HEAD BLIGHT OF BARLEY IN SOUTH AFRICA IS CAUSED BY *FUSARIUM GRAMINEARUM* WITH A 15-ADON CHEMOTYPE

A.-L. Boutigny, I. Beukes and A. Viljoen

University of Stellenbosch, Department of Plant Pathology, Private Bag X1, Matieland 7602, South Africa

SUMMARY

In the past 4 years, the emergence of Fusarium head blight (FHB) of irrigated barley in the Northern Cape Province (NCP) became a concern to the malting and brewing industries, and the food and feed companies in South Africa. This motivated an epidemiological survey whereby 320 single-spored *Fusarium* isolates were obtained from diseased barley kernels collected in the NCP over 2 consecutive crop years (2008-2009) on two barley cultivars (potential malting varieties) at several locations. A multiplex PCR using previously published species-specific primers was utilized to simultaneously detect the most frequently encountered species of the FHB complex, including *Fusarium graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae*. For isolates that could not be identified using PCR, further characterization was performed through sequence analyses. *Fusarium graminearum* (91.9%) was the dominant species, while *F. boothii* (6.25%), one of the species within the *F. graminearum* species complex, *F. equiseti* (1.25%) and *F. poae* (0.6%) were isolated far less frequently. Within the *F. graminearum* species complex, most of the isolates (99.7%) belonged to the 15-ADON chemotype. A real-time PCR assay based on SYBR Green technology was used to accurately quantify *F. graminearum* in barley samples. This is the first report on *Fusarium* species and their mycotoxin profiles associated with FHB of barley in South Africa.

Key words: Fusarium head blight, cereal, *Fusarium*, trichothecene, real-time PCR.

INTRODUCTION

Fusarium head blight (FHB) of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) is a disease of great economic significance across the world (Parry *et al.*, 1995; Goswani and Kistler, 2004). In the USA alone, the estimated wheat and barley losses due to reduced yield and quality caused by FHB during the 1990s amount to more than $3 billion (Windels, 2000). In South Africa, FHB was reported on irrigated wheat for the first time in 1988 (Marasas *et al.*, 1988a; Scott *et al.*, 1988). With the substantial increase in wheat and barley production in recent years, FHB has become an important emerging disease, particularly in irrigated fields.

Barley was historically grown on dry land in the Western Cape Province which is unfavourable to the development of FHB. However, in the past 4 years, barley cultivation expanded to the Northern Cape Province (NCP) where the crop is now grown under irrigation and in rotation with wheat and maize. Barley in the NCP is intended for use by the malting and beer brewing industries of South Africa. Severe contamination with FHB can render the crop technologically unsuitable for malt production by altering kernel plumpness, malt quality and germination, which are all important malting properties, and can also alter brewing performance and beer quality (off-flavours in beer, problems in the brew-house, beer gushing) (Schwartz, 2003).

Up to 17 different *Fusarium* species have been associated with FHB. These species vary in importance depending on the crop species involved, the region and the season (Parry *et al.*, 1995; Bottalico and Perrone, 2002; Xu, 2003). The *Fusarium* species predominantly found in association with FHB in small-grain cereals are *Fusarium graminearum* Schwabe, *Fusarium avenaceum* (Fries) Saccardo and *Fusarium culmorum* (W.G. Smith) Saccardo (Bottalico and Perrone, 2002). However, *Fusarium poae* (Peck) Wollenweber, *Fusarium tricinctum* (Corda) Saccardo, *Fusarium sporotrichioides* Sherbakoff, *Fusarium equiseti* (Corda) Saccardo and *Fusarium langsethiae* Torp and Nirenberg are also often associated with the disease (Bottalico and Perrone, 2002). *Fusarium graminearum* was previously thought to be a single species, but has recently been divided into a complex of 13 species (O’Donnell *et al.*, 2000; Yli-Mattila *et al.*, 2009).

