Dry Heat Disinfection versus Seed Borne *Fusarium* – Six Years' Practical Experience

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Since 2001 the Agriculture and Agri-Food (AAFC) Seed Increase Unit (SIU) has eradicated *Fusarium graminearum* from >60 tonnes of Breeder seed using a method of dry heat disinfection developed by researchers with the Canadian Grain Commission and AAFC. To the best of the author's knowledge the SIU is the only pedigreed seed facility in Canada using this technique on a routine basis.

The data in this report is not from replicated scientific experiments but has been collated from legal documents: Certificates of Seed Analysis and Reports of Seed Analysis, which were obtained from accredited seed laboratories for certification purposes. Apart from the calculation of arithmetic means no statistical analyses have been performed on the data.

The AAFC Seed Increase Unit at Indian Head, Saskatchewan produces Breeder seed of AAFC-developed crop varieties. This Breeder seed is distributed to Select seed growers across Canada to initiate the production of pedigreed seed which is, in turn, planted by commercial farmers. The unit's current inventory comprises 269 varieties of 43 crop kinds including 183 varieties of cereal crops. These cereal varieties constitute a significant portion of commercial grain production in Canada.

The SIU is located in southeastern Saskatchewan where Fusarium Head Blight (FHB) is endemic. Seed borne *Fusarium* in Breeder seed became a major issue for the SIU in the mid 1990's when it was apparent that FHB was causing major economic losses to grain producers in several areas of Canada and that the causal fungi could be spread by infected seed.

The initial steps to control FHB in Breeder seed produced by the SIU were agronomic practices. Since 1996 these have included: seed treatment, multiple applications of foliar fungicides during the pre-anthesis to soft dough stages, mixtures of contact and systemic fungicides, fungicide application with double nozzles to target the heads of cereal crops, post emergent broadcast application of KCl fertilizer and reduced use of glyphosate herbicide.

Although agronomic practices have reduced FHB in Breeder seed to generally low levels they have been unable to eliminate seed borne infection in years favourable to disease development (Tables 1 & 2).

Fusarium Status Prior to Seed Disinfection

Before 2001 Breeder seed of cereals was tested only for the presence of *Fusarium spp.* In 2000, a year favourable to FHB development at Indian Head, all 23 lots of cereal Breeder seed produced by the SIU were positive for *Fusarium spp.* (Table 1). In 2001 conditions were less favourable for FHB still, 29 of 32 cereal lots were positive for *Fusarium spp.* Since 2000, 184 of the 210 retained cereal Breeder seed lots produced by the SIU have been positive for *Fusarium spp.* (Table 1).

In 2001 commercial seed labs in western Canada introduced testing for individual *Fusarium species* including *F. graminearum*. Since then all cereal Breeder seed lots produced by the SIU have been analyzed for both *F. gr.* and "other" *Fusarium spp.* Of the 187 retained cereal Breeder seed lots produced since 2001, 100 have tested positive for *F. graminearum* (Table 2). These 100 *F. gr.*- positive seed lots represent 53.5% of all retained cereal Breeder seed lots and 62.5% of the *F. sp.*- positive lots produced since 2001 (Tables 1 & 2).

Alberta *Fusarium graminearum* Management Plan

In 2002 the *Alberta Fusarium graminearum Management Plan* declared *F. gr.* a "regulated pest" and outlawed the importation or planting of cereal seed infected with *F. gr.* Coincidentally, the SIU began routine screening of Breeder seed for individual *Fusarium* species. Nine of the 29 *Fusarium*-positive Breeder seed lots produced at Indian Head in 2001 were found to be infected with *F. gr.* (Table 2).

It was immediately apparent that without an effective means of seed disinfection the SIU would be unable to meet the conditions of the Alberta *Fusarium graminearum* Management Plan. Breeder seed distribution to seed growers in Alberta was in jeopardy.

A Solution at Hand?

