Control of Cereal Seed-borne Diseases by Hot Humid Air Seed Treatment

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Abstract

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Treatment of cereal seed using hot, humid air, or aerated steam, was investigated as a method for control of seed-borne diseases.

The influence of important treatment parameters on the vitality of the seed and of the pathogen was determined. Optimum strategies of treatment time and air humidity were proposed for effective disinfestation with maintained seed viability. The process of heat and moisture transfer between the treatment air and the seed was clarified and it was concluded that quick heating with humid air for a short time, immediately followed by rapid cooling, gives a partly selective heating of external layers of the seed where most of the important cereal seed-borne pathogens are located. By taking the discussed physical relations into account, the improved viability equation of Ellis & Roberts was modified for prediction of post-treatment germinability and infestation rate with considerably reduced error.

Tolerance to high temperatures was tested for a large number of seed lots. Different species were differently influenced by high temperatures. Due to variations in growing and storage history among seed lots, individual lots differ in heat tolerance. Due to such factors, the tolerance to high temperatures varies also within seed lots. However, the optimum temperature for thermal treatment of a seed lot can be found by pre-testing procedures.

The influence of seed storage on the effect of the aerated steam treatment was investigated, both for treatments performed after storage and for seed stored after the treatment. Long-term storage of seed infested with pathogens persistent to storage reduced the disinfestation rate obtained from the treatment both when the seeds were stored before and after the treatment. When long-term storage is required before or after the treatment, the seeds should be stored at low temperature and at low moisture content.

The optimized treatment method was evaluated in extensive experiments in six European countries. It was concluded that the method is capable of controlling most cereal seed-borne diseases equally with chemical seed dressing, exceptions being those where the pathogen is located deeply within the seed. The method can also control many important seed-borne diseases on other crops.

Keywords: Heat treatment, pathogen, disinfestation, sanitization, aerated steam, heat transfer, storage, selectivity

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Résumé

Forsberg, G. 2004. Thermothérapie à la vapeur de semences céréalières contre les maladies séminicoles. Dissertation de doctorat.
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Le traitement de semences céréalières par de l'air chaud et humide a été défini comme un méthode pouvant combattre les maladies séminicoles.

L'influence de paramèters importants sur la vitalité de la semence et des champignons pathogènes a été déterminée. En optimisant la durée de traitement et l'humidité de l'air, la semence est désinfectée tout en préservant sa germination. L'analyse du transfert de chaleur et d'eau entre l'air de traitement et la semence montre que le chauffage rapide et court à la vapeur d'eau, immédiatement suivi d'un refroidissement rapide, donne lieu à un traitement sélectif des couches externes de la semence, d'où se trouve la majorité des microbes pathogènes. En prenant en compte ces relations physiques, l'équation améliorée de Ellis et Roberts a été modifiée pour précisément prédire la germination post-traitement et le taux d'infestation.

La tolérance aux températures élevées, testée sur de nombreux lots de semence, varie entre les éspèces testées. Dû aux variations des conditions de croissance de la plante et celles liées au stockage, cette tolérance diffère entre les lots individuels. Du fait de ces différents facteurs, la tolérance aux traitements thermiques varie également au sein d'un même lot. Il est néanmoins possible de trouver la température optimale d'un lot défini en effectuant un test de pré-traitement.

L'influence du stockage, sur l'efficacité du traitement thermique, a été examiné pour à la fois les traitements effectués après le stockage et le stockage post-traitement. Le stockage à long-terme de semences infestées de microbes pathogènes résistents au stockage a réduit le taux de désinfection obtenu par le traitement aussi bien pour les semences stockées avant ou après celui ci. Lorsqu'un stockage à long-terme est nécessaire avant ou après traitement, un stockage aux températures et teneurs en eau réduites des semences est recommendé.

L'évaluation étendue dans 6 pays européens du méthode optimisé a montré que le méthode peut combattre la plupart des maladies séminicoles céréalières équivalant aux traitements chimiques, à la seule exception près, lorsque le microbe pathogène est situé en profondeur de la graine. Le travail a montré que le méthode est aussi efficace pour d'autres cultures que les céréales.

Mots clé: Pathogène, désinfection, assainir, transfert de chaleur, stockage, sélectivité

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"Tout le mérite d'une bonne friture provient de la ***surprise***; c'est ainsi qu'on appelle l'invasion du liquide bouillant qui carbonise ou roussit, à l'instant même de l'immersion, la surface extérieure du corps qui lui est soumis."

("The whole merit of frying consists of the ***surprise***; for such is the name given to the sudden action of the boiling liquid which carbonizes or scorches the surface of the substance in question, at the very moment of its immersion.")

Jean-Anthelme Brillat-Savarin (1825a, b)

To Lovisa, Ludvig and Klara

Contents

Appendix

List of papers I – V

The present thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Forsberg, G., Andersson, S. & Johnsson, L. Evaluation of hot, humid air seed treatment in thin layers and fluidized beds for seed pathogen sanitation. *Journal of Plant Diseases and Protection, 109* (4), 357-370, 2002.

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

III. Forsberg, G., Johnsson, L. & Gerhardson, B. Seed age influence on efficiency of seed sanitation by aerated steam treatment. *Submitted (Seed Technology).*

IV. Forsberg, G., Johnsson, L. & Lagerholm, J. Effects of aerated steam seed treatment on seed-borne diseases and crop yield. *Submitted (Journal of Plant Diseases and Protection).*

V. Forsberg, G. Modified equation predicting seed germinability and infection rate after aerated steam seed treatment. (Manuscript).

1. Introduction

Seed-borne diseases are an important threat to crop yield and quality. At present, in the struggle to control these diseases, large quantities of cereal seed are treated by seed dressing with chemical pesticides. However, an extensive use of pesticides has been found to pose risks for pollution of the environment with sometimes well-known, sometimes poorly known consequences, not only for leaving residuals in food products but also for negative influences on the health of people regularly working with them. Another constraint is the development of pathogen resistance to commonly used chemical compounds. During recent decades, thus, there has been increasing public demand from consumers, politicians, environmental organisations, etc., for non-chemical methods of plant pest and disease control.

Especially in organic farming there is a demand for alternatives to chemicals. In lacking efficient methods for control of, particularly, seed-borne diseases, and when based on organically produced seed, organic farming faces serious problems. Because of this, only a small percentage of the certified seed (Agrista, 2004) is produced by following the rules of the EU for organic agriculture (EEG, 1991, modified by EEG, 1999).

One way of solving these problems is by using biological seed treatments, for example with bacterial antagonists (Hökeberg 1997; Gerhardson, 2002), which so far have been commercialized for various kinds of seeds in some countries, for cereals particularly by BioAgri AB (www.bioagri.se). Another possibility is to use physical methods, like washing-off fungal spores from seeds, proposed already in 1755 by Tillet (1755), or by different kinds of irradiation or thermal treatments (Maude, 1996).

The recently developed electron beam treatment (Burth *et al*., 1991) has been commercialized in Germany for treatment of organic cereal seed. Radioactive irradiation has also in a few cases been reported to be successful (Cuero *et al*., 1986; Bagegni *et al*., 1990), but has not been widely used because exposures sufficient to control pathogens often also kill the seeds. Even laser treatment has been reported to be effective (Bel'skii & Mazulenko, 1984), although since laser beams are narrow and the whole surface of the seed should be evenly exposed for good effect it is of limited practical interest.

Thermal seed treatment has been practically applied in different ways. A simple way of thermal treatment is solarization, where the seeds are heated by irradiation from the sun (Luthra & Sattar, 1934; Luthra, 1953), which is sometimes applied in warm countries, but is of little interest in industrial agriculture due to low precision and difficulties with large-scale application. Dry hot air has been developed for use against insects in grain stocks (Dermott & Evans, 1978; Evans *et al*., 1983; Thorpe *et al*., 1983; Thorpe, 1987) and is applied in Australia at capacities up to 150 tons/hour (Banks, 1998), but in most cases it has not shown good potential against fungal infections in seeds (Baker, 1969, 1972; Couture & Sutton, 1980).

Also hot water treatment has been used in practice for sanitization of seed from seed-borne diseases - for cereals since the beginning of the 20th century and up to

the 1960's (Tapke, 1924; Neergaard, 1977; Johnsson, 1990; Olvång, 2000). However, hot water treatment suffers from important problems: ***1.*** Being submerged first for a certain time in hot water followed by cooling in cold water, extensive post-treatment drying of the seed is required, and this is expensive due to the requirement of energy and special drying facilities. ***2.*** Handling of seeds during the process is often impractical since the wet seeds stick together and become more sensitive to mechanical stress. ***3.*** For high precision control of the temperature of the heating medium, the medium should be transported at high velocity related to the exposed material. For water, this is complicated to achieve since either the moving water may transport the seeds (the density of water, 1 kg/l, is similar to the specific gravity of cereal grain, 0.95-1.06, 1.13-1.33 and 1.29-1.32 kg/l for oats, barley and wheat, respectively – ASAE, 1988), or, if the seeds are immobilised, for example by a net, a high driving pressure would be required. Actually, when applied commercially, the treatment often caused reduced seed germinability (Lier & Jørstad, 1948).

Due to these problems, the hot water treatment was almost completely abandoned for cereals after 1960 since cheap and efficient chemicals for seed dressing had become available. Perhaps some of the problems with hot water treatment could be solved with modern technology and new approaches. However, for many reasons, instead of using hot water it seems that the problems could be even better solved by using hot, humid, air, or "aerated steam", as a heating medium. Commercial applications using aerated steam have been developed for treatment of lobelia seeds against *Alternaria* infection (Hall & Taylor, 1983), and for treatment of sugarcane stalks against ratoon stunting disease and other sett-borne infections (Edison & Ramakrishnan, 1972; Cochran *et al*., 1975, 1978; Cochran, 1976; Srivastava *et al*., 1977; Singh *et al*., 1980; Damann, 1983). However, since the mid 1990's these approaches have been developed towards full-scale commercial use for cereal grain, in collaboration between SLU and Acanova AB (www.acanova.se; Bergman, 1993; Bergman & Forsberg, 2000; Forsberg, 2003; Lagerholm, 2003). With new knowledge improving the properties of aerated steam treatment, there was a vision and a hypothesis that it could present a new efficient and low cost alternative to chemical seed dressing also for cereals. This thesis reports on our testing of this hypothesis.

2. Aims and outline of the study

Before I joined the project, experiments had been performed by the research team with seed treated by using a simple steaming device. The experiments were successful since efficient disinfestation of infected seeds was achieved with maintained germinability. The explanation of the good effects was thought to be the use of air humidity sufficient to give moisture equilibrium between the seed and the treatment air and therefore avoiding drying during the process. Based on these ideas, in 1998, a new high-precision laboratory treatment device was constructed by Acanova AB where the treatment process was minutely regulated by modern sensor and computer control technology, combined with real-time documentation

of all treatment parameters. The device was designed to repeat and improve the results from the earlier experiments, assumed to be permitted by the use of well-known equations for moisture equilibrium. These new ideas were to be evaluated by extensive testing of treated seed within an EU-financed project, "Demonstration of a biologically sustainable and environmentally friendly high precision thermal seed treatment method", acronym DEST (Bergman & Forsberg, 2000; Hartl & Girch, 2000; DEST, 2001), involving partners from Austria (LBG), Denmark (KVL), Germany (BBA and LPP), Italy (UNITO) and Sweden (SLU and Acanova AB). In July, 1998, I came into the project as a PhD student, and since then many challenges have been faced.

I performed the first treatments for the DEST project evaluation according to assumption above. The results of these treatments were deceptive and it was clear that the whole secret behind the good effects obtained in earlier tests was not yet known (DEST, 2001). The initial objective of the work therefore became:

1st Aim: To investigate the influence of important treatment parameters on the survival of the seed and of the pathogen and draw conclusions about the heating process using different kinds of heating.

After an intensive summer with extensive testing of a large number of different treatment strategies, the clue to the right recipe of treatment parameters was found. This work continued during subsequent years by performing iterative experiments optimizing the process step by step.

