Fusarium graminearum: not the only *Fusarium* species that should be of concern for Canada's grain industry

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Microbiology Program, Grain Research Laboratory

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Other species than *Fusarium graminearum*?

**Fusarium slowly invading the Prairies**

It was identified in Saskatchewan for the first time last summer.

By Laura Rance
Western Producer staff

BRANDON, Man. — The *Fusarium* head blight disease which wreaked havoc with Manitoba cereal crops last year is gradually migrating west, officials with the Canadian Grain Commission say.

Strains of the disease were first identified in southern Manitoba and in irrigated areas of southern Alberta a decade ago. For the first time, it was identified in Saskatchewan fields last summer.

"It has been expanding gradually across southern Manitoba until now we have it growing on susceptible varieties of durum wheat in southern Saskatchewan," biologist Louise Cooke said last week.

"There was so much moisture, the spores and moulds reproduced very quickly. That may have advanced the spread," Cooke said.

"We have it here and that's all there is to it."

*Fusarium* head blight causes what are called "tombstone kernels" — mouldy, bleached and shrivelled grain which can contain vomitoxin, a mycotoxin which makes the grain unfit for human consumption.

Weather and climate conditions are major factors affecting the disease's impact on production and quality, said Randy Clear, a mycologist with the commission.

It's unclear whether the disease could survive the dry zone through the Palliser Triangle. But moisture conditions across the northern Prairies — the so-called canola belt — are prime for the disease to thrive.

**Prevention techniques**

The best way farmers can manage their risk is through crop rotations and variety selection, Clear said.

Studies conducted at North Dakota State University last year found semi-dwarfed wheats were more susceptible to the disease than the taller Canadian hard red spring wheat varieties.

"Marshall and Grandin have been found in particular to be more susceptible than hard red spring wheat varieties," he said.

Durum varieties and Canadian prairie spring varieties were also more susceptible than hard red spring varieties such as Katapowa. But Clear said although some varieties were more susceptible than others, none were completely resistant.

**"Trend"**

The Lethbridge test area showed signs of both *Fusarium culmorum* and *Fusarium graminearum*.

**Heading west**

Western Producer Jan 20, 1994
Distribution of *F. graminearum*

**1985**

- **black soil zone**
- **dark brown soil zone**
- **brown soil zone**
Distribution of *F. graminearum*

1993

- **black soil zone**
- **dark brown soil zone**
- **brown soil zone**
Distribution of *F. graminearum* 2002

- **black soil zone**
- **dark brown soil zone**
- **brown soil zone**
## Frequency of Isolation of *Fusarium* species from Seed

<table>
<thead>
<tr>
<th>Species</th>
<th>CWAD</th>
<th>CWRS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. graminearum</em></td>
<td>0.003</td>
<td>0.004</td>
</tr>
<tr>
<td><em>F. avenaceum</em></td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td><em>F. culmorum</em></td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td><em>F. sporotrichioides</em></td>
<td>--</td>
<td>0.003</td>
</tr>
<tr>
<td><em>F. poae</em></td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td><em>F. acuminatum</em></td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td><em>F. equiseti</em></td>
<td>0.07</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Samples with <em>Fusarium</em></strong></td>
<td><strong>38.6</strong></td>
<td><strong>39.3</strong></td>
</tr>
</tbody>
</table>

**Manitoba 1937-42**

Gordon (1944)
Frequency of Isolation of *Fusarium* species from Seed

**Western Manitoba 1986**

<table>
<thead>
<tr>
<th>Species</th>
<th>CWAD</th>
<th>CWRS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. graminearum</em></td>
<td>1.2</td>
<td>--</td>
</tr>
<tr>
<td><em>F. avenaceum</em></td>
<td>2.6</td>
<td>0.4</td>
</tr>
<tr>
<td><em>F. culmorum</em></td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td><em>F. sporotrichioides</em></td>
<td>2.9</td>
<td>0.3</td>
</tr>
<tr>
<td><em>F. poae</em></td>
<td>0.7</td>
<td>1.0</td>
</tr>
<tr>
<td><em>F. acuminatum</em></td>
<td>1.5</td>
<td>0.06</td>
</tr>
<tr>
<td><em>F. equiseti</em></td>
<td>0.6</td>
<td>--</td>
</tr>
<tr>
<td><strong>Samples with <em>Fusarium</em></strong></td>
<td><strong>100</strong></td>
<td><strong>75.8</strong></td>
</tr>
</tbody>
</table>
Fusarium Head Blight (FHB)

