The distribution and type B trichothecene chemotype of <i>Fusarium</i> species associated with head blight of wheat in South Africa

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Keywords: Fusarium head blight; Wheat; South Africa; Fusarium species; type B trichothecene chemotype

Abstract: Fusarium head blight (FHB) is a common disease of wheat grown in the irrigation regions of South Africa but is less common in dryland production regions. <i>Fusarium</i> species causing the disease were morphologically identified in the 1980s and more recently, but changes in production practices, the availability of new cultivars, and a better understanding of species limits necessitated a contemporary and comprehensive characterisation of FHB pathogens in the country. Symptomatic wheat heads were, therefore, collected from irrigated fields in the Northern Cape, KwaZulu-Natal (KZN), the Bushveld and the eastern part of the Free State in 2008 and 2009, and from a dryland field in the Western Cape in 2009. <i>Fusarium</i> isolates were identified using species-specific primers or by analysis of partial <i>EF-1α</i> sequences. A representative subset of isolates was also characterized morphologically. In total, 1047 <i>Fusarium</i> isolates were collected, comprising 24 species from seven broad species complexes. The <i>F. sambucinum</i> species complex (FSAMSC) and the <i>F. incarnatum-equiseti</i> species complex (FIESC) were the most common, accounting for 83.5% and 13.3% of isolates respectively. However, isolates from the <i>F. chlamydosporum</i> species complex (FCSC), the <i>F. fujikuroi</i> species complex (FFSC), the <i>F. oxysporum</i> species complex (FOSC), the <i>F. solani</i> species complex (FSSC), and the <i>F. tricinctum</i> species complex (FTSC) were also observed. Within the FSAMSC, 90.7% of isolates were identified as members of a subgroup known as the <i>F. graminearum</i> species complex (FGSC), which accounted for 75.7% of all isolates collected in South Africa. The type B trichothecene chemotype of FGSC isolates and related species was inferred via a chemotype-specific PCR assay. Chemotype diversity was limited (15-ADON = 90.1%) and highly structured in relation to species differences. These results greatly expand the known species diversity associated with FHB in South Africa, and include the first report of <i>F. acuminatum</i>, <i>F. armeniacum</i>, <i>F. avenaceum</i>, <i>F. temperatum</i>, and <i>F. pseudograminearum</i> from wheat heads in South Africa. Globally, it is also the first report of <i>F. brachygibbosum</i>, <i>F. lunulosporum</i>, and <i>F. transvaalense</i> from wheat. In addition, potentially novel species were identified within the FCSC, FFSC, FOSC, FSAMSC, FIESC and FTSC.

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The distribution and type B trichothecene chemotype of *Fusarium* species associated with head blight of wheat in South Africa

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Abstract

Fusarium head blight (FHB) is a common disease of wheat grown in the irrigation regions of South Africa but is less common in dryland production regions. *Fusarium* species causing the disease were morphologically identified in the 1980s and more recently, but changes in production practices, the availability of new cultivars, and a better understanding of species limits necessitated a contemporary and comprehensive characterisation of FHB pathogens in the country. Symptomatic wheat heads were, therefore, collected from irrigated fields in the Northern Cape, KwaZulu-Natal (KZN), the Bushveld and the eastern part of the Free State in 2008 and 2009, and from a dryland field in the Western Cape in 2009. *Fusarium* isolates were identified using species-specific primers or by analysis of partial EF-1α sequences. A representative subset of isolates was also characterized morphologically. In total, 1047 *Fusarium* isolates were collected, comprising 24 species from seven broad species complexes. The *F. sambucinum* species complex (FSAMSC) and the *F. incarnatum-equiseti* species complex (FIESC) were the most common, accounting for 83.5% and 13.3% of isolates respectively. However, isolates from the *F. chlamydosporum* species complex (FCSC), the
F. fujikuroi species complex (FFSC), the F. oxysporum species complex (FOSC), the F. solani species complex (FSSC); and the F. tricinctum species complex (FTSC) were also observed. Within the FSAMSC, 90.7% of isolates were identified as members of a subgroup known as the F. graminearum species complex (FGSC), which accounted for 75.7% of all isolates collected in South Africa. The type B trichothecene chemotype of FGSC isolates and related species was inferred via a chemotype-specific PCR assay. Chemotype diversity was limited (15-ADON = 90.1%) and highly structured in relation to species differences. These results greatly expand the known species diversity associated with FHB in South Africa, and include the first report of F. acuminatum, F. armeniacum, F. avenaceum, F. temperatum, and F. pseudograminearum from wheat heads in South Africa. Globally, it is also the first report of F. brachygibbosum, F. lunulosporum and F. transvaalense from wheat. In addition, potentially novel species were identified within the FCSC, FFSC, FOSC, FSAMSC, FIESC and FTSC.

Introduction

Fusarium head blight (FHB) is a major disease of wheat (Triticum aestivum) worldwide. The disease reduces grain yield and causes the production of discoloured, shrivelled kernels contaminated with mycotoxins [1]. In the late 1990s, FHB resulted in losses estimated at US$ 2.7 billion in parts of the USA [2], while about 7 million ha have been affected by FHB epidemics in China [3]. The disease has also been damaging to wheat production in South America [4 – 7], Canada [8, 9] and Europe [10, 11]. In South Africa, wheat production has been negatively affected by the disease, although little information is available on its financial impact.

Studies conducted globally to identify the causal agents of FHB of wheat have demonstrated the Fusarium graminearum species complex (FGSC) to be widespread and predominant in many regions [9, 10, 12 – 14]. The FGSC, which is a subgroup within the Fusarium sambucinum species complex (FSAMSC) [15], consists of at least 16 phylogenetically distinct species [16, 17]. Members of the FGSC display significant biogeographic structure due to geographic speciation and host selection [12, 18 – 20]. Members of the FGSC can also infect barley (Hordeum vulgare), maize (Zea mays) and soybean (Glycine max); all crops that are grown in rotation with wheat in South Africa [21.
Other *Fusarium* species associated with FHB around the world include *F. chlamydosporum* [member of the *F. chlamydosporum* species complex (FCSC)]; *F. cerealis* (syn. *F. crookwellense*), *F. culmorum*, *F. poae*, and *F. pseudograminearum*, (members of the FSAMSC); *F. equiseti*, [member of the *F. incarnatum–equiseti* species complex (FIESC)]; *F. avenaceum* and *F. tricinctum* [members of the *F. tricinctum* species complex (FTSC)] and *F. proliferatum*, *F. subglutinans* and *F. verticillioides* [part of the *Fusarium fujikuroi* species complex (FFSC)] [10, 14, 15, 24 – 30].

*Fusarium* species associated with FHB produce mycotoxins, which are toxic secondary metabolites harmful to humans and animals. The most important among these include the type A and B trichothecene mycotoxins, and zearalenone (ZEA) [31]. Important type A trichothecene mycotoxins include diacetoxyscirpenol (DAS), neosolaniol (NEO), and T-2 and HT-2 toxins, while important type B trichothecene mycotoxins include deoxynivalenol (DON), and nivalenol (NIV) [31]. DON and ZEA (a nonsteroidal estrogen), are widely considered as the most important for wheat and barley [32], although NIV is also found in these crops [26]. The trichothecenes are potent inhibitors of eukaryotic protein synthesis and immunomodulatory [31] and are phytotoxic [33]. DON has two acetylated forms, namely 3-acetyldenoxynivalenol (3-ADON) and 15-acetyldenoxynivalenol (15-ADON) [32], while NIV has an acetylated form called fusarenon X (Fus-X) [10]. ZEA, on the other hand, has estrogenic properties associated with hyperestrogenism and infertility in pigs [31].

There are three major wheat production areas in South Africa [34]. These include the Western Cape Province (Mediterranean climate with mostly winter rainfall); the summer rainfall areas of Gauteng, Limpopo, Mpumalanga, and the North-West Province, the Northern Cape Province and KwaZulu-Natal (KZN); and the Free State Province (also summer rainfall). Wheat is also produced on small scale in the Eastern Cape Province. Historical reports of FHB in South Africa emanated from the irrigated production regions of the eastern Free State, North West Province and KZN during the 1980s [35], with only one report from dryland fields in the Western Cape [36]. FHB of wheat in KZN and the North West Province was shown to be caused by *Gibberella zeae* (now FGSC), and in the eastern Free State by *F. crookwellense* (*F. cerealis*) [35]. The Northern Cape was considered disease-free. This changed a few years later when FHB reached epidemic proportions in irrigated wheat fields near the Orange River in the Northern Cape during the early 1990s [37]. At one locality, 28% of the grain samples were infected with *Fusarium* species, including *F. graminearum* (member
of FGSC (48.4%), *F. moniliforme* (36.3%) (now *F. verticillioides*) and *F. subglutinans* (1.6%) (members of FFSC), *F. equiseti* (member of FIESC) (9.7%), *F. chlamydosporum* (member of FCSC) (3.2%), and *F. oxysporum* (member of *F. oxysporum* species complex, FOSC) (0.8%). *Fusarium poae* was reported from glume spot of wheat heads in South Africa in 1996, in association with a mite species (*Siteroptes avenae*) [38]. A later study [19] identified a total of 277 *Fusarium* isolates, designated FGSC in the current study, and found that *F. graminearum* s.s. with the 15-ADON chemotype was the dominant member associated with FHB in South Africa. The largest species diversity occurred in KZN, where five of the six FGSC members in South Africa was found. A study published in 2017 [39] reported *F. graminearum* (member of FGSC) as the most common *Fusarium* spp. causing FHB of wheat in seven localities across four South African provinces. Only five other *Fusarium* species were identified, including *F. chlamydosporum* (member of FCSC) and *F. equiseti* (member of FIESC) in four localities in the Northern Cape, and *F. cerealis* and *F. culmorum* (members of FSAMSC), and *F. semitectum* (member of FIESC) at one locality each in the Eastern Cape Province, KZN and North West Province. The only records of FHB from dryland fields in the Western Cape Province was on diseased wheat heads under dryland conditions from three farms in the George-district and one farm in the Swellendam-district, where the causal organism was identified as *F. graminearum* Group 2 (now FGSC) [36]. *Fusarium* spp. of wheat can differ between regions and fields due to a combination of climatic factors; agronomical practices such as crop rotation, tillage practices and the amount and type of stubble; host genotype; and disease management practices, which include host resistance and chemical control [10, 40–44]. Consequently, the *Fusarium* species occurring on wheat in a country or region can change in response to these drivers. For instance, *F. avenaceum*, *F. culmorum* and *F. poae* were historically common in the colder regions of northern Europe, but the FGSC has become more dominant in some of these regions in recent years, believed to be due to an increase in maize production and climate change [11, 25, 45]. In Italy, on the other hand, *F. graminearum* is replaced by *F. poae*, believed to be due to variation in environmental conditions [46]. Furthermore, the homothallic nature of the FGSC allows for the mass production of ascospores, which may aid in the epidemiology of the disease [47].
The commercial release of new spring wheat cultivars able to complete its life cycle in a shorter period than older cultivars enabled producers in the irrigation regions to sequentially cultivate crops like wheat in the winter and maize in the summer on the same fields, a practice known as double-cropping [48]. Irrigation practices like flood-irrigation have also been replaced with centre-pivot irrigation, which creates a more suitable microclimate for FHB development [49]. Conservation agriculture, which includes minimum / no-tillage practices and crop rotation with barley, oats and broad-leaf crops like canola replaced conventional tillage and monoculture in the Western Cape [34, 50]. All of these practices may have impacted the occurrence of FHB and associated *Fusarium* species in South Africa. With the availability of powerful molecular techniques, numerous new *Fusarium* species and species complexes were described [51]. These techniques facilitated reassessments of species identity among isolates from major reference collections [52] and enabled accurate identification of fusaria via curated databases of DNA sequence data [53]. With the exception of one study, which focused only on the FGSC [19], all previous studies of *Fusarium* species associated with FHB of wheat in South Africa described isolates using morphological characteristics only. Previous surveys, furthermore, collected diseased wheat heads from only a limited number of localities within large and geographically diverse production regions. As such, there is a need to have both an updated and phylogenetically broader understanding of FHB pathogen diversity within South Africa. The aim of this study, therefore, was to conduct a survey to determine the identity, distribution and type B trichothecene chemotype of *Fusarium* species associated with FHB of wheat in South Africa.

**Materials and methods**

**Wheat production areas in South Africa**

Spring wheat cultivars are grown under dryland conditions in the Western Cape Province and under centre-pivot irrigation in the summer rainfall irrigation regions and Free State Province. Winter wheat is grown under dryland (rain-fed) conditions in the Free State Province (Fig 1). The Western
Cape Province is the largest production area in the country, although it yields less grain ha\(^{-1}\) than the irrigation areas. There are two production regions in the Western Cape, namely the Swartland (western) and the Overberg (southern) regions. Regions within the summer rainfall irrigation area differ greatly in terms of climate and soil type. It consists of the Bushveld; which comprises parts of Gauteng, Limpopo, Mpumalanga and the North West Province; the eastern part of the Free State Province (referred to hereafter as the Free State); parts of KZN; and areas in the Northern Cape Province in the vicinity of the Orange-, Vaal- and Modder River (referred to hereafter as the Northern Cape).

**Collection of wheat heads**

**Irrigation production areas**

Wheat heads with FHB symptoms were collected at 15 localities in the Free State, KZN and the Northern Cape during 2008, and at 14 localities in the Bushveld, Free State, KZN, and Northern Cape during 2009 (Fig 1; Table 1). Collections were done in the irrigation spring wheat cultivar evaluation trials of the Agricultural Research Council’s Small Grain (ARC-SG). A total of 20 samples were collected from each of four cultivars (Baviaans, Duzi, Kariega and PAN3434), which were planted in a randomised block design with four replicates, to provide 80 samples per locality. Additionally, 40 symptomatic wheat heads were sampled randomly from one locality each in the Free State in 2008 (Frankfort), and KZN in 2009 (Greytown). The former was from a commercial wheat field, where collections were made from the spring cultivar SST835, while the latter was from a field demonstration trial from a local seed company, where collections were made from the spring cultivar PAN3434.

**Western Cape Province**

The dryland spring wheat cultivar evaluation trials of the ARC-SG were inspected each year at three localities each in the Overberg and Swartland production regions of the Western Cape Province (dryland production) for the presence of FHB, but no visible disease was found. Visible
FHB symptoms was, however, found in a commercial dryland wheat field (Vissershok) during 2009, where 40 symptomatic wheat heads were randomly sampled from the spring cultivar SST027.