*Fusarium* species not only cause crop losses and poor grain quality, but also release toxic secondary metabolites, referred to as mycotoxins, in the grain (Parry *et al.*, 1995; Goswani and Kistler, 2004; Miller, 2008). These
mycotoxins constitute an important food safety concern, as they have health implications to both humans and animals (Bennett and Klich, 2003). Most of the Fusarium species are able to produce one or more mycotoxins with varying degree of toxicity (Bottalico and Perrone, 2002). Type B trichothecenes constitute the largest group of mycotoxins produced by Fusarium in small-grain cereals. Type B trichothecenes include deoxynivalenol (DON) and its acetylated forms 3-acetyldeoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON), and nivalenol (NIV) and its acetylated form 4-acetyl-nivalenol or fusarenone X (FX). Among the type B trichothecene-producing Fusarium, three chemotypes have been defined: the NIV chemotype produces NIV and acetylated derivatives, the 3-ADON chemotype produces DON and 3-ADON, and the 15-ADON chemotype produces DON and 15-ADON (Miller et al., 1991).

Although a slight difference exists between these compounds in the pattern of hydroxylation or acetylation, their toxicity and activity differ greatly, indicating that these chemotype differences may have important health consequences (Kimura et al., 1998). NIV is generally regarded as more toxic than DON to humans and animals (Ryu et al., 1988). Since type B trichothecenes are heat stable molecules and are not eliminated by current food manufacturing processes (Hazel and Patel, 2004), mycotoxins such as DON present in Fusarium-infected malt barley may ultimately end up in the beer (Steffenson, 2003).

Fusarium species involved in FHB differ in their pathogenicity, fungicide sensitivity and ability to produce mycotoxins (Xu et al., 2005). Their accurate identification, the characterization of their mycotoxin profile and their quantification in planta, therefore, are of great importance. Visual estimation of FHB infection in the field is time consuming and does not differentiate species, whereas plating methods and morphological identification of the pathogens are time consuming and require taxonomic expertise for species identification (Leslie and Summerell, 2006).

Advanced molecular methods, such as gene sequencing, PCR and quantitative real-time PCR today provide scientists with tools to rapidly and accurately identify Fusarium species associated with FHB, characterize their chemotype and quantify them in planta. Several PCR assays using species-specific primers have been successfully developed and used in order to identify Fusarium species (Waalwijk et al., 2003; Nicholson et al., 2004; Goertz et al., 2010). Waalwijk et al. (2003) developed a multiplex PCR with seven primer pairs to detect the most common species of Fusarium occurring on wheat [F.avenaceum, F. culmorum, F. graminearum, F. poae, F. proliferatum (Matsushima) Nirenberg, Microdochium nivale var. majus and M. nivale var. nivale]. PCR assays based on the amplification of portions of genes that code for key enzymes involved in trichothecene biosynthesis have been successfully developed (Ward et al., 2002; Chandler et al., 2003; Quarta et al., 2006; Wang et al., 2008; Suzuki et al., 2010; Zhang et al., 2010) and used (Jennings et al., 2004; Ward et al., 2008; Prodi et al., 2009; Scoz et al., 2009; Kammoun et al., 2010; Pasquali et al., 2010) to accurately predict type B trichothecene-producing Fusarium chemotype. In addition, a number of quantitative real-time PCR assays have been successfully developed and offer an accurate estimation of the biomass of individual species in planta (Reischer et al., 2004; Waalwijk et al., 2004; Sarlin et al., 2006; Yli-Mattila et al., 2008; Nicolson et al., 2009). These assays can be used to monitor a range of Fusarium species during epidemiological studies, to understand the interactions between species and to estimate the effects of preventive or curative measures on disease development.

Despite all the international research on FHB of wheat and barley, little information is available on the disease in South Africa; the Fusarium species involved as well as their mycotoxin profiles. To our knowledge, there are no previous reports on the South African Fusarium isolates involved in head blight of barley and their chemotypes. In this study, Fusarium species involved in FHB of irrigated barley in the NCP of South Africa, and their trichothecene chemotype, were determined. A quantitative real-time PCR assay was then used to quantify F. graminearum, as the predominant species involved in the disease, in local barley samples.