- 3 *Fusarium* spp. are typically associated with FHB in Canada

- *Fusarium graminearum*
- *Fusarium culmorum*
- *Fusarium avenaceum*
Mycotoxigenic *Fusarium* spp. (Trichothecenes)

- **Type B (Nivalenol, 15-Acetyl & 3-Acetyl Deoxynivalenol)**
  - *Fusarium pseudograminearum* VPI19289
  - *Fusarium pseudograminearum* DAOM235707
  - *Fusarium culmorum* BBA64223
  - *Fusarium graminearum* BBA65356
  - *Gibberella zeae* BBA62720
  - *Fusarium asiaticum* BBA69078
  - *Fusarium coenophialum* BBA64545
  - *Fusarium avenaceum* BBA65929

- **Type A (HT-2, T-2, Diacetoxyscirpenol)**
  - *Fusarium sporotrichioides* BBA71933
  - *Fusarium langsethiae* BBA70945
  - *Fusarium musarum* BBA68458
  - *Fusarium poae* VPI15323
  - *Fusarium kyushuense* BBA70845
  - *Fusarium sp.* BBA72296
  - *Fusarium sp.* NRRL13924
  - *Fusarium sp.* DAOM167768
  - *Fusarium robustum* BBA63687
  - *Fusarium sambucinum* BBA63572
  - *Fusarium brachygibbosum* BBA64675
  - *Fusarium longipes* CBS186.35
  - *Fusarium compactum* CBS121682
**Fusarium pseudograminearum**
(aka *F. graminearum* group I)

- 1999 formally separated
- Morphology similar to *Fusarium graminearum*
- Crown and root rot pathogen
- Rarely associated with FHB in Canada
- Limited distribution & almost exclusively 3-ADON chemotype
Species-specific Detection Methods

**F. graminearum** selective media (FGA) - Pouleur et al. 2005

**F. pseudograminearum** specific PCR assay - Aoki & O’Donnell 1999

PCR primers Fp1-1 / Fp1-2

523 bp
Frequency of *F. pseudogramearinearum* isolated from FDK in 2004

- not detected
- 0-1%
- 1-2%

Map showing distribution of frequency in Alberta, Saskatchewan, and Manitoba across all wheat classes.
Frequency of *F. pseudograminearum* isolated from FDK in 2010

- **not detected** 0-1 %
- **1-2 %**

All wheat classes

- **Alberta**
- **Saskatchewan**
- **Manitoba**
**Fusarium culmorum**

- Morphological ID straight forward
- Commonly associated with root and stem rot of small cereals
- Southern AB in 1987/1988: Wheat contaminated with DON infected by *Fusarium culmorum*
- Variable distribution pattern
- Exclusively 3-ADON chemotype
Frequency of *Fusarium culmorum* isolated from FDK in 1995

- **Alberta**
  - Not detected
  - 0-10%
  - 10-30%
  - >30%

- **Saskatchewan**
  - Not detected
  - 0-10%
  - 10-30%
  - >30%

- **Manitoba**
  - Not detected
  - 0-10%
  - 10-30%
  - >30%

All wheat classes

**Map legend:**
- **Yellow:** not detected
- **Light brown:** 0-10%
- **Dark brown:** 10-30%
- **Blue:** >30%
Frequency of *Fusarium culmorum* isolated from FDK in 1998

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Alberta</th>
<th>Saskatchewan</th>
<th>Manitoba</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10 %</td>
<td>not detected</td>
<td>10-30 %</td>
<td>&gt; 30 %</td>
</tr>
<tr>
<td>10-30 %</td>
<td>63%</td>
<td>63%</td>
<td></td>
</tr>
</tbody>
</table>

All wheat classes
Frequency of *Fusarium culmorum* isolated from FDK in 2002

**Map showing the frequency of *Fusarium culmorum* in Alberta, Saskatchewan, and Manitoba.**

- **Yellow** (not detected): 0-10%
- **Brown** (10-30%)
- **Blue** (>30%)

