Seed treatments against fusarium in organic spring wheat

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Abstract
The results of 2003 and preliminary results of 2004 of a research on non-chemical seed treatments against fusarium in organic spring wheat are presented. Treatments in 2003 were hot water treatment, electron beam treatment and grading. In 2004 seeds were treated with hot water and aerated steam. Trials were sown at different dates (2003) and different sowing densities (2004). We looked at the effect of the treatments on seed infection, seed emergence in the field and grain yield. Also the development of the disease from the infected seeds to higher plant parts was studied. In 2003 the hot water treatment was highly effective for the early sowing date. A feasibility study also showed its practical and economical potential. In 2003 hot water treatment was executed at laboratory scale. Seeds treated at "industrial" scale in 2004 did not confirm these positive results. This might be due to technical problems with the application of this method at larger scale (and if so, could be overcome with more experience). In 2004 the aerated steam treatment gave good results, but as this treatment was applied at laboratory scale, future trials at "industrial" scale are needed to confirm these results.

Keywords: *Fusarium* spp, *Microdochium nivale*, Spring Wheat, Seed treatment, Organic Farming

Introduction
In the Netherlands the organic spring wheat seed harvest contains moderate to high levels of fusarium disease in one out of two years (Agrifirm, major Dutch organic cereal seed producer, pers. comm.). To be able to supply all farmers with sufficient seeds, batches of sub-optimal quality are put on the market. Dutch seed regulations allow a contamination with fusarium of up to 25% in certified seed, but between 10 and 25% the seed label should state that seed treatment is required. However, organic cereal seeds are not treated, because of lack of available methods.

Fusarium disease of wheat may be caused by a number of fungi. In the Netherlands *Fusarium graminearum* is the dominating species, followed by *F. culmorum* and *Microdochium nivale* (Waalwijk et al., 2003). When seeds are infected these may show no or poor germination with weakened seedlings. Such seedlings may overcome the disease when weather conditions after sowing are good, but in rainy and cold springs a considerable number of seedlings of infested seeds are lost. In such years, seed lots with a contamination of 20-25% result in poor crop establishments. The current practice of organic farmers is to increase seed density (e.g. sow 20% more if seed lot contains 20% fusarium) and to postpone seeding until the end of March, when temperatures are higher.

These practices increase costs (more seeds) and reduce yield (shorter cropping period). Despite these measures, farmers too often are confronted with disappointing crop stands. Therefore a group of farmers asked the authors to study seed treatments, which are appropriate to organic farming and economically viable. Seed treatments are compared at different sowing dates (in 2003) and seeding rates (in 2004). We also studied the possible effect of seed contamination with *Fusarium* spp. on the amount of fusarium in the harvested grain. This information is important to determine whether a low amount of seed infection may be tolerated or not.

Seed treatments against fusarium
In literature thermal treatments show the most promising results. Before the sixties of the last century, when the use of synthetic fungicides became wide spread, cereal seeds were treated with hot water. In the Netherlands knowledge on the practical application of this method disappeared with the people who applied it. In a recent research in Switzerland Winter et al. (1998) applied this method at farm scale level. They submerged 25 kg sacks of seeds in a container, regularly used for cheese making, with water of 45°C for two hours and afterwards dried the seeds back with forced air of 35 °C with equipment available at any commercial farm. This method proved to be effective against fusarium. However, when this method would be applied at

a larger scale (seed company level) drying back large quantities of seeds in a short period may be a drawback. Forsberg (2001) proposed the aerated steam method. In this method seeds are exposed to moist air with a high temperature (>60°C) for a few minutes. Because seeds are not immersed in water, drying is not a problem. The high temperature also may damage the seeds and hence the exact temperature and exposure time should be determined in a pre-test for each separate seed lot. A prototype treatment system with a capacity of 1.3 tons/hour is operative in Sweden.

Besides these treatments the German company E-ventus has commercially developed the electron beam treatment (e-dressing) (www.e-ventus.de). This method is based on treating the seeds with low-energy electrons and was developed during the 1980's for control of seed-borne fungal pathogens (Burth et al 1991). In recent years the method was developed for large scale treatments of cereal seeds (Röder and Schröder 1998).

According to Dutch organic farmers grading might be a solution. This is supported by Hare et al. (1999) who showed that in fractions with bigger seeds less seeds were contaminated with *F. culmorum*. Grading also implies higher losses during seed cleaning and therefore an increase in seed price. Other options would be applications of beneficial micro-organism by seed treatment (Harman 1991).

Research in 2003

The project started in 2003 and continued in 2004. The research consisted of a field trial and a study on the practical and economic feasibility of the best treatment of 2003. The field trial was located at an experimental field station, that was recently converted to organic (conversion started in 2001). The first year the field trial was sown in a complete block design with three replicates, the second year the trial was sown in four replicates. All seeds were of the spring wheat variety *Lavett,* which is grown by most organic farmers in the Netherlands. Normal sowing rate for organic spring wheat is 180 kg/ha or 475 plants/m^2.

