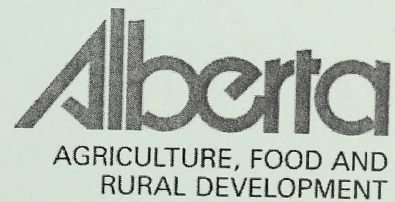


# **Alberta**

## *Fusarium graminearum*

### **Management Plan**







# **Alberta *Fusarium graminearum* Management Plan**

**Developed by the Provincial Fusarium Action Committee  
August 2002**

**Contact: Alberta Agriculture, Food and Rural Development  
Pest Risk Management Unit**

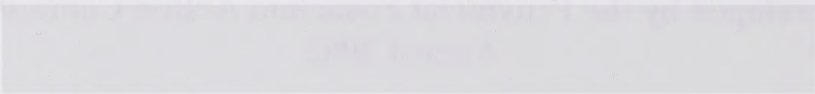
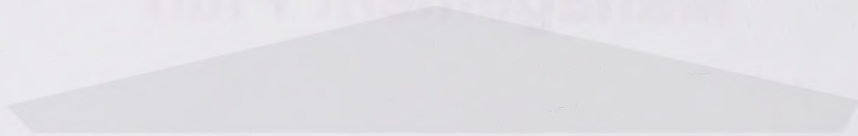
**Shaffeeq Ali**

Head, Pest Risk Management Unit  
Chair, Fusarium Action Committee  
Alberta Agriculture, Food Development  
304, J.G. O'Donoghue  
7000 – 113 Street,  
Edmonton, Alberta  
Tel: 780 422-4909  
Email: shaffeeq.ali@gov.ab.ca

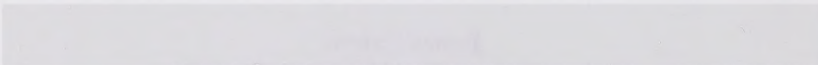
**James Calpas**

Provincial Integrated Pest Management Specialist  
Pest Risk Management Unit  
Alberta Agriculture, Food and Rural Development  
304, J.G. O'Donoghue Building  
7000 – 113 Street,  
Edmonton, Alberta  
Tel: 780 422-4911  
Email: james.calpas@gov.ab.ca

Alberta Fusarium Management Plan



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## Table Of Contents

<b>I. Background .....</b>	<b>3</b>
<b>II. Objectives .....</b>	<b>4</b>
<b>III. Regulatory Status.....</b>	<b>4</b>
<b>IV. Risk of the spread of <i>Fusarium graminearum</i> into Alberta .....</b>	<b>5</b>
<b>V. Mycotoxin production .....</b>	<b>5</b>
i. Importance to livestock.....	5
ii. Importance in food .....	6
<b>VI. Management plan rationale .....</b>	<b>6</b>
<b>VII. Management of <i>Fusarium graminearum</i>.....</b>	<b>7</b>
1. Cereal grains intended for use as seed.....	7
2. Management of <i>Fusarium graminearum</i> in cereal grains and cereal grain products intended for use as feed.....	8
3. Management of <i>Fusarium graminearum</i> infested fields.....	9
<b>VIII. Transportation of cereal grain.....</b>	<b>10</b>
<b>IX. Responsibilities .....</b>	<b>11</b>
1. Alberta Agriculture, Food and Rural Development (AAFRD).....	11
2. Agricultural Service Boards.....	11
3. Landowner/Occupant.....	12
4. Feed Lot Operators.....	12
5. Trucking Industry.....	12
6. Fusarium Action Committee.....	12
<b>Appendix I Biology of <i>Fusarium graminearum</i> .....</b>	<b>13</b>
<b>Appendix II Field Sampling for Fusarium Head Blight.....</b>	<b>16</b>
<b>Appendix III Protocol for Testing Cereal Seed.....</b>	<b>18</b>
<b>Appendix IV Protocol for Sampling Cereal Seed.....</b>	<b>19</b>
<b>Appendix V Management of <i>Fusarium graminearum</i> in feed cereal grains...27</b>	<b>27</b>
<b>Appendix VI Fusarium Action Committee Contact List.....</b>	<b>31</b>
<b>Glossary.....</b>	<b>32</b>





## I. Background

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Caused by the fungus *Fusarium graminearum*, fusarium head blight (FHB), is the most destructive fungal disease of barley and wheat in Canada. The occurrence and higher severity of FHB in cereal crops in western Manitoba and eastern Saskatchewan is cause for concern. The prevalence and severity of fusarium head blight in Manitoba and eastern Saskatchewan has caused major economic losses to producers and the grain export industry. *Fusarium graminearum* infection greatly decreases yield, seed quality and produces mycotoxins (deoxynivalenol and zearalenone). It is estimated that Manitoba now loses 50 to 100 million dollars annually in wheat and barley due to loss of yield, access to malt and hog feed markets, increased transportation costs associated with sourcing mycotoxin-free grain and other impacts on end-use processing. Further movement west by this disease would be disastrous for the grain producing regions of western Saskatchewan and Alberta.

Alberta is currently free from any fusarium head blight outbreaks caused by *Fusarium graminearum*. Trace levels of *Fusarium graminearum* have been found in Alberta in a few instances and actions have been taken to eliminate it. If it were to establish in this province, this fungal disease would become a major economic consequence to Alberta's cereal and animal feeding industries.

This management plan defines the prevention and control strategy to reduce the risk of *Fusarium graminearum* becoming established in Alberta. It provides the minimum acceptable standards/requirements for the management of this pest within the province.

## II. Objectives

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1. Prevent the establishment of *Fusarium graminearum* in Alberta.
2. Prevent the increase and spread of *Fusarium graminearum* should it be found in Alberta.

## III. Regulatory Status

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Alberta's Agricultural Pests Act is the legislative authority for enforcement of control measures for declared pests in Alberta. The Minister of Alberta Agriculture, Food and Rural Development is responsible for this Act however the enforcement is the responsibility of the Municipality. *Fusarium graminearum* has been a declared pest under Alberta's Agricultural Pests Act since 1999.

Pest inspectors may be appointed by the local municipality or by the Minister of Agriculture, Food and Rural Development. By virtue of the office, an Agricultural Fieldman is a pest inspector under the Agricultural Pests Act. Pest inspectors have the power to enter land at a reasonable hour, without permission, to inspect for pests and collect samples.

The owner or occupant of land has the responsibility of taking measures to prevent the establishment of pest on land, property and livestock and to control or destroy all pests on or in the land property or livestock.

Enforcement of pest control measures is the responsibility of the municipal authority; the Agricultural Fieldman is responsible for enforcing pest control measures in their municipality.

The control measures for *Fusarium graminearum* are specified in this management plan. It is important to understand that these control measures represent the minimum standard that is to be applied in all municipalities across the province. Municipalities can enhance the standard within their own jurisdictions.



#### **IV. Risk of the spread of *Fusarium graminearum* into Alberta**

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The presence of a virulent pathogen in sufficient quantity is a requirement for the development of disease. All survey information currently available demonstrates that *Fusarium graminearum* is not commonly found in Alberta.

A progressive field-to-field yearly spread of *Fusarium graminearum* could theoretically eventually bring large quantities of *Fusarium graminearum* into Alberta however this process would likely take 10 to 20 years. The long distance spread of wind borne ascospores in “spore clouds” into Alberta from eastern Saskatchewan and Manitoba is also improbable. Dispersal of ascospores occurs over relatively short distances. Ascospore survival is significantly reduced after exposure to the UV radiation that would be encountered by an airborne ascospore. It is expected that less than 1% ascospore survival would occur after 3 days of field exposure to natural UV radiation from the sun.

