 2.5 years in 10 °C before the aerated steam treatment pre-tests.

The results from this study show that aerated steam treatment has deteriorative effects on the seed, which should be considered especially if the seed is stored more than one season before or after the aerated steam treatment. After validation by natural ageing, these results can be used to better adjust the aerated steam treatment intensity for individual seed lots based on storage time before or after the aerated steam treatment.

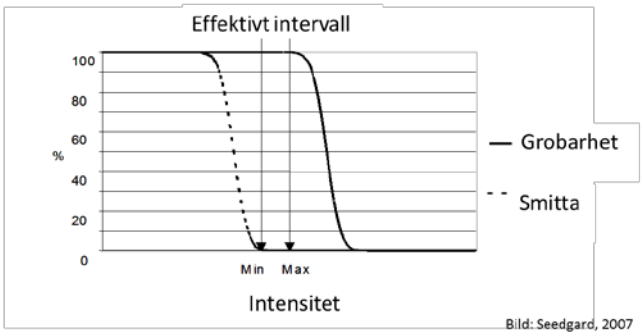
Key words: seed ageing, accelerated ageing, seed viability equation, aerated steam treatment, storability, commercial seed sanitation, shelf-life, *Triticum aestivum* L., seed vigour

Svensk sammanfattning

En av grundförutsättningarna för ett välfungerade jordbruk är ett utsäde med hög kvalitet, vilket innebär att det har hög grobarhet och låga halter av smittor som kan skada plantorna. För att framställa ett bra utsäde krävs noggrann odling samt att utsädet behandlas mot eventuella smittor och lagras till nästa odlingssäsong eller längre utan att grobarheten minskar.

Under lagringen åldras fröna, vilket kan leda till att grobarheten sänks. Hur snabbt utsäde åldras styrs av hur varmt och fuktigt det lagras, och under hur lång tid. Ju fuktigare och varmare fröna lagras, desto snabbare sker de skadliga processer som leder till att fröet åldras. Åldrandet leder på sikt till att fröna förlorar sin grobarhet. Vanligtvis lagras bara utsädet till nästa odlingssäsong innan det sås, men ibland lagras det länge tid på grund av överskott eller minskad efterfrågan.

För att behandla utsädet mot smittor som kan skada plantan görs ofta en kemisk betning. Ett alternativ till kemisk betning av utsäde är värmebehandling. Fröet hettas upp med het ånga, och behandlingen optimeras för att samtidigt få bästa möjliga skjutkraft och effekt mot smittan (figur 1). Denna metod ger ett lika bra resultat eller bättre som den kemiska betningen, men dess främsta fördelar är en mindre miljöpåverkan och bättre arbetsmiljö.

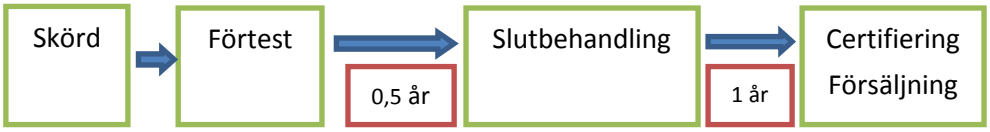


Figur 1. Genom att utsädet kan klara högre intensitet på värmebehandling än skadliga organismer kan det saneras från smitta.

Metoden används kommersiellt under namnet ThermoSeed. Sedan 2005 används ThermoSeed i Sverige och varje år behandlas cirka 11 000 ton spannmål.

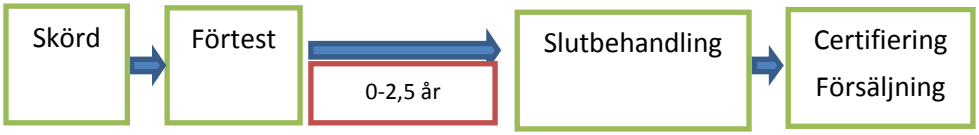
Normal sett sker tillvägagångssättet vid behandling enligt figur 2. Ett representativt prov tas på utsädespartiet. Det förtestas genom att delprov behandlas med olika behandlingsintensiteter, och därefter testas grobarheten på delproven. Baserat på resultatet från förtestet bestäms en intensitet som hela partiet slutbehandlas med. Därefter certifieras och säljs utsädet. Det får maximalt gå ett halvt år mellan förtest och slutbehandling, och maximalt ett år mellan slutbehandling och certifiering enligt nuvarande användarvillkor. Syftet med denna studie är att undersöka sambandet mellan lagring och värmebehandling, för att kunna sätta bättre underbyggda

tidsgränser och kunna anpassa intensiteten på behandlingen till den förväntade lagringstiden.



Figur 2. Processtegen vid kommersiell värmebehandling. Lagringstiderna mellan förtest, slutbehandling och certifiering är maximalt tillåtna lagringstider enligt nuvarande användningsvillkor.

Den första av studiens två frågeställningar handlar om hur utsädets tolerans för värmebehandling minskar mellan förtestet och slutbehandlingen om utsädet lagras däremellan (figur 3). För att undersöka detta har utsädet först förtestats, och den optimala behandlingsintensiteten har beräknats utifrån resultatet. Därefter har obehandlat utsäde från samma fröparti åldrats på konstgjord väg med en metod kallad snabbåldrande (Rapid ageing). Det har sedan förtestats igen, och resultaten från de förtesten har sedan jämförts med det första förtestet. Sambandet mellan snabbåldrande och verkligt åldrande har sedan beräknats med en modell som ofta används för att beräkna åldrande, kallad Ellis-Roberts seed viability equation.



Figur 3. I den första frågeställningen undersöks det hur lagringstider på upp till 2,5 år mellan förtest och slutbehandling påverkar utsädets värmebehandlingstolerans, genom att förtest gjorda innan och efter utsädet åldrats jämförts. Resultaten visar att utsädets värmebehandlingstolerans sjunker desto längre det lagras.

Jämförelsen mellan förtesten visar tydligt att värmebehandlingstoleransen sjunker ju längre utsädet åldrats. Toleransen sjunker snabbare för utsäde med högre vattenhalt, vilket är väntat eftersom det åldras snabbare. Sambandet är linjärt mellan ålder och minskad tolerans för värmebehandling. Om dessa resultat visar sig stämma även för utsäde som åldrats på naturlig väg, innebär det att resultatet kan användas till att räkna ut hur tolerans hos ett fröparti förändrats efter en viss lagringstid i en viss lagringsmiljö.

Den andra frågeställningen handlar om hur grobarheten påverkas av att utsädet lagras i upp till 2,5 år efter att det har slutbehandlats (figur 4). För att undersöka detta har utsäde behandlats vid tre olika behandlingsintensiteter, och sedan åldrats med Snabbåldrande. Därefter har utsädets grobarhet testats för att se om den har förändrats av lagringen.

Figur 4. I den andra frågeställningen undersöks det hur lagringstider på upp till 2,5 år mellan slutbehandling och certifiering samt försäljning påverkar utsädets grobarhet. Utsädet behandlades vid tre olika intensiteter och åldrades, och utsädets grobarhet testades sedan. Resultatet visar att värmebehandlat utsäde har sämre lagringsbarhet än obehandlat utsäde.

Resultatet visar att ju högre intensitet utsädet behandlas med och ju längre tid det lagras, desto mer försämras grobarheten. Fuktigt utsäde har också i detta försök sämre lagringsbarhet än utsäde med lägre vattenhalt. Utsädet kan dock lagras i ett år innan grobarheten faller under acceptabla nivåer. Konsekvensen av dessa resultat är att vid kommersiell behandling bör utsädet värmebehandlas så sent som möjligt innan det säljs för att bevara utsädets grobarhet, alternativt behandlas vid lägre intensiteter. En orsak till det påskyndande åldrandet kan vara de små vattenmängder som tillförs fröet under behandlingen. Genom att utöka torkningsfasen av behandlingen skulle vattenhalten sänkas, vilket skulle leda till ökad lagringsbarhet på utsädet.

Studien innehåller också en längre genomgång av vetenskaplig litteratur om frön hur frön åldras under naturliga förhållanden, olika sätt att på konstgjord väg åldra frön samt olika sätt att förutsäga hur snabbt åldrande kommer att ske i olika lagringsmiljöer.

Sammantaget visar studien att de maximala lagringstider som gäller idag enligt användarvillkoren är rimliga, men det öppnar också upp för att sätta mer specifika gränser beroende på lagringsmiljön. Studien visar också hur intensiteten på värmebehandlingen kan anpassas beroende på hur länge utsädet lagrats innan slutbehandlingen, och hur länge det ska lagras efteråt.

Content

Glossary and abbreviations

AS	Aerated steam
AST	Aerated steam treatment
Carry-over	Storage of seeds for more than until the next growing season
E	Energy content in the aerated steam treatment (kJ/m^3)
m	Mass of water in the aerated steam treatment ($g\ H_2O/m^3$)
m.c.	Moisture content of the seed (wet base) (%)
N.T	Not treated
R.H.	Relative humidity of the air (%)
Storability	The ability of the seed to be stored without quality losses
Storage longevity	How long the seeds can be stored without quality loss
Viability	Here defined as: The ability to germinate
Vigour	The potential of the seed lot for rapid, uniform emergence and normal seedlings under a wide range of field conditions
C_H, C_Q	Seed viability constants, universal for all species
K_E, C_W	Seed viability constants, unique for each seed lot or species
K_I	Initial viability of the seed lot in probits
LD_{50}, LD_1, $LD_{0.1}$	A relative measure tolerance; the storage time or AST intensity that reduces the viability to 50, 99 or 99.9% of the initial viability
p	Storage time in hours, days or years
P_{50}, P_{85}	An absolute measure of tolerance; the storage time or AST intensity that reduces the viability to 50 % or 85%
Probits	A unit of measurement of statistical probability based on deviations from the mean of a normal distribution, in this case standard deviations from mean viability (P_{50})
σ	Standard deviation of the distribution of seed death in time
t	Temperature (°C)
ν	Probit percentage viability at the time p

1

Introduction

Viable seeds are a key factor for the survival of flowering plants from one generation to the next. The seeds typically have to survive in a resting stage when conditions are unfavourable for plant development, and germinate in a more suitable environment. Seeds from some species only have to survive until the next growing season, whereas others have to wait several years for favourable conditions for germination. Even for seeds where suitable germination conditions are likely to occur soon, it is favourable to have seeds that maintain viability for longer periods in cases of changed environmental conditions.

On the other hand, time in combination with high humidity and heat, inevitably deteriorates seeds. The fraction of viable seeds in a seed lot decreases with time, until no viable seeds are left. This is generally referred to as ageing, describing the physiological age rather than the chronological age of the seeds.

Viable seeds are also important for the farmer. High germination of the seeds is essential for dense plant stand, which reduces the weed growth and gives a high yield. The utilization of resources for crop production, as nutrients, water or sunlight, will be lower in a field with fewer crop plants than anticipated.

High viability of the seeds is also important for seed companies, who stores and sells the seeds to farmers. The seeds must have enough viability to be able to be stored until next growing season or longer, without large viability losses. Treatment of the seeds is sometimes performed, for e.g. priming or sanitation of pathogens, and must be able to be carried out without reducing the germination below acceptable levels.

1.1 Sanitation of seeds with aerated steam treatment (AST)

Infection by seed-borne fungal pathogens is a large problem in farming, since it lowers both the yield and quality of the harvest. Seed-borne pathogens can also infest previously un-infested areas, if infected seed is used. To avoid this, conventional seed treatment by chemical pesticides is performed on many seed lots before sale. However, chemical seed treatments suffer from a number of drawbacks; most notably the associated risks of negative environmental impact and hazards in the work environment during application and handling of the treated seed. Furthermore, chemical seed treatment by pesticides is not allowed in organic farming, thus reducing the means to control pathogens in these cultivation systems.

An alternative method for seed treatment has been developed and is used commercially for seed treatment of both conventional and organic seed under the name

ThermoSeed™. It is based on a specially controlled thermal process that seeds can endure better than the seed-borne pathogens do, although the seed are also sensitive to heat (Forsberg *et al*., 2003). The seeds are treated with carefully regulated aerated steam (AS), at an intensity where the pathogens are killed by the treatment while the seeds are not harmed (figure 5). After the aerated stem treatment (AST), the seeds are dried and cooled.

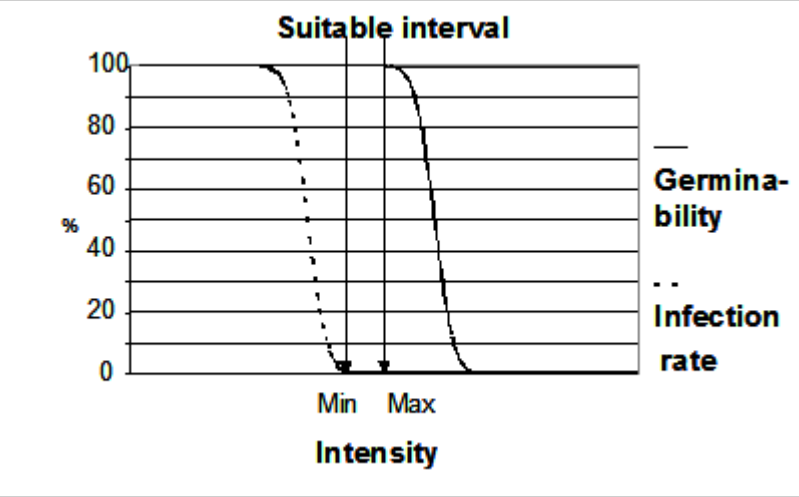


Figure 5. Schematic drawing of the optimum aerated steam treatment (AST) intensity. Within the suitable treatment intensity interval, the seeds maintain full viability and the plants developed from the treated seeds are free from seed-borne infection (Incotec, 2011).

The AST consists of two parts. First, a pre-test is performed on a representative sample from the seed lot. The pre-tests exposes the seeds to a range of AST intensities, called recipes, and the AST tolerance for the seed batch is measured as the highest intensity the seeds can withstand without loss of viability compared to the viability of untreated seeds. The recipes are composed of seed-lot specific combinations of factors like energy content, treatment time, air moisture content and airflow rate. Second, based on the results of the pre-test, an optimal AST recipe for the seed lot is calculated and the whole seed lot is treated.

The AST performed on wheat has shown equal sanitation effect compared to chemical seed treatment for sanitation of common bunt (*Tilletia caries*), leaf and glume blotch (*Stagonospora nodorum*), snow mold (*Microdochium nivale*) and *Fusarium* spp., but limited effect on loose smut *(Ustilago tritici)* (Forsberg *et al.*, 2005; Incotec, 2011).

1.2 Natural ageing of seeds

As seeds age, they deteriorate and eventually die. The reasons for the deterioration are complex and difficult to study since each seed in a seed lot behave uniquely (Copeland & McDonald, 2001). The ability of a seed to germinate can only be measured by a germination test that in its nature is destructive.

The ageing of seeds is characterized by a sigmoid relationship between viability and storage time (e.g. Walters *et al.*, 2010; Ellis & Roberts, 1980a). A long period in which very few seeds die is followed by a breaking point, and continued by a period of rapid decline of viability until most seeds are dead. This curve has also been described as a negative cumulated normal distribution of seed death in time (Ellis & Roberts, 1980a) (figure 6).

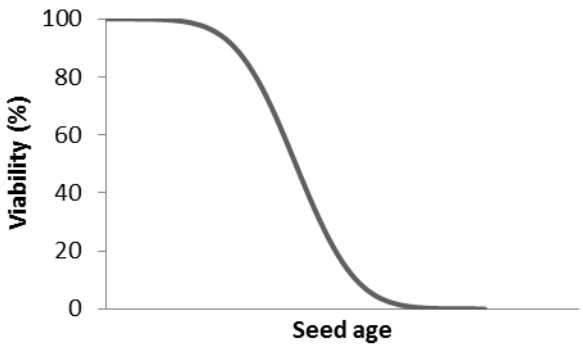


Figure 6. The negative cumulative relationship between seed age and viability in a storage environment with constant temperature and humidity (after Ellis and Roberts, 1980a).

Walters *et al*. (2010) suggest that the deterioration is caused by a combination of irreversible biochemical and structural changes of the seed. The rate of the deterioration is determined by the accumulation of damaging substances as reactive oxygen species (ROS) caused by outer and inner factors as radiation or seed metabolism, and the physiological state of the seed which affects the movement of the damaging substances in the seeds. The physiological state of the seed is in return determined by moisture and temperature.

A high moisture content of the seeds allows respiration and fungal growth which may rapidly deteriorate the seed, whereas very low moisture content causes damages to the membranes within the seed. The temperature determines the rate of the reactions, i.e. a high temperature accelerates the deterioration of the seed. Seed moisture is considered as the main factor of deterioration, since high temperature alone has little effect on deterioration (Copeland and McDonald, 2001).

1.3 Seed quality

Seed quality is often described in the terms of vigour and viability. Viability is the ability to germinate, which means that the seed has a living embryo. Vigour is a term describing quality of the seed or seed lot, which includes viability as well as the ability to give normal seedlings and uniform and rapid emergence under field conditions. To test vigour, seeds are exposed to stressful conditions, as cold, heat, moisture, chemicals, or mechanical resistance (i.e. packed soil) prior to or during a germination test.

Other symptoms of deterioration than seed death can be measured with chemical methods. For wheat seeds, many studies have been made on genetic and biochemical alterations during ageing. For example, wheat seeds (*Triticum aestivum*, cv. Sonalika) aged naturally for 1, 7 and 10 years in dry conditions in room temperature showed that the frequency of damaged chromosomes increase and the frequency of dividing cells decreases in the root tip with increasing age (Akther *et al.*, 1992). Durum wheat seeds (*Triticum durum* cv. Cappelli) naturally aged in 10 °C in up to 35% relative humidity (RH) for 0-36 years, showed a decrease in antioxidants and a deterioration of the gluten, whereas free radicals was most abundant after 13 to 15 years (Pinzino *et al*., 1999).

1.4 Storage of cereal seeds

The aim of storage is to store the seeds while maintaining viability. Since both high temperatures and high humidities deteriorate the seed, it is desirable to store seeds in cold and dry conditions. This reduces the biological activity of the seed and the risk of fungal growth. However, seeds are usually warmer and wetter at harvest than is appropriate for long-term storage.

To lower the moisture content (m.c.) of the seeds, they are dried. There are several types of dryers, using either warm outdoor air or heated air. The warm air reduces the exterior water activity and causes evaporation at the surface, which builds up a moisture gradient in the seed that generates water diffusion from inside to the outside. The continuous energy consumption by evaporation contributes to keep the temperature at an acceptable level limiting damage. If the drying process is too quick, the moisture content in the seeds becomes very unevenly distributed, which can cause physiological damages. Since drying is energy consuming and thereby costly, seeds are not dried to lower moisture contents than necessary, in Sweden mostly to 13-14% for cereals. After the drying, the seeds are cooled to ambient temperature.

An alternative and sometimes cheaper method is cooling of grain down to low temperatures where the viability is less affected by high moisture content, but this is seldom practiced. Dried cereal seeds generally maintain their viability well in ambient temperature, making a temperature controlled environment unnecessary, whereas cooled seeds must be kept cool during the whole storage period to maintain their viability. Cereal seeds are normally stored in bins or silos at the farm or at the seed company after harvest and drying. The smaller the storage container, the faster the temperature of the seed adapts to the surrounding temperature, but in large-volume bins, the equilibration of the temperature with the surroundings can take several months if the grain is not cooled by circulating air.

Cereal seed are often stored until next growing season. However, sometimes leftover seed from last season or changed demands of the farmers due to weather or changed market conditions for a crop can lead to a surplus of seed. These are stored to next growing season, and can be used if the viability is still acceptable. This is called over-carrying of seeds.

