Journal of Microbiological Methods 157 (2019) 21–30

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Real-time PCR quantification of *Fusarium avenaceum* in soil and seeds

Anne T. Pollard[a], Patricia A. Okubara[b,*]

[a] Department of Crop and Soil Sciences, Washington State University, Pullman, WA 6420, USA
[b] USDA-ARS, Wheat Health, Genetics and Quality Research Unit, Pullman, WA 99164-6430, USA

ARTICLE INFO

Keywords:
Fusarium avenaceum
Fusarium arthrosporioides
Primer design
Real-time PCR
Seeds
Soil
Translation elongation factor

ABSTRACT

The pathogenic fungus *Fusarium avenaceum* infects a broad range of plant hosts across the globe. While primarily soilborne, *F. avenaceum* can colonize all plant tissues, including buds, seeds, fruits, stems, crowns, and roots, resulting in significant crop yield reductions and economic losses for growers. In addition to its impact on crop productivity, *F. avenaceum* produces toxic metabolites that can be transferred to humans and livestock through consumption of infected foods. The ability of *F. avenaceum* to cause seed decay may be utilized to deplete the weed seedbank in soil, an important integrated weed management strategy. We developed a SYBR Green I-based real-time polymerase chain reaction (qPCR) assay to efficiently detect and quantify *F. avenaceum* in soil, wild oat (*Avena fatua* L.) seed caryopses, and wild oat seed hulls. The primer pair was designed from the translation elongation factor 1-alpha (*TEF1*) gene. *In silico* and wet lab testing were done to assess the ability of the primers to bind *TEF1* sequences from *Fusarium* spp. and common soil fungi. The findings indicated that the primers were specific to *F. avenaceum*, and also recognized GenBank *TEF1* accessions annotated as *F. arthrosporioides*, which has been listed as a foliar pathogen of wheat in Oregon, and conspecific with *F. avenaceum*. Standard curves of *F. avenaceum* DNA diluted with soil, caryopsis, or hull extracts indicated primer amplification efficiency was not significantly affected by PCR inhibitors. This real-time PCR assay effectively assesses the presence and abundance of *F. avenaceum* and its close relative *F. arthrosporioides*, if present, in soil and seed tissues. The assay can be used for endpoint PCR as well.

1. Introduction

The globally significant fungal pathogen *Fusarium avenaceum* causes various diseases in a wide range of staple food crops, including head blight in wheat, barley, and maize (Logrieco et al. 2002; Uhlig et al. 2007; Vogelgsang et al. 2008); root rot in legumes (Persson et al. 1997; Hwang et al. 2000; You et al. 2005; Feng et al. 2010; Holtz et al. 2011; Chang et al. 2014a), seedling blight and root rot in canola (Chen et al. 2014); and dry rot in potato (Peters et al. 2008). It often co-occurs with other *Fusarium* species, such as in the Fusarium head blight disease complex that may include *F. culmorum*, *F. graminearum*, and *F. poae*, among many others (Bottalico and Perrone 2002; Champeil et al. 2004; Köhl et al. 2007). *Fusarium avenaceum* infection can significantly reduce crop yields. Inoculation of 14 hard red spring wheat varieties in Switzerland resulted in up to 25% yield reductions (Vogelgsang et al. 2008) and seed inoculation of 19 lupine cultivars in Canada resulted in losses as great as 52% (Chang et al. 2014b).

This cosmopolitan generalist, which exists as a saprophyte, weak parasite, or strong pathogen (Yli-Mattila et al. 1996; Leslie and Summerell 2006), is broadly adapted across host species and implicated in plant diseases across the globe. Although most prevalent in temperate regions of the USA, Northern and Central Europe, Australia, and South Africa, *F. avenaceum* is also present in the arctic and sub-arctic regions of Norway, Siberia, and Canada (Abbas et al. 1989; Köhl et al. 2007; Muthomi et al. 2008; Summerell et al. 2011; Tewoldemedhin et al. 2011; and Stakheev et al. 2016). It has also been identified in the tropical highlands of Malaysia (Manshor et al. 2012) and the tropical rainforests of China (Luo et al. 2014). *Fusarium avenaceum* is typically isolated from soil, but can also be seedborne (McGee and Kellock 1974; Axelrood et al. 1995).

Pathogenic fungi, such as *F. avenaceum,* are virulent to economically significant crop plants in part through production of diverse degradative enzymes and toxic secondary metabolites (Gómez and Nosanchuk 2003; Abdellatif et al. 2010; Khaledi et al. 2017; Pollard 2018). Mycotoxin profiles vary among and within *Fusarium* species. *Fusarium avenaceum* has been shown to produce several lesser known fungal secondary metabolites, so called “emerging mycotoxins” (Ivanova et al. 2006; Jestoi 2008). Comparative analysis of the *F. avenaceum* genome to that of other sequenced *Fusarium* spp. suggests it is enriched with genes involved in “toxin biosynthetic processes” (Lysøe

* Corresponding author.
E-mail address: okubara@ars.usda.gov (P.A. Okubara).

https://doi.org/10.1016/j.mimet.2018.12.009
Received 20 October 2018; Received in revised form 11 December 2018; Accepted 11 December 2018
Available online 17 December 2018
0167-7012/

et al. 2014). Indeed, the metabalome of *F. avenaceum* commonly contains more secondary metabolites than other *Fusarium* spp. (Hansen et al. 2015). An examination of 53 *F. avenaceum* strains showed that enniatins and 2-amino-14,16-dimethyloctadecan-3-ol (2-AOD-3-ol) were the primary metabolites to produce a cytotoxic response in animal tissues, with the magenta pigment aurofusarin also demonstrating cytotoxicity, although to a lesser extent (Uhlig et al. 2006). Another study found that 10 of 13 Finnish strains of *F. avenaceum* infecting wheat heads produced beauvericin and all strains produced enniatins (Logrieco et al. 2002); both compounds have shown significant toxicity *in vitro* (Jestoi 2008; Fraeyman et al. 2017; Gruber-Dorninger et al. 2017). Moniliformin, aurofusarin, enniatins, and 2-AOD-3-ol were isolated from diseased apples infected with *F. avenaceum*, the primary causal agent of wet apple core rot (Sørensen et al. 2009). Phytotoxic effects of *Fusarium* mycotoxins include inhibition of seed germination, inhibition of root and shoot growth throughout plant development, and effects associated with disease symptoms such as wilting, necrosis, and chlorosis (Köhl et al. 2007; Ismaiel and Papenbrock 2015). Mycotoxin production can be influenced by external factors including climate, environment, and host (Miller et al. 1991; Starkey et al. 2007; Vogelgsang et al. 2008; Vujanovic et al. 2017).

The phytotoxic properties of pathogenic fungi may be harnessed for the development of alternative weed management strategies, specifically those aiming to deplete the weed seedbank in soils (Davis et al., 2006). Weed seeds are densely distributed in soils, with up to nearly 1 million seeds m^{-2} detected in agricultural soil seedbanks (Baskin and Baskin 2006). Microbial seed decay, which commonly occurs *via* mycotoxin production, cell wall degrading enzymes, and hyphal penetration, is a major cause of seed loss from soil (Buhler et al. 1997; Menalled et al. 2001; Bastiaans et al. 2008; Pollard 2018). Seed fatality of the common weed downy brome (*Bromus tectorum*) due to the fungal seed pathogen *Pyrenophora semeniperda* has been extensively researched (Beckstead et al. 2007; Finch et al. 2013; Finch-Boekweg et al. 2013; Meyer et al. 2014). *Fusarium avenaceum* isolate *F.a.*1, which was used in this study, colonized dormant seeds of the noxious weed wild oat (*Avena fatua* L.), and elicited their rapid and pronounced decay in soil incubation experiments (de Luna et al. 2011). Isolate *F.a.*1 produces a magenta pigment likely to be the toxin aurofusarin (Vujanovic et al. 2017). Subsequent agar media-based studies on the interaction between isolate *F.a.*1 and wild oat seeds at the biochemical and molecular level indicated that wild oat seeds mount a complex enzymatic defense response to *F.a.*1 (Anderson et al. 2010; Fuerst et al. 2011, 2014, 2018). The influence of soil properties and conditions on the weed seed-soil microbe interaction, however, remains unclear (Wagner and Mitschunas 2008; Gallagher et al. 2013; Abedi et al. 2014). In order to develop integrated weed management techniques that utilize the inherent ability of soil fungi to cause weed seed mortality, a deeper mechanistic understanding of fungal-weed seed interactions must be attained.

A sensitive, specific and reproducible method for detecting and quantifying *F. avenaceum* in soil and plant tissue is an important tool for studying its application in weed management, its role in global crop disease, and its potential toxicity to humans and animals. Accurate identification of *Fusarium* species is complex and controversial. *Fusarium* species are defined using morphological, biological, and especially phylogenetic species concepts, yet the three approaches frequently do not yield the same result (Leslie and Summerell 2006; Summerell et al. 2010; Aoki et al. 2014). Moreover, rapid genome evolution from horizontal gene transfer may further complicate phylogenetic analyses (Ma et al. 2010; Watanabe et al. 2011; O'Donnell et al. 2015). Accurate quantification of fungi using conventional culture-based methods is likewise labor-intensive and prone to inaccuracy (Filion et al. 2003). Since its inception in the mid-1990s, real-time, or quantitative, PCR (qPCR) has become the laboratory standard for detection and quantification of specific DNA targets.

The fungal nuclear ribosomal internal transcribed spacer (ITS) region, widely accepted as the universal barcode for fungal identification (Schoch et al. 2012), is not sufficiently diverse to identify *Fusarium* to the species level (O'Donnell and Cigelnik, 1997). Intronic regions of protein-coding genes, such as the translation elongation factor 1-alpha (*TEF1*) gene, frequently evolve at a faster rate than ITS regions, which enables them to more precisely distinguish between related species (O'Donnell et al. 1998a; Watanabe et al. 2011). The *TEF1* sequence displays an even higher degree of polymorphism among closely related species compared to other intron-rich protein-coding genes (Rahjoo et al. 2008), making it an effective alternative target region for phylogenetic discrimination of *Fusarium* at the species level. Moreover, unlike ITS, non-orthologous copies of *TEF1* have not been identified in the genus (Geiser et al. 2004; O'Donnell et al. 2015). Single copy qPCR assays allow for more accurate quantification compared to qPCR of multi-copy genes, but the sensitivity of detection is greater in the latter (Churro et al. 2012; Gil-Serna et al. 2017).

Our aim was to develop a cost-effective molecular assay that could discriminate and quantify *F. avenaceum* from soil and seed samples. Previous studies have identified real-time PCR primers for quantifying *F. avenaceum* using either TaqMan or SYBR Green I technology (Waalwijk et al. 2004; Nicolaisen et al. 2009). However, in neither study were the primers tested against DNA extractions from soil samples, which is significant because it is primarily a soilborne fungus. Moreover, in order to utilize *F. avenaceum* to biologically control weed seeds in the soil seedbank we need an accurate and reproducible method for its specific detection and quantification in wild weed seeds, which commonly display different physiological traits than seeds from their cultivated relatives (Tanksley and McCouch 1997; Paz and Vazquez-Yanes 1998). Here, we report a SYBR Green I-based real-time PCR assay targeting the *TEF1* gene region for the specific detection and quantification of *F. avenaceum* DNA isolated from pure culture, from soil, and from seeds of the noxious weed wild oat incubated in soil.