The second challenge was the results obtained in investigations of heat tolerance of a wide number of seed lots, measured as the tolerance to high temperatures, that were performed on the same time as the first evaluations (DEST, 2001). The objective of the second investigation was:

2nd Aim: To investigate if the same treatment temperature could be used for all seed lots – do all seed lots have the same heat tolerance?

We were hoping to find that the use of standard equations describing the relations between heat tolerance and measurable parameters, such as moisture content (mc) and germinability, would be possible to generalize for the aerated steam application, possibly with some correction for crop and cultivar differences. The results showed, in contrast, that no important generalizations could be made. Instead, all seed lots have individual heat tolerance that could not be predicted with sufficient precision from standard seed data. For the evaluations in the following years, in order to find the accurate temperatures, the heat tolerance of each seed lot had to be pre-tested.

Due to the findings that individual seed lots differ in heat tolerance, the objective of the following investigation became:

3rd Aim: To investigate if there are significant variations in heat tolerance within seed lots and if such variations can be detected by pre-tests of representative samples.

The pre-test of heat tolerance was implemented routinely. However, it took a long time and a lot of effort to calibrate these tests for good accuracy of the

predicted optimum temperature compared with the optimum temperature for field conditions. Therefore parts of the field test evaluations of the treatment method in the first years of the DEST project suffered from poor predictions of the optimum temperatures (DEST, 2001).

At the end of the DEST project, when the treatment parameters had been optimized and the pre-test predictions were increased, most of the evaluations gave good results. However, in some cases unexpectedly low disinfestation effects were obtained. After extensive analysis of close to 100 field tests that had been performed during the period, it was found that the tests where the results were unexpected were performed using old seed lots. For research on seed-borne diseases, it is important to have highly infected seed material. When a highly infected seed lot is found, researchers are keen to keep samples of this seed lot as a reserve for later testing in subsequent years. Many of the tested seed lots therefore were up to four years old. This also raised questions about the effects of post-treatment seed storage. The aim of the next investigation therefore became:

4th Aim: To test the influence of seed storage on the effect of thermal treatment, both when the treatment is performed after a long storage period and when treated seeds are stored after the treatment.

Based on the knowledge achieved, an evaluation under practical conditions using fresh seed became interesting. The objective of the last investigation therefore was:

5th Aim: To investigate the effects of the optimized treatment method with the accurate pre-test on fresh seed treated on a large scale and tested under field conditions.

The different objectives stated here resulted in the research reported in the papers forming the base of this thesis.

3. Thermal seed sanitization – general principles and methods

3.1 Previous research

As explained in the introduction above, different kinds of thermal seed treatment like solarization, dry heat, hot water treatment and aerated steam have been applied practically. Seed sanitization from diseases by heat treatment is possible in cases where the pathogens have a lower tolerance to high temperatures than the infected seeds. Optimally, an interval of treatment temperatures can be found where plants developing from treated seeds are free from symptoms of many diseases, but without any plant injury as schematically outlined in Fig. 1). Baker (1962a) formulated that “The basic principle of thermotherapy is that parasitic

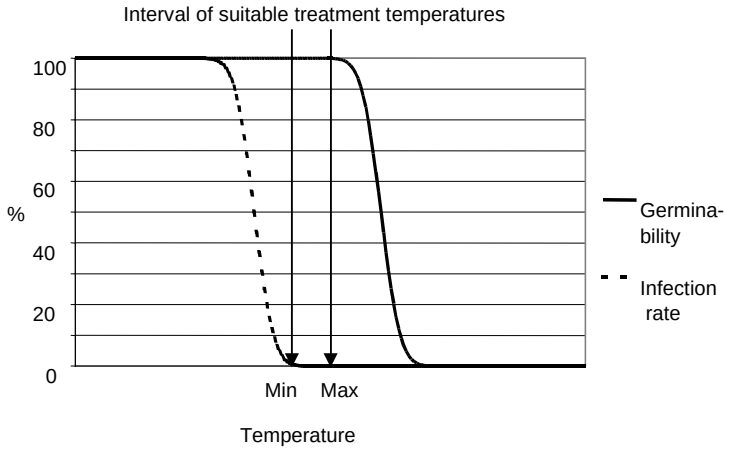

Fig. 1. Schematic drawing of the optimum case for thermal treatment: For treatment temperatures within the interval [Min; Max], here also called the "treatment window" the seeds have full germinability and the plants developed from treated seeds are free from seed-borne infection (Forsberg, 2001 a).

microorganisms often are killed, or viruses inhibited, at temperature-times only slightly injurious to the host".

In the late 19th century, Jensen (1888) found hot water treatment of seeds to be an efficient method for sanitization of seed-borne pathogens. In different forms the method has shown effects against many seed-borne fungal diseases and some seed-transmitted phytopathogenic bacteria and nematodes (Will Brinck, 1923; Russel & Tyner, 1954; Batts, 1956; Baker, 1962a; Doling, 1965; Smilanick *et al*., 1988; Da Silveira *et al*., 1989; Grondeau & Samson, 1994; Winter *et al*., 1996, 1997; Singh, 1997; Tenente *et al*., 1999; Garcia *et al*., 2000). However, conventional hot water treatment is laborious (Miller & McWhorter, 1948; Baker, 1969) and, because of the subsequent drying needed, it is also very energy-demanding. Good effects of treatment with dry hot air against seed-borne diseases caused by fungi, bacteria, nematodes and viruses on cereals and other crops with no or little lowering of germinability have only been obtained occasionally (Atanasoff & Johnson, 1920; Lehman, 1925; Miller & McWhorter, 1948; Baker, 1962a; Nakagawa & Yamaguchi, 1989; Fourest *et al*., 1990; Grondeau & Samson, 1994; Androsova & Sadkovskii, 1995; Trigo *et al*., 1998; Tenente *et al*., 1999; Garcia *et al*., 2000), but often at the price of long exposures and/or high temperatures. Solarization can be applied either as a form of hot water treatment, for which it has been documented for effects against loose smut on wheat (*Ustilago segetum* var. *tritici*) (Luthra and Sattar, 1934; Luthra, 1953), or as dry heat treatment but with the difference compared with ordinary such treatments that the sun is used as heating source. Solarization suffers from poor control of treatment temperature. Microwave irradiation has been tested (Hankin & Sands, 1977; Cavalcante & Muchovej, 1993; Hörsten, 1996; Stephenson *et al*., 1996; Forsberg, 1998), but mainly due to unreliable effects it has not yet been developed towards commercialization.

As a way of solving the constraints of the hot water or dry heat methods, some researchers have tried to develop methods using hot humid air, often called aerated steam or vapour-heat treatment (For cereals: Tapke, 1926; Navaratnam *et al*., 1980; For other crops: Latta, 1932, 1939; McWhorter & Miller, 1944; Miller & McWhorter, 1948; Smith, 1966; Bertus, 1967, 1972; Baker, 1969, 1972). Jensen, who was the first to test treatment using hot water, also seems to have been the first to test the effect of "heating in moist air" (Jensen, 1888) and succeeded in controlling *Ustilago avenae*, causal agent of loose smut, in infested oat seed. However, the effect was obtained at the expense of lowering the seed germinability which was reported to have been seriously affected probably due to insufficiently controlled temperature. Reports on practical use of aerated steam treatment for disinfestation of seed-borne diseases has been very limited. To my knowledge, there is only one example of the practical use of steaming of cereal seed. It is from North Korea where especially skilled persons traditionally treat cereal seed for control of diseases by placing them on a net over a water bath which is heated on an open fire. However, the process is difficult to control and it often results in reduced germinability (pers. comm.).

3.2 Mechanisms

Some of the mechanisms involved in the effect of the heat treatment have been summarised by Baker (1962b), where denaturation of enzymes and other proteins and lipid liberation are the most relevant for seeds. These are chemical properties that, by differing between host and parasite, could be the cause of the differential heat sensitivity. Other examples are autooxidation of fatty acids (Flood & Sinclair, 1981), genetic changes (Roberts, 1978; Orlova & Soldatova, 1980), various kinds of cell damage (Roberts, 1973) in organelles like the nucleus, ribosomes or endoplasmic reticulum (Berjak & Villiers, 1970), mitochondria (Abu-Shakra & Ching, 1967), or cell membranes (Simak *et al*., 1957). The pattern of seed deterioration preceding death is the same whether the seed survives for seconds in hot-air drying or for decades in long-term storage at sub-zero temperatures (Roberts, 1981).

In order to test whether the aerated steam treatment would cause a detectable chemical change in the seed or in the pathogen inoculum, we made an experiment (Brishammar *et al*., 2001, so far unpublished) where we subjected treated seeds to HPLC (High Performance Liquid Chromatography) analysis. Barley seed infested with *D. teres*, oat seed infested with *U. avenae* and wheat seed infested with *T. caries* were treated with aerated steam in a thin layer at 95 % rh (relative humidity) at temperatures that gave good disinfestation without lowering germination. Whole untreated and treated barley seeds were milled, suspended in ethanol, centrifuged and the supernatant was concentrated using rotation evaporation and saved for analysis. For the oat and wheat seeds, spores were separated from untreated and treated seeds in ethanol and centrifuged. The supernatant was concentrated using rotation evaporation. The obtained solutions were cleared by filtering and then subjected to HPLC analysis. Substances in the analysed solution were separated in the device and detected with an on-line spectrophotometer at 205 and 225 nm wavelength. For the barley seeds, the obtained HPLC curves from treated seeds did

not differ from those of untreated seeds. Since the pathogen inoculum is such a small part of the whole grain, a chemical change in the pathogen would not be visible in the HPLC diagrams. For the isolated and concentrated spores, however, clear differences were observed since the diagrams from untreated spores of both fungi had large peaks that, in the curves from the treated spores, had been broken down into several small peaks surrounding the point where the large peak was observed in the curve from the untreated spores. The retention times were the same for these peaks for the two fungi and for both wavelengths, indicating that the treatment had caused an important change in the chemical composition of the treated fungal material.

Looking at *Tilletia* spores on treated seed in the microscope, we were surprised to observe that the spores were strongly deformed and tightly adhered to the seed surface, looking just as if they had melted in the hot air. If this is what had happened, the observation would be a drastic example of the lipid liberation discussed by Baker (1962b) obtained from the treatment by influencing the viscosity of membrane lipids.

4. Principles of the aerated steam treatment devices used

4.1 Basic principles

Basically, the thermal treatment method used consists of two phases: The heating phase, where the seeds are heated for a certain time with air having a certain temperature and relative humidity calculated for good disinfestation, followed by a cooling phase that interrupts the treatment process before seeds are injured. The devices were constructed to permit precise control of important parameters (temperature, air humidity, treatment time, air flow and treatment and cooling durations). Control was achieved by computer-based systems using on-line measurements of relevant data at different locations in the devices and permitting the choice of treatment strategies arbitrarily within wide ranges. PID strategies (Proportional, Integrated and Derivative; control system, see Glad & Ljung, 1989) were used for control of temperature and air humidity. All devices were constructed to permit the following: ***1.*** Short treatment times; ***2.*** Equal heat exposure of all seeds; ***3.*** Heating with large air volumes per kg of seed and per unit of time.

Three different types of aerated steam treatment systems were tested: Batchwise treatment in thin-layer and fluid bed and continuous fluid bed treatments. The tested aerated steam treatment procedures are basically described in Figure 2.

4.2 The tested types of treatment processes

Batchwise treatment: In batchwise treatment a certain volume, or batch, of the material is treated together and at exactly the same time. The treatment of the batch must be finished and removed from the treatment system before a new batch can be started.

Closed-loop heating: During the heating phase, hot humid air is circulating in a closed-loop in order to save energy. The seeds are placed in the seed flow which is forced to penetrate and pass through the seed mass which, therefore, is heated by convection and conduction from the air. The temperature and humidity of the treatment air are continuously measured and controlled by a computer which gives signals to a heater and a steam generator that are precisely powered to compensate for the heat lost from the air to the seed mass, so that after a cycle in the loop the parameters of the air exposing the seed in subsequent air cycles are re-established to the desired values.