In Sweden, it is recommended that cereals have moisture contents at storage of 14% or below. The temperature of the seeds should not exceed outdoor temperature at harvest with more than 4-5 °C. If possible, the seeds should be cooled to 10 °C during winter using outdoor air for cooling. If the seeds should be over-carried, a maximum of 13% m.c. and a temperature of 15 °C is recommended (Råsberg, 1998). Viability after the storage must be at least 85%, otherwise the seeds cannot be certified and sold (SJVFS, 2011).

1.5 Accelerated ageing

Accelerated ageing is a method in which the seed's natural ageing is accelerated by exposure to heat, sometimes in combination with high humidity. Since the aging process occurs during a few days up to some weeks, the quality parameters related to longevity and viability can quickly be estimated. The technique is based on the assumption that accelerated ageing mimics natural ageing. However, very few studies has been made comparing seeds aged naturally and accelerated (Galleschi *et al.*, 2002).

There is a variety of methods and applications for accelerated ageing. Most methods is mainly used for vigour testing as Traditional Accelerated Ageing (TAA), Controlled Deterioration (CD) and Saturated Salt Accelerated Ageing (SSAA). In these methods, the seeds are often aged in very high humidities, causing an increase of seed moisture. The exposure to stressing conditions separates seed lots with high and low vigour, but the accelerated ageing treatment time is not

correlated to how long the seeds can survive in storage conditions. The goal of these methods is to predict how the seed will perform under real field conditions.

The methods mentioned above are approved for vigour testing of several species by The International Seed Testing Association (ISTA) and Association of Official Seed Analysts (AOSA). For wheat, TAA is approved for vigour testing by both associations.

Predictions of how long the seeds will survive in storage conditions can be made with the accelerated ageing method sometimes known as rapid ageing (RA). The seeds maintain their initial moisture content during the testing since the seeds are kept in sealed vials, which makes it possible to assess the influence of both moisture content and heat on deterioration. This method is not approved by ISTA or AOSA for any purpose, and is mostly used for scientific reasons or by seed banks.

1.5.1 Traditional Accelerated Ageing (TAA)

In the first developed method for accelerated ageing, the ageing of the seeds take place over an open water surface, resulting in 100 % RH in the air (Delouche & Baskin, 1973). The moisture content of the seeds increases during the treatment, resulting in a faster deterioration since the moisture further accelerates the ageing process.

The seeds are placed in a wire basket on a stand 6-8 cm above the water surface in a water bath. The water bath is placed in a temperature-regulated chamber, for an accurate temperature control. After two to three days at 40 °C to 45 °C in the chamber, the seeds are removed and a germination test is performed (Baskin, 1981). Various time and temperature combinations are recommended for wheat; 45°C /48h (Baskin, 1981), 41°C /72 h (Hampton & TeKrony, 1995; AOSA, 1983 in Modarresi *et al*., 2002), 43°C /72h, 45°C /72h or 41°C /96 h (Modarresi *et al*., 2002). The aim of the combinations of treatment temperature and time is to achieve maximal separation between the seed lots in terms of vigour.

This method is used primarily to provide a measure of the difference in viability between seed lots and between fresh and old seeds from the same batch. It is also used to estimate the relative storability of seed lots. No comparisons are made between the time of TAA and real storage. However, Galleschi *et al*. (2002) concluded that for their investigated seed lots of durum wheat (Triticum durum cv. Capelli), seeds aged with TAA in 40 °C for 3, 4, 6, 10 and 14 days had similar germination capacities as the seeds from the same seed lot aged naturally in a dark 10 °C room with 35% RH (Pinzino *et al*., 1999) for 15, 30, 33, 35 and 36 years respectively.

Galleschi *et al.* (2002) also studied how the content of radicals, anti-oxidants and storage proteins changed during traditional accelerated ageing compared to natural ageing of the same seed lot. Although the germination rate was reduced in a similar way as during the natural ageing, the investigated substances did not change in the same way. Lutein, an antioxidant, decreased less than in natural aged seeds, whereas radicals increased in TAA seeds instead of decrease as in the naturally aged seeds.

The TAA method has been criticized for the large and uncontrolled water uptake during the treatment. Since dry seeds are placed in high humidity, it causes an increase of moisture content of the seed during the test period. Wheat with initial moisture content between 11.1 % and 13.2 % increased to 28.1 % moisture content (m.c.) after the first 48 h, and with another 4 percentage after 48 additional hours (Modarresi *et al*., 2002). High moisture content of the seed makes the seed much less able to withstand the deteriorative effect of high storage temperature, compared to seeds with lower moisture content (Copeland and McDonald, 2001). Hence, the deteriorative effect of the accelerated ageing treatment will increase during the test, but exactly how is not measured, which makes the TAA test difficult to interpret (Ellis and Roberts, 1979). High humidity in the water bath during treatment is difficult to measure and keep stable, and therefore the amount of absorbed water will be difficult to estimate. Different seed lots will absorb different amounts of water, depending on initial moisture content, also adding to the difficulties in interpreting the results of a TAA test. The water uptake is larger for small seeds, causing poor correlation between germination after TAA and seed vigour during natural conditions (Zhang & McDonald, 1997).

If the seeds are aged for longer times with TAA, the moist and warm conditions can cause growth of microorganisms, which can influence the germination and biochemical content of the seeds (Galleschi *et al*., 2002).
Ellis and Roberts (1979, 1980a) also pointed out that this method only gives one treated sample, and a single sample germination test easily suffers from sampling errors.

These weaknesses can however be avoided. The methods described in the following sections; Controlled Deterioration, Salt Saturated Accelerated Ageing and Rapid Ageing are all examples of methods without some or all of these drawbacks.

1.5.2 Controlled Deterioration (CD)

Controlled deterioration (CD) is a method similar to the TAA, but the moisture of the seeds is increased to a pre-determined moisture content before the high temperature treatment. The seeds are kept in sealed vials during the 24 h test period in

a 45 °C water bath to ensure consistent moisture content (Powell & Matthews, 1981). Different combinations of moisture content, temperature and time have later been introduced for a variety of crops, where 18% m.c, 45 °C and 72 h has been recommended for wheat (Modarresi & Van Damme, 2003).

1.5.3 Saturated Salt Accelerated Ageing (SSAA)

The uptake of water during the accelerated ageing is reduced in the method Saturated Salt Accelerated Ageing (SSAA). The same equipment as in a TAA can be used, but the water in the water bath is replaced with saturated salt solutions. The salt solutions give a reduced relative humidity compared to water. KCl, NaCl and NaBr gives relative humidities of 87%, 76% and 55% respectively. The lower relative humidity reduces the water uptake of the seed, prevents microbial growth and prolongs the ageing period for a better separation of seed lots (Zhang & McDonald, 1997).

The SSAA test was developed for small vegetable seeds (Zhang & McDonald, 1997) but has also been tested on large seeded crops such as corn (Bennett *et al.*, 2004) and on wheat seeds, using NaCl solution (Meriaux *et al.*, 2007). The wheat seeds (approx. 13 % m.c.) were aged in 45 °C for 7 days. SSAA gave a one-percentage moisture content increase compared to 17 percentages with 5 days/45 °C TAA and better separation between the tested seed lots. The tests were reproduced in another lab, and the results from SSAA had higher reproducibility than the TAA results. SSAA has also been used for scientific purposes (Hay *et al.*, 2003).

1.5.4 Rapid ageing (RA)

Yet another method of accelerated ageing is rapid ageing (RA) with control of water uptake was used by seed scientists Ellis and Roberts (1979). It is closely linked to the seed viability equations also developed by them (1980a). Data from these tests can be used to predict initial viability and storage behaviour, which is discussed in section 1.6, “Seed viability equations”.

During the rapid ageing, the seeds are kept in sealed vials, which maintain the original moisture content of the seeds. The sealed vials are kept in hot air or in water baths at a constant temperature. Vials are removed at several times during the test and the viability of the seeds is tested, giving a curve reflecting how time causes deterioration.

Since original moisture content is preserved, seeds can be dried to different moisture contents before the accelerated ageing treatment. After treatment, the survival of the same seed lot at different moisture content can be compared. Dif-

ferent temperatures for the same seed lot can also be used, giving an estimation of the temperature effect on survival.

This technique is not approved by either ISTA or AOSA as an official test method, and has not been developed and evaluated to the same extent as the others. Nevertheless, this method is widely used for scientific purposes (Stoyanova *et al.*, 2007; Mead & Gray, 1999; Tang *et al.*, 1999b); and for seed bank purposes (Hong & Ellis 1996, Probert *et al.*, 2009).

1.6 Seed viability equations

An equation for prediction of viability of seeds after storage was developed by seed scientists Ellis and Roberts (1980a) with barley as a model crop. Previously, models for prediction of storage time had been limited to predict behaviour only for some species and some storage conditions. The equations are based on an empirical model, and merely describe the rate of deterioration in a constant environment. This equation applies to a vast number of species with orthodox seed and storage conditions. These seeds can be dried to moisture contents around 5%, tolerate freezing temperatures and are generally long-lived. Most crops, including cereals, have this type of seeds. The other type of seeds is recalcitrant seed, often from perennial trees in tropical climate, which cannot be dried or tolerate freezing temperature, and are short-lived (Copeland and McDonald, 2001). Species with orthodox seeds have seeds which can be dried to low moisture contents and stored at low temperatures, and includes most species.

The model is linked to the accelerated ageing procedure described in the previous section, since it demands viability data from the investigated seed lot stored under different moisture conditions. The simplest way to obtain seeds of different moisture content from the same seed lot, is to dry or wet seeds from the same seed lot and age the seeds by rapid ageing in a high constant temperature.

1.6.1 The equations

The distribution of seed deaths over time is assumed to be normally distributed, and the rate of the deterioration is assumed to be the same for all seed lots of a species stored under identical storage conditions by Ellis and Roberts (1980a; 1980b). The normal distribution is described by two measures; mean and standard deviation (σ), or variance (figure 7). In the seed viability equations, a normal distribution with a mean set to the time when the germination rate has fallen to 50%, denoted $P_{50,}$ is used. The standard deviation of the distribution of seed death in time, σ, is specific for each seed lot and storage condition.

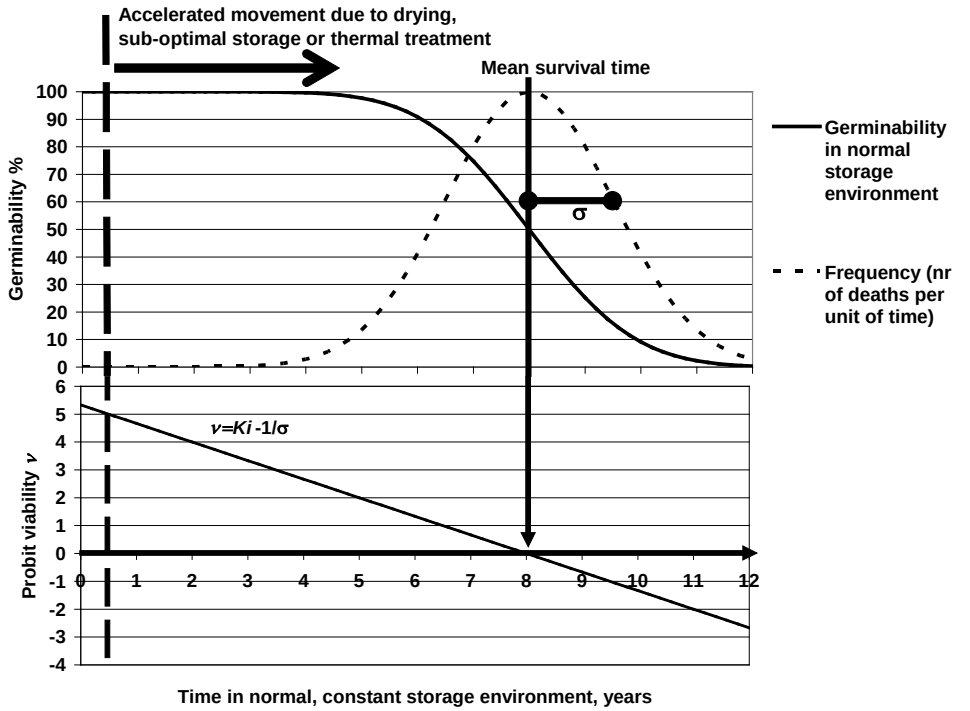


Figure 7. The relationship between cumulative viability decrease in normal storage environment, the distribution of seed death in time (frequency of seed death per unit of time) and the viability decrease expressed as probits. Ageing, AS treatment or suboptimal storage environment accelerates the viability decrease (Forsberg, 2004a).

The deterioration can also be described as a negative cumulative curve showing remaining viable seeds (figure 7). This curve can be converted to a straight line if the viability is expressed as standard deviations from mean, also known as probits. The equation for this curve is

$$\upsilon = K_i - p/\sigma \qquad \text{(Equation 1)}$$

where υ is probit percentage viability after p days of storage at a constant temperature t (°C) and moisture content (m.c.) (% wet base) of the seeds. K_i is the initial viability expressed in probits, and is the intercept of the curve (viability at the time 0). σ can also be describes as the time in days it takes for the seed lot to lose one probit in viability in a certain constant environment, whereas 1 / σ describe the rate of the deterioration in probits. A larger σ means that the lifespan of the seed lot will be longer, since the deterioration takes place during a longer time period (Hay, 2004). For a specific species, σ depends on the storage environment as

$$\log \sigma = K_E - C_W \log \text{m.c.} - C_H t - C_Q t^2 \qquad \text{(Equation 2)}$$

where K_E, C_W, C_H and C_Q are constants. According to Dickie *et al.* (1990) C_H and C_Q is universal for all seed lots, whereas K_E and C_W are predicted to be universal for a species (Ellis & Roberts, 1980a).

C_W is calculated by plotting the logarithmic values of σ from seeds from the same seed lot that were stored under different moisture conditions but in the same storage temperature, against the log value of moisture content of the seeds (log m). The equation of the straight line obtained is

$$\log \sigma = K - C_W \log \text{m.c.} \qquad \text{(Equation 3)}$$

where K is the intercept of the curve and the slope is C_W. K_E is calculated using K and the universal constants of C_H and C_Q, by the equation

$$K = K_E - C_H t - C_Q t^2 \qquad \text{(Equation 4)}$$

When all constants have been determined for a seed lot, the storage time can be predicted for almost any combination of moisture content, storage temperature and desired viability after storage (exceptions are discussed in section 1.6.2, "Limitations of the model"). This is preferably done using a combination of equation (1) and (2):

$$\upsilon = K_i\text{-}p/10\text{^} (K_E - C_W \log \text{m.c.} - C_H t - C_Q t^2) \qquad \text{(Equation 5)}$$

For a comprehensive guide on how to use the seed viability equations including illustrative examples, "Seed viability equations" (Hay, 2004) is recommended.

Viability constants have been calculated for a large number of species, including wheat (Ellis *et al.* 1990) where K_E was calculated to 9.42 and C_W to 5.859. The values for C_H and C_Q have been determined to 0.0329 and 0.000478 respectively, and is considered to apply to all orthodox species (Dickie *et al.*, 1990). Filho and Ellis (1992) investigated RA regimes with temperatures of 30 °C and 40 °C and moisture contents of 13-17 % m.c., and found that the combination 40 °C and 15 % m.c. provided the most reliable combination for determining K_i for wheat.

1.6.2 Limitations of the model

There are a few limitations for the model. Firstly, the equations only apply to constant storage conditions. Some attempts have been made to use it for predicting storage time during warehouse conditions, where humidity and temperature vary,

which will be discussed in section 1.6.5, “Modifications of the seed viability equations”.

Secondly, it only applies to orthodox seeds (Ellis & Roberts, 1980a), since recalcitrant seeds cannot be dried and cooled to any larger extent.

Thirdly, there are limitations to the moisture content and storage temperatures where these equations apply. For all seeds, there is a lower limit of moisture content, below which the seed suffers from damage due to disrupted membranes. This limit is species specific and determined to 5.5% for wheat (Ellis *et al.*, 1990). The maximum moisture content for this model also differs between species. Above this moisture content, repair processes start in the seeds that can prolong the life-span, if oxygen is available. For cereals, this limit is estimated to be around 26-28% (Roberts, 1986). For temperature, the model only applies to storage temperatures between -20 °C and +90 °C. Below -20 °C, the longevity is overestimated, whereas most seeds cannot survive more than 90 °C (Dickie *et al.*, 1990).

1.6.3 The reliability of assumptions in the model

The assumption of normal distribution of seed death in a seed lot has been confirmed in several tests performed by the inventors of the seed viability equation, for example in the summarizing article “The quantification of ageing and survival of orthodox seeds” (Ellis and Roberts, 1981). The determination of normal distribution was made by a visual judging of the fit. Others who used statistical means such as χ^2 –tests to determine if data is normally distributed more frequently have reported lack of fit to a normal distribution (Tang *et al.*, 1999b). The deviation from normal distribution might be the result of errors in handling or sampling of the seed lots during the experiment, as well as invalidity of the assumption of normal distribution. A reason for not fitting to a normal distribution curve might also be that the viability of the seed lot never was 100%, since a part of the seed is unable to germinate under current conditions, due to damaged seeds or dormancy (Mead & Gray, 1999). Difficulties to determine K_i have been pointed out as another reason for the inability to fit a normal distribution. Several determinations of K_i from a seed lot by rapid-ageing tests gave substantial variation between the attempts, thus resulting in inaccurate predictions of storage longevity (Fabrizius *et al.*, 1999).

The model also assumes that the rate of deterioration ($1/\sigma$) for all seed lots of a species will be the same under identical storage conditions (Ellis & Roberts, 1980a). In detail, this assumption means that all seed lots of a species should be equally affected by temperature and moisture, i.e. that the same values for K_E, C_W, C_H and C_Q apply for all seed lots of the species. Many tests have been carried out

on a variety of crop species, as on barley (Ellis & Roberts, 1980b), that confirm this assumption (Tang *et al.*, 1999a). Results contradicting the assumption have also been published, for example from soybean (Fabrizius *et al.*, 1999) and hybrid corn (Tang *et al.*, 1999a; Tang *et al.*, 1999b). The rate of deterioration in hybrid corn was affected by both genotype and initial vigour and was only slightly improved when truncated germination data (i.e. considering only the germination values between 5% and 95%) were used.

1.6.4 Prediction of storage length in the model

There have been very few studies testing the predictions of storage longevity estimated with the seed viability equations (Fabrizius *et al.*, 1999). Especially long-term studies are scarce. There are however a few note-worthy studies.

One way of validating the model is to apply it to seed data from previously made long time storage experiments. If initial viability and storage conditions are known, predicted viabilities can be calculated and compared to the viabilities observed in the experiment. This approach has been taken for cottonseed (Usberti *et al.*, 2006), barley (Ellis & Roberts, 1980a) and many more, generally providing a good fit. A similar approach is also common, where the constants derived from one cultivar during a certain storage environment is used to predict the viability for another cultivar stored in the same environment (e.g. Demir *et al.*, 2009).

The model has also been used to predict storage longevity for corn in warehouse storage, where temperature varies. Fabrizius *et al.* (1999) calculated viabilities for 30-days intervals with average temperatures of the period, and the predicted germination at the end of the previous 30-day period as initial viability for the next period. However, the model failed to predict viabilities after more than one year's carry-over.

Studies that found that the assumptions underlying the model was not valid, discussed above in section 1.6.3, "The reliability of the seed viability equations", would probably have reported that their data could not predict storage longevity accurately, if they would have used their data for predictions. However, the model's wide-spread use in seed-banks indicates its usefulness for these storage environments. Sapra *et al* (2003) pointed out the need for careful measuring of the storage conditions when seeds are stored under cryogenic conditions (low temperature and moisture) as in seed banks, since small measuring errors of moisture or temperature can cause large prediction errors.

1.6.5 Modifications of the seed viability equations

There have been several attempts to modify and develop the Ellis and Robert's seed viability equations (1980a; b) to make them more reliable and useful.