Alberta’s environment is not a barrier to the establishment of *Fusarium graminearum* in this province. Once the pathogen establishes it will readily overwinter on infected crop residue.

***Fusarium graminearum* is a seedborne pathogen and infected seed or feed grain represents the greatest risk of introducing *Fusarium graminearum* in quantities sufficient to cause disease development.**

#### **V. Mycotoxin production**

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*Fusarium graminearum* produces mycotoxins including deoxynivalenol (DON) and zearalenone. The presence of these mycotoxins reduces the marketability of grain. DON can cause reduced feed intake and food refusal. Livestock and poultry are susceptible to DON. Zearalenone has estrogenic effects, and depending on the concentration, ingestion can result in reproductive dysfunctions. Lightweight shriveled *Fusarium* damaged kernels, may contain high concentrations of the mycotoxin DON. Levels as high as 30 parts per million (ppm) in wheat and barley and barley have been detected.

##### **i. Importance to livestock**

In non-ruminants, such as hogs, contamination of feed grain with 1 ppm of DON can result in reduced feed consumption and consequently a reduction in growth. At high concentrations of 5 ppm or more, feed refusal can occur. Young pigs are more susceptible to the effects of DON and may exhibit feed refusal with dietary concentrations of less than 1 ppm. Most hog producers now have a zero tolerance for DON.

Adult beef cattle can tolerate higher levels of DON without known detrimental effects. Some studies have shown that beef cattle can feed on grain that has up to 12 ppm of DON, but calves may have problems at lower levels of contamination.

Agriculture and Agri-Food Canada guidelines for acceptable feed are 1 ppm for swine, dairy cattle and horses and 5 ppm for beef cattle, sheep and poultry.

## ii. Importance in food

The presence of DON will also affect the production of beer. The mycotoxin affects the taste of beer and causes gushing or excess foaming. Malting companies will reject barley lots suspected of containing detectable levels of DON. Most malting companies now have a zero tolerance for DON and test for DON before purchasing.

Bread making is also affected by the mycotoxin DON. The flour changes colour and the bread does not rise normally. The baking process does not destroy DON. Several methods, both chemical and physical, have been studied as potential methods of detoxifying DON. There is no easy way to reduce the toxicity of the contaminated kernels.

## VI. Management plan rationale

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The most desirable approach to managing *Fusarium graminearum* in Alberta is through a proactive program designed to prevent the entry of this pathogen in the province. This would require a virtual zero tolerance to all infected grain coming into the Province.

In an attempt to minimize the risk of the establishment of *Fusarium graminearum* in the province and to address the needs of all sectors of the industry a zero tolerance approach will not be applied to the entire industry. In light of Alberta's current feed barley deficit situation, the zero tolerance approach in feed grain is not feasible. There will be a zero tolerance for *Fusarium graminearum* in cereal seed. There will be management practices for handling cereal grain and cereal grain products including screenings and pellet feed used as feed that **have not** been tested and found to be free of *Fusarium graminearum*.

The long-term goal of this response plan is to prevent or delay the establishment of *Fusarium graminearum* in Alberta at least until *Fusarium graminearum* resistant wheat and barley varieties are developed.



## VII. Management of *Fusarium graminearum*

Where reference is made to a certificate of laboratory analysis certifying that that a grain sample was found to be free of *Fusarium graminearum*, it is recognized that this does guarantee that *Fusarium graminearum* was not present, only that it was not found. For this reason pre-plant fungicide seed treatments for *Fusarium graminearum* are also required for seed imported into Alberta.

### 1. Cereal grains intended for use as seed.

To prevent the movement of *Fusarium graminearum* infected seed and the introduction of *Fusarium graminearum* into Alberta fields from infected seed.

- a. A laboratory certificate, showing that the seed lot in question was tested and found to be free of *Fusarium graminearum*, must accompany all cereal grain intended for use as seed in Alberta.
- b. All imported cereal grain intended for use as seed in Alberta must also be treated with a registered fungicide, prior to planting, that includes the genus *Fusarium* on the label list of fungi that are controlled.
- c. It is recommended that cereal grain intended for use as seed that is grown in Alberta also be treated with a registered fungicide, prior to planting, that includes the genus *Fusarium* on the label list of fungi that are controlled.
- d. A certificate, of laboratory analysis, must accompany all lots of cereal grain intended for use as seed. This certificate must certify that a representative sample of the lot was tested according to the accepted protocol and found to be free of *Fusarium graminearum*. This certificate must be available for inspection at any time during the transportation and handling of the lot.
- e. The end user of the cereal grain intended for use as seed must maintain the reports of laboratory analysis certifying that each lot of cereal grain intended for use as seed brought in by the end user, was found to be free of *Fusarium graminearum*. These certificates must be readily available for inspection.
- f. Cereal grain intended for use as seed that tests positive for *Fusarium graminearum* must be properly and effectively disposed of. These lots may be sold for food or feed but treated (fungicide or insecticide) lots must be disposed of and buried in a landfill. If the cereal grain is sold as feed it must be handled in accordance with Section VII-2.



- g If cereal fields are found to have been planted with cereal seed infected with *Fusarium graminearum*, the fields will be subject to the control measures outlined in Section VII-3 – Management of *Fusarium graminearum* infested fields.

NOTE - field control strategies are initiated as soon as a field infection or infestation is detected.

## 2. **Management of *Fusarium graminearum* in cereal grains and cereal grain products intended for use as feed.**

The best management practice to prevent the establishment of *Fusarium graminearum* in Alberta through feed grain is to ensure that all out of province feed grain has been tested and certified to be free of *Fusarium graminearum* before being allowed for use the province. With Alberta's feed deficit situation, feed importation is necessary and testing of all feed is impractical. To minimize the risk of spread of *Fusarium graminearum* through imported grain it is imperative that the following management practices be adopted.

**These management practices are the control measures to be followed for handling *Fusarium graminearum* infected cereal grain and cereal grain products (including screenings and pellet feed) at unloading, loading, storage and feeding sites. The definition of "grain" in the following control measures includes cereal grain (including corn) and cereal grain products (including screenings and pellet feed).**

These measures apply only to those operations handling feed grain that **has not** been tested and found to be free of *Fusarium graminearum*. Operations that only handle fusarium-free feed grain do not have to follow these control protocols as long as they retain laboratory certificates for all lots of grain handled by their facilities, demonstrating that the grain was found to be free of *Fusarium graminearum*.

- a. Out-of-province feed grain must not be stored in uncovered piles or in contact with the soil.
- b. All loading/unloading sites handling out-of-province feed grain must use both a wind fence and drop sock when loading or unloading grain to prevent grain or grain dust blow-off from the loading/unloading site. A covered loading/unloading facility is preferred.
- c. Out-of-province feed grain must be unloaded in such a manner such that spillage does not occur. Grain must not come into contact with the soil.