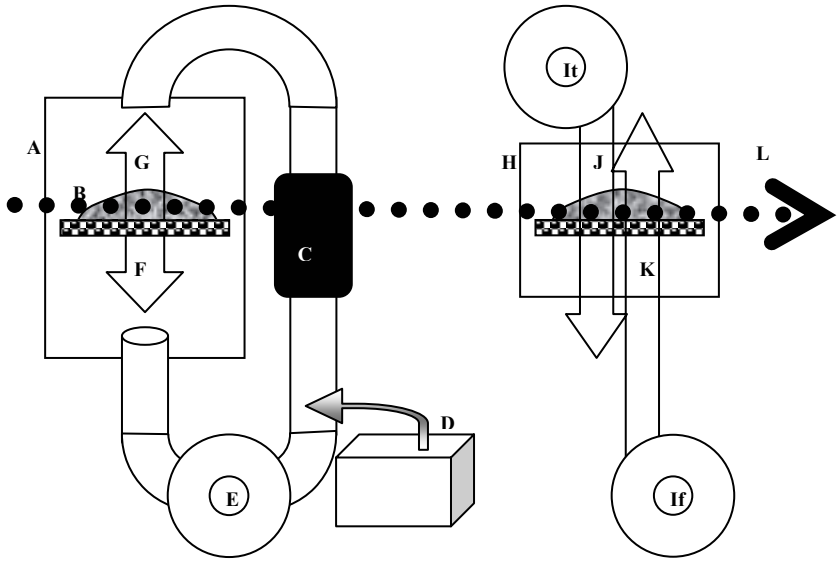

Fig. 2. Principle of the procedure used. I) The heating phase. A: Inside a treatment chamber (A) the seeds are placed for a certain time on a net (thin layer) or perforated plate (fluid bed) (B). Treatment air temperature is controlled by a heater (C) and vapour is injected from a steam generator (D). A fan (E) blows the air to circulate in a closed-loop system that re-uses the injected energy. The direction of the air is counter-clockwise for thin layer treatment (F) where the immobile seed layer is penetrated from above, and clockwise for fluidised bed treatment (G) where the seed layer is mixed by the air penetrating from below. II) The cooling phase. The seeds are placed for a certain time in a cooling chamber (H) similar to the treatment chamber into which cold and dry air is blown from a fan (It or If). For thin layer treatment, the seed layer is immobile because the air penetrates from above (J), and for fluidised bed treatment the seed layer is mixed because the air penetrates from below (K). For continuous treatment, seed is constantly flowing from left to right according to the dotted arrow (L) and thus successively passing through both chambers.

Thin layer treatment: For thin layer treatment, the seeds are evenly distributed on a net. The air flow is directed from above the seed layer and penetrates the layer while going downwards. The seed is pressed towards the net and is therefore immobile. For treatment of a thick layer in this way, immobile seeds in upper levels of the layer would cool the air before it reaches the lower levels, and the seeds at different levels would therefore not be uniformly treated. In order to minimise this effect, the treatments should be performed with thin seed layers, preferably no more than 1 cm. thick. Thin layer treatment generally gives uniform heating, since large air volumes per second are used per kg of seed and therefore only little energy of the air would be lost by the exposure of the initially cold seed, which explains why temperature control of the system is normally easily achieved. However, if for some reason control of the treatment temperature is disturbed, the heating process would be influenced. The potential for large-scale application is limited for thin layer treatment since a very large treatment surface would be required compared with other kinds of systems permitting thick layer treatment. The batchwise thin layer treatment system tested is described in detail and evaluated in **I**.

Fluid bed heating: For fluid bed treatment (Zabrodsky, 1966), the seeds are placed on a plate perforated with drill-holes. The air flow is directed from below and penetrates the seed layer while going upwards. For accurately chosen air velocities (normally 1-2.5 m/s as an average over the surface of a horizontal section of the treatment chamber) the seeds are lifted by the air and mixed without being removed from the chamber by the air. This treatment can be performed in thick seed layers (up to 15-20 cm) because the seeds constantly change position in the treatment chamber through the continuous mixing, which gives uniform exposure. Thus fluidised bed systems have the potential for large-scale application. Since thicker seed layers are used and the air velocity must be limited in order not to remove the seeds, the air flow rate per kg of seed is lower than for thin layer treatment and the air loses more temperature while passing through the seed layer, which also results in increased relative air humidity and water loss due to condensation of water on the initially cold seed. The consequence is that the control and power system rapidly has to compensate for this in order to avoid the air having incorrect temperature and humidity values after a cycle in the closed-loop system. For batchwise closed-loop treatment systems, perfect compensation is difficult to achieve and at the beginning of the heating phase the parameters may oscillate before stabilising on the desired values. However, the process is less sensitive to external disturbance of the treatment parameters than thin layer treatment because the large seed mass has a higher thermal inertia compared with the air and because the parameters are physically stabilized at the dew-point of the air. The tested batchwise fluid bed treatment system is described in detail in **I** and evaluated in **I** and **IV**.

Continuous treatment: For continuous treatment, a large seed lot is not divided into smaller parts that are treated separately from each other as for batch treatment systems. Instead, the seed material is continuously streaming successively through the treatment and the cooling chambers, which are formed like rectangular channels. A constant flow of untreated seed enters the heating chamber and treated seed exits from the cooling chamber at the same flow rate. The average heating

duration is equal to the volume of the channel (bed thickness × channel length × width) divided by the seed flow rate. The continuous treatment has three main advantages: ***1.*** The whole seed lot is treated without interruption, which is why treatment of a large seed lot needs little supervision. ***2.*** In a batchwise treatment system, the heating power required varies over time. The first part of the heating phase requires more energy because the treatment air looses most energy to the seeds when the seeds are cold. For continuous treatment, the power required is constant over time if the seed flow rate and the seed temperature are constant, and less powerful heating and steaming facilities are required. ***3.*** Since the required power is constant, precise parameter control is considerably facilitated. Continuous treatment in the form of a fluidised bed is possible because fluidising material behaves like a liquid in a recipient, see Fig. 3.

One inconvenience with continuous fluid bed treatment is the phenomenon called back-mixing. The mixing motion induced on the seeds by the air is mainly vertical, but random horizontal motion is also induced. Therefore, although the average flow is constant, the individual seeds do not flow at a constant velocity. This may be a problem because a result of this is that individual seeds do not stay exactly the same time in the treatment chamber, and the variation in residence time is normally distributed. For a material like the seed, where a certain treatment precision is needed, the variation in residence time must be limited. One way of doing this is by inducing an inclined vibrating motion to the fluid bed with a frequency and amplitude sufficient to induce a controlled horizontal motion of the seed material (Fig. 3). The continuous treatment system tested was not equipped with vibrating facilities. It is described in detail in **IV** (see Fig. 4).

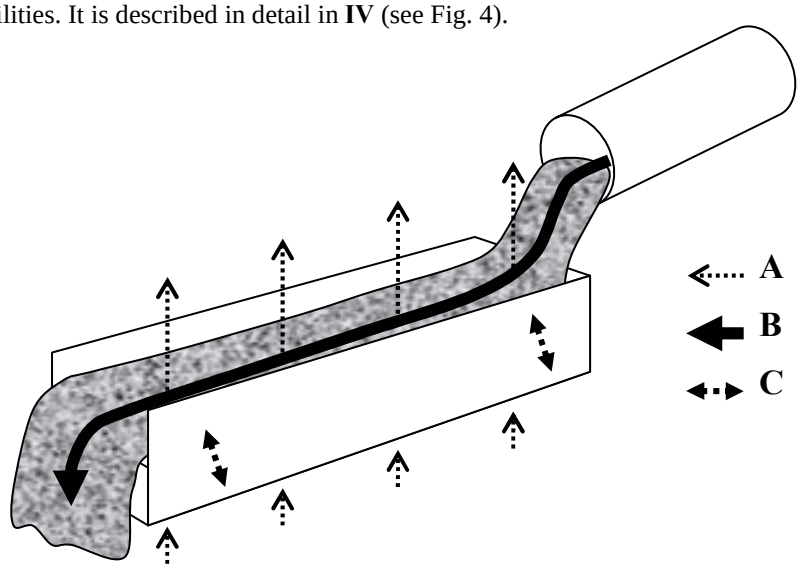

Fig. 3. Continuous treatment in a fluidised bed. Seed continuously enters the treatment chamber at one end. Because air flow (indicated by the arrows A) makes the material fluidise, it runs like a liquid (in the direction indicated by the arrow B) to the other end of the chamber where it exits. The arrows marked C indicate the oscillating motion of the treatment chamber in vibrating fluidised beds.

The advantages and disadvantages of the different treatment principles are summarized in Table 1.

Table 1. *A summary of the advantages (+) and disadvantages (-) of the four types of procedures discussed for aerated steam seed treatment*

Equipment type **Quality**	***Thin layer*** ***Batchwise***	***Fluidized bed*** ***Batchwise***	***Fluidized bed*** ***Continuous***	***Vibrating fluidized*** ***bed. Continuous***
Temperature stability	-	+	+	+
Uniform heating phase	+	-	+	+
Even exposure through seed layer	+	+	+	+
Exact treatment time	+	+	-	+
Suitability for large-scale treatments	-	+	+	+
Low power requirements/kg	-	-	+	+

Fig. 4. A CAD drawing of the tested large-scale continuous treatment system operating at 1.3-2 tons/h capacity. The seed flows through the rectangular heating channel bent to a spiral-formed circular treatment chamber at the upper half of the device and then down through the similarly circular cooling chamber before it is filled into a 700 kg bag. The height of the system is about 4 m. (Published with the kind permission of Acanova AB, Sweden).

4.3 Important characteristics of aerated steam treatment in a fluidised bed

1. *Water absorption.* For practical reasons, most characteristics of the fluidized bed, and also water absorption, were studied using the smaller equipment. A large number of experiments were performed, treating one kg samples of barley seed

either at 95 % relative air humidity at 63 °C, or at 75 % rh at 64 ºC. The treatments were interrupted after different durations in the treatment cycle, the treated sample was taken out, and its weight was measured immediately. Then the sample was cooled to room temperature in the cooling phase and its weight was measured again. The quantity of water absorbed by the seeds at different points in the treatment cycle was measured by comparing the total mass of the content in the treatment container with the mass of the content before the treatment. The mass before and after cooling gave a measure of the character of the water absorption: Water evaporated during the cooling phase was less strongly bound to the grain than the water that remained after the cooling.

The results of measuring the temperature of barley seed with IR- (Infra-Red) sensors over a treatment cycle with set-point temperature at 63 °C and air humidity 90 % are shown in Fig. 5. The heating was very rapid thanks to condensation of water on the cold seeds. The temperature top after 30-60 seconds during the heating phase and the dip in the early cooling phase are the result of the automatic internal calibration of the IR-sensor which does not measure accurately when the temperature changes quickly. After these points, however, measurements were accurate. Since the air is cooled down to the dew point while passing through the seed layer, the seeds do not reach temperatures higher than the dew point, which in the experiment was close to 60 °C. The instants when the samples in the performed experiments at 63 and 64 degrees were taken out for measurements of weight are indicated with arrows in Fig. 5.

The mass of the treated samples, changing over the treatment cycle due to water absorbed on the seeds, is shown for the measurements performed before and after cooling of the sample in treatments using 95 and 75 % relative air humidity in Fig. 6 - 7.

