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# Species-specific PCR-based assays for the detection of *Fusarium* species and a comparison with the whole seed agar plate method and trichothecene analysis

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**Abstract**

Species-specific PCR was used for the identification of nine *Fusarium* species in pure mycelial culture. A PCR-based method was compared with the whole seed agar plate method and trichothecene analysis for three toxin-producing *Fusarium* species using 85 grain samples of wheat, barley, oat, corn and rye. A simple SDS-based DNA extraction system followed by potassium acetate precipitation resulted in consistent PCR amplification of DNA fragments from cultures and grain samples. The species-specific PCR assays correctly identified pure cultures of *Fusarium avenaceum* ssp. *avenaceum* (9 isolates), *Fusarium acuminatum* ssp. *acuminatum* (12 isolates), *Fusarium crookwellense* (7 isolates), *Fusarium culmorum* (12 isolates), *Fusarium equiseti* (11 isolates), *Fusarium graminearum* (77 isolates), *Fusarium poae* (10 isolates), *Fusarium pseudograminearum* (23 isolates), and *Fusarium sporotrichioides* (10 isolates). Multiplex PCR was developed for the simultaneous detection of *F. culmorum*, *F. graminearum* and *F. sporotrichioides*, the three most important trichothecene producing species in Canada. In grain samples, results of PCR assays for these same three species related well with whole seed agar plate method results and determination of Fusarium trichothecenes. The PCR assay described in this study can be used for routine detection and identification of *Fusarium* spp. in Canada.


*Keywords:* *F. graminearum*; deoxynivalenol; Identification; Cereal grains; Multiplex PCR; Mycotoxins

## 1. Introduction

Fusarium head blight (FHB), caused by several *Fusarium* species, is one of the major fungal diseases of cereals worldwide. In Manitoba alone, economic losses to FHB from 1993 to 1998 are estimated at US $300 million (Windels, 2000). All cereal cultivars presently grown in Canada are susceptible to FHB. Infection can adversely affect the quantity, quality, and marketability of the grain by reducing yield, discoloring and shriveling seeds, reducing vigor and contaminating the grain with

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trichothecenes and other mycotoxins. The most common trichothecene found in affected grain in Canada is deoxynivalenol (DON, also known as vomitoxin) (Clear et al., 2000a,b), although other compounds such as 15-acetyl DON (15-ADON), 3-acetyl DON (3-ADON), 3,15-diacetyl DON, diacetoxyscirpenol (DAS), T-2 toxin, and HT-2 toxin also have been detected in naturally infected grain samples in Canada (Abramson et al., 1987, 1998; Usleber et al., 1996; Clear et al., 2000a). All *Fusarium* species capable of producing trichothecenes carry the *tri5* or *tox*5 gene (Niessen and Vogel, 1998). *Fusarium graminearum* Schwabe is the predominant FHB pathogen in Canada, although in western Canada it is primarily found in Manitoba and southeastern Saskatchewan (Clear and Patrick, 2000). The other principal FHB pathogens in Canada are *Fusarium avenaceum* (Fr.) Sacc. ssp. *avenaceum* Sangalang et al. and *Fusarium culmorum* (W.G. Smith) Sacc. Several other common *Fusarium* species also infect cereal grains in Canada, including *Fusarium acuminatum* Ell. and Ev. ssp. *acuminatum* Burgess et al., *Fusarium equiseti* (Corda) Sacc., *Fusarium poae* (Peck) Wollenw., and *Fusarium sporotrichioides* Sherb. (Clear et al., 2000a,b, Clear and Patrick, 1993).

Traditional diagnostic methods for detection and identification of *Fusarium* species in pure culture or in infected grain are based on micro- and macromorphological features developing on a non-selective agar medium. This process is time consuming and requires extensive training. In addition, it can often be difficult to distinguish between species having similar morphological characteristics. This is especially true when using growth characteristics and spore morphology (Aoki and O'Donnell, 1999) to reliably distinguish between *F. graminearum* and *Fusarium pseudograminearum* O'Donnell and Aoki, and to a lesser extent *Fusarium crookwellense* Burgess, Nelson and Toussoun. With the placement of *F. graminearum* on Alberta's list of declared pests in 1999, and the requirement that all cereal seed planted in the province be tested to ensure that the grain does not contain a detectable level of *F. graminearum* (Ali and Calpas, 2002), accurate and rapid differentiation of these species is required. It is also challenging to distinguish between two commonly found species on cereal grain, *F. acuminatum* ssp. *acuminatum* and *F. avenaceum* ssp. *avenaceum*. As these species are frequently encountered by seed analysts in Canada, a quick and reliable method for distinguishing them would be useful. Polymerase chain reaction (PCR) is a sensitive and rapid method that can be used for the detection and screening of *Fusarium* species in infected grain samples. Species-specific primers have been used for PCR detection and screening of *F. acuminatum* (Williams et al., 2002), *F. avenaceum* (Schilling et al., 1996; Doohan et al., 1998; Waalwijk et al., 2003, 2004), *F. crookwellense* (Yoder and Christianson, 1998), *F. culmorum* (Nicholson et al., 1998), *F. equiseti* (Mishra et al., 2003), *F. graminearum* (Nicholson et al., 1998; Waalwijk et al., 2003, 2004), *F. poae* (Parry and Nicholson, 1996), and *F. pseudograminearum* (Aoki and O'Donnell, 1999). However, the use of species-specific PCR for the detection and screening of the primary trichothecene producing *Fusarium* species in cereals in Canada has not been reported. The objectives of this study were to (1) apply species-specific PCR analysis for the identification of nine *Fusarium* species in pure culture and to test for the presence of the Tox5 gene in these isolates, (2) develop a multiplex PCR method for the detection of the three most important trichothecene producing Fusaria (*F. culmorum*, *F. graminearum* and *F. sporotrichioides*), and (3) compare PCR methods with the traditional whole seed agar plate method for detecting the three most important trichothecene producing Fusaria in 85 grain samples of wheat, barley, corn, oat and rye and to evaluate those same samples for trichothecenes.

## 2. Materials and methods

### 2.1. Grain samples

Each fall, the Canadian Grain Commission (CGC) conducts a survey of the new crop in which samples weighing 250 to 500 g are sent by producers and grain companies to the CGC for various analyses. In 2002, 25-g subsamples were removed from Canada western red spring (CWRS) and most Canada western amber durum (CWAD) samples and bulked according to crop district (CD). There are 12 CDs in Manitoba, although CDs 9 and 10 are usually

reported together, 20 CDs in Saskatchewan, and 7 CDs in Alberta (Clear et al., 2000a). All CWRS composites were comprised of 10 samples, whereas the CWAD composites ranged from 1 to 10 samples per CD. Consequently, in order to have enough grain for testing, more than 25 g was taken from some CWAD samples when the number of grain samples from a crop district was small. The other grain samples tested were individual producer samples collected between 1994 and 2003, except for a single oat cargo sample collected at the port of Vancouver. The amount of the 85 grain samples ranged between 100 and 500 g. All 85 samples were stored from the date of collection at −15 °C until analyzed.