2. Materials and methods

Laboratory soil microcosms were established using pasteurized or nonpasteurized soil, infested with *F.a.*1 or non-infested. Three organ fractions derived from wild oat seeds were used in the experiments: 1) the whole seed, consisting of the intact caryopsis (grain) and its attached outer hull (lemma and palea); 2) the caryopsis, or grain without the hull; and 3) the dissected hull, consisting of the lemma and palea but no caryopsis. In the Soil-Caryopsis experiments, wild oat caryopses were incubated in the soils for 3 to 28 days. In the Soil-Whole Seed experiments, intact whole wild oat seeds were incubated in the soils for 2 to 9 weeks.

2.1. Fungal isolates

Fusarium avenaceum isolate *F.a.*1 was used to initiate development of the qPCR assay. It was originally isolated from the surface of dormant wild oat caryopses (seeds without hulls) buried in field soil at the Palouse Conservation Field Station in Pullman, Washington, located in the inland Pacific Northwest, USA. A thorough description of the isolation and origin of this isolate, also known as “223a”, can be found in de Luna et al. (2011) and Fuerst et al. (2011). Genomic DNA was extracted from the following additional fungal isolates for assessing *F. avenaceum* primer specificity: *F. oxysporum* f. sp. *spinaciae* isolate 001 (GenBank FJ972801.1) (Okubara et al. 2013); *F. culmorum* isolate 70,110,023, a Pacific Northwest native isolate originally collected in a 2011 field survey (Thompson et al. 2017); *F. pseudograminearum* Gp-2, causal agent of Fusarium crown rot of wheat (Okubara et al. 2017); *Rhizoctonia solani* AG-8 isolate C1 (Weller et al. 1986; Smith et al. 2003); and *Gaeumannomyces graminis* var. *tritici* (*Ggt*).

The isolates were cultured on potato dextrose agar (PDA) plates. For all fungal isolates except *Ggt*, cultures were started using stocks of fungi that had been archived on sterile cellulose Whatman No. 1 filter papers

at −20 °C (Okubara et al. 2013). One 0.5 cm^2 filter disc was placed on the center of a PDA plate. *Ggt* cultures were started by transferring an agar plug from the margin of an actively growing *Ggt* culture onto a fresh ⅕ × PDA plate. All plates were incubated at 25 °C in the dark.

2.2. F.a.1-oat inoculum

The *F.a.*1 inoculum used for infesting soil was made as described by Paulitz and Schroeder (2005). In summary, 250 mL of clean whole oat (*Avena sativa* L.) seed were mixed in an Erlenmeyer flask with 175 mL deionized (DI) water and autoclaved on two consecutive days (60 min @ 120 °C). Oats were infested with three PDA plates (100 mm) of 10–14 d *F.a.*1 cultures, cut into 0.5 cm^2 pieces, and incubated at 25 °C for four weeks. Flasks were gently shaken weekly to evenly distribute the *F.a.*1 mycelia throughout the oats. Inoculated oats were dried, ground with a coffee grinder, and sequentially passed through #35 (500 μm) and #60 (250 μm) sieves; the fraction retained on the latter sieve was used to infest soil. Population density (CFU g^{-1} of ground inoculum) of the *F.a.*1-oat inoculum was estimated from 1:10 serial dilutions of 1 g inoculum in sterile DI water plated on water agar (Okubara et al. 2008).

2.3. Soil treatments and seeds

The soil used was a Shano sandy loam (coarse-silty, mixed, superactive, mesic Xeric Haplocambids), collected from a non-agricultural site near Quincy, Washington that is covered in native vegetation. The physical and chemical properties of the soil have been previously described (Raaijmakers et al. 1997). It was collected from the upper 30 cm of the soil profile, air dried for one week, passed through a 0.5 cm sieve, and stored at room temperature (Raaijmakers et al. 1997; Landa et al. 2002).

The four soil treatments used in the study were: 1) pasteurized; 2) pasteurized and infested with ground *F.a.*1 inoculum (described below); 3) nonpasteurized; and 4) nonpasteurized and infested with ground *F.a.*1 inoculum. Soil infestation rates were 1500 CFU *F.a.*1 per gram soil for the whole seed experiments and 500 CFU *F.a.*1 per gram soil for the caryopsis experiments, which was equivalent to approximately 2.25 g and 0.75 g, respectively, per replicated soil microcosm (described below). To clarify, the “whole seed experiments” used intact wild oat seeds. Pasteurization was carried out on two consecutive days at 60 °C for 60 min, followed by air drying in a laminar flow hood for 48 h. Sterile DI water was added to each soil treatment to attain 9.31% gravimetric water content equivalent to −0.11 megapascals (Mpa) water potential, which is an approximate water activity for optimal *F. avenaceum* vegetative growth (Magan and Lacey 1984).

Seeds used in the experiments were harvested from the highly dormant wild oat isoline Montana 73 (M73) (Naylor and Fedec 1978) grown under greenhouse conditions at Washington State University (Pullman, Washington, USA) in 2016 and 2017. For the experiments in which caryopses were used, the hulls (lemma and palea) were removed by hand to avoid caryopsis damage.

2.4. Soil microcosm set-up and seed infection assays

Two different wild oat seed infection assays were established by constructing experimental soil microcosms from Magenta™ GA-7 boxes (Sigma Aldrich, St. Louis, MO, USA) using the four previously described soil treatments. In one experiment, whole seeds were sampled at two-week intervals for eight weeks; in the second experiment, caryopses were sampled at six times over a 28-d interval. The four treatments were replicated 5 times per time-point.

Autoclaved Magenta™ GA-7 boxes (7.7 cm × 7.7 cm × 9.7 cm) were loosely filled halfway with 150 g moistened soil (described previously in section 2.3). Two nylon mesh bags (Gcircus, Shenzhen, China, amazon.com) (6 cm × 7 cm), each filled with 10 g soil and either 15 whole seeds or 20 caryopses for the whole seed and caryopsis experiments, respectively, were positioned vertically upright side-by-side on the soil surface. An additional 100 g soil was added to cover the bags and keep them separated from each other. Each mesh bag represented one time-point within each treatment, such that a single microcosm was used for two consecutive time-points. Boxes were covered with lids to prevent cross-contamination and incubated in the dark at 25 °C. After 2, 4, 6, and 8 weeks (for whole seeds) or 3, 7, 10, 14, 21, and 28 d (for caryopses), one mesh bag was removed from each microcosm and the soil and seed material contained within were collected. The soil was air-dried and stored at −20 °C. In the whole seed experiment, after seeds were retrieved from the mesh bags, hulls were carefully removed from the caryopses. This allowed us to evaluate *F.a.*1 primers on the hull and caryopsis fractions separately. All collected seed material was stored at −20 °C.

2.5. DNA extraction from F.a.1 culture, soil, and wild oat seeds

Total genomic DNA was extracted from cultured *F.a.*1 using the FastDNA™ Kit (MP Biomedicals™, Santa Ana, California, USA) according to the manufacturer's instructions. Mycelia were harvested from *F.a.*1 cultures after incubation for 5 to 7 d in potato dextrose broth. Mycelia were washed with sterile DI water over gentle vacuum filtration on a Buchner funnel and total genomic DNA was extracted from 0.25 g of partially dried mycelia. The samples were homogenized twice at speed four for 45 s using the FastPrep®-24 homogenizer (MP Biomedicals). DNA concentration was determined using the Fluorescent DNA Quantitation Kit (Bio-Rad, Hercules, California) and a Synergy 2 microplate reader (BioTek Instruments, Winooski, Vermont, USA), and with a NanoDrop® spectrophotometer (Thermo-Fisher Scientific, Waltham, Massachusetts, USA). DNA quality was assessed from $A_{260/280\ nm}$ using a NanoDrop®.

Total *F.a.*1 pure culture genomic DNA was also extracted using the DNeasy PowerLyzer PowerSoil DNA extraction kit (Qiagen, Hilden, Germany) and the DNeasy PowerPlant Pro Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Mycelia was harvested and DNA extracted and quantified as described above, except for different bead beating parameters. Homogenization with the FastPrep®-24 (MP Biomedicals) was performed twice at speed 5 for 45 s and once at speed 4.5 for 2 min for the soil and plant kit, respectively. Pure culture *F.a.*1 DNA extracted with the soil and plant kits (Qiagen) were used to create *F.a.*1 standard curves, which were in turn applied when quantifying *F.a.*1 in soil and seed DNA extractions *via* qPCR.

Total genomic DNA was extracted from samples of ~0.25 g of air-dried soil, five wild oat caryopses (~0.10 g), and five hulls (~0.03 g) using the soil and plant kits (Qiagen) as previously described. Caryopses and hulls were finely-ground using a mortar and pestle prior to DNA extraction.

2.6. F.a.1 TEF1 gene sequencing and identification

The *TEF1* gene region from *F.a.*1 total genomic DNA extracts was amplified by conventional PCR using the EF-1 forward primer (5′ ATG GGT AAG GAR GAC AAG AC 3′) and the EF-2 reverse primer (5′ GGA RGT ACC AGT SAT CAT G 3′) (Eurofins Genomics, Louisville, Kentucky, USA) (O'Donnell et al. 1998b, 2012). Amplification reactions were performed in a total volume of 25 μL containing 1 × Green GoTaq Flexi Buffer (Promega, Madison, Wisconsin, USA), 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 5 μM EF-1 forward primer, 5 μM EF-2 reverse primer, 1.25 U GoTaq Flexi DNA polymerase, and 75 ng of genomic DNA. The amplification program was modified from Haapalainen et al. (2016) and included an initial denaturation step at 94 °C for 60 s, 30 cycles of denaturation at 94 °C for 30 s, primer annealing at 58 °C for 30 s, elongation at 72 °C for 60 s, and a final 5 min extension period at 72 °C. The PCR amplifications were performed with a T100 thermal cycler (Bio-Rad). The size and quality of the DNA were analyzed by horizontal gel electrophoresis of a 5 μL aliquot of the PCR product on a 1% agarose

gel in 1× Tris-acetate-EDTA (TAE) buffer, pH 8.0. Gels were stained with ethidium bromide and visualized using the AlphaImager HP System (Protein Simple, Santa Clara, California, USA). PCR products were purified using QIAquick PCR Purification Kit (Qiagen, Valencia, California, USA) and DNA (10–15 ng μL^{-1}) was sequenced by Eurofins Genomics using the EF-1 and EF-2 primers. The *F.a.*1 forward and reverse nucleotide sequences were edited and assembled into a contig using SeqMan II (Version 5.0, DNASTAR, Inc. Madison, Wisconsin, USA). The consensus sequence was queried against the GenBank non-redundant nucleotide database using MegaBLAST (Altschul et al. 1990; Zhang et al. 2000) and open reading frames were analyzed using NCBI's Open Reading Frame Finder (https://www.ncbi.nlm.nih.gov/orffinder/), both with the default settings.