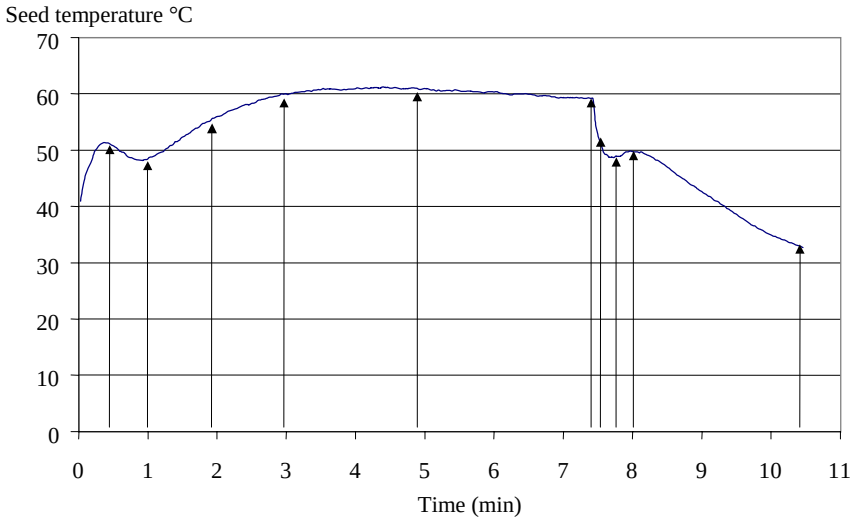

Fig. 5. Temperature on seed surface during treatment at 60 °C, measured by an IR-sensor. The arrows mark the instants when the samples were weighed, in order to monitor the seed moisture content during the treatment.

In order to limit post-treatment moisture content using the tested treatment device, according to the results, low air humidity should be used for short durations followed by a long cooling phase. An alternative, giving more freedom in the choice of air humidity and treatment duration, would be to use a two-step cooling. The first step would be a fluid bed cooler, quickly reducing the temperature sufficiently to interrupt the treatment process. The second would be similar to a dryeration cooling system (Brooker *et al*., 1992) which uses low air flow rates through the warm seed, giving efficient convective drying by using the heat remaining in the seeds for evaporation.

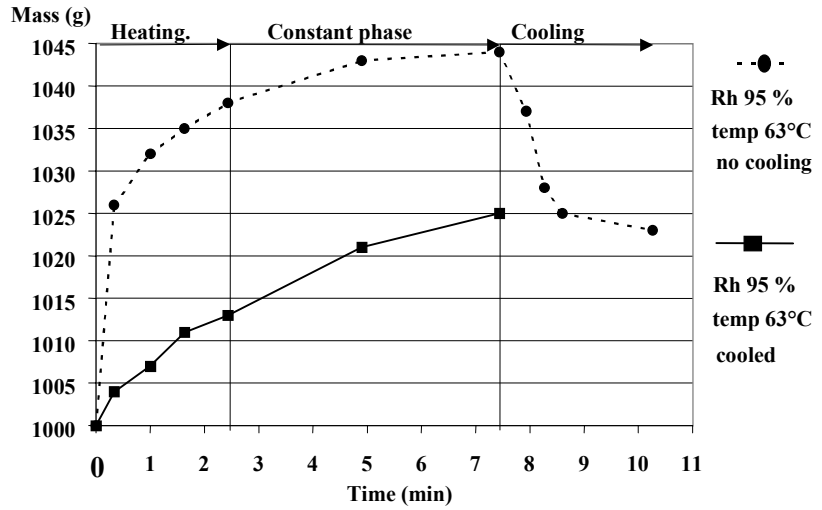

Fig. 6. Sample weight during treatment as function of treatment time (63 °C, 95 % rh). The measurements were performed at the instants and at the temperatures indicated in Figure 5.

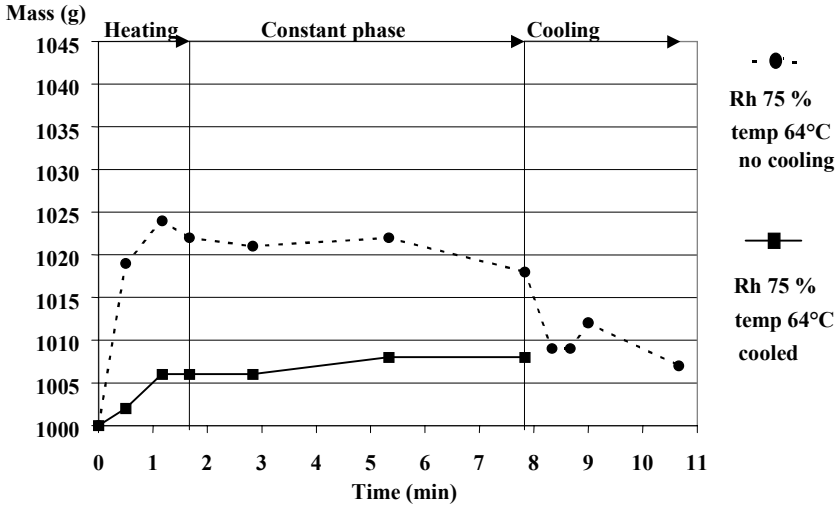

Fig. 7. Sample weight during treatment as a function of treatment time (64 °C, 75 % rh). The measurements were performed at the instants and at the temperatures indicated in Figure 5.

2. *Back-mixing at continuous treatment.* For the continuous large-scale treatments equipment, the variation in treatment time, caused by back-mixing in the heating chamber, was examined (Boisman, 1999). The distribution was studied during treatment of barley seed by momentanously injecting a 500 g sample of coloured barley seed at the loading entrance of the chamber, studying their distribution in time by taking out 150 g seed samples after certain time intervals from the exit of the heating chamber, running the equipment at a capacity of 500 kg/hour and 10 cm bed depth. The percentage of coloured kernels in each 150 g sample was counted and their distribution in time for their transport through the chamber was analysed.

The distribution in treatment time is shown in Fig. 8, as percentage of coloured seeds in each sample at the corresponding sampling time. 90 % of the seeds were treated within 5+/-1 min.

The resulting variation in influence on seed germinability can be calculated from the variation in treatment time using the Ellis & Roberts improved equation for prediction of seed viability (Ellis & Roberts, 1980a, b). From this, a theoretical variation in treatment temperature can be calculated that, during a constant treatment time, would have caused the same variation in influence on germinability as the observed variation in time at constant temperature. For treatments of ordinary cereal seed around 63 °C, the seeds treated at constant duration but influenced by a variation in temperature corresponding to the observed variation in treatment time would have been treated at temperatures between 61.96 and 63.84 °C. The measured variation in treatment times should, therefore, give good results on 90 % of the seeds for crop-pathogen combinations having an interval of suitable treatment temperatures wider than 2 °C for treatments during five minutes. For evaluations of this kind of treatment chamber, see Table 2 in Section 5.2.3 and **IV**. Using a fluid bed treatment system equipped with facilities for inclined vibrating motion inducing a uniform seed velocity in the continuous treatment chamber, the back-mixing would be considerably reduced.

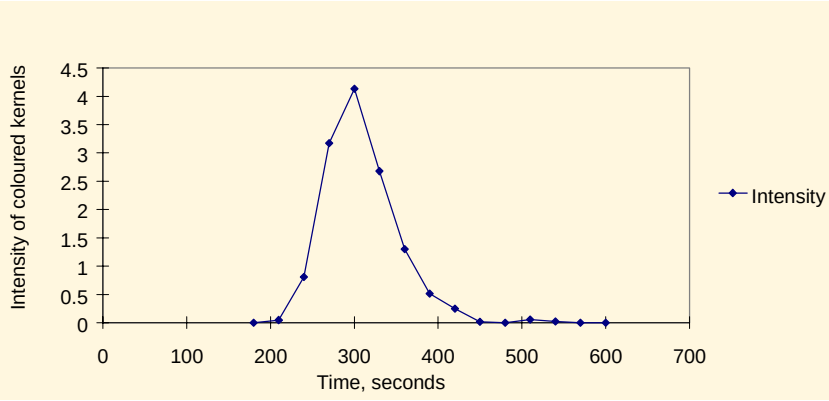

Fig. 8. The distribution of heating time in the continuous fluid bed treatment chamber. 500 g of coloured seed were injected momentaneously through the inlet and the frequency in g/s of coloured seeds counted at the outlet is plotted for the time passed after the injection (reproduced from Boisman, 1999).

Optimum treatment of more than 90 % of the seeds would be permitted as well as successful treatments of crop-pathogen combinations having an optimal treatment temperature interval narrower than 2 °C.

5. Biological effects of aerated steam seed treatment

5.1 Effects on germination of cereals

5.1.1 Seed storage, survival time and heat tolerance

Germinability is one of the fundamental properties of a seed – the ability to wake up from a non-active state to give a seedling, the first stage in plant development. The germinability of a seed lot depends on the physiological condition of the seeds which, in addition to genetic factors and growth conditions before the seed was harvested is also influenced by its drying, storage and handling history. A seed that is handled in an unfavourable way is exposed to stress that might lower its ability to germinate. For normally recommended storage environments - storage at low mc in seed bins, silos, etc. - the stress is reduced but still present and is regarded as an ageing process. In the following text, when quantified, the moisture content (mc) is expressed on a wet base and measured according to ASAE (1995). Ageing can be defined as “an increased probability of death of an individual per unit time as age increases” (Ellis & Roberts, 1981).

Seed ageing is accelerated at high storage temperatures and at high mc. For example, seed drying causes stress to the seed, resulting in accelerated ageing (Nellist, 1981; Roberts, 1981). Seed ageing is irreversible and each stress that the seed is exposed to reduces additively its viability, of which germinability is a measure. The survival time of seeds in a seed lot is considered to be normally distributed around the mean survival time, LD50, and the standard deviation in survival time is a measure of the length of the period when most of the seeds loose the ability to germinate (Ellis & Roberts, 1980 a), see Fig. 9. The viability is often expressed in the probit scale, which is a transformation giving linearization of the cumulative Normal function Φ, with average 0 and standard deviation 1 (Fig. 9). The probability for survival is $p = \Phi(\nu)$, where ν is the probit viability. A highly viable seed lot normally has a longer mean survival time and also a larger spread in survival time within the seed lot. However, limitations in the relevance of this relation have been discussed (Ellis & Roberts, 1977; Priestley *et al*., 1985; Kraak & Vos, 1987; Roos & Davidson, 1992; Fabrizius *et al*., 1999; Mead & Gray, 1999; **II**).

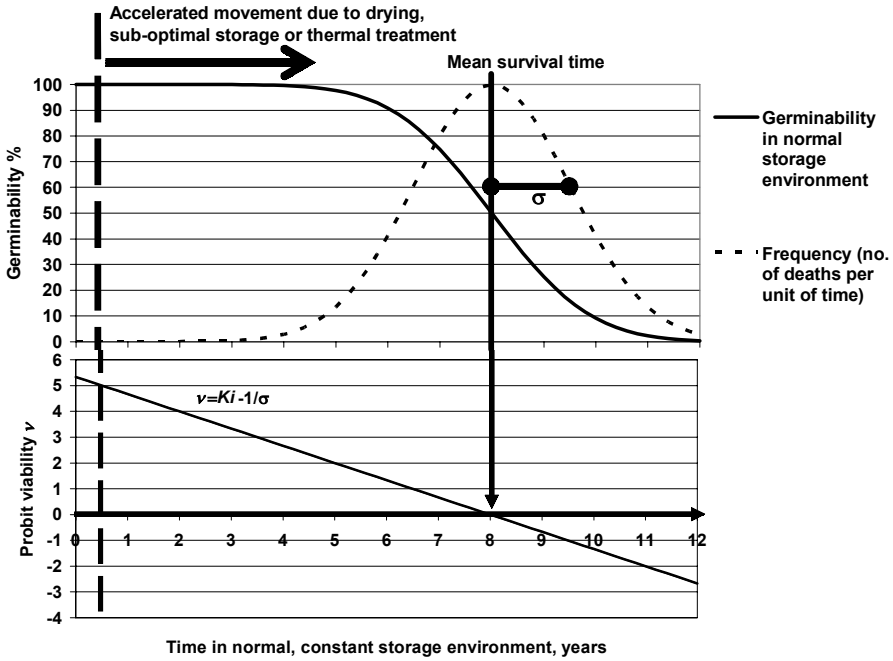

Fig. 9. Example illustrating the relation between the time of storage in a particular, constant storage environment and the viability of a seed lot, of which the germinability is a measure. The frequency of deaths is considered to be linearly distributed over time with standard deviation σ. The lower part of the figure shows the viability in probit scale, which is a straight line. In the example the initial probit viability is 5.3 and σ = 1.5 years.