- d. All modes of transport of out-of-province grain must be securely covered to prevent spillage of grain during transport.
- e. All transport vehicles/units hauling out-of-province grain must have the box/trailers/cars thoroughly swept clean of any residual grain and gates closed before being allowed to leave the unloading site. The swept material must be placed in a compost site so that the material will reach a temperature of 60 to 70 °C for two weeks. This ensures that any *Fusarium graminearum* is killed.
- f. Out of province grain must not come in contact with the soil during feeding. Range feeding livestock is not recommended. Bunk feeding is the preferred method.
- g. If grain is spilled at anytime during the feeding/handling process it must be completely recovered and composted so that the material will reach a temperature of 60 to 70 °C for two weeks. This ensures that any *Fusarium graminearum* is killed.

### 3. **Management of *Fusarium graminearum* infested fields.**

If *Fusarium graminearum* is found in a cereal/grass crop, the following procedures will apply immediately.

- a. Prior to maturity, infested crops must be cut and ensiled immediately. Ensure that the load is securely covered to prevent spillage during transport. The silage can then be fed to cattle in such a way as to prevent spillage of the silage onto the soil.
- b. In mature crops, the grain can be harvested and fed to cattle. Ensure that the load is securely covered to prevent spillage during transport.
- c. Remove any crop residue from all equipment before leaving an infested field.

- d. Incorporate cereal/grass residue in the soil after harvest. If soil erosion is a problem on the land, cultivation may be delayed to just prior to planting a non-cereal/non-grass crop in early spring.
- e. The following season, use shallow tillage or direct seeding of non-cereal/non-grass crops to avoid bringing infested crop residue to the surface.
- f. Control volunteer cereals and grassy weeds on infested land, including headlands.
- g. Keep *Fusarium graminearum* infested land in non-host crops such as canola, alfalfa, clover or pea for a minimum of three years following the detection of the disease.
- h. Do not use corn in rotation with small grain cereals. Corn is also a host of *Fusarium graminearum*, which causes seed rots, seedling blight, root rot, stalk rot and ear rot. Research has shown that *Fusarium graminearum* and mycotoxin levels in harvested grain are higher for small grain cereals grown in rotation with corn.
- i. After a three-year rotation, disease-free cereal seed from a cultivar that has low susceptibility to, or resistance to *Fusarium graminearum* and is treated with a recommended fungicide can be planted.

### **VIII. Transportation of cereal grain.**

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- a. All cereal grains, including seed, transported in the province must be securely covered. No grain is to be allowed to blow off the vehicle while in-transport. Vehicles with unsecured loads of grain will be impounded until the load is securely covered.
- b. If imported cereal grain intended for use as seed, is found to be either not treated with a fungicide or not accompanied with a certificate, the seed will be impounded. The seed will remain impounded until such time as a certificate of laboratory analysis is produced or the grain is treated or both.
- c. Cereal grain intended for use as seed that is trans-shipped through Alberta is exempt from the requirement of a certificate of laboratory analysis if the load is not handled or redistributed from a facility in Alberta and the load is securely covered.



- d. Cereal grain intended for use as feed that is trans-shipped through Alberta and is handled for redistribution at a facility in Alberta must be handled in accordance with the management practices for feed grain (Section VII-2).

## **IX. Responsibilities**

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### **1. Alberta Agriculture, Food and Rural Development (AAFRD).**

- a. Pest Risk Management Unit will co-ordinate the Alberta *Fusarium graminearum* Management Program.
- b. Pest Risk Management Unit will provide regulatory consultation and training.
- c. Prepare and provide technical information on *Fusarium graminearum* control recommendations to inspectors and field staff.
- d. Provide training in disease identification and management.
- e. Will evaluate the Alberta *Fusarium graminearum* Management Program as required.

### **2. Agriculture Service Boards.**

- a. Provide support and resources to the Agricultural Fieldmen in carrying out their duties.
- b. Agricultural Fieldmen will monitor their municipality for *Fusarium graminearum*, particularly in areas of trace infestation or around cattle feed lots
- c. Enforce control measures as necessary to meet the objectives of the Alberta *Fusarium graminearum* Management Program.
- d. Provide recommendations and information to farmers on disease prevention and control.
- e. Conduct field surveys and maintain records of infestations.

**3. Landowner/Occupant**

Observe and practice all management practices to meet the objectives of the Alberta *Fusarium graminearum* Management Program.

**4. Feed Lot Operators**

Observe and practice all management practices to meet the objectives of the Alberta *Fusarium graminearum* Management Program.

**5. Trucking Industry**

Observe and practice all management practices to meet the objectives of the Alberta *Fusarium graminearum* Management Program.

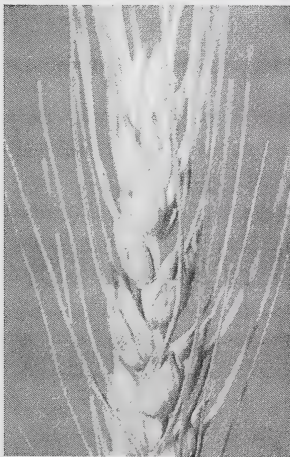
**6. Fusarium Action Committee**

- a. Provide a forum to represent the interests and views of the Alberta agriculture industry regarding the management of *Fusarium graminearum*.
- b. Recommend management strategies for *Fusarium graminearum* for inclusion in the Alberta *Fusarium graminearum* Management Program.
- c. Educate the Alberta agriculture industry about *Fusarium graminearum* and the threat that it represents to Alberta

## Appendix I

### Biology of *Fusarium graminearum*

The fungus overwinters mainly on infected crop residue (Gilbert and Tekauz, 2000, Tekauz et al. 2000) but can also be seed-borne. *Fusarium graminearum* is capable of causing seed rot, seedling blight and root rot in barley and wheat (Mathre 1997, Weise 1987) and seed rot, seedling blight, root rot, stalk rot and ear rot in corn (White 1999). In addition, seed to seedling transmission has been demonstrated in wheat and in corn (Duthie & Hall 1987, Golzar 1989, Halfon-Meir et al. 1979, and Kabeere et al. 1997). Duthie & Hall (1987) demonstrated significant positive relationships between levels of seed infection and the incidence of infection of tillers and stem bases. Transmission rates of 0.55 to 0.94% from seed to tiller or shoots were demonstrated under field conditions. The authors indicated that these transmission rates occurred under conditions that were not conducive to seedling blight and suggested that rates may be higher under more conducive conditions. Under greenhouse conditions Kabeere et al. 1997 demonstrated transmission rates of up to 52% from maize seed to seedlings. Under growth chamber conditions, Halfon-Meir (1979) found that pre-emergence mortality and incidence of diseased wheat seedlings were significantly correlated to the level of seed infection. The authors also found *Gibberella zeae*, the sexual stage of *Fusarium graminearum*, on infected portions of a few wheat seedlings. More recent studies have indicated the potential for seedling infection of several crop species from *Fusarium graminearum* infected head tissues (Chongo et al. 1999).



Although infected seed can cause seedling blight and root rot, it typically does not directly give rise to head blight symptoms in one growing season. Infected seed may help to establish the fungus in fields free of this pathogen as a result of infection of seedlings and cereal root and crown tissues, thus producing a source of infected crop residue for the following growing season. Thus, producers should to avoid planting seed that originates in areas where fusarium head blight caused by *Fusarium graminearum* is a problem. Effective seed treatment and the use of seed that is free or essentially free from infection should be used to help minimize the risk of seedling blight caused by this and other pathogens. Pathogen-free seed and the use of appropriate seed treatment will serve to reduce the risk of introducing *Fusarium graminearum* into uninfected fields via infected seed.