**MATERIALS AND METHODS**

**Fungal reference isolates.** Reference isolates of F. graminearum (NRRL 28439) and F. culmorum (NRRL 3288) were provided by Dr. Kerry O’Donnell (USDA-ARS Peoria, IL, USA), and those of F. avenaceum (MRC 3227) and F. poae (MRC 8486) by Prof. Wally Marasas (Department of Plant Pathology, University of Stellenbosch, South Africa). Isolates for which toxin profiles have been ascertained were provided by Léaëtia Pinson-Gadais (French National Institute for Agricultural Research, Villenave d’Ornon, France). These include F. culmorum with a 3-ADON chemotype (INRA 233), F. graminearum with a 15-ADON chemotype (INRA 156) and F. graminearum with a NIV chemotype (INRA 91). The reference isolates were used as positive controls in PCR assays and for standard curve in real-time PCR assays.

**Barley samples.** Fifty barley heads with visual symptoms of FHB were collected individually at maturity in each of three different fields in 2008 and six different fields in 2009 in the NCP in South Africa. In total, 450 barley heads were collected over 2 consecutive crop
years (2008-2009) from two cultivars (Puma and Cock-tail, potential malting varieties). After harvesting, barley heads were kept at -4°C until isolation procedure. From each diseased head, two diseased kernels were taken for isolation of *Fusarium* species and the remaining kernels were placed at -20°C until DNA extraction. Kernels were surface-disinfected by washing them in 70% ethanol for 1.5 min and then in 2% sodium hypochlorite for 3 min. The kernels were then extensively rinsed with sterile distilled water and placed on potato dextrose agar (PDA) plates containing 40 mg l⁻¹ streptomycin. Plates were incubated for 5 days at 25°C. *Fusarium*-like mycelium was sub-cultured on new PDA plates using a single spore technique (Leslie and Summerell, 2006). In this way, a total of 320 isolates were obtained. These cultures are all maintained in 15% glycerol at -80°C at the facilities of the Department of Plant Pathology, Stellenbosch University, South Africa.

**Species identification.** Isolates were grown on PDA plates for 5 days and genomic DNA was extracted from aerial fresh mycelia using the Wizard SV Genomic DNA Purification System Kit (Promega, South Africa). *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae* were identified using a multiplex PCR assay using previously published species-specific primers (Table 1). Multiplex PCR assays were conducted using 20 ng of fungal DNA in a total volume of 25 μl containing 2.5 mM MgCl₂, 1.5 U Taq DNA polymerase, 0.4 mM dNTPs, 1 mg ml⁻¹ BSA, 125 nM of the primers Fg11F/Fg11R, FaF/FaR, FP82F/FP82R and 250 nM of the primers Fc01F/Fc01R. PCR amplification consisted of an initial step at 94°C for 2 min, followed by 35 cycles of 94°C for 45 sec, 60°C for 30 sec and 72°C for 45 sec, with a final extension step at 72°C for 5 min. Reference cultures were used as positive controls. Resulting PCR products were separated by gel electrophoresis, stained with ethidium bromide and visualized under UV light.

For isolates that did not generate PCR products using the multiplex PCR assay, a standard PCR protocol was used to amplify the translation elongation factor (*EF1α*) gene region using ef1 and ef2 primers (Table 1). PCR assays were conducted using 20 ng of fungal DNA in a total volume of 40 μl containing 2 mM MgCl₂, 1.5 U Taq DNA polymerase, 0.4 mM dNTPs, 1 mg ml⁻¹ BSA, 400 nM of each primer. PCR amplification consisted of an initial step at 94°C for 2 min, followed by 40 cycles of 94°C for 45 sec, 51°C for 45 sec and 72°C for 1 min, with a final extension step at 72°C for 7 min. Resulting PCR products were separated by gel electrophoresis, stained with ethidium bromide and visualized under UV light. The *EF1α* PCR product generated using the ef1 and ef2 primers was used as a template for DNA sequencing, after purification using the MSB Spin PCRapace Kit (Invitrek, South Africa). Sequencing of both strands was performed at the sequencing facility of the University of Stellenbosch. Sequences were edited using Geneious software. Once edited, the *EF1α* sequences from the isolates of interest were blasted for comparison to the NCBI GenBank database and to the FUARIUM-ID database (Geiser et al., 2004). DNA sequence data generated for 24 isolates from South Africa have been deposited in GenBank under accession Nos HQ889137-HQ889160.