### 2.2. Mycological analysis

For each grain sample 200 seeds were surface disinfested with a 0.3% sodium hypochlorite (NaOCl) solution and placed onto potato dextrose agar (PDA) for 5 days according to the method of Clear and Patrick (2000). Three corn samples from Ontario were ground prior to receival, precluding mycological analysis.

### 2.3. Fungal cultures

The location and source of fungal cultures are presented in Table 2. Nomenclature of several of the *Fusarium* species has been revised in recent years. Our identifications were based on Nelson et al. (1983) except where the species have been further subdivided. For these species we used the identification system of Burgess et al. (1994). For nearly all cultures in this study, initial fungal isolations were made by transferring mycelia growing from infected cereal seed to a Petri plate containing potato dextrose agar (PDA) (Difco, Detroit, MI.). The exceptions were three isolates of *F. pseudograminearum*, which were recovered from straw, and three *F. crookwellense* isolates from Ontario and Quebec, which were provided by Dr. Allen Xue, Eastern Cereal and Oilseed Research Centre, Ottawa. After 5 to 10 days of growth at room temperature and under UV light, a spore suspension was prepared and spread onto a fresh PDA plate and incubated for 18 h at room temperature. A single germinating conidium was then removed from the PDA plate, transferred to an SNA (Spezieller Nährstoffarmer Agar) plate (Nirenberg, 1981), and after 7 days growth stored in a plastic bag at 4 °C for up to 1 year. For DNA extraction, mycelia from the SNA plates were transferred to PDA plates and incubated in the dark at room temperature for 7 to 10 days. The mycelia were then harvested with sterile needles, placed into 50-mL Falcon tubes, and freeze-dried for 3 days or placed into 2-mL microcentrifuge tubes then frozen at −70 °C. Information on *Fusarium* spp. isolates and grain samples is presented in Tables 2 and 3.

### 2.4. DNA extraction

For cereal samples, 20 g was ground in a coffee grinder for 90 s and then 0.2-g ground grain was weighed into a 2-mL microcentrifuge tube. Freeze-dried mycelial extracts and the extracts frozen at −70 °C were placed into 2-mL microcentrifuge tubes until the tubes were 1/4 to 1/2 full. Two 0.25-in. ceramic spheres (Qbiogene, CA) were added and the samples were pulverized to a fine powder with Retsch Mixer Mill (F. Kurt Retsch, Haan, Germany) for 1 min at a speed of 30 Hz. One milliliter sodium dodecyl sulphate (SDS) extraction buffer (200 Mm Tris–HCl, pH 7.5; 288 mM NaCl; 25 mM EDTA, pH8.0; 0.5% SDS) was added to each ground sample and homogenization was carried out on the Mixer Mill for 30 s at 30 Hz. Samples were then centrifuged at 12,000 rpm for 5 min and 750 μL of the supernatant was transferred to a new microcentrifuge tube. A 215-μL aliquot of a solution of potassium acetate (3 M potassium and 5 M acetate) was added to the supernatant. The solution was mixed and incubated on ice for 30 min, and then centrifuged at 12,000 rpm for 15 min at 4 °C. A 700-μL aliquot of the supernatant was transferred to a new microcentrifuge tube and 500 μL of cold isopropanol was added to precipitate the DNA. The resulting pellet was washed with 70% ethanol, dried in a 120 SpeedVac Thermo Savant (Savant Instruments, Holbrook, NY) and dissolved in 200 μL Tris–EDTA.

### 2.5. Polymerase chain reaction

PCR for single species detection was performed in 96-well plates containing 25 μL of a reaction mixture consisting of 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM

Tris–HCl (pH8.3), 0.2 mM of each of the four dNTPs, 0.4 μM of each oligonucleotide primer (Table 1), and 0.75 units of *Taq* DNA polymerase (Applied Biosystems, Foster, CA). The 25-μL multiplex PCR reaction contained 1X AccuPrime™ buffer (20 mM Tris–HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 mM of each of the four dNTPs, 0.4 μM of each oligonucleotide primer and 2.5 units of AccuPrime *Taq* DNA polymerase (Invitrogen, Burlington, Canada). For the mycelial and ground grain samples, 1.5 μL DNA (out of the 200-μL stock, ~30 to 50 ng DNA) was used directly in the 25 μL PCR reaction. DNA amplification (including multiplex PCR) was performed in an MJ Research PTC-200 Thermal Cycler using an initial 3.0-min denaturation at 95 °C; and then 38 cycles of 30 s at 95 °C, 20 s at 62 °C, and 45 s at 72 °C, followed by a final extension of 5 min at 72 °C. Annealing temperatures of 56 °C and 57 °C were used for *F. acuminatum* ssp. *acuminatum* and *F. pseudograminearum*, respectively. Amplification products were separated by electrophoresis in 2.0% (wt/vol) agarose gels stained with 0.2 μg/mL ethidium bromide.

### 2.6. Fusarium trichothecene analysis

For the analysis of Fusariotoxins, one-half of each grain sample was ground in a Ditting bench top coffee grinder (model KFA 903, Elpack, Toronto, Ontario) previously adjusted so that at least 90% of the ground grain would pass through a No. 20 mesh US standard sieve. Extraction and analyses for DON, nivalenol (NIV), 3-ADON, 15-ADON, fusarenon X (FX), DAS, HT-2 toxin, and T-2 toxin were carried out following a modification of the method published by Tacke and Casper (1996). A 10-g portion of the ground sample was extracted for 1 h with 40 mL of acetonitrile+water (84+16, v/v) on a reciprocating shaker. A 6-mL extract was eluted through a 1.5-g cleanup column containing C18+aluminum oxide (1+1, w/w). The eluate was evaporated to dryness and derivatized with a TMSI–TMCS (trimethylsilylimidazole–trimethylchlorosilane) mix. The TMS (trimethylsilyl) derivatives of the trichothecenes were identified and quantified using an Agilent 6890 Plus capillary column gas chromatograph, Agilent Model No.