Head infections in wheat typically take place during flowering (anthesis) via wind-borne spores produced from infected crop residue. About 2 weeks later symptoms become visible on infected heads. Moist conditions resulting from high relative humidity, dew, rainfall or irrigation, and moderate temperatures during flowering are critical for infection to occur. Symptoms initially appear as dead, prematurely ripened portions of the cereal head. With severe infections, the entire head may prematurely ripen and there will be a brownish discoloration of the stem directly below the head. An orange or pinkish discoloration on affected portions of the head may also be apparent and is the result of spore production by the head blight fungus. After harvest infected kernels can range in appearance from shriveled and chalky white (called fusarium damaged kernels) to symptomless, depending on the time of infection.



Although typical symptoms in wheat are quite distinct they may still be confused with diseases, insects or physiological conditions that cause premature ripening of the plant. For example, take-all root rot destroys the root system causing the flow of water and nutrients to be cut off, resulting in premature ripening of the entire plant. The key difference for fusarium head blight is that the head is prematurely ripened but the rest of the plant is still green. Take-all causes premature ripening of the head and stem with a black discoloration of the roots and stem base. Furthermore, if the weather has been humid the fusarium head blight pathogen may also produce orange or pinkish mycelium and spore masses in crevices and on bases of affected spikelets of infected heads. These symptoms will help to distinguish it from other diseases or physiological conditions such as the premature

tip ripening observed in AC Barrie and other wheat varieties during 1999. Eventually small black fruiting bodies of the sexual stage of *Fusarium graminearum* may be produced on affected portions of heads. These structures will appear as discreet small black bumps or appear as a crust where their production has been extensive. However, there is potential to confuse these black fruiting bodies with sporulation by saprophytic sooty molds, which affect ripening plant tissue, giving them a dusty grayish-black appearance.

Head and kernel symptoms in barley are much less distinct and can be easily confused with diseases such as spot blotch, kernel smudge, or perhaps even hail damage. Typical symptoms in barley are premature ripening and a brownish discoloration of the affected portions of the head. As in wheat, orange or pinkish mycelium and spore masses may be produced in crevices and on

bases of affected spikelets. In addition, the black fruiting bodies of the sexual stage of *Fusarium graminearum* may be produced on infected heads and kernels. These tend to be more common in barley than in wheat.

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## Appendix II

### Field Sampling For Fusarium Head Blight

The Canadian Grain Commission surveys for *Fusarium graminearum* and other *Fusarium spp* from grain samples collected and tested annually across the prairies. With this survey information, we can identify localised FHB presence where more intensive in-crop surveys can be conducted. More intensive surveys could be conducted after suitable training by a combination of AAFRD Crop Specialists, local Agricultural Fieldmen, and individual plant pathologists.

#### Survey Protocol

Assess and collect grain head samples along a diamond-shaped path that covers the field and starting at least 25 m (82 ft) in from the edge of the field. At three sites along a diamond-shaped path non-selectively examine 100 heads for FHB-like symptoms (300/field) when plants are at the late milk to early dough stage of development (Feekes G.S. 11.1-11.2, Zadoks et al. 77-85). **It is critical to assess and collect samples at this stage** since diseased heads turn yellow to white and stand out from the unripe green heads, particularly in wheat. At each site count and record the number of heads with any typical symptoms of Fusarium head blight (e.g. 10 out of 100, 0 out of 100 etc.). Mechanical clicker counters help to make this easier since you can mentally count the number of required heads and click the counter each time an infected head is encountered. At each site collect 5 infected heads (if 5 are present) and put in labeled paper bag(s). It is not critical that samples from the three sites within an individual field remain separate, however placing samples from each site in separate bags make them less bulky.

Record the legal location of each field, the stage of growth (soft dough, etc.) and when disease was assessed and the samples were collected. Use the survey form provided. If you observe Fusarium head blight symptoms on heads that are not included as part of your assessments or samples in a particular field, collect them, label accordingly.

Sampled heads can then be brought indoors for drying or refrigeration and storage. Allow the bags and head contents to dry thoroughly and as rapidly as naturally possible. (Artificial heat may kill the fungus mycelium). For shipping, place sampled heads into a sturdy cardboard box. Lightly pack the samples to avoid crushing the dried heads. Use more than one box if required.



## **Collecting Specimens (preferably at Feekes stage 11.1-11.2 or Zadoks 77-85)**

Submit cereal specimens with suspected *Fusarium graminearum* infection as follows:

- a. Place infected plant tissues (typically heads) in a paper bag. Some drying can occur. (Do not place plant material in a sealed plastic bag because they will ROT in transit). The fungus will remain viable as long as the heads are dried quickly and naturally.
- a. Send several heads showing the suspected infection (if possible).
- c. Ensure if at all possible that the problem causing the bleached head to stand out among the green heads is not wheat stem maggot, Hessian fly or take-all.

## **Forwarding Specimens for Diagnosis**

- a. Specimens must reach the laboratory in good condition. Send specimens early in the week; avoid late-week mailing as specimens may deteriorate in transit; use the most direct route possible, e.g. Government courier, bus or private courier service.
- b. Wrap specimens in dry paper bags, label accurately and place in a sturdy mailing container.
- c. The fungus remains viable in fully dried heads.

## Appendix III

### Protocol for Testing Cereal Seed for *Fusarium graminearum* Using the Whole Seed Testing Method

Susan Patrick and Randy Clear  
Grain Research Laboratory  
1404-303 Main St.  
Winnipeg, Manitoba  
R3C 3G8  
Ph. (204) 983-7797 Fax: (204) 983-0724  
Email: spatrick@cgc.ca or rclear@cgc.ca

The following is a protocol of the Mycology lab of the Grain Research Laboratory. The authors recommend that one also know the regional source of the seed to be tested. This knowledge can be used to assess the likelihood of finding infected seeds in a seed lot. The farther west the source of the seed, the less likely one will find detectable levels of *Fusarium graminearum*.

Obtain a representative sample of the lot to be tested. A subsample of 250g to 500g is sufficient.

1. Store the subsample at -15°C.
2. Mix the subsample then remove a random selection of 200+ seeds.
3. Examine the remainder of the subsample and select those seeds with a shriveled, chalky white appearance (applicable to wheat only).
4. Surface disinfect the random and selected seeds in steps 3 and 4 by soaking them in a solution of 25ml Javex bleach and 500 ml H<sub>2</sub>O. Swirl the seeds in the solution for 1 minute, pour off the liquid through a strainer, and place the seeds onto filter paper in an open petri dish. Dry the seeds using a laminar flow hood. (Note: Failure to completely dry the seeds can result in bacterial problems).
5. Place 200 randomly selected seeds plus the selected seeds onto Potato Dextrose agar (Difco) in 100mm X 15mm plastic petri plates, Plate a maximum of 10 seeds per plate. (Note: Agar plates should have been poured 2 to 3 days earlier and allowed to dry before being used. Using wet plates can cause bacterial problems). One should get about 60 plates per liter of agar, with each plate having an agar thickness of about 4mm. Avoid using plates that are too thick or thin.)
6. Incubate the plates at 23°C for 5 days. Plates are exposed to a daily cycle of 12 hrs of light, consisting of 1 long wave UV light and 4 fluorescent lights 50cm above the plates, and 12hrs of darkness.
7. Examine the cultures with a dissecting microscope. Make slides of the spores and examine under the compound microscope. *Fusarium graminearum* should sporulate under the above conditions. The most diagnostic spores will be found in the aerial mycelium around the seed (if there are any spores there) or in wet sporodochia (occasionally formed by this species). Dry sporodochia are more typically formed than wet sporodochia on the agar around the seed. However, diagnostic spores are infrequent in the dry sporodochia. Both wet and dry sporodochia formed by *Fusarium graminearum* are exclusively or predominantly red. On rare occasions, one can find perithecia forming on the seed or on the agar after 5 days. Slashing the culture and returning it to the incubation chamber for another few days often results in perithecial production.