The probit analysis of viability data to fit a linear curve in Equation (1) is very sensitive to very high and very low viabilities. Attempts have been made to use truncated data sets (germination between 5% and 95%) to get normally distributed curves, but this resulted in only slight improvement since most germination data lies outside of this range (Tang *et al.*, 1999b). The use of truncated data can only be recommended if five or more germination data points lie in the range of 5-95%.

Mead and Gray (1999) pointed out that the seed viability equations (Ellis & Roberts, 1980a) assume that the viability has been 100% for the seed lot some time prior to storage, which is not true for seed lots with damaged, infested or unripe seeds that never have been viable. They are nevertheless assumed to be a part of the normal distribution curve in the original model, causing overestimations of viability after short and very long storage periods and underestimation for moderate storage periods around P_{50} (Mead & Gray, 1999). They suggest an introduction of a 'control viability parameter', which takes the non-viable seeds into account, providing better fitted curves for seed lots with low initial viability.

Another way of obtaining better fitted curves is suggested by Hay *et al.* (2003). As described above, the constants are normally fitted in two steps; first a probit analysis to determine K_I and σ, followed by multiple regression analysis based on the estimate of σ to fit the constants K_E, C_W, C_H and C_H if these are not known. This study suggests that all constants are to be fitted in one step, which provides better fit of curves since the variability of the data is accounted for, for all constants. This approach was made possible by the development of more powerful statistical methods.

A more radical approach to modify the seed viability equations was taken by Tang *et al*. (2000) to predict the deterioration during storage for corn. They propose an alternative model, based on the seeds response to a change of moisture and temperature between a rapid ageing test and storage environment. This approach solves the problems earlier accounted by the authors when testing the seed viability equation on hybrid corn (Tang *et al.*, 1999a; Tang *et al.*, 1999b); that the deterioration of all seed lots was not normally distributed, and that the rate of deterioration differed between seed lots stored in the same environment. The model is described by the equation

$$P_{1;50} = P_{2;50}\, 10\text{^}(-C_1\Delta m.c. - C_3\Delta t - C_5\Delta(t*m.c.) - C_6(t \ln m.c.)) \qquad \text{(Equation 6)}$$

where $P_{1;50}$ is the time of storage in storage environment 1 until half the seed lot is viable, $P_{2;50}$ is the time of storage in storage environment 2 (rapid ageing) until half the seed lot is viable. P_{50} can be replaced by an arbitrarily chosen P, as long as it is the same for both environments. Δm.c. is the difference in moisture content of the seeds between the two storage environments; Δt is the difference in temperature between the two storage environments. C_1, C_3, C_5 and C_6 are constants, which are species specific.

This model has three advantages, compared to the seed viability equation (Equation 5). First, the distribution of seed death in time does not have to be normally distributed; any distribution (e.g. logistic distribution) can be used, as long as the same distribution describes the deterioration of the seed lot in both environments. Secondly, it does not require that all seed lots deteriorate at the same rate in the same environment. The P_1 and P_2 values are seed lot specific, and P_2 has to be determined by a carefully made rapid ageing test. Thirdly, the equation does not include the constants K_i and K_E, which are difficult to determine and therefor can cause incorrect estimations of storage time and viability (Hay *et al.*, 2003; Fabrizius *et al.*, 1999).

The model has however not yet been used for other species than corn, and has only been used for storage temperatures between 30 °C and 50 °C.

A similar model, where the rate of deterioration is determined as the difference between viabilities after a certain storage time in a warehouse, has been used to predict viability losses in fungicide treated wheat, compared to un-treated seeds of the same seed lot (Marcondes *et al.*, 2011). The rate of deterioration was calculated as

$$\sigma = (V_p - K_i)/p \qquad \text{(Equation 7)}$$

with Vp as viability after p days. However, this method has several weaknesses. First, the estimation relies on only two values, instead of a linear regression based on several values. Secondly, it assumes that the environment in the open storage is the same the whole storage time as during the time period *p*. This model nevertheless correctly estimated viabilities during the tested 300 days for all of four investigated fungicide treated seed lots and for one of four non-treated seed lots in a relatively constant climate in Brazil.

Another model for predicting longevity during warehouse storage has been developed for corn stored under open warehouse in Brazil (Andreoli, 2007). By determining the time for the viability of the seed lot to decrease to the same viability as after a TAA test (41 °C/100% RH/96 h), in this case 120 days, a chart was constructed in which just a simple TAA test and a germination test on the seeds before

storage can be used to predict storability of the seeds. However, this chart will only be valid under these storage conditions that cannot vary too much between years, and the data for the chart is time-consuming to obtain. This chart was tested for 360 days and correctly predicted viability losses for all five seed lots of corn that was tested.

Yet another model has been developed by Sinicio (2004), a generalized longevity model based on the seed viability equations (Ellis & Roberts, 1980a). The model uses data on seed composition instead of viability constants for determining σ, based on the following equation

$$\log \sigma = A + B \log(\text{m.c.}) + Ct + Dt^2 \qquad \text{(Equation 8)}$$

where the constants K_E, C_W, C_H and C_Q in Equation 2 are replaced with the constants A, B, C and D, based on the concentrations of ash, carbohydrates, lipids and proteins in the seeds. This type of generalized longevity model has been developed for soy bean, chickpea and cowpea (Sinicio, 2004), and can be a successful way of predicting storage longevity for a large number of species (e.g. in a seed bank) where it would be too costly to determine viability constants for all species but where the composition of the seeds are known.

1.7 Aim and objectives

The purpose of this study was to investigate how the storage of winter wheat seed in combination with heat treatment affects the germination of the seed. Heat, especially in combination with moisture, ages the seed and thereby reduces the germination rate, as earlier discussed. Aerated steam treatment (AST) for disinfection of seeds is carried out with both heat and high humidity, and might therefore accelerate the ageing of the seed and thereby reduce the possibility to carry over the seed. To be able to carry over the seed for use in a subsequent, or later, year is an important tool for seed companies to manage changes in demand, and for the seed companies that treat their seeds with AST, the storage effects of the treatment need to be known.

This study will investigate how the heat tolerance changes with physiological age, and will be performed on wheat seeds of different moisture content and physiological age. This work has two objectives; to investigate the effects of AST before and after storage of winter wheat seed.

Objective 1: *How is the AS treatment tolerance of winter wheat seed affected by storage prior to the AS treatment?*

The first part of the thesis examines whether a pre-test made when the seed was relatively newly harvested can be used to determine the optimum AST recipe more than half a year later. Samples from seed batches of different quality were pre-tested to determine heat tolerance. Thereafter, seed from the seed batches were aged with accelerated aging to three different ages. Finally, the aged seed were pre-tested, and the results compared with the pre-tests made prior to the accelerated ageing. In this way differences in heat tolerance in each seed batch after four different storage times could be determined.

The hypothesis was that the AST tolerance of the seed decreases with storage time and therefor, the recommended treatment recipe needs to be adjusted, or the pre-test repeated.

Objective 2: *How is viability affected by storage after AS treatment?*

The second part of the thesis examines for how long seeds treated with AS can be stored without the viability decreasing below acceptable levels. Based on the results from the pre-tests of the seed batches in Objective 1 the seeds in each batch were split into four parts. Three of them were treated with different AST intensities and one was left as control. Thereafter, each part was artificially aged to four physiological ages. Finally, the seeds were sown in a soil germination test and differences in the viability between untreated and treated seeds aged to different ages are compared.

The hypothesis for this objective was that the viability decreases faster for AS treated seed compared to untreated, that the viability decreases faster for moist seed compared to dry seed, and that the optimum recipe could be different depending on the required storability for the individual lot.

1.7.1 Previous research of aerated steam treatment

Storage effects of seeds treated with AST has only been the subject in one study on cereals up to now. Forsberg *et al.* (2004b) showed that a lot of barley (*Hordeum vulgare* L. cv. Svani) heavily infected with *Drechslera teres* aged 3 to 6 years in paper bags at room temperature had decreased tolerance to high treatment temperatures with increased age. The sanitation effect of the AST was also considerably lower in the aged seeds. It was also proven that seed lots of barley (*Hordeum vulgare* L. cv. Kinnan) infested with *Drechslera teres* and oats *(Avena sativa* L. cv. Sanna*)* infested with *Drechslera avenae* performed well 17 month after treatment, however oat seed treated with one of the highest treatment temperatures showed a

decreased emergence. From these experiments, the authors recommended that seeds should not be stored long prior to treatment, or for more than one year after. Corresponding tests have not been conducted on winter wheat, or with storage for up to 3 years after harvest prior to the pre-test or on storage after treatment, nor with fresh seed, all which was done in this study.

The tolerance to AST has been investigated in seeds with different moisture contents, where dry seed was wetted to the desired moisture content and then treated at different intensities (Forsberg *et al.*, 2003). The seed material in that experiment included fresh wheat seeds (cv. Kosack). The investigation showed that drier seeds can withstand higher treatment temperatures without decreased germination.

Reduced germination after storage of AS treated seeds has not been reported in the commercial ThermoSeed production (personal communication, Forsberg, 2011). A reason for this may be the strict license rules for commercial ThermoSeed™ treatment. According to them, only seed with a viability of at least 85 % after treatment in pre-tests may be treated and seeds used more than one year after treatment must be tested again to ensure at least 85 % viability. The use of an established seed lot recipe is not allowed later than 6 months after the pre-test. This thesis opens up for more precise time limits.

2 Materials and Methods

The practical work of this thesis consists of five major parts; manipulation of the original seed lot into four seed batches, determination of aerated stream treatment (AST) tolerance before storage, determination of seed viability constants for the seed lot, testing of the shelf-life of pre-tests (objective 1) and testing of storage longevity of AST treated seeds (objective 2). Each of these parts will be described in a separate section below.

To a great extent, materials and methods already used for AST have been used in these trials. This choice has been made to utilize the experience that already exists regarding AST, and to enable comparisons with previous experiments conducted with AST. In other respects, the experiments have been designed so that similar methods and materials are used to fulfil the two objectives in the study.

2.1 Manipulation of the seed lot into four seed batches of different quality

The common material for all parts was a seed lot of 150 kg of wheat seeds (cv. Ellvis). The choice of winter wheat as model crop in this experiment was made because of its widespread use, and the seed lot was chosen for its high germination and soundness. Seeds with high viability could be deteriorated in a controlled way, without decreasing the viability below 85%.

The seeds were delivered from Lantmännen in six sealed bags, consisting of an outer paper bag and an inner plastic bag. The bags were stored in a dark 6 ºC chamber for three months before the start of the trial. The seeds have consistently been kept in sealed bags or containers in a dark 6 ºC chamber during the trial, unless when stated otherwise. The moisture content of the seeds from each plastic bag was determined in a grain moisture meter (Aquamatic 5100, v. 3.12L, Perten Instruments). All samples had a moisture content of 13.6 % or 13.7 %.

The seed lot was by drying and ageing was transformed into four seed batches (figure 8, table 1). By ensuring that all seeds have the same origin and in addition to the drying and ageing have been treated exactly the same, all the viability differences between the seed batches can be attributed to the differences in age and moisture content.

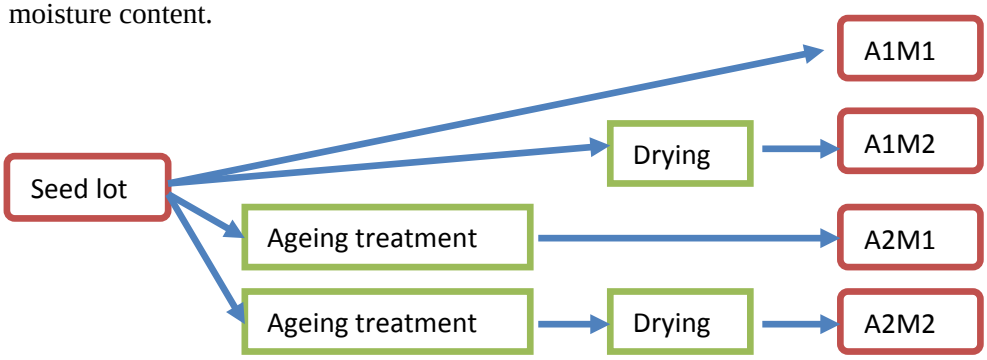


Figure 8.Schematic description of the treatment steps during the manipulation of the original seed lot into four new seed batches A1M1,A1M2, A2M1 and A2M2.The ageing treatment was performed by AST, recipe K (see table 2 for details of treatment intensity). The drying was performed in a hot air dryer.

Table 1. Summary of the treatments steps and the characteristics of the four seed batches. For details of recipe K ageing treatment, see table 2.

Seed batch	Description	Ageing treatment	Drying	m.c. (%)
A1M1	Untreated	None	None	13.7
A1M2	Low moisture	None	Approx. 1,25 h	11.8
A2M1	Low longevity	Recipe K	Approx. 0,4 h	13.8
A2M2	Low longevity and moisture	Recipe K	Approx. 2 h	11.6

Eight boxes of seed, two for every treatment, were filled with 9 kg seeds from the plastic bags, and thoroughly mixed. A plastic bag was put between the box and the lid to prevent air exchange.

Half of the seeds were treated with aerated stem to age them. The treatment exposes the seed to both heat and high humidity during a limited time, thus ageing the seeds. Based on the data from the pre-test on seed batch A1M1 (see Results), it was decided that ageing treatment of seeds in seed batch A2M1 and A2M2 should be performed with an AST recipe with an energy level of 62.9 kJ/m^3 and moisture content of 15.8 g H_2O/m^3 (recipe K in table 2) equivalent to 96 % germination. The seeds were treated in 1 kg batches, and then thoroughly mixed. All the boxes were stored in room temperature one week prior to the treatment, to avoid different storage conditions for treated and untreated seeds.

Half of these seeds and half of the untreated seeds were then dried to two percentages lower moisture content. Drying was chosen over wetting as method for

altering the moisture content, compared to Forsberg *et al*. (2004b), as it is the method used in agriculture for adjusting the seed moisture. Two percentages difference in moisture content was chosen to obtain a sufficient difference between the batches still within the range of seed moisture in commercial seed lots. Seeds from seed batch A2M1, which had increased their moisture content during the ageing treatment, were dried to a moisture content close to the original 13.7 %. The drying was performed in a hot air dryer at 45 ºC in 8 kg batches. The seeds were then thoroughly mixed, and cooled in the 6 ºC chamber and the moisture content was measured after 18 hours.

2.2 Determination of aerated steam treatment tolerance before storage

Pre-tests were performed on the seed batches, as described in section 1.1, "Sanitation of seeds with aerated steam treatment (AST)", with seeds stored at room temperature for 2 days prior to pre-testing (figure 9). The pre-tests were performed in AST intensity intervals likely to cover the viability decrease caused by AST in every seed batch. The AST recipes used for seed batch A1M1 was A-O, A-S for seed batch A1M2 and A2M2, and B-S for seed batch A2M1 (table 2).

Figure 9. Schematic description of the treatment step during the determination of AST tolerance before storage. The seed batches A1M1, A1M2, A2M1 and A2M2 were pre-tested prior to a soil germination test.

Table 2. Energy (E) and mass (m) data for the AST recipes used for pre-tests of the seed batches. A-O was used for seed batch A1M1, A-S for seed batch A1M2 and A2M2, and B-S for seed batch A2M1.

AST recipes/intensities	E (kJ/m3)	m (g H2O/m3)
A	57,7	14,3
B	58,2	14,5
C	58,7	14,6
D	59,2	14,8
E	59,8	14,9
F	60,3	15,1
G	60,8	15,2
H	61,3	15,4
I	61,8	15,5
J	62,4	15,7
K	62,9	15,8
L	63,4	16,0
M	63,9	16,1
N	64,5	16,3
O	65,0	16,5
P	65,5	16,6
Q	66,0	16,8
R	66,6	16,9
S	67,1	17,1

Immediately after treatment, a soil germination test was performed. The seeds were sown in pots with 50 seeds in each pot at 3 cm depth, with three repetitions. The soil used in the study was specially commissioned from Hasselfors Garden. A detailed description of its properties is given in Appendix A. The pots were stored with lids on in a 6 ºC chamber for 10 days, and then moved to a 20 ºC growing chamber with lids removed. Every pot was also watered with approximately 200 ml tap water. Four days later, i.e. 14 days after sowing, the number of seedlings in each pot was determined.

The test is a vigour test, aiming to provide stressful conditions only high vigour seeds can cope with. Low vigour seeds can germinate, but cannot penetrate the soil and reach the soil surface during the test time, and are therefore not included in the germination percentage. Germination test on filter paper which is a standard method that is used by seed companies has been found to poorly predict the field emergence of AS treated seed (Forsberg, 2004a).

2.3 Determination of seed viability constants

To determine the initial viability and σ for the seed batches A1M1 and A1M2, a rapid ageing (RA) treatment was performed prior to a soil germination test (figure 10).

Figure 10. Schematic description of the treatment step during the determination of seed viability constants. The seed batches A1M1 and A1M2 were treated by rapid ageing (RA) prior to a soil germination test.

Rapid ageing as accelerated ageing technique was chosen because it allows comparisons between batches of different moisture content. Hence, both the effect of moisture content of the seed and storage temperature on the ageing process can be assessed using this method. Other methods of accelerated ageing affects the moisture content of the seeds, since moisture equilibrium establishes between the seeds and the air surrounding with high relative humidity during the treatment period. By having sealed vessels the seed cannot take up or release water, except by exchange with the air surrounding the seeds in the closed vessel, which is so small that no moisture content change should take place. The air flow was therefore minimized in this experiment by using plastic bags sealed as tightly as possible around the seed samples.

The rapid ageing was carried out in a Nüve ST 402 water bath to obtain uniform temperature transfer. An oven or equivalent could have been used, but it was deemed more difficult to maintain an even temperature in all parts of the oven so that all seeds would be given the same treatment. The intensity of 50 °C was chosen based on the 50 °C water bath for barley (*Hordeum vulgare* L.) with approximately 14% moisture content (m.c.) resulted in a P_{50}-time of about 5 days (Ellis & Roberts, 1980b), which meant that the experiment could be performed within the time frame of this study.

The water bath temperature was controlled against a calibrated thermometer (Swedac, most recent control 6 months earlier). 30 sealed plastic bags were prepared, containing 30g of seeds from both seed batches and a weight. The bags were put into the water bath at three occasions. First, nine bags were put into the water bath, 8 h later 10 bags were added, and after another 8 h the last 10 bags were added. The last bag served as control and was placed directly in a 6 ºC chamber. Three bags, one from each adding occasion, were removed from the water bath once per day at the same time of the day as the first adding occasion. The ef-

fect of this was the same as if all bags had been put in the water bath at the same time and one bag removed every 8th hour.

The removed bags were cooled in cold water, and then stored in the 6 ºC chamber. After all bags were removed and cooled, a soil germination test was performed, and the result was used to determine the seed viability constants K_i, σ, C_W and K_E for this seed lot. The sowing of water bath treated seeds have been carried out in the same manner as after the pre-tests, but with one day longer period in the growing chamber to ensure that seeds with delayed germination time would reach the soil surface.

2.4 Shelf-life of pre-tests

To test how the optimal treatment intensity and viability after treatment is affected by the age of the seed lots, the seeds from each seed batch were aged in water bath prior to pre-testing (figure11).

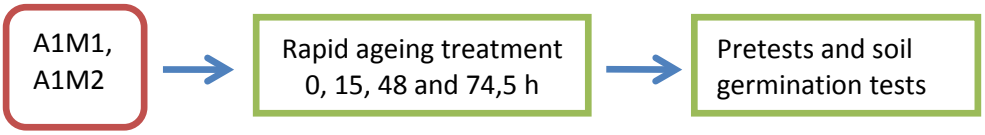


Figure 11. Schematic description of the treatment step during the testing of shelf-life of pre-tests. The seed batches A1M1 and A1M2 were treated by rapid ageing (RA) for 0, 15, 48 and 74.5 h respectively prior to pre-testing to determine if the AST tolerance is affected by ageing.