Treatments in 2003

In 2003 we compared hot water treatment, seed application of a commercially available antagonist (*Bacillus subtilis*), grading seeds (fraction >2.5 mm and >2.8 mm, normal fraction >2.4 mm) with untreated seeds of the same seed lot (23% fusarium), sowing the same seed lot at greater density (23% more seeds) a "healthy" seed lot (8% fusarium) and Farmers' seed (farmer multiplication for his own use). The trial was sown at two different sowing dates (18 March, 11 April). Also the seed supplier offered us a separate lot of seeds which were treated with electron beams. This lot together with its untreated control (32% fusarium) was added to the first sowing date. Hot water treatment was carried out at laboratory scale. Seeds were immersed in water of 35 °C for two hours (starting at the moment after immersion when water temperature reached 35°C again) and dried back for two hours with air of 40°C. *Bacillus subtilis* was applied by mixing 1 gram of the commercial product Terranal with 1 kg of seeds. Grading was done by hand using split sieves. For e-dressing seeds were sent to the German company E-ventus.

Analyses and field measurements

Infection rate of the sowing seeds and harvested grains were analysed in the laboratory both using a blotter test (de Tempe, 1958) and incubating seeds on Potato Dextrose Agar (PDA) Medium. The amount of *Fusarium avenaceum*, *F. graminearum*, *F. culmorum*, *F. poae* and *M. nivale* was quantified in seeds, in various plant parts before harvest and the harvested grain. For quantification of these fungi, real-time PCR using species-specific TaqMan primers and probes was applied (Waalwijk et al., in press).

In the field we observed seed emergence (number of plants/m^2 and a visual score/plot) and grain yield.

Table 1: Seed infestation with fusarium according to analysis in a blottertest of two different seed lots of the spring wheat variety Lavett before and after seed treatment.

Seed lot	Infestation with Fusarium (% of seeds)
Untreated control 1	24
Treatments	
hot water	0
Bacillus subtilis	29
Grading Fraction>2.5mm	28
Grading Fraction>2.8mm	33
Untreated control 2	32
Treatment	
E-dressing	21

Economic and practical feasibility study

Farmers, companies involved in seed treatment and manufacturers of the necessary equipment were interviewed.

Results in 2003

Seed treatments

According to the blotter test (Table 1) the treatment with hot water reduced the original fusarium infection of 24% to 0. In the PDA test we found that still a small amount (1,5%) of the seeds of the warm water treatment was infected with fusarium. The effect of e-dressing was too small to be of practical use. The other treatments had no effect on the fusarium infection. Results of the TaqMan analysis show that *Fusarium graminearum* and *Microdochium nivale* were present in the seeds (Table 2). In the early sowing the laboratory results were confirmed: number of plants/m² after hot water treatment was more than 40% higher than the untreated control (Figure 1), while the other treatments had no effect. In the late sowing date the hot water treatment still gave the best results, but differences were not statistically significant. This was because the germination of the other treatments had improved, while the hot water treatment remained as high as in the first sowing. This confirms that with better weather conditions during germination infected wheat seedlings are able to overcome the disease.

Table 2: Fusarium species present in hot water-treated and untreated seed according to TaqMan analysis

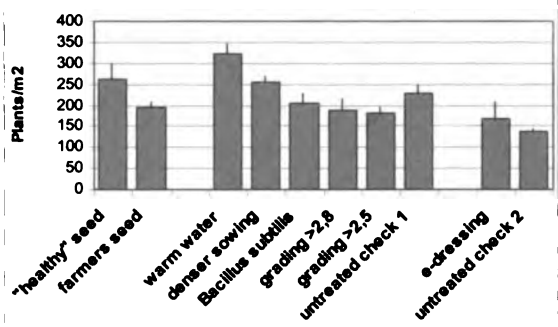
	Amount of fungus (picogram DNA/mg dry weight)	
Fusarium species	**Untreated control 1**	**Hot water treatment**
Fusarium avenaceum	1	0
Fusarium culmorum	0	0
Fusarium graminearum	119	34
Fusarium poae	0	0
Microdochium nivale	58	9

Although the hot water treatment improved crop stand, we did not find differences in yield between seed treatments at harvest time. Between sowing dates we did find a considerable difference: the average yield of 8500 kg/ha in the early sowing was 1600 kg/ha higher than in the late sowing date. The climate in 2003 was exceptionally favourable for (organic) wheat production, resulting in high yields all over the country. This probably enabled the crop to recover from the poor stand in the beginning of the season.

Economic and practical feasibility of hot water treatment

Although in 2003 the hot water treatment did not result in higher yields, it did show other benefits. First of all a better stand in the early stage, which for organic farmers is very important because this helps to suppress weeds. Secondly it allows farmers to sow earlier, which indirectly may increase yield. Thirdly seeding rates could be reduced. Currently Dutch organic farmers sow at least 12.5% denser than the recommended seeding rate. With an effective hot water treatment this amount can be saved on expenditure on seeds and so if the hot water treatment would not increase seed costs with more than 12.5%, it would be economically beneficial.