Table 1
List of primer sequences, expected DNA fragment length and sources of primers

| Primer name | Target | Sequence (5′–3′) | Size (bp) | Source[a] |
|---|---|---|---|---|
| FAC F | *F. acuminatum* | GGGATATCGGGCCTCA | 600 | A |
| FAC R | | GGGATATCGGCAAGATCG | | |
| FaF | *F. avenaceum* | CAAGCATTGTCGCCACTCTC | 920 | B |
| FaR | | GTTTGGCTCTACCGGGACTG | | |
| J1AF | *F. avenaceum* | GCTAATTCTTAACTTACTAGGGGCC | 220 | C |
| J1AR | | CTGTAATAGGTTATTTACATGGGCG | | |
| CroA F | *F. crookwellense* | CTCAGTGTCCACCGCGTTGCGTAG | 842 | D |
| CroA R | | CTCAGTGTCCCAATCAAATAGTCC | | |
| FC01F | *F. culmorum* | ATGGTGAACTCGTCGTGGC | 570 | E |
| FC01R | | CCCTTCTTACGCCAATCTCG | | |
| FEF1 | *F. equiseti* | CATACCTATACGTTGCCTCG | 400 | F |
| FER1 | | TTACCAGTAACGAGGTGTATG | | |
| Fg16F | *F. graminearum* | CTCCGGATATGTTGCGTCAA | 450 | E |
| Fg16R | | GGTAGGTATCCGACATGGCAA | | |
| FP82F | *F. poae* | CAAGCAAACAGGCTCTTCACC | 220 | G |
| FP82R | | TGTTCCACCTCAGTGACAGGTT | | |
| AF330109CF | *F. sporotrichioides* | AAAAGCCCAAATTGCTGATG | 332 | H |
| AF330109CR | | TGGCATGTTCATTGTCACCT | | |
| FP1-1 | *F. pseudograminearum* | CGGGGTAGTTTCACATTTCYG | 523 | I |
| FP1-2 | | GAGAATGTGATGASGACAATA | | |
| Tox5/1 | Trichothecene | GCTGCTCATCACTTTGCTCAG | 685 | J |
| Tox5/2 | | CTGATCTGGTCACGCTCATC | | |

[a] Sources of primers: A—Williams et al., 2002; B—Doohan et al., 1998; C—Turner et al., 1998; D—Yoder and Christianson, 1998; E—Nicholson et al., 1998; F—Mishra et al., 2003; G—Parry and Nicholson, 1996; H—Genbank AF330109 (primer designed by authors); I—Aoki and O'Donnell, 1999; J—Niessen and Vogel, 1998.

6890, equipped with an autoinjector, mass selective detector, Agilent Model No. 5973N, and an Agilent 30 m×0.25 mm×0.25 μm DB35-MS column. Isobenzan, also known as Telodrin® (Shell Chemical, 99%+ pure, New York, N.Y.), was used as an internal standard. DON, NIV, 3-ADON, 15-ADON, FX, DAS, HT-2 toxin, and T-2 toxin standards were purchased from Sigma (Oakville, Ontario). Minimum limits of quantitation were 0.1 μg/g. The recovery rates for DON, 15-ADON and HT-2 were 96%, 97% and 124%, respectively. The recovery rates were consistent regardless of matrices. The Fusarium trichothecene results shown in Table 3 are based on single extractions.

### 2.7. Statistical analysis

McNemar's Chi-square test with continuity correction (Conover, 1980) was used to determine if the results of PCR and whole seed agar plate were statistically the same.

## 3. Results and discussion

### 3.1. PCR detection of Fusarium species in pure mycelial cultures

Both freeze-dried and frozen mycelial samples produced DNA suitable for PCR amplification. *F. acuminatum* ssp. *acuminatum* species specific PCR using FACF/R primer set amplified the expected 600-bp DNA fragment in all 12 isolates tested (Table 2). PCR performed on the DNA isolates of the other eight *Fusarium* species did not result in amplification of the expected 600-bp DNA fragment. PCR performed with Tox5 primer set produces a 658-bp DNA fragment in trichothecene producing *Fusarium* species (Niessen and Vogel, 1998), and thus could be used for group specific PCR detection of potential trichothecene-producing *Fusarium* species. Only two of the 12 isolates produced the 658-bp *tri5* DNA fragment. Logrieco et al. (1992) reported considerable variability in the ability of isolates of *F. acuminatum* from several countries to produce trichothecenes, with 7 of 25 producing detectable levels in culture. Abramson et al. (1993) detected trichothecenes in culture extracts of three Manitoba isolates but at very low levels. Further testing of a wider selection of isolates is required to evaluate the role of this species in mycotoxin production in Canada.

All nine *F. avenaceum* ssp. *avenaceum* isolates yielded the expected 220-bp DNA fragment with the primer set J1AF/R (Table 2). None of the other species produced a product with this primer set. However, PCR performed with another primer, reported to be specific to *F. avenaceum* (Doohan et al., 1998), amplified all isolates of both *F. avenaceum* ssp. *avenaceum* and *F. acuminatum* ssp. *acuminatum*. However, the FaF/R primer was more sensitive than the J1AF/R primer pair when testing ground grain for the presence of *F. avenaceum* ssp. *avenaceum* (data not shown), but it was unable to distinguish between these two morphologically similar species. None of the *F. avenaceum* ssp. *avenaceum* isolates resulted in the amplification of the 658-bp *tri5* DNA fragment. This is consistent with in vitro studies of *F. avenaceum* ssp. *avenaceum* from western Canada in which none of 42 isolates of *F. avenaceum* ssp. *avenaceum* produced trichothecenes on autoclaved rice (Abramson et al., 2001) and by other studies (Niessen and Vogel, 1998).

*F. crookwellense* species specific primer (CroAF/R) amplified the expected 842-bp DNA fragment in the seven isolates tested (Table 2), and PCR performed with the other eight *Fusarium* species isolates did not result in the amplification of the 842-bp DNA fragment. All seven *F. crookwellense* isolates resulted in the amplification of the 658-bp *tri5* DNA fragment. All four isolates used by Niessen and Vogel (1998) also produced the 658-bp *tri5* DNA fragment.

*F. culmorum* species-specific PCR using FC01F/R primer set produced the expected 570-bp DNA fragment in all 12 isolates collected in western Canada (Table 2). PCR performed on DNA isolates of the other eight *Fusarium* species did not result in the amplification of the 570-bp DNA fragment, indicating the specificity of the FC01F/R primer set. Eleven of the 12 isolates also produced the expected DNA fragment with Tox5 PCR indicating the presence of the trichothecene-forming gene in these isolates. This observation agrees with in vitro studies of *F. culmorum* from western Canada in which all 38 isolates produced trichothecenes on autoclaved rice (Abramson et al., 2001).