3 sealed bags of 400g seeds were prepared from each seed batch A1M1 and A1M2. The bags were treated in the water bath for 15, 48 and 74.5 hours respectively, corresponding to 0.5, 1.5 and 2.5 years in a 10 ºC storage according to the Ellis and Roberts viability equations (1980a) (see calculations in Results, section 3.2). The removed bags were cooled in cold water, and then stored in the 6 ºC chamber.

After all bags had been removed and cooled, the seeds from each bag were pre-tested. The pre-test intensities ranged between 58.7136 kJ/m^{3}and 14.6179 g H_2O/m^3, and 64.9860 kJ/m^3 and 16.4551 g H_2O/m^3, in steps of 1.0454 kJ/m^3, 0.3062 g H_2O/m^3 (recipes C, E, G, I, K, M, O in table 2). Soil germination tests, as described earlier, were performed on the treated seeds, and per cent viability was assessed 15 days later. The results from this pre-test on the artificially aged seeds were then compared to the results from the initial pre-tests.

For validation of the results from this trial, seeds from seed batch A1M1 and A1M2 are stored in outdoor ambient temperature in Uppsala in sealed plastic bags, to enable future pre-tests after natural ageing.

2.5 Storability of seeds treated with aerated steam

To test how the viability in AS treated seed lots decreases after storage, seeds were AS treated and afterwards aged by accelerated ageing prior to soil germination tests (figure 12).

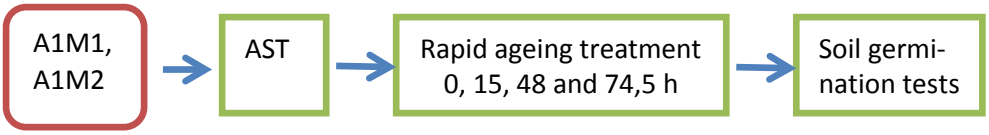


Figure 12. Schematic description of the treatment steps during the testing of storage longevity. The seed batches A1M1 and A1M2 were treated with aerated steam treatment (AST) intensities F, H, J and H, J, L respectively prior to artificial ageing by rapid ageing (RA) for 0, 15, 48 and 74.5 h. The viability of the seeds was then measured in a soil viability test to determine how the AS treated seeds tolerated ageing.

Seeds from seed batches A1M1 and A1M2 were used in this test. The seeds were treated with tree recipes; F, H, J for seed batch A1M1 and H, J, L for seed batch A1M2 (table 3). The treatment was conducted with 50 g per treatment with four replications.

The same length of rapid ageing treatment as above (0, 15, 48 and 74.5 h) were used to age the AS-treated seeds. The seeds from each treatment were packed in sealed bags immediately after treatment, and were either subjected rapid ageing in a water bath or stored in a 6°C chamber. One bag from each AS-treatment intensity was kept in the water bath for every accelerated ageing treatment time (table 3). After removal from the water bath, the seeds were cooled in cold water and stored at 6°C before the soil germination test.

Table 3. Summary of AST recipes and accelerated ageing treatment times of the treated seed samples for testing the storability of AS treated seeds. Details for treatment recipes are found in table 2.

Accelerated ageing times	**Treatment recipes**	
(50 °C water bath)	**Seed batch A1M1**	**Seed batch A1M2**
0 h (stored in 6 °C)	Not treated, F, H, J	Not treated, H, J, L
15 h	Not treated, F, H, J	Not treated, H, J, L
48 h	Not treated, F, H, J	Not treated, H, J, L
74,5 h	Not treated, F, H, J	Not treated, H, J, L

After the ageing treatment, the seeds were sown in a soil germination test as described above. Viability was assessed 16 days after sowing.

For comparison of the results from this trial, treated and untreated seeds from seed batch A1M1 and A1M2 were also sent to the seed testing laboratory LABOSEM for accelerated ageing treatment with the saturated salt accelerated ageing

(SSAA) method. The SSAA test was carried out over NaCl solution, giving 75% relative humidity (RH), in 45 °C for 7 days. Germination tests were performed on the seeds before and after the SSAA test, using 200 seeds on sand in 20 °C. After 7 days, the number of normal and abnormal seedlings and non-germinated seeds were recorded.

Treated seeds from all four seed batches are also stored in Uppsala at outdoor ambient temperature for future testing of viability after natural storage. The seeds were treated with the same AST recipes as described above.

2.6 Statistical methods

Determination of AST tolerance before storage: A negative accumulated normal distribution function was fitted to the pre-tests curves using a least squared error procedure. The statistical model to interpret the pre-test was designed for eight viability values. Therefore, values close to the steep viability decrease were chosen to better describe the deterioration. σ, LD_{50}, P_{85}, LD_{99}, and $LD_{99.9}$ were calculated. LD values (lethal dose) are relative to the initial viability, whereas P_{85} is 85% viability.

Shelf-life of pre-tests: A negative accumulated normal distribution function was fitted to the pre-tests curves using a least squared error procedure, and σ, LD_{50}, P_{85}, LD_{99}, and $LD_{99.9}$ was calculated. LD values (lethal dose) are relative the initial viability, whereas P_{85} is 85% viability.

2.7 Mathematical methods – Seed viability equations

In order to correlate RA treatment length to natural storage times and predict potential longevity in a variety of storage environments, the seed viability equations were used. The viability data obtained in the RA test was converted to probits, and a straight line was fitted to the data from each seed batch by the least square fit method in Excel 2010, which gave values of σ and Ki. Equations 1-5 were used to calculate υ, σ, K_E and C_W as described in section 1.6.1, "The Equations". Values for C_H and C_Q of 0.0329 and 0.000478 respectively (Dickie *et al.*, 1990) were assumed.

A rough estimate of RA storage time corresponding to 0, 0.5, 1.5 and 2.5 years in 10 °C natural storage was based on previously obtained RA data from the seed lot (not published). These viability constants were used to estimate which treatment time (p) in the 50 °C water bath that would give the same viability (i.e. the

same ageing) as storage for 0, 0.5, 1.5 and 2.5 years, using the same equation and solving for p.

To calculate the relationship between the RA treatment in this study and natural storage environments, the seed viability equation (2) and the obtained seed viability data from the RA test is used. The storage time in 5, 10 and 15 °C natural storage, which corresponds to 0, 15, 48 and 74.5 h in RA was calculated, assuming the same moisture content of the seed.

A comparison between the estimated viabilities based on the seed viability data obtained in this trial, the estimated viabilities calculated using C_W and K_E from Ellis *et al.* (1990) and the actual viabilities obtained in section 3.3 and 3.4 of “Results“ was also made.

3 Results

The germination results from each pot are found in Appendix B.

3.1 Determination of aerated steam treatment tolerance before storage

Pre-tests were performed on the four seed batches used in this trial to determine their aerated steam treatment (AST) tolerance. AST tolerance is defined in this study as the highest AST intensity the seeds can tolerate without more than a certain viability loss. Mathematically, these certain viability losses are describes by LD and P values in this study. The LD_{50} AST tolerance means that at this intensity, 50 % of the original viability is lost. This AST intensity is higher than the LD_1 AST tolerance, which is the intensity giving a 1 % viability loss.

The pre-tests showed a similar pattern of heat tolerance for all batches (figure 13). The seeds maintained a viability close to the viability of the untreated sample up to a breaking point temperature, thereafter the viability decreases sharply until all or most seeds were dead at approximately recipe O. The un-aged seed batches had higher initial viability and a higher heat tolerance. The un-aged seed batches also had steeper slopes of viability loss compared to the aged seed batches (i.e. lower σ values).

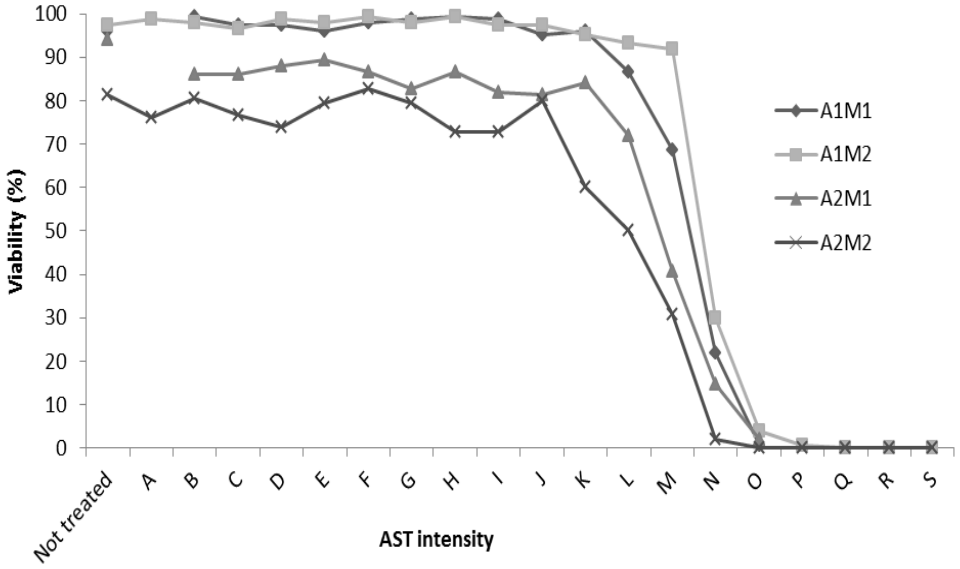


Figure 13. The results from the pre-tests of the four seed batches. The figure shows how the viability depends on the aerated steam treatment (AST) intensity. The AST tolerance for the seed batch is measured as the highest intensity the seeds can withstand without loss of viability compared to the viability of untreated seeds. Every point represents the mean of viability for 3 pots with 50 seeds in each. Seed batch A1M1, un-aged, 13.7 % m.c (untreated); seed batch A1M2, un-aged. 11.7% m.c.; seed batch A2M1, aged, 13.8% m.c.; seed batch A2M2, aged, 11.6 % m.c. A list of the energy and mass of the AST intensities is found in table 2.

By adapting a normal distribution to the data, LD and P values describing the temperature tolerance was calculated. The breaking point of heat tolerance, after which the steep decrease starts, is described by the LD_1 value (table 4). The LD_1 values differ between the seed batches; where the aged seed lots A2M1 and A2M2 had lower LD_1 values (mean of the two calculated LD_1 values) than the un-aged seed batches A1M1 and A1M2.

The P_{85} values shows how high AST intensity the seeds can tolerated before the viability decreases to 85%. The dry, un-aged seeds in seed batch A1M2 can tolerated the most, followed by moist, un-aged A1M1 seeds and the aged, moist seeds in seed batch A1M1. Since the viability of the untreated seeds in seed batch A2M2 was 81.3 % and the treatment decreases the viability, the P_{85} value could not be estimated for this seed batch.

Table 4. AST tolerance for seed batches A1M1, A1M2, A2M1 and A2M2, calculated by adapting a normal distribution to the pre-test series in figure 13 for seed batch A1M1. Germ. N.T is germination of non-treated seeds from the four seed batches, σ is the standard deviation of the distribution of seed death by temperature; LD_{50}, LD_1, $LD_{0.1}$ is the temperature which decreases the viability to 50%, 99% and 99.9% of initial viability, P_{85} is the temperature which decrease the viability to 85%. The AST intensity is described by two measures; E is energy in kJ/m^3 and m is mass of water in g H_2O/m^3.

Seed batch	**Germ. N.T**	**Recipes**	**σ**		**LD_{50}**		**P_{85}**		**LD_1**		**$LD_{0.1}$**	
	(%)		E	m	E	m	E	m	E	m	E	m
A1M1	96,0	I-O	0,51	0,15	64,13	16,20	63,51	16,02	62,94	15,86	62,55	15,74
A1M2	97,3	J-P	0,32	0,09	64,34	16,27	63,97	16,16	63,59	16,04	63,34	15,97
A2M1	94,0	H-N	0,95	0,28	63,78	16,10	62,54	15,74	61,57	15,45	60,84	15,24
A2M1	94,0	I-O	0,92	0,27	63,78	16,10	62,58	15,75	61,64	15,47	60,93	15,27
A2M2	81,3	H-N	0,79	0,23	63,49	16,02	na	na	61,65	15,48	61,04	15,30
A2M2	81,3	G-M	1,15	0,34	63,73	16,09	na	na	61,07	15,31	60,19	*15,05*

3.2 Determination of seed viability constants

RA treatment of the seeds followed by soil viability tests were performed on seed batch A1M1 and A1M2 to determine initial viability (K_i), σ and the constants C_W and K_E for the seed viability equations (Ellis and Roberts, 1980a). These were later used to correlate the RA temperature and treatment time in the shelf-life and storability tests of this study (section 3.3 and 3.4) to the temperature and storage time of natural storage.

The results show a similar pattern as the pre-tests, with high viability until a breaking point, followed by a steep decrease of viability (figure 14). The drier seeds maintains high viability for a longer time than the moister seeds.

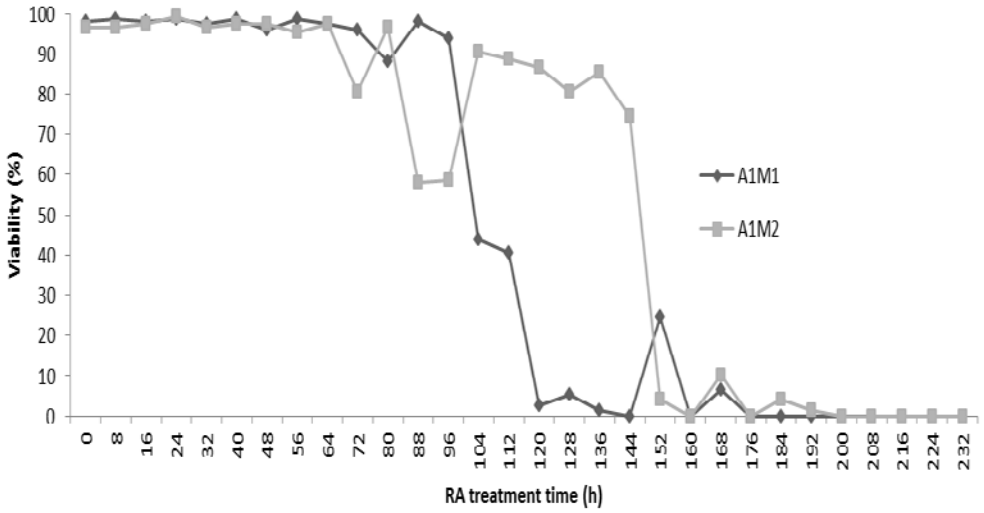


Figure 14. Viability of seed batch A1M1 and A1M2 measured by a soil viability test after the RA treatement performed in a 50 °C water bath. Each viability data point represents the mean of three pots from the soil viability test.

To be able to calculate K_i and σ from this viability data, the viabilities were transformed to probit percentage viability and a straight line was adapted to the data from each seed batch (figure 15). Some data were excluded from the adaption of the curve since they deviated from nearby data and the expected result. For seed batch A1M1, the viability data corresponding to water bath times of 80 h, 152 h and 168 h was removed, and for seed batch A1M2 viability data from the treatment times 72 h, 88 h, 96 h, and 168 h was removed. Possible explanations for the deviations are discussed in Discussion, section 4.2, "Determination of seed viability constants".

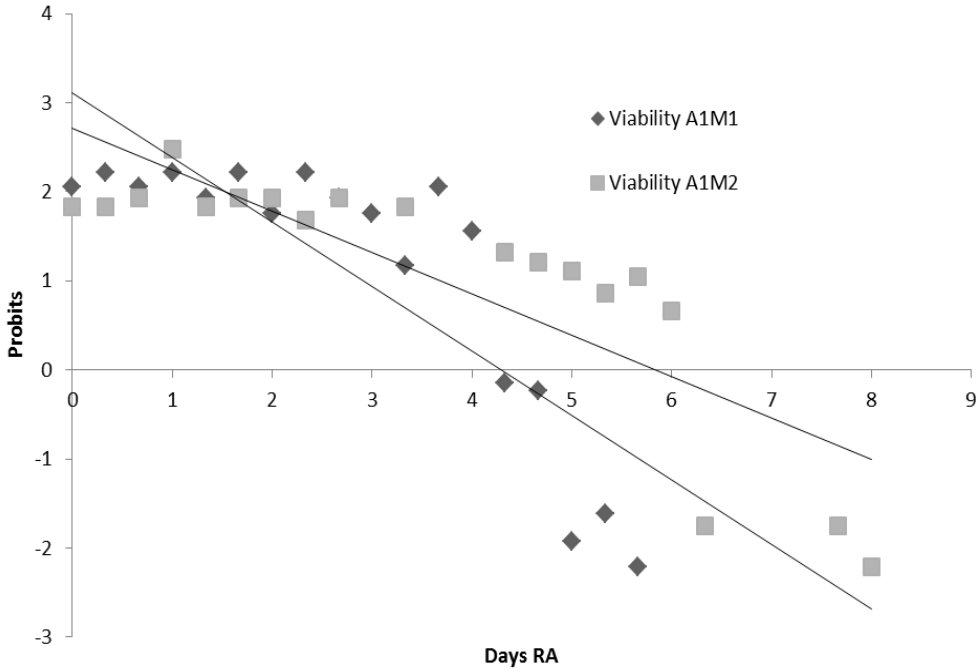


Figure 15. The viability data from the soil viability test on RA treated seeds from seed batch A1M1 and A1M2 expressed in probit percentage viability, with a straight line adapted to the data by least square fit method. The equation describing the viability decrease in A1M1 is v = 3.1067 - p/ 1.37969 (R^2 = 0.6887) and the equation describing the viability decrease in A1M2 is v = 2.712 - p/ 2.14776 (R^2 = 0.707), with v as predicted viability (probit percentage) at the time p (days).

The equation of the straight line in figure 15 is described by Equation (1); $v = K_i - p/\sigma$. The R^2 values of the fits are 0.6887 and 0.707 for A1M1 and A1M2 respectively. From these equations, the values of Ki and σ were obtained and used to calculate the constants K_E and C_W with equations (3) and (4) (table 5). The universal values C_H=0.0329, C_Q=0.000478 (Dickie *et al.*, 1990) were used in these calculations.

Table 5. Seed viability data for seed batch A1M1 and A1M2. m.c. is the moisture content of the seeds, K_i is the initial viability calculated from the straight line adaption in figure 15 expressed in probit percentage, σ is days to lose one probit percentage viability and C_W, K_E, C_H and C_Q are constants.

Seed batch	m.c.	K_i	σ (days)	C_W	K_E	C_H	C_Q
A1M1	13,7	3,0167	1,37969	2,6598	6,00323	0,0329	0,000478
A1M2	11,6	2,712	2,14776	2,6598	6,00323	0,0329	0,000478

The viability data (table 5) was used to calculate the relationship between RA and normal storage environments (table 6). The estimated viabilities after 15, 48 and 74.5 h RA was found to correspond to the estimated viabilities after 0.50, 1.59 and

2.47 years of 10 °C storage, assuming the same moisture content of the seeds. The corresponding storage times in 5 °C and 15 °C were also calculated.