The thermal seed treatment increases the temperature of the seed to high levels during a short time. This induces a stress in the seed similar to the accelerated ageing occurring in a hot-air grain dryer and therefore it causes accelerated ageing of the seed (Nellist, 1981; Roberts, 1981). If the thermal treatment is performed during a too long time, or at a too high temperature, the accelerated ageing will reduce the viability so much that the seed lot in the example in Fig. 9 would react as if it would be more than 4.5 years old and the germinability would be reduced. If post-harvest seed drying has been performed at a high temperature for a long time with seeds at high initial moisture content, the ageing process will accelerate and the ageing state of the seed lot will advance quickly on the time-axis of the ageing curve.

The effect of thermal sanitization treatment is based on the principle that pathogens are heat-sensitive (**I**). For maximum treatment effect, the treatment temperature should therefore be maximized, which is why the seed ageing should be pushed as far as possible by the treatment without adventuring the germinability. If the seed ageing state is pushed to the right, either by accelerated ageing or by normal ageing in storage, this will reduce the efficacy of thermal treatment, since in order to eliminate the risk for reduced germinability, lower temperatures giving unsatisfactory disinfestation would have to be used (**III**). However, if thermal treatment is performed on seed stored for a limited time, the treatment can be performed at temperatures that are fully effective for disinfestation (**I**; **IV**).

The heat tolerance of the subjected seed is, therefore, an important property for thermal treatment applications. For a particular application (heating medium, treatment time etc.) practical measures of heat tolerance are the tolerance to (or rate of survival at) high temperatures and the LDx temperature (Lethal Dose), being the temperature that reduces the rate of survival by x % (**II**).

The optimum treatment temperature for application of aerated steam depends on the heat tolerance and could be defined as "the maximum temperature for a given duration and air humidity that does not reduce the yield potential of the crop established from the treated seeds". Since yield can be difficult to predict, a simpler way to define the optimum temperature could be: "The highest temperature for a given duration and air humidity that does not significantly reduce germinability or delay plant emergence" (**I**). This definition will be used in the following text. If the aerated steam treatment would be performed at a higher temperature than the optimum or during a longer time than for which the optimum temperature was determined, the seeds would be injured (Fig. 1). Analogically, if seed treated at the optimum temperature is not used within a certain time after the treatment, the ageing process would continue and after a while the emergence speed and the germinability are at risk to be reduced (**III**). In order to obtain a seed that should be storable for a certain time after the treatment, the treatments should be performed at sub-optimum temperatures or the storage should be arranged in a way that reduces the speed of the ageing process.

5.1.2 Variation in heat tolerance between seed lots

For aerated steam treatment, it would be desirable if the treatment could be performed in the same way for all seed lots, or at least if the optimum treatment parameters could be possible to calculate from information about the seed that is known or measurable. It is well established that the physiological condition of the seed, that can be measured in terms of germinability, is the basic factor determining its potential to survive exposure to high temperatures (Ellis & Roberts, 1980c; Roberts, 1981). It is also well known that seed moisture content, which is measurable instantly using modern conductivity sensors, influences the heat tolerance (Edwards & Colin, 1834). Ellis & Roberts (1980a, b) developed an equation for prediction of seed viability for wide ranges of storage conditions using seed germination and mc data. If this information would be sufficient for calculation of the optimum treatment temperature, any seed lot could be successfully treated at a temperature easily predictable by the equation using the mc and germinability (**II**) that anyway are tested routinely for most commercial seed, or at least by using an accelerated ageing test (Ellis & Roberts, 1980c).

In order to verify this and in order to investigate whether specific crops or cultivars should be treated at specific temperatures, 10 oat seed lots, 14 of rice, 12 of rye, 16 of wheat and 17 of barley were collected in five European countries from locally used cultivars for which tolerance for a range of temperatures during 5 minutes was tested at two different moisture contents with a thin-layer aerated steam laboratory treatment system (**II**). It was concluded from the results obtained that different crops differ in heat tolerance and rice was very little influenced by

different moisture contents, although it could not be determined whether different cultivars differ. However, the most important conclusion was that using the accessible information concerning mc and germinability was not sufficient for prediction of the optimum treatment temperature with high precision, and that even different seed lots of the same cultivar behaved differently in this respect. Not even germination data obtained after accelerated ageing performed in the same aerated steam treatment system gave sufficient prediction precision using the Ellis & Roberts equation other than for low moisture contents for seed lots with very high heat tolerance. Because the tested seed lots differed individually, the solution proposed (**II**) for optimum treatment was to perform pre-tests of heat tolerance under the specific treatment conditions for every seed lot to be treated (**I**).

5.1.3 Variation in heat tolerance within seed lots

Since different seed lots of the same cultivar vary in heat tolerance even though they have been harvested the same year and stored under similar conditions (**II**), this raises questions whether there might be differences in heat tolerance within a seed lot. One example of this was discussed by Fabrizius *et al.* (1999), having found that the initial viability constant, *Ki*, varied between samples of a seed lot. Several reasons for such differences could be discussed: ***1.*** The seed lot might have been harvested at different locations where the crop might have been established on *different sowing dates* and therefore all seeds might not have reached the same maturity state (Hay *et al*., 1997). ***2.*** The seed lot might have been *harvested on different dates* and during the meantime the crop might have been exposed to warm and rainy weather initiating decomposition of the seed. ***3.*** Seeds harvested in the same field during the same day might have *differences in moisture content*, for example resulting from dew or light rain in the morning followed by warm and sunny weather in the afternoon. Therefore one part of the seed lot might have been dried with warm air at a high initial moisture content when the seed is heat sensitive, whereas the other part might have been dried with a lower initial moisture content at which the ageing process is not pushed as far by the drying, or drying might not be needed at all.

In order to investigate the importance of variation in heat tolerance within seed lots, extensive experiments were performed. Commercial seed lots were selected in a seed treatment plant in Uppsala, Sweden. The following seed lots were selected: ***1.*** Barley cvs. Vanja 62 and 80 tons, Filippa 80 and 105 tons, Mentor 80 tons, and Pongo 434 tons. ***2.*** Oats cvs. Sang 80 tons, Belinda 80, 185 and 244 tons, and Svala 178 tons. ***3.*** Spring wheat cv. Curry 52 tons and winter wheat cv. Stava 105 and 140 tons. Each seed lot was sampled at 10 equidistant locations within each respective lot. The heat tolerance was tested for each individual sample by treating sub-samples at 10 different temperatures including an untreated control in the thin layer treatment device for five minutes at 95 % rh (**I**). The optimum treatment temperature was calculated from fitting an accumulated normal distribution function to germination data from each sample (**II**) (Fig. 10).

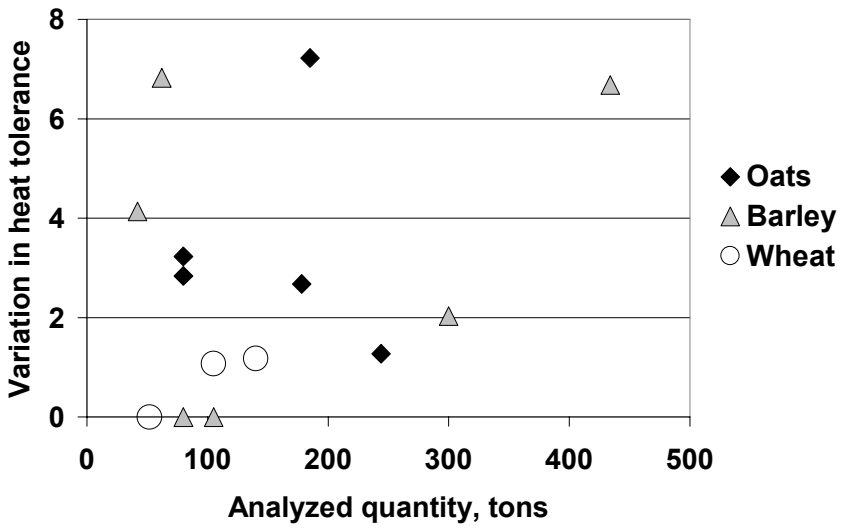

Fig. 10. The variation in heat tolerance, expressed as the LD50 temperature obtained for treatments at 95 % rh for 2 minutes, plotted against the size of the analyzed seed lot. The results are based on 110 treatments and germination tests per plotted point.

For each seed lot, the variation in heat tolerance was defined as "the LD50 temperature for the most tolerant sample" minus "the LD50 temperature for the least tolerant sample" because the LD50 temperature is the LD temperature that can be calculated with the highest precision. The same tests were performed on a representative sample of the seed lot produced by mixing all samples from the seed lot immediately before the test treatment. The standard deviation σ of the thermal death temperature was specially analysed for the representative sample in order to investigate if it could be used for determination of the variation in heat tolerance, see Fig. 12.

5.1.4 What is the optimum treatment temperature for an individual seed lot?

Since seed lots differ in heat tolerance, the optimum treatment temperature must be determined in pre-tests of individual seed lots (**II**). The proposed pre-tests should be done by treating small representative sub-samples of the seed lot with aerated steam at a range of temperatures covering the temperature interval where the optimum and super-optimum temperatures would be expected to be found, and then testing the germinability of the treated sub-samples plus an untreated control (**I**). An accumulated normal distribution function can be fitted to germination data (**II**), from which the temperature giving a desired germinability can be calculated. One measure of the maximum treatment temperature that does not affect germination could be the LD1 temperature predicted to be the Lethal Dose for 1 % of the seeds. If a sensitive germination test is used, LD1 does not necessarily mean that 1 % of the seeds are dead, but that the germination is delayed for 1 % of the seeds. Such a small reduction in germination speed does not significantly affect

seed quality, even though a 0 % reduction would be desirable. However, such a requirement would not permit any storage, treatment or handling of the seed. An acceptable reduction rate must be determined in order to reach effective treatment temperatures.

For optimum treatment effect it is very important to be able to predict the optimum treatment temperature with high precision. The optimum strategy should, of course, be defined as the strategy giving maximum economic outcome for the producer and the user of the seed. This means that the treatment should add maximum value to the seed for the actor performing the treatment implicating that it should also have maximum value for the farmer who buys it. The maximum value for the farmer is if he can produce a high-yielding crop of good quality. As far as this concerns the seed, it means high and quick field emergence at varying climate conditions and healthy plants. However, for thermally treated seed, in-house experiments show that standard germination tests often give poor predictions of field emergence. This might result in a choice of a final treatment temperature that either might seriously reduce field emergence (see the example in Fig. 11) or give insufficient disinfestation effect of the infesting pathogens. Extensive work was done from 1999-2002 in order to develop a germination test method that gives good predictions of the optimum treatment temperature both concerning field emergence and the post-treatment infection rate. Special attention was paid to adjustment of parameters such as germination temperature and medium, which have been shown to be of significant importance (Diethardt *et al.*, 2000). The final test is based on germination assays including recordings of both germinability, germination speed and early visible symptoms of seed-borne diseases. The test has given predictions with high precision in extensive evaluations in field tests of barley, oats and winter and spring wheat at up to six locations in Sweden during two years.

This test takes 6-8 days to complete. This is sufficiently fast for most situations, because the seed lot can often be sampled some time before the treatment. However, tests giving the results immediately would be desirable, since this would make it possible to sample and test the seed lot just before treatment, which would reduce the logistic requirements on sampling and testing routines. The ability to germinate is a chemically determined property. Therefore, if key substances that influence the ability to germinate could be found where the quantity or other measurable property of the particular substances would be well correlated with germinability, a quick method designed for detection of these substances could replace the germination test.