In early November 2001 Clear, Patrick, Turkington and Wallis (2001) presented a paper *Effect of Dry Heat Treatment on Fusarium graminearum* at the Canadian Workshop on Fusarim Head Blight (CWFHB). This paper described a method of dry heat disinfection versus seed borne *F. gr.* The research showed that *Fusarium graminearum* was eliminated from wheat seed after 15 days at 60˚C, 5 days at 70˚C or 2 days at 80˚C. *F. gr.* was eliminated from barley seed after 21 days at 60˚C, 9 days at 70˚C or 5 days at 80˚C. Germination rates in wheat were relatively unaffected by the treatments while barley heated at 80˚C showed a slight decrease in viability.

The Head of the SIU learned of the heat disinfection from the authors of this paper. By the end of November, 2001 SIU staff had treated samples from two Breeder seed lots with known FHB infection. 1 kg samples of Superb hard red spring wheat and AC Ranger six row barley were disinfected.

The SIU's method was similar to that of Clear et. al. with the exception that **a pretreatment phase** was added to minimize "cooking" of the seed. The treatment cycle had two distinct phases: 1) a 2 day pretreatment at 38˚C and 2) a 5 day disinfection at 70˚C. The pretreatment phase was designed to dry the bagged samples to <3% moisture content before raising the temperature to 70˚C. 38˚C is the maximum temperature recommended for drying commercial seed without damage to germination. The initial drying phase was added because moisture in the 1 kg bagged samples could not escape as readily as from the 200 seeds in open Petri plates used by Clear et. al. It was reasoned that higher moisture content in seed at the centre of the bags could cause seed damage by protein denaturation at 70˚C = "cooking".

Samples of both lots were taken: 1) before treatment, 2) after the initial 38˚C drying phase and 3) after the 70˚C disinfection phase. The samples we submitted to a local commercial seed lab where they were tested for germination and seed borne *Fusaria*. The results from these initial tests were very encouraging (Table 3).

Dry Heat Disinfection of Larger Seed Lots

Based on the results from the 1 kg wheat and barley samples, the SIU began a program of dry heat disinfection for cereal Breeder seed lots with known seed borne *Fusarium gr.*

Cereal Breeder seed is bagged in 15 kg units immediately after cleaning. To prevent contamination and for other practical reasons the seed was heat treated in the 15 kg bags. The facilities available for this were a large cabinet-type, forced-air sample dryer and a larger walk-in sample dryer. The smaller unit had electric heat with excellent controls and cross ventilation giving uniform temperature. However, its 400 kg batch capacity was insufficient to treat the amount of Breeder seed requiring disinfection. The larger shop-built, walk-in dryer was heated by a gas fired infra-red radiant emitter. It had a much higher batch capacity of 2.4 tonnes but poor air circulation in the chamber caused spatial temperature variability of 5˚C when operated at 70˚C. Both dryers were located in buildings >250 metres from the Seed Plant where Breeder seed was conditioned and stored so the seed had to be loaded onto a truck or trailer, hauled and manually unloaded and placed on racks or sample carts.

The larger walk-in dryer was chosen as the main heat treatment facility. To overcome the problem of uneven temperatures the carts on which the bagged seed was placed for treatment were manually rotated each day. To further compensate for uneven temperatures and the time taken up by daily movement of the seed the 38˚C drying phase and the 70˚C disinfection phase were lengthened by one and two days, respectively. Thus, the initial drying phase became 3 days followed by a 7 day disinfection phase for a total treatment cycle of 10 days.

During the winter of 2001/ 2002, 9 cereal Breeder lots (2398 kg) from the 2001 crop were disinfected in the walk-in sample dryer. These included 6 lots of spring wheat and 1 lot each of durum wheat, barley and oats. Testing by a commercial seed lab after disinfection confirmed that *F. gr.* was eradicated by the dry heat treatment (Table 4).

The following winter, 32 lots (16458 kg) of Breeder Seed produced in 2002 and 3 lots (1022kg) produced in 2000 were disinfected in the walk-in dryer. Heat treatment eradicated seed borne *F. gr.* from all but one lot; a 178 kg lot of oat seed from the 2000 crop had 1% *F. gr.* after treatment (Table 4).

During the winter of 2003/ 2004, 16 lots (3814 kg) from the 2003 crop were treated. *F. gr.* was eradicated in all (Table 4).