**All wheat classes**

Alberta, Saskatchewan, Manitoba
Frequency of *Fusarium culmorum* isolated from FDK in 2008

- Alberta: not detected
- Saskatchewan: 0-10%
- Manitoba: 10-30%
- > 30 %
Frequency of *Fusarium culmorum* isolated from FDK in 2010

**Map showing the frequency of *Fusarium culmorum* in different provinces in Canada:**

- **Alberta:**
  - Not detected
  - 0-10%
  - 10-30%
  - >30%

- **Saskatchewan:**
  - Not detected
  - 0-10%
  - 10-30%
  - >30%

- **Manitoba:**
  - Not detected
  - 0-10%
  - 10-30%
  - >30%

**Legend:**
- not detected
- 0-10%
- 10-30%
- >30%
EFSA requested opinions on other *Fusarium* toxins (e.g. beauvericin, enniatins, moniliformin, nivalenol, diacetoxyscirpenol)

Scandinavia: ENNs detected in *Fusarium* infected small cereals (rarely at ppm levels)

Moniliformin production strain specific → no correlation with FDK counts!

Canada: *Fusarium avenaceum* most potent producer on FHB infected cereal grain
Fusarium avenaceum

- Morphological species complex
- In drier or cooler crop districts commonly associated with FHB
- Weak pathogen or saprophyte on plants
- Poland: < 40 ppm MON in contaminated oats
  (Sharman et al. 1991)
Frequency of *Fusarium avenaceum* isolated from FDK in 1995

- Alberta: 50-75%
- Saskatchewan: 25-50%
- Manitoba: < 1 %

All wheat classes
Frequency of *Fusarium avenaceum* isolated from FDK in 1998

- Alberta: >75%
- Saskatchewan: 50-75%
- Manitoba: 25-50%

**All wheat classes**
Frequency of *Fusarium avenaceum* isolated from FDK in 2002

- Alberta: 50-75%
- Saskatchewan: 25-50%
- Manitoba: <1%

All wheat classes
Frequency of *Fusarium avenaceum* isolated from FDK in 2005

- Alberta: 50-75%
- Saskatchewan: 25-50%
- Manitoba: >75%

Map showing frequency distribution across different regions.
Frequency of *Fusarium avenaceum* isolated from FDK in 2010

- Alberta: 50-75%
- Saskatchewan: 75%
- Manitoba: <1%

All wheat classes
Durum Wheat Area in Western Canada

http://www.fas.usda.gov/remote/Canada/can_whd.htm
### Percentage of *Fusarium* species from FDK in Saskatchewan

#### Percent of Average Precipitation (Prairie Region)

<table>
<thead>
<tr>
<th></th>
<th>2010</th>
<th>2005</th>
<th>2002</th>
<th>1999</th>
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<tbody>
<tr>
<td>FDK</td>
<td>14.4</td>
<td>3.4</td>
<td>3.1</td>
<td>--</td>
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<tr>
<td>Fg</td>
<td>4</td>
<td>14</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Faven</td>
<td>67</td>
<td>44</td>
<td>47</td>
<td>65</td>
</tr>
<tr>
<td>Fculm</td>
<td>3</td>
<td>5</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>Snod</td>
<td>15</td>
<td>24</td>
<td>18</td>
<td>12</td>
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April 1, 2010 to September 30, 2010

**CWAD Saskatchewan CD 2-4 & 6-7**
### Percentage of *Fusarium* species from FDK in Alberta

<table>
<thead>
<tr>
<th>Species</th>
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<th>2002</th>
<th>1999</th>
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</thead>
<tbody>
<tr>
<td>FDK</td>
<td>15.4</td>
<td>1</td>
<td>1.6</td>
<td>--</td>
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<tr>
<td><em>Fg</em></td>
<td>35</td>
<td>33</td>
<td>37</td>
<td>54</td>
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<tr>
<td>Faven</td>
<td>17</td>
<td>25</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>Fculm</td>
<td>12</td>
<td>9</td>
<td>25</td>
<td>3</td>
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<tr>
<td>Snod</td>
<td>21</td>
<td>22</td>
<td>9</td>
<td>20</td>
</tr>
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</table>
Percentage of *Fusarium* species from FDK in Saskatchewan in %