Table 1. Production region, cultivar, crop history and geographical information of localities where wheat heads with Fusarium head blight symptoms were sampled.

| Sampling year | Production region | Locality     | Previous crop | GPS coordinates                      | Elevation (m) |
|---------------|-------------------|--------------|---------------|--------------------------------------|---------------|
| 2008          | Free State        | Bethlehem    | Fallow        | 28.161474° S 28.304801° E            | 1643          |
|               |                   | Frankfort    | Maize         | 27.178925° S 28.405266° E            | 1489          |
|               |                   | Ladybrand    | Cabbage       | 29.184615° S 27.553087° E            | 1536          |
|               |                   | Villiers     | Maize         | 27.038234° S 28.662245° E            | 1528          |
|               | KwaZulu-Natal     | Bergville    | Soybean       | 28.753716° S 29.342924° E            | 1127          |
|               |                   | Winterton    | Maize         | 28.885823° S 29.471612° E            | 1119          |
|               |                   | Dundee       | Maize         | 28.104323° S 30.262652° E            | 1214          |
|               | Northern Cape     | Douglas      | Groundnut     | 29.012244° S 23.960668° E            | 1006          |
|               |                   | Hartswater   | Maize         | 27.795774° S 24.779891° E            | 1105          |
|               |                   | Modderrivier | Maize         | 29.104098° S 24.579221° E            | 1128          |
|               |                   | Prieska      | Maize         | 29.609747° S 22.856582° E            | 938           |
|               |                   | Orania 1     | Maize         | 29.790727° S 24.423845° E            | 1098          |
|               |                   | Orania 2     | Maize         | 29.881946° S 24.585153° E            | 1123          |
|               |                   | Remhoogte    | Maize         | 29.537154° S 22.993977° E            | 980           |
|               |                   | Vaalharts    | Maize         | 27.965495° S 24.836811° E            | 1176          |
| 2009          | Bushveld          | Brits        | Sunflower     | 25.593013° S 27.768504° E            | 1087          |
|               |                   | Groblersdal  | Peppers       | 25.178062° S 29.389781° E            | 936           |
|               |                   | Koedoeskop   | Soybean       | 25.011394° S 27.562786° E            | 955           |
|               |                   | Marble Hall  | Cabbage       | 25.041904° S 29.221597° E            | 927           |
|               | Free State        | Bethlehem    | Soybean       | 28.161434° S 28.305021° E            | 1643          |
Ladybrand  Cabbage  29.181017° S 27.556070° E  1545
KwaZulu-Natal  Dundee  Maize  27.984337° S 30.349992° E  1168
Greytown  Fallow  29.084172° S 30.603934° E  1028
Newcastle  Soybean  27.643130° S 29.979236° E  1192
Winterton  Soybean  28.839872° S 29.467234° E  1097
Northern Cape  Barkly West  Onions  28.507932° S 24.593006° E  1109
Bull Hill  Maize  28.048725° S 24.579655° E  1060
Hopetown  Maize  29.636905° S 24.176112° E  1071
Remhoogte  Maize  29.538249° S 22.969875° E  961
Western Cape  Vissershok  Canola\textsuperscript{b}  33.785813° S 18.555610° E  12

\textsuperscript{a} Crop grown during the previous summer growing season, except where indicated
\textsuperscript{b} Crop grown during the previous winter growing season (summer fallow)

**Isolations from diseased kernels**

For the first year, two visually scabby kernels per sample were surface-disinfected by washing in 70% ethanol for 1 min, followed by 1 min in a 1% sodium hypochlorite solution. The kernels were then rinsed with sterile distilled water and left to air dry in the laminar flow cabinet on sterile tissue paper. One kernel was plated onto potato dextrose agar (PDA) (Biolab Diagnostics, Midrand, South Africa) amended with 40 mg L\textsuperscript{-1} streptomycin sulphate, and the other kernel onto selective *Fusarium* agar (SFA) [54]. During the second year, only one visually scabby kernel per sample was surface-disinfected as described above, and isolated onto PDA only.

Plates were incubated for 5 days at 21˚C in the dark. Developing *Fusarium* colonies were purified and single-spored. Single-spore cultures were plated onto PDA to harvest mycelium for DNA extraction, and onto divided plates containing PDA amended with 40 mg L\textsuperscript{-1} streptomycin sulphate and carnation leaf agar (CLA) [54], which was incubated underneath cool white and near-UV lights with a photoperiod of 12 hrs for 21 days, for morphological identification [54]. Single-spore cultures were stored in 15% glycerol at -80˚C at the Department of Plant Pathology, Stellenbosch University, South Africa.
**Fungal reference cultures**

Reference isolates of *F. graminearum* (NRRL28439) and *F. culmorum* (NRRL3288) were obtained from Dr K. O'Donnell (USDA-ARS Peoria, IL, USA), while isolates of *F. avenaceum* (MRC 3227) and *F. poae* (MRC 8486) were provided by Prof W.F.O Marasas (MRC-PROMEC, Tygerberg, South Africa). The reference isolates for *F. cerealis* (MRC 8399; CAV359) and FIESC (MRC 1813; CAV367) were provided by Prof A. Viljoen (Department of Plant Pathology, Stellenbosch University, South Africa), while the positive control for *F. pseudograminearum* (WCA3532) was obtained in this study following identification with multilocus genotyping (MLGT) by Dr T.J. Ward (USDA-ARS Peoria, IL, USA).

Isolates with known type B trichothecene chemotype identities were provided by Laëtitia Pinson-Gadais (French National Institute for Agricultural Research, Villenave d’Ornon, France). These included *F. culmorum* with a 3-ADON chemotype (INRA 233), FGSC with a 15-ADON chemotype (INRA 156) and FGSC with a NIV chemotype (INRA 91). These isolates served as positive controls in PCR assays to determine the chemotype of B-trichothecene isolates obtained in this study.

**Identification of Fusarium isolates**

Single-spore cultures were grown on PDA plates for 7 days, where after genomic DNA was extracted from mycelia using the Wizard® SV Genomic DNA Purification System Kit (Promega, South Africa). Isolates of *F. avenaceum*, *F. culmorum*, FGSC and *F. poae* were identified in a multiplex PCR with known species-specific primers (Table 2). Amplifications were carried out with an initial denaturation step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 30 s and extension at 72°C for 45 s, with a final extension step of 72°C for 5 min [22]. Isolates of *F. cerealis* and *F. pseudograminearum* were also identified by PCR with species-specific primers listed in Table 2, using the same reaction conditions [22]. Isolates that did not generate PCR products were identified by sequencing of the translation elongation factor-1 alpha (*EF-1α*) gene [55]. These included members of the FGSC. The *EF-1α* gene was amplified in a PCR...
reaction that consisted of an initial denaturation step of 94°C for 5 min, followed by 30 cycles of
denaturation at 94°C for 30 s, primer annealing at 57°C for 45 s and primer extension at 72°C for 1 min, followed by a final extension of 72°C for 7 min [22]. Generated EF-1α products were purified and sequenced, and edited sequences were compared to sequences available on the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/genbank/), the CBS-KNAW Fungal Biodiversity Centre’s Fusarium MLST website (http://www.cbs.knaw.nl/Fusarium), and the FUSARIUM-ID database [56]. Isolates with less than 99% sequence similarity to reference sequences in Fusarium MLST were annotated as unknown species (F. sp.) and may represent novel species-level diversity. If the same Fusarium species was obtained from the two kernels of the same sample on the different growing media, only one isolate was selected to represent the sample. DNA sequence data generated for 45 isolates from FHB in South Africa have been deposited in GenBank under accession numbers MG588054 – MG588069, MG588071 – MG588087, MK617767 – MK617769, and MK629641 – MK629649. The identification of FIESC isolates by EF-1α gene sequencing were confirmed via PCR with species-specific primers listed in Table 2. Amplifications were carried out with an initial denaturation step at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 60°C for 30 s and extension at 72°C for 1 min, with a final extension step of 72°C for 2 min [22].
Table 2. Primer names, sequences and expected sizes of PCR products of *Fusarium* species associated with head blight of wheat.

| Target                          | Primer | Primer sequence (5’ – 3’)                          | Annealing T (°C) | Amplicon size (bp) | Reference |
|--------------------------------|--------|----------------------------------------------------|------------------|-------------------|-----------|
| *F. avenaceum*                 | FaF    | CAAGCATTGTCGCCACTCTC                                | 60               | 920               | [57]      |
|                                | FaR    | GTTTGGCTCTACCGGGACTG                                |                  |                   |           |
| *F. cerealis*                  | CroAF  | CTCAGTGTCCACCACGGTTGCGTAG                          | 60               | 842               | [58]      |
|                                | CroAR  | CTCAGTGTCCCAATCAATAGTAGG                           |                  |                   |           |
| *F. culmorum*                  | Fc01F  | ATGGTGAACTCGTGCGTTGGC                               | 60               | 570               | [59]      |
|                                | Fc01R  | CCCCCACGCAATGCGAGTC                                 |                  |                   |           |
| FGSCa                          | Fg11F  | CTCCGGATATGTTGCGTCAA                                | 60               | 450               | [59]      |
|                                | Fg11R  | GGTAGGTATCCGACATTGGCA                               |                  |                   |           |
| FIESCb                         | FeF1   | CATACCTATACGTTGCGTCC                                | 60               | 400               | [60]      |
|                                | FeR1   | TTACACGTAACGAGGTGTATG                               |                  |                   |           |
| *F. poae*                      | Fp82F  | CAAGCAAACAGGCTCACC                                 | 60               | 220               | [61]      |
|                                | Fp82R  | TGCTCCACCTCAGTGACAGGTT                              |                  |                   |           |
| *F. pseudograminearum*         | FpgF   | GTCGCCGTCACTATC                                     | 60               | 779               | [62]      |
|                                | FpgR   | CACTTTATCTCTGTTGCAG                                 |                  |                   |           |
| EF1α                           | EF1    | ATGGGTAAGGA(A/G)GACAAGAC                            | 57               | 648               | [55]      |
|     |     | ![Sequence](https://example.com/sequence.png) |   |     |
|-----|-----|-----------------------------------------------|---|------|
| EF2 | GGA(G/A)GTACCAGT(G/C)ATCATGTT | TRI3 | 3CON | TGGCAAGACTGGTTCAC | 58 | 243 (3-ADON) |
|     |     | 3NA | GTGCACAGAATAATACGAGC | 610 | 15-ADON |
|     |     | 3D15A | ACTGACCAGCTGCATC | 840 | NIV |
|     |     | 3D3A | CGCATTGGCTAACAATG |     |     |

*a* *Fusarium graminearum* species complex

*b* *Fusarium incarnatum–equiseti* species complex
The identities of a representative group of type B trichothecene-producing isolates were confirmed at the USDA-ARS (Peoria, IL, USA) using a multilocus genotyping assay (MLGT) [63]. These included *F. cerealis* (five isolates), *F. culmorum* (one isolate), *F. pseudograminearum* (two isolates), *Fusarium lunulosporum* (three isolates), while 277 isolates of the FGSC obtained in this study were previously identified [19]. Of the 277 FGSC isolates, 85.2% were identified as *F. graminearum* s.s. An additional 32 FGSC isolates were also identified at the USDA-ARS (Peoria, IL, USA) as *F. boothii*, *F. graminearum* s.s. and *F. meridionale* using the MLGT assay. In the current study, FGSC isolates were only identified using the FGSC-specific primer-pair mentioned earlier [59], and the FGSC species will therefore not be referred to by their phylogenetic species names. The molecular identities of FHB isolates from South Africa were linked to prior morphological species definitions by studying the morphology of 85 isolates representing all fusaria obtained [54]. The identity of six isolates could not be determined morphologically. These included three isolates of *F. transvaalense*, one isolate of *F. brachygibbosum*, and one isolate of an unknown *Fusarium* species (FSAMSC), as well as one isolate of *F. temperatum* (FFSC).

**Chemotype identification**

The NIV, 3-ADON and 15-ADON chemotypes of FGSC and related species within clade 1 of the FSAMSC (FSAMSC-10) [64] were identified using a multiplex PCR that amplified portions of the *Tri3* gene [63] (Table 2). The PCR reaction conditions consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer-annealing at 58°C for 30 s and extension at 72°C for 30 s, with a final extension step of 72°C for 5 min.

**Results**

**Fusarium species**

A total of 1047 *Fusarium* isolates were identified in this study (S1 Table), which included 24 *Fusarium* species from seven major *Fusarium* species complexes (Table 3). The FSAMSC
accounted for 83.5% of all isolates, with most FSAMSC isolates belonging to the FGSC subgroup. The FGSC comprised 69.1% ($n = 439$) of *Fusarium* isolates obtained in 2008 and 85.9% ($n = 354$) in 2009. The FIESC accounted for 13.3% of all isolates, and the other species complexes (FFSC, FTSC, FOSC, FCSC, and FSSC) each accounted for less than 1.5% of the FHB isolates. Due to the use of clade-specific primers in this study, 487 of the 874 FSAMSC isolates were identified only to the level of the FGSC. However, 14 named species were identified among the remaining 387 FSAMSC isolates. Among the FCSC, we identified two informally named species (FCSC 1 and FCSC 5). Among the FFSC, we identified *F. subglutinans*, *F. temperatum*, and *F. verticillioides*. *F. oxysporum* and the informally named species FSSC 5 were identified from the FOSC and FSSC respectively. *F. acuminatum* and *F. avenaceum* were identified from the FTSC. All but two of the 139 FIESC isolates were identified with clade-specific primers that did not permit species level identification. The two FIESC isolates identified via DNA sequence analyses, as well as 11 additional isolates from the other species complexes lacked similarity to reference sequences in the Fusarium MLST database sufficient for species identification (F. sp) and may represent novel species diversity (Table 3).
| Locality / region | FCSC<sup>a</sup> | FFSC<sup>b</sup> | FIESC<sup>c</sup> | FOSC<sup>d</sup> | FSAMSC<sup>e</sup> | FSSC<sup>f</sup> | FTSC<sup>g</sup> | Total<sup:h</sup> |
|------------------|----------------|---------------|----------------|---------------|----------------|-------------|-------------|--------------|
|                  | FCSC | FCSC | F. sp. | sub | temp | vert | F. sp. | oxy | F. sp. | arm | bra | cer | cul | FGSC | lun | poae | pse | tvl | F. sp. | FSSC | acu | ave | F. sp. |
| **2008**         |      |      |        |     |      |      |        |     |        |     |     |     |     |      |     |      |     |     |        |     |     |      |        |
| Free State (FS)  |      |      |        |     |      |      |        |     |        |     |     |     |     |      |     |      |     |     |        |     |     |      |        |
| Bethlehem        | 0    | 0    | 0      | 0   | 0    | 0    | -      | 27  | 0      | 1   | -   | -   | 23  | 3    | 0   | 0    | 0   | 39  | 0      | 0   | 3   | 0   | 51    |
| Frankfort        | 0    | 0    | 0      | 0   | 0    | 0    | -      | 0   | 0      | 0   | -   | -   | 8   | 0    | 91  | 0    | 0   | 0   | 0      | 0   | 0   | 0   | 24    |
| Ladybrand        | 0    | 0    | 0      | 0   | 0    | 0    | -      | 66  | 0      | 2   | -   | -   | 4   | 2    | 13  | 0    | 0   | 0   | 0      | 0   | 0   | 0   | 45    |
| Villiers         | 0    | 0    | 0      | 0   | 0    | 0    | -      | 6   | 0      | 0   | -   | -   | 0   | 90   | 0   | 0    | 0   | 0   | 0      | 1   | 0   | 0   | 80    |
| **Total FS**     | 0    | 0    | 0      | 0   | 0    | 0    | -      | 24  | 0      | 1   | -   | -   | 8   | 1    | 50  | 0    | 0   | 10  | 0      | 1   | 0   | 0   | 200   |
| KwaZulu-Natal (KZN) |      |      |        |     |      |      |        |     |        |     |     |     |     |      |     |      |     |     |        |     |     |      |        |
| Bergville        | 0    | 0    | 0      | 0   | 0    | 0    | -      | 5   | 0      | 0   | -   | -   | 0   | 0    | 86  | 0    | 2   | 0   | 0      | 0   | 2   | 0   | 36    |
| Dundee           | 0    | 0    | 0      | 0   | 0    | 0    | -      | 9   | 0      | 0   | -   | -   | 0   | 90   | 0   | 0    | 0   | 0   | 0      | 0   | 0   | 0   | 32    |
| Winterton        | 0    | 0    | 0      | 0   | 0    | 0    | -      | 36  | 0      | 0   | -   | -   | 0   | 63   | 0   | 0    | 0   | 0   | 0      | 0   | 0   | 0   | 11    |
| **Total KZN**    | 0    | 0    | 0      | 0   | 0    | 0    | -      | 11  | 0      | 0   | -   | -   | 0   | 84   | 0   | 1    | 0   | 0   | 0      | 0   | 1   | 1   | 79    |
| Northern Cape (NC) |      |      |        |     |      |      |        |     |        |     |     |     |     |      |     |      |     |     |        |     |     |      |        |
| Douglas          | 0    | 0    | 0      | 0   | 0    | 0    | -      | 30  | 0      | 0   | -   | -   | 0   | 57   | 0   | 0    | 7   | 0   | -      | 2   | 0   | 0   | 40    |
| Hartswater       | 0    | 0    | 0      | 0   | 0    | 0    | -      | 12  | 0      | 0   | -   | -   | 0   | 62   | 0   | 0    | 0   | 0   | 0      | 0   | 12  | 0   | 8     |
| Modderrivier     | 0    | 0    | 0      | 0   | 0    | 0    | -      | 53  | 0      | 0   | 6   | -   | 0   | 88   | 0   | 0    | 0   | 0   | 0      | 0   | 6   | 0   | 15    |
| Orania 1         | 1    | 0    | 0      | 0   | 0    | 0    | -      | 5   | 0      | 0   | -   | -   | 0   | 94   | 0   | 0    | 0   | 1   | -      | 0   | 0   | 1   | 75    |
| Orania 2         | 0    | 0    | 0      | 0   | 0    | 0    | -      | 3   | 0      | 0   | -   | -   | 0   | 90   | 0   | 0    | 0   | 0   | 1      | 0   | 0   | 0   | 55    |
| Prieska          | 0    | 0    | 0      | 0   | 0    | 0    | -      | 17  | 0      | 0   | -   | -   | 0   | 75   | 5   | 0    | 0   | 0   | 0      | 0   | 0   | 1   | 52    |
| Remhooge         | 0    | 0    | 0      | 0   | 0    | 0    | -      | 1   | 0      | 0   | 0   | -   | 0   | 98   | 0   | 0    | 0   | 0   | 0      | 0   | 0   | 0   | 59    |
| Vaalharts        | 0    | 0    | 0      | 0   | 0    | 0    | -      | 38  | 0      | 0   | -   | -   | 0   | 55   | 0   | 0    | 0   | 0   | 0      | 0   | 0   | 1   | 52    |
| **Total NC**     | 0.3  | 1    | 0.3    | 0   | 0    | 0    | -      | 16  | 0.3    | 0   | 0   | -   | 0   | 76   | 0.8 | 0    | 0.8 | 0.8 | -      | 0.3 | 0.3 | 0.8 | 0.8 | 356 |
| **Total 2008+**  | 1    | 5    | 1      | 3   | 1    | 1    | -      | 115 | 1      | 2   | 1   | -   | 16  | 3    | 439 | 3    | 1    | 23  | 3      | 2   | 2   | 10  | 3    | 635 |
| **2009**         |      |      |        |     |      |      |        |     |        |     |     |     |     |      |     |      |     |     |        |     |     |      |        |