The first three years of dry heat treatment proved the effectiveness of the method. *F. gr.* was eliminated from 59 Breeder seed lots with a combined total of 23514 kg. While this was a significant accomplishment it pushed both staff and equipment capacity to the limit. It was time for an upgrade.

In the spring of 2004 the SIU acquired a new walk-in oven for heat disinfection. The oven is located in the Seed Plant and is accessible to the unit's forklift thereby greatly improving its ease of use. Its 3.6 tonne batch capacity is sufficient to keep pace with seed conditioning. Temperature control and cross ventilation within the chamber are excellent thus eliminating daily rotation of the seed and making it possible to reduce the disinfection phase by one day so that the treatment cycle could be shortened to 9 days. This oven enabled the SIU to incorporate heat disinfection as a routine procedure in its Breeder seed operation. Since purchase in 2004 the walk-in oven has been used to disinfect 60 seed lots with a combined total of 36407 kg. Its use has eliminated seed borne *F. gr.* from all seed lots excepting one 65 kg lot of barley in 2004 (Table 4). It has been a very valuable acquisition.

Effect of Dry Heat Disinfection on Seed Viability

To evaluate the effect of the dry heat treatment on seed viability, the initial germination of Breeder seed lots and their retention of viability over time were examined. Germination records of both treated and untreated lots were used.

The SIU is legally required to maintain current (annual) germination analyses for all Breeder seed lots on inventory. This provides a germination history of each lot from shortly after harvest until the lot is either expended or removed from inventory. Germination analyses were not routinely performed on untreated sub-samples from heat treated lots so direct comparison of germination histories of treated versus untreated seed from the same lots was not possible. However, it was possible to compare the initial germination and subsequent germination histories of treated versus untreated lots. For each crop kind arithmetic mean germinations were calculated based on years post-treatment or years post-harvest for treated and untreated lots, respectively (Tables 5, 6 & 7). Weather-damaged and sprouted lots were omitted because damaged seed is unsuitable for disinfection by dry heat, has substandard germination and loses viability rapidly under normal circumstances.

In most cases, germination testing indicated minimal effects of dry heat treatment on germination and retention of viability in ambient storage. After 3 years the mean germination of 5 disinfected wheat lots was only 0.4% lower than shortly after treatment (Table 5A). Similarly, after 3 years the mean germination of 10 untreated wheat lots was 0.4% lower than the initial post-harvest germination (Table 5B). Heat treatment did not damage the initial germination of wheat, barley or oats (Tables 5, 6 & 7). Viability of stored barley decreased more rapidly than that of wheat or oats for both treated and untreated lots (Tables 6A & 6B). Dry heat disinfection did not damage the viability or storage longevity of wheat, barley and oat seed lots with high initial germination. Dry heat treatment induced secondary dormancy in several seed lots. In these cases the germination 1 year post-treatment was >10% lower than the initial germination but recovered to near initial values by the third year (data not shown).

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Reference

Clear, R.M., S.K. Patrick, T.K. Turkington and R. Wallis. Effect of Dry Heat Treatment on *Fusarium graminearum* in seed. Proceedings, Canadian Workshop on Fusarium Head Blight. Ottawa, Ontario, November 3-5, 2001: 84-87. http://res2.agr/ca/ecorc/fusarium01/index.htm

Table 1. **Initial *Fusarium** status of retained cereal Breeder seed lots from the AAFC Seed Increase Unit, Indian Head, Saskatchewan**

Year	Lots positive for *Fusarium spp.**	Mean Infestation, %	Range of Infestation, %
2000	23: 23	5.1	1.0 - 13.5
2001	29: 32	1.2	0.0 - 7.0
2002	32: 32	3.3	0.5 - 12.0
2003	14: 34	1.1	0.0 - 2.0
2004	27: 30	2.0	0.0 - 5.5
2005	32: 32	3.0	0.5 - 9.0
2006	27: 27	5.5	0.5 - 20.0
sum	184: 210 (87.7%)	3.0	

* Includes: *F. graminearum, F. avenaceum, F. poae, F. culmorum, F. sporotrichioides, F. equiseti, F. acuminatum and F. proliferatum*