2.7. Sequence alignments and primer design

Potential *TEF1* qPCR primers specific to *F. avenaceum* were identified by aligning multiple *TEF1* sequences using Clustal Omega (EMBL-EBI; Sievers et al., 2011) and sequence differences were visualized using GeneDoc version 2.6.001 (Nicholas et al. 1997). Sequences from isolate *F.a.*1 were aligned with sequences retrieved from GenBank that represented a wide range of fungal genera and species, geographic origins, and isolate source. These included diverse *F. avenaceum* accessions, various *Fusarium* spp. prevalent in Pacific Northwest soils, and other common non-*Fusarium* soil fungi (Table 1). Nine unique regions in the multiple sequence alignment were manually identified for potential primer design based on melting temperature, GC content, primer and amplicon length, and predicted homo- and hetero-dimer formation determined using GeneRunner 6.2.07 (Edgar 2004; Untergasser et al. 2012) and OLIGO 7 (Molecular Biology Insights, Inc., Colorado Springs, Colorado, USA) (Rychlik 2007).

2.8. Conventional PCR and gel electrophoresis

Conventional PCR and gel electrophoresis were performed with the potential primer pairs to confirm amplification of the target *F.a.*1 region, to check for possible primer dimer formation, and to assess annealing temperature. Primer pairs were tested against DNA extracted from pure *F.a.*1 culture, non–*F. avenaceum* fungi (Table 1), and non-pasteurized non-infested soil (no added *F.a.*1 inoculum); sterile nanopure water was the negative control. Each 25 µL amplification reaction was as described previously for the EF-1 /EF-2 primers, but with the following changes: 10 µM each forward and reverse primer and 2 µL DNA. The thermocycling program included an initial denaturation step at 95 °C for 90 s, followed by 40 cycles of denaturation at 95 °C for 30 s, primer annealing at 61 °C for 60 s, elongation at 72 °C for 90 s, and a final 5 min extension period at 72 °C. PCR amplifications were performed with a T100 thermal cycler (Bio-Rad). Five µL of PCR product from each reaction were analyzed by horizontal gel electrophoresis on a 2% agarose gel in 1× TAE buffer. Gels were stained, visualized, and processed as previously described.

Table 1
Non-target soil fungi included in primer testing.[a]

Sample	pg target *F.a.*1 DNA/pg total gDNA[b]	Ct
Negative control (water)	0	na
F. avenaceum (10^{-2} standard)	1.0 E+00	21.8
F. culmorum isolate 70,110,023	3.0 E-04	36.5
F. culmorum isolate 728	8.2 E-05	37.3
F. pseudograminearum Gp-2	6.3 E-04	35.8
Gaeumannomyces graminis var. *tritici*	na	na
Rhizoctonia solani AG-8 isolate C1	7.3 E-04	35.7
Chaetomium jodhpurense	1.4 E-04	36.1

na = no amplification.
[a] Ct and DNA values generated with primer pair 3.
[b] pg of target *F.a.*1 DNA per pg of total genomic DNA used per qPCR reaction.

2.9. F.a.1 standard curve and qPCR cycling conditions

All qPCR-based evaluation of potential qPCR primers was performed in triplicate using a 96-well plate-based LightCycler 480 II (Roche Diagnostics, Penzberg, Germany) and KAPA SYBR FAST qPCR Kit Master Mix (2×) (Kapa Biosystems, Wilmington, Massachusetts, USA). Efficiency and sensitivity of the three primer pairs were evaluated using ten-fold serial dilutions in water of *F.a.*1 genomic DNA ranging from 0.0736 to 73,600 pg genomic DNA per reaction. Standard curves were produced by plotting the average cycle threshold (Ct) values against the log DNA concentration. Sterile nanopure water and soil and seed samples not infested with *F.a.*1 were used as the negative controls. Amplification efficiencies were calculated from the slope of Ct plotted against log DNA concentration (Ginzinger 2002). Primer specificity was assessed using qPCR melt curve analysis of target and non-target genomic DNA extracted from: 1) the four previously described soil treatments, 2) various prevalent non–*F. avenaceum* soil fungi (Table 1), and 3) wild oat caryopses and hulls.

Amplification reactions were performed in final volumes of 10 µL consisting of 1× Kapa Mix, 0.4 µM each forward and reverse primer, and 1.0 µL of DNA template or sterile nanopure water for negative controls. The qPCR cycling conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 50 cycles of 95 °C for 10 s, annealing temperature for 5 s, and 72 °C for 10 s. Melting curves were produced by increasing the reaction temperature from 65 °C to 97 °C. Annealing temperatures between 61 °C and 64 °C were evaluated. The primer pairs that exhibited a single melting curve peak, high endpoint fluorescence, and efficiency > 80% were chosen for additional testing.

2.10. In silico analysis

Specificity of the most favorable candidate primer pairs was additionally tested *via in silico* analysis using NCBI Primer-BLAST (Altschul et al. 1990; Ye et al. 2012) with the default settings combined with duplex stability (delta G) predictions of primer-target and non-target sequences using PrimerSelect (DNASTAR, Inc. Madison, Wisconsin, USA). Delta G values were calculated using PrimerSelect and default settings of 50 mM salt concentration, 25 °C, and 0.76 kcal mol^{-1} bp^{-1} average stability cut-off (Okubara et al. 2013). For the non–*F. avenaceum* accessions whose delta G values indicated potential mis-priming, additional sequences of those species were retrieved from GenBank and analyzed *in silico*.

2.11. Agricultural samples

To determine if the soil or caryopsis and hull fractions contained compounds that could inhibit qPCR amplification (Wilson 1997), DNA extracted from pure *F.a.*1 culture was serially diluted ten-fold with pooled DNA extracts from four replicated samples of 1) 0.25 g non-pasteurized non-infested soil; 2) five non-infested caryopses; and 3) five non-infested hulls. DNA standard curves were created with each set of serial dilutions, and PCR inhibition was assessed by comparison with the *F.a.*1 water standard curve. Initial *F.a.*1 DNA concentrations were 7.36×10^4 pg μL^{-1} for the soil extract, and 1.32×10^5 pg μL^{-1} for the caryopsis and hull extracts. DNeasy PowerLyzer PowerSoil kit (Qiagen) and DNeasy PowerPlant Pro Kit (Qiagen) were used according to previously described conditions for extracting DNA from soil samples and seed caryopsis and hull fractions, respectively.

In addition, a soil spiking experiment was conducted to assess the relationship between the concentration of *F.a.*1-oat inoculum added to soil and the quantity of *F.a.*1 detected with qPCR, as well as to evaluate the limit of quantification and detection of *F.a.*1 in soil. Increasing concentrations (0, 50, 100, 250, 500, 1000, and 2000 CFU g^{-1} soil) of

*F.a.*1-oat inoculum were added to 10.00 g aliquots of pasteurized, air-dried non-infested soil and thoroughly mixed to evenly distribute inoculum. Four replicate soil samples (0.25 g) were collected from each inoculum level (total $n = 28$) and genomic DNA was extracted from each using the DNeasy PowerLyzer PowerSoil kit (Qiagen). Triplicate qPCR reactions were performed on all DNA extractions and *F.a.*1 quantified using an *F.a.*1 standard curve generated from *F.a.*1 pure culture extracted with the soil kit.

3. Results

3.1. DNA extraction and amplification

The FastDNA™ Kit (MP Biomedicals™) produced a high yield and purity of *F.a.*1 DNA from pure culture, with an average concentration of 134.97 ng μL^{-1} and average $A_{260/280\ nm}$ of 1.81. Gel electrophoresis following PCR amplification with EF-1 and EF-2 primers produced a single strong band of approximately 700 nucleotides, suggesting the DNA was of high quality and the target locus on the *TEF1* gene was amplified. Forward and reverse amplicon sequences of the purified PCR product (11 ng μL^{-1}) was sequenced by Eurofins Genomics.

Genomic DNA extraction from pure *F.a.*1 culture using the DNeasy PowerLyzer PowerSoil kit (Qiagen) and the DNeasy PowerPlant Pro Kit (Qiagen) likewise produced high yields of high quality and purity DNA (Table 2). These DNA extraction results are consistent with what was observed in our larger comprehensive studies (unpublished).

3.2. F.a.1 TEF1 gene sequencing and alignment

The text and chromatogram files as generated by Eurofins Genomics were utilized in tandem to analyze the forward and reverse *F.a.*1 sequences. Poor quality sequencing or weak signal strength at the strand ends were trimmed as needed and uncalled "n" bases were predicted if possible. Alignment of the two sequences produced a high-quality consensus sequence of 689 base pairs. A MegaBLAST query identified the *F.a.*1 contig as *F. avenaceum* and the sequence was deposited into GenBank (accession no. MK000734). The 100 most significantly aligned GenBank accessions share 99–100% sequence identity with *F.a.*1. and 94–100% sequence coverage. The *F.a.*1 *TEF1* sequence aligned almost perfectly with the top 100 BLAST hits, except for nine single nucleotide polymorphisms (SNPs) located within three introns (bps 61–152, 216–453, and 593–648) (Table S1). Five of the nine *F.a.*1 SNPs mismatched with > 38% of the 100 GenBank accessions, possibly indicating sites of *F. avenaceum* isolate variations. The other four *F.a.*1 SNPs mismatched with < 16% of the other sequences, suggesting those base transitions may reflect sequencing errors rather than genetic differences.

Table 2
Average total genomic DNA concentration[a] and purity[b] from each extraction source.

Source	DNA (ng μL^{-1})	$A_{260/280\ nm}$
*F.a.*1 culture[c]	78.827	1.875
*F.a.*1 culture[d]	94.609	1.705
Pasteurized soil	6.00	1.88
Nonpasteurized soil	15.20	1.94
Caryopses, pasteurized soil	87.10	1.85
Caryopses, nonpasteurized soil	89.40	1.84
Hulls, pasteurized soil	25.30	1.80
Hulls, nonpasteurized soil	30.20	1.79

[a] DNA concentration measured with a Synergy 2 microplate reader (BioTek Instruments, Winooski, Vermont, USA) and with a NanoDrop® spectrophotometer (Thermo-Fisher Scientific, Waltham, Massachusetts, USA).
[b] DNA purity assessed from $A_{260/280\ nm}$ using a NanoDrop®.
[c] *F.a.*1 DNA extracted using PowerLyzer PowerSoil kit (Qiagen, Hilden, Germany).
[d] *F.a.*1 DNA extracted using PowerPlant Pro kit (Qiagen, Hilden, Germany).