<table>
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<tr>
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<th>2005</th>
<th>2002</th>
<th>1999</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDK</td>
<td>12.9</td>
<td>2.3</td>
<td>1.1</td>
<td>--</td>
</tr>
<tr>
<td><em>Fg</em></td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>13</td>
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<tr>
<td><em>Faven</em></td>
<td>35</td>
<td>25</td>
<td>16</td>
<td>22</td>
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<tr>
<td><em>Fcultm</em></td>
<td>3</td>
<td>3</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td><em>Snod</em></td>
<td>43</td>
<td>51</td>
<td>43</td>
<td>33</td>
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CWRS Saskatchewan CD 2-4 & 6-7
Percentage of *Fusarium* species from FDK in Alberta

<table>
<thead>
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<tbody>
<tr>
<td>FDK</td>
<td>3.9</td>
<td>0.6</td>
<td>0</td>
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</tr>
<tr>
<td>Fg</td>
<td>23</td>
<td>19</td>
<td>17</td>
<td>46</td>
</tr>
<tr>
<td>Faven</td>
<td>6</td>
<td>16</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Fculm</td>
<td>5</td>
<td>6</td>
<td>47</td>
<td>9</td>
</tr>
<tr>
<td>Snod</td>
<td>60</td>
<td>43</td>
<td>16</td>
<td>28</td>
</tr>
</tbody>
</table>
“Emerging” Mycotoxins – HT-2 & T-2 toxins

- EFSA call for HT-2/T-2 toxin data closed
- Assessment of chemistry, occurrence, exposure, toxicity
- Maximum limit in EU for HT-2 + T-2 in raw grain?
- Canada: *Fusarium sporotrichioides*
- Europe: *Fusarium langsethiae*
**Fusarium langsethiae**

Morphology vs. Phylogeny

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**Sections**

- Discolor
- Gibbosum
- Sporotrichiella
- Arthrosporiella

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**Fusarium austroamericanum** BBA65929
**Fusarium graminearum** BBA65356
**Gibberella zeae** BBA62720
**Fusarium asiaticum** BBA69078
**Fusarium cerealis** BBA64545
**Fusarium culmorum** BBA64223
**Fusarium lunulosporum** BBA62459
**Fusarium pseudograminearum** VPRI19289
**Fusarium pseudograminearum** DAOM235707

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**Fusarium armeniacum** DAOM235626
**Fusarium sp. 2008M-023**
**Fusarium sp. BBA62170**
**Fusarium sporotrichioides** BBA71933
**Fusarium langsethiae** BBA70945
**Fusarium musarum** BBA68458

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**Fusarium sambucinum** BBA63572
**Fusarium robustum** BBA63667
**Fusarium sp. DAOM167768**
**Fusarium sp. NRRL13924**
**Fusarium sp. BBA72296**
**Fusarium kyushuense** BBA70845
**Fusarium poae** VPRI15323

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**Fusarium brachygibbosum** BBA64675
**Fusarium longipes** CBS186.35
**Fusarium compactum** CBS121682
**Fusarium compactum** BBA65671

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**F. sporotrichioides**
**F. poae**

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**F. langsethiae**
**Fusarium langsethiae**

Species specific PCR

- Comprehensive validation essential to ensure specificity of the assay
- Validation on numerous isolates of *F. langsethiae* from various locations and plant hosts
- High sensitivity of PCR assay: DNA concentrations <0.1 pg/µl detected
**Fusarium sporotrichioides**

**RealTime PCR Assay**

- TaqMan assay specific for *F. sporotrichioides*
- Assay is negative for *F. langsethiae* and other closely related species
- Validated on 150 strains from 5 continents and many different hosts
- Estimation of HT-2 and T-2 toxin contamination in raw grain

![Standard curve for efficiency calculation derived from dilution series](Image)

Fluorescence signals of samples
Conclusions

- Significant changes in frequency of FHB pathogens depending on year & region
- Situation can change annually (except for dominance of *F. graminearum* in Manitoba)
- Durum wheat, barley, oats – higher risk of contamination with emerging mycotoxins
- Bio-molecular assays may complement chemical analyses in risk determination