Table 3. Incidence<sup>a,b</sup> of *Fusarium* isolates obtained from diseased wheat heads in South Africa during 2008 and 2009.
|          | Vissershok | Bull Hill | Barkly West | Total KZN | Greytown | Bethlehem | Ladybrand | Total FS | Total KZN | Northern Cape | Total NC | Western Cape (WC) | Total 200 |
|----------|------------|-----------|-------------|-----------|----------|-----------|-----------|----------|-----------|--------------|----------|------------------|-----------|
| Brits    | -          | -         | 2           | 5         | 0        | 0         | 0         | 7        | 0         | -            | 0        | -                | 1         |
| Groblersdal | -          | -         | 0           | 0         | 0        | 1         | 0         | 0        | 1         | -            | 0        | -                | 1         |
| Koedoeskop | -          | -         | 0           | 0         | 0        | 7         | 0         | 0        | 1         | -            | 0        | -                | 1         |
| Marble Hall | -          | -         | 0           | 0         | 0        | 2         | 0         | 0        | 0         | -            | 0        | -                | 1         |
| Total Bushveld | 0.5       | 0         | 9           | 0         | 0        | 1         | 0         | 89       | 0         | 0           | 0        | -                | 200       |
| Free State |            |           |             |           |          |           |           |          |           |              |          |                  |           |
| Bethlehem | -          | -         | 0           | 0         | -        | 0         | 0         | 11       | -         | 11           | 0        | -                | 9         |
| Ladybrand | -          | -         | 0           | 0         | 20       | -         | 0         | 0        | 0         | -            | 0        | -                | 5         |
| Total FS  | 0          | 0         | 7           | 0         | 0        | 7         | 0         | 35       | 0         | 50           | 0        | -                | 14        |
| KZN      |            |           |             |           |          |           |           |          |           |              |          |                  |           |
| Dundee   | -          | -         | 0           | -        | 0        | 0         | 0         | 100      | 0         | 0           | 0        | -                | 29        |
| Greytown | -          | -         | 0           | 0         | 4        | 13        | 0         | 0        | 0         | -            | 0        | -                | 22        |
| Newcastle | -          | -         | 0           | 0         | 5        | -         | 0         | 0        | 0         | -            | 0        | -                | 20        |
| Winterton | -          | -         | 0           | 0         | 0        | -         | 0         | 0        | 0         | -            | 0        | -                | 16        |
| Total KZN | 0          | 1         | 4           | 0         | 0        | 0         | 0         | 87       | 3         | 0           | 3        | -                | 87        |
| Northern Cape |          |           |             |           |          |           |           |          |           |              |          |                  |           |
| Barkly West | -         | -         | 0           | -        | 0        | 0         | 0         | 0        | 0         | -            | 0        | -                | 52        |
| Bull Hill | -          | -         | 0           | -        | 0        | 0         | 0         | 0        | 3         | -            | 0        | -                | 33        |
| Hopetown | -          | -         | 0           | -        | 0        | 20        | -         | 0        | 0         | -            | 0        | -                | 5         |
| Remhoogte | -          | -         | 0           | -        | 0        | 0         | 0         | 0        | 100       | 0            | 0        | -                | 2         |
| Total NC  | 0          | 0         | 1           | 0         | 0        | 1         | 97        | 0        | 0         | 0            | 0        | -                | 92        |
| Western Cape (WC) |          |           |             |           |          |           |           |          |           |              |          |                  |           |
| Vissershok | -          | -         | 0           | -        | 0        | 0         | 0         | 0        | 0         | -            | 26       | -                | 73        |
| Total WC  | -          | -         | 0           | -        | 0        | 0         | 0         | 0        | 0         | -            | 26       | -                | 73        |
| Total 200 | -          | -         | 1           | -        | 1        | 24        | -         | 1        | 2         | 2            | 354      | -                | 412       |

| 289 | Incidence for a locality was calculated as follows: (number of isolates of a *Fusarium* species obtained at a locality / number of *Fusarium* isolates obtained at the locality) x 100 |
| 290 |
Incidence for a production region was calculated as follows: (number of isolates of a *Fusarium* species obtained in a production region / number of *Fusarium* isolates obtained in the production region) x 100

FCSC = *Fusarium chlamydosporum* species complex: FCSC 1, FCSC 5 = *F. chlamydosporum* clade 1 and clade 5 (O'Donnell *et al.*, 2009); F. sp. = unknown *Fusarium* species within the FCSC

FFSC = *Fusarium fujikuroi* species complex: sub = *F. subglutinans*; temp = *F. temperatum*; vert = *F. verticillioides*; F. sp. = unknown *Fusarium* species within the FFSC

FIESC = *Fusarium incarnatum-equiseti* species complex

FOSC = *Fusarium oxysporum* species complex: oxy = *Fusarium oxysporum*; F. sp. = unknown *Fusarium* species within the FOSC

FSAMSC = *Fusarium sambucinum* species complex: arm = *F. armeniacum*; bra = *F. brachygibbosum*; cer = *F. cerealis*; cul = *F. culmorum*; FGSC = *Fusarium graminearum* species complex, species observed include *F. graminearum*, *F. boothii*, *F. acaciae-mearnsii*, *F. brasilicum*, and *F. cortaderiae*

FSAMSC = *Fusarium sambucinum* species complex: arm = *F. armeniacum*; bra = *F. brachygibbosum*; cer = *F. cerealis*; cul = *F. culmorum*; FGSC = *Fusarium graminearum* species complex, species observed include *F. graminearum*, *F. boothii*, *F. acaciae-mearnsii*, *F. brasilicum*, and *F. cortaderiae*

FSSC = *F. solani* species complex: FSSC 5 = *F. solani* clade 5 (O'Donnell *et al.*, 2008; Zhang *et al.*, 2006)

FTSC = *Fusarium tricinctum* species complex: acu = *F. acuminatum*; ave = *F. avenaceum*; F. sp. = unknown *Fusarium* species within the FTSC

Total number of *Fusarium* isolates

*Fusarium* species not obtained in specific year
Considerably more *Fusarium* isolates were obtained in 2008 than in 2009, even though the same number of diseased wheat heads were collected in both years. This can be attributed to the high incidence of *Alternaria* isolates found in wheat kernels at localities like Brits (Bushveld), Remhoogte, Bull Hill and Hopetown (Northern Cape), KZN, the Free State and the Western Cape in 2009 (data not presented). Many symptomatic kernels from these regions yielded no *Fusarium* isolates during 2009. All members of the FCSC and FTSC, *F. temperatum* , *F. verticillioides*, *F. oxysporum*, *F. armeniacum*, *F. culmorum*, *F. lunulosporum*, *F. transvaalense*, and FSSC 5 were collected only in 2008. *F. subglutinans*, the *F*. sp. isolate from the FFSC, *F. brachygibbosum*, and the *F*. sp. isolates from the FSAMSC were collected only in 2009 (Table 3).

Co-occurrence of *Fusarium* species (when more than one *Fusarium* species was obtained from the same wheat head or same kernel), was very low and occurred only in 0.9% of all isolations performed. Of these cases, the FIESC co-occurred 48.4% (*n* = 16) with the FGSC, and 16.1% (*n* = 5) with other fusaria.

**Geographical distribution of *Fusarium* species**

The FGSC and FIESC were the most widely distributed fusaria in South Africa (Fig 1). The FGSC was found in all production regions and localities, apart from Modderrivier (Northern Cape) and Bethlehem (Free State) in 2008 (Table 3). *Fusarium pseudograminearum* was predominant at the one locality in the Western Cape. The incidence of the FGSC was highest at Remhoogte (98.3%) and Orania 2 (94.6%) in the Northern Cape, at Frankfort (91.7%) and Villiers (90%) in the Free State, and at Dundee (90.6%) in KZN in 2008. In 2009, its incidence was highest at Dundee and Winterton in KZN, and Barkly West in the Northern Cape, all at 100%, followed by Groblersdal (98.4%) and Marble Hall (98%) in the Bushveld. Ladybrand and Bethlehem in the Free State had the lowest incidence of the FGSC in 2008 and 2009, at 13.3% and 11.1% respectively (Table 3). In 2009, Remhoogte had an incidence of 100%, compared to 98.3% in 2008, although a total of 58 *Fusarium* isolates were obtained there in 2008 compared to just two isolates in 2009 (S1 Table).
FSAMSC:
FGSC
F. pseudograminearum
Other FSAMSC
FCSC
FFSC
FIESC
FOSC
FSSC
FTSC

n = 200
Bushveld

n = 448
Northern Cape

n = 19
Western Cape

n = 166
KwaZulu-Natal

n = 214
Free State
Fig 1. Geographical distribution of *Fusarium* species obtained from diseased wheat heads in South Africa during 2008 and 2009, according to the total number of *Fusarium* isolates obtained (n).

Bushveld: a = Marble Hall, b = Groblersdal, c = Brits, d = Koedoeskop; Free State: e = Bethlehem, f = Frankfort, g = Ladybrand, h = Villiers; KwaZulu-Natal, i = Bergville, j = Dundee, k = Greytown, l = Newcastle, m = Winterton; Northern Cape: n = Barkly West, o = Bull Hill, p = Douglas, q = Hartswater, r = Hopetown, s = Modderriver, t = Orania 1, u = Orania 2, v = Prieska, w = Remhoogte, x = Vaalharts; Western Cape: y = Vissershok.

FSAMSC = *F. sambucinum* species complex. FGSC = *Fusarium graminearum* species complex, species observed include *F. graminearum*, *F. boothii*, *F. meridionale*, *F. acaciae-mearnsii*, *F. brasilicum*, and *F. cortaderiae*.

Other FSAMSC = members of the FSAMSC other than FGSC and *F. pseudograminearum*: *F. armeniacum*, *F. brachygibbosum*, *F. cerealis*, *F. culmorum*, *F. lunulosporum*, *F. poae*, and unknown *Fusarium* species within the FSAMSC.

FCSC = *Fusarium chlamydosporum* species complex: *F. chlamydosporum* clade 1 and clade 5 (O'Donnell et al., 2009), and unknown *Fusarium* species within the FCSC.

FFSC = *Fusarium fujikuroi* species complex: *F. subglutinans*, *F. temperatum*, *F. verticillioides*, and unknown *Fusarium* species within the FFSC.

FIESC = *Fusarium incarnatum-equiseti* species complex

FOSC = *Fusarium oxysporum* species complex: *Fusarium oxysporum*, and unknown *Fusarium* species within the FOSC.

FSSC = *F. solani* species complex: *F. solani* clade 5 (O'Donnell et al., 2008; Zhang et al., 2006).

FTSC = *Fusarium tricinctum* species complex: *F. acuminatum*, *F. avenaceum*, and unknown *Fusarium* species within the FTSC.
The FIESC was obtained in all wheat production regions except the Western Cape. The fungus was not isolated at Frankfort (Free State) in 2008, and at several localities in 2009, including Bethlehem (Free State), Marble Hall (Bushveld), Dundee and Winterton (KZN), and Barkly West, Bull Hill and Remhoogte (Northern Cape) (Table 3). In the Free State, KZN, and the Northern Cape, FIESC comprised 24.5, 11.4 and 16% of isolates collected in 2008, respectively (Table 3). In 2009, the FIESC comprised 9, 7.1, 4.6 and 1.1% of isolates collected in the Bushveld, Free State, KZN and the Northern Cape, respectively. The highest incidence in 2008 was obtained at Ladybrand in the Free State (66.7%), followed by Modderrivier (53.3%) and Vaalharts (38.5%) in the Northern Cape (Table 3). The incidence of the FIESC was substantially reduced in 2009, with the highest incidence found at Brits in the Bushveld (38.2%). Where present, the lowest incidence of the FIESC in 2008 was at Remhoogte in the Northern Cape (1.7%), and at Groblersdal in the Bushveld (1.6%) in 2009 (Table 3).

The Northern Cape was the wheat production region with the highest FHB species diversity, with 16 *Fusarium* species from all seven species complexes obtained there (Fig 1). Ten *Fusarium* species from six species complexes were collected from wheat in the Free State, and seven *Fusarium* species from four species complexes in KZN. Five *Fusarium* species from four species complexes were obtained from wheat in the Bushveld, while only two species from one species complex (FSAMSC) were obtained at the locality in the Western Cape (Fig 1).

*Fusarium* species other than those in the FGSC and FIESC were mostly obtained at low incidences (Table 3). The members of the FCSC, *F. oxysporum* within the FOSC, *F. armeniacum*, *F. lunulosporum*, *F. transvaalense* and an unknown *Fusarium* species within the FTSC were obtained only in the Northern Cape in 2008. *Fusarium temperatum* and FSSC 5 were obtained only in the Free State and Northern Cape in 2008, while *F. verticillioides* and *F. culmorum* were obtained only in the Free State in 2008. *Fusarium acuminatum* and *F. avenaceum* was obtained from KZN and the Northern Cape, and from the Free State, KZN and Northern Cape in 2008 respectively. *Fusarium subglutinans* and *F. brachygibbosum* were only obtained in 2009 in the Bushveld, while unknown *Fusarium* spp. within the FFSC and FSAMSC were obtained only in 2009 in KZN. An unknown *Fusarium* sp. within the FOSC was obtained in the Free State in 2008, and from the Bushveld in 2009. *Fusarium cerealis* was obtained from the Free State in 2008 and 2009, and from
the Northern Cape in 2009 only, while *F. poae* was only obtained in KZN, where it occurred both years. *Fusarium pseudograminearum* was obtained both years in the Free State, and in the Northern and Western Cape during 2009 (Table 3).

The greatest species diversity at individual localities in 2008 was found at Ladybrand (Free State) and Orania 1 (Northern Cape) with seven species each, and the lowest at Dundee and Winterton (KZN), Frankfort (Free State), and Remhoogte (Northern Cape), with two species each. In 2009, the highest species diversity was at Greytown (KZN) with five species, and the lowest at Dundee and Winterton (KZN), and Barkly West and Remhoogte (Northern Cape), where only the FGSC was obtained (Table 3).