Table 6. The estimated viabilities (calculated from data in table 5) after 0, 15, 48 and 74.5 h, and the corresponding storage time (p) to obtain the same viability decrease in 5, 10 and 15 °C, assuming the same moisture content of the seeds. ν is percentage viability at the time p, σ is days to lose one probit percentage viability.

t (°C)	**p** (h)	**p** (days)	**p** (years)	**A1M1**			**A1M2**		
				σ (days)	**υ** (probit)	**υ** (%)	**σ** (days)	**υ** (probit)	**υ** (%)
50	0	0	0	1,37969	3,02	99,9	2,1478	2,71	99,7
50	15	0,625	0,0017	1,37969	2,56	99,5	2,1478	2,42	99,2
50	48	2,000	0,0055	1,37969	1,57	94,1	2,1478	1,78	96,3
50	74,5	3,104	0,0085	1,37969	0,77	77,8	2,1478	1,27	89,7
5		0	0	636	3,02	99,9	990	2,71	99,7
5		288	0,79	636	2,56	99,5	990	2,42	99,2
5		922	2,53	636	1,57	94,1	990	1,78	96,3
5		1431	3,92	636	0,77	77,8	990	1,27	89,7
10		0	0	401	3,02	99,9	624	2,71	99,7
10		182	0,50	401	2,56	99,5	624	2,42	99,2
10		581	1,59	401	1,57	94,1	624	1,78	96,3
10		902	2,47	401	0,77	77,8	624	1,27	89,7
15		0	0	239	3,02	99,9	372	2,71	99,7
15		108	0,30	239	2,56	99,5	372	2,42	99,2
15		347	0,95	239	1,57	94,1	372	1,78	96,3
15		538	1,47	239	0,77	77,8	372	1,27	89,7

3.3 Shelf-life of pre-tests

How the AST tolerance of the seeds changes as the seed age was tested by ageing the seeds by RA in a 50 °C water bath prior to pre-testing. The pre-tests showed a decreased AST tolerance (figures 16 and 17), compared to un-aged seeds of the seed batches previously tested.

Seeds of seed batch A1M1 and A1M2 were aged for 15, 48 and 74.5 h, corresponding to 0.5, 1.6, and 2.5 years of storage at 10 °C, according to the calculations presented above. The viability at low temperatures was unchanged regardless of rapid ageing treatment time, except for 74.5 h RA in seed batch A1M1, which

was approximately 10 percentages lower. The AST tolerance decreases with age for both seed batches.

For seed batch A1M1, LD_{50} values decreases with age from 64.1 kJ/m^3, 16.2 g H_2O/m^3 for unaged seeds to 62.6 kJ/m^3, 15.8 g H_2O/m^3 for seeds aged 74,5 h (table 7). A similar pattern is seen for LD_1 and $LD_{0.1}$, however the difference in temperature is slightly larger at lower LD values. P_{85}, showing how high AST intensity the seeds can tolerated without the viability decreasing below 85 % does also decrease the longer the seeds are treated. The σ values are higher for the aged seeds, compared to the non-aged.

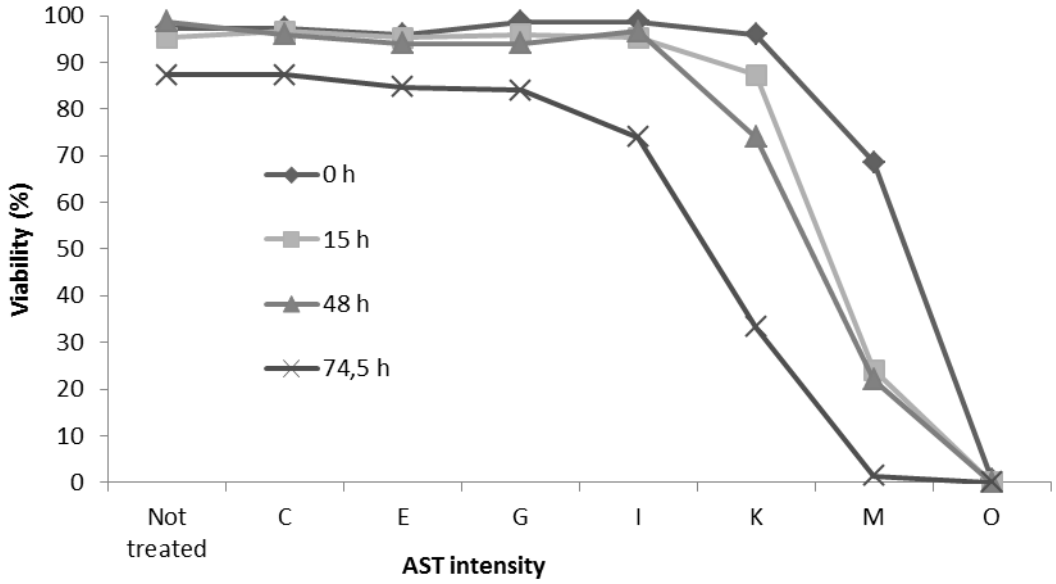


Figure 16. Viability results from the pre-tests on seed batch A1M1 performed after the accelerated ageing in 50 °C for 15, 48 and 74.5 h respectively. A list of the energy and mass of the AST intensities is found in table 2.

Table 7. Viability constants for seeds aged 0, 15, 48 and 74,5 h in 50 °C, calculated by adapting a normal distribution to the pre-test series in figure 16 for seed batch A1M1. Germ NT is the germination of the untreated seeds from the seed lot, σ is the standard deviation of the distribution of seed death by intensity; LD_{50}, LD_1, $LD_{0.1}$ is the AST intensity which decreases the germination/viability to 50%, 99% and 99.9% of initial viability, P_{85} is the intensity that decreases the viability to 85%. The AST intensity is described by two measures; E is energy in kJ/m³ and m is mass of water in g H_2O/m^3.

RA treatment	**0 h**		**15 h**		**48 h**		**74.5 h**	
Germ. N.T	97,3%		95,3%		98,7%		87,3%	
AST intensity	E (kJ/m³)	m (g H_2O/m^3)	E (kJ/m³)	m (g H_2O/m^3)	E (kJ/m³)	m (g H_2O/m^3)	E (kJ/m³)	m (g H_2O/m^3)
$LD_{0.1}$	62,4	15,7	61,7	15,5	60,8	15,2	60,0	15,0
LD_1	62,8	15,8	62,1	15,6	61,5	15,4	60,7	15,2
P_{85}	63,5	16,0	62,8	15,8	62,5	15,7	61,0	15,3
LD_{50}	64,1	16,2	63,6	16,0	63,4	16,0	62,6	15,8
σ	0,570	0,167	0,624	0,183	0,822	0,241	0,832	0,244

The same pattern is seen in the results for seed batch A1M2, however all pre-tests have similar viabilities for low AST intensities, 95.3 % to 98.3 % (table 8). LD_{50} values range from 64.4 kJ/m³, 16.3g H_2O/m^3 for untreated seeds to 63.3 kJ/m³, 15.9 g H_2O/m^3 for seeds treated 74.5 h, and similar differences are found for LD_1 and $LD_{0.1}$ values. The P_{85} value, below which seed cannot be certified, lies between 64.0 kJ/m³, 16.2 g H_2O/m^3 and 62.7 kJ/m³, 15.8 g H_2O/m^3. The σ values increases with increasing age of the seeds.

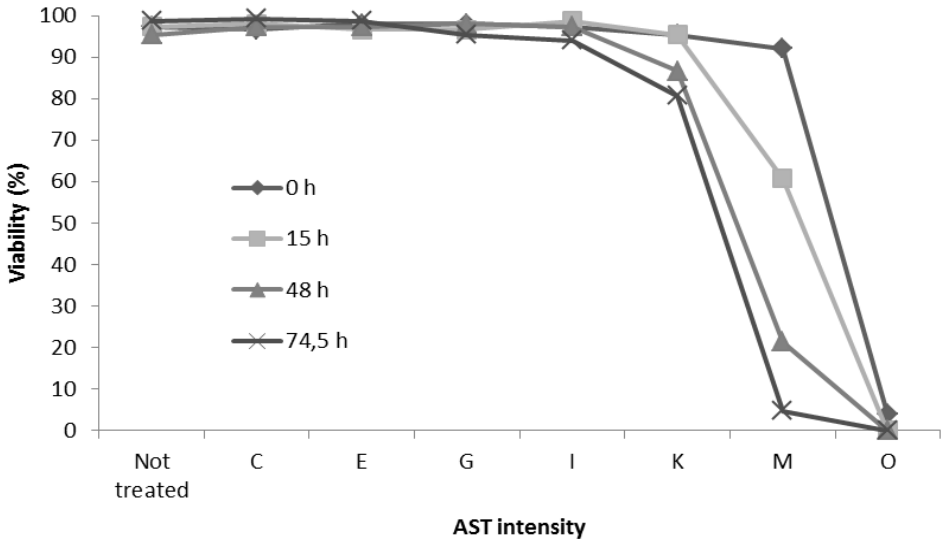


Figure 17. Viability results from the pre-tests on seed batch A1M2 performed after the accelerated ageing in 50 °C for 15, 48 and 74.5 h respectively. A list of the energy and mass of the AST intensities is found in table 2.

Table 8. Viability constants for seeds aged 0, 15, 48 and 74,5 h in 50 C, calculated by adapting a normal distribution to the pre-test series in figure 17 for seed batch A1M2. σ is the standard deviation of the distribution of seed death by intensity; LD_{50}, LD_1, $LD_{0.1}$ is the AST intensity which decreases the germination/viability to 50%, 99% and 99.9% of initial viability, P_{85} is the intensity that decreases the viability to 85%. The AST intensity is described by two measures; E is energy in kJ/m^3 and m is mass of water in g H_2O/m^3.

RA treatment	**0 h**		**15 h**		**48 h**		**74.5 h**	
N.T germ.	97,3%		97,3%		95,3%		98,7%	
AST intensity	E (kJ/m^3)	m (g H_2O/m^3)	E (kJ/m^3)	m (g H_2O/m^3)	E (kJ/m^3)	m (g H_2O/m^3)	E (kJ/m^3)	m (g H_2O/m^3)
$LD_{0.1}$	63,3	16,0	63,3	15,6	61,6	15,5	61,6	15,5
LD_1	63,6	16,0	62,6	15,8	62,1	15,6	62,0	15,6
P_{85}	64,0	16,2	63,3	16,0	62,8	15,8	62,7	15,8
LD_{50}	64,4	16,3	64,0	16,2	63,5	16,0	63,3	15,9
σ	0,362	0,106	0,609	0,178	0,617	0,181	0,548	0,161

Comparing the two seed batches, the drier seed batch A1M2 has consistently higher AST tolerance. This seed batch tolerates treatment intensities approximately 0.5227 kJ/m^3 and 0.1531 g H_2O/m^3 higher than A1M1 for all RA treatment times, except for A1M2 74.5 h, which is significantly higher than A1M1 74.5h.

The negative relationship between AST tolerance and length of RA treatment is shown in figure 18, with AST tolerance $LD_{0.1}$, LD_1, LD_{50} and P_{85} shown as a function of RA treatment times. Equations describing the relationships and adjoining R^2 values are found in table 9. The AST tolerance decrease between 0.015 and 0.030 kJ/m^3 per hour RA and the R^2 values lies between 0.758 and 0.988.

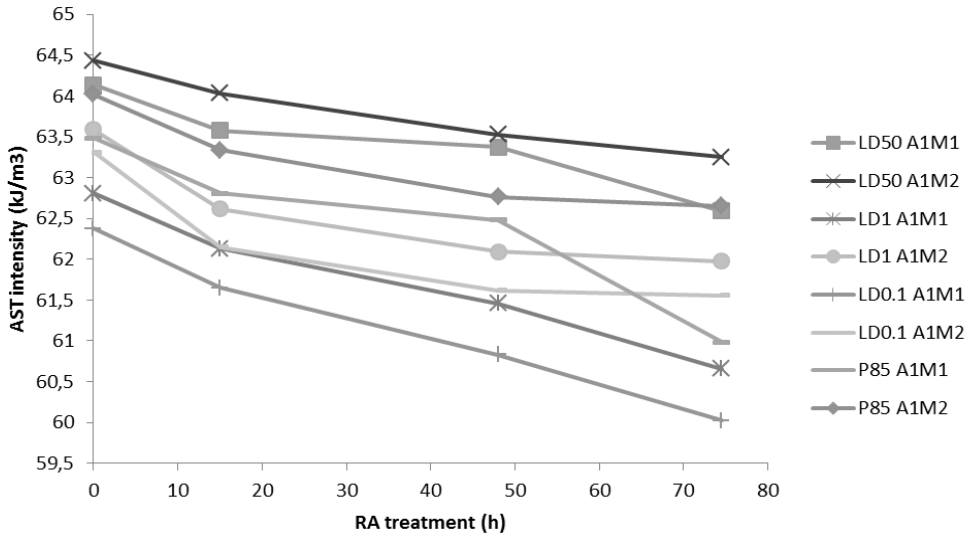


Figure 18. $LD_{0.1}$, LD_1, LD_{50} and P_{85} values after rapid ageing (RA) treatment for 0h, 15h, 48h and 74,5h. The RA causes $LD_{0.1}$, LD_1, LD_{50} and P_{85} values to decrease in seed batches A1M1 and A1M2.

Table 9. Linear regression analysis by least square fit for changes in $LD_{0.1}$, LD_1, LD_{50} and P_{85} over time, based on the values in figure 18 above. y = treatment energy intensity (kJ/m3), x = RA treatment time (h). The best explaniation of the linear relationship between RA time and viability is provided by $LD_{0.1}$ and LD_1 for A1M1, and LD_{50} for A1M2, however most regressions have high R^2 values.

Seed batch	AST tolerance	Equation	R^2
A1M1	$LD_{0.1}$	y = -0,0302x + 62,258	0,988
A1M1	LD_1	y = -0,0273x + 62,701	0,983
A1M1	LD_{50}	y = -0,0183x + 64,049	0,923
A1M1	P_{85}	y = -0,0301x + 63,472	0,908
A1M2	$LD_{0.1}$	y = -0,0212x + 62,888	0,758
A1M2	LD_1	y = -0,0198x + 63,248	0,807
A1M2	LD_{50}	y = -0,0155x + 64,343	0,971
A1M2	P_{85}	y = -0,0175x + 63,749	0,870

The correlation between σ and RA treatment duration (figure 19), provides less good fit than LD- and P-values and RA treatment times presented above.

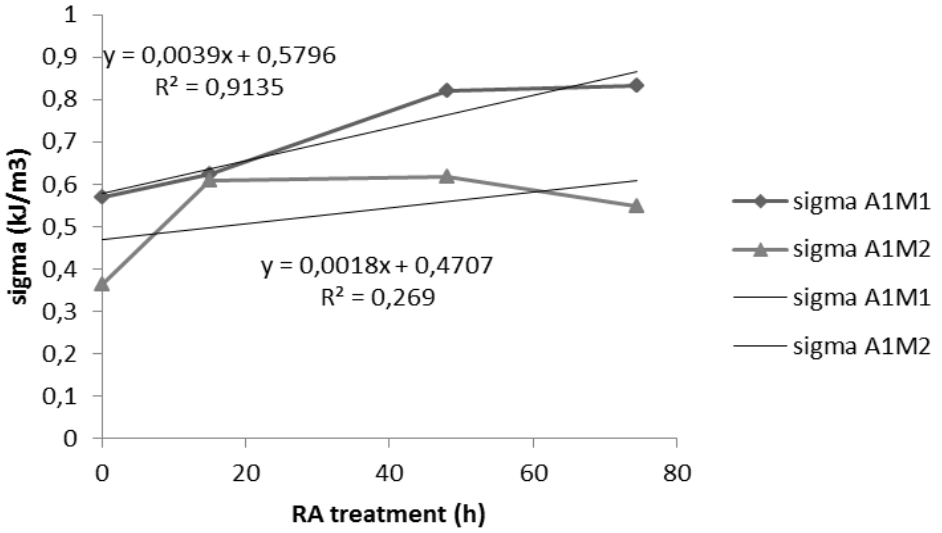


Figure 19. Relationship between the time in 50 °C raipd ageing (RA) in hours and the standard deviation of the distribution of seed death by energy intensity in kJ/m^3 (σ).

The moisture content in the seeds increased during the RA treatment (table 10). The correlation between LD- and P-values and moisture content after RA treatment (figure 20), provided less good fit than LD- and P-values and the length of RA treatment presented in figure 18 and table 9.

Table 10. Moisture content (m.c.) of the seeds before and after 15, 48 or 74.5h rapid ageing treatment in 50 °C.

Prior to RA m.c. (%)	RA 15 h m.c. (%)	RA 48 h m.c. (%)	RA 74,5 h m.c. (%)
13.7	14.4	14.2	14.5
11.8	12.1	12.3	12.7

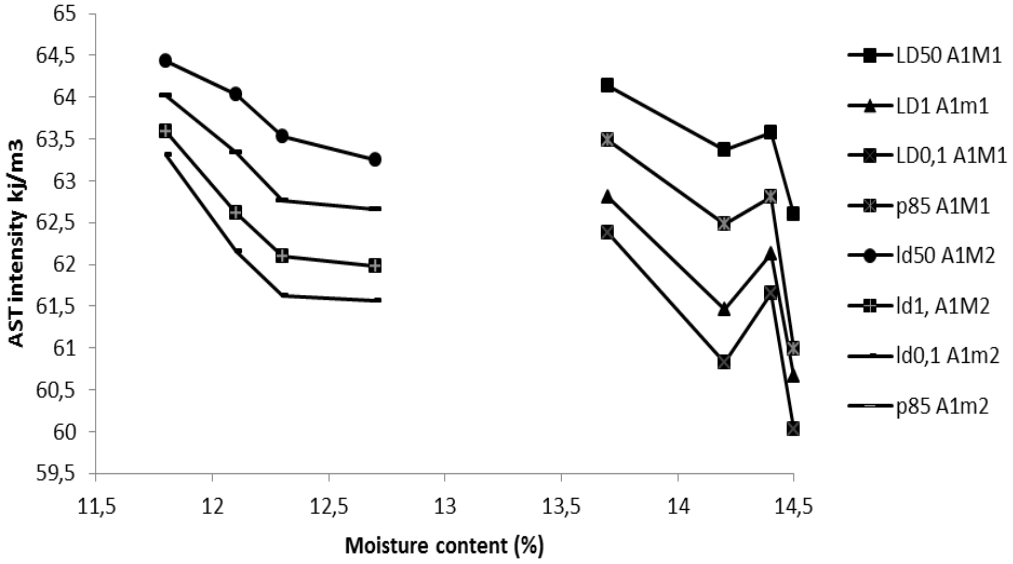


Figure 20. Relationship between the moisture content of the seeds after rapid ageing treatment and the aerated steam treatment (AST) tolerance of $LD_{0.1}$, LD_1, LD_{50} and P_{85} (kJ/m^3) after rapid ageing.

3.4 Storability of seeds treated with aerated steam

The storability of AST treated seeds was tested by treating seeds at different TS treatment intensities prior to rapid ageing treatment to simulate the effects of natural ageing. A soil viability test was performed on the seeds. The results consistently show that the viability of the seeds decreased more during storage if they were treated with a high AST intensity compared to a lower intensity (figure 21 and 22). The storability of AST treated seeds was considerably lower than untreated seeds.

The AST intensities used in this part of the study can be related to the LD values for the non-RA-treated seeds (table 4), since these LD values describes the AST tolerance of fresh seeds. The AST intensities F, H and J for seed batch A1M1 can thereby be described as $LD_{0.1}$-2.3kJ/m^3, $LD_{0.1}$-1.2 kJ/m^3 and $LD_{0.1}$-0.2 kJ/m^3, and for seed batch A1M2 the AST intensities H, J and L as $LD_{0.1}$-2.0 kJ/m^3, $LD_{0.1}$-1.0 kJ/m^3 and $LD_{0.1}$+0.0 kJ/m^3, in terms of energy intensity. Compared to the $LD_{0.1}$

values of each seed lot, seed batch A1M2 was thus treated by slightly higher intensity than seed batch A1M1.

After the AS treatment, the seeds were treated by rapid ageing to simulate natural ageing. Seeds of seed batch A1M1 and A1M2 were aged for 15, 48 and 74.5 h, corresponding to 0.5, 1.6, and 2.5 years of storage at 10 °C, according to the calculations presented above. In seed batch A1M1, the seeds maintained the initial viability after 15 h rapid ageing. Thereafter, the viability decreases fast for the AS treated seeds, resulting in viabilities below the 85% certification limit after 48 h, and below 10% after 74.5 h. The viability of the untreated seeds decreased slightly, but was still above 85% after 74.5 h.