Table 2
Sources of Fusarium isolates collected in Canada and PCR results for Tox5 gene

| Species | Location[a] | Source[b] | Year | TOX5-1/TOX5-2 primers |
|---|---|---|---|---|
| *F. acuminatum* ssp. *acuminatum* | Hamiota, MB | Barley seed | 2000 | − |
| *F. acuminatum* ssp. *acuminatum* | Hamiota, MB | Barley seed | 2000 | − |
| *F. acuminatum* ssp. *acuminatum* | Hamiota, MB | Barley seed | 2000 | − |
| *F. acuminatum* ssp. *acuminatum* | Hamiota, MB | Barley seed | 2000 | − |
| *F. acuminatum* ssp. *acuminatum* | Hamiota, MB | Barley seed | 2000 | + |
| *F. acuminatum* ssp. *acuminatum* | Hamiota, MB | Barley seed | 2000 | − |
| *F. acuminatum* ssp. *acuminatum* | Brandon, MB | Barley seed | 2000 | − |
| *F. acuminatum* ssp. *acuminatum* | Brandon, MB | Barley seed | 2000 | + |
| *F. acuminatum* ssp. *acuminatum* | Brandon, MB | Barley seed | 2000 | − |
| *F. acuminatum* ssp. *acuminatum* | Brandon, MB | Barley seed | 2000 | − |
| *F. acuminatum* ssp. *acuminatum* | Brandon, MB | Barley seed | 2000 | − |
| *F. acuminatum* ssp. *acuminatum* | Brandon, MB | Barley seed | 2000 | − |
| *F. avenaceum* ssp. *avenaceum* | Edmonton, AB | FDK | 2003 | − |
| *F. avenaceum* ssp. *avenaceum* | Vermilion, AB | FDK | 2003 | − |
| *F. avenaceum* ssp. *avenaceum* | Falher, AB | FDK | 2003 | − |
| *F. avenaceum* ssp. *avenaceum* | Humboldt, SK | FDK | 2003 | − |
| *F. avenaceum* ssp. *avenaceum* | Nokomis, SK | FDK | 2003 | − |
| *F. avenaceum* ssp. *avenaceum* | Kamsack, SK | FDK | 2003 | − |
| *F. avenaceum* ssp. *avenaceum* | Davidson, SK | FDK | 2003 | − |
| *F. avenaceum* ssp. *avenaceum* | Prairie River, SK | FDK | 2003 | − |
| *F. avenaceum* ssp. *avenaceum* | Somerset, MB | FDK | 2003 | − |
| *F. crookwellense* | Manitoba | Wheat seed | 2001 | + |
| *F. crookwellense* | Ottawa, Ont | Wheat seed | 1996 | + |
| *F. crookwellense* | Ottawa, Ont | Wheat seed | 1991 | + |
| *F. crookwellense* | Ste. Anne de Bellevue, PQ | Wheat seed | 1990 | + |
| *F. crookwellense* | New Brunswick | Barley seed | 2003 | + |
| *F. crookwellense* | New Brunswick | Barley seed | 2003 | + |
| *F. crookwellense* | New Brunswick | Barley seed | 2003 | + |
| *F. culmorum* | Vulcan, AB | FDK | 2003 | + |
| *F. culmorum* | Dalmeny, SK | FDK | 2003 | + |
| *F. culmorum* | Bow Island, AB | FDK | 2003 | + |
| *F. culmorum* | Raymore, SK | FDK | 2003 | + |
| *F. culmorum* | Hague, SK | FDK | 2003 | + |
| *F. culmorum* | Burdett, AB | FDK | 2003 | + |
| *F. culmorum* | Wadena, SK | FDK | 2003 | + |
| *F. culmorum* | Francis, SK | FDK | 2003 | + |
| *F. culmorum* | Indian Head, SK | FDK | 2003 | + |
| *F. culmorum* | Grimshaw, AB | FDK | 2003 | + |
| *F. culmorum* | Coaldale, AB | FDK | 2003 | + |
| *F. culmorum* | Hawk Hills, AB | FDK | 2003 | − |
| *F. equiseti* | Quebec | Cereal seed | 2003 | + |
| *F. equiseti* | Quebec | Cereal seed | 2003 | − |
| *F. equiseti* | Quebec | Cereal seed | 2003 | − |
| *F. equiseti* | Quebec | Cereal seed | 2003 | − |
| *F. equiseti* | Quebec | Cereal seed | 2003 | − |
| *F. equiseti* | Quebec | Cereal seed | 2003 | − |
| *F. equiseti* | Stonewall, MB | Barley seed | 2001 | − |
| *F. equiseti* | Stonewall, MB | Barley seed | 2001 | − |
| *F. equiseti* | Stonewall, MB | Barley seed | 2001 | − |
| *F. equiseti* | Stonewall, MB | Barley seed | 2001 | + |
| *F. equiseti* | Stonewall, MB | Barley seed | 2001 | − |
| *F. graminearum* | Selkirk, MB | FDK | 2003 | + |

Table 2 (*continued*)