**Type B trichothecene chemotype**

The B-trichothecene chemotypes of 861 isolates from FSAMSC-1 were assessed via a chemotype-specific PCR assay. 15-ADON was the dominant type (90.1%) associated with these *Fusarium* isolates collected in South Africa (S1 Table). Isolates with the 3-ADON and NIV types comprised 5.4 and 4.5%, respectively, of the FSAMSC-1 isolates in the country. The 15-ADON type was only observed among the FGSC, where it was predominant (97.4%). Less than 0.5% of this species complex had the 3-ADON type, while 2.3% had the NIV type (S1 Table). *Fusarium cerealis* and *F. lunulosporum* were exclusively of the NIV type, and *F. culmorum* and *F. pseudograminearum* were exclusively of the 3-ADON type (Fig 2).
B-trichothecene chemotype:
- 3-ADON
- 15-ADON
- NIV
- No B-trichothecene chemotype detected

Northern Cape

- *F. cerealis*
  - 3-ADON: 1
  - 15-ADON: 362

- *F. lunulosporum*
  - NIV: 3

- *F. pseudograminearum*
  - NIV: 178

Bushveld

- *F. cerealis*
  - 3-ADON: 17
  - 15-ADON: 3

- *F. culmorum*
  - NIV: 105

Free State

- *F. cerealis*
  - 3-ADON: 17
  - 15-ADON: 3

- *F. pseudograminearum*
  - NIV: 26

Western Cape

- *F. pseudograminearum*
  - 3-ADON: 5
  - 15-ADON: 11

KwaZulu-Natal

- *F. pseudograminearum*
  - 3-ADON: 143

No B-trichothecene chemotype detected detected.
Fig 2. Incidence of type B trichothecene chemotypes of different members of the *Fusarium sambucinum* species complex (FSAMSC) obtained from diseased wheat heads in South Africa during 2008 and 2009, according to the total number of FSAMSC isolates obtained (*n*).

Bushveld: a = Marble Hall, b = Groblersdal, c = Brits, d = Koedoeskop; Free State: e = Bethlehem, f = Frankfort, g = Ladybrand, h = Villiers; KwaZulu-Natal, i = Bergville, j = Dundee, k = Greytown, l = Newcastle, m = Winterton; Northern Cape: n = Barkly West, o = Bull Hill, p = Douglas, q = Hartswater, r = Hopetown, s = Modderrivier, t = Orania 1, u = Orania 2, v = Prieska, w = Remhoogte, x = Vaalharts; Western Cape: y = Vissershok.

FGSC = *Fusarium graminearum* species complex, species observed include *F. graminearum*, *F. boothii*, *F. meridionale*, *F. acaciae-mearnsii*, *F. brasilicum*, and *F. cortaderiae*.

B-trichothecene chemotype: 3-ADON = 3-acetyldeoxynivalenol, 15-ADON = 15-acetyldeoxynivalenol, NIV = Nivalenol, no B-trichothecene chemotype detected = *F. pseudograminearum* isolates that did not produced a result with PCR to indicate its chemotype.
Geographic distribution of type B trichothecene chemotypes

*Fusarium* species representing all three B-trichothecene chemotypes were present in all wheat production regions of South Africa, except for KZN and the Bushveld, where the 3-ADON type was absent (Fig 2). Four *Fusarium* species or species complexes with B-trichothecene chemotypes were collected in the Northern Cape (*F. cerealis*, FGSC, *F. lunulosporum*, *F. pseudograminearum*) and Free State (*F. cerealis*, *F. culmorum*, FGSC and *F. pseudograminearum*) (Fig 2). FGSC was the sole fusaria with a B-trichothecene chemotype among the FSAMSC-1 isolates in the Bushveld and KZN, while both the FGSC and *F. pseudograminearum* in the Western Cape had B-trichothecene chemotypes (Fig 2).

15-ADON was most dominant type found in all production regions, mainly due to the widespread occurrence of the FGSC in South Africa. Of the FGSC isolates obtained in the Northern Cape and Bushveld, more than 99% were of the 15-ADON type, while more than 95% of FGSC isolates obtained in the Free State and more than 90% of FGSC isolates obtained in KZN were of the 15-ADON type. Since *F. pseudograminearum* dominated at Vissershok in the Western Cape, the 3-ADON type was dominant there (78.6%) (Fig 2).

Discussion

Twenty-four *Fusarium* species from seven of the major *Fusarium* species complexes were associated with FHB of wheat in South Africa. Species from the FGSC (part of FSAMSC) were most common. This confirms previous reports on the dominance of FGSC as FHB pathogens in South Africa [35 – 37, 39] and internationally [1, 9 – 11, 13, 14, 26, 45]. In the 1980s, only the FGSC was obtained from diseased wheat heads in South Africa in KZN and parts of the Bushveld, while *F. cerealis* was found in the eastern parts of the Free State [35]. Seed batches from FHB-infected wheat fields at Prieska (Northern Cape) collected 10 years later provided the first reports of *F. verticillioides* (formerly *F. moniliforme*) and *F. subglutinans*, *F. equiseti*, *F. chlamydosporum* and *F. oxysporum* associated with FHB in South Africa [37]. *F. poae* was reported from glume spot of wheat heads in South Africa in 1996 [38]. *F. culmorum* and *F. semitectum* was added to the list of *Fusarium* species associated with wheat heads in a more recent report [39].
The current study reported six *Fusarium* species associated with FHB in South Africa for the first time. These include *F. acuminatum*, *F. armeniacum*, *F. avenaceum*, *F. temperatum*, *F. poae* and *F. pseudograminearum*. Some *Fusarium* species from wheat heads were also reported for the first time in certain production regions. *Fusarium cerealis* was found for the first time in the Northern Cape; *F. culmorum* in the Free State; the FIESC in the Bushveld, Free State and KZN; and *F. oxysporum* (FOSC) in the Northern Cape. Although this is the first report of FCSC 1 and FCSC 5 [28], and FSSC 5 [65, 66] from wheat grain globally, the species complexes to which they belong (FCSC and FSSC) have been reported from wheat previously, including in South Africa [10, 37]. Based on sequencing data of the EF1-α gene-area, unknown *Fusarium* species were also obtained from FCSC, FFSC, FOSC, FSAMSC, and FTSC. The identity of these species will be determined in subsequent studies.

All members of FCSC, FFSC, FOSC, FSSC and FTSC, as well as *F. armeniacum*, *F. brachygibbosum*, *F. culmorum*, *F. lunulosporum*, *F. poae*, *F. transvaalense* (FSAMSC) and an unknown species within the FSAMSC were obtained at low frequencies in this study, which indicate them to be of minor importance as FHB pathogens in South Africa. All these species, apart from *F. brachygibbosum*, *F. lunulosporum* and *F. transvaalense* have previously been associated with wheat globally [10, 24 – 26, 29, 67]. *Fusarium brachygibbosum* has been reported to cause stalk rot of maize in China [68] and has been obtained from diseased human tissue [28]. *Fusarium lunulosporum* was first isolated from grapefruit exported to Europe from South Africa in 1968, and the species was formally described in 1977 [69]. Although this species has a type B trichothecene (NIV) chemotype, its infrequent occurrence on wheat in South Africa makes it potentially a less important FHB-pathogen. *Fusarium transvaalense* was recently described from rhizosphere soil in the Kruger National Park in South Africa [70], and the present study is, to our knowledge, the first report of this species from wheat globally.

In the eastern Free State, *F. cerealis* has been replaced as the primary pathogen of wheat [35] by the FGSC in the north and *F. pseudograminearum* and the FIESC in the south. This may be due to an increase in maize production and warmer temperatures in the area, which has been shown to favour the FGSC over cold-weather pathogens such as *F. culmorum* [11, 25, 45]. The FGSC was also shown to be a more effective DON producer than the closely related *F. culmorum* [71], and is
homothallic, which may aid in the epidemiology of the pathogen [47]. Double-cropping of wheat and
maize may have introduced the FGSC into the region. *Fusarium cerealis* was still found in the eastern
Free State, and more frequently than in any other part of South Africa. The eastern Free State also
yielded the highest levels of the FIESC, which was partly due to the high incidence of the species
complex at Ladybrand, especially during 2008, when 66.7% of isolates obtained at this location
belonged to the FIESC. The dominance of this species complex at Ladybrand may be ascribed to
the cropping history, which consisted of wheat rotated with cabbage, since mulch of cruciferous
crops like white mustard (*Sinapis alba*) and Indian mustard (*Brassica juncea*) have been reported to
suppress *Fusarium* infection and decrease mycotoxin contents in wheat grain [72]. The dominance
of FIESC at this locality may, therefore, be due to the relative absence of the FGSC, brought about
by the crop rotation practice, since this was the only locality where wheat was rotated with a
cruciferous crop. The FIESC did, however, occur at several other localities, at frequencies varying
from 1.56 – 53.33%. The FIESC was first reported from FHB in South Africa in grain samples
obtained from FHB infected wheat fields near Prieska in the Northern Cape [37]. The FIESC was
also the Fusaria co-occurring most frequently with other species, although co-occurrence of Fusaria
in the same wheat head or kernel was very low (0.9% of isolations performed). The reason for the
relative high occurrence of the FIESC is unclear, but may be due to sampling conducted at the dough
stage (Zadoks growth stage 83 – 85), when FHB symptoms are most visible, but kernels are not fully
developed. When performing isolations, it can be unclear which kernels are diseased when they are
dry a few days after sampling. A subsequent study on the FIESC isolates obtained in this study
revealed high species diversity, but low toxigenic potential (unpublished data), indicating that this
species complex may be less important as FHB pathogens in South Africa.

An interesting observation was the dominance of *F. pseudograminearum* as an FHB
pathogen at one locality in the eastern Free State and the one locality in the Western Cape, although
it must be taken into account that sample size in the Western Cape was quite small, which may not
be representative of the larger Western Cape. However, isolates obtained from wheat heads
exhibiting FHB symptoms from three localities in the Western Cape in a subsequent study, revealed
that *F. pseudograminearum* was the dominant species at all three localities, constituting more than
80% of ~300 isolates obtained (unpublished data). *Fusarium pseudograminearum* is best known as
the cause of Fusarium crown rot (FCR) of wheat [73, 74]. Its dominance at the locality in the Western Cape can be ascribed to the prevalence of FCR in this region as well as the use of minimum / no till practices, which results in a build-up of inoculum levels in stubble [74]. The *F. pseudograminearum* isolates obtained in this study belong exclusively to the 3-ADON chemotype, which reflects results from Australia [27], Canada [75] and China [14]. It is, however, unclear whether the difference in chemotype may be the reason why *F. pseudograminearum* dominated over the FGSC (15-ADON) at Bethlehem. The superior ability of *F. pseudograminearum* to cause FCR has been ascribed to its ability to produce higher levels of DON than *F. culmorum* and *F. graminearum* in the stem base, while *F. culmorum* and *F. graminearum* produced high levels of DON in grains to cause FHB [76]. However, an outbreak of FHB in Australia was shown to be caused by both *F. graminearum* and *F. pseudograminearum*, indicating a lack of specialisation for FHB among these species [27]. Since the epidemiology of FHB and FCR differs drastically [8, 73], the question arises whether some level of specialisation is not present in the genetically highly diverse *F. pseudograminearum* population [77].

The vast majority of FGSC isolates in this study had the 15-ADON chemotype, with a few exceptions. This corresponds to results obtained from barley in the Northern Cape of South Africa [22], and from wheat in Argentina [5], Brazil [78], parts of Europe, and China [25, 45]. FGSC isolates with the NIV chemotype dominated at Greytown in KZN, while 40% of the FGSC isolates collected at Vissershok in the Western Cape had the NIV chemotype. FGSC isolates with the 3-ADON chemotype were found in three adjacent localities in the Northern Cape. It is important that Fusarium mycotoxins and their acetylated forms be determined during surveillance studies, as these might provide insights on the distribution of toxigenic forms of the fungus. Between 1999 and 2000, a small, localised populations of the FGSC with the 3-ADON chemotype was discovered in the Midwestern-USA, which might have been introduced to this region [79]. A 14-times increase in *F. graminearum* s.s. with the 3-ADON chemotype was subsequently reported in western Canada between 1998 and 2004 [63]. Strains from this introduced population produced significantly more DON and had a higher growth rate and fecundity than the population characterised by the 15-ADON type, therefore posing a significant threat to food safety and security. This difference in toxin accumulation and aggressiveness between *F. graminearum* s.s. isolates with the 3-ADON vs 15-ADON chemotype is,
however, likely related to differences in the genetics of the two populations, and not a direct result of trichothecene chemotype differences [63, 80].

Crop rotation and tillage practices can partly account for the differences in *Fusarium* species composition and diversity within production regions and localities in this study. Double cropping of maize and wheat is standard practice under conventional tillage in most summer rainfall wheat production regions, while wheat and maize / soybean are frequently produced under no-till conditions in KZN [81, 82]. Minimum / no-till practices, which include minimum soil disturbance, crop rotation and soil coverage with stubble or living plants [50], is also common in the Western Cape [34]. Although minimum / no-till practices hold various advantages for producers and the environment, it does result in an increase in the amount of stubble left on the soil, which can subsequently increase the risk of stubble-borne diseases like FHB and FCR [42, 44, 83]. In a study on the colonization of residues of different plant species by *F. graminearum* and their contribution to Fusarium head blight inoculum in Uruguay [84], it was found that the FGSC was more frequently isolated from residues of wheat and barley than residues of sunflower or *Festuca arundinacea* (tall fescue). The FGSC produced more ascospores, the primary source of inoculum for FHB, in wheat and barley residues than maize or other gramineous hosts, while not producing any on sunflower residues. The FGSC furthermore survived longer on wheat and barley residues under no-tillage production compared to reduced tillage production. Finally, some level of specialisation in the association between *Fusarium* species and type of stubble was found. *Fusarium avenaceum* and *F. sambucinum*, for example, was isolated from wheat, barley and gramineous stubble, but not from sunflower or tall fescue. In this study, FGSC was sometimes found to be the dominant species at localities where the previous crop was not maize, and where conventional tillage was practiced. These included all localities in the Bushveld, where the FGSC was abundant and the previous crops at the respective sites were sunflower, peppers, soybean and cabbage. A comprehensive study to determine the incidence and severity of FHB in different crop rotation systems and tillage regimes is, therefore, recommended. This, along with the use of host resistance and chemical control, can form part of an integrated disease management approach.

The absence of FHB in the Northern Cape during the 1980s [35] can partly be attributed to the practice of wheat production followed by a fallow-period in the summer, coupled with removal of...
stubble and conventional tillage, in addition to flood irrigation. The introduction of FHB of wheat to
the Northern Cape is unknown. The replacement of old with new wheat cultivars from 1988 to 2008
[34], coupled with the introduction of double-cropping, could have introduced the disease with
infected seed [35, 85]. A population genetics study of the most important members of the FGSC
population in all production regions of South Africa, as was done in a study of *F. graminearum*
isolates from Canada and the USA [86] may elucidate the origin of the disease in the Northern Cape
region and the rest of South Africa.

FGSC and FIESC isolates were more abundant in the Free State in 2008 compared to 2009.
This also happened for the FGSC in the Northern Cape. In KZN, the occurrence of the FGSC
remained almost unchanged during the two years. The presence of all members of FCSC, *F.
temperatum* and *F. verticillioides*, *F. oxysporum* (FOSC), *F. armeniacum*, *F. culmorum*, *F.
lunulosporum*, *F. transvaalense*, FSSC 5, *F. acuminatum*, *F. avenaceum*, and an unknown *Fusarium*
sp. within FTSC in 2008 but not 2009, and *F. subglutinans*, *F. brachygibbosum*, and unknown
*Fusarium* spp. within FFSC and FSAMSC in 2009 but not 2008, may have been coincidental since
the incidence of all of these species was very low. The high incidence of *Alternaria* species obtained
in all production regions in 2009 might have contributed to lower *Fusarium* levels in the 2009
production season, while differences in climate, cropping history and agronomic practices of the
collection sites in the two years might have also contributed to the discrepancy in *Fusarium* species
composition between years [40, 41, 43]. Variation in the timing and the amount of water provided
through irrigation, especially near anthesis, could also have influenced the resultant disease intensity
and associated *Fusarium* species between years [87]. Reliable disease forecasting models to aid
producers in managing the disease [1], therefore, need to be developed for South African wheat
producers.