Table 2. **Initial *Fusarium graminearum* status of retained cereal Breeder seed lots from the AAFC Seed Increase Unit, Indian Head, Saskatchewan**

Year	Lots positive for *F. graminearum*	Mean Infestation, %	Range of Infestation, %
2001	9: 32	1.1	0.0 - 3.5
2002	22: 32	1.1	0.0 - 3.0
2003	12: 34	1.0	0.0 - 2.0
2004	12: 30	1.1	0.0 - 2.5
2005	21: 32	1.2	0.0 - 4.0
2006	24: 27	3.8	0.5 -17.5
sum	100: 187 (53.5%)	1.8	

Table 3. Effects of Dry Heat Treatment on 1kg Samples of Superb wheat and AC Ranger Barley, November 2001

Variety	Treatment	*Fusarium spp.* %	*Fusarium gr.* %	Germination %
AC Ranger barley	None	0.5	0.5	91
	2 days @ 38˚C	0.0	0.0	95
	2 days @ 38˚C+ 5 days @ 70˚C	0.0	0.0	93
Superb wheat	None	4.5	0.0	99
	2 days @ 38˚C	4.0	0.0	98
	2 days @ 38˚C+ 5 days @ 70˚C	0.0	0.0	96

Table 4. Fusarium status of cereal Breeder seed lots after dry heat disinfection

Year	Lots disinfected # (kg)	Lots positive for *F. spp.* after treatment	Lots positive for *F. gr.* after treatment
2000	3* (1022)	1	1**
2001	9 (2398)	2	0
2002	32 (16458)	8	0
2003	16 (3814)	NA	0
2004	12 (3116)	12	1***
2005	32 (20871)	12	0
2006	27 (12420)	NA	0
sum	131 (60099)	35: 88	2:131

* 3 lots from the 2000 harvest were disinfected during the winter of 2002 / 2003.
** 1 -178 kg lot of standard oats tested 1.0% *F. gr.* after treatment; 2.5% before.
*** 1 - 65 kg lot of six row barley tested 0.5% *F. gr.* after treatment: 0.5% before.

Table 5. Germination Histories of Wheat Breeder Seed Lots

A. Mean Germination of Heat Treated Lots, % (# lots used to calculate means)

Years Post-treatment

0	1	2	3	4
98.4 (43)				
96.1 (28)	95.25			
95.9 (14)		96.4		
96.4 (5)			96.0	
93.0 (1)				90.0

B. Mean Germination of Untreated Seed Lots, % (# lots used to calculate means)

Years Post-harvest

0	1	2	3	4	5
94.3 (19)					
94.3 (17)	94.1				
93.2 (11)		93.4			
93.1 (10)			92.7		
93.4 (5)				95.6	
93.4 (5)					91.2

Table 6. Germination Histories of Barley Breeder Seed Lots

A. Mean Germination of Heat Treated Lots, % (# lots used to calculate means)

Years Post-treatment

0	1	2	3	4
95.9 (13)				
96.25 (12)	89.9			
96.8 (5)		94.6		
97.5 (2)			89.5	
96.0 (1)				83.0

B. Mean Germination of Untreated Seed Lots, % (# lots used to calculate means)

Years Post-harvest

0	1	2	3
97.0 (6)			
96.6 (5)	95.4		
96.6 (5)		90.4	
99.0 (2)			81.5

Table 7. Germination Histories of Oat Breeder Seed Lots

A. Mean Germination of Heat Treated Lots*,% (# lots used to calculate means)

Years Post-treatment					
0	**1**	**2**	**3**	**4**	**5**
96.3 (14)					
96.1 (11)	93.7				
95.5 (6)		96.2			
95.4 (5)			94.6		
94.0 (1)				96.0	
94.0 (1)					95.0

B. Mean Germination of Untreated Lots*, % (# lots used to calculate means)

Years Post-harvest					
0	**1**	**2**	**3**	**4**	**5**
95.4 (16)					
96.3 (13)	94.8				
95.4 (9)		92.6			
96.0 (11)			95.5		
94.6 (5)				79.2	
94.6 (5)					87.2

* Does not include hulless oat varieties.