### Table 1. Primer identification, sequences and expected amplicon sizes used in this study.

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer designation</th>
<th>Primer sequence</th>
<th>Amplicon (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. avenaceum</em></td>
<td>Fg11F, Fg11R</td>
<td>CAAGCCATTTGTCGCCACTTTC</td>
<td>920</td>
<td>Dharm et al., 1998</td>
</tr>
<tr>
<td></td>
<td>Fg11F, Fg11R</td>
<td>CTTGCGCTCTAAGGGACCTT</td>
<td>920</td>
<td>Dharm et al., 1998</td>
</tr>
<tr>
<td><em>F. culmorum</em></td>
<td>FaF, FaR</td>
<td>ATGGTGAAACTCTCTCCTC</td>
<td>570</td>
<td>Nicholson et al., 1998</td>
</tr>
<tr>
<td></td>
<td>Fg11F, Fg11R</td>
<td>CCGTCGCTCTAAGGGACCTT</td>
<td>920</td>
<td>Nicholson et al., 1998</td>
</tr>
<tr>
<td><em>F. graminearum</em></td>
<td>Fg11F, Fg11R</td>
<td>CTCCGCGATTTTGGCTGCA</td>
<td>920</td>
<td>Nicholson et al., 1998</td>
</tr>
<tr>
<td><em>F. poae</em></td>
<td>Fg11F, Fg11R</td>
<td>CAGACACCCAGCTCTCTCCA</td>
<td>920</td>
<td>Perry and Nicholson, 1996</td>
</tr>
<tr>
<td><em>EF1α</em></td>
<td>ef1, ef2</td>
<td>ATGGGTAAAGAGAAGGGAACAC</td>
<td>~700</td>
<td>O'Donnell et al., 1998</td>
</tr>
<tr>
<td></td>
<td>ef1, ef2</td>
<td>CGAAAGATCGACAGGCGGAC</td>
<td>~700</td>
<td>O'Donnell et al., 1998</td>
</tr>
<tr>
<td><em>Trx3</em></td>
<td>3CON</td>
<td>TGGCGAGCTGCTGACCTC</td>
<td>240</td>
<td>Ward et al., 2008</td>
</tr>
<tr>
<td></td>
<td>3NA</td>
<td>CGTGACGCAATATGCAAGA</td>
<td>610</td>
<td>Ward et al., 2008</td>
</tr>
<tr>
<td></td>
<td>3DHA</td>
<td>ACGCGCAAGAGAGAGAGAGA</td>
<td>840</td>
<td>Ward et al., 2008</td>
</tr>
<tr>
<td></td>
<td>3DIA</td>
<td>GGCGTGTATGCTGCTGAG</td>
<td>840</td>
<td>Ward et al., 2008</td>
</tr>
<tr>
<td><em>F. graminearum</em></td>
<td>Pf03R</td>
<td>CCTATCTGGTGGGCT</td>
<td>~60</td>
<td>Nicolea et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Pf03R</td>
<td>CCTATCTGGTGGGCT</td>
<td>~60</td>
<td>Nicolea et al., 2009</td>
</tr>
<tr>
<td><em>Plant. EF1</em></td>
<td>Horf</td>
<td>TCTCTGGTGGTGTGAGGAC</td>
<td>~60</td>
<td>Nicolea et al., 2009</td>
</tr>
<tr>
<td></td>
<td>HorCr</td>
<td>GGGCCTGTTAGCCCTTCTC</td>
<td>~60</td>
<td>Nicolea et al., 2009</td>
</tr>
</tbody>
</table>
Chemotype identification. NIV, 3-ADON and 15-ADON chemotypes were identified using a multiplex PCR assay to amplify portions of the Tri3 gene (Ward et al., 2008). With the Tri3 primer set (Table 1), PCR products of 243, 610 and 840 bp are produced for 3-ADON, 15-ADON and NIV chemotypes, respectively (Ward et al., 2008). Multiplex PCR assays were conducted using 20 ng of fungal DNA in a total volume of 25 µl containing 2.5 mM MgCl₂, 1.5 U Taq DNA polymerase, 0.4 mM dNTPs, 1 mg ml⁻¹ BSA, 250 nM of each primer. PCR amplification consisted of an initial step at 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 58°C for 30 sec and 72°C for 30 sec, with a final extension step at 72°C for 5 min. Reference cultures were used as positive controls. Resulting PCR products were separated by gel electrophoresis, stained with ethidium bromide and visualized under UV light.