Using MLGT, the identity and type B trichothecene chemotype of 277 FGSC isolates obtained
in this study was determined [19]. This study, however, extends and places into context the previous
results by reporting on the identity of all the Fusaria associated with FHB of wheat in South Africa. It
showed that FHB pathogens of wheat were wide-spread in South Africa, and that the diversity of
*Fusarium* species associated with FHB was greater than previously reported [19, 35 – 39]. This study
also reported on the type B trichothecene chemotype profile (3-ADON, 15-ADON and NIV) of *F.*
cerealis, F. culmorum, FGSC, F. lunulosporum and F. pseudograminearum associated with FHB of wheat in South Africa. FGSC was the dominant contributor to FHB and contained the only isolates with the 15-ADON type, the most prevalent trichothecene type observed. The dominance of the FGSC at almost every locality sampled in South Africa indicates that the local grain industry is at risk of contamination of grain with well-known mycotoxins such as DON, NIV and ZEA [10]. Legislation on Maximum Tolerated Levels of DON was introduced in South Africa in 2016 [88]. More research is thus needed to determine the amount of DON and ZEA in harvested grain over different seasons and at different localities across South Africa. This could be achieved by quantifying fungal biomass of type B trichothecene producing Fusarium species under natural conditions in South Africa using real-time quantitative PCR, and by correlating this with mycotoxin levels in harvested grain [23]. The type B trichothecene mycotoxins and ZEA are, however, not the only important Fusarium mycotoxins occurring in harvested grain in South Africa. Follow-up studies must therefore also be conducted to determine the level of contamination of harvested grain with other mycotoxins like the type A trichothecenes DAS and NEO [31], as well as mycotoxins produced by Alternaria species [89].

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Supporting information

S1 Table. Strain data for 1047 Fusarium isolates obtained from wheat plants with FHB symptoms in different production areas in South Africa

(XLSX)
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Supporting Information
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The distribution and type B trichothecene chemotype of *Fusarium* species associated with head blight of wheat in South Africa

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Abstract

Fusarium head blight (FHB) is a common disease of wheat grown in the irrigation regions of South Africa but is less common in dryland production regions. *Fusarium* species causing the disease were morphologically identified in the 1980s and more recently, but changes in production practices, the availability of new cultivars, and a better understanding of species limits necessitated a contemporary and comprehensive characterisation of FHB pathogens in the country. Symptomatic wheat heads were, therefore, collected from irrigated fields in the Northern Cape, KwaZulu-Natal (KZN), the Bushveld and the eastern part of the Free State in 2008 and 2009, and from a dryland field in the Western Cape in 2009. *Fusarium* isolates were identified using species-specific primers or by analysis of partial EF-1α sequences. A representative subset of isolates was also characterized morphologically. In total, 1047 *Fusarium* isolates were collected, comprising 24 species from seven broad species complexes. The *F. sambucinum* species complex (FSAMSC) and the *F. incarnatum-equiseti* species complex (FIESC) were the most common, accounting for 83.5% and 13.3% of isolates respectively. However, isolates from the *F. chlamydosporum* species complex (FCSC), the
F. fujikuroi species complex (FFSC), the F. oxysporum species complex (FOSC), the F. solani species complex (FSSC); and the F. tricinctum species complex (FTSC) were also observed. Within the FSAMSC, 90.7% of isolates were identified as members of a subgroup known as the F. graminearum species complex (FGSC), which accounted for 75.7% of all isolates collected in South Africa. The type B trichothecene chemotype of FGSC isolates and related species was inferred via a chemotype-specific PCR assay. Chemotype diversity was limited (15-ADON = 90.1%) and highly structured in relation to species differences. These results greatly expand the known species diversity associated with FHB in South Africa, and include the first report of F. acuminatum, F. armeniacum, F. avenaceum, F. temperatum, and F. pseudograminearum from wheat heads in South Africa. Globally, it is also the first report of F. brachygibbosum, F. lunulosporum and F. transvaalense from wheat. In addition, potentially novel species were identified within the FCSC, FFSC, FOSC, FSAMSC, FIESC and FTSC.

Introduction

Fusarium head blight (FHB) is a major disease of wheat (*Triticum aestivum*) worldwide. The disease reduces grain yield and causes the production of discoloured, shrivelled kernels contaminated with mycotoxins [1]. In the late 1990s, FHB resulted in losses estimated at US$ 2.7 billion in parts of the USA [2], while about 7 million ha have been affected by FHB epidemics in China [3]. The disease has also been damaging to wheat production in South America [4 – 7], Canada [8, 9] and Europe [10, 11]. In South Africa, wheat production has been negatively affected by the disease, although little information is available on its financial impact.

Studies conducted globally to identify the causal agents of FHB of wheat have demonstrated the *Fusarium graminearum* species complex (FGSC) to be widespread and predominant in many regions [9, 10, 12 – 14]. The FGSC, which is a subgroup within the *Fusarium sambucinum* species complex (FSAMSC) [15], consists of at least 16 phylogenetically distinct species [16, 17]. Members of the FGSC display significant biogeographic structure due to geographic speciation and host selection [12, 18 – 20]. Members of the FGSC can also infect barley (*Hordeum vulgare*), maize (*Zea mays*) and soybean (*Glycine max*); all crops that are grown in rotation with wheat in South Africa [21.
Other *Fusarium* species associated with FHB around the world include *F. chlamydosporum* [member of the *F. chlamydosporum* species complex (FCSC)]; *F. cerealis* (syn. *F. crookwellense*), *F. culmorum*, *F. poae*, and *F. pseudograminearum*, (members of the FSAMSC); *F. equiseti*, [member of the *F. incarnatum–equiseti* species complex (FIESC)]; *F. avenaceum* and *F. tricinctum* [members of the *F. tricinctum* species complex (FTSC)] and *F. proliferatum*, *F. subglutinans* and *F. verticillioides* [part of the *Fusarium fujikuroi* species complex (FFSC)] [10, 14, 15, 24–30].

*Fusarium* species associated with FHB produce mycotoxins, which are toxic secondary metabolites harmful to humans and animals. The most important among these include the type A and B trichothecene mycotoxins, and zearalenone (ZEA) [31]. Important type A trichothecene mycotoxins include diacetoxyscirpenol (DAS), neosolaniol (NEO), and T-2 and HT-2 toxins, while important type B trichothecene mycotoxins include deoxynivalenol (DON), and nivalenol (NIV) [31]. DON and ZEA (a nonsteroidal estrogen), are widely considered as the most important for wheat and barley [32], although NIV is also found in these crops [26]. The trichothecenes are potent inhibitors of eukaryotic protein synthesis and immunomodulatory [31] and are phytotoxic [33]. DON has two acetylated forms, namely 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) [32], while NIV has an acetylated form called fusarenon X (Fus-X) [10]. ZEA, on the other hand, has estrogenic properties associated with hyperestrogenism and infertility in pigs [31].

There are three major wheat production areas in South Africa [34]. These include the Western Cape Province (Mediterranean climate with mostly winter rainfall); the summer rainfall areas of Gauteng, Limpopo, Mpumalanga, and the North-West Province, the Northern Cape Province and KwaZulu-Natal (KZN); and the Free State Province (also summer rainfall). Wheat is also produced on small scale in the Eastern Cape Province. Historical reports of FHB in South Africa emanated from the irrigated production regions of the eastern Free State, North West Province and KZN during the 1980s [35], with only one report from dryland fields in the Western Cape [36]. FHB of wheat in KZN and the North West Province was shown to be caused by *Gibberella zeae* (now FGSC), and in the eastern Free State by *F. crookwellense* (*F. cerealis*) [35]. The Northern Cape was considered disease-free. This changed a few years later when FHB reached epidemic proportions in irrigated wheat fields near the Orange River in the Northern Cape during the early 1990s [37]. At one locality, 28% of the grain samples were infected with *Fusarium* species, including *F. graminearum* (member
of FGSC (48.4%), *F. moniliforme* (36.3%) (now *F. verticillioides*) and *F. subglutinans* (1.6%) (members of FFSC), *F. equiseti* (member of FIESC) (9.7%), *F. chlamydosporum* (member of FCSC) (3.2%), and *F. oxysporum* (member of *F. oxysporum* species complex, FOSC) (0.8%). *Fusarium poae* was reported from glume spot of wheat heads in South Africa in 1996, in association with a mite species (*Sitereptes avenae*) [38]. A later study [19] identified a total of 277 *Fusarium* isolates, designated FGSC in the current study, and found that *F. graminearum* s.s. with the 15-ADON chemotype was the dominant member associated with FHB in South Africa. The largest species diversity occurred in KZN, where five of the six FGSC members in South Africa was found. A study published in 2017 [39] reported *F. graminearum* (member of FGSC) as the most common *Fusarium* spp. causing FHB of wheat in seven localities across four South African provinces. Only five other *Fusarium* species were identified, including *F. chlamydosporum* (member of FCSC) and *F. equiseti* (member of FIESC) in four localities in the Northern Cape, and *F. cerealis* and *F. culmorum* (members of FSAMSC), and *F. semitectum* (member of FIESC) at one locality each in the Eastern Cape Province, KZN and North West Province. The only records of FHB from dryland fields in the Western Cape Province was on diseased wheat heads under dryland conditions from three farms in the George-district and one farm in the Swellendam-district, where the causal organism was identified as *F. graminearum* Group 2 (now FGSC) [36].

*Fusarium* spp. of wheat can differ between regions and fields due to a combination of climatic factors; agronomical practices such as crop rotation, tillage practices and the amount and type of stubble; host genotype; and disease management practices, which include host resistance and chemical control [10, 40 – 44]. Consequently, the *Fusarium* species occurring on wheat in a country or region can change in response to these drivers. For instance, *F. avenaceum, F. culmorum* and *F. poae* were historically common in the colder regions of northern Europe, but the FGSC has become more dominant in some of these regions in recent years, believed to be due to an increase in maize production and climate change [11, 25, 45]. In Italy, on the other hand, *F. graminearum* is replaced by *F. poae*, believed to be due to variation in environmental conditions [46]. Furthermore, the homothallic nature of the FGSC allows for the mass production of ascospores, which may aid in the epidemiology of the disease [47].
The commercial release of new spring wheat cultivars able to complete its life cycle in a shorter period than older cultivars enabled producers in the irrigation regions to sequentially cultivate crops like wheat in the winter and maize in the summer on the same fields, a practice known as double-cropping [48]. Irrigation practices like flood-irrigation have also been replaced with centre-pivot irrigation, which creates a more suitable microclimate for FHB development [49]. Conservation agriculture, which includes minimum / no-tillage practices and crop rotation with barley, oats and broad-leaf crops like canola replaced conventional tillage and monoculture in the Western Cape [34, 50]. All of these practices may have impacted the occurrence of FHB and associated Fusarium species in South Africa. With the availability of powerful molecular techniques, numerous new Fusarium species and species complexes were described [51]. These techniques facilitated reassessments of species identity among isolates from major reference collections [52] and enabled accurate identification of fusaria via curated databases of DNA sequence data [53]. With the exception of one study, which focused only on the FGSC [19], all previous studies of Fusarium species associated with FHB of wheat in South Africa described isolates using morphological characteristics only. Previous surveys, furthermore, collected diseased wheat heads from only a limited number of localities within large and geographically diverse production regions. As such, there is a need to have both an updated and phylogenetically broader understanding of FHB pathogen diversity within South Africa. The aim of this study, therefore, was to conduct a survey to determine the identity, distribution and type B trichothecene chemotype of Fusarium species associated with FHB of wheat in South Africa.

Materials and methods

Wheat production areas in South Africa

Spring wheat cultivars are grown under dryland conditions in the Western Cape Province and under centre-pivot irrigation in the summer rainfall irrigation regions and Free State Province. Winter wheat is grown under dryland (rain-fed) conditions in the Free State Province (Fig 1). The Western
Cape Province is the largest production area in the country, although it yields less grain ha\(^{-1}\) than the irrigation areas. There are two production regions in the Western Cape, namely the Swartland (western) and the Overberg (southern) regions. Regions within the summer rainfall irrigation area differ greatly in terms of climate and soil type. It consists of the Bushveld; which comprises parts of Gauteng, Limpopo, Mpumalanga and the North West Province; the eastern part of the Free State Province (referred to hereafter as the Free State); parts of KZN; and areas in the Northern Cape Province in the vicinity of the Orange-, Vaal- and Modder River (referred to hereafter as the Northern Cape).

**Collection of wheat heads**

**Irrigation production areas**

Wheat heads with FHB symptoms were collected at 15 localities in the Free State, KZN and the Northern Cape during 2008, and at 14 localities in the Bushveld, Free State, KZN, and Northern Cape during 2009 (Fig 1; Table 1). Collections were done in the irrigation spring wheat cultivar evaluation trials of the Agricultural Research Council’s Small Grain (ARC-SG). A total of 20 samples were collected from each of four cultivars (Baviaans, Duzi, Kariega and PAN3434), which were planted in a randomised block design with four replicates, to provide 80 samples per locality. Additionally, 40 symptomatic wheat heads were sampled randomly from one locality each in the Free State in 2008 (Frankfort), and KZN in 2009 (Greytown). The former was from a commercial wheat field, where collections were made from the spring cultivar SST835, while the latter was from a field demonstration trial from a local seed company, where collections were made from the spring cultivar PAN3434.

**Western Cape Province**

The dryland spring wheat cultivar evaluation trials of the ARC-SG were inspected each year at three localities each in the Overberg and Swartland production regions of the Western Cape Province (dryland production) for the presence of FHB, but no visible disease was found. Visible
FHB symptoms was, however, found in a commercial dryland wheat field (Vissershok) during 2009, where 40 symptomatic wheat heads were randomly sampled from the spring cultivar SST027.

Table 1. Production region, cultivar, crop history and geographical information of localities where wheat heads with Fusarium head blight symptoms were sampled.

| Sampling year | Production region | Locality | Previous crop | GPS coordinates | Elevation (m) |
|---------------|-------------------|----------|---------------|-----------------|--------------|
| 2008          | Free State        | Bethlehem| Fallow        | 28.161474° S 28.304801° E | 1643         |
|               |                   |          |               | 28.161434° S 28.305021° E | 1643         |
|               |                   |          |               | 27.795774° S 24.779891° E | 1105         |
|               |                   |          |               | 29.790727° S 24.423845° E | 1098         |
|               |                   |          |               | 29.881946° S 24.585153° E | 1123         |
|               |                   |          |               | 29.537154° S 22.993977° E | 980          |
|               |                   |          |               | 27.965495° S 24.836811° E | 1176         |
|               |                   |          |               | 25.593013° S 27.768504° E | 1087         |
|               |                   |          |               | 25.178062° S 29.389781° E | 936          |
|               |                   |          |               | 25.011394° S 27.562786° E | 955          |
|               |                   |          |               | 25.041904° S 29.221597° E | 927          |
|               |                   |          |               | 28.161434° S 28.305021° E | 1643         |
|               |                   |          |               | 28.161474° S 28.304801° E | 1643         |
|               |                   |          |               | 28.161434° S 28.305021° E | 1643         |
| Location            | Crop          | Latitude       | Longitude      | ISPM NHS 2501 |
|---------------------|---------------|----------------|----------------|---------------|
| Ladybrand           | Cabbage       | 29.181017° S   | 27.556070° E   | 1545          |
| KwaZulu-Natal       | Dundee        | 27.984337° S   | 30.349992° E   | 1168          |
|                     | Greytown      | 29.084172° S   | 30.603934° E   | 1028          |
|                     | Newcastle     | 27.643130° S   | 29.979236° E   | 1192          |
|                     | Winterton     | 28.839872° S   | 29.467234° E   | 1097          |
| Northern Cape       | Barkly West   | 28.507932° S   | 24.593006° E   | 1109          |
|                     | Bull Hill     | 28.048725° S   | 24.579655° E   | 1060          |
|                     | Hopetown      | 29.636905° S   | 24.176112° E   | 1071          |
|                     | Remhoogte     | 29.538249° S   | 22.969875° E   | 961           |
| Western Cape        | Vissershok    | 33.785813° S   | 18.555610° E   | 12            |

\textsuperscript{a} Crop grown during the previous summer growing season, except where indicated

\textsuperscript{b} Crop grown during the previous winter growing season (summer fallow)

**Isolations from diseased kernels**

For the first year, two visually scabby kernels per sample were surface-disinfected by washing in 70% ethanol for 1 min, followed by 1 min in a 1% sodium hypochlorite solution. The kernels were then rinsed with sterile distilled water and left to air dry in the laminar flow cabinet on sterile tissue paper. One kernel was plated onto potato dextrose agar (PDA) (Biolab Diagnostics, Midrand, South Africa) amended with 40 mg L\(^{-1}\) streptomycin sulphate, and the other kernel onto selective *Fusarium* agar (SFA) \([54]\). During the second year, only one visually scabby kernel per sample was surface-disinfected as described above, and isolated onto PDA only.