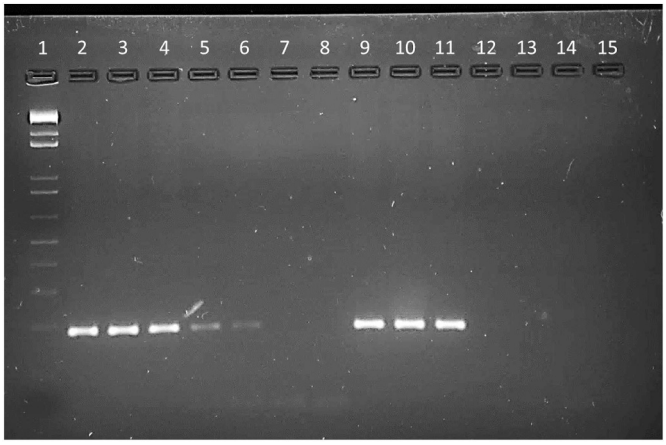

Fig. 1. PCR gel electrophoresis of endpoint PCR amplification using primer pairs 2 and 3. Wells: Lane 1) 1KbDNA ladder; Lanes 2-4) Primer 2 + *F.a.*1; Lanes 5-6) Primer 2 + soil DNA extract; Lanes 7-8) Primer 2 + water (neg. control); Lanes 9-11) Primer 3 + *F.a.*1; Lanes 12-13) Primer 3 + soil DNA extract; Lanes 14-15) Primer 3 + water (neg. control).

An alignment of *F.a.*1 with 82 *TEF1* fungal sequences retrieved from GenBank indicated that the *F.a.*1 *TEF1* gene amplicon contained sufficient diversity to discriminate *F. avenaceum* from other *Fusarium* species, with the exception of *F. arthrosporiodes* (Table S2). Nine potential primer pairs were manually identified, and three primer pairs with the most optimal characteristics were selected for oligonucleotide synthesis (Eurofins Genomics) and primer evaluation (Table S3).

3.3. Screening of candidate primer pairs and standard curves

Candidate primer pairs were screened for efficiency, specificity, and sensitivity *via* conventional PCR and gel electrophoresis, real-time PCR, and *in silico* analysis, with annealing temperatures between 61 °C and 64 °C. Conventional PCR of *F.a.*1 DNA with primer pairs 2 and 3 produced the expected target amplicons of ~190 base pairs, as seen in the distinctly visible agarose gel bands (Fig. 1). When primer pair 2 was tested against DNA extracted from non-infested nonpasteurized soil, thin and low intensity bands of similar size to the *F.a.*1 target DNA and faint bands of small sized DNA (< 50 bp) were observed. Faint bands of small sized DNA (< 50 bp) were also produced when primer pair 2 was tested on the negative water control. Since the non-infested nonpasteurized soil is believed to contain no *F. avenaceum*, these gel staining observations suggest that pair 2 may not specifically amplify the target sequence, and indicate it forms primer dimers. In contrast, primer pair 3 did not produce any bands indicative of nonspecific binding or primer dimer formation; moreover, the *F.a.*1 bands produced with pair 3 were larger and more intense than those from pair 2, suggesting pair 3 amplifies more of the target sequence than pair 2. DNA from other soil fungi did not amplify when PCR was performed with primer pair 3.

Real-time PCR was performed on 7 ten-fold serial dilutions in water of *F.a.*1 genomic DNA ranging from 96.42 fg DNA μL^{-1} to 96.42 ng DNA μL^{-1} for the three candidate primer pairs at annealing temperatures between 61 °C and 64 °C. Melting curve analysis suggested that pair 1 produced primer dimers and non-uniform melting temperatures of the ten-fold dilution series, indicating possible nonspecific binding. Pair 1 was not tested further.

In addition to ten-fold serial dilutions of *F.a.*1 genomic DNA, primer pairs 2 and 3 were tested on ten-fold serial dilutions of genomic DNA extracted from non-infested nonpasteurized soil ranging in

Table 3
Real-time PCR data for primer pair 3 tested against total genomic DNA extracted from soil, caryopsis, and hull samples.[a]

Incubation time in soil[b]	Soil	Treatment	Extract source[c]	Ct	CV [d] of Ct	DNA	CV of DNA
					(%)	(pg μl^{-1})	(%)
3	Pasteurized	*F.a.*1	soil	23.74	0.05	3.53E + 02	0.58
14	Pasteurized	*F.a.*1	soil	23.51	0.16	4.14E + 02	2.50
21	Pasteurized	*F.a.*1	soil	21.95	0.28	1.20E + 03	4.52
28	Pasteurized	Negative	soil	43.00	3.30	5.86E-03	42.72
14	Nonpasteurized	*F.a.*1	soil	24.55	0.12	2.24E + 02	1.84
28	Nonpasteurized	*F.a.*1	soil	25.08	0.10	1.55E + 02	1.61
21	Nonpasteurized	Negative	soil	42.55	2.25	4.92E-03	42.55
4	Pasteurized	*F.a.*1	caryopsis	23.05	0.10	4.51E + 02	1.78
9	Pasteurized	*F.a.*1	caryopsis	19.86	0.19	3.98E + 03	2.36
6	Pasteurized	Negative	caryopsis	37.33	0.49	2.04E-01	6.03
2	Nonpasteurized	*F.a.*1	caryopsis	25.70	0.16	7.31E + 01	2.86
6	Nonpasteurized	*F.a.*1	caryopsis	27.91	0.06	1.97E + 01	1.10
4	Nonpasteurized	Negative	caryopsis	37.62	0.72	1.46E-01	8.13
4	Pasteurized	*F.a.*1	hull	21.41	0.15	1.15E + 03	2.06
9	Pasteurized	*F.a.*1	hull	23.62	0.07	2.52E + 02	1.17
9	Pasteurized	Negative	hull	37.83	1.57	1.17E-01	18.51
2	Nonpasteurized	*F.a.*1	hull	20.01	0.02	2.97E + 03	0.32
6	Nonpasteurized	*F.a.*1	hull	21.85	0.08	8.04E + 02	1.25
9	Nonpasteurized	Negative	hull	38.41	0.88	8.65E-02	9.72

[a] Annealing temperature was 61 °C. Cycle threshold (Ct) and DNA values are averaged from three technical replicates.
[b] Unit of time is 'days' when extraction source is soil and 'weeks' when extraction source is caryopsis or hull.
[c] *Soil* samples were collected from within the mesh seed bag in the Soil-Caryopsis experiment, and *caryopsis* and *hull* samples were obtained from the Soil-Whole Seed experiment.
[d] CV, coefficient of variation.

concentration from 0.001–100 pg total soil DNA μL^{-1}, soil genomic DNA extracted from the four previously described soil treatments, and on DNA extracted from pure cultures of additional soil fungi (Table 1). Efficiency of primer pair 2 as determined from the water standard curve was 98% when annealing temperatures was 61 °C. However, non-specific binding and primer dimer formation at each non-target soil serial dilution and with no template water controls was observed with pair 2. Considering the conventional and real-time PCR results, primer pair 2 was no longer evaluated.

When tested on ten-fold serial dilutions of *F.a.*1 genomic DNA, primer pair 3 produced a single melting peak with tightly aligned dilutions, indicating amplification of a single product with uniform melting temperature. It did not amplify DNA from non-target soil fungi. Primer pair 3 did not produce primer dimers when evaluated on serial dilutions of total genomic DNA from non-infested soil nor with water controls. Primer pair 3 demonstrated sensitive quantification and detection of *F. avenaceum* DNA from the *F.a.*1-treated soils. The limit of quantification was 70 fg μL^{-1} and Ct values among technical replicates were within 0.5 Ct (Table 3). Primer pair 3 did not detect or amplify *F. avenaceum* from the pasteurized or nonpasteurized non-infested soils; average *F. avenaceum* DNA concentration in non-infested samples was 5.5 fg μL^{-1} and Ct values were ≥ 42.00. From the conventional and real-time PCR screening results, we concluded that primer pair 3 was an efficient, specific, and sensitive *F. avenaceum* primer pair with an experimentally optimized annealing temperature of 61 °C.

3.4. Primer efficiency and sensitivity

Primer pair 3 is described in Table 4. Using ten-fold water serial dilutions of *F.a.*1 genomic DNA extracted with the DNeasy PowerLyzer PowerSoil DNA extraction kit (Qiagen) and three technical replicates per dilution, the limits of detection and quantification were 73.6 fg DNA μL^{-1} (Table 5). Primer amplification efficiency was 98.0%, and R^2 was 0.999. Amplification of replicated ten-fold water serial dilutions of *F.a.*1 genomic DNA extracted with the DNeasy PowerPlant Pro Kit (Qiagen) had a limit of detection of 13.2 fg DNA μL^{-1} and a limit of quantification of 0.13 pg DNA μL^{-1}. Primer amplification efficiency was 98.3%, and R^2 was 0.999 (Table 5).

Table 5
Standard curve equations and amplification efficiencies from plots of log of DNA concentration *vs.* cycle threshold (Ct) obtained in real-time PCR assays for isolate *F.a.*1 DNA in water and agricultural extracts.

Diluent	m[a]	b[b]	E (%)[c]	Ct[d]	Dilution range (pg μl^{-1})
Water[e]	−3.372	20.95	98	35	7.36×10^{-4} to 7.36×10^{4}
Water[f]	−3.364	20.84	98	35	1.32×10^{-2} to 1.32×10^{5}
Soil extract	−3.422	21.54	96	32	7.36×10^{-4} to 7.36×10^{4}
Hull	−3.345	21.18	99	33	1.32×10^{-2} to 1.32×10^{5}
Caryopsis	−3.493	20.88	93	34	1.32×10^{-2} to 1.32×10^{5}

[a] m = slope of the line
[b] b = y-intercept
[c] Amplification efficiency = [10^(−1/slope)] − 1 (Ginzinger, 2002).
[d] Ct = Ct value beyond which quantification becomes unreliable.
[e] *F.a.*1 DNA extracted using PowerLyzer PowerSoil kit (Qiagen, Hilden, Germany).
[f] *F.a.*1 DNA extracted using PowerPlant Pro kit (Qiagen, Hilden, Germany).

3.5. In silico analysis

In silico analysis of primer pair 3 using NCBI Primer-BLAST tool yielded 481 total accession matches based on the default settings. The first 286 hits were *F. avenaceum* accessions, suggesting that our selected

Table 4
Primer pair 3.

Pair	Primer Name	Strand	Sequence (5' → 3')	Length (bp)	% GC	Tm (°C)	Position on *F.a.*1	Amplicon Length (bp)	Amplicon% GC
3	Fa-8f	Forward	CACGACTCGCTCCCTCATTCG	21	62	63.1	258-278	188	53
	Fa-13R	Reverse	CCAGTGGTTAGTGACTGCAAGACATAG	27	48	63.3	445-419		

primer pair was potentially highly specific for *F. avenaceum*. Further analysis of the specificity and duplex stability of the non-*F. avenaceum* accessions using PrimerSelect indicated the forward and reverse primers may bind non-specifically to *F. acuminatum, F. arthrosporioides*, and *F. equiseti,* and potential mispriming by only the forward primer was possible against *F. flocciferum, F. tricinctum, F. torulosum,* and *F. heterosporum* (Table S4). However, further *in silico* testing with 70 additional GenBank accessions identified as these species (Table S4) indicated that in addition to *F. avenaceum*, only *F. arthrosporioides* consistently demonstrated strong binding to our primer pair. Delta G values were identical for the two *Fusarium* species: $-47.5\ \text{kcal mol}^{-1}$ and $-45.9\ \text{kcal mol}^{-1}$ for forward and reverse primer sequences, respectively. Only two of 16 *F. acuminatum* GenBank accessions tested *in silico* showed strong binding to primer pair 3 (Table S4), suggesting those *F. acuminatum* accessions may be misidentified (Stavrou et al. 2018).