Figure 1: Emergence (plants/m²) of different treatments after early sowing in 2004



Winter et al. (1998) suggest to submerge 25 kg sacks of seeds in containers used for small scale cheese making and ordinary hot air blowers for drying back the seeds. In the Netherlands this equipment is available for affordable prices on the second hand market and could be an option for farmers who do not count the economic value of the labour they invert. If labour costs are included, seed price would increase considerably more than the 12.5% we calculated above.

Another option in the Netherlands is using equipment which is used for hot water treatment of bulbs and onion sets. Commercial companies (in the Netherlands known as "bulb boilers") treat complete wooden crates of 1m³ in a container with hot water and use forced air to dry the bulbs back. A preliminary price estimate is about 12.5% of the current organic seed price. As these companies have no experience with wheat yet an exact price only can be established after practical experience.

Relationship Fusarium in seeds and in the field

For the detection and identification of fusarium species we only analysed the samples of the hot water treatment, the untreated control and the "healthy" seed lot, with respectively 0%, 23% and 8% fusarium (*Fusarium graminearum, Microdochium nivale*) in the seeds.

Using TaqMan, no *Fusarium* spp. were found in the ear or in the harvested grains. This can be explained by the dry weather during anthesis, not favourable for infections by *Fusarium* spp. In samples collected one month before harvest, *M. nivale* was present in the stembase, stem and in necrotic and green leaves. *F. graminearum*, found on seeds before, was not present on such plant parts. *F. culmorum* and *F. avenaceum* occurred in the stembase and in dead leaves. No clear treatment effects could be detected.

Research in 2004

The second year we only continued with the hot water treatment and added the Thermoseed™ (aerated steam) method to the trial. The hot water treatment is able to eliminate fusarium, but its adoption depends on its costs. One way to diminish the costs is by reducing the seeding rate. Therefore we added two different seeding rates to our research (normal = 475 and reduced = 350 plants/m^2). These treatments were applied to a seed lot with 16% fusarium and compared with the untreated control, this control sown 16% denser and a "healthy" seed lot.

The other important difference with 2003 was the execution of the hot water treatment. Based on our results of 2003 the organic seed trader decided to send two tons of seeds to a commercial "bulb boiler" for treatment. So we used these seeds, instead of the seeds treated at laboratory scale. Thermoseed™ was applied at laboratory scale by Acanova in Sweden. Laboratory and field measurements are identical to 2003

Preliminary results of 2004

At the moment of this publication only the field results are available. The Thermoseed™ treatment showed a better stand than the hot water treatment and the untreated control at both seed densities. This difference was statistically significant (Anova followed by LSD, $p<0.01$). The hot water treatment showed no effect compared to the untreated control.

The stand of the Thermoseed™ treatment sown at 350 plants/m^2 was equal to the stand of the untreated seeds sown at 475 plants/m^2. So, after application of this treatment one could reduce sowing density with 25%.

Discussion and Conclusions

In 2003 we found good results with the hot water treatment at the early sowing date. The trial of 2004 did not confirm these results. The better plant emergence in 2003 only was statistically significant at the early sowing date, when weather conditions were more unfavourable for germination. This confirms the knowledge that fusarium disease only causes problems under unfavourable germination conditions.

In 2004 the Thermoseed™ treatment had a positive effect on seed emergence, while the hot water treatment showed no effect. This contradictory result might be explained by a different way of executing the hot water treatment. In 2003 the seeds were treated at laboratory scale, while in 2004 the treatment was done at "industrial" scale. As this was done without prior experience a proper working protocol still had to be developed. The major difficulty that was encountered was drying back the seeds within an acceptable period. This might have caused damage to the seeds. If so, with more practical experience these difficulties probably will be overcome. The difference in results between the treatment at laboratory scale and practical scale underlines the importance of doing this type of research also at practical scale. As the Thermoseed™ treatment in 2004 also was executed at laboratory scale, it is still too early to draw conclusions from these results.

We also quantified populations of different *Fusarium* spp., for a part brought into the field with the seeds. Due to the unfavourable weather conditions in 2003 for these fungi, infection rates in the field were low and no fusarium was found in the ears. However, we found *M. nivale* at lower plant parts including green leaves and dead leaves. Leaves from plots sown with hot water-treated seeds tended to be less infected with this fungus. On the other hand we found more *F. culmorum* and *F. avenaceum* on the halmbase and leaves of such plants. However, such differences were statistically not significant, possibly due to the relatively low quantities of *Fusarium* spp. found and the variation between plots, estimated only for three replicates.

Ackowledgement

The European Regional Development Fund and the Dutch Ministry of Agriculture, Nature and Food Quality have made this research possible

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