Plates were incubated for 5 days at 21°C in the dark. Developing *Fusarium* colonies were purified and single-spored. Single-spore cultures were plated onto PDA to harvest mycelium for DNA extraction, and onto divided plates containing PDA amended with 40 mg L\(^{-1}\) streptomycin sulphate and carnation leaf agar (CLA) \([54]\), which was incubated underneath cool white and near-UV lights with a photoperiod of 12 hrs for 21 days, for morphological identification \([54]\). Single-spore cultures were stored in 15% glycerol at -80°C at the Department of Plant Pathology, Stellenbosch University, South Africa.
**Fungal reference cultures**

Reference isolates of *F. graminearum* (NRRL28439) and *F. culmorum* (NRRL3288) were obtained from Dr K. O'Donnell (USDA-ARS Peoria, IL, USA), while isolates of *F. avenaceum* (MRC 3227) and *F. poae* (MRC 8486) were provided by Prof W.F.O Marasas (MRC-PROMEC, Tygerberg, South Africa). The reference isolates for *F. cerealis* (MRC 8399; CAV359) and FIESC (MRC 1813; CAV367) were provided by Prof A. Viljoen (Department of Plant Pathology, Stellenbosch University, South Africa), while the positive control for *F. pseudograminearum* (WCA3532) was obtained in this study following identification with multilocus genotyping (MLGT) by Dr T.J. Ward (USDA-ARS Peoria, IL, USA).

Isolates with known type B trichothecene chemotype identities were provided by Laëtitia Pinson-Gadais (French National Institute for Agricultural Research, Villenave d’Ornon, France). These included *F. culmorum* with a 3-ADON chemotype (INRA 233), FGSC with a 15-ADON chemotype (INRA 156) and FGSC with a NIV chemotype (INRA 91). These isolates served as positive controls in PCR assays to determine the chemotype of B-trichothecene isolates obtained in this study.

**Identification of Fusarium isolates**

Single-spore cultures were grown on PDA plates for 7 days, where after genomic DNA was extracted from mycelia using the Wizard® SV Genomic DNA Purification System Kit (Promega, South Africa). Isolates of *F. avenaceum*, *F. culmorum*, FGSC and *F. poae* were identified in a multiplex PCR with known species-specific primers (Table 2). Amplifications were carried out with an initial denaturation step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 30 s and extension at 72°C for 45 s, with a final extension step of 72°C for 5 min [22]. Isolates of *F. cerealis* and *F. pseudograminearum* were also identified by PCR with species-specific primers listed in Table 2, using the same reaction conditions [22]. Isolates that did not generate PCR products were identified by sequencing of the translation elongation factor-1 alpha (*EF-1α*) gene [55]. These included members of the FGSC. The *EF-1α* gene was amplified in a PCR
reaction that consisted of an initial denaturation step of 94°C for 5 min, followed by 30 cycles of
denaturation at 94°C for 30 s, primer annealing at 57°C for 45 s and primer extension at 72°C for 1
min, followed by a final extension of 72°C for 7 min [22]. Generated EF-1α products were purified
and sequenced, and edited sequences were compared to sequences available on the NCBI
GenBank database (https://www.ncbi.nlm.nih.gov/genbank/), the CBS-KNAW Fungal Biodiversity
Centre’s Fusarium MLST website (http://www.cbs.knaw.nl/Fusarium), and the FUSARIUM-ID
database [56]. Isolates with less than 99% sequence similarity to reference sequences in Fusarium
MLST were annotated as unknown species (F. sp.) and may represent novel species-level diversity.
If the same Fusarium species was obtained from the two kernels of the same sample on the different
growing media, only one isolate was selected to represent the sample. DNA sequence data
generated for 45 isolates from FHB in South Africa have been deposited in GenBank under
accession numbers MG588054 – MG588069, MG588071 – MG588087, MK617767 – MK617769,
and MK629641 – MK629649. The identification of FIESC isolates by EF-1α gene sequencing were
confirmed via PCR with species-specific primers listed in Table 2. Amplifications were carried out
with an initial denaturation step at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for
45 s, annealing at 60°C for 30 s and extension at 72°C for 1 min, with a final extension step of 72°C
for 2 min [22].
Table 2. Primer names, sequences and expected sizes of PCR products of *Fusarium* species associated with head blight of wheat.

| Target                  | Primer | Primer sequence (5’ – 3’) | Annealing T (°C) | Amplicon size (bp) | Reference |
|-------------------------|--------|---------------------------|------------------|--------------------|-----------|
| *F. avenaceum*          | FaF    | CAAGCATTGTGCCACTCTC       | 60               | 920                | [57]      |
|                         | FaR    | GTTTGGCTCTACCGGGACTG      |                  |                    |           |
| *F. cerealis*           | CroAF  | CTCAGTGTCACCACGCTTGCCTAG | 60               | 842                | [58]      |
|                         | CroAR  | CTCAGTGTCACCAATCAAGTCC    |                  |                    |           |
| *F. culmorum*           | Fc01F  | ATGGTGAACTCGTCTGTCG       | 60               | 570                | [59]      |
|                         | Fc01R  | CCGCTTTACGAAATCTCG        |                  |                    |           |
| FGSC<sup>a</sup>        | Fg11F  | CTCCGGATATGTGGCCTGCA      | 60               | 450                | [59]      |
|                         | Fg11R  | GGTAGGTATCCGACATGGCAA     |                  |                    |           |
| FIESC<sup>b</sup>       | FeF1   | CATACCTATACGTTGCTCG       | 60               | 400                | [60]      |
|                         | FeR1   | TTACCAGTAACGAGGTATG        |                  |                    |           |
| *F. poae*               | Fp82F  | CAAGCAACAGGCTCTTCAACC     | 60               | 220                | [61]      |
|                         | Fp82R  | TGTTCCACCTCAGTGGACAGT     |                  |                    |           |
| *F. pseudograminearum*  | FpgF   | GTCGCCGTCACATTC           | 60               | 779                | [62]      |
|                         | FpgR   | CACTTTATCTCTTGTTGCA       |                  |                    |           |
| *EF1α*                  | EF1    | ATGGGTAAGGA(A/G)GACAAGAC  | 57               | 648                | [55]      |
| Tri3  | 3CON    | TGGCAAAGACTGCTTCAC | 58 | 243 (3-ADON) | [63] |
|-------|---------|---------------------|----|-------------|------|
| 3NA   |         | GTGCACAGAAATACGAGC  |    | 610 (15-ADON) | [63] |
| 3D15A |         | ACTGACCAAGCTGCATC   |    | 840 (NIV)   | [63] |
| 3D3A  |         | CGCATTGCTAACAATG    |    |             | [63] |

\(^{a}\) *Fusarium graminearum* species complex

\(^{b}\) *Fusarium incarnatum–equiseti* species complex
The identities of a representative group of type B trichothecene-producing isolates were confirmed at the USDA-ARS (Peoria, IL, USA) using a multilocus genotyping assay (MLGT) [63]. These included *F. cerealis* (five isolates), *F. culmorum* (one isolate), *F. pseudograminearum* (two isolates), *Fusarium lunulosporum* (three isolates), while 277 isolates of the FGSC obtained in this study were previously identified [19]. Of the 277 FGSC isolates, 85.2% were identified as *F. graminearum* s.s. An additional 32 FGSC isolates were also identified at the USDA-ARS (Peoria, IL, USA) as *F. boothii*, *F. graminearum* s.s. and *F. meridionale* using the MLGT assay. In the current study, FGSC isolates were only identified using the FGSC-specific primer-pair mentioned earlier [59], and the FGSC species will therefore not be referred to by their phylogenetic species names. The molecular identities of FHB isolates from South Africa were linked to prior morphological species definitions by studying the morphology of 85 isolates representing all fusaria obtained [54]. The identity of six isolates could not be determined morphologically. These included three isolates of *F. transvaalense*, one isolate of *F. brachygibbosum*, and one isolate of an unknown *Fusarium* species (FSAMSC), as well as one isolate of *F. temperatum* (FFSC).

**Chemotype identification**

The NIV, 3-ADON and 15-ADON chemotypes of FGSC and related species within clade 1 of the FSAMSC (FSAMSC-10) [64] were identified using a multiplex PCR that amplified portions of the *Tri3* gene [63] (Table 2). The PCR reaction conditions consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer-annealing at 58°C for 30 s and extension at 72°C for 30 s, with a final extension step of 72°C for 5 min.

**Results**

**Fusarium species**

A total of 1047 *Fusarium* isolates were identified in this study (S1 Table), which included 24 *Fusarium* species from seven major *Fusarium* species complexes (Table 3). The FSAMSC...
accounted for 83.5% of all isolates, with most FSAMSC isolates belonging to the FGSC subgroup. The FGSC comprised 69.1% (n = 439) of Fusarium isolates obtained in 2008 and 85.9% (n = 354) in 2009. The FIESC accounted for 13.3% of all isolates, and the other species complexes (FFSC, FTSC, FOSC, FCSC, and FSSC) each accounted for less than 1.5% of the FHB isolates. Due to the use of clade-specific primers in this study, 487 of the 874 FSAMSC isolates were identified only to the level of the FGSC. However, 14 named species were identified among the remaining 387 FSAMSC isolates. Among the FCSC, we identified two informally named species (FCSC 1 and FCSC 5). Among the FFSC, we identified F. subglutinans, F. temperatum, and F. verticillioides. F. oxysporum and the informally named species FSSC 5 were identified from the FOSC and FSSC respectively. F. acuminatum and F. avenaceum were identified from the FTSC. All but two of the 139 FIESC isolates were identified with clade-specific primers that did not permit species level identification. The two FIESC isolates identified via DNA sequence analyses, as well as 11 additional isolates from the other species complexes lacked similarity to reference sequences in the Fusarium MLST database sufficient for species identification (F. sp) and may represent novel species diversity (Table 3).
| Locality / region | FCSC¹ | FFSC² | FIESC³ | FOSC⁴ | FSAMSC⁵ | FSSC⁶ | FTSC⁷ | Total¹ |
|-------------------|-------|-------|--------|-------|---------|-------|-------|--------|
|                   | FCSC  | FCSC  | F. sp. | oxy   | F. sp.  | arm   | bra   | cul    | FGSC   | lun    | poae   | pse   | tvl   | F. sp. | FSSC | acu | ave | F. sp. |
|                   | 1     | 5     |        |       |         |       |       |        |        |        |        |       |       |       |      |     |    |       |
| 2008              |       |       |        |       |         |       |       |        |        |        |        |       |       |       |      |     |    |       |
| Free State (FS)   |       |       |        |       |         |       |       |        |        |        |        |       |       |       |      |     |    |       |
| Bethlehem         | 0.00  | 0.00  | 0.00   | -     | 0.00    | 1.26  | 0.00  | -     | 23.83  | 3.02   | 0.00   | 0.00  | 0.00  | 39.22 | 0.00 | -   |     | 51   |
| Frankfurt         | 0.00  | 0.00  | 0.00   | -     | 0.00    | 0.00  | 0.00  | -     | 8.33   | 0.00   | 91.67  | 0.00  | 0.00  | 0.00  | 0.00 | -   |     | 24   |
| Ladybrand         | 0.00  | 0.00  | 0.00   | -     | 0.00    | 2.22  | 0.00  | -     | 4.44   | 2.22   | 13.33  | 0.00  | 0.40  | 0.00  | 0.00 | -   |     | 45   |
| Villiers          | 0.00  | 0.00  | 0.00   | -     | 2.50    | 0.00  | 0.00  | -     | 0.00   | 0.00   | 90.00  | 0.00  | 0.00  | 0.00  | 0.00 | -   |     | 125  |
| Tota FS           | 0.00  | 0.00  | 0.00   | -     | 1.00    | 1.00  | 0.00  | -     | 8.00   | 1.50   | 50.00  | 0.00  | 0.00  | 10.00 | 0.00 | -   |     | 50   |
| KwaZulu-Natal (KZN) |       |       |        |       |         |       |       |        |        |        |        |       |       |       |      |     |    |       |
| Bergville         | 0.00  | 0.00  | 0.00   | -     | 0.00    | 5.56  | 0.00  | 0.00  | -     | 0.00   | 0.00   | 86.44 | 0.00  | 2.78  | 0.00  | 0.00 | -   |     | 2.78 |
| Dunoon            | 0.00  | 0.00  | 0.00   | -     | 0.00    | 9.38  | 0.00  | 0.00  | -     | 0.00   | 0.00   | 90.63 | 0.00  | 0.00  | 0.00  | 0.00 | -   |     | 0.00 |
| Winterton         | 0.00  | 0.00  | 0.00   | -     | 0.00    | 36.36 | 0.00  | 0.00  | -     | 0.00   | 0.00   | 63.64 | 0.00  | 0.00  | 0.00  | 0.00 | -   |     | 0.00 |
| Tota KZN          | 0.00  | 0.00  | 0.00   | -     | 0.00    | 11.39 | 0.00  | 0.00  | -     | 0.00   | 0.00   | 84.81 | 0.00  | 1.27  | 0.00  | 0.00 | -   |     | 1.27 |
| Northern Cape (NC)|       |       |        |       |         |       |       |        |        |        |        |       |       |       |      |     |    |       |
| Douglas           | 0.00  | 0.00  | 0.00   | -     | 0.00    | 30.00 | 0.00  | 0.00  | -     | 0.00   | 0.00   | 57.00 | 0.00  | 0.00  | 7.50  | 0.00 | -   |     | 7.50 |
| Hartswater        | 0.00  | 0.00  | 0.00   | -     | 0.00    | 12.50 | 12.50 | 0.00  | -     | 0.00   | 0.00   | 62.50 | 0.00  | 0.00  | 0.00  | 0.00 | -   |     | 12.50|
| Modderrivier      | 0.00  | 20.00 | 6.67   | -     | 53.33   | 6.67  | 0.00  | 0.00  | -     | 0.00   | 0.00   | 94.00 | 0.00  | 0.00  | 1.92  | 0.00 | -   |     | 1.92 |
| Orana 1           | 1.33  | 0.00  | 0.00   | -     | 1.33    | 5.33  | 0.00  | 0.00  | -     | 88.00  | 0.00   | 0.00  | 0.00  | 0.00  | 1.33 | 0.00 | -   |     | 1.33 |
| Orana 2           | 0.00  | 0.00  | 0.00   | -     | 0.00    | 3.64  | 0.00  | 0.00  | -     | 94.00  | 0.00   | 0.00  | 0.00  | 1.92  | 0.00 | 0.00 | -   |     | 1.92 |
| Prickla           | 0.00  | 0.00  | 0.00   | -     | 0.00    | 17.31 | 0.00  | 0.00  | -     | 0.00   | 0.00   | 75.00 | 5.72  | 0.00  | 0.00  | 0.00 | -   |     | 5.72 |
| Remhoogte         | 0.00  | 0.00  | 0.00   | -     | 0.00    | 1.69  | 0.00  | 0.00  | -     | 0.00   | 0.00   | 98.31 | 0.00  | 0.00  | 0.00  | 0.00 | -   |     | 0.00 |
| Vaalharts         | 0.00  | 3.85  | 0.00   | -     | 38.46   | 0.00  | 0.00  | 0.00  | -     | 0.00   | 0.00   | 55.77 | 0.00  | 0.00  | 0.00  | 0.00 | -   |     | 0.00 |
| Tota NC           | 0.32  | 1.40  | 0.32   | -     | 0.32    | 0.28  | 0.00  | 0.00  | -     | 76.40  | 0.84   | 0.00  | 0.00  | 0.84  | 0.84 | 0.32 |     | 0.32 |
| 2008              | 1     | 5     | 1      | -     | 3       | 1     | 1     | 16    | 3     | 439    | 3      | 1     | 23    | 3     | 2    | 2    | 10   | 3    | 635  |
| Year | Location | Brits | Groblersdal | Kookoeskop | Marble Hall | Winterton | Newcastle | Ladybrand | Total NC | Bushveld | Total FS | Free State | Bushveld | Free State | KZN | Northern Cape | NC | Western Cape (WC) | WC | Total 2009 |
|------|----------|-------|-------------|------------|-------------|-----------|----------|-----------|----------|----------|-----------|------------|----------|------------|----|---------------|----|-------------|
| 2009 |          | 2.54  | 0.00        | 0.00       | 0.00        | 2.04      | 0.00     | 0.00      | 0.00     | 0.50     | 0.00     | 0.00      | 0.00      | 0.00      | 0.00 | 1.15          | 0.00     | 0.00        | 0.00 | 1.60        | 0.00 | 412        |