DNA extraction for real-time PCR. *F. graminearum* (isolate NRRL 28439) was used to produce DNA standards for the quantification of *F. graminearum* in barley samples. To produce fungal mycelia, the isolate was grown on potato dextrose broth (PDB) in 250-ml Erlenmeyer flasks at 25°C for 14 days under shaking. Mycelium was harvested by filtration through filter paper and quantified using the Nanodrop ND-1000 Spectrophotometer (Inqaba biotechnical industries, South Africa). DNA purity was evaluated by comparing the absorbance ratios A260/280 and A260/230.

DNA from environmental samples, a mixed DNA standard curve was prepared by diluting pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water. For direct comparisons with environmental samples, a mixed DNA standard curve was prepared by diluting the pathogen DNA (11.6 ng µl⁻¹) and host DNA (33.8 ng µl⁻¹) 4X, 16X, 64X, 256X and 1024X in water.
4X, 16X, 64X, 256X, 4096X and 16384X (0.0007 ng µl⁻¹) in host DNA (15 ng µl⁻¹). Real-time PCR reactions were performed in triplicate on each standard curve sample. Standard curves were analyzed and the cycle threshold (Ct) values of the undiluted and diluted samples were plotted against the logarithm of the known (given by the Nanodrop Spectrophotometer) DNA concentration. To assess the presence of inhibitors, the Ct value for the undiluted sample was extrapolated from the equation calculated by linear regression. Subsequently, the extrapolated Ct was compared with the measured Ct. Criteria for DNA quality acceptance are: slope between -3.1 and -3.6 and linearity above 0.98. In addition, the ∆Ct between extrapolated and measured Ct number on the undiluted sample should be less than 0.5 (CRL-EM-01/08).

Real-time PCR reactions were also used to determine the reproducibility of the DNA extraction method to be used from contaminated barley and to test the intra-run variability of the PCR assays. DNA, therefore, was initially extracted from five ground barley samples. For four of these samples, DNA was extracted from three independent sub-samples, and for one of the barley sample, DNA was extracted from six independent sub-samples. DNA yields were then estimated using the real-time PCR assay specific for the plant EF1α gene. Real-time PCR reactions were performed in triplicate on each sub-sample.

To quantify F. graminearum in barley samples collected from the nine field locations, the real-time PCR assay contained a triplicate of each barley sample and a triplicate of the standard (pathogen DNA diluted 16X in host DNA free of contamination). Using the standard curves obtained from the mixed DNA samples, the Ct value was transformed into DNA concentration. An infection coefficient (IC) was determined by calculating the ratio of the amount of pathogen DNA (estimated by real-time PCR) by the host DNA concentration (Mideros et al., 2009).

**RESULTS**

**Species and chemotype identification of Fusarium infecting barley.** A total of 164 and 156 Fusarium-like isolates were obtained in 2008 and 2009 corresponding, respectively, to 55% and 26% of the total number of isolates obtained after kernel plating. In 2008, 162 of the isolates were identified as F. graminearum, one isolate as F. boothii and one isolate as F. equiseti. In 2009, 132 of the isolates were identified as F. graminearum, 19 isolates as F. boothii, three isolates as F. equiseti and two isolates as F. poae. Over the 2 years, within the F. graminearum species complex, 313 isolates were identified as being from the 15-ADON chemotype and only one isolate was identified as being from the 3-ADON chemotype in 2008.