3.6. *F. avenaceum* quantification in agricultural samples

*F.a.*1 standard curves were made with DNA extracts from soil, hulls, and caryopses and compared to water standard curves of *F.a.*1 DNA to assess if amplification efficiency decreased as a result of potential PCR inhibitors (Yan et al. 2012) (Table 5). Isolate *F.a.*1 was detectable and quantifiable to 7.4 fg DNA μL^{-1} and 0.74 pg DNA μL^{-1}, respectively, when applying the *F.a.*1 standard curve made by serially diluting with soil DNA extract. Amplification efficiency of the soil standard curve was 0.96% with a slope of −3.422 and an R^2 of 0.99. Isolate *F.a.*1 was detectable and quantifiable to 13.2 fg DNA μL^{-1} and 0.13 pg DNA μL^{-1}, respectively, when applying the *F.a.*1 standard curve made by serially diluting with hull DNA extract. Amplification efficiency of the hull standard curve was 0.99% with a slope of −3.345 and an R^2 of 0.99. The detectable and quantifiable sample limits were 13.2 fg DNA μL^{-1} and 0.13 pg DNA μL^{-1}, respectively, when applying the standard curve made by serially diluting with caryopsis DNA extract. Amplification efficiency of the caryopsis standard curve was 0.93% with a slope of −3.493 and an R^2 of 0.99.

To detect and quantify *F. avenaceum* in soil and seeds, qPCR was performed on samples of total genomic DNA that had been extracted from the four described soil treatments and the seeds contained therein (Table 3). Real-time PCR was also performed on caryopses and hulls from fresh wild oat seeds that were not exposed to any soil treatments. No *F. avenaceum* amplification products were detected from the pasteurized or nonpasteurized non-infested soils, from the seeds incubated in those soils, or from fresh non-infested wild oat seeds. *Fusarium avenaceum* was detected in all DNA extractions from pasteurized and nonpasteurized soil infested with *F.a.*1 and from the wild oat seeds incubated in the infested soils.

Results from the soil spiking experiment showed a significant linear relationship between log CFU g^{-1}*F.a.*1 inoculum and Ct values ($R^2 = 0.998$) (Fig. 2a), as well as between *F.a.*1 inoculum concentration (CFU g^{-1}) and *F. avenaceum* DNA (pg μL^{-1}) ($R^2 = 0.977$) (Fig. 2b). The < 99% R^2 value of the latter resulted from the *F. avenaceum* DNA measured for the 500 CFU g^{-1} inoculum level, which was lower than expected based on the DNA concentrations for the other five inoculum levels. When the 500 CFU g^{-1} inoculum level is omitted from the inoculum *vs* DNA regression, R^2 is 0.994. The departure from linearity at this inoculum level illustrates the inherent variability in soil biosystems, including heterogeneity of pathogen distribution and density in soils. Furthermore, R^2 of 0.994 when including 2000 CFU g^{-1} *F.a.*1 inoculum concentration suggests that the upper limit of qPCR quantification is > 2000. Average *F. avenaceum* DNA concentration and Ct from pasteurized soil samples with zero added inoculum averaged 2.9 fg ng^{-1} total soil DNA (166% CV), but with a high Ct of 41.3, this DNA concentration is not reliable. Real-time PCR results for the next lowest inoculum level of 50 CFU g^{-1} was 1.03×10^3 fg ng^{-1} total soil DNA (24% CV) and 27.8 Ct. The lower limit of quantification and detection for *F.a.*1 inoculum in soil is likely between zero and 50 CFU g^{-1} soil.

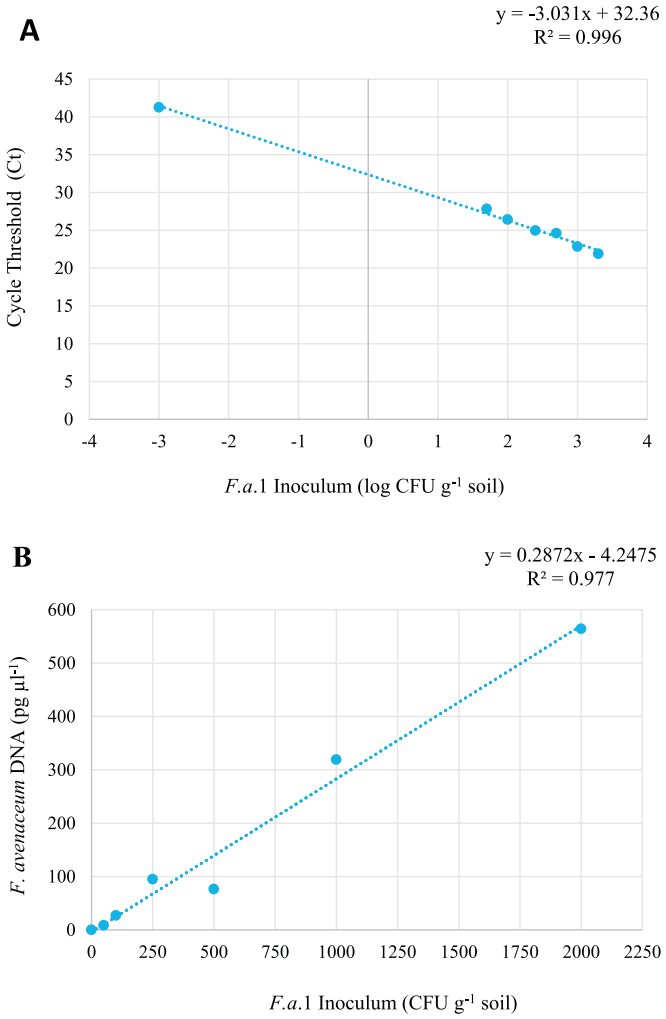

Fig. 2. Real-time PCR assay of pasteurized Quincy soil infested with *F.a.*1-oat inoculum at 0, 50, 100, 250, 500, 1000, and 2000 CFU per gram dry weight of soil.

4. Discussion

The goal of this research was to develop a real-time PCR assay targeting the *TEF1* gene to specifically detect and quantify *F. avenaceum* from culture, soil, and soil-incubated seeds of the noxious weed wild oat. We demonstrate that our qPCR primers designed from the *TEF1* gene efficiently detect and quantify *F. avenaceum* from artificially infested pasteurized and nonpasteurized soil samples. Furthermore, these primers effectively amplify *F. avenaceum* present in genomic DNA extracted from wild oat caryopses and hulls previously incubated in *F.a.*1 infested soil. Development of this qPCR assay for evaluating *F. avenaceum* in the noxious weed species wild oat broadens the plant hosts from which *F. avenaceum* can be quantified.

Although previous publications have identified real-time PCR primers specific to *F. avenaceum* (Waalwijk et al. 2004; Nicolaisen et al. 2009), they were applied only to wheat or maize. Considering that *F. avenaceum* is found in soils worldwide, it is imperative to accurately detect and quantify it from soil, which presents a uniquely complex matrix for efficient molecular applications and is crucial to understanding the species' behavior in the environment. Furthermore, the amplicon characteristics described for primer pair 3 differ significantly from the target amplicon of the *F. avenaceum*-specific primers identified in Nicolaisen et al. (2009), which is only 52 nucleotides long and positioned from 593 to 644 bp near the 3′ end of the *TEF1* gene region. Short target amplicons (< 80 bp) in SYBR Green assays may be

challenging to distinguish from primer dimers and can result in larger Ct values and false positives (Bustin et al. 2009; Bustin and Huggett 2017).

Fusarium species are challenging to accurately identify using morphological criteria and even molecular diagnostics. The top BLAST matches upon querying GenBank may be inaccurate or misleading due to originally misidentified accessions and/or updated taxonomy (O'Donnell et al. 2015). The taxonomy of *F. arthrosporioides*, the one non-target species to which primer pair 3 is predicted to anneal, is contested in the literature and it is often considered to be in fact conspecific with *F. avenaceum* (Kristensen et al. 2005; Leslie and Summerell 2006; Triest et al. 2015; Stakheev et al. 2016). Niessen et al. (2012) found that the majority of the *F. arthrosporioides* reference cultures used in their study phylogenetically identified as *F. avenaceum*. *Fusarium arthrosporioides* is not known to exist in the Pacific Northwest, USA (T.C. Paulitz and L.J. du Toit, Washington State University, personal communication). An extensive search of the U.S. National Fungus Collections Fungus-Host Database (https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm), which includes samples from around the globe, produced 23 *F. arthrosporioides* records, of which two listed the species in the Pacific Northwest (Oregon), yet without a description (USDA, 1960; Rossman and Lu 1980). For comparison, the database search for *F. avenaceum* and *F. acuminatum* produced 403 and 596 records, respectively. *Fusarium arthrosporioides* was listed as associated with wheat (USDA, 1960) and red alder (*Alnus rubra* Bong.) (Rossman and Lu 1980). In the latter publication, it was morphologically identified following isolation from a mixed culture of dozens of fungi on red alder leaves. Changes to *Fusarium* taxonomy and systematics with the development of molecular techniques since 1960 and 1980 undoubtedly throw into question the identification of these two isolates as *F. arthrosporioides.* A more conclusive updated taxonomy of *F. arthrosporioides* could not be found in the literature at the time of this writing. If *F. arthrosporioides* is indeed a different species from *F. avenaceum,* this real-time assay will be limited in cases where they co-occur. Nevertheless, this remains an effective, sensitive, and relatively specific tool to detect and quantify *F. avenaceum* in soil spiking experiments or in natural soils where *F. arthrosporioides* is not prevalent.

While our research group is applying this real-time assay to analyze a larger set of comprehensive experiments on the interaction between *F.a.*1 and dormant wild oat weed seeds for sustainable weed management solutions, such a discriminating molecular tool may also benefit pathogen survey studies, plant disease diagnostics and management, and mycotoxin research (Feng et al. 2010; Sankaran et al. 2010; Sanzani et al. 2014). Moreover, *F. avenaceum* primers that utilize SYBR Green I qPCR chemistry will be more widely accessible because of their lower start-up and running costs and ease of use compared to TaqMan-based systems (Ponchel et al. 2003; Law et al. 2015). While significant reductions in crop yield and quality (Hwang et al. 2000; Kiecana et al. 2002) resulting from *F. avenaceum* infection impact producers worldwide, human and animal mycotoxicoses are especially problematic in developing countries where commodity crops susceptible to *F. avenaceum* infection comprise major dietary staples (Strosnider et al. 2006). The greater negative impact of *Fusarium* infection in developing nations is compounded by the limited funds for basic infrastructure of plant diagnosis laboratories and a shortage of skilled human resources with access to technology (Muthomi et al. 2008; Miller et al. 2009; Mailafia et al. 2017).