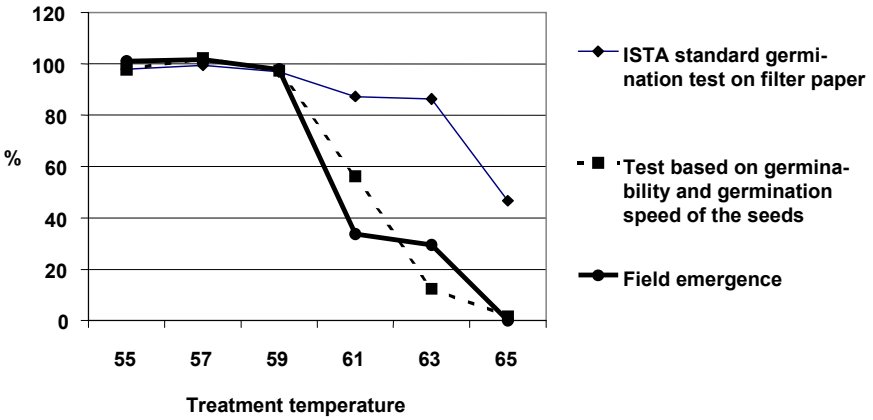

Fig. 11. Example of comparisons of different germination test methods for prediction of field emergence after aerated steam treatment. Samples of oats cv. Freja were treated in fluidized bed at 90 % rh and at six different temperatures. The standard ISTA filter paper test (ISTA, 1996) predicted too high germinability for high temperatures. The test developed gives a good prediction of the optimum treatment temperature.

A seed lot with large variation in heat tolerance is more difficult to treat optimally than a seed lot that is perfectly uniform in this respect. If the "treatment window", the width of the interval of treatment temperatures giving satisfactory disinfestation without affecting seed germinability, is wider than the variation in heat tolerance within the seed lot, then the whole seed lot can be successfully treated with the same treatment temperature. If the "treatment window" is narrow, however, more knowledge would be required for successful treatment. For example:

1. The seed lot can be successfully treated if the *heat tolerance of all individual parts of the seed lot are known in advance*, for example from a heat tolerance test of all parts. The basic requirement to be successful with this strategy is that the variation should be smaller for small sub-seedlots and therefore also for small seed lots. This is possible if the parts of a seed lot handled in different ways (for example harvested on different days) can be handled separately all the way from the farmer to a thermal seed treatment plant. For practical conditions this is impossible to achieve perfectly. Normally, due to limitations in available storage and transportation facilities on farms, sub-seedlots of the same cultivar that have not been handled in the same way have to be stored in the same bins, where they become mixed and unseparable. Therefore, there will always exist a certain variation in heat tolerance, although for small seed lots the risk for large variation would be lower.

2. The whole seed lot could be accurately treated if it would be possible to *predict heat tolerance from on-line measurements* during the treatment (if the heat tolerance depends exclusively on measurable parameters such as mc, seed size, chemical composition, etc.) so that the treatment parameters could be continuously adapted accordingly. On-line measurements of seed size and mc could be practically performed. However, variations in heat tolerance caused by, for

example, differences in seed mc prior to hot air grain drying would not be possible to detect using such measurements. In order to detect such variations, chemical measurements would be required for detection of viability as discussed above. On-line measurements of chemical composition are delicate. One practical approach could be measurements by irradiation (for example by NIT, Near Infra-Red Transmittance) because it would not require the use of a chemical laboratory. Such methods are used, for example, for analysis of mc and protein content in grain (www.foss.dk). However, this type of measurement is complicated even for substances that are present in a large quantity in the seeds, such as water or protein, because it requires extensive calibration of a neural network pattern recognition system including the analysis with NIT and reference methods for thousands of samples. For analysis of the substances determining heat tolerance, which are probably in smaller amounts in the seed, both the detectors and the pattern recognition system would have to be much more sensitive and probably much larger numbers of samples and reference analyses would be required for the calibration.

However, the experiments performed indicate that the value of σ obtained from a pre-test of a representative sample of the seed lot can be used as a predictor of the variation in heat tolerance (Fig. 12). If the relation between σ and the variation in heat tolerance is determined for a large number of seed lots, a heat tolerance test of a representative sample of a seed lot would give sufficient information to reveal if the variation is within acceptable ranges (smaller than the specific "treatment window" of the infecting disease) or if it is too large for successful thermal treatment of the whole lot at constant temperature.

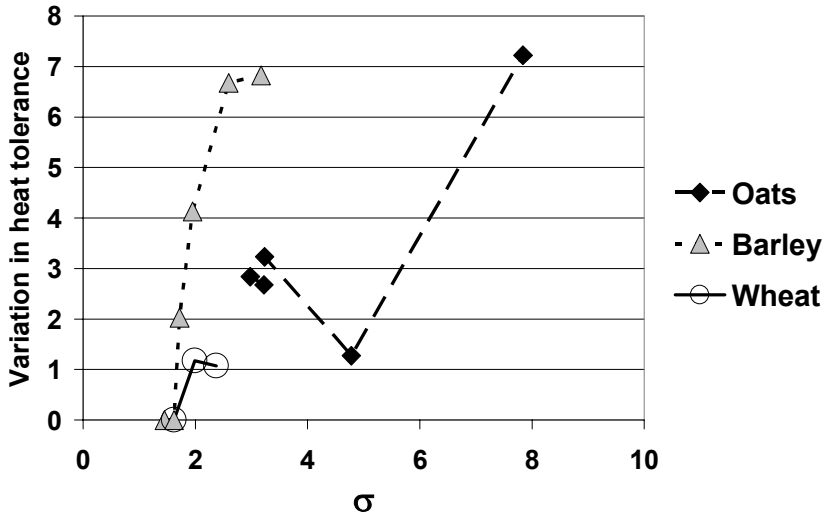

Fig. 12. The variation in heat tolerance plotted against the standard deviation σ of the thermal death temperature of the tested seed lot obtained by testing the heat tolerance of a representative sample of the seed lot.

5.1.5 Prediction of post-treatment germinability and its connection with physical relations

The equation developed by Ellis & Roberts (1980a, b) for prediction of seed viability after storage in a wide range of treatment environments was evaluated for aerated steam treatment conditions (**II**). As discussed above, the prediction precision was not found to be satisfactory. One important reason discussed was that the equation was developed based on experiments where the seeds were kept in sealed glass vials in order to avoid drying. In the glass vials, the seeds were heated by thermal irradiation and by conduction from slowly moving hot air through the glass and the enclosed and poorly mobile air to the seed.

Heating under the tested aerated steam treatment conditions is very rapid (**I**;**II**;**V**) since: ***1.*** Humid air *contains more energy* than dry air; ***2.*** The treatment air moves at high velocity, heating the seeds with *large air volumes per kg of seed*; ***3.*** *Condensation of the humid air* releases evaporation heat on the seed surface. Therefore, in order to improve predictions by using the equation, this difference should be considered. An attempt to do this was done in **V**, where assumed differences in heat transfer rates and seed wetting between the experimental conditions of Ellis & Roberts and those of aerated steam treatment were used to improve the prediction of the of the Ellis & Roberts equation. Corrections were added to the temperature and mc components in the equation. The assumption was evaluated in an experiment with treatment of barley seed at 95 % rh in thin layer at durations from 0.5 to 10 minutes and at temperatures up to 80 °C. It turned out that this adjustment improved the predictions considerably and the error was reduced by 75 %. In combination with the analysis of *D. teres* infection (see Section 5.2.2 below) of the treated seeds (a pathogen situated close to the seed surface), even though simulations or measurements of heat conduction were not performed, this experiment, in addition, gave a picture of the heat and moisture transfer in a seed and how these are influenced by the method used for heating (**V**). The temperature corrections were also adjusted by an empirically developed function that further reduced the prediction error by about 50 % (**V**).

5.2 Effects on cereal diseases

5.2.1 "The surprise" – a mechanism behind the disease suppressing effect?

Baker (1962a) stated that "The basic principle of thermotherapy is that parasitic microorganisms often are killed, or viruses inhibited, at temperature-times only slightly injurious to the host". However, different types of thermotherapy (dry hot air, micro-wave, hot water, aerated steam) are not equally effective (**I**). If the basic principle of thermotherapy, as described by Baker, would be the only mechanism, then the different types of thermal treatment would not differ in efficiency (**V**).

The initial hypothesis of the mechanism behind the improved effect of the aerated steam treatment compared with dry hot air treatment was that: "By using dry air the seed would begin to dry which would require evaporation heat and limit the temperature increase in the seed shell, and in order to avoid this and in order to

avoid increase of seed moisture content, higher air humidity, exactly determined for establishment of moisture equilibrium between the seed and the surrounding air, should be used." The modified Henderson equation (Henderson, 1952) was developed for calculation of moisture equilibrium levels for particles stored in air at a certain temperature and relative air humidity. Thompson (1967) modified this equation for cereal grains. In using this equation for determination of treatment parameters, theoretically both drying and wetting would be avoided (**I**). However, tests using this strategy showed that the seeds actually were dried by the treatment, indicating that the equation was not suitable for the treatment conditions used. In addition, which was very deceptive, the obtained disinfestation rates were far from satisfactory (DEST, 2001). I did not try to further modify the Henderson equation for accurate calculations for this particular application, bacause this was not one of the primary aims.

Instead I worked with the hypothesis that: "Since hot water treatment is effective, then treatment at very high air humidities, which would be equivalent to hot water treatment, would give good effects without critical wetting". This hypothesis was tested and confirmed by tests of thin layer treatments of barley seed infested with *D. teres* for 5 minutes (**I**) and of wheat seed infested with *Tilletia caries* for 1-9 minutes (Kristensen & Forsberg, 2000) by comparing the effects of using the modified Henderson equation with those obtained by maximizing the air humidity (**I**). From these results it was concluded that treatment at high temperature and air humidity gives rapid heat transfer by the high heat content of moist air and by condensation of hot water on the cold seed and the release of evaporation heat. The air humidity for thin-layer treatments should preferable exceed 90 % (**I**), though some cases (unpublished) good effects can be obtained down to 85 %. During this kind of treatment, the moisture content in the seed surface increases, which increases the heat sensitivity of pathogens located close to the seed surface or just under the seed shell (**I**; Baker, 1962b). As the duration is short, the seed is cooled before the temperature increases to dangerous levels in the deeper situated embryo, whereas close to the surface the pathogen with increased sensitivity is exposed to the highest temperatures. This means that, similarly with the "surprise" as explained by Brillat-Savarin to be the "merit of frying" (1825a, b), by the rapid heating with aerated steam, a treatment is achieved that is partly selective for the surface, where many important pathogens are located (**I**;**V**).

This, in turn, would also mean that shorter treatment durations would give even higher selectivity and would permit even higher treatment temperatures without harming the embryo (**I**). This idea was also tested for barley seed infested with *D. teres* and for oat seed infested with *D. avenae* for durations ranging from 30 seconds to 10 minutes (**V**; Forsberg, 2001a). These thoughts were partly confirmed because the shortest tested durations, 30 and 60 seconds, were the most effective for infested barley treated in a single-seed layer. However, for thicker seed layers the results for 30 seconds duration were not as good (**V**), probably due to uneven exposure of the thicker immobile seed layer. For the infested oats seed, even for the single-seed layer the results were less satisfactory for the 30 seconds duration. The question arose whether the thick and heat-isolating seed shell of the oats seed would make the exposure uneven under the seed shell for short durations (**V**). This could be expected on account of the air flow in an immobile seed layer not being

perfectly uniform around the seeds. The seed might be disinfested on one side but not on the other. The consequence for seed disinfestation in immobile seed layers would be the presence of an optimum treatment duration. Similar arguments are probably valid for seeds treated in a fluidised bed. Even though the seeds are not immobile, a certain time is required for mixing of seed before all the seeds within the seed layer have received the same exposure (**V**). Similar experiences (unpublished) were made on winter wheat seed cv. Kosack infested with *Tilletia caries*, causal agent of common bunt, and tested after treatment in a fluid bed for 0.5-5 minutes. In these experiments, also the shortest duration gave good disinfestation indicating that the optimum duration might be even shorter.

In the barley experiments it was also found that disinfestation was slightly more effective for seeds at lower mc (**V**). This confirmed the earlier experience of Baker (1962a), who noticed that the "spread in differential heat sensitivity" between germinability and pathogen, which is analogical to what here is referred to as the "treatment window", was increased for lower seed moisture content. The probable explanation is that seeds having low mc are less sensitive to high temperatures, whereas the pathogen is wetted during treatment with aerated steam or hot water and therefore it is not protected by the low initial mc.