Seeds treated with the highest treatment intensity, recipe J, decreased the most in viability with time, compared to seeds treated with recipe F and H, which had close to identical viabilities (figure 21).

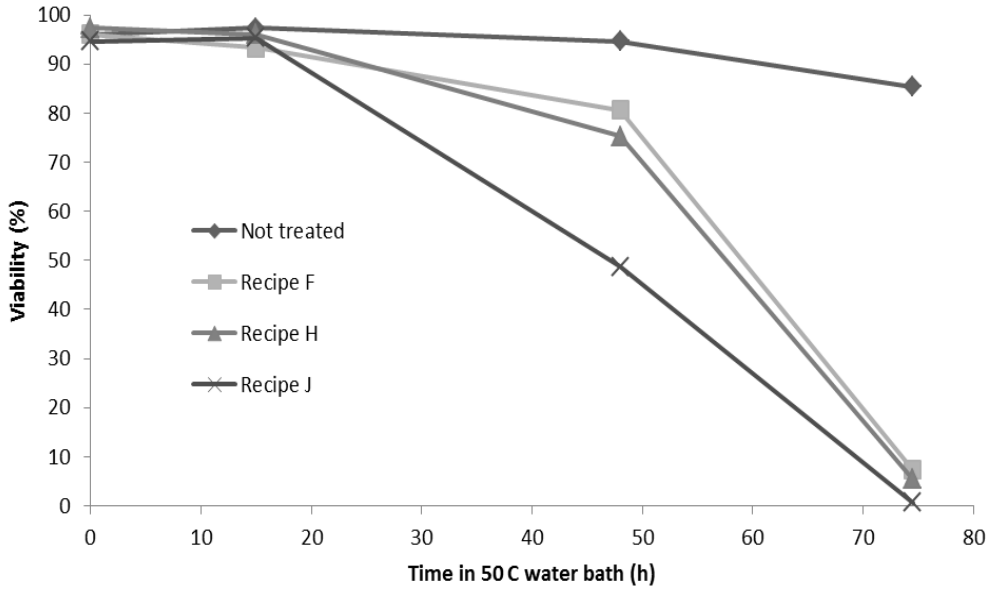


Figure 21. Seed batch A1M1. Viability for seeds treated with AST at three intensities and an untreated control after accelerated ageing in 50 °C water bath for 0, 15, 48 and 74.5 h, corresponding to 0.5, 1.6 and 2.5 years of natural ageing in a 10 °C storage. A list of the energy and mass of the AST intensities F, H and J is found in table 2.

The same pattern for viability after rapid ageing as for A1M1 (13.7 % m.c.) could be seen for the drier seed batch A1M2 (11.7 % m.c.) (figure 22). The untreated control maintains a high viability, whereas the viability for seeds treated with AST decreased below 85% germination after some storage time. Seeds treated with recipe H or J maintained a high viability (approx. 97-99%) after 48h accelerated ageing, thereafter the viability was reduced.

Seeds treated with recipe J had higher viability after 74.5 h RA treatment than seeds treated with recipe H. Possible reasons for this is discussed in the Discussion, section 4.4, “Storability of seeds treated with aerated steam treatment”. Seeds treated with the highest treatment intensity, recipe L, had a larger decrease of viability than seeds treated with recipe H or J. Even if the viability still was above the 85% limit after 15 h rapid ageing treatment for the seeds treated with recipe L, the figure 22 shows that the decrease of viability has started. The untreated seeds maintain viability above 95 % for all rapid ageing treatment times.

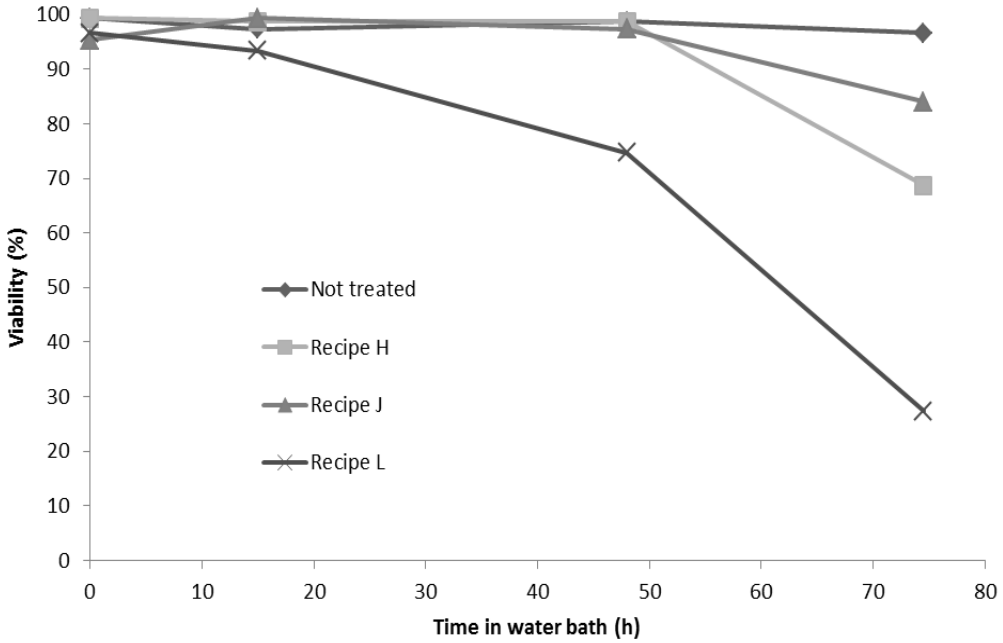


Figure 22. Seed batch A1M2. Viability for seeds treated with AST at three intensities and an untreated control after accelerated ageing in 50 °C water bath for 0, 15, 48 and 74.5 h corresponding to 0.5, 1.6 and 2.5 years of natural ageing in a 10 °C storage. A list of the energy and mass of the AST intensities H, J and L is found in table 2.

The storability of the AS treated seeds was also assessed by an external laboratory, LABOSEM. The measurement of moisture content of the seeds at LABOSEM show a 0.3-0.4 percentage lower moisture content than measured at the INCOTEC lab with the grain moisture meter (Aquamatic 5100, v 3.12L, Perten Instruments). Since storability of seeds depends on the moisture content, storability based on both measurements of each seed batch are presented in table 11.

The SSAA test showed that seeds from batch A1M1 treated with AST have higher risk of viability losses over 10 % after 5 months of warehouse storage, compared to untreated seeds from the seed batch. The risk of viability losses increased with higher treatment temperatures. Based on the moisture content meas-

ured at LABOSEM, seeds treated with recipe F or H had average risk of more than 10% viability losses, whereas seeds treated with recipe J had high risk of viability loss. Assuming the INCOTEC lab moisture content, the risks of viability losses were increased to high and very high for seeds treated with recipe H or J, respectively.

For seed batch A1M2, based on the LABOSEM moisture content measurement, untreated seed and seed treated with recipe H or J had a low risk of more than 10% viability decrease after 5 months of warehouse storage. Seeds treated with AST recipe L had high risk of viability losses. Assuming the INCOTEC moisture content, the risk rises from low to average for seeds treated with recipe H and from high to very high for seeds treated with recipe L.

All results from LABOSEM, including abnormal seedlings and non-germinated seeds, are found in Appendix C.

Table 11. The risk of more than 10% viability loss after the period January – June 2012 for untreated and AS treated seeds from seed batch A1M1 and A1M2, based on a saturated salt accelerated ageing test (SSAA) performed by LABOSEM. A list of the energy and mass of the AST intensities F, H, J and L is found in table 2. The moisture content measured at LABOSEM differed from the moisture content measured at INCOTEC, and since the risk of viability loss depends on the moisture content of the seeds, the predicted risk of viability loss based on both moisture contents is shown.

Seed batch	**AST recipe**	**Moisture content (%)**		**Viability (%)**		**Risk of >10% viability loss jan - jun**	
		INCOTEC	LABOSEM	Before SSAA	After SSAA	INCOTEC m.c.	LABOSEM m.c.
A1M1	n.t	13,9	13,5	89	65	low	low
A1M1	F	14,7	14,3	97	59	average	average
A1M1	H	14,8	14,4	99	50	high	average
A1M1	J	14,9	14,5	97	28	very high	high
A1M2	n.t	11,9	11,5	98	71	low	low
A1M2	H	12,9	12,5	99	28	average	low
A1M2	J	13,0	12,6	97	4	low	low
A1M2	L	13,0	12,7	94	12	very high	high

3.5 Comparison of predicted and measured viabilities

A comparison between the estimated and actual viabilities after RA treatment was made to test the accuracy of the predictions calculated by the seed viability equations. The estimates made with the constants obtained in this study and the values of C_W and K_E obtained from Ellis *et al.* (1990), and the actual viabilities after RA obtained in the shelf-life and storability tests were compared (table 12).

For seed batch A1M1, the calculations based on the constants obtained in this study slightly overestimates the initial viability and the rate of deterioration, whereas the rate of deterioration is much overestimated in the calculations based on the Ellis *et al.* (1990) constants. After 74.5 h RA it is estimated that the viability should be 77.8 % (based on this study's constants) or 15.4 % (based on Ellis *et al.* 1990), but the actual viabilities were 87.3 % and 85.3%. The estimates and the actual values differs less for seed batch A1M2. No decrease in viability can be seen based on the actual viability values, whereas the estimated values predict a decrease from 99.7 % to 89.7% and 90.5% respectively between 0 and 74.5 h RA.

Table 12. Comparison between the estimated viabilities after RA treatment calculated with the K_E and C_W obtained in this study (1) and calculated with the K_E and C_W from Ellis et al. *(1990) (2), and the actual viabilities obtained in the shelf-life study (3) and storability study (4) for seed batches A1M1 and A1M2.*

t (°C)	**p (h)**	**A1M1 viability (%)**				**A1M2 viability (%)**			
		1	2	3	4	1	2	3	4
50	0	99,9	99,7	97,3	96,0	99,7	99,7	97,3	99,3
50	15	99,5	97,5	95,3	97,3	99,2	99,2	97,3	97,3
50	48	94,1	62,1	98,7	94,7	96,3	96,4	95,3	98,7
50	74,5	77,8	15,4	87,3	85,3	89,7	90,4	98,7	96,7

4 Discussion

4.1 Determination of aerated steam treatment tolerance before storage

The pre-tests performed on the four seed batches before aerated steam treatment (AST) and ageing showed higher viability for seed batch A1M1 and A1M2, than the two aged seed batches A2M1 and A1M2. This result is consistent with previous research on seed deterioration (Copeland and McDonald, 2001), which clearly shows that ageing; caused by exposure to heat man moisture during a period of time, results in reduced viability.

The difference in temperature tolerance between the un-aged seed batches, A1M1 and A1M2 was also expected, since moister seeds deteriorate faster. The drier seeds had a higher temperature tolerance than the moister seeds, probably since the moister seeds have higher thermal conductivity, thus heating the seed embryo more and accelerate chemical reactions within the seed, causing deterioration.

The aged seed batches A2M1 and A2M2 had lower initial viabilities than expected. They were treated with recipe L that would give 96 % germination, and then pre-tested. However, when germination tested, the viability of the seeds was much lower. This indicates that AST with subsequent drying can reduce viability. The drying of the seeds in this study was performed at a low temperature, 45 °C, which is well below the recommended 60 °C (Råsberg, 1998), and should not damage the seeds. However, the dryer had a very high drying capacity, probably due to a large specific air flow, which may have mechanic tension an microscopic caused cracks in the seed surface due to the fast drying, causing viability loss. Vigour tests performed on a spring wheat lot before and after drying from 16 % to 14.9 % moisture content (m.c.) showed a reduction of vigour from 70% to 50% (not published), indicating that the viability loss may be caused by the drying. The saturated salt accelerated ageing (SSAA) test results show lower viability for the

dried but not aged seeds from seed batch A1M2, providing further evidence that the drying and not the AST for ageing caused the damage.

The aged seed batches A2M1 and A2M2 were not used for further testing in the storability or shelf-life tests, since it was not the purpose of this study to test properties of seed with drying damages.

Normally, the aerated steam treatment gives improved viability of the seed lot. However, in these pre-tests, no improvement compared to untreated seeds was seen. The used seed lot of Ellvis was free from diseases; hence no sanitation effect can be seen in the pre-tests since there are no fungal pathogens to control on these seeds.

The data from these pre-tests were used for comparison with pre-tests performed on aged seed, discussed in section 4.3, “Shelf-life of pre-test”.

4.2 Determination of seed viability constants

A rapid ageing test (RA) was performed for seed batches A1M1 and A1M2, which was used to determine the seed viability constants in the Ellis and Roberts’s seed viability equation (1980a).

The curves of the RA tests are uneven, with measured some values that clearly deviate from the expected (figure 14). The values that deviate downwards are probably due to leaking bags causing increased moisture content of the seeds and thereby decreased heat tolerance. The machine used for sealing had a damaged sealing strip causing damaged seals. Unfortunately the same machine was used for the shelf-life of pre-tests test, but a different sealing machine was used for the storability test. The values deviating upwards can be explained by that some bags might not have been sufficiently immersed in the water bath, by temperature differences within the water bath (+- 0.3 °C was measured) or by uneven exposure of the bags to the warm water due to lack of space between the bags. These values were excluded from the mathematical and statistical analysis.

The straight lines adapted to the probit viabilities have R^2-values of 0.6887 and 0.707 (figure 15), showing that the decrease of probit percentage viability is not linear, meaning that the distribution if seed death in time is not normally distributed. It is likely that all bags had a slight leak, which can explain the deviation from normal distribution. Increasing moisture content during the rapid ageing would have accelerated the ageing, giving a faster viability decrease towards the end of the test period. Difficulties to fit a straight line to the curve have also been experienced by Tang *et al*. (1999b), indicating that the assumption of normal distributions of all seed lots may not be valid. To achieve more accurate viability predic-

tions, the RA test could be repeated with tighter seals and with more samples in the region close to P_{50}. This would provide a new set of viability data that could be normally distributed.

Despite of the less good fit of the lines in this test, the K_i and σ obtained was used for further calculations. The correlation between natural storage and RA, and predictions of storage longevity in natural storage conditions based on these calculations should be seen as very rough estimates. The RA test does also give tools for relative comparison between the seed batches, showing the greater relative storability of seed batch A1M2 compared to A1M1, which can be seen in many of the tests.

A disadvantage of RA is the difficulty in relating the results to natural ageing. Several methods have been discussed in this study for relating the viability losses caused by artificial ageing to the losses caused by natural ageing. They should all be seen as values for rough predictions, and few long-time studies have been made to evaluate them. Differences in the changes in other properties apart from viability, as chemical and physical properties, between artificial and natural ageing is much less researched. Galleschi *et al*. (2002) showed that the changes in biochemical content between seeds aged with Traditional accelerated ageing compared to natural ageing had different rates of the studied metabolites at the same viability.

The original seed viability equation (Ellis & Roberts, 1980a) was used for determining the seed viability constants, without any of the modifications discussed in section 1.6.5, “Modifications of the seed viability equations”. The reliability of the assumptions underlying the model and the seed longevity predictions based on it has been questioned, however no more precise alternative for predicting wheat viability was found in the literature. The model was developed on the closely related crop barley, and has been used previously on wheat. Generally, the tests to determine the viabilities of the aged seeds are filter paper test, which allows even weak and damaged seeds to germinate. In these trials, the more severe soil germination test was used to determine viability, as this much better predicts field emergence for AST treated seeds (Forsberg, 2004a). A correlation test between 5 days filter paper germination test and 16 days soil germination test with RA treated seeds from this study showed a consistently lower germination for the soil germination test (not published). This will result in consistently lower figures for K_i and ν in this study, compared to studies where the germination is tested with viability tests.

The modification suggested by Mead and Grey (1999) using a control viability parameter to account for non-viable seeds in low viability seed lots, was not used due to the high viability of the seed lot. The seed viability equations as proposed

by Ellis and Roberts (1980a) have been reported to fail to predict viabilities after storage for seed lots with low viability (Tang *et al.*, 2000). Since all seed lots investigated in this study had high viability this would not apply here.

Full data sets were used, since use of truncated data would have left very few data points for calculation of viability constants, which would have resulted in very uncertain values for Ki and σ. The one-step fit suggested by Hay *et al*.(2003), would perhaps have been more suitable to use for the calculation of viability constants than the used two-step method described by Hay (2004). However, due to time constraints this was not done. If further analysis of the results of this study is performed, the one-step method could provide viability constants that would better predict the viability decrease of the seed batches.

Alternative models to predict storage, as described by Marcondes *et al.*, 2011; Sinicio, 2004; Tang *et al.*, 2000; Andreoli, 2007 were not used either. Many of the models have never been used on cereals, and only been used once for scientific purposes. The original seed viability equations have been widely used and concluded to accurately describe the deterioration in a variety of species.

Based on the results from the RA test, RA times corresponding to 0.5, 1.6 and 2.5 years in 10 °C were calculated. The choice of these storage times were based on the existing shelf-life for pre-test used in commercial AS treatment, 0.5 year. Storage times of the additional one and two years were chosen to simulate one and two years of over-carrying. Over-carrying more than two years cannot be expected for commercial wheat seeds lots. The storage temperature 10 °C is likely to be lower than the mean temperature of wheat seed. When stored in late summer, after harvesting and drying, the seeds have higher temperature, and the adaption to surrounding ambient temperature is slow. If over-carried, the seed will heated in the summer and cooled during the winter. Since the seed viability equations are developed for a constant environment, they will only roughly predict the deterioration in a changing environment. Attempts to use the model in a changing climate by modelling for 30 day intervals has been made by Fabrizius *et al.* (1999), thus failing to predict more than one year's over-carriage for corn. However, this approach would be interesting to test on the naturally aged seeds that will validate the results from this trial.

If the seeds are stored at other temperatures than 10 °C, e.g. in other countries or climates, the storage longevity of the seeds will be different. Knowledge of the relationship between AST intensity and storability will make it possible to fine-tune the AST to optimize it for both sanitation efficacy and sufficient storage longevity.

Based on the seed viability parameters from the RA test, the ageing in the cold chamber during the study could be calculated. Between the pre-tests performed in October until the storability tests in January, both seed batches A1M1 and A1M2 had estimated viability losses of 0.1%. This decrease is considered negligible since it is much smaller than the variation between the pots from the same treatment in the soil germination tests.

To address the difficulties in relating artificial ageing to natural ageing, as well as the bad linear fit of the RA test, validation of the results by natural ageing will be performed. Both AST treated and un-treated seeds will be aged in natural storage environment, and the viability will be tested regularly. The validation needs to be done before the results of the study can be implemented in the commercial AST treatment, since commercial seed lots represent a large economic value. The predictions of storage longevity based on the RA test and seed viability equations have are also compared to the results from the SSAA test. The SSAA test on the seeds gives a relative comparison between the seed lots, and LABOSEM also relates the test results to predicting the risk of viability loss after a half year in conventional storage environment. The results show roughly the same as the seed viability equation predictions.

Comparing the estimated viabilities based on them with the actual viabilities obtained in the shelf-life test and storability test, it is clear that the estimated viabilities calculated with K_i and σ from the RA test does not deviate much from the actual viabilities. It shows that seed viability data from RA tests can roughly predict storage longevity despite bad fit. The rate of the deterioration is slightly overestimated, which makes the prediction of storage maximal storage time before the seed lot has lower than 85% viability to short. If the C_W and K_E constants calculated by Ellis *et al.* (1990) are used, the deviation from actual viabilities is much larger for A1M1 but not for A1M2. The difference between the K_E and C_W values from Ellis *et al.* (1990) compared to the values obtained in this study proves that these parameters are not species specific as originally suggested by, but seed lot specific. C_W and K_E values are obtained from the difference of viability between seed lots with different moisture contents, thus the different methods to measure germination between their study and this study should not affect the values.