Author contributions

The project was carried out by ATP. ATP and PAO contributed to the analysis, content and editing of the manuscript. PAO was a doctoral co-advisor of ATP.

Acknowledgements

We are grateful for Dr. E. Patrick Fuerst for securing funding for this project and for providing feedback and support during creation of this manuscript. The authors thank Lindsey duToit and Tim Paulitz for valuable advice on *F. arthrosporioides*. We recognize the technical assistance of Karen Adams in the operation of the LightCycler 480 II. There are no conflicts of interest.

Funding

This work was supported by the United States Department of Agriculture, National Institute of Food and Agriculture, Agriculture and Food Research Initiative Foundational Program award number 2014-67013-21575 (E. Patrick Fuerst and PAO) and CRIS Project Number 2090-22000-017-00D (PAO).

Declarations of interest

All authors: none to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2018.12.009.

References

Abbas, H.K., Mirocha, C.J., Gunther, R., 1989. Mycotoxins produced by toxic Fusarium isolates obtained from agricultural and nonagricultural areas (Arctic) of Norway. Mycopathologia 105, 143–151.

Abdellatif, L., Fernandez, M.R., Bouzid, S., Vujanovic, V., 2010. Characterization of virulence and PCR-DGGE profiles of Fusarium avenaceum from western Canadian Prairie ecozone of Saskatchewan. Can. J. Plant Pathol. 32, 468–480. https://doi.org/10.1080/07060661.2010.510643.

Abedi, M., Bartelheimer, M., Poschlod, P., 2014. Effects of substrate type, moisture and its interactions on soil seed survival of three Rumex species. Plant Soil 374, 485–495. https://doi.org/10.1007/s11104-013-1903-x.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Anderson, J.V., Fuerst, E.P., Tedrow, T., Hulke, B., Kennedy, A.C., 2010. Activation of polyphenol oxidase in dormant wild oat caryopses by a seed-decay isolate of Fusarium avenaceum. J. Agric. Food Chem. 58, 10597–10605. https://doi.org/10.1021/jf102625a.

Aoki, T., O'Donnell, K., Geiser, D.M., 2014. Systematics of key phytopathogenic Fusarium species: current status and future challenges. J. Gen. Plant Pathol. 80, 189–201. https://doi.org/10.1007/s10327-014-0509-3.

Axelrood, P.E., Neumann, M., Trotter, D., Radley, R., Shrimpton, G., Dennis, J., 1995. Seedborne Fusarium on Douglas-fir: pathogenicity and seed stratification method to decrease Fusarium contamination. New For. 9, 35–51.

Baskin, C.C., Baskin, J.M., 2006. The natural history of soil seed banks of arable land. Weed Sci. 54, 549–557. https://doi.org/10.1614/WS-05-034R.1.

Bastiaans, L., Paolini, R., Baumann, D.T., 2008. Focus on ecological weed management: what is hindering adoption? Weed Res. 48, 481–491. https://doi.org/10.1111/j.1365-3180.2008.00662.x.

Beckstead, J., Meyer, S.E., Molder, C.J., Smith, C., 2007. A race for survival: can Bromus tectorum seeds escape Pyrenophora semeniperda-caused mortality by germinating quickly? Ann. Bot. 99, 907–914. https://doi.org/10.1093/aob/mcm028.

Bottalico, A., Perrone, G., 2002. Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. In: Logrieco, A., Bailey, J.A., Corazza, L., Cooke, B.M. (Eds.), Mycotoxins in Plant Disease. Springer, Netherlands, Dordrecht, pp. 611–624. https://doi.org/10.1007/978-94-010-0001-7_2.

Buhler, D.D., Hartzler, R.G., Forcella, F., 1997. Implications of weed seedbank dynamics to weed management. Weed Sci. 45, 329–336.

Bustin, S., Huggett, J., 2017. qPCR primer design revisited. Biomol. Detection Quantification 14, 19–28. https://doi.org/10.1016/j.bdq.2017.11.001.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., Wittwer, C.T., 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55, 611–622. https://doi.org/10.1373/clinchem.2008.112797.

Champeil, A., Doré, T., Fourbet, J., 2004. Fusarium head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by Fusarium in wheat grains. Plant Sci. 166, 1389–1415. https://doi.org/10.1016/j.plantsci.2004.02.004.

Chang, K.F., Conner, R.L., Hwang, S.F., Ahmed, H.U., McLaren, D.L., Gossen, B.D.,