| Species | Location[a] | Source[b] | Year | TOX5-1/TOX5-2 primers |
|---|---|---|---|---|
| *F. graminearum* | Souris, MB | FDK | 2003 | + |
| *F. graminearum* | Winkler, MB | FDK | 2003 | + |
| *F. graminearum* | Teulon, MB | FDK | 2003 | + |
| *F. graminearum* | Rosser, MB | FDK | 2003 | + |
| *F. graminearum* | Roland, MB | FDK | 2003 | + |
| *F. graminearum* | Oakville, MB | FDK | 2003 | + |
| *F. graminearum* | Stonewall, MB | FDK | 2003 | + |
| *F. graminearum* | Pilot Mound, MB | FDK | 2003 | + |
| *F. graminearum* | Rathwell, MB | FDK | 2003 | + |
| *F. graminearum* | Estevan, SK | FDK | 2003 | + |
| *F. graminearum* | Pelly, SK | FDK | 2003 | + |
| *F. graminearum* | Glenella, MB | FDK | 2003 | + |
| *F. graminearum* | Vulcan, AB | FDK | 2003 | + |
| *F. graminearum* | Bow Island, AB | FDK | 2003 | + |
| *F. graminearum* | Bow Island, AB | FDK | 2003 | + |
| *F. graminearum* | Cassils, AB | FDK | 2003 | + |
| *F. graminearum* | High Prairie, AB | FDK | 2003 | + |
| *F. graminearum* | Redcoat, AB | FDK | 2003 | + |
| *F. graminearum* | Weyburn, SK | FDK | 2003 | + |
| *F. graminearum* | North Star, AB | FDK | 2003 | + |
| *F. graminearum* | North Star, AB | FDK | 2003 | + |
| *F. poae* | Raymore, SK | FDK | 2003 | – |
| *F. poae* | Westlock, AB | FDK | 2003 | + |
| *F. poae* | Birtle, MB | FDK | 2003 | + |
| *F. poae* | Tangent, SK | FDK | 2003 | + |
| *F. poae* | Grande Prairie, AB | FDK | 2003 | + |
| *F. poae* | Canora, SK | FDK | 2003 | + |
| *F. poae* | Vulcan, AB | FDK | 2003 | + |
| *F. poae* | Bow Island, AB | FDK | 2003 | + |
| *F. poae* | Edenwold, SK | FDK | 2003 | + |
| *F. poae* | Glengarry, ON | Cereal seed | 2003 | + |
| *F. pseudograminearum* | Dunmore, AB | FDK | 2002 | – |
| *F. pseudograminearum* | Bateman, SK | FDK | 2002 | – |
| *F. pseudograminearum* | Bateman, SK | FDK | 2002 | – |
| *F. pseudograminearum* | Swift Current, SK | FDK | 2002 | – |
| *F. pseudograminearum* | Swift Current, SK | FDK | 2002 | – |
| *F. pseudograminearum* | Cudworth, SK | FDK | 2002 | – |
| *F. pseudograminearum* | Central Alberta | Barley straw | 2002 | – |
| *F. pseudograminearum* | Central Alberta | Barley straw | 2002 | – |
| *F. pseudograminearum* | Central Alberta | Barley straw | 2002 | – |
| *F. pseudograminearum* | Redcoat, AB | FDK | 2002 | – |
| *F. pseudograminearum* | Vauxhall, AB | FDK | 2002 | – |
| *F. pseudograminearum* | Bow Island, AB | FDK | 2002 | – |
| *F. pseudograminearum* | Coaldale, AB | FDK | 2003 | + |
| *F. pseudograminearum* | Vulcan, AB | FDK | 2003 | + |
| *F. pseudograminearum* | Mundare, AB | Wheat seed | 2003 | + |
| *F. pseudograminearum* | Saskatchewan | Wheat seed | 2003 | + |
| *F. pseudograminearum* | Vanguard, SK | FDK | 2002 | – |
| *F. pseudograminearum* | Speers, SK | FDK | 2003 | + |
| *F. sporotrichioides* | Birtle, MB | FDK | 2003 | – |
| *F. sporotrichioides* | Glenella, MB | FDK | 2003 | – |
| *F. sporotrichioides* | Edenwold, SK | FDK | 2003 | – |

*(continued on next page)*

Table 2 (*continued*)

| Species | Location[a] | Source[b] | Year | TOX5-1/TOX5-2 primers |
|---|---|---|---|---|
| *F. sporotrichioides* | Coaldale, AB | FDK | 2003 | + |
| *F. sporotrichioides* | Manning, AB | FDK | 2003 | + |
| *F. sporotrichioides* | Prescott, ON | CERS | 2003 | + |
| *F. sporotrichioides* | Glengarry, ON | CERS | 2003 | + |
| *F. sporotrichioides* | Glengarry, ON | CERS | 2003 | + |
| *F. sporotrichioides* | Red River South, MB | CWRS | 2003 | + |
| *F. sporotrichioides* | Fannystelle, MB | CWRS | 2003 | + |

[a] AB—Alberta; MB—Manitoba; ON—Ontario; SK—Saskatchewan.
[b] FDK—*Fusarium* damaged wheat kernels; CERS—Canada eastern red spring wheat; CWRS—Canada western red spring wheat.

*F. equiseti* species-specific PCR using the primer set FEF1/FER1 produced the expected 400-bp DNA fragment in all 11 isolates (Table 2). No PCR amplification was observed from the isolates of the other eight *Fusarium* species. Only two of the 11 *F. equiseti* isolates had the 658-bp Tox5 PCR product (Table 2). In the study by Niessen and Vogel (1998) only one of the two *F. equiseti* strains collected from barley was positive with Tox5 PCR, and Hestbjerg et al. (2002) reported that 8 of 57 isolates from soils in northern Europe produced trichothecenes. Abramson et al. (1993) tested culture extracts of three Manitoba isolates and detected very low levels of trichothecenes in all three. Although the results for the Tox5 gene indicate that Canadian isolates of *F. equiseti* from seed are not frequently able to form trichothecenes, testing of a wider selection of isolates is required to further evaluate the role of this species in mycotoxin production in Canada.

*F. graminearum* species-specific PCR amplification was obtained for all 22 isolates with Fg16F/Fg16R primer set (Table 2). No amplification was obtained from the other eight *Fusarium* species. To further test the primers, five *F. graminearum* isolates from Australia (provided by Dr. V. Mitter, CSIRO, Australia) and 50 other isolates recovered from FDK in Canada (data not shown) were correctly identified with the Fg16F/R primer set. These additional isolates were not challenged with the primers from the other eight species nor tested for the presence of the Tox5 gene. O'Donnell et al. (2004) proposed the existence of nine phylogenetically distinct species within the *F. graminearum* clade. It is possible that PCR performed with the Fg16F/R primer set can amplify DNA fragments from many of the suggested *F. graminearum* species. According to Waalwijk et al. (2003) *F. graminearum* lineages 1, 2, 6 and 7 (lineage 7 was the only lineage recovered from North American crops and O'Donnell et al. have retained the name *F. graminearum* for this group) were amplified using the Fg16F/R primer set, whereas no PCR products were found in lineages 3, 4 and 5. All 22 *F. graminearum* isolates produced the 658-bp *tri5* DNA fragment (Table 2). Although Abramson et al. (2001) reported that 14 of 42 western Canadian isolates grown on autoclaved rice did not produce detectable amounts of trichothecenes, it may be that the substrate or growth conditions were not suitable for these isolates to produce measurable levels of trichothecenes.

*F. poae* species-specific PCR using the primer set FP82F/R produced the expected 220-bp DNA fragment in all the 10 isolates (Table 2). PCR performed on DNA isolates of the other eight *Fusarium* species did not result in the amplification of the 220-bp DNA fragment, indicating the specificity of the FP82F/R primer set. In addition, Tox5 PCR resulted in the amplification of the 658-bp *tri5* DNA fragment in nine of the 10 isolates (Table 2). In 1993, Abramson et al. reported that 6 of 6 Manitoba isolates of *F. poae* produced significant amounts of Type A trichothecenes in liquid culture.