5.2.2 Prediction of post-treatment infestation rate - connections with physical relations

As mentioned above (Section 5.1.5), the use of the Ellis & Roberts improved equation (Ellis & Roberts, 1980a, b) was modified for better prediction of germinability of treated seeds (**V**). The same kinds of temperature and mc adjustments were also tested as for prediction of the post-treatment rate of Drechslera teres infestation of the investigated barley seeds (**V**). Here, the equations adjusted for prediction of infestation rate gave even better prediction precision than those for prediction of germinability. The most interesting thing with the equations and the developed adjustments was that, together, they gave a picture of the mechanism for the selective treatment effect discussed above (**V**). This showed how both the temperature and the moisture content increased faster in the seed surface where the pathogen was located than in the deeper-lying seed embryo. The results do not prove that the effect is obtained in this way, but the fact that the adjustments considerably improved predictions of both germinability and infection rate gives a good indication. The results also indicate that using the conditions of the Ellis & Roberts experiments, in which sealed dry hot air was used, the temperature difference between the seed surface and the temperature in the embryo is very small which explains why treatment selectivity for the pathogen is absent (**V**). For flowing dry hot air, the selectivity would be expected to be even more reduced due to the evaporation heat "stolen" from the seed surface by the drying process, and also because during drying the seed surface has the lowest moisture content. Hot water treatment could theoretically give effects similar to those obtained from aerated steam treatment if used for short durations followed by rapid cooling. However, during hot water treatment it is difficult to reach a perfectly uniform treatment, since the water velocity through a seed bed would be limited due to the mechanical properties of water, which would lead to increased risks for

temperature gradients within the bed at short duration treatments. For hot water treatment, there is also so much excess water on the seed surface that it is difficult to re-establish the initial moisture content of the seeds after treatment.

5.2.3 Evaluations of treatment effect in disease-infested cereal seeds

An initial evaluation of the aerated steam treatment method using the new principle for treatment devices was done within the EU-financed project DEST (see Chapter 2 above). Seed lots of wheat, barley, oats, rye, triticale, spelt wheat and rice, highly infested with important seed-borne diseases were collected in the participating countries among locally used cultivars. Samples from the infested seed lots were sent to Uppsala where they were pre-tested and treated with the laboratory treatment device that was under development (**I**; DEST, 2001). The treated samples were returned to the country of origin for evaluation together with untreated and chemically treated samples in standardised laboratory, greenhouse and in field tests. Totally 96 field tests were conducted in the five countries during the period 1998-2001 (DEST, 2001). At the end of the project, the participating researchers made common conclusions concerning treatment effects of the method (DEST, 2001; Krauthausen *et al*., 2002; Forsberg, 2003), which are summarized in Table 2. The effects against most tested diseases were equivalent to those achieved by chemical seed dressing. The exceptions were *Ustilago nuda* and *U. nuda* var. *tritici*, causing loose smut in barley and wheat, respectively, and snow mould caused by *Fusarium (Microdochium) nivale*. In the first mentioned two cases, the infection is situated within the heat-sensitive seed embryo.

The treatment in most cases showed excellent effects against seed-borne *Fusarium nivale* infestation, but in some experiments performed in Germany (DEST, 2001) it showed somewhat lower efficacy than for chemical treatment. One reason for this might have been infections originating from soil or plant debris since thermal treatment does not protect the seed from external post-treatment infections. Another reason might be a *F. nivale* infection located deeply within the seed where, as is the case for some smuts, it would be partly protected from heat exposure. However, in later tests (see below and Table 2), we saw no such tendency as was noticed in the tests in Germany that the aerated steam treatment should have inferior effects compared with those obtained by using chemicals for *F. nivale* in winter wheat.

Further evaluations of effects against pathogens were continued in Sweden during 2002 (unpublished) and 2003 (**IV**). In both years field trials were performed at 2-6 locations, where 28 infected seed lots, most of which were heavily infested, were tested: 2+2 of winter wheat, 2+3 of spring wheat, 7+7 of barley and 3+4 of oats each year, respectively. The effects of the developed aerated steam treatment method were compared with those of chemical treatments and with untreated control (**IV**). The treatments during 2002 were performed in the fluidised bed laboratory device (**I**), but in most of the experiments in 2003, the treatments were carried out in the large-scale demonstration treatment system with a treatment capacity of 1.3-2 tons/hour (**IV**), see Fig. 4. Statistical tests of results from these evaluations confirmed that the treatment effects, except for loose smut in barley, are equivalent to those obtained by using chemical seed dressing, both concerning

the disinfestation effect and concerning the harvest yield obtained from the different plots (**IV**).

During 2003, evaluations were also performed in Norway in collaboration with Høgskolen i Hedmark (Sund & Myromslien, 2003; Tobiasson *et al*., 2004). Three seed lots of each of barley, oats and spring wheat of Norwegian cultivars infected with common pathogens were tested: *Fusarium* spp., *D. teres* and *D. graminea* in barley, *Fusarium* spp., *D. avenae* and *U. avenae* in oats and *Fusarium* spp. and *S. nodorum* on wheat. Treatments were performed in the fluidised bed laboratory device (**I**) and treated and untreated seeds were tested in laboratory germination and health tests (ISTA, 1996) and in field trials. It was concluded in accordance with previous experience that the treatment effects obtained from the developed treatment method were very good (Sund & Myromslien, 2003; Tobiasson *et al*., 2004).

A summary of the treatment effects obtained in the evaluations on infected cereal seed with the method developed is shown in Table 2.

Table 2. *Results obtained in evaluation of the treatment method for cereal and rice seed*

Crop	Pathogen	Common name	Results close to or as good as chemical	Better than untreated, less good than chemical	Insufficient results	Evaluations on which the classifications are based (see note below)
Wheat	*Tilletia caries*	Common bunt	X*			D,S
(winter and	*Septoria nodorum*	Leaf and glume blotch	X*			D,S,N
summer)	*Fusarium* spp.	Fusarioses	X*			D,S,N
	Fusarium nivale	Snow mould	X (S)	X (D)		D,S
	Fusarium culmorum		X			D
	Ustilago nuda var. *Tritici*	Loose smut			X	D
Barley	*Drechslera graminea*	Leaf stripe	X			D,S,N
	Drechslera teres	Net blotch	X*			D,S,N
	Fusarium spp.	Fusarioses	X[1)]*			S,N
	Bipolaris sorokiniana		X*			S
	Ustilago nuda	Loose smut			X	D,S
Oats	*Drechslera avenae*	Leaf spot	X (S)*	X (D)		D,S,N
	Fusarium spp.	Fusarioses	X[1)]			N
	Ustilago avenae	Loose smut	X*			D,S,N
Rye	*Fusarium nivale*	Snow mould		X		D
	Urocystis occulta	Stem smut	X			D
Triticale	*Fusarium nivale*	Snow mould		X		D
	Septoria nodorum	Leaf and glume blotch	X			D
Spelt	*Tilletia caries*	Common bunt			X	D
Rice	*Magnaporthe grisea*		X			D,I
	Cochliobolus miyabeanus		X			D,I
	Gibberella fujikuroi		X			D,I

[1)]Limited experience *Results obtained in evaluation of large-scale treatments

D = DEST project, Weinhappel ***et al*** ., 2000; DEST, 2001; Krauthausen ***et al.*** , 2002; **I**; Forsberg, 2001a, b, 2003.
S = Sweden 2002 (unpublished) and 2003 (**IV**),
N = Norway 2003 (Sund & Myromslien, 2003; Tobiasson ***et al.*** , 2004),
I = Italy 2002 (Titone ***et al*** ., 2003) and 2003 (unpublished)

For spelt wheat, the threshing of the seed lots was incompletely done and the seeds were not fully separated from the ears. Therefore, the *Tilletia* spores were well insulated from heat exposure within the ears. For rice, see Section 5.3 “Experience from other crops” below.

Also the effect against seed-borne *Tilletia contraversa*, dwarf bunt, has been tested using methods described by Johnsson (1991). Infection of a plant shoot with *T. contraversa* occurs when spores germinate in the presence of light and then infect the plant when it is emerging through the soil surface. Sowing infected seeds does not give infected plants, since the spores cannot germinate in the absence of light. In subsequent years, however, thanks to tilling, the spores reach the soil surface where they can infect new crops as a soil-borne infection. In order to test seed-borne infection, the seeds that are subjected to testing should be distributed on the ground so that emerging plants get into contact with the spores germinating on the spread seeds. The effect of thermal treatment against seed-borne infection with *T. contraversa* was tested in field tests located in Uppsala and Västerås. In the tests, no infection was achieved even from untreated infected seeds which is why we still don't know whether the method is effective against seed-borne dwarf bunt. However, since the spores, as for *T. caries*, are situated externally on the seed surface, it is likely that the treatment would affect *T. contraversa* spores similarly as for *T. caries* spores.

5.2.4 Temperature interval where effective treatment is achieved

The width of the temperature interval where effective treatments are achieved, here called the "treatment window", is an important property influencing the possibility for successful treatment when the heat tolerance varies within a seed lot. The "treatment window" can be defined in many ways. One way to define it may be "the optimum treatment temperature minus the highest temperature where the infection rate is significantly higher than at the optimum temperature". In some of our experiments, a sufficient number of treatment temperatures have been tested for determination of the width of the "treatment window" according to this definition (unpublished). Most of the tests were performed using a 5-minute treatment duration and with seed at 12-15 % mc. Treatments were performed either in a thin layer with relative air humidities around 95 % or in a fluid bed using air humidities near 90 %. The typical values for tested crop-pathogen combinations according to the above definition of the "treatment window" are shown in Table 3.

The values can be regarded as representative for each type of infection, except for *D. graminea*, where only one complete determination of "treatment window" has been made. Some factors influencing the efficiency of the treatment, and therefore also influencing the width of the "treatment window", can be identified or assumed: ***1.*** *Variation of treatment parameters.* For example, imprecise control of treatment parameters (temperature, air humidity, etc.) and non-uniform embryo and pathogen heat tolerance increases the standard deviation of the lethal temperature for seed and pathogen. See Fig. 13. ***2.*** *Air humidity.* As discussed above (5.2.1) and in **I**. ***3.*** *Seed storage history.* For aged seed, the heat tolerance is lowered for the seed, but not necessarily for the pathogen (**III**). ***4.*** *Treatment duration.* Correctly chosen treatment duration increases the selectivity for intensive exposure of the pathogen (**V**). ***5.*** *Seed mc.* Dry seeds resist higher temperatures whereas the pathogen is rendered sensitive by wetting obtained through the treatment (**V**). ***6.*** *Location of the pathogen inoculum.* If the inoculum is situated deep in the seed, the possibilities for selective treatment with intensive exposure of the pathogen with reduced exposure of the embryo are reduced (**I**;**V**). This is the case particularly for

U. nuda and *U nuda* var. *tritici* (DEST, 2001; **IV**). In some cases also *Fusarium* infection might penetrate deep into the seed (Scheinpflug & Duben, 1988). However, since from own experience *Fusarium* spp. in wheat often seems to have a low heat tolerance, this does not necessarily affect the possibility for effective thermal pathogen control. ***7.*** *Variation in seed shell thickness.* This is assumed to be the reason why the variation in heat tolerance is normally larger for oats seed than for barley, and that the variation seems to be even lower for wheat (Fig. 10).

No investigations of the variation in heat tolerance of the pathogen within a seed lot have been performed. However, for small seed samples, the values given for the ”treatment window” seem to be typical for the respective pathogen. The limited experiments performed do not indicate a large variation since the heat tolerance of the pathogen seems to be well correlated with the heat tolerance of the seeds within the seed lot, indicating a constant ”treatment window” for small sub-samples within a seed lot. The correlation in heat tolerance between the seed and the pathogen for small samples also is logical since they have a similar handling and storage history. Unpublished experiments performed with representative samples of large seed lots with documented variation in heat tolerance confirm this assumption.