4.3 Shelf-life of pre-tests

The shelf-life test was performed with seed batches A1M1 and A1M2, and showed that the AST tolerance decreases the longer the seeds were aged artificially with RA. The heat deteriorates the seed, as previous research show, thereby lowering

the tolerance to stress such as high temperatures, i.e. lower vigour. The RA treatment has however not lowered the viability of the seed, since all treatments except A1M1 74.5 h have maintained their initial viability. This deterioration will thus not be detected by a germination test of the untreated seeds after storage. To detect these changes in AST tolerance, a new pre-test needs to be made prior to treatment.

The analysis of LD and P values reveal very strong linear relationships between AST tolerance and RA treatment times. This answers Objective 1; how the AST tolerance is affected by storage prior to the treatment. By translating the RA treatment times to storage in conventional storage environment for 0, 0.5, 1.5 and 2.5 respectively, based on the seed viability equations, we can discuss shelf-life for the AST pre-test. The AST tolerance decreases by time for all investigated seed lots, and a year of storage decreases $LD_{0.1}$ AST tolerance for these seed lots with 0.62-0.85 kJ/m^3. The decrease of AST tolerance can be measured by LD or P values, giving a linear decrease with high R^2 fit.

If these relationships can be validated by pre-tests of naturally aged seeds from several seed lots, these relationships can be used to predict AST tolerance changes after natural storage. A pre-test on newly harvested seed and one or more pre-test on the same seed lot after RA treatment or natural ageing can be used to calculate the linear relationship, thus optimal AST intensity can be predicted for any storage time. However, this is only valid for constant storage without pests that could decrease viability. All R^2 values are high, indicating that very few data points (storage times) are needed for finding an accurate equation, but since seed lots represents large economic value, several pre-tests forming a more reliable relationship is preferred.

The nature of the relationship between age and AST tolerance can also be different for other seed lots. If the pre-test performed on a seed lot show a rapidly decreasing viability already at low AST intensities in the pre-test, this indicates that the seed lot is not suitable for long-time storage even if a recipe providing 85% viability or more can be found. The low tolerance to low AST intensities can be the result of a fast ageing process or damages on the seed that will give low storage longevity.

The σ value has a weak but positive correlation with RA treatment time, indicating an increased σ after storage. This means that the seed lot has less uniform behaviour after longer storage periods which can be explained by that the seed lot approaches the mean lifespan after storage, and both the age and the treatment will kill the seeds.

The water content increased during the treatment, probably due to leaking seals of the plastic bags that the seeds were kept in. This might have caused an even more accelerated ageing than intended. It would also cause a lowered AST tolerance, since moister seeds have lower heat tolerance. Therefore, the relationship between LD- and P-values and moisture content was investigated. Since these relationships were very weak, it can be concluded that the changes in LD and P-values most likely were caused by the deterioration by the heat, as intended in RA. Regardless of this, the changed moisture content of the seeds did probably play a small role in the changed tolerance. If there was more time available for this project, these tests would be repeated.

Why the initial viability of A1M1 74.5 h is so low compared to the other A1M1 treatments is not clear, and the 10 % viability decrease cannot be explained by increasing moisture content. Further testing is needed. Thus, the LD-values from this treatment fit with the other RA treatments of the same seed batch, indicating that the low initial viability might be a true effect of ageing.

Based on the linear decrease of AST tolerance with age, it is better to calculate a new optimal treatment intensity based on the storage time between the pre-test and AST treatment, than to decide a shelf-life for the pre-tests. The rate of AST tolerance decrease is larger the first 0.5 years than the following 2 years compared to the pre-test of un-aged seed, thus indicating that extra caution should be taken when the AST treatment intensity is set on seeds stored a half year between pre-test and AST treatment, since the tolerance will have decreased more than the equation based on the 0-2.5 year interval will predict. This must however be validated on other seed lots and species before commercial implementation.

4.4 Storability of seeds treated with aerated steam

The AST intensities used for treating the seeds in the storability trial was chosen based on the LD values from pre-tests on non-aged seeds described in table 4. Since it is essential for the success of AST to not cause a large viability reduction, the LD values show how high intensities that can be used without large damages to the seed lot.

The best way of interpreting these results, is therefor by relating the AST intensities to the LD values for the non-RA-treated seeds, since these LD values describes the AST tolerance of fresh seeds. Seed batch A1M2 was treated with higher AST intensity compared to the $LD_{0.1}$ values, compared to the seed batch A1M1 AST intensities.

However, the A1M2 seed batch was capable to maintain viability above 85% up to 48 h RA for AST recipes H and J, equivalent to 1.6 years of storage in 10 °C, which was superior to the storability of the A1M1 seeds. The seed from both seed batches treated with the other intensities only maintains >85% viability for 15h, equivalent to 0.5 years in 10 °C. Drier seeds can both tolerate higher treatment intensities, and maintain viability after treatment for longer storage periods. This answers Objective 2 in this study, how the viability is affected by storage after AS treatment.

The lower viability after SSAA for AS treated A1M2 seeds compared to seeds AS treated seeds from A1M1 is most like caused by the drying. During the SSAA treatment, the seeds increase the moisture content, thus eliminating the storability advantage the drier seeds have. For storage in conventional storage, the low moisture content is predicted by the SSAA test to give the seed much improved storage longevity.

In both the RA tests and the SSAA test, the AST recipe J intensity performed better than recipe H in seed batch A1M2. It is very unlikely that these seed, treated with higher intensity, should have higher storability. Most likely, these have been mixed up after the AST, probably by incorrect labelling. New AST with the same intensities will be performed on the seeds that will be naturally aged, which should clarify this anomaly.

The data set from this trial is too small to calculate a general prediction model for storability of AS treated seeds, as done on the pre-test shelf-life. However, decreasing viability after storage can easily be detected by a viability test, preferably performed in soil since it better predicts field emergence compared to filter paper tests. A number of viability tests on natural aged seeds from the investigated seed lot will validate the results from this study. For commercial use, continuous viability testing of AST treated seeds will detect seed lots with high viability and low rate of viability decrease that can be stored for longer periods.

According to the seed viability equations, the seed moisture affects the storability much more than the seed temperature. The AST treatments performed increased the moisture content of the seeds with 0.5-1.0 percentages. Since 13% m.c. is recommended for seeds that are over-carried and AS treatment increases the moisture content with 0.5- 1%, seeds which are planned to be over-carried should not have moisture contents above 12-12.5 % before AST treatment.

5 Conclusion

The overall objective of this study has been to clarify the interdependence between seed age and aerated steam treatment (AST) intensities.

Regarding the shelf-life of the pre-tests that measures AST tolerance of a seed lot; it has been found that the older the seeds are, the lower the AST intensity tolerance of the seed. Further this relationship has been shown to be linear. Based on these findings, there are no advantages of deciding for a shelf-life of the pre-tests. The decrease in AST tolerance after longer storage periods should instead be taken into account when the AST intensity is chosen for a seed lot.

It is also shown that the AST-treated seeds in this study deteriorate faster than the untreated seeds. The closer to $LD_{0.1}$ intensity the AST intensity is, the lower the storability. Drier seed can tolerate higher AST intensities and intensities closer to $LD_{0.1}$ than moister seeds without large viability losses. Based on this study, all AS treated seeds maintain a high viability after 0.5 years storage in 10 °C. Since predictions made with the seed viability equations overestimate the deterioration compared to actual viabilities measured in this study, it is likely that the seeds can be stored for longer periods. Validation of the deterioration rate by viability testing of naturally aged seeds will validate these results.

AST increases the moisture content (m.c.) of the seeds in this study with 0.5-1.0 percentage. Since 13 % m.c. or less is recommended to over-carried seed, seeds that are to be over-carried should have a moisture content of 12-12.5 % or less prior to AST. The moisture increase could also be addressed by prolonged drying as a part of the AST.

If a seed lot is planned to be stored for longer times, it is better to perform AST at the end of the storage period than prior to the storage. Since AST exposes the seed for both heat and moisture, which accelerates the ageing, such treatment should be performed as late as possible. However, the seed-borne diseases do not suffer the same decrease in AST tolerance with age (Forsberg, 2004b), so in order to treat at sufficiently high temperatures, this should be done before the seed lot is

too old to ensure sufficient sanitation. In Forsberg *et al*. (2004b), the seeds were stored in approximately 20 °C for up to 6 years, so this effect is not expected to be as big in conventional storage environments.

Validation of the results from this study will be performed by natural ageing of AS treated and untreated seeds from the seed batches used in this trial at Uppsala in outdoor ambient conditions. Pre-tests and soil germination tests will be performed on the untreated seed and soil germination tests on the AS treated seed, as in this study. The temperature changes will be measured with a temperature log. To examine whether the findings from this study are generalizable to other seed lots and crops, the corresponding storage tests should be performed on a variety of seed material. The same approach should be taken to test if the results from this study apply in other climates as well.

References

Akhter F. N., Kabir G., Mannan M. A. & Saheen N. N. (1992). Ageing effect of wheat and barley seeds upon germination, mitotic index and chromosomal damage cytology. J. Islamic Acad. Sci. 5(1), 44-48.

Andreoli, C. & De Andrade, R.V. (2007). Seed longevity chart to predict viability of corn seed during open storage. Revista Brasiliera de Milho et Sorgo 6(2), 247-255.

Baskin, C.C. (1981). Accelerated aging test. In: *Handbook of vigour test methods*, ed: Perry, D.A., pp. 43-48. The international seed testing association, Switzerland.

Bennett, M.A., Grassbaugh, E.M., Evans, A.F. & Kleinhenz, M.D. (2004). Saturated salt accelerated aging (SSAA) and other vigor tests for vegetable seeds. In: Guerke, W.R. (Ed.) *Seed Technology*. pp. 67-74; 26). ISBN 1096-0724.

Copeland, L. O. & McDonald M. B. (2001). *Principles of seed science and technology*, 4th ed. ISBN: 0792373227. Kulwer academic publishers, London

Delouche, J.C. & Baskin, C.C. (1973). Accelerated aging techniques for predicting the relative storability of seed lots. *Seed Science and Technology* 1(2), 427-452.

Demir, I., Kenanoglu, B.B., Mavi, K., Celikkol, T., Hay, F. & Sariyildiz, Z. (2009). Derivation of Constants (K(E), C(W)) for the Viability Equation for Pepper Seeds and the Subsequent Test of Its Applicability. *Hortscience* 44(6), 1679-1682.

Dickie, J.B., Ellis, R.H., Kraak, H.L., Ryder, K. & Tompsett, P.B. (1990). Temperature and seed storage longevity. *Annals of Botany* 65(2), 197-204.

Ellis, R.H. & Roberts E.H. (1979). Towards a rational basis for seed testing quality. In: Hebblewaithe, P.D. (Ed.) *Seed Production*. Pp. 605-635. Butterworths, London

Ellis, R.H. & Roberts, E.H. (1980a). Improved equations for the prediction of seed longevity. *Annals of Botany* 45(1), 13-30.

Ellis, R.H. & Roberts, E.H. (1980b). Influence of temperature and moisture on seed viability period in barley (Hordeum-distichum L). *Annals of Botany* 45(1), 31-37.

Ellis, R.H. & Roberts, E.H. (1981). The quantification of aging and survival in orthodox seeds. *Seed Science and Technology* 9(2), 373-409.

Ellis, R.H., Hong, T.D., Roberts, E.H. & Tao, K. (1990). Low moisture-content limits to relations between seed longevity and moisture. *Annals of Botany* 65(5), 493-504.

Fabrizius, E., TeKrony, D., Egli, D.B. & Rucker, M. (1999). Evaluation of a viability model for predicting soybean seed germination during warehouse storage. *Crop Science* 39(1), 194-201.

Filho, C.P. & Ellis, R.H. (1992). Estimating the value of the seed lot constant (Ki) of the seed viability equation in barley and wheat. *Seed Science and Technology* 20(1), 93-99.

Forsberg, G. (2004a). Control of cereal seed-borne diseases by hot humid air seed treatment. Diss. Uppsala : Sveriges lantbruksuniv., Acta Universitatis agriculturae Sueciae. Agraria, 1401-6249 ; 443 ISBN 91-576-6496-X

Forsberg, G., Johansson, L. & Gerhardsson, B. (2004b). Seed age influence on efficiency of seed sanitation by areated steam treatment. *In:* Forsberg, G. (2004a). Control of cereal seed-borne diseases by hot humid air seed treatment. Diss. Uppsala : Sveriges lantbruksuniv., Acta Universitatis agriculturae Sueciae. Agraria, 1401-6249 ; 443 ISBN 91-576-6496-X

Forsberg, G., Johnsson, L. & Lagerholm, J. (2005). Effects of aerated steam seed treatment on cereal seed-borne diseases and crop yield. *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection* 112(3), 247-256.

Forsberg, G., Kristensen, L., Eibel, P., Titone, P. & Hartl, W. (2003). Sensitivity of cereal seeds to short duration treatment with hot, humid air. *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection* 110(1), 1-16.

Forsberg, Gustaf. Product manager ThermoSeed™, Incotec Sweden. Personal communication, (2012).

Galleschi, L., Capocchi, A., Ghiringhelli, S. & Saviozzi, F. (2002). Antioxidants, free radicals, storage proteins, and proteolytic activities in wheat (Triticum durum) seeds during accelerated aging. *Journal of Agricultural and Food Chemistry* 50(19), 5450-5457.

Hay, F.R. (2004). *The seed viability equations*. Seed conservation department, Kew Royal botanical gardens. Available: http://data.kew.org/sid/viability/SeedViabilityEquationsFHDec04.pdf [2012-01-17]

Hay, F.R., Mead, A., Manger, K. & Wilson, F.J. (2003). One-step analysis of seed storage data and the longevity of Arabidopsis thaliana seeds. *Journal of Experimental Botany* 54(384), 993-1011.

Hong, T.D. & Ellis, R.H. (1996). A protocol to determine seed storage behaviour. IPGRI Technical Bullentin, 1. ISBN 92-9043-279-9 Available: http://www.cbd.int/doc/case-studies/tttc/SeedStorage.pdf 2011-11-03

Incotec. (2011). ThermoSeed™ the cleanest seed in the world. [Brochure]

Khan, A.Z., Shah, P., Mohd, F., Khan, H., Amanullah, S.P., Nigar, S., Khalil, S.K. & Zubair, M. (2010). Vigor tests used to rank seed lot quality and predict field emergence in wheat. *Pakistan Journal of Botany* 42(5), 3147-3155.

Krishnan, P., Nagarajan, S. & Moharir, A. (2004). Thermodynamic characterisation of seed deterioration during storage under accelerated ageing conditions. *Biosystems Engineering* 89(4), 425-433.

Marcondes, M.C., Andreoli, C. & Miglioranza, E. (2011). Viability equation to determine the longevity of fungicide-treated seeds of wheat stored in a conventional warehouse. *Acta Scientiarum-Agronomy* 33(3), 539-544.

Mead, A. & Gray, D. (1999). Prediction of seed longevity: a modification of the shape of the Ellis and Roberts seed survival curves. *Seed Science Research* 9(1), 63-73.

Meriaux, B., Wagner, M.H., Ducournau, S., Ladonne, F. & Fougereux, J.A. (2007). Using sodium chloride saturated solution to standardize accelerated aging test for wheat seeds. *Seed Science and Technology* 35(3), 722-732.

Modarresi, R. & Van Damme, P. (2003). Application of the controlled deterioration test to evaluate wheat seed vigour. *Seed Science and Technology* 31(3), 771-775.

Modarresi, R., Rucker, M. & Tekrony, D.M. (2002). Accelerating ageing test for comparing wheat seed vigour. *Seed Science and Technology* 30(3), 683-687.

Pinzino, C., Nanni, B. & Zandomeneghi, M. (1999). Aging, free radicals, and antioxidants in wheat seeds. *Journal of Agricultural and Food Chemistry* 47(4), 1333-1339.

Powell, A.A. & Matthews, S. (1981). Evaluation of controlled deterioration, a new vigor test for small seeded vegetables. *Seed Science and Technology* 9(2), 633-640.

Probert, R.J., Daws, M.I. & Hay, F.R. (2009). Ecological correlates of ex situ seed longevity: a comparative study on 195 species. *Annals of Botany* 104(1), 57-69.

Roberts, E. H. (1986). Quantifying seed deterioration. p.101-123. In: M.B. McDonald, Jr.and C.J. Nelson (eds.), Physiology of seed deterioration. CSSA Special Pub. No.11. CSSA Inc. Madison WI.

Råsberg, A. (1998). Lagring och torkning av spannmål. In: Kvalitetsguide för ekologisk spannmål – avseende hygienisk kvalitet. Ed: Svantesson A, Sjelin K. Swedish board of agriculture.

Sapra, R.L., Narain, P., Bhat, S.R., Lal, S.K. & Jain, S.K. (2003). Prediction of seed longevity in the genebank: How reliable are the estimates? *Current Science* 85(11), 1612-1616.

Sinicio, R. (2004). Generalised longevity model for orthodox seeds. *Biosystems Engineering* 89(1), 85-92.

SJVFS. (2011) Föreskrifter om ändring i Statens jordbruksverks föreskrifter (SJVFS 1994:22) om certifiering m.m. av utsäde av stråsäd; beslutade den 27 oktober 2011 (SJVFS 2011:45). Jönköping.

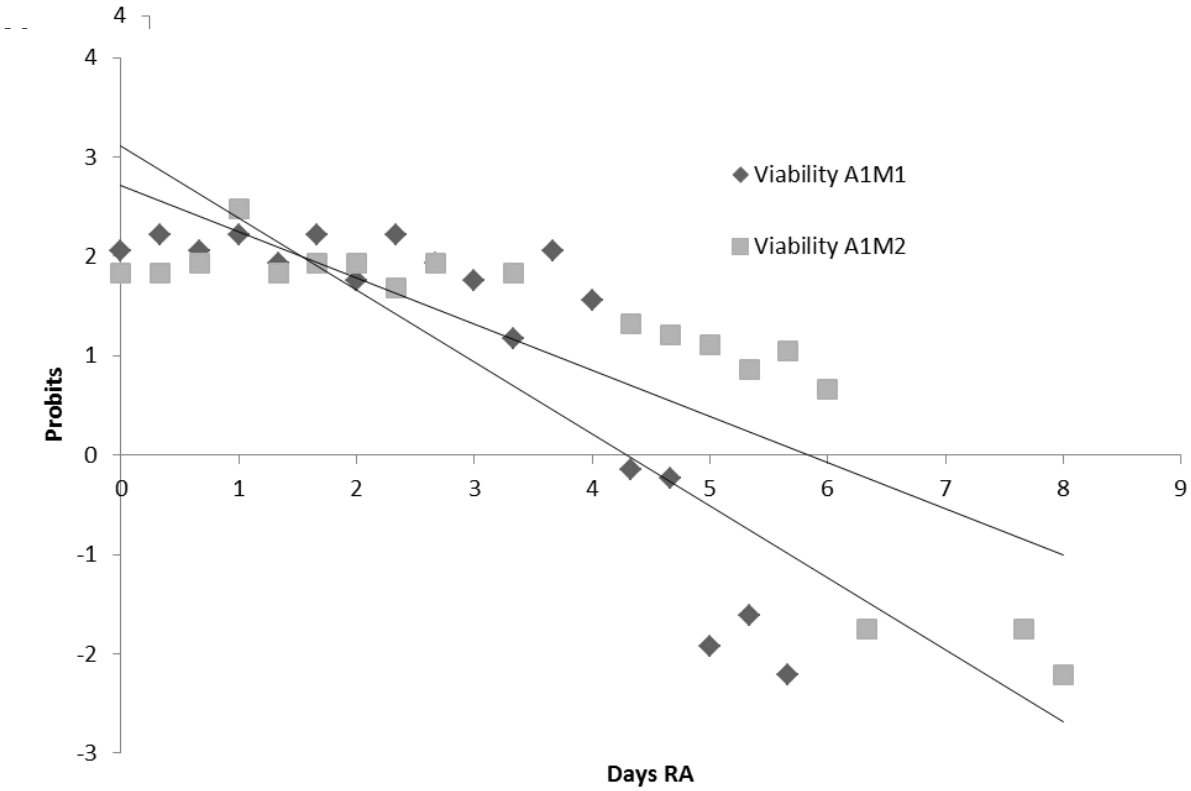
Stoyanova, S.D., Odzhakova, G.N. & Menkov, N.D. (2007). Drying of wheat seeds to low seed moisture for genebank storage. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 6(10), 2490-2499.