## Appendix IV

### Protocol for Sampling Cereal Seed for *Fusarium graminearum* for testing using the Whole Seed Testing Method

The procedures described below were adapted from those of the Canadian Seed institute (CSI), the Canadian Food Inspection Agency (CFIA), or the United States Department of Agriculture Grain Inspection, Packers and Stockyards Administration (GIPSA).

1. Definitions (Anonymous 2000)
  - 1.1. Primary Sample: A small portion taken from one point in the lot. Each primary sample is obtained by passing the sampling equipment through the seed stream once (including hand grab samples).
  - 1.2. Composite Sample: A sample obtained by combining and mixing all of the primary samples taken from a seed lot.
  - 1.3. Retained Sample: A sample taken from a lot of seed and held for a certain time period by the BSF as backup in case additional testing is required.
  - 1.4. Stream Sample: A sample obtained by moving a container through the entire cross-section of a moving flow of seed.

2. Sampling from carriers

- 2.1. Equipment (Anonymous 1995)

- 2.1.1. Probe. Probes are constructed of brass or aluminum and come in various sizes, with standard lengths of 5, 6, 8, 10, and 12 feet. The depth of the carrier or container dictates the length of probe that is used to draw the sample. Probes consist of two tubes, one inside the other. The inner tube is divided into compartments. Depending on its length, a probe may have 11, 12, 16, or 20 compartments. The outer tube has slots that match the compartment openings of the inner tube. When the slots in the tubes are aligned, grain can enter into and be emptied from the compartments.

Carriers	Probe Lengths	Compartments
Hopper Cars	10- or 12-foot	20 compartments
Boxcars	6-foot	12 compartments
Trucks	5- or 6-foot	11 or 12 compartments
Hopper-Bottom Trucks	6-, 8-, or 10-foot	12, 16, or 20 compartments

- 2.1.2. Sampling Canvas or Cloth. Sampling canvases, which are usually made of flat duck cloth or similar material, must be longer than the probe used to draw the sample. This "extra length" is needed so that the grain from the entire length of each probe may be placed on the canvas and examined without being spilled. Always keep sampling canvases clean, dry, and free of holes. Half sections of pipe or troughs (e.g., rain gutters) may be used instead of sampling canvases. Troughs must be longer than the probe used to draw the sample.

Other Containers - Use grain probes that will reach the bottom of the container.



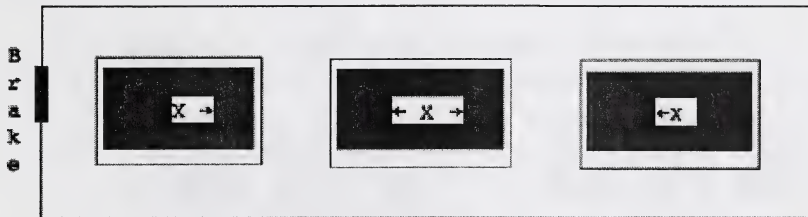
## 2.2. General Procedures

- 2.2.1. Drawing the Primary Sample (Anonymous 1995). Prior to sampling, record the carrier's identification number on the sample label. Next, spread the canvas on a level surface. Make sure the probe and canvas are clean and dry.
- 2.2.2. Sample size. In accordance with CSI procedures ((Anonymous 2000)) a retained sample of at least 2.0 kg should be retained for appeal and retesting. Since the *Fusarium graminearum* whole seed testing method protocol calls for a 250-500g sample, The composite sample weigh at least 2.25 kg. The working component used for *Fusarium graminearum* determinations should then be divided from the composite sample using one of the CSI-approved diving methods: coffee can, mechanical divider, seed pan or quarter down (Anonymous 2000). The size of a seed lot (e.g. truck vs. railcar) has an insignificant impact on the required composite sample size, since sample size will always be very small in relation to the size of the lot. Consequently, a 2.5 kg composite sample can be collected from all carrier or storage types.
- 2.2.3. For each type of carrier, there is an established sampling pattern (see section 2.3). Probe the carrier in the areas identified for the particular type of carrier. There are many techniques for using a probe. Regardless of which technique is used, follow these general rules to obtain a representative sample:
- Insert the probe at a 10-degree angle from the vertical, with the slots facing upward and completely closed. Keep the slots closed until the probe is inserted as deeply as possible into the grain. If the slots are not kept closed, a disproportionate amount of grain from the top of the lot will fall into the probe's compartments as it is being inserted.
  - If the grain contains sand or grit, it is permissible to insert the probe with the slots facing downward to avoid "freezing" the probe. After the probe is inserted, turn the slots upward before opening.
  - After the probe is fully inserted (with the slots facing upward), open the slots and move the probe up-and-down in two quick, short motions. When sampling grains, such as oats and barley, additional up-and-down movements may be necessary to fill the probe.
  - Close the slots completely. Then, grasp the probe by the outer tube and withdraw it from the grain. Do not pull the probe by the wooden handle. This can cause the inner tube to be pulled out of the outer tube. When this occurs, the probe must be emptied, reassembled, and the area reprobbed.
  - Empty the probe on the canvas or trough and transfer to the sample bag, taking care not to spill any portion of the sample or allow fine material to be blown away.
  - After placing the sample and completed sample ticket into the sample bag,
  - Tighten the drawstrings at the top of the bag so that it is closed securely.
  - Carefully remove the bag from the carrier so that none of the sample is lost or spilled. Do not throw or drop the sample to the ground.

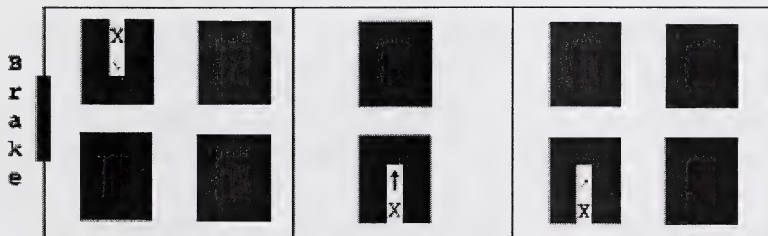
2.3. Sampling patterns (Anonymous 1995). The following diagrams show the standard sampling patterns. Insert the probe at the points marked (X), with the tip of the probe pointed toward the direction of the arrowhead. When two arrowheads are shown, the tip of the probe may be pointed in either direction.