Table 3. *The width of the interval of treatment temperatures that gives satisfactory disinfestations of seed from pathogens without affecting germinability, also called the “treatment window”, for the diseases where this was tested*

Crop	Pathogen	Width of the "treatment window", °C
Wheat	*Fusarium culmorum*	6
	Fusarium spp.	6
	Tilletia caries	6
	Septoria nodorum	6
Barley	*Drechslera teres*	4
	Drechslera graminea	1-2*
	Bipolaris sorokiniana	4-9
	Fusarium spp.	No data
Oats	*Ustilago avenae*	4
	Drechslera avenae	4
	Fusarium spp.	No data

*Limited data

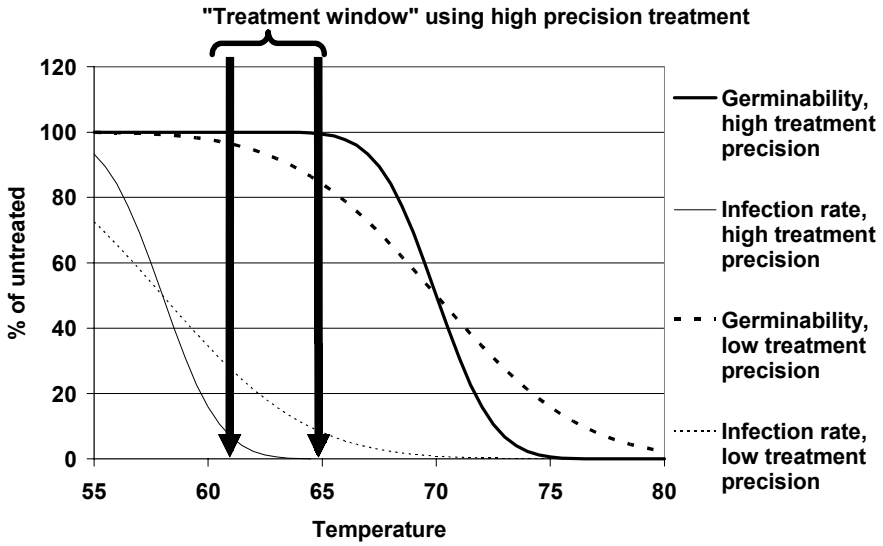

Fig. 13. The variation in treatment parameters influences the efficiency and the width of the interval of temperatures where good treatment effects are achieved, here also called "treatment window". In the example, high precision treatment (σ=2) gives a window of 5 °C whereas the low precision treatment (σ=5) does not permit any effective treatment without lowering germinability.

5.2.5 Seed storage influences on thermal seed treatment efficacy

As discussed above in Section 5.1.1, seed ageing is an important factor influencing the thermal treatment effects. These aspects were investigated also concerning pathogen effects for model seed lots of barley and oats (**III**).

1. *Long-term storage reduces the heat tolerance* of the seed and therefore also the maximum exposure intensity permitted that does not affect seed germinability (**III**). If a pathogen is aged similarly to the seeds, the reduction in heat tolerance of the seed would not cause any problem since the ageing of the pathogen would increase the efficacy of treatment at low temperatures. However, in investigations of barley seed infested with *D. teres* it was shown that for this particular pathogen, the viability was not reduced to the same extent as the viability of the infected seeds for storage at room temperature (about 20 ºC) for up to six years between harvest and aerated steam treatment (**III**). In fact, tendencies were observed that seeds rendered weaker by ageing seemed to be more susceptible to infection. Therefore, for seed stored at room temperature infested with such diseases, treatment should be performed preferably within one year after harvest of the seed and that long-term storage risks to reduce the treatment effect (**III**). However, for storage at lower temperatures and lower seed mc, the influence of storage on the efficacy of the disinfestation treatment effect would be reduced.

2. *Heat treatment causes accelerated ageing* of the seed. If the ageing caused by the treatment would be pushed close to the limit giving full germinability, supplementary storage would give a risk for lowering of germinability. This was tested in experiments (**III**) with one barley and one oats seed lot, stored for 0.5 –

2.5 years respectively at room temperature, were treated with aerated steam and then stored for another 17 months at room temperature (about 20 °C) after the heat treatment. After the complete storage period, the emergence and infection rate of plants sown from the subjected seeds were recorded in greenhouse tests. It was observed that the stored seeds could still perform well, although the infection rate of plants sown from the seeds stored 17 months after treatment was higher than for plants sown just after the treatment. This indicates either increased aggressiveness of the pathogen or, more probably, increased susceptibility of the seed to infection as the viability is reduced by ageing. This also is analogous to experiences from treatment of aged seed, as mentioned above (**III**). It was noted in these tests that seeds treated at the highest temperatures not affecting germinability before the storage had delayed emergence and weaker plants after 17 months of post-treatment storage whereas those treated at lower temperatures seemed to be unaffected by the storage. If post-treatment storage for several years would be required, reduced treatment temperatures would increase seed longevity. However, this would also increase the risk of plant infection for seed lots having a narrow ”treatment window”. Treated seed lots could thus be regarded as a fresh product and for storage at room temperature they should preferably be sown within one year after treatment (**III**). However, just like for storage preceding the treatment as discussed above, for storage at lower temperatures and lower seed mc, the influence of storage on the efficacy of the disinfestation treatment effect would be reduced.

5.3 Experience of treating seeds from other crops than cereals

The effect of aerated steam treatment has also been tested on a number of seeds from non-cereal crops. The results obtained from the tested crop-pathogen combinations are summarized in Table 4. The results were obtained from treatments where the optimum temperatures were determined in pre-tests of the individual seed lots (**I**). The treatments were performed using a fluid bed or thin layer laboratory treatment systems (**I**) and the treatment strategy (batch size, air humidity, treatment and cooling times and air flow) varied depending on species.

Table 4. *Summary of the results obtained from experiments with various non-cereal species of seed, treated using the developed aerated steam laboratory devices. The listed treatment effects were obtained with seed germinability equivalent or superior to the germinability of the untreated control*

Crop	Pathogen	Disease control	Yield effect	Kind of test	Reference
Cabbage	*Alternaria brassicicola*	II		Laboratory	Unpublished
	Xanthomonas campestris	III		-"-	-"-
	Xanthomonas campestris	I		Greenhouse	
Carrot	*Alternaria radicina*	II		Laboratory	-"-
	Alternaria dauci	II		-"-	-"-
	Xanthomonas campestris pv. *carotae*	I		Greenhouse	-"-
Onion	*Botrytis aclada*	III		Laboratory	-"-
	Stemphylium	II		-"-	-"-
Parsley	*Septoria*	C+	C	Field, 1999-2002	-"-
Pea	*Ascochyta pisi*	II		Laboratory	-"-
Red clover	*Phoma medicaginis* var. *Pinodella*	C+	C+	Field	Lager & Johnsson, 2002
Rice	*Magnaporthe grisea*	C	C	-"-	Titone *et al.*, 2003
	Cochliobolus miyabeanus,	C	C	-"-	Titone *et al.*, 2003
	Gibberella fujikuroi.	C	C	-"-	Titone *et al.*, 2003
Tomato	*Pseudomonas syringae* pv. *tomato*	I-III[1)]		Greenhouse	Tinivella, 2001
	Xanthomonas campestris pv. *vesicatoria*	I-III[1)]		-"-	-"-

I Better than untreated
II Good effect
III Complete eradication

When compared with chemical treatment:
C Equivalent with chemical treatment
C+ Better than chemical treatment

[1)] Very low control infection

6. Conclusions

- For good efficacy of seed sanitization by aerated steam treatment, a relative air humidity exceeding 85 % is necessary.
- For seed lots subjected to aerated steam treatment, a treatment duration can be found that gives optimum pathogen sanitization with maintained germinability.
- Quick heating with humid air for a short time immediately followed by rapid cooling gives a partly selective heating of external layers of the seed where most of the important cereal seed-borne pathogens are located.
- By taking the above aspects of the heating process into account, the error in prediction of germinability found by using the Ellis & Roberts viability equation for seeds treated with quick heating using aerated steam can be considerably reduced.
- By taking the above aspects of the heating process into account, the Ellis & Roberts viability equation can also be used for prediction of post-treatment pathogen viability for seeds treated with quick heating using aerated steam with a low error.
- Aerated steam treatment can successfully control cereal seed-borne diseases when these are situated close to the seed surface.

- Tolerance to high temperatures varies among species and seed lots depending on genetic factors and the production and storage history of the seed.
- Optimum temperature for thermal treatment of a seed lot can be found by pre-testing procedures.
- Tolerance to high temperatures varies within seed lots, and pre-tests of representative samples of a seed lot can be used for analysis of the variation in heat tolerance within the seed lot.

- Efficacy of aerated steam seed treatment is reduced with increased time in storage before treatment of the seed. For seed storage at low mc and temperature, longer pre-treatment storage time could be permitted without affecting the efficacy of the aerated steam seed treatment.
- Efficacy of aerated steam seed treatment is reduced with increased time in storage after the seed treatment. For seed storage at low mc and temperature, longer post-treatment storage time could be permitted without affecting the efficacy of the aerated steam seed treatment.
- For optimum effect, for storage at room temperature, the storage length should preferably be limited to one year before and one year after the treatment.

- Aerated steam seed treatment is capable of controlling many important seed-borne diseases on other crops than cereals.

7. References

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8. “The choice of the sciences”

“La découverte d'un mets nouveau fait plus pour le bonheur du genre humain que la découverte d'une étoile.”

“The discovery of a new dish does more for the happiness of mankind than the discovery of a star.”

Le choix des sciences

“Ne poursuivons plus la gloire:
Elle vend cher ses faveurs;
Tâchons d’oublier l’histoire:
C’est un tissue de malheurs.
Mais appliquons-nous à boire
Ce vin qu’aimaient nos aïeux.
Qu’il est bon, quand il est vieux! (*Bis.*)

J’ai quitté l’astronomie,
Je m’égarais dans les cieux;
Je renounce à la chimie,
Ce goût devient trop coûteux.
Mais pour la gastronomie
Je veux suivre mon penchant.
Qu’il est doux d’être gourmand! (*Bis.*)

Jeune, je lisais sans cesse;
Mes cheveux en sont tous gris:
Les sept sages de la Grèce
Ne m’ont pourtant rien appris.
Je travaille la pareses:
C’est un aimable péché.
Ah! comme on est bien couché! (*Bis.*)

J’étais fort en medicine,
Je m’en tirais à plaisir:
Mais tout ce qu’elle imagine
Ne fait qu’aider à mourir.
Je préfère la cuisine:
C’est un art réparateur:
Quel grand homme qu’un traiteur! (*Bis.*)

Ces travaux sont un peu rudes,
Mais sur le decline du jour,
Pour égayer mes études,
Je laisse approcher l’amour.
Malgré les caquets des prudes,
L’amour est un joli jeu:
Jouons-le toujours un peu. (*Bis.*)”

Poème

“Let us fame no more pursue,
For she sells her favours dear;
History we’ll forfeit too,
With her string of tales so drear.
Like our ancient forbears who
Drank mightily when nights were bold,
Let us drink a wine that’s old. (*Twice*)

I have left Astronomy
With her highways in the sky;
Chemistry is not for me,
The cost is far too high.
But for dear Gastronomy
I feel love I know is true.
Gourmandise, I worship you! (*Twice*)

Reading did I never cease
Till my hair turned steely grey;
Yet the sages that were Greece
Had not much of note to say.
Now I spend my days in peace,
Learning laziness instead.
Ah, what blizz to lie in bed! (*Twice*)

I was once a doctor grave,
Then I bade my drugs goodbye.
Drugs and physics do not save,
Only help a man to die.
So to food my heart I gave
Cooking does much more than books:
There are no better men than cooks.
(*Twice*)

This my work is somewhat rude,
But as night invades the sky,
Lest melancholy should intrude,
I let love come stealing nigh.
For despite the sharp-tongued prude,
Love’s a pretty game to play:
Let us play it while we may! (*Twice*)”

Jean-Anthelme Brillat-Savarin (1825a, b)

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