All 18 *F. pseudograminearum* isolates produced the expected 523-bp FP1-1/FP1-2 DNA fragment (Table 2). In addition, five *F. pseudograminearum* isolates from Australia (courtesy of Dr. V. Mitter, CSIRO, Australia) were also correctly identified (data not shown). No amplification was obtained from the other eight *Fusarium* species isolates indicating the specificity of the FP1-1/FP1-2 primer set. Five of the 18 isolates also resulted in the amplification of the 658-bp *tri5* DNA fragment. The ability of Canadian isolates of

*F. pseudograminearum* to produce trichothecenes has yet to be studied, but they do appear to cause FHB as 13 of the 18 were recovered from FDK.

The *F. sporotrichioides* species-specific primer set (AF330109CF/R) was designed in our laboratory based on sequence data available in Genbank (Table 2). All ten *F. sporotrichioides* isolates produced the expected 332-bp DNA fragment. None of the DNA from the other eight *Fusarium* species was amplified with the *F. sporotrichioides* specific primer indicating the specificity of the AF330109CF/R primer set. In addition, seven of the ten isolates produced the 658-bp *tri5* DNA fragment. According to Niessen and Vogel (1998) one out of four *F. sporotrichioides* strains collected from barley and wheat did not amplify the 658-bp PCR product using the Tox5 primer set. Abramson et al. (1993) found 6 of 6 Manitoba isolates of *F. sporotrichioides* to be capable producers of several Type A trichothecenes in liquid culture, including T-2 toxin, HT-2 toxin, and DAS.

### 3.2. Development of multiplex PCR

Multiplex PCR was developed for the simultaneous detection of the three most important mycotoxin producing *Fusarium* species (*F. culmorum*, *F. graminearum* and *F. sporotrichioides*) in Canada. Mycelial DNA from the three *Fusarium* species as well as DNA extracted from the grain samples resulted in the amplification of species-specific DNA fragments (Fig. 1). AccuPrime Taq DNA polymerase worked more consistently than Ampli*Taq* DNA polymerase for the multiplex PCR (data not shown).

### 3.3. Comparison of PCR and whole seed agar plate methods in the detection of F. graminearum, F. culmorum and F. sporotrichioides and the presence of trichothecenes in 85 grain samples

The primary toxigenic species, *F. graminearum*, was detected by species-specific PCR amplification in 34 of 64 wheat, three of six corn, nine of ten barley, three of four oat, and the single rye sample, demonstrating that the method is suitable for the major cereal grains grown in Canada (Table 3). *F. culmorum* was detected in the fewest samples by PCR (17 samples) and by whole seed agar plating (16 samples) (Table 3). *F. sporotrichioides* was detected in 48 of 85 grain samples by PCR and in 53 of 82 grain samples by whole seed plating. There was no significant difference ($P>0.999$) between the two detection methods for *F. culmorum* and *F. sporotrichioides* (data not shown). Although *F. pseudograminearum* was not detected in grain by whole seed or PCR testing (data not shown) and *F. crookwellense* was present in only one grain sample, the morphological similarity of these two species to *F. graminearum* and the presence of these species in Canada emphasizes the need to be able to reliably distinguish them from *F. graminearum* in order to properly enforce the Alberta legislation. Overall, over 83% of the results for the whole seed plate method were the same as the PCR results (Table 3). In all samples where a discrepancy was noted the level of the target species in the plating results was very low. Most importantly, *F. graminearum* was detected more often by PCR (50 of 85 samples) than by whole seed agar plating (35 of 82 samples), and there was a statistically significant difference ($P=0.024$) between the two methods. The PCR-based method was able to accurately distinguish between *F. graminearum*, *F.*

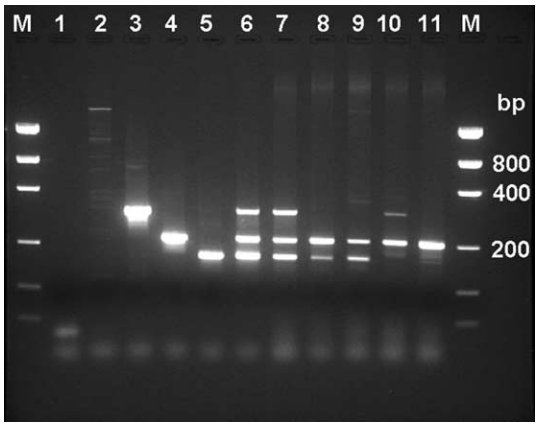


Fig. 1. Multiplex PCR system for amplification of three toxin producing Fusaria (*F. culmorum*, *F. graminearum*, and *F. sporotrichioides*) in wheat. Lanes. 1—no DNA control; 2—non-*Fusarium* infected wheat DNA; 3—*F. culmorum* mycelial DNA; 4—*F. graminearum* mycelial DNA; 5—*F. sporotrichioides* mycelial DNA; 6—mixtures of the three mycelial DNA; 7 to 11—DNA from wheat composite samples of Canada western amber durum (CWAD), SK 5B (7), Canada western red spring (CWRS), SK 1B (8), CWRS, MB 6 (9), CWAD, AB 2 (10), CWRS, SK 7B (11). Low mass DNA ladder (Invitrogen, CA) is shown in the first and last lanes.

Table 3
Detection of *F. graminearum*, *F. culmorum* and *F. sporotrichioides* in grain samples using PCR, whole seed agar plate and mycotoxin analysis