2.3.1. Sampling Patterns for Hopper Cars.

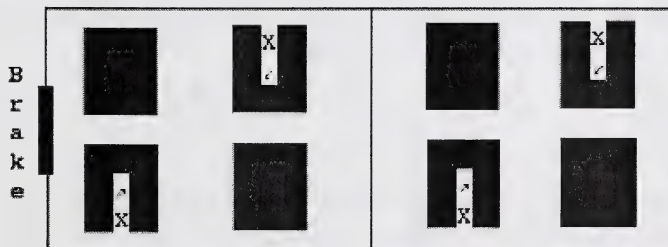
- 3-Compartment, Trough or Door Type Hopper Cars. Insert probe in the direction of the arrow at an approximately 10 degree angle, the probe may be inserted either in the center of each hopper or slightly off center in order to miss the crossbeams



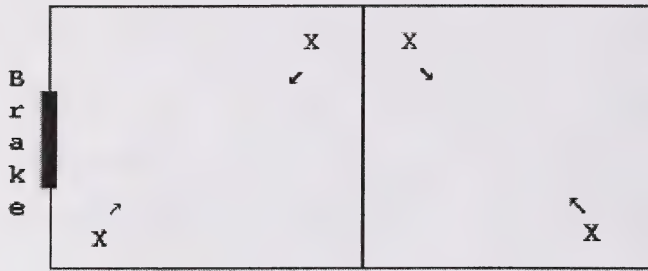
- 3-Compartment, 10-Hatch Type Hopper Cars. Insert probe in the direction of the arrow at an approximately 10-degree angle.



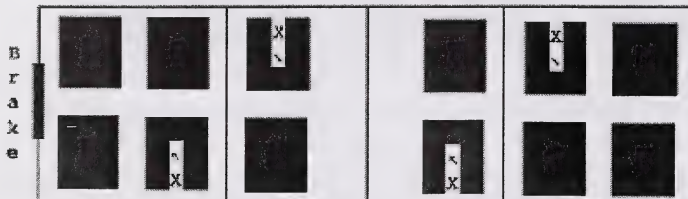
- 2-Compartment, 8-Hatch Type Hopper Cars. Insert probe in the direction of the arrow at an approximately 10-degree angle.



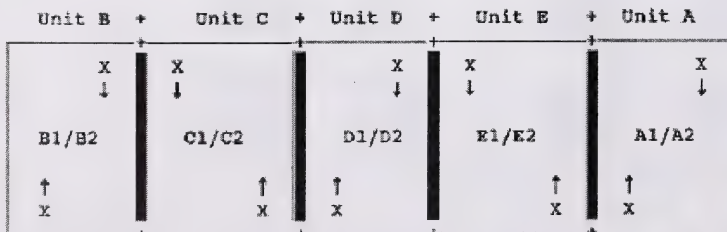
- 2-Compartment, Open Top Type Hopper Cars. Insert probe in the direction of the arrow at an approximately 10-degree angle.



- 4-Compartment, 12-Hatch Type Hopper Cars. Insert probe in the direction of the arrow at an approximately 10-degree angle.



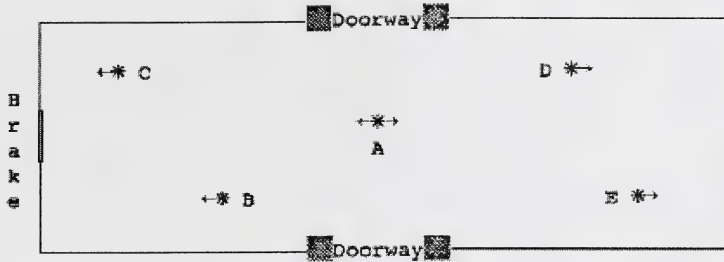
- Articulated Type Hopper Cars. Insert probe in the direction of the arrow at an approximately 10-degree angle. Articulated type hopper cars (e.g., "Super Hoppers") are easily recognized because of their configuration. The cars design permanently mounts five, 2-hatch type hopper cars onto 6 sets of wheels. The five car units carry the equivalent of three jumbo hopper cars. Since articulated hopper cars are unique in design, samplers should also be aware that their identification system is different from that of standard hopper cars. The manufacturer labels often articulated hopper car units. If they are, use this information for identification. If they are not labeled, identify one end unit of the car as the A unit and identify the other end unit as the B unit. Then, identify the three middle units as C, D, and E, going from unit B to unit A. Each unit has two compartments within a unit is identified as 1 and the A-end unit within the same compartment is identified as 2.



- Other Types of Hopper Cars. When sampling other types of hopper cars, use the sampling pattern which will provide the most representative sample.



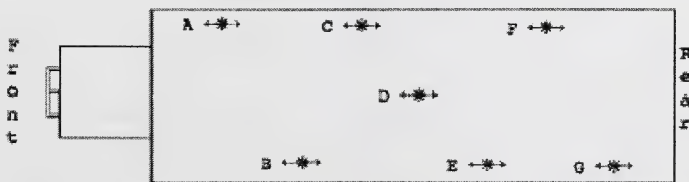
- Sampling Pattern for Box Cars. Insert the probe at an approximately 10-degree angle in the direction of the arrows shown in the diagram. The probe pattern shown may also be used in reverse of the one shown.



- Site A - Draw a sample from the center of the car. The probe may be taken with the slots facing toward either end of the car.
- Site B - Draw a sample approximately 3 - 5 feet back from the doorpost and approximately 2 - 4 feet out from the side of car. The slots in the probe should face toward the end of the car.
- Site C - Draw a sample approximately 3 - 5 feet from the same end of the car and approximately 2 - 4 feet from the opposite side of the car from site B. The slots in the probe face toward the end of the car.
- Site D - Draw a sample approximately 3 - 5 feet back from the doorpost and approximately 2 - 4 feet out from the side of car opposite of site B. The slots in the probe face toward the end of the car.
- Site E - Draw a sample approximately 3 - 5 feet from the same end of the car and approximately 2 - 4 feet from the opposite side of the car from site D. The slots in the probe face toward the end of the car.

2.3.2. Sampling Patterns for trucks. Insert the probe at an approximately 10-degree angle in the direction of the arrows shown in the diagram. The probe pattern shown may also be used in reverse of the one shown.

- Flat-Bottom Trucks or Trailers Containing Grain More than 4-Feet Deep or 8 Filled Probe Compartments.



- Site A - Draw a sample approximately 2 feet from the front and side.
- Site B - Draw a sample from the opposite side of site A, approximately halfway between the front and center of the carrier, and approximately 2 feet from the side.
- Site C - Draw a sample from the same side as site A, approximately: (three-fourths) of the distance between the front and center of the truck and approximately 2 feet from the side.

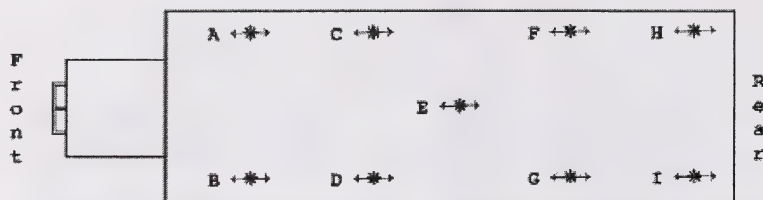
Site D - Draw a sample from the center of the carrier.

Site E - Draw a sample from the side opposite site C, approximately: (three-fourths) of the distance between the rear and center, approximately 2 feet from the side.

Site F - Draw a sample from the side opposite site E, approximately one-half the distance between the rear and center, approximately 2 feet from the side.

Site G - Draw a sample from the same side as site E, approximately 2 feet from the rear and side of the carrier.

- Flat-Bottom Trucks or Trailers Containing Grain Less than 4 Feet Deep or Fewer than 8 Filled Probe Compartments.



Site A - Draw a sample approximately 2 feet from the front and side.

Site B - Draw a sample from the opposite side of site A, approximately 2 feet from the side.

Site C - Draw a sample from the same side as site A, approximately: (three-fourths) of the distance between the front and center of the truck and approximately 2 feet from the side.