**Explanation**
- **Western Cape (WC)**: Brits, Groblersdal, Kookoeskop, Marble Hall, Winterton, Newcastle, Ladybrand.
- **Free State**: Bethlehem, Ladybrand.
- **Bushveld**: Total Bushveld, Total FS, Ladybrand, Winterton.
- **KZN**: Dundee, Greytown, Newcastle, Winterton, Total KZN.
- **Northern Cape**: Barkly West, Bull Hill, Hope Town, Remouchegle, Total NC.
- **Western Cape (WC)**: Total WC.
Incidence for a locality was calculated as follows: (number of isolates of a *Fusarium* species obtained at a locality / number of *Fusarium* isolates obtained at the locality) x 100

Incidence for a production region was calculated as follows: (number of isolates of a *Fusarium* species obtained in a production region / number of *Fusarium* isolates obtained in the production region) x 100

FCSC = *Fusarium chlamydosporum* species complex: FCSC 1, FCSC 5 = *F. chlamydosporum* clade 1 and clade 5 (O'Donnell *et al*., 2009); F. sp. = unknown *Fusarium* species within the FCSC

FFSC = *Fusarium fujikuroi* species complex: sub = *F. subglutinans*; temp = *F. temperatum*; vert = *F. verticillioides*; F. sp. = unknown *Fusarium* species within the FFSC

FIESC = *Fusarium incarnatum-equiseti* species complex

FOSC = *Fusarium oxysporum* species complex: oxy = *Fusarium oxysporum*; F. sp. = unknown *Fusarium* species within the FOSC

FSAMSC = *Fusarium sambucinum* species complex: arm = *F. armeniacum*; bra = *F. brachyginbosum*; cer = *F. cerealis*; cul = *F. culmorum*; FGSC = *Fusarium graminearum* species complex, species observed include *F. graminearum*, *F. boothii*, *F. acaciae-mearnsii*, *F. brasilicum*, and *F. cortaderiae*; lun = *F. lunulosporum*; poae = *F. poae*; pse = *F. pseudograminearum*; tvl = *F. transvaalense*; F. sp. = unknown *Fusarium* species within the FSAMSC

FSSC = *F. solani* species complex: FSSC 5 = *F. solani* clade 5 (O'Donnell *et al*., 2008; Zhang *et al*., 2006)

FTSC = *Fusarium tricinctum* species complex: acu = *F. acuminatum*; ave = *F. avenaceum*; F. sp. = unknown *Fusarium* species within the FTSC

Total number of *Fusarium* isolates

*Fusarium* species not obtained in specific year
Considerably more *Fusarium* isolates were obtained in 2008 than in 2009, even though the same number of diseased wheat heads were collected in both years. This can be attributed to the high incidence of *Alternaria* isolates found in wheat kernels at localities like Brits (Bushveld), Remhoogte, Bull Hill and Hopetown (Northern Cape), KZN, the Free State and the Western Cape in 2009 (data not presented). Many symptomatic kernels from these regions yielded no *Fusarium* isolates during 2009. All members of the FCSC and FTSC, *F. temperatum*, *F. verticillioides*, *F. oxysporum*, *F. armeniacum*, *F. culmorum*, *F. lunulosporum*, *F. transvaalense*, and FSSC 5 were collected only in 2008. *F. subglutinans*, the *F*. sp. isolate from the FFSC, *F. brachyribbosum*, and the *F*. sp. isolates from the FSAMSC were collected only in 2009 (Table 3).

Co-occurrence of *Fusarium* species (when more than one *Fusarium* species was obtained from the same wheat head or same kernel), was very low and occurred only in 0.9% of all isolations performed. Of these cases, the FIESC co-occurred 48.4% (*n* = 16) with the FGSC, and 16.1% (*n* = 5) with other fusaria.

### Geographical distribution of *Fusarium* species

The FGSC and FIESC were the most widely distributed fusaria in South Africa (Fig 1). The FGSC was found in all production regions and localities, apart from Modderrivier (Northern Cape) and Bethlehem (Free State) in 2008 (Table 3). *Fusarium pseudograminearum* was predominant at the one locality in the Western Cape. The incidence of the FGSC was highest at Remhoogte (98.3%) and Orania 2 (94.6%) in the Northern Cape, at Frankfort (91.7%) and Villiers (90%) in the Free State, and at Dundee (90.6%) in KZN in 2008. In 2009, its incidence was highest at Dundee and Winterton in KZN, and Barkly West in the Northern Cape, all at 100%, followed by Groblersdal (98.4%) and Marble Hall (98%) in the Bushveld. Ladybrand and Bethlehem in the Free State had the lowest incidence of the FGSC in 2008 and 2009, at 13.3% and 11.1% respectively (Table 3). In 2009, Remhoogte had an incidence of 100%, compared to 98.3% in 2008, although a total of 58 *Fusarium* isolates were obtained there in 2008 compared to just two isolates in 2009 (S1 Table).
FSAMSC:
- FGSC
- F. pseudograminearum
- Other FSAMSC
- FCSC
- FFSC
- FIESC
- FOSC
- FSSC
- FTSC

- Bushveld: $n = 200$
- Free State: $n = 214$
- KwaZulu-Natal: $n = 166$
- Western Cape: $n = 19$
- Northern Cape: $n = 448$

Bushveld:
- Northern Cape:
- Free State:
- KwaZulu-Natal:
- Western Cape:
Fig 1. Geographical distribution of *Fusarium* species obtained from diseased wheat heads in South Africa during 2008 and 2009, according to the total number of *Fusarium* isolates obtained (*n*).

Bushveld: a = Marble Hall, b = Groblersdal, c = Brits, d = Koedoeskop; Free State: e = Bethlehem, f = Frankfort, g = Ladybrand, h = Villiers; KwaZulu-Natal, i = Bergville, j = Dundee, k = Greytown, l = Newcastle, m = Winterton; Northern Cape: n = Barkly West, o = Bull Hill, p = Douglas, q = Hartswater, r = Hopetown, s = Modderrivier, t = Orania 1, u = Orania 2, v = Prieska, w = Remhoogte, x = Vaalharts; Western Cape: y = Vissershok.

FSAMSC = *F. sambucinum* species complex. FGSC = *Fusarium graminearum* species complex, species observed include *F. graminearum*, *F. boothii*, *F. meridionale*, *F. acaciae-mearnsii*, *F. brasilicum*, and *F. cortaderiae*.

Other FSAMSC = members of the FSAMSC other than FGSC and *F. pseudograminearum*: *F. armeniacum*, *F. brachygibbosum*, *F. cerealis*, *F. culmorum*, *F. lunulosporum*, *F. poae*, and unknown *Fusarium* species within the FSAMSC.

FCSC = *Fusarium chlamydosporum* species complex: *F. chlamydosporum* clade 1 and clade 5 (O’Donnell et al., 2009), and unknown *Fusarium* species within the FCSC.

FFSC = *Fusarium fujikuroi* species complex: *F. subglutinans*, *F. temperatum*, *F. verticillioides*, and unknown *Fusarium* species within the FFSC.

FIESC = *Fusarium incarnatum-equiseti* species complex

FOSC = *Fusarium oxysporum* species complex: *Fusarium oxysporum*, and unknown *Fusarium* species within the FOSC.

FSSC = *F. solani* species complex: *F. solani* clade 5 (O’Donnell et al., 2008; Zhang et al., 2006)

FTSC = *Fusarium tricinctum* species complex: *F. acuminatum*, *F. avenaceum*, and unknown *Fusarium* species within the FTSC.
The FIESC was obtained in all wheat production regions except the Western Cape. The fungus was not isolated at Frankfort (Free State) in 2008, and at several localities in 2009, including Bethlehem (Free State), Marble Hall (Bushveld), Dundee and Winterton (KZN), and Barkly West, Bull Hill and Remhoogte (Northern Cape) (Table 3). In the Free State, KZN, and the Northern Cape, FIESC comprised 24.5, 11.4 and 16% of isolates collected in 2008, respectively (Table 3). In 2009, the FIESC comprised 9, 7.1, 4.6 and 1.1% of isolates collected in the Bushveld, Free State, KZN and the Northern Cape, respectively. The highest incidence in 2008 was obtained at Ladybrand in the Free State (66.7%), followed by Modderrivier (53.3%) and Vaalharts (38.5%) in the Northern Cape (Table 3). The incidence of the FIESC was substantially reduced in 2009, with the highest incidence found at Brits in the Bushveld (38.2%). Where present, the lowest incidence of the FIESC in 2008 was at Remhoogte in the Northern Cape (1.7%), and at Groblersdal in the Bushveld (1.6%) in 2009 (Table 3).

The Northern Cape was the wheat production region with the highest FHB species diversity, with 16 *Fusarium* species from all seven species complexes obtained there (Fig 1). Ten *Fusarium* species from six species complexes were collected from wheat in the Free State, and seven *Fusarium* species from four species complexes in KZN. Five *Fusarium* species from four species complexes were obtained from wheat in the Bushveld, while only two species from one species complex (FSAMSC) were obtained at the locality in the Western Cape (Fig 1).

*Fusarium* species other than those in the FGSC and FIESC were mostly obtained at low incidences (Table 3). The members of the FCSC, *F. oxysporum* within the FOSC, *F. armeniacum*, *F. lunulosporum*, *F. transvaalense* and an unknown *Fusarium* species within the FTSC were obtained only in the Northern Cape in 2008. *Fusarium temperatum* and FSSC 5 were obtained only in the Free State and Northern Cape in 2008, while *F. verticillioides* and *F. culmorum* were obtained only in the Free State in 2008. *Fusarium acuminatum* and *F. avenaceum* was obtained from KZN and the Northern Cape, and from the Free State, KZN and Northern Cape in 2008 respectively. *Fusarium subglutinans* and *F. brachygibbosum* were only obtained in 2009 in the Bushveld, while unknown *Fusarium* spp. within the FFSC and FSAMSC were obtained only in 2009 in KZN. An unknown *Fusarium* sp. within the FOSC was obtained in the Free State in 2008, and from the Bushveld in 2009. *Fusarium cerealis* was obtained from the Free State in 2008 and 2009, and from
the Northern Cape in 2009 only, while *F. poae* was only obtained in KZN, where it occurred both years. *Fusarium pseudograminearum* was obtained both years in the Free State, and in the Northern and Western Cape during 2009 (Table 3).

The greatest species diversity at individual localities in 2008 was found at Ladybrand (Free State) and Orania 1 (Northern Cape) with seven species each, and the lowest at Dundee and Winterton (KZN), Frankfort (Free State), and Remhoogte (Northern Cape), with two species each. In 2009, the highest species diversity was at Greytown (KZN) with five species, and the lowest at Dundee and Winterton (KZN), and Barkly West and Remhoogte (Northern Cape), where only the FGSC was obtained (Table 3).

**Type B trichothecene chemotype**

The B-trichothecene chemotypes of 861 isolates from FSAMSC-1 were assessed via a chemotype-specific PCR assay. 15-ADON was the dominant type (90.1%) associated with these *Fusarium* isolates collected in South Africa (S1 Table). Isolates with the 3-ADON and NIV types comprised 5.4 and 4.5%, respectively, of the FSAMSC-1 isolates in the country. The 15-ADON type was only observed among the FGSC, where it was predominant (97.4%). Less than 0.5% of this species complex had the 3-ADON type, while 2.3% had the NIV type (S1 Table). *Fusarium cerealis* and *F. lunulosporum* were exclusively of the NIV type, and *F. culmorum* and *F. pseudograminearum* were exclusively of the 3-ADON type (Fig 2).
Northern Cape

F. cerealis

n = 1

FGSC

n = 362

F. lunulosporum

n = 3

F. pseudograminearum

Bushveld

FGSC

n = 178

Free State

F. cerealis

n = 17

F. culmorum

n = 3

FGSC

n = 105

F. pseudograminearum

n = 26

Western Cape

F. pseudograminearum

FGSC

n = 5

n = 11

B-trichothecene chemotype:

- Red: 3-ADON
- Yellow: 15-ADON
- Blue: NIV
- Green: No B-trichothecene chemotype detected

KwaZulu-Natal

FGSC

n = 143

23
Fig 2. Incidence of type B trichothecene chemotypes of different members of the *Fusarium sambucinum* species complex (FSAMSC) obtained from diseased wheat heads in South Africa during 2008 and 2009, according to the total number of FSAMSC isolates obtained (*n*).

Bushveld: a = Marble Hall, b = Groblersdal, c = Brits, d = Koedoeskop; Free State: e = Bethlehem, f = Frankfort, g = Ladybrand, h = Villiers; KwaZulu-Natal, i = Bergville, j = Dundee, k = Greytown, l = Newcastle, m = Winterton; Northern Cape: n = Barkly West, o = Bull Hill, p = Douglas, q = Hartswater, r = Hopetown, s = Modderrivier, t = Orania 1, u = Orania 2, v = Prieska, w = Remhoogte, x = Vaalharts; Western Cape: y = Vissershok.

FGSC = *Fusarium graminearum* species complex, species observed include *F. graminearum*, *F. boothii*, *F. meridionale*, *F. acaciae-mearnsii*, *F. brasilicum*, and *F. cortaderiae*.

B-trichothecene chemotype: 3-ADON = 3-acetyldeoxynivalenol, 15-ADON = 15-acetyldeoxynivalenol, NIV = Nivalenol, no B-trichothecene chemotype detected = *F. pseudograminearum* isolates that did not produced a result with PCR to indicate its chemotype.
Geographic distribution of type B trichothecene chemotypes

Fusarium species representing all three B-trichothecene chemotypes were present in all wheat production regions of South Africa, except for KZN and the Bushveld, where the 3-ADON type was absent (Fig 2). Four Fusarium species or species complexes with B-trichothecene chemotypes were collected in the Northern Cape (F. cerealis, FGSC, F. lunulosporum, F. pseudograminearum) and Free State (F. cerealis, F. culmorum, FGSC and F. pseudograminearum) (Fig 2). FGSC was the sole fusaria with a B-trichothecene chemotype among the FSAMSC-1 isolates in the Bushveld and KZN, while both the FGSC and F. pseudograminearum in the Western Cape had B-trichothecene chemotypes (Fig 2).