**Real-time PCR assay validation.** The DNA extraction method and real-time PCR assay used to assess F. graminearum in five barley samples were highly reproducible (Table 2). The DNA extracted from three independent sub-samples from four barley samples, and from six independent sub-samples from one barley sample, showed a very small variation in CT-values (SD between 0.02 and 0.22, n = 5). Triplicate PCR on the same extracted DNA preparation within a single run also showed a very small variation in CT-values (mean SD =

<table>
<thead>
<tr>
<th>Sample</th>
<th>Linearity (R²)</th>
<th>Slope</th>
<th>Efficiency</th>
<th>∆Ct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium graminearum (NRRL 28439)</td>
<td>0.99</td>
<td>-3.38</td>
<td>0.97</td>
<td>0.001</td>
</tr>
<tr>
<td>Fusarium graminearum (NRRL 28439) diluted in barley DNA</td>
<td>0.99</td>
<td>-3.56</td>
<td>0.90</td>
<td>0.06</td>
</tr>
<tr>
<td>Barley</td>
<td>0.99</td>
<td>-3.31</td>
<td>1.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Table 3.** Linearity and inhibition test results of the DNA extracted for subsequent applications of real-time PCR.

<table>
<thead>
<tr>
<th>Barley samples</th>
<th>Cultivar</th>
<th>Farm</th>
<th>Coordinates</th>
<th>IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>Puma</td>
<td>1</td>
<td>S 29° 00.578’ / E 23° 57.187’</td>
<td>2.13</td>
</tr>
<tr>
<td>2008</td>
<td>Cocktail</td>
<td>1</td>
<td>S 29° 00.822’ / E 23° 56.837’</td>
<td>2.66</td>
</tr>
<tr>
<td>2008</td>
<td>Cocktail</td>
<td>2</td>
<td>S 29° 57.204’ / E 24° 15.634’</td>
<td>3.21</td>
</tr>
<tr>
<td>2009</td>
<td>Puma</td>
<td>3</td>
<td>S 28° 55.297’ / E 24° 16.138’</td>
<td>0.35</td>
</tr>
<tr>
<td>2009</td>
<td>Cocktail</td>
<td>4</td>
<td>S 29° 16.033’ / E 23° 47.237’</td>
<td>1.26</td>
</tr>
<tr>
<td>2009</td>
<td>Cocktail</td>
<td>5</td>
<td>S 28° 51.557’ / E 24° 01.090’</td>
<td>1.80</td>
</tr>
<tr>
<td>2009</td>
<td>Cocktail</td>
<td>6</td>
<td>S 28° 58.244’ / E 24° 07.542’</td>
<td>0.60</td>
</tr>
<tr>
<td>2009</td>
<td>Cocktail</td>
<td>1</td>
<td>S 29° 00.107’ / E 23° 57.118’</td>
<td>0.00</td>
</tr>
<tr>
<td>2009</td>
<td>Puma</td>
<td>7</td>
<td>S 28° 55.513’ / E 24° 16.202’</td>
<td>0.07</td>
</tr>
</tbody>
</table>

**Table 4.** Infection coefficient (IC) with Fusarium graminearum of nine barley samples from the Northern Cape Province, South Africa.
DNA samples with or without barley DNA. Cycle thresholds (CT) were plotted against the known amount of *F. graminearum* DNA expressed on a logarithmic scale. The error bars indicate standard deviations.

### DISCUSSION

The emergence of FHB and contamination of malting barley with mycotoxins was the cause of great concern to the beer brewing industries in South Africa. This resulted in a 2-year epidemiological survey of the occurrence and cause of FHB in the country. Our findings showed that *F. graminearum* was the dominant *Fusarium* species associated with FHB of irrigated barley fields in the NCP of South Africa for two consecutive years. We also found that *F. boothii*, one of the species within the *F. graminearum* species complex, *F. equiseti* and *F. poae* were associated with FHB. To our knowledge, this is the first report on *Fusarium* species associated with FHB of barley in South Africa.