Site D - Draw a sample from the same side as site B, and opposite of site C, approximately: (three-fourths) of the distance between the front and center, approximately 2 feet from the side.

Site E - Draw a sample from the center.

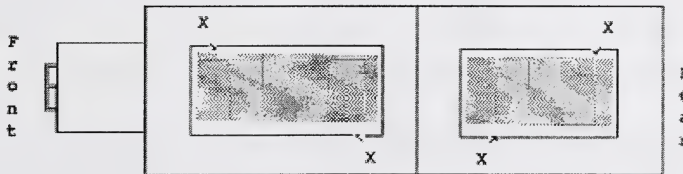
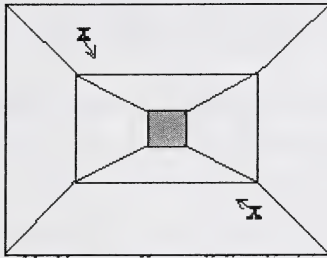
Site F - Draw a sample from the same side as site C, approximately: (three-fourths) of the distance between the center and rear of the truck and approximately 2 feet from the side.

Site G - Draw a sample from the same side as site D, approximately: (three-fourths) of the distance between the center and rear of the truck and approximately 2 feet from the side.

Site H - Draw a sample from the same side as site F, approximately 2 feet from the rear and side of the carrier.

Site I - Draw a sample from the same side as site G, approximately 2 feet from the rear and side of the carrier.

- Sampling Pattern for Hopper-Bottom Containers, Trucks, and Trailers. Insert the probe at an approximately 10-degree angle in the direction of the arrows shown in the diagram.



### 3. Grain stored in bins (Anonymous 2000)

A minimum composite sample size of at 2.5 kg is required. It is recommended that composite be taken from the stream of a seed lot whenever possible, as opposed to using a bin probe to obtain a sample. Stream samples may be taken as the seed is transferred into the storage bin, or as it is being transferred from bin to bin. To obtain a stream sample, the sample container may be moved through the entire seed stream either by hand or automatically.

#### 3.1. Stream samples

3.1.1. Manual sampling. An appropriate container such as a grain scoop, pan stream handler or sample tray may be used. It is run through the entire cross-section of the seed stream so that the sample is uniform, and the process is carried out at least seven times for a lot of seed.

3.1.2. Automatic sampler. This type of sampler is frequently mounted at the discharge of a receiving leg. It allows for the interval of sampling and sample size to be regulated much more precisely than when sampling by hand. The sampler is also placed in a position where there is no de-mixing of seed.

#### 4. Drop samples(Anonymous 1999)

A tray (e.g., a metal pan large enough to collect a 1 kg sample and that will not break under pressure); small plastic bags (with approximately 2.5 kg capacity and measuring approximately 43 cm long by 13 cm wide by 0.15 cm thick); and big bags to transport many of the samples enclosed in the small bags will be required. All equipment shall be clean, i.e., free of products and organisms previously sampled. For each bin selected, do as follows.

- Locate the bottom valve (or try hole) of the bin.
- Ensure that the respective belt is locked before sampling.
- If sampling from the valve, position the tray on the belt under the spout, open the valve, let the bin content flow onto the tray, and, when at least 2.5 kg is on the tray, close the valve.



- If sampling from the try hole, position the tray against the try hole, open the valve, let the bin content flow onto the tray, and, when at least 2.5 kg is on the tray, close the valve.
- Put at least 2.5 kg of the bin content in a plastic bag. Close and label the bag.

#### 4.3 Top Samples (Anonymous 1999)

A cylindrical sampler (divided bulk probe) (approximately 1 to 3 m long by 4 cm in diameter, with about 3 slots of each approximately 8 cm long by 2 cm large) or an electrically operated suction sampler\*; small plastic bags; and big bags to transport many of the samples enclosed in the small bags are required. All equipment shall be clean, i.e., free of products and organisms previously sampled. Locate the bin top. Have the top cover of the bin removed, and probe the bin content. The sampling pattern can be adapted to the most relevant carrier sampling pattern (2.3). Put at least 2.5 kilogram of the bin content in a plastic bag. Close and label the bag.

\* The Probovac probe draws a grain sample from the bin through suction in a double walled pipe; this unit is effective to a depth of about 40 feet and a representative sample can be obtained from the center of the grain mass.

#### Reference List

1. Anonymous. 1995. Grain Inspection Handbook, Book 1. Washington, D.C.: United States Department of Agriculture.
2. \_\_\_\_\_. 1999. Procedure PI-002: Sampling Grains and Field Crops, their Residues, and Associated Small Organisms. Nepean, ON: Plant Products Directorate, Plant Health and Products Division, Canadian Food Inspection Agency.
3. \_\_\_\_\_. 2000. CSI Technical Manual For Bulk Storage Facilities. Ottawa, ON: Canadian Seed Institute.

## Appendix V

### Management of *Fusarium graminearum* in cereal grains\* and cereal grain products intended for use as feed.

\*Note: The term “grain” includes corn and processed cereal products, such as screening and pellet feed.

Purpose: To prevent the introduction and spread of fusarium head blight (FHB) caused by *Fusarium graminearum*.

Measures to be followed during transport, loading, unloading, storage and feeding of cereal grains.

Monitoring Procedures	Critical Limit	Suggested Deviation & Corrective Action	Records (Suggested)
<b>Purchasing Feed:</b>			
User/buyer requests that the seller guarantee the grain (barley, wheat, oats and corn) is tested and found to be <i>Fusarium graminearum</i> -free, based on standard testing protocols.	Grain is <i>Fusarium graminearum</i> -free.	If user/buyer does not purchase certified feed grains, then follow the standard operating procedures below for transport, storage, processing and feeding to reduce FHB risks.	Results of analysis from a qualified laboratory.
<b>Feed Storage Off-Site or Trans-Loading Sites:</b>			
Out-of-province grain that has not been tested and shown to be <i>Fusarium graminearum</i> -free must not be stored in uncovered piles, or in contact with soil.	No uncovered ground storage; no direct contact with soil.	Cover pile or move into covered storage until grain can be probed, tested and demonstrated to be <i>Fusarium graminearum</i> -free, or properly utilized or disposed of.	Province of origin Laboratory test results.
All loading/unloading sites handling out-of-province grain must have wind fence and drop socks and/or a covered loading bay.	Eliminate spillage and fungal spore aerosolization.	Cease transloading until truck or rail cars can be moved to a suitable facility, or until the proper equipment can be brought to the loading site. Report drivers to trucking company if they refuse to clean and disinfect trucks.	Record of complaints sent to trucking companies.
Out-of-province grain must be unloaded in such a manner to eliminate spillage. It is important the grain not come into contact with soil.	No spillage.	If feed is spilled, it is cleaned up immediately so that all grain is removed. Workers are educated on how to reduce spillage and how to recover spilled grain. Grain from cleanup must be salvaged and composted.	Ag Fieldman records of noncompliance.