15-ADON was most dominant type found in all production regions, mainly due to the widespread occurrence of the FGSC in South Africa. Of the FGSC isolates obtained in the Northern Cape and Bushveld, more than 99% were of the 15-ADON type, while more than 95% of FGSC isolates obtained in the Free State and more than 90% of FGSC isolates obtained in KZN were of the 15-ADON type. Since F. pseudograminearum dominated at Vissershok in the Western Cape, the 3-ADON type was dominant there (78.6%) (Fig 2).

Discussion

Twenty-four Fusarium species from seven of the major Fusarium species complexes were associated with FHB of wheat in South Africa. Species from the FGSC (part of FSAMSC) were most common. This confirms previous reports on the dominance of FGSC as FHB pathogens in South Africa [35 – 37, 39] and internationally [1, 9 – 11, 13, 14, 26, 45]. In the 1980s, only the FGSC was obtained from diseased wheat heads in South Africa in KZN and parts of the Bushveld, while F. cerealis was found in the eastern parts of the Free State [35]. Seed batches from FHB-infected wheat fields at Prieska (Northern Cape) collected 10 years later provided the first reports of F. verticillioides (formerly F. moniliforme) and F. subglutinans, F. equiseti, F. chlamydosporum and F. oxysporum associated with FHB in South Africa [37]. F. poae was reported from glume spot of wheat heads in South Africa in 1996 [38]. F. culmorum and F. semitectum was added to the list of Fusarium species associated with wheat heads in a more recent report [39].
The current study reported six *Fusarium* species associated with FHB in South Africa for the first time. These include *F. acuminatum*, *F. armeniacum*, *F. avenaceum*, *F. temperatum*, *F. poae* and *F. pseudograminearum*. Some *Fusarium* species from wheat heads were also reported for the first time in certain production regions. *Fusarium cerealis* was found for the first time in the Northern Cape; *F. culmorum* in the Free State; the FIESC in the Bushveld, Free State and KZN; and *F. oxysporum* (FOSC) in the Northern Cape. Although this is the first report of FCSC 1 and FCSC 5 [28], and FSSC 5 [65, 66] from wheat grain globally, the species complexes to which they belong (FCSC and FSSC) have been reported from wheat previously, including in South Africa [10, 37]. Based on sequencing data of the EF1-α gene-area, unknown *Fusarium* species were also obtained from FCSC, FFSC, FOSC, FSAMSC, and FTSC. The identity of these species will be determined in subsequent studies.

All members of FCSC, FFSC, FOSC, FSSC and FTSC, as well as *F. armeniacum*, *F. brachygibbosum*, *F. culmorum*, *F. lunulosporum*, *F. poae*, *F. transvaalense* (FSAMSC) and an unknown species within the FSAMSC were obtained at low frequencies in this study, which indicate them to be of minor importance as FHB pathogens in South Africa. All these species, apart from *F. brachygibbosum*, *F. lunulosporum* and *F. transvaalense* have previously been associated with wheat globally [10, 24 – 26, 29, 67]. *Fusarium brachygibbosum* has been reported to cause stalk rot of maize in China [68] and has been obtained from diseased human tissue [28]. *Fusarium lunulosporum* was first isolated from grapefruit exported to Europe from South Africa in 1968, and the species was formally described in 1977 [69]. Although this species has a type B trichothecene (NIV) chemotype, its infrequent occurrence on wheat in South Africa makes it potentially a less important FHB-pathogen. *Fusarium transvaalense* was recently described from rhizosphere soil in the Kruger National Park in South Africa [70], and the present study is, to our knowledge, the first report of this species from wheat globally.

In the eastern Free State, *F. cerealis* has been replaced as the primary pathogen of wheat [35] by the FGSC in the north and *F. pseudograminearum* and the FIESC in the south. This may be due to an increase in maize production and warmer temperatures in the area, which has been shown to favour the FGSC over cold-weather pathogens such as *F. culmorum* [11, 25, 45]. The FGSC was also shown to be a more effective DON producer than the closely related *F. culmorum* [71], and is
homothallic, which may aid in the epidemiology of the pathogen [47]. Double-cropping of wheat and maize may have introduced the FGSC into the region. *Fusarium cerealis* was still found in the eastern Free State, and more frequently than in any other part of South Africa. The eastern Free State also yielded the highest levels of the FIESC, which was partly due to the high incidence of the species complex at Ladybrand, especially during 2008, when 66.7% of isolates obtained at this location belonged to the FIESC. The dominance of this species complex at Ladybrand may be ascribed to the cropping history, which consisted of wheat rotated with cabbage, since mulch of cruciferous crops like white mustard (*Sinapis alba*) and Indian mustard (*Brassica juncea*) have been reported to suppress *Fusarium* infection and decrease mycotoxin contents in wheat grain [72]. The dominance of FIESC at this locality may, therefore, be due to the relative absence of the FGSC, brought about by the crop rotation practice, since this was the only locality where wheat was rotated with a cruciferous crop. The FIESC did, however, occur at several other localities, at frequencies varying from 1.56 – 53.33%. The FIESC was first reported from FHB in South Africa in grain samples obtained from FHB infected wheat fields near Prieska in the Northern Cape [37]. The FIESC was also the Fusaria co-occurring most frequently with other species, although co-occurrence of Fusaria in the same wheat head or kernel was very low (0.9% of isolations performed). The reason for the relative high occurrence of the FIESC is unclear, but may be due to sampling conducted at the dough stage (Zadoks growth stage 83 – 85), when FHB symptoms are most visible, but kernels are not fully developed. When performing isolations, it can be unclear which kernels are diseased when they are dry a few days after sampling. A subsequent study on the FIESC isolates obtained in this study revealed high species diversity, but low toxigenic potential (unpublished data), indicating that this species complex may be less important as FHB pathogens in South Africa.

An interesting observation was the dominance of *F. pseudograminearum* as an FHB pathogen at one locality in the eastern Free State and the one locality in the Western Cape, although it must be taken into account that sample size in the Western Cape was quite small, which may not be representative of the larger Western Cape. However, isolates obtained from wheat heads exhibiting FHB symptoms from three localities in the Western Cape in a subsequent study, revealed that *F. pseudograminearum* was the dominant species at all three localities, constituting more than 80% of ~300 isolates obtained (unpublished data). *Fusarium pseudograminearum* is best known as...
the cause of Fusarium crown rot (FCR) of wheat [73, 74]. Its dominance at the locality in the Western Cape can be ascribed to the prevalence of FCR in this region as well as the use of minimum / no till practices, which results in a build-up of inoculum levels in stubble [74]. The *F. pseudograminearum* isolates obtained in this study belong exclusively to the 3-ADON chemotype, which reflects results from Australia [27], Canada [75] and China [14]. It is, however, unclear whether the difference in chemotype may be the reason why *F. pseudograminearum* dominated over the FGSC (15-ADON) at Bethlehem. The superior ability of *F. pseudograminearum* to cause FCR has been ascribed to its ability to produce higher levels of DON than *F. culmorum* and *F. graminearum* in the stem base, while *F. culmorum* and *F. graminearum* produced high levels of DON in grains to cause FHB [76]. However, an outbreak of FHB in Australia was shown to be caused by both *F. graminearum* and *F. pseudograminearum*, indicating a lack of specialisation for FHB among these species [27]. Since the epidemiology of FHB and FCR differs drastically [8, 73], the question arises whether some level of specialisation is not present in the genetically highly diverse *F. pseudograminearum* population [77].

The vast majority of FGSC isolates in this study had the 15-ADON chemotype, with a few exceptions. This corresponds to results obtained from barley in the Northern Cape of South Africa [22], and from wheat in Argentina [5], Brazil [78], parts of Europe, and China [25, 45]. FGSC isolates with the NIV chemotype dominated at Greytown in KZN, while 40% of the FGSC isolates collected at Vissershok in the Western Cape had the NIV chemotype. FGSC isolates with the 3-ADON chemotype were found in three adjacent localities in the Northern Cape. It is important that *Fusarium* mycotoxins and their acetylated forms be determined during surveillance studies, as these might provide insights on the distribution of toxigenic forms of the fungus. Between 1999 and 2000, a small, localised populations of the FGSC with the 3-ADON chemotype was discovered in the Midwestern-USA, which might have been introduced to this region [79]. A 14-times increase in *F. graminearum* s.s. with the 3-ADON chemotype was subsequently reported in western Canada between 1998 and 2004 [63]. Strains from this introduced population produced significantly more DON and had a higher growth rate and fecundity than the population characterised by the 15-ADON type, therefore posing a significant threat to food safety and security. This difference in toxin accumulation and aggressiveness between *F. graminearum* s.s. isolates with the 3-ADON vs 15-ADON chemotype is,
however, likely related to differences in the genetics of the two populations, and not a direct result of trichothecene chemotype differences [63, 80].

Crop rotation and tillage practices can partly account for the differences in Fusarium species composition and diversity within production regions and localities in this study. Double cropping of maize and wheat is standard practice under conventional tillage in most summer rainfall wheat production regions, while wheat and maize / soybean are frequently produced under no-till conditions in KZN [81, 82] (Richard Findlay, personal communication). Minimum / no-till practices, which include minimum soil disturbance, crop rotation and soil coverage with stubble or living plants [50], is also common in the Western Cape [34]. Although minimum / no-till practices hold various advantages for producers and the environment, it does result in an increase in the amount of stubble left on the soil, which can subsequently increase the risk of stubble-borne diseases like FHB and FCR [42, 44, 843].

In a study on the colonization of residues of different plant species by F. graminearum and their contribution to Fusarium head blight inoculum in Uruguay [824], it was found that the FGSC was more frequently isolated from residues of wheat and barley than residues of sunflower or Festuca arundinacea (tall fescue). The FGSC produced more ascospores, the primary source of inoculum for FHB, in wheat and barley residues than maize or other gramineous hosts, while not producing any on sunflower residues. The FGSC furthermore survived longer on wheat and barley residues under no-tillage production compared to reduced tillage production. Finally, some level of specialization in the association between Fusarium species and type of stubble was found. Fusarium avenaceum and F. sambucinum, for example, was isolated from wheat, barley and gramineous stubble, but not from sunflower or tall fescue. In this study, FGSC was sometimes found to be the dominant species at localities where the previous crop was not maize, and where conventional tillage was practiced. These included all localities in the Bushveld, where the FGSC was abundant and the previous crops at the respective sites were sunflower, peppers, soybean and cabbage. A comprehensive study to determine the incidence and severity of FHB in different crop rotation systems and tillage regimes is, therefore, recommended. This, along with the use of host resistance and chemical control, can form part of an integrated disease management approach.

The absence of FHB in the Northern Cape during the 1980s [35] can partly be attributed to the practice of wheat production followed by a fallow-period in the summer, coupled with removal of
stubble and conventional tillage, in addition to flood irrigation. The introduction of FHB of wheat to the Northern Cape is unknown. The replacement of old with new wheat cultivars from 1988 to 2008 [34], coupled with the introduction of double-cropping, could have introduced the disease with infected seed [35, 835]. A population genetics study of the most important members of the FGSC population in all production regions of South Africa, as was done in a study of *F. graminearum* isolates from Canada and the USA [846] may elucidate the origin of the disease in the Northern Cape region and the rest of South Africa.

FGSC and FIESC isolates were more abundant in the Free State in 2008 compared to 2009. This also happened for the FGSC in the Northern Cape. In KZN, the occurrence of the FGSC remained almost unchanged during the two years. The presence of all members of FCSC, *F. temperatum* and *F. verticillioides*, *F. oxysporum* (FOSC), *F. armeniacum*, *F. culmorum*, *F. lunulosporum*, *F. transvaalense*, FSSC 5, *F. acuminatum*, *F. avenaeum*, and an unknown *Fusarium* sp. within FTSC in 2008 but not 2009, and *F. subglutinans*, *F. brachygibbosum*, and unknown *Fusarium* spp. within FFSC and FSAMSC in 2009 but not 2008, may have been coincidental since the incidence of all of these species was very low. The high incidence of *Alternaria* species obtained in all production regions in 2009 might have contributed to lower *Fusarium* levels in the 2009 production season, while differences in climate, cropping history and agronomic practices of the collection sites in the two years might have also contributed to the discrepancy in *Fusarium* species composition between years [40, 41, 43]. Variation in the timing and the amount of water provided through irrigation, especially near anthesis, could also have influenced the resultant disease intensity and associated *Fusarium* species between years [857]. Reliable disease forecasting models to aid producers in managing the disease [1], therefore, need to be developed for South African wheat producers.

Using MLGT, the identity and type B trichothecene chemotype of 277 FGSC isolates obtained in this study was determined [19]. This study, however, extends and places into context the previous results by reporting on the identity of all the Fusaria associated with FHB of wheat in South Africa. It showed that FHB pathogens of wheat were wide-spread in South Africa, and that the diversity of *Fusarium* species associated with FHB was greater than previously reported [19, 35 – 39]. This study also reported on the type B trichothecene chemotype profile (3-ADON, 15-ADON and NIV) of *F.
cerealis, F. culmorum, FGSC, F. lunulosporum and F. pseudograminearum associated with FHB of wheat in South Africa. FGSC was the dominant contributor to FHB and contained the only isolates with the 15-ADON type, the most prevalent trichothecene type observed. The dominance of the FGSC at almost every locality sampled in South Africa indicates that the local grain industry is at risk of contamination of grain with well-known mycotoxins such as DON, NIV and ZEA [10]. Legislation on Maximum Tolerated Levels of DON was introduced in South Africa in 2016 [868]. More research is thus needed to determine the amount of DON and ZEA in harvested grain over different seasons and at different localities across South Africa. This could be achieved by quantifying fungal biomass of type B trichothecene producing Fusarium species under natural conditions in South Africa using real-time quantitative PCR, and by correlating this with mycotoxin levels in harvested grain [23]. The type B trichothecene mycotoxins and ZEA are, however, not the only important Fusarium mycotoxins occurring in harvested grain in South Africa. Follow-up studies must therefore also be conducted to determine the level of contamination of harvested grain with other mycotoxins like the type A trichothecenes DAS and NEO [31], as well as mycotoxins produced by Alternaria species [879].

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Supporting information

S1 Table. Strain data for 1047 Fusarium isolates obtained from wheat plants with FHB symptoms in different production areas in South Africa

(XLSX)
Response to reviewers

Journal Requirements:

Comment: Please review your reference list to ensure that it is complete and correct. If you have cited papers that have been retracted, please include the rationale for doing so in the manuscript text, or remove these references and replace them with relevant current references. Any changes to the reference list should be mentioned in the rebuttal letter that accompanies your revised manuscript. If you need to cite a retracted article, indicate the article’s retracted status in the References list and also include a citation and full reference for the retraction notice.

Response: The reference list is complete and correct. All references were checked on the PubMed database or the journal’s website to confirm that it has not been retracted. To the best of our knowledge, none of the references cited has been retracted. The personal communication with Richard Findlay (p. 29, line 535) has been replaced with two scientific articles (references 81 and 82), and the reference list has been updated accordingly. All Journal names have been edited to their correct abbreviations according to NCBI database, and all URLs and doi’s have been removed.

Additional Editor Comments

Comment: There is still one minor comment, in regard to table 3: the calculated incidence: "then calculated as follows: For FGSC: (30/35) x 100 = 85.71%, and for FIESC: (5/35) x 100 = 14.29%. This is also indicated in the subscript of Table 3." But my point is that when you have 100 samples, you can never be more precise than 1%, so for example 1.567% is impossible. This also accounts when you work with less samples, so everything lower than 1 should definitely be removed....85.71% should be 85%...this is as precise as you can be when doing these types of calculation.

Response: This has been addressed accordingly.

Reviewer’s Comments

Comment: While revising your submission, please upload your figure files to the Preflight Analysis and Conversion Engine (PACE) digital diagnostic tool, https://pacev2.apexcovantage.com/. PACE helps ensure that figures meet PLOS requirements. To use PACE, you must first register as a user. Registration is free. Then, login and navigate to the UPLOAD tab, where you will find detailed instructions on how to use the tool. If you encounter any issues or have any questions when using PACE, please email PLOS at figures@plos.org. Please note that Supporting Information files do not need this step.

Response: Figure files (Fig. 1 and Fig. 2) has been uploaded to the PACE digital diagnostic tool and has been archived.