The NCP is known as a warm, dry area near the Kalahari Desert in the northern central part of South Africa. All barley fields in this region are cultivated under pivot irrigation. The dominance of *F. graminearum* in the region, therefore, was to be expected, as the fungus is well known to be the major *Fusarium* species involved in FHB of barley in similar climates in other parts of the world (Bottalico and Perrone, 2002). Other common FHB pathogens such as *F. culmorum* and *F. avenaceum* were not isolated in this study, and *F. poae* was only associated with the disease in 2009. These *Fusarium* species are known to be more often associated with FHB in cooler regions such as Northern Europe (Bottalico and Perrone, 2002). In a study by Hudec and Rohacik (2009), *F. poae* was found to be the most common *Fusarium* species found on barley kernels in Slovakia over 2 consecutive crop years (2004-2005). *Fusarium boothii* was previously collected from maize in South Africa by P. Martin at an unknown date (O'Donnell et al., 2004), while *F. equiseti* is commonly found on agricultural crops and in soil in the country (Marasas et al., 1988b).

It is important to notice that the *F. graminearum*-specific primers used in this study did not give a product for the *F. boothii* isolates, which were subsequently identified by means of sequencing. In their study, Waalwijk et al. (2003) reported that when isolates representing the seven species within the *F. graminearum* species complex (O'Donnell et al., 2000) were tested, only *Fg11F/R* products were obtained for *F. avenaceum*, *F. meridionale*, *F. asiaticum* and *F. graminearum*, but not for *F. boothii*, *F. mesoamericanum* and *F. acaciae-mearnsii*.

Most of the *F. graminearum* isolates from barley in South Africa (99.7%) belonged to the 15-ADON chemotype. To our knowledge, this is the first time that the complete mycotoxin profile of *F. graminearum* from barley has been characterized in South Africa. This finding is of great significance as the dominant chemotype in the FHB fungal population provides important information about which toxins might be produced into the grains. DON-producing isolates of *F. graminearum* also appear to occur more frequently than NIV-produc-
ing isolates in Europe (Bottalico and Perrone, 2002; Waalwijk et al., 2003; Jenings et al., 2004; Szecsi et al., 2005; Prodi et al., 2009; Pasquale et al., 2010), North America and Canada (Gale et al., 2007; Guo et al., 2008; Ward et al., 2008), South America (Ramirez et al., 2006; Scoz et al., 2009) and China (Ji et al., 2007).

In a recent study evaluating wheat resistance to NIV and DON chemotypes, Milus et al. (2011) reported that wheat lines with resistance to isolates of the DON chemotype had even higher levels of resistance to isolates of the NIV chemotype. Von der Ohe et al. (2010) compared isolates of F. graminearum with a 3-ADON chemotype and a 15-ADON chemotype for their ability to produce DON and to cause FHB in wheat. They reported that the FHB incidences caused by 3-ADON and 15-ADON isolates were not significantly different for any wheat genotype, but that the average DON production by 3-ADON isolates was significantly higher than for the 15-ADON isolates for moderately resistant and susceptible lines. These results indicate that 3-ADON isolates could pose a greater risk to food safety. However, on highly resistant lines, the aggressiveness and DON production of 3-ADON and 15-ADON chemotypes was similar; suggesting that the use of highly resistant cultivars is still the most effective way of reducing the risks associated with DON contamination in cereals.

In this study, a real-time PCR assay based on SYBR Green technology was used to accurately quantify F. graminearum in barley samples. This F. graminearum assay was previously developed and used by Nicolaïsen et al. (2009) to quantify F. graminearum in wheat and maize samples. According to Nicolaïsen et al. (2009), this assay will detect all species in the F. graminearum complex except for F. cortaderiae and F. brasilicum. In our assay, F. graminearum was quantifiable between 23.1 ng and 1.4 pg. The estimation of contamination by F. graminearum of barley by real-time PCR could be used to monitor the disease, as F. graminearum has been shown to be the predominant species involved in FHB of barley in South Africa.

ACKNOWLEDGEMENTS

The authors would like to thank Frikkie Lubbe from SAB Maltings (South Africa) for helping to collect barley samples. Dr. Kelly O’Donnell (USDA-ARS Peoria, IL., USA), Prof. Wally Marasas (Department of Plant Pathology, University of Stellenbosch, South Africa) and Laëtitia Pinson-Gadais (INRA, Villeneuve d’Ornon, France) are gratefully acknowledged for the supply of reference isolates. Prof. Wally Marasas is acknowledged for reviewing this manuscript. This work was financed by the University of Stellenbosch, the Winter Cereal Trust and THRIP.

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