| Sample information[a] | | Mycotoxins[b] | | | *F. culmorum* | | F. graminearum | | F. sporotrichioides | |
|---|---|---|---|---|---|---|---|---|---|---|
| Crop and province | CD or town | Ppm DON | ppm 15-ADON | ppm HT-2 | PCR | % | PCR | % | PCR | % |
| CWRS, MB | 1 | 0.6 | ND | ND | – | 0.0 | + | 6.5 | + | 1.5 |
| CWRS, MB | 2 | 0.1 | ND | ND | – | 0.5 | + | 1.5 | + | 2.0 |
| CWRS, MB | 3 | 0.8 | ND | ND | – | 0.0 | + | 11.0 | + | 1.5 |
| CWRS, MB | 4 | 0.2 | ND | ND | – | 0.0 | + | 4.0 | + | 2.0 |
| CWRS, MB | 5 | ND | ND | ND | + | 0.0 | – | 0.0 | + | 3.0 |
| CWRS, MB | 6 | 0.2 | ND | ND | – | 0.0 | + | 1.5 | + | 0.0 |
| CWRS, MB | 7 | 2.0 | ND | ND | – | 0.0 | + | 22.0 | + | 1.0 |
| CWRS, MB | 8 | 0.5 | ND | ND | – | 0.0 | + | 7.5 | + | 1.0 |
| CWRS, MB | 9 | 1.2 | ND | ND | – | 0.0 | + | 6.5 | + | 0.5 |
| CWRS, MB | 11 | 1.0 | ND | ND | – | 0.0 | + | 10.0 | + | 2.5 |
| CWRS, MB | 12 | 1.3 | ND | ND | – | 0.5 | + | 10.5 | + | 2.5 |
| CWRS, SK | 1A | 0.7 | ND | ND | – | 0.0 | + | 4.5 | + | 1.0 |
| CWRS, SK | 1B | 0.3 | ND | ND | – | 0.0 | + | 3.5 | + | 2.5 |
| CWRS, SK | 2A | 0.1 | ND | ND | – | 0.0 | + | 0.0 | + | 2.5 |
| CWRS, SK | 2B | ND | ND | ND | – | 0.0 | + | 0.0 | + | 0.5 |
| CWRS, SK | 3AN | ND | ND | ND | – | 0.5 | + | 0.0 | + | 1.5 |
| CWRS, SK | 3AS | ND | ND | ND | + | 0.0 | – | 0.0 | + | 0.0 |
| CWRS, SK | 3BN | ND | ND | ND | + | 0.0 | – | 0.0 | – | 0.0 |
| CWRS, SK | 3BS | ND | ND | ND | – | 0.5 | – | 0.0 | – | 0.0 |
| CWRS, SK | 4A | ND | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| CWRS, SK | 4B | ND | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| CWRS, SK | 5A | ND | ND | ND | – | 0.0 | + | 0.5 | + | 1.5 |
| CWRS, SK | 5B | ND | ND | ND | – | 0.0 | – | 0.5 | + | 1.5 |
| CWRS, SK | 6A | ND | ND | ND | – | 0.0 | – | 0.0 | + | 1.0 |
| CWRS, SK | 6B | ND | ND | ND | + | 0.0 | – | 0.0 | – | 0.0 |
| CWRS, SK | 7A | ND | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| CWRS, SK | 7B | ND | ND | ND | – | 0.0 | + | 0.0 | – | 0.0 |
| CWRS, SK | 8A | ND | ND | ND | – | 0.0 | – | 0.0 | + | 2.0 |
| CWRS, SK | 8B | ND | ND | ND | + | 0.0 | – | 0.0 | + | 0.0 |
| CWRS, SK | 9A | ND | ND | ND | – | 0.0 | + | 0.0 | – | 0.0 |
| CWRS, SK | 9B | 0.1 | ND | ND | – | 0.0 | + | 0.0 | – | 0.0 |
| CWRS, AB | 1 | ND | ND | ND | – | 0.0 | + | 0.0 | – | 0.0 |
| CWRS, AB | 2 | ND | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| CWRS, AB | 3 | ND | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| CWRS, AB | 4 | ND | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| CWRS, AB | 5 | ND | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| CWRS, AB | 6 | ND | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| CWRS, AB | 7 | ND | ND | ND | – | 0.0 | + | 0.0 | – | 0.0 |
| CWAD, MB | 1 | 1.1 | ND | ND | – | 0.0 | + | 5.0 | + | 6.0 |
| CWAD, MB | 2 | 0.7 | ND | ND | – | 0.0 | + | 13.0 | + | 4.5 |
| CWAD, MB | 5 | 0.1 | ND | ND | – | 0.0 | – | 0.5 | + | 3.5 |
| CWAD, SK | 1A | 1.3 | ND | ND | – | 0.0 | + | 8.0 | + | 2.5 |
| CWAD, SK | 1B | 0.8 | ND | ND | – | 0.0 | + | 5.5 | + | 3.5 |
| CWAD, SK | 2A | 0.2 | ND | ND | – | 0.0 | + | 1.5 | + | 1.5 |
| CWAD, SK | 2B | ND | ND | ND | – | 0.0 | + | 1.0 | + | 1.0 |
| CWAD, SK | 3AN | Trace | ND | ND | + | 0.0 | + | 0.0 | – | 0.5 |
| CWAD, SK | 3AS | ND | ND | ND | – | 0.0 | – | 0.0 | – | 1.5 |
| CWAD, SK | 3BN | ND | ND | 0.11 | – | 0.0 | – | 0.0 | – | 0.5 |
| CWAD, SK | 3BS | ND | ND | ND | – | 0.5 | – | 0.0 | + | 0.5 |
| CWAD, SK | 4A | ND | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| CWAD, SK | 4B | ND | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| CWAD, SK | 5A | 0.1 | ND | ND | – | 0.0 | + | 0.5 | + | 4.5 |

Table 3 (*continued*)

| Sample information[a] | | Mycotoxins[b] | | | *F. culmorum* | | F. graminearum | | F. sporotrichioides | |
|---|---|---|---|---|---|---|---|---|---|---|
| Crop and province | CD or town | Ppm DON | ppm 15-ADON | ppm HT-2 | PCR | % | PCR | % | PCR | % |
| CWAD, SK | 5B | 0.2 | ND | 0.1 | + | 2.5 | + | 1.0 | + | 6.0 |
| CWAD, SK | 6A | 0.1 | ND | ND | + | 0.5 | + | 1.0 | + | 1.5 |
| CWAD, SK | 6B | ND | ND | ND | + | 0.5 | – | 0.0 | + | 1.0 |
| CWAD, SK | 7A | 0.1 | ND | ND | – | 0.5 | – | 0.0 | + | 0.5 |
| CWAD, SK | 7B | Trace | ND | ND | + | 0.0 | – | 0.0 | – | 0.0 |
| CWAD, SK | 8A | ND | ND | ND | + | 0.0 | – | 0.0 | + | 0.5 |
| CWAD, SK | 8B | 0.3 | ND | ND | + | 2.0 | + | 0.0 | + | 4.0 |
| CWAD, SK | 9A | 0.1 | ND | ND | + | 0.5 | – | 0.0 | – | 0.0 |
| CWAD, SK | 9B | ND | ND | ND | + | 0.5 | – | 0.0 | – | 0.0 |
| CWAD, AB | 1 | ND | ND | ND | + | 0.5 | – | 0.0 | – | 0.0 |
| CWAD, AB | 2 | Trace | ND | ND | + | 1.0 | + | 0.0 | – | 0.5 |
| CWAD, AB | 3 | ND | ND | ND | – | 0.0 | + | 0.0 | – | 0.0 |
| Barley, MB | Hamiota | 1.4 | 0.1 | ND | – | 0.0 | + | 26.5 | + | 3.5 |
| Barley, MB | Hamiota | 1.1 | 0.1 | ND | – | 0.0 | + | 15.0 | + | 2.0 |
| Barley, MB | Brandon | 0.4 | ND | ND | – | 0.0 | + | 7.0 | + | 1.0 |
| Barley, MB | Brandon | 0.5 | ND | ND | – | 0.0 | + | 9.5 | + | 1.5 |
| Barley, MB | Hargrave | 2.0 | 0.13 | ND | – | 0.0 | + | 16.5 | + | 7.5 |
| Hulless Barley, MB | Boissevain | 2.8 | 0.1 | ND | – | 0.0 | + | 22.5 | + | 4.0 |
| Barley, MB | Glenlea | 1.2 | Trace | ND | – | 0.0 | + | 4.5 | + | 3.0 |
| Barley, NB | NA | 7.2 | ND | ND | – | 0.0 | + | 38.5 | + | 9.5 |
| Barley, SK | Redvers | 6.4 | 0.61 | 0.15 | – | 0.0 | + | 47.5 | + | 5.0 |
| Barley, AB | Alix | ND | ND | ND | – | 0.5 | – | 0.0 | – | 0.0 |
| Oat, MB | Pilot Mound | 0.9 | 0.1 | 0.53 | – | 0.0 | + | 46.5 | + | 8.0 |
| Oat, MB (100 seeds) | Emerson | 3.6 | 0.16 | ND | – | 0.0 | + | 64.0 | + | 5.0 |
| Oat, SK | Wadena | ND | ND | ND | + | 0.5 | + | 0.0 | + | 2.5 |
| Oat, Cargo | Vancouver | 0.1 | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| Corn, ON. | Petrolia | 1.3 | 0.19 | ND | – | NA | + | NA | – | NA |
| Corn, ON | Essex | 8.7 | 1.8 | ND | – | NA | + | NA | – | NA |
| Corn, ON | Essex | 13.0 | 2.6 | ND | – | NA | + | NA | – | NA |
| Corn, AB | Lethbridge | ND | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| Corn, AB | Lethbridge | ND | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| Corn, AB | Lethbridge | ND | ND | ND | – | 0.0 | – | 0.0 | – | 0.0 |
| Rye, SK | Armour | 0.1 | ND | ND | – | 0.0 | + | 3.5 | + | 2.0 |