Turnbull, G.D., 2014a. Effects of seed treatments and inoculum density of Fusarium avenaceum and Rhizoctonia solani on seedling blight and root rot of faba bean. Can. J. Plant Sci. 94, 693–700. https://doi.org/10.4141/cjps2013-339.
Chang, K.F., Hwang, S.F., Ahmed, H.U., Strelkov, S.E., Gossen, B.D., Turnbull, G.D., Blade, S.F., 2014b. Disease reaction to Fusarium avenaceum and yield losses in narrow-leafed lupin lines. Can. J. Plant Sci. 94, 1211–1218. https://doi.org/10.4141/cjps2013-243.
Chen, Y., Zhou, Q., Strelkov, S.E., Hwang, S.-F., 2014. Genetic diversity and aggressiveness of Fusarium spp. isolated from canola in Alberta, Canada. Plant Dis. 98, 727–738. https://doi.org/10.1094/PDIS-01-13-0061-RE.
Churro, C., Pereira, P., Vasconcelos, V., Valério, E., 2012. Species-specific real-time PCR cell number quantification of the bloom-forming cyanobacterium Planktothrix agardhii. Arch. Microbiol. 194, 749–757. https://doi.org/10.1007/s00203-012-0809-y.
Davis, A.S., Anderson, K.I., Hallett, S.G., Renner, K.A., 2006. Weed seed mortality in soils with contrasting agricultural management histories. Weed Sci. 54, 291–297. https://doi.org/10.1614/WS-05-54.2.291.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797. https://doi.org/10.1093/nar/gkh340.
Feng, J., Hwang, R., Chang, K.F., Hwang, S.F., Strelkov, S.E., Gossen, B.D., Conner, R.L., Turnbull, G.D., 2010. Genetic variation in Fusarium avenaceum causing root rot on field pea: genetic variation in Fusarium avenaceum. Plant Pathol. 59, 845–852. https://doi.org/10.1111/j.1365-3059.2010.02313.x.
Filion, M., St-Arnaud, M., Jabaji-Hare, S.H., 2003. Direct quantification of fungal DNA from soil substrate using real-time PCR. J. Microbiol. Methods 53, 67–76. https://doi.org/10.1016/S0167-7012(02)00225-7.
Finch, H., Allen, P.S., Meyer, S.E., 2013. Environmental factors influencing Pyrenophora semeniperda-caused seed mortality in Bromus tectorum. Seed Sci. Res. 23, 57–66. https://doi.org/10.1017/S0960258512000244.
Finch-Boekweg, H., Allen, P., Meyer, S., 2013. Exposure to low water potentials and seed dormancy. Plant Prot. Sci. 49, S15–S20.
Fraeyman, S., Croubels, S., Devreese, M., Antonissen, G., 2017. Emerging Fusarium and Alternaria mycotoxins: occurrence, toxicity and toxicokinetics. Toxins 9 (7), 228. https://doi.org/10.3390/toxins9070228.
Fuerst, E.P., Anderson, J.V., Kennedy, A.C., Gallagher, R.S., 2011. Induction of polyphenol oxidase activity in dormant wild oat (Avena fatua) seeds and caryopses: a defense response to seed decay fungi. Weed Sci. 59, 137–144. https://doi.org/10.1614/WS-D-10-00123.1.
Fuerst, E.P., Okubara, P.A., Anderson, J.V., Morris, C.F., 2014. Polyphenol oxidase as a biochemical seed defense mechanism. Front. Plant Sci. 5. https://doi.org/10.3389/fpls.2014.00689.
Fuerst, E.P., James, M.S., Pollard, A.T., Okubara, P.A., 2018. Defense enzyme responses in dormant wild oat and wheat caryopses challenged with a seed decay pathogen. Front. Plant Sci. 8. https://doi.org/10.3389/fpls.2017.02259.
Gallagher, R., Granger, K., Snyder, A., Pittmann, D., Fuerst, E., 2013. Implications of environmental stress during seed development on reproductive and seed bank persistence traits in wild oat (*Avena fatua* L.). Agronomy 3, 537–549. https://doi.org/10.3390/agronomy3030537.
Geiser, D.M., del Mar Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T.J., Zhang, N., Kuldau, G.A., O'Donnell, K., 2004. FUSARIUM-ID v. 1.0: a DNA sequence database for identifying Fusarium. Eur. J. Plant Pathol. 110, 473–479. https://doi.org/10.1023/B:EJPP.0000032386.75915.a0.
Gil-Serna, J., Patiño, B., Jurado, M., Mirete, S., Vázquez, C., González-Jaén, M.T., 2017. Targeting conserved genes in Fusarium species. In: Moretti, A., Susca, A. (Eds.), Mycotoxigenic Fungi. Methods in Molecular Biology. Vol. 1542. Humana Press, New York, NY, pp. 141–147.
Ginzinger, D.G., 2002. Gene quantification using real-time quantitative PCR. Exp. Hematol. 30, 503–512. https://doi.org/10.1016/S0301-472X(02)00806-8.
Gómez, B.L., Nosanchuk, J.D., 2003. Melanin and fungi. Curr. Opin. Infect. Dis. 16, 91–96. https://doi.org/10.1097/00001432-200304000-00005.
Gruber-Dorninger, C., Novak, B., Nagl, V., Berthiller, F., 2017. Emerging mycotoxins: beyond traditionally determined food contaminants. J. Agric. Food Chem. 65, 7052–7070. https://doi.org/10.1021/acs.jafc.6b03413.
Haapalainen, M., Latvala, S., Kuivainen, E., Qiu, Y., Segerstedt, M., Hannukkala, A.O., 2016. Fusarium oxysporum, F. proliferatum and F. redolens associated with basal rot of onion in Finland. Plant Pathol. 65, 1310–1320. https://doi.org/10.1111/ppa.12521.
Hansen, F.T., Gardiner, D.M., Lysøe, E., Fuertes, P.R., Tudzynski, B., Wiemann, P., Sondergaard, T.E., Giese, H., Brodersen, D.E., Sørensen, J.L., 2015. An update to polyketide synthase and non-ribosomal synthetase genes and nomenclature in Fusarium. Fungal Genet. Biol. 75, 20–29. https://doi.org/10.1016/j.fgb.2014.12.004.
Holtz, M.D., Chang, K.F., Hwang, S.F., Gossen, B.D., Strelkov, S.E., 2011. Characterization of Fusarium avenaceum from lupin in Central Alberta: genetic diversity, mating type and aggressiveness. Can. J. Plant Pathol. 33, 61–76. https://doi.org/10.1080/07060661.2011.536651.
Hwang, S.F., Gossen, B.D., Turnbull, G.D., Chang, K.F., Howard, R.J., Thomas, A.G., 2000. Effect of temperature, seeding date, fungicide seed treatment and inoculation with Fusarium avenaceum on seedling survival, root rot severity and yield of lentil. Can. J. Plant Sci. 80, 899–907. https://doi.org/10.4141/P99-177.
Ismaiel, A., Papenbrock, J., 2015. Mycotoxins: producing fungi and mechanisms of phytotoxicity. Agriculture 5, 492–537. https://doi.org/10.3390/agriculture5030492.
Ivanova, L., Skjerve, E., Eriksen, G.S., Uhlig, S., 2006. Cytotoxicity of enniatins a, A1, B, B1, B2 and B3 from Fusarium avenaceum. Toxicon 47, 868–876. https://doi.org/10.1016/j.toxicon.2006.02.012.
Jestoi, M., 2008. Emerging Fusarium -mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin—a review. Crit. Rev. Food Sci. Nutr. 48, 21–49. https://doi.org/10.1080/10408390601062021.
Khaledi, N., Taheri, P., Falahati Rastegar, M., 2017. Identification, virulence factors characterization, pathogenicity and aggressiveness analysis of Fusarium spp., causing wheat head blight in Iran. Eur. J. Plant Pathol. 147, 897–918. https://doi.org/10.1007/s10658-016-1059-7.
Kiecana, I., Mielniczuk, E., Kaczmarek, Z., Kostecki, M., Golinski, P., 2002. Scab response and moniliformin accumulation in kernels of oat genotypes inoculated with Fusarium avenaceum in Poland. Eur. J. Plant Pathol. 108, 245–251.
Köhl, J., de Haas, B.H., Kastelein, P., Burgers, S.L.G.E., Waalwijk, C., 2007. Population dynamics of Fusarium spp. and Microdochium nivale in crops and crop residues of winter wheat. Phytopathology 97, 971–978. https://doi.org/10.1094/PHYTO-97-8-0971.
Kristensen, R., Mona, T., Kosiak, B., Holst-Jensen, A., 2005. Phylogeny and toxigenic potential is correlated in Fusarium species as revealed by partial translation elongation factor 1 alpha gene sequences. Mycol. Res. 109, 173–186.
Landa, B.B., de Werd, H.A., McSpadden Gardener, B.B., Weller, D.M., 2002. Comparison of three methods for monitoring populations of different genotypes of 2, 4-diacetylphloroglucinol-producing Pseudomonas fluorescens in the rhizosphere. Phytopathology 92, 129–137.
Law, J.W.-F., Ab Mutalib, N.-S., Chan, K.-G., Lee, L.-H., 2015. Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. Front. Microbiol. 5. https://doi.org/10.3389/fmicb.2014.00770.
Leslie, J.F., Summerell, B.A., 2006. The Fusarium Laboratory Manual. Blackwell Publishing. Ames, Iowa. In: USA.
Logrieco, A., Rizzo, A., Ferracane, R., Ritieni, A., 2002. Occurrence of beauvericin and enniatins in wheat affected by Fusarium avenaceum head blight. Appl. Environ. Microbiol. 68, 82–85. https://doi.org/10.1128/AEM.68.1.82-85.2002.
de Luna, L.Z., Kennedy, A.C., Hansen, J.C., Paulitz, T.C., Gallagher, R.S., Fuerst, E.P., 2011. Mycobiota on wild oat (Avena fatua L.) seed and their caryopsis decay potential. Plant Health Progress. https://doi.org/10.1094/PHP-2011-0210-01-RS.
Luo, J., Walsh, E., Naik, A., Zhuang, W., Zhang, K., Cai, L., Zhang, N., 2014. Temperate pine barrens and tropical rain forests are both rich in undescribed fungi. PLoS One 9, e103753.
Lysøe, E., Harris, L.J., Walkowiak, S., Subramaniam, R., Divon, H.H., Riiser, E.S., Llorens, C., Gabaldón, T., Kistler, H.C., Jonkers, W., 2014. The genome of the generalist plant pathogen Fusarium avenaceum is enriched with genes involved in redox, signaling and secondary metabolism. PLoS One 9, e112703.
Ma, L.-J., van der Does, H.C., Borkovich, K.A., Coleman, J.J., Daboussi, M.-J., Di Pietro, A., Dufresne, M., Freitag, M., Grabherr, M., Henrissat, B., Houterman, P.M., Kang, S., Shim, W.-B., Woloshuk, C., Xie, X., Xu, J.-R., Antoniw, J., Baker, S.E., Bluhm, B.H., Breakspear, A., Brown, D.W., Butchko, R.A.E., Chapman, S., Coulson, R., Coutinho, P.M., Danchin, E.G.J., Diener, A., Gale, L.R., Gardiner, D.M., Goff, S., Hammond-Kosack, K.E., Hilburn, K., Hua-Van, A., Jonkers, W., Kazan, K., Kodira, C.D., Koehrsen, M., Kumar, L., Lee, Y.-H., Li, L., Manners, J.M., Miranda-Saavedra, D., Mukherjee, M., Park, G., Park, J., Park, S.-Y., Proctor, R.H., Regev, A., Ruiz-Roldan, M.C., Sain, D., Sakthikumar, S., Sykes, S., Schwartz, D.C., Turgeon, B.G., Wapinski, I., Yoder, O., Young, S., Zeng, Q., Zhou, S., Galagan, J., Cuomo, C.A., Kistler, H.C., Rep, M., 2010. Comparative genomics reveals mobile pathogenicity chromosomes in Fusarium. Nature 464, 367–373. https://doi.org/10.1038/nature08850.
Magan, N., Lacey, J., 1984. Water relations of some Fusarium species from infected wheat ears and grain. Trans. Br. Mycol. Soc. 83, 281–285.
Mailafia, S., God'spower Richard Okoh, H.O., Olabode, K., Osanupin, R., 2017. Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria. Vet. World 10, 393–397.
Manshor, N., Rosli, H., Ismail, N.A., Salleh, B., Zakaria, L., 2012. Diversity of Fusarium species from highland areas in Malaysia. Tropical Life Sci. Res. 23, 1–15.
McGee, D.C., Kellock, A.W., 1974. Fusarium avenaceum, a seed-borne pathogen of subterranean clover roots. Aust. J. Agric. Res. 25, 549–557.
Menalled, Fabián D., Gross, K.L., Hammond, M., Menalled, Fabian D., 2001. Weed aboveground and seedbank community responses to agricultural management systems. Ecol. Appl. 11, 1586–1601. https://doi.org/10.2307/3061080.
Meyer, S.E., Franke, J.-L., Baughman, O.W., Beckstead, J., Geary, B., 2014. Does Fusarium-caused seed mortality contribute to Bromus tectorum stand failure in the Great Basin. Weed Res. 54, 511–519. https://doi.org/10.1111/wre.12094.
Miller, J.D., Greenhalgh, R., Wang, Y., Lu, M., 1991. Trichothecene chemotypes of three Fusarium species. Mycologia 83, 121–130. https://doi.org/10.2307/3759927.
Miller, S.A., Beed, F.D., Harmon, C.L., 2009. Plant disease diagnostic capabilities and networks. Annu. Rev. Phytopathol. 47, 15–38.
Muthomi, J.W., Ndung'u, J.K., Gathumbi, J.K., Mutitu, E.W., Wagacha, J.M., 2008. The occurrence of Fusarium species and mycotoxins in Kenyan wheat. Crop Prot. 27, 1215–1219.
Naylor, J.M., Fedec, P., 1978. Dormancy studies in seed of Avena fatua. 8. Genetic diversity affecting response to temperature. Can. J. Bot. 56, 2224–2229.
Nicholas, K.B., Nicholas Jr., H.B., Deerfield II, D.W., 1997. GeneDoc: analysis and visualization of genetic variation. EMBnet News 4, 1–4.
Nicolaisen, M., Supronienė, S., Nielsen, L.K., Lazzaro, I., Spliid, N.H., Justesen, A.F., 2009. Real-time PCR for quantification of eleven individual Fusarium species in cereals. J. Microbiol. Methods 76, 234–240. https://doi.org/10.1016/j.mimet.2008.10.016.
Niessen, L., Gräfenhan, T., Vogel, R.F., 2012. ATP citrate lyase 1 (acl1) gene-based loop-mediated amplification assay for the detection of the Fusarium tricinctum species complex in pure cultures and in cereal samples. Int. J. Food Microbiol. 158, 171–185. https://doi.org/10.1016/j.ijfoodmicro.2012.06.021.
O'Donnell, K., Cigelnik, E., 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are nonorthologous. Mol. Phylogenet.