Tang, S.D., TeKrony, D.M., Egli, D.B. & Cornelius, P.L. (1999a). Survival characteristics of corn seed during storage: II. Rate of seed deterioration. *Crop Science* 39(5), 1400-1406.

Tang, S.D., TeKrony, D.M., Egli, D.B. & Cornelius, P.L. (2000). An alternative model to predict corn seed deterioration during storage. *Crop Science* 40(2), 463-470.

Tang, S.D., TeKrony, D.M., Egli, D.B., Cornelius, P.L. & Rucker, M. (1999b). Survival characteristics of corn seed during storage: I. Normal distribution of seed survival. *Crop Science* 39(5), 1394-1400.

Usberti, R., Roberts, E.H. & Ellis, R.H. (2006). Prediction of cottonseed longevity. *Pesquisa*



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Appendix A. Soil recipe (Swedish)

Eget recept — Hasselfors Garden AB

Kund, namn: **SEEDGARD AB**

Ort: **Uppsala**

Kundnr: **7712**

Användning: **Spannmålstester**

Datum	2006-05-17
Lev vecka:	21
Ordernr:	77858
Fabrik:	MOSÅS
Kopia av tidigare order nr	
Godkänt:	CSB / 060517
Godkänt per tel:	
Säljare:	AEH
Mängd, m3:	18
Artikelnummer:	2005
Antal säck(ca):	360
Antal pall:	10
Antal storsäck:	

Övrigt:

Text på säck:

Råvaror

Råvaror	Volyminnehåll	
Blocktorv		%
Ljus torv, normalsiktad	60	%
Ljus torv, finsiktad		%
Master 2 - 10 mm		%
Master 2 - 35 mm		%
Mörk torv		%
Svarttorv,grävd	25	%
Fibertorv		%
PeatNuggets		%
Sand 0,5 - 4 mm	15	%
Leca 2 - 6 mm		%
Perlit 0,5 - 6 mm		%
Bark 0 - 10 mm		%
Bark 10 - 40 mm		%
Kogödselkompost		%
Premiumtorv		%
Soft Peat Nuggets		%
		%
		%
		%
		%
Granulerad lera		kg

Tillsatser per kubikmeter

Pelleterad hönsgödsel		kg
Multimix/PG-mix, NPK 14-7-15	1,30	kg
Kalksalpeter, N 15		kg
Superfosfat, P 20		kg
Pro Magna NPK 11-5-18 mikro		kg
Multispor/FTE 36	0,050	kg
Järnsulfat		kg
Optifer		kg
Microsilica		kg
Vialgin		kg
Kalkstensmjöl	6,0	kg
Dolomitmjöl	2,0	kg
Gips, S 20		kg
Multicote mikro 4 mån		kg
Multicote mikro 8 mån		kg
Kaliumsulfat		kg
		kg
		kg
		kg
		kg

Version: CSB/051122

Dokumenterade kundkrav

Siktning: **Normal (Fin - Med)**

Fuktas: **Ingen tillsats**

6

d

Appendix B. Viability results per pot

Part of study	Seed batch	RA (h)	AST intensity		Germinated seeds		
			E (kJ/m3)	m (g H2O/m3)	Pot 1	Pot 2	Pot 3
In. Pretests	A1M1		N.T.	N.T.	47	46	49
In. Pretests	A1M1		N.T.	N.T.	50	48	48
In. Pretests	A1M1		58,1909	14,4648	49	50	50
In. Pretests	A1M1		58,7136	14,6179	49	49	48
In. Pretests	A1M1		59,2363	14,771	49	48	49
In. Pretests	A1M1		59,759	14,9241	49	45	50
In. Pretests	A1M1		60,2817	15,0772	48	50	49
In. Pretests	A1M1		60,8044	15,2303	48	50	50
In. Pretests	A1M1		61,3271	15,3834	50	48	50
In. Pretests	A1M1		61,8498	15,5365	49	49	50
In. Pretests	A1M1		62,3725	15,6896	48	47	48
In. Pretests	A1M1		62,8952	15,8427	44	50	50
In. Pretests	A1M1		63,4179	15,9958	42	48	40
In. Pretests	A1M1		63,9406	16,1489	36	33	34
In. Pretests	A1M1		64,4633	16,302	13	11	9
In. Pretests	A1M1		64,986	16,4551	0	1	0
In. Pretests	A1M2		N.T.	N.T.	46	50	50
In. Pretests	A1M2		57,6682	14,3117	48	50	50
In. Pretests	A1M2		58,1909	14,4648	48	50	49
In. Pretests	A1M2		58,7136	14,6179	48	49	48
In. Pretests	A1M2		59,2363	14,771	50	48	50
In. Pretests	A1M2		59,759	14,9241	48	49	50
In. Pretests	A1M2		60,2817	15,0772	50	50	49
In. Pretests	A1M2		60,8044	15,2303	50	48	49
In. Pretests	A1M2		61,3271	15,3834	50	49	50
In. Pretests	A1M2		61,8498	15,5365	50	49	47
In. Pretests	A1M2		62,3725	15,6896	49	47	50
In. Pretests	A1M2		62,8952	15,8427	48	48	47

In. Pretests	A1M2	63,4179	15,9958	48	44	48
In. Pretests	A1M2	63,9406	16,1489	46	45	47
In. Pretests	A1M2	64,4633	16,302	16	20	9
In. Pretests	A1M2	64,986	16,4551	3	1	2
In. Pretests	A1M2	65,5087	16,6082	0	1	0
In. Pretests	A1M2	66,0314	16,7613	0	0	0
In. Pretests	A1M2	66,5541	16,9144	0	0	0
In. Pretests	A1M2	67,0768	17,0675	0	0	0
In. Pretests	A2M1	N.T.	N.T.	50	46	45
In. Pretests	A2M1	58,1909	14,4648	41	44	44
In. Pretests	A2M1	58,7136	14,6179	43	44	42
In. Pretests	A2M1	59,2363	14,771	43	46	43
In. Pretests	A2M1	59,759	14,9241	43	45	46
In. Pretests	A2M1	60,2817	15,0772	46	41	43
In. Pretests	A2M1	60,8044	15,2303	41	42	41
In. Pretests	A2M1	61,3271	15,3834	41	46	43
In. Pretests	A2M1	61,8498	15,5365	34	49	40
In. Pretests	A2M1	62,3725	15,6896	42	37	43
In. Pretests	A2M1	62,8952	15,8427	34	49	43
In. Pretests	A2M1	63,4179	15,9958	37	36	35
In. Pretests	A2M1	63,9406	16,1489	22	20	19
In. Pretests	A2M1	64,4633	16,302	10	5	7
In. Pretests	A2M1	64,986	16,4551	1	1	1
In. Pretests	A2M2	N.T.	N.T.	42	42	38
In. Pretests	A2M2	57,6682	14,3117	39	38	37
In. Pretests	A2M2	58,1909	14,4648	40	42	39
In. Pretests	A2M2	58,7136	14,6179	37	38	40
In. Pretests	A2M2	59,2363	14,771	35	39	37
In. Pretests	A2M2	59,759	14,9241	41	43	35
In. Pretests	A2M2	60,2817	15,0772	44	38	42
In. Pretests	A2M2	60,8044	15,2303	39	39	41
In. Pretests	A2M2	61,3271	15,3834	36	37	36
In. Pretests	A2M2	61,8498	15,5365	41	37	31
In. Pretests	A2M2	62,3725	15,6896	35	42	43
In. Pretests	A2M2	62,8952	15,8427	38	26	26
In. Pretests	A2M2	63,4179	15,9958	26	24	25
In. Pretests	A2M2	63,9406	16,1489	17	16	13
In. Pretests	A2M2	64,4633	16,302	0	3	0
In. Pretests	A2M2	64,986	16,4551	0	0	0
In. Pretests	A2M2	65,5087	16,6082	0	0	0

In. Pretests	A2M2		66,0314	16,7613	0	0	0
In. Pretests	A2M2		66,5541	16,9144	0	0	0
In. Pretests	A2M2		67,0768	17,0675	0	0	0
Viab. const	A1M1	0			50	48	49
Viab. const	A1M1	8			50	49	49
Viab. const	A1M1	16			49	48	50
Viab. const	A1M1	24			49	49	50
Viab. const	A1M1	32			49	48	49
Viab. const	A1M1	40			50	49	49
Viab. const	A1M1	48			48	47	49
Viab. const	A1M1	56			49	50	49
Viab. const	A1M1	64			49	48	49
Viab. const	A1M1	72			50	47	47
Viab. const	A1M1	80			46	43	43
Viab. const	A1M1	88			48	49	50
Viab. const	A1M1	96			43	49	49
Viab. const	A1M1	104			24	17	25
Viab. const	A1M1	112			22	17	22
Viab. const	A1M1	120			0	2	2
Viab. const	A1M1	128			3	3	2
Viab. const	A1M1	136			2	0	0
Viab. const	A1M1	144			0	0	0
Viab. const	A1M1	152			10	15	12
Viab. const	A1M1	160			0	0	0
Viab. const	A1M1	168			2	5	3
Viab. const	A1M1	176			0	0	0
Viab. const	A1M1	184			0	0	0
Viab. const	A1M1	192			0	0	0
Viab. const	A1M1	200			0	0	0
Viab. const	A1M1	208			0	0	0
Viab. const	A1M1	216			0	0	0
Viab. const	A1M1	224			0	0	0
Viab. const	A1M1	232			0	0	0
Viab. const	A1M2	0			50	45	50
Viab. const	A1M2	8			48	49	48
Viab. const	A1M2	16			49	49	48
Viab. const	A1M2	24			50	49	50
Viab. const	A1M2	32			49	47	49
Viab. const	A1M2	40			49	48	49
Viab. const	A1M2	48			47	50	49

Viab. const	A1M2	56			48	48	47
Viab. const	A1M2	64			48	49	49
Viab. const	A1M2	72			40	36	45
Viab. const	A1M2	80			49	48	48
Viab. const	A1M2	88			34	27	26
Viab. const	A1M2	96			35	26	27
Viab. const	A1M2	104			43	46	47
Viab. const	A1M2	112			47	41	45
Viab. const	A1M2	120			45	44	41
Viab. const	A1M2	128			40	42	39
Viab. const	A1M2	136			39	47	42
Viab. const	A1M2	144			38	35	39
Viab. const	A1M2	152			5	0	1
Viab. const	A1M2	160			0	0	0
Viab. const	A1M2	168			6	7	2
Viab. const	A1M2	176			0	0	0
Viab. const	A1M2	184			3	0	3
Viab. const	A1M2	192			1	1	0
Viab. const	A1M2	200			0	0	0
Viab. const	A1M2	208			0	0	0
Viab. const	A1M2	216			0	0	0
Viab. const	A1M2	224			0	0	0
Viab. const	A1M2	232			0	0	0
Shelf-life	A1M1	15	N.T.	N.T.	49	49	45
Shelf-life	A1M1	15	58,7136	14,6179	50	46	49
Shelf-life	A1M1	15	59,2363	14,771	47	48	48
Shelf-life	A1M1	15	59,759	14,9241	48	49	47
Shelf-life	A1M1	15	60,2817	15,0772	47	49	47
Shelf-life	A1M1	15	60,8044	15,2303	45	45	41
Shelf-life	A1M1	15	61,3271	15,3834	13	12	11
Shelf-life	A1M1	15	61,8498	15,5365	0	0	0
Shelf-life	A1M1	48	N.T.	N.T.	48	50	50
Shelf-life	A1M1	48	58,7136	14,6179	50	46	48
Shelf-life	A1M1	48	59,2363	14,771	47	49	45
Shelf-life	A1M1	48	59,759	14,9241	47	47	47
Shelf-life	A1M1	48	60,2817	15,0772	48	49	48
Shelf-life	A1M1	48	60,8044	15,2303	31	38	42
Shelf-life	A1M1	48	61,3271	15,3834	18	6	9
Shelf-life	A1M1	48	61,8498	15,5365	0	0	0
Shelf-life	A1M1	74,5	N.T.	N.T.	42	45	44

Shelf-life	A1M1	74,5	58,7136	14,6179	45	47	39
Shelf-life	A1M1	74,5	59,2363	14,771	39	45	43
Shelf-life	A1M1	74,5	59,759	14,9241	44	42	40
Shelf-life	A1M1	74,5	60,2817	15,0772	34	39	38
Shelf-life	A1M1	74,5	60,8044	15,2303	15	21	14
Shelf-life	A1M1	74,5	61,3271	15,3834	2	0	0
Shelf-life	A1M1	74,5	61,8498	15,5365	0	0	0
Shelf-life	A1M2	15	N.T.	N.T.	49	49	48
Shelf-life	A1M2	15	58,7136	14,6179	48	49	50
Shelf-life	A1M2	15	59,2363	14,771	46	49	50
Shelf-life	A1M2	15	59,759	14,9241	47	49	49
Shelf-life	A1M2	15	60,2817	15,0772	49	49	50
Shelf-life	A1M2	15	60,8044	15,2303	49	49	45
Shelf-life	A1M2	15	61,3271	15,3834	30	32	29
Shelf-life	A1M2	15	61,8498	15,5365	0	0	0
Shelf-life	A1M2	48	N.T.	N.T.	48	45	50
Shelf-life	A1M2	48	58,7136	14,6179	50	48	48
Shelf-life	A1M2	48	59,2363	14,771	50	47	49
Shelf-life	A1M2	48	59,759	14,9241	49	49	49
Shelf-life	A1M2	48	60,2817	15,0772	49	47	50
Shelf-life	A1M2	48	60,8044	15,2303	40	46	44
Shelf-life	A1M2	48	61,3271	15,3834	14	8	10
Shelf-life	A1M2	48	61,8498	15,5365	0	0	0
Shelf-life	A1M2	74,5	N.T.	N.T.	48	50	50
Shelf-life	A1M2	74,5	58,7136	14,6179	50	50	49
Shelf-life	A1M2	74,5	59,2363	14,771	50	49	49
Shelf-life	A1M2	74,5	59,759	14,9241	47	49	47
Shelf-life	A1M2	74,5	60,2817	15,0772	46	46	49
Shelf-life	A1M2	74,5	60,8044	15,2303	42	42	37
Shelf-life	A1M2	74,5	61,3271	15,3834	1	2	4
Shelf-life	A1M2	74,5	61,8498	15,5365	0	0	0
Storability	A1M1	0	N.T.	N.T.	49	48	47
Storability	A1M1	0	60,2817	15,0772	47	49	48
Storability	A1M1	0	61,3271	15,3834	48	49	49
Storability	A1M1	0	62,3725	15,6896	47	47	48
Storability	A1M1	15	N.T	N.T	48	49	49
Storability	A1M1	15	60,2817	15,0772	48	45	47
Storability	A1M1	15	61,3271	15,3834	50	47	47
Storability	A1M1	15	62,3725	15,6896	48	47	48
Storability	A1M1	48	N.T	N.T	48	48	46

Storability	A1M1	48	60,2817	15,0772	43	40	38
Storability	A1M1	48	61,3271	15,3834	37	38	38
Storability	A1M1	48	62,3725	15,6896	16	29	28
Storability	A1M1	74,5	N.T	N.T	40	44	44
Storability	A1M1	74,5	60,2817	15,0772	6	3	2
Storability	A1M1	74,5	61,3271	15,3834	1	3	4
Storability	A1M1	74,5	62,3725	15,6896	0	0	1
Storability	A1M2	0	N.T	N.T	50	50	49
Storability	A1M2	0	61,3271	15,3834	50	50	49
Storability	A1M2	0	62,3725	15,6896	48	49	46
Storability	A1M2	0	63,4179	15,9958	48	49	48
Storability	A1M2	15	N.T	N.T	49	47	50
Storability	A1M2	15	61,3271	15,3834	50	50	48
Storability	A1M2	15	62,3725	15,6896	50	49	50
Storability	A1M2	15	63,4179	15,9958	47	47	46
Storability	A1M2	48	N.T	N.T	50	49	49
Storability	A1M2	48	61,3271	15,3834	49	50	49
Storability	A1M2	48	62,3725	15,6896	50	47	49
Storability	A1M2	48	63,4179	15,9958	38	38	36
Storability	A1M2	74,5	N.T	N.T	49	49	47
Storability	A1M2	74,5	61,3271	15,3834	32	35	36
Storability	A1M2	74,5	62,3725	15,6896	45	42	39
Storability	A1M2	74,5	63,4179	15,9958	18	9	14

RA = rapid ageing
AST = aerated steam treatment
N.T = not treated seeds

Appendix C. Results from SSAA test (French)

RESULTATS DE TEST DE VIEILLISSEMENT ACCELERÉ

Brain sur l'Authion, le 20-févr.-12

Etabli à l'intention de : Maria SCHÖCK
Svalövsvägen 3
75671 UPPSALA
Sweden

Réf. Labosem :	D1171
Réf. Client :	
Contact client :	M. SCHÖCK
Nbre d'échantillon :	8
Espèce :	Blé tendre

Vieillissement accéléré : méthode
Effectif de 200 graines
75% d'humidité relative (utilisation de NaCl)
Bain Marie : 45°C
7 jours

Faculté germinative : méthode
Effectif : 200 graines
Substrat : sable
Prérefrigeration : non
Température : 20°C
Durée : 7 jours

Réf. Labosem	Référence établissement	Humidité Ets	Humidité Labosem	Faculté Germinative après vieillissement accéléré			Risque perte FG >10% d'ici à fin juin 2012
				Germes normaux (%)	Germes anormaux (%)	Semences non germées (%)	
101	1	13,9	13,5	65	18	17	faible
102	2	14,7	14,3	59	23	18	moyen
103	3	14,8	14,4	50	27	23	élevé
104	4	14,9	14,5	28	38	34	très élevé
105	5	11,9	11,4	71	12	17	faible
106	6	12,9	12,5	28	24	48	moyen
107	7	13	12,6	40	33	27	faible
108	8	13	12,7	12	49	39	très élevé

L'interprétation du risque de perte de faculté germinative prend en compte la teneur en eau de stockage de l'etablissement (cf.verso)

D. ROUSSEAU
Service Expérimentation

LABOSEM - Laboratoire d'analyses de Semences - Impasse du Verger - 49800 BRAIN sur L'AUTHION
Tél : 02.41.68.93.50 - Fax : 02.41.54.99.49 - contact@labosem.fr

RESULTATS DE TEST DE VIEILLISSEMENT ACCELERÉ

Brain sur l'Authion, le 20-févr.-12

Etabli à l'intention de : Maria SCHÜCK
Svalövsvägen 3
75671 UPPSALA
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Faculté germinative : méthode
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Substrat : sable
Prérréfrigeration : non
Température : 20°C
Durée : 7 jours

Réf. Labosem	Référence établissement	Humidité Ets	Humidité Labosem	Faculté Germinative après vieillissement accéléré			Risque perte FG >10% d'ici à fin juin 2012
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106	6	12,9	12,5	28	24	48	moyen
107	7	13	12,6	40	33	27	faible
108	8	13	12,7	12	49	39	très élevé

L'interprétation du risque de perte de faculté germinative prend en compte la teneur en eau de stockage de l'etablissement (cf.verso)

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