NA—not available.

Percentage values are based on 200 surface disinfested seeds per sample plated onto potato dextrose agar except for the Emerson oat sample, which is based on 100 seeds.

[a] CWRS—Canada western red spring wheat; CWAD—Canada western amber durum wheat; AB—Alberta; MB—Manitoba; NB—New Brunswick; ON—Ontario; SK—Saskatchewan; CD—Crop District. All wheat samples are CD composites. Other grains are samples from individual producers or a cargo sample.

[b] ND—not detected at a level equal to or greater than 0.10 ppm. Trace—detected at a level less than 0.10 ppm.

*pseudograminearum* and *F. crookwellense*. Failure of whole seed agar plating to detect species that PCR detected may be due to the removal of fungal material during surface disinfestations, failure to detect the Fusaria due to competition on the plate, or lack of viability of the target species. Differences between PCR and whole seed agar plating results may also be due to variation in the two sampling techniques and to low levels of the target species on the grain. Mulfinger et al. (2000) reported that sampling technique has a major influence on the analytical results of *Fusarium* contamination studies. In this trial, whole seed agar plating was done on 200 individual kernels, which would weigh about 7 or 8 g, whereas a 0.2-g subsample from 20-g ground seed was used for DNA extraction and PCR analyses. A negative PCR result in samples where whole seed plating was positive could be due to absence of an infected kernel

in the ground material or to a level of fungal growth that is below the sensitivity of the PCR but which becomes apparent when the fungi are allowed to grow during incubation on the agar plate.

Of the eight trichothecenes tested for, only three (DON, 15-ADON, and HT-2 toxin) were present at detectable levels. Wheat composite samples from Manitoba had a higher concentration of DON and *F. graminearum* infection than samples from Alberta or Saskatchewan, which is consistent with previous reports for barley and oat composite samples (Clear et al., 2000a,b). Forty-five samples had detectable DON levels. Forty-two of these, when analyzed by PCR, also had detectable levels of either *F. graminearum*, *F. culmorum*, or both, whereas in the same 42 analyzed by whole seed agar plating, 36 samples recorded one or both of these two species. PCR-based detection was also more sensitive when the 40 samples without DON were tested. Nineteen of the 40 samples had either *F. graminearum*, *F. culmorum* or both species-specific DNA fragments, whereas with whole seed agar plating 11 of 40 samples had one or both of these species detected (Table 3). *F. graminearum*, the most likely producer of 15-ADON (Abramson et al., 2001) was detected by PCR in all 11 samples having measurable levels of this toxin and in all 8 samples tested by whole seed agar plating. *F. sporotrichioides*, the most likely producer of HT-2 toxin, was detected by PCR in 3 of 4 samples and by whole seed agar plating in 4 of 4 samples that contained measurable levels of this toxin. Recently, Nicholson et al. (2004) have reported the development of PCR methods for the detection of different types of trichothecenes, which should be helpful for screening grain samples.

The PCR-based method, which can take as little as one day to complete, is quicker and more sensitive than whole seed agar plating, which takes a minimum of 5 days. PCR-based methods also can distinguish between morphologically similar species such as *F. graminearum* and *F. pseudograminearum* and *F. acuminatum* ssp. *acuminatum* and *F. avenaceum* ssp. *avenaceum*. The ability to detect *Fusarium* species commonly infecting Canadian grain, some of which are involved in mycotoxin production, will be valuable for the detection and screening of *Fusarium* spp. contamination in cereal grains in Canada. However, whole seed plating is likely superior to qualitative PCR-based testing when assessing the risk of mycotoxin contamination as whole seed agar plating provides an estimate of the degree of fungal infection in the sample, whereas a qualitative PCR-based method can only indicate the presence or absence of the target species. Development of a quantitative real-time PCR method (Waalwijk et al., 2004) would allow a better assessment of fungal mass than whole seed agar plating and could, therefore, be a better estimate of the risk of mycotoxin contamination by toxigenic *Fusarium* species. When screening for prohibited pathogens, a PCR-based test is preferable to whole seed agar plating because of greater sensitivity. Only where the pathogen has been eradicated by thermotherapy (Clear et al., 2002) or other fungicidal procedures would it be desirable to test the seed using whole seed plating as this method will reveal if the pathogen is still viable.

At present, the simple DNA extraction system coupled with the species-specific PCR-based test described in this work appears to be superior to morphological-based identifications of the common Fusaria infecting cereal grain in Canada. The method is also more suitable than whole seed plating for the screening of cereal samples for several trichothecene-producing species of Fusaria, notably *F. graminearum*.

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