Evol. 7, 103–116. https://doi.org/10.1006/mpev.1996.0376.
O'Donnell, K., Cigelnik, E., Nirenberg, H.I., 1998a. Molecular systematics and phylogeography of the Gibberella fujikuroi species complex. Mycologia 90, 465–493. https://doi.org/10.2307/3761407.
O'Donnell, K., Kistler, H.C., Cigelnik, E., Ploetz, R.C., 1998b. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. 95, 2044–2049. https://doi.org/10.1073/pnas.95.5.2044.
O'Donnell, K., Humber, R.A., Geiser, D.M., Kang, S., Park, B., Robert, V.A.R.G., Crous, P.W., Johnston, P.R., Aoki, T., Rooney, A.P., Rehner, S.A., 2012. Phylogenetic diversity of insecticolous fusaria inferred from multilocus DNA sequence data and their molecular identification via FUSARIUM-ID and *Fusarium MLST*. Mycologia 104, 427–445. https://doi.org/10.3852/11-179.
O'Donnell, K., Ward, T.J., Robert, V.A., Crous, P.W., Geiser, D.M., Kang, S., 2015. DNA sequence-based identification of Fusarium: current status and future directions. Phytoparasitica 43, 583–595.
Okubara, P.A., Schroeder, K.L., Paulitz, T.C., 2008. Identification and quantification of Rhizoctonia solani and R. oryzae using real-time polymerase chain reaction. Phytopathology 98, 837–847. https://doi.org/10.1094/PHYTO-98-7-0837.
Okubara, P.A., Harrison, L.A., Gatch, E.W., Vandemark, G., Schroeder, K.L., du Toit, L.J., 2013. Development and evaluation of a TaqMan real-time PCR assay for Fusarium oxysporum f. sp. spinaciae. Plant Dis. 97, 927–937. https://doi.org/10.1094/PDIS-03-12-0317-RE.
Okubara, P., Kumar, N., Hohenwarter, L., Graham, D., Kandel, S., Doty, S.L., Micknass, U., Kogel, K.-H., Imani, J., 2017. Inhibition of fungal and bacterial plant pathogens by VEG'LYS®, an Allium-based antimicrobial formulation. J. Biol. Nat. 8, 40–51.
Paulitz, T.C., Schroeder, K.L., 2005. A new method for the quantification of Rhizoctonia solani and R. oryzae from soil. Plant Dis. 89, 767–772.
Paz, L., Vazquez-Yanes, C., 1998. Comparative seed ecophysiology of wild and cultivated Carica papaya trees from a tropical rain forest region in Mexico. Tree Physiol. 18, 277–280. https://doi.org/10.1093/treephys/18.4.277.
Persson, L., Bødker, L., Larsson-Wikström, M., 1997. Prevalence and pathogenicity of foot and root rot pathogens of pea in southern Scandinavia. Plant Dis. 81, 171–174.
Peters, J.C., Lees, A.K., Cullen, D.W., Sullivan, L., Stroud, G.P., Cunnington, A.C., 2008. Characterization of Fusarium spp. responsible for causing dry rot of potato in Great Britain. Plant Pathol. 57, 262–271.
Pollard, A.T., 2018. Seeds vs fungi: an enzymatic battle in the soil seedbank. Seed Sci. Res. 28, 197–214. https://doi.org/10.1017/S0960258518000181.
Ponchel, F., Toomes, C., Bransfield, K., Leong, F.T., Douglas, S.H., Field, S.L., Bell, S.M., Combaret, V., Puisieux, A., Mighell, A.J., Robinson, P.A., Inglehearn, C.F., Isaacs, J.D., Markham, A.F., 2003. Real-time PCR based on SYBR-Green I fluorescence: an alternative to the TaqMan assay for a relative quantification of gene rearrangements, gene amplifications and micro gene deletions. BMC Biotechnol. 3, 18.
Raaijmakers, J.M., Weller, D.M., Thomashow, L.S., 1997. Frequency of antibiotic-producing Pseudomonas spp. in natural environments. Appl. Environ. Microbiol. 63, 881–887.
Rahjoo, V., Zad, J., Javan-Nikkhah, M., Gohari, A.M., Okhovvat, S.M., Bihamta, M.R., Razzaghian, J., Klemsdal, S.S., 2008. Morphological and molecular identification of Fusarium isolated from maize ears in Iran. J. Plant Pathol. 90, 463–468.
Rossman, A.Y., Lu, K.C., 1980. Filamentous fungi associated with leaf surfaces of red alder and Douglas fir seedlings in western Oregon. Mycotaxon 10 (2), 369–371.
Rychlik, W., 2007. OLIGO 7 primer analysis software. In: Yuryev, A. (Ed.), PCR Primer Design, Methods in Molecular Biology. vol. 402. Humana Press, pp. 35–39.
Sankaran, S., Mishra, A., Ehsani, R., Davis, C., 2010. A review of advanced techniques for detecting plant diseases. Comput. Electron. Agric. 72, 1–13.
Sanzani, S.M., Li Destri Nicosia, M.G., Faedda, R., Cacciola, S.O., Schena, L., 2014. Use of quantitative PCR detection methods to study biocontrol agents and phytopathogenic fungi and oomycetes in environmental samples. J. Phytopathol. 162, 1–13. https://doi.org/10.1111/jph.12147.
Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Fungal Barcoding Consortium, 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc. Natl. Acad. Sci. 109, 6241–6246. https://doi.org/10.1073/pnas.1117018109.
Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Soding, J., Thompson, J.D., Higgins, D.G., 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol. Syst. Biol. 7, 539. https://doi.org/10.1038/msb.2011.75.
Smith, J.D., Kidwell, K.K., Evans, M.A., Cook, R.J., Smiley, R.W., 2003. Evaluation of spring cereal grains and wild Triticum germplasm for resistance to Rhizoctonia solani AG-8. Crop Sci. 43, 701–709.
Sørensen, J.L., Phipps, R.K., Nielsen, K.F., Schroers, H.-J., Frank, J., Thrane, U., 2009. Analysis of Fusarium avenaceum metabolites produced during wet apple core rot. J. Agric. Food Chem. 57, 1632–1639. https://doi.org/10.1021/jf802926u.
Stakheev, A.A., Khairulina, D.R., Zavriev, S.K., 2016. Four-locus phylogeny of Fusarium avenaceum and related species and their species-specific identification based on partial phosphate permease gene sequences. Int. J. Food Microbiol. 225, 27–37. https://doi.org/10.1016/j.ijfoodmicro.2016.02.012.
Starkey, D.E., Ward, T.J., Aoki, T., Gale, L.R., Kistler, H.C., Geiser, D.M., Suga, H., Toth, B., Varga, J., O'Donnell, K., 2007. Global molecular surveillance reveals novel Fusarium head blight species and trichothecene toxin diversity. Fungal Genet. Biol. 44, 1191–1204.
Stavrou, A.A., Mixão, V., Boekhout, T., Gabaldón, T., 2018. Misidentification of genome assemblies in public databases: the case of Naumovozyma dairenensis and proposal of a protocol to correct misidentifications. Yeast 35, 425–429. https://doi.org/10.1002/yea.3303.
Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R.V., Breiman, R., Brune, M.-N., Decock, K., Dilley, A., Groopman, J., Hell, K., Henry, S.H., Jeffers, D., Jolly, C., Jolly, P., Kibata, G.N., Lewis, L., Liu, X., Luber, G., McCoy, L., Mensah, P., Miraglia, M., Misore, A., Njapau, H., Ong, C.-N., Onsongo, M.T.K., Page, S.W., Park, D., Patel, M., Phillips, T., Pineiro, M., Pronczuk, J., Rogers, H.S., Rubin, C., Sabino, M., Schaafsma, A., Shephard, G., Stroka, J., Wild, C., Williams, J.T., Wilson, D., 2006. Public health strategies for reducing aflatoxin exposure in developing countries: a workgroup report. Environ. Health Perspect. 114, 1898–1903. https://doi.org/10.1289/ehp.9302.
Summerell, B.A., Laurence, M.H., Liew, E.C.Y., Leslie, J.F., 2010. Biogeography and phylogeography of Fusarium: a review. Fungal Divers. 44, 3–13. https://doi.org/10.1007/s13225-010-0060-2.
Summerell, B.A., Leslie, J.F., Liew, E.C., Laurence, M.H., Bullock, S., Petrovic, T., Bentley, A.R., Howard, C.G., Peterson, S.A., Walsh, J.L., 2011. Fusarium species associated with plants in Australia. Fungal Divers. 46, 1–27.
Tanksley, S.D., McCouch, S.R., 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277, 1063–1066. https://doi.org/10.1126/science.277.5329.1063.
Tewoldemedhin, Y.T., Mazzola, M., Botha, W.J., Spies, C.F.J., McLeod, A., 2011. Characterization of fungi (Fusarium and Rhizoctonia) and oomycetes (Phytophthora and Pythium) associated with apple orchards in South Africa. Eur. J. Plant Pathol. 130, 215–229. https://doi.org/10.1007/s10658-011-9747-9.
Thompson, A.L., Mahoney, A.K., Smiley, R.W., Paulitz, T.C., Hulbert, S., Garland-Campbell, K., 2017. Resistance to multiple soil-borne pathogens of the Pacific Northwest, USA is colocated in a wheat recombinant inbred line population. Genes Genomes Genet. 7, 1109–1116. https://doi.org/10.1534/g3.116.038604.
Triest, D., Stubbe, D., De Cremer, K., Piérard, D., Normand, A.-C., Piarroux, R., Detandt, M., Hendrickx, M., 2015. Use of matrix-assisted laser desorption ionization–time of flight mass spectrometry for identification of molds of the Fusarium genus. J. Clin. Microbiol. 53, 465–476. https://doi.org/10.1128/JCM.02213-14.
Uhlig, S., Jestoi, M., Knutsen, A.K., Heier, B.T., 2006. Multiple regression analysis as a tool for the identification of relations between semi-quantitative LC-MS data and cytotoxicity of extracts of the fungus Fusarium avenaceum (syn. F. Arthrosporioides). Toxicon 48, 567–579.
Uhlig, S., Jestoi, M., Parikka, P., 2007. Fusarium avenaceum- the north European situation. Int. J. Food Microbiol. 119, 17–24. https://doi.org/10.1016/j.ijfoodmicro.2007.07.021.
United States Department of Agriculture Crops Research Division, Agricultural Research Service, 1960. Plant Pests of Importance to North American Agriculture. Index of Plant Diseases in the United States, Agriculture Handbook. Superintendent of Documents, U.S. Government Printing Office, Washington, D.C, pp. 165.
Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., Rozen, S.G., 2012. Primer3- new capabilities and interfaces. Nucleic Acids Res. 40, e115. https://doi.org/10.1093/nar/gks596.
Vogelgsang, S., Sulyok, M., Hecker, A., Jenny, E., Krska, R., Schuhmacher, R., Forrer, H.-R., 2008. Toxigenicity and pathogenicity of Fusarium poae and Fusarium avenaceum on wheat. Eur. J. Plant Pathol. 122, 265–276. https://doi.org/10.1007/s10658-008-9279-0.
Vujanovic, V., Daida, M.A., Daida, P., 2017. qPCR assessment of aurofusarin gene expression in mycotoxigenic Fusarium species challenged with mycoparasitic and chemical control agents. Biol. Control 109, 51–57. https://doi.org/10.1016/j.biocontrol.2017.03.010.
Waalwijk, C., van der Heide, R., de Vries, I., van der Lee, T., Schoen, C., Costrel-De Corainville, G., Häuser-Hahn, I., Kastelein, P., Köhl, J., Lonnet, P., Demarquet, T., Kema, G.H.J., 2004. Quantitative detection of Fusarium species in wheat using TaqMan. Eur. J. Plant Pathol. 110, 481–494.
Wagner, M., Mitschunas, N., 2008. Fungal effects on seed bank persistence and potential applications in weed biocontrol: a review. Basic Appl. Ecol. 9, 191–203. https://doi.org/10.1016/j.baae.2007.02.003.
Watanabe, M., Yonezawa, T., Lee, K., Kumagai, S., Sugita-Konishi, Y., Goto, K., Hara-Kudo, Y., 2011. Molecular phylogeny of the higher and lower taxonomy of the Fusarium genus and differences in the evolutionary histories of multiple genes. BMC Evol. Biol. 11, 322. https://doi.org/10.1186/1471-2148-11-322.
Weller, D.M., Cook, R.J., MacNish, G., Bassett, E.N., Powelson, R.L., Petersen, R.R., 1986. Rhizoctonia root rot of small grains favored by reduced tillage in the Pacific Northwest. Plant Dis. 70, 70–73.
Wilson, I.G., 1997. Inhibition and facilitation of nucleic acid amplification. Appl. Environ. Microbiol. 63, 3741–3751.
Yan, G., Smiley, R.W., Okubara, P.A., 2012. Detection and quantification of Pratylenchus thornei in DNA extracted from soil using real-time PCR. Phytopathology 102, 14–22. https://doi.org/10.1094/PHYTO-03-11-0093.
Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., Madden, T.L., 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinforma. 13, 134.
Yli-Mattila, T., Paavanen, S., Hannukkala, A., Parikka, P., Tahvonen, R., Karjalainen, R., 1996. Isozyme and RAPD-PCR analyses of Fusarium avenaceum strains from Finland. Plant Pathol. 45, 126–134.
You, M.P., Barbett, M.J., Nichols, P.G.H., 2005. New sources of resistance identified in Trifolium subterraneum breeding lines and cultivars to root rot caused by Fusarium avenaceum and Pythium irregulare and their relationship to seedling survival. Australas. Plant Pathol. 34, 237–244.
Zhang, Z., Schwartz, S., Wagner, L., Miller, W., 2000. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. 7, 203–214. https://doi.org/10.1089/10665270050081478.