Monitoring Procedures	Critical Limit	Suggested Deviation & Corrective Action	Records (Suggested)
<p>If transport vehicle is subsequently to be used to transport <i>Fusarium graminearum</i> free grain or seed, it must be cleaned before leaving the unloading site.</p>	<p>This is to eliminate cross contamination between loads.</p>		
<b>Transport:</b>			
<p>All modes of transport from source to feeding site are covered.</p>	<p>All transport vehicles covered.</p>	<p>If not covered, inform operator to cover load and inform trucking company for corrective action.</p>	<p>Provincial Trucking Standards for loading/unloading and hauling grain. Record of complaints to trucking company.</p>
<p>During transport, care is taken to avoid any spillage.</p>	<p>No spillage.</p>	<p>Report to transport company.</p>	<p>Record of complaints to trucking company. Grain Hauler Truck sign at feedlot at unloading sites.</p>
<p>All transport vehicles hauling out-of-province grain must have the box/trailers cleaned and gates closed before being allowed to leave the unloading site.</p>	<p>No residual feed grain in transport vehicle.</p>	<p>Persons from business receiving the grain will check transport vehicle for compliance. Receiving site will contain an area and supplies for proper clean-up. Uncooperative operators will be reported to transport company and/or grain seller. Transport company educates drivers so problem doesn't reoccur.</p>	<p>Record of complaints to trucking company.</p>
<b>Unloading Transport Unit:</b>			
<p>All transport units hauling out-of-province grain must during unloading have tarps covering trailers and must remain covered during unloading</p>	<p>Control grain dust.</p>	<p>Persons from business receiving the grain will check transport vehicle for compliance. Receiving site will contain an area and supplies for proper clean-up. Uncooperative operators will be reported to transport company and/or grain seller. Transport company educates operators so problem doesn't reoccur.</p>	<p>Record of complaints to trucking company.</p>



Monitoring Procedures	Critical Limit	Suggested Deviation & Corrective Action	Records (Suggested)
<b>Storage On-Site:</b>			
Out of province feed grain that cannot be proven to be <i>Fusarium graminearum</i> -free must not be stored in uncovered piles, nor come into direct contact with the soil.	No uncovered ground storage	Cover pile or move into covered storage until grain can be probed, tested and demonstrated to be <i>Fusarium graminearum</i> -free, or properly utilized or disposed of.	Province of origin Laboratory test results.
<b>Storage/Processing/Feeding:</b>			
Out-of-province grain must be unloaded and handled to minimize spillage. Grain is not to come in contact with the soil.	No spillage.	If feed is spilled, it is cleaned up immediately so that all grain is removed, and the area is cleaned. Workers are educated on how to reduce spillage and how to clean contaminated areas. Grain from cleanup must be salvaged and composted.	Feeding site sanitation plan including management practices for in-pen composting. Grain Truck Unloading sign at feedlot for proper clean-out procedures. Manure management plan records.
Facilities receiving and processing any out-of-province grain must properly handle scalplings and dust collections.	No spillage.	If feed is spilled, it is cleaned up immediately so that all grain is removed, and the area is disinfected with an approved disinfectant. Workers are educated on how to reduce spillage and how to clean contaminated areas. Grain from cleanup must be salvaged and composted.	Feeding site sanitation plan. Manure management plan records.

Follow the Same management practices from Feeding Site above for the following:

Trucking management practices- direct from infected out of province areas

- coverage
- spillage
- cleaning

Off Loading Sites (Terminal Storage: off loading; rail off loading)

- wind fence and drop socks

## Example of Grain Trucking Standards for Feeding Site

### CLEAN TRAILERS

- If trailers/tractors/boxcars need to be swept (i.e. they carried uncertified, out-of-province grain), it must be done while grain is being unloaded - over the pit.
- Grain spillage must be cleaned off trailers/tractors/boxcars and trailers/tractors/boxcars must be disinfected.
- During unloading, tarps are not to be rolled until the grain stops flowing from the loading facility.
- Hopper gates and tarps can not be left open to let the wind clean out any kernels or dust.
- If trucks are used to haul different commodities, they must be cleaned completely before loading new commodity.

Reference: TransMark Ltd/R.K. Heggie Grain Ltd.

## Appendix VI.

### Fusarium Action Committee Contact List

Name & Organization	Phone	Fax	E-mail
Shaffeek Ali, Chair AAFRD	780-422-4909	780-422-0783	shaffeek.ali@gov.ab.ca
Jim Anderson Agricore United	403-216-0116	403-216-0121	janderson2@agricoreunited.com
Ron Axelson Alberta Cattle Feeders' Assoc.	403-250-2509	403-244-2340	cattle@cadvision.com
Dee Ann Bernard Alberta Research Council	780-632-8230	780-632-8612	benard@arc.ab.ca
Brenda Brindle Alberta Grain Commission	780-427-3077	780-422-9690	brenda.brindle@gov.ab.ca
Jim Calpas AAFRD	780-422-4911	780-422-0783	james.calpas@gov.ab.ca
Bob Coppock American Board of Toxicology	780-632-6122	780-632-4492	bobc@digitalweb.net
Audrey Gall AAAF	780-322-3745	780-322-3000	agall@telusplanet.net
Erin Holowach Alberta Grain Commission	780-422-0878	780-422-9690	erin.holowach@gov.ab.ca
Andrew Horton AAFRD	780-422-0153		andrew.horton.gov.ab.ca
Mark Kaun Canadian Seed Growers Assoc.	403-886-4562	403-886-5283	markkaun@telusplanet.net
Paul King AAAF	780-672-4765	780-672-4997	pking@county.camrose.ab.ca
Ralph Lange Alberta Research Council	780-632-8226	780-632-8612	ralph@arc.ab.ca
Hartman Nagel Canadian Grain Commission	780-774-2190	780-774-2021	hnagel@telusplanet.net
Richard Nordstrom Alberta Barley Commission	780-336-4875	780-336-4030	tholak@telusplanet.net
Ed Schultz Alberta Pork	780-491-3527	780-479-5128	ed.schultz@albertapork.com
Kelley Turkington Agriculture & Agri-Food Canada	403-782-8138	403-782-6120	turkingtonk@em.agr.ca
Len Vogelaar Alberta Cattle Commission	403-627-2078	403-627-2078	lbvogel@telusplanet.net
Bill Witbeck AAFRD	403-782-4641	403-782-5514	billy.witbeck@gov.ab.ca
Terry Young Alberta Barley Commission	403-347-7664	403-347-7664	tctyoung@telusplanet.net



## Glossary of Terms

- Alberta *Fusarium graminearum* Management Plan** – the document that defines the province wide approach to managing *Fusarium graminearum* and the specific control actions that must be taken to control the disease which are enforceable under the authority of the Alberta Agricultural Pests Act.
- Ascospore** – a spore produced as a result of the sexual process.
- Cereal** – a grass grown for its edible seed, includes corn.
- Conidia** – a spore produced as a result of an asexual process.
- Deoxynivalinol (DON)** – a mycotoxin produced by *Fusarium graminearum*; also referred as vomitoxin.
- Feed** – any product that is used as food for animals; can include food for human consumption.
- Fusarium** – A genus of fungi having sickle-shaped multicelled conidia; includes many important plant pathogens.
- Fusarium graminearum** – A species within the genus *Fusarium*, the plant pathogen/causal agent of the disease fusarium head blight.
- Fusarium head blight (FHB)** – The name of the disease caused by the plant pathogen *Fusarium graminearum*.
- Mycotoxin** – a non-enzymatic metabolite produced by a fungus that has toxic effects; especially affecting humans or animals.
- ppm** – parts per million
- Producer** – any person involved in primary agricultural production.
- Spore** - a general term for a fungal reproductive structure involved in the spread of a fungus. May be a produced as a result of a sexual or asexual process.
- Residue** – remainder or whatever is left over of the crop plant material.
- Zearalenone** – a mycotoxin produced by *Fusarium graminearum*.



