Infectivity of the *Fusarium fujikuroi* Species Complex (FFSC) on Maize (*Zea mays* Lam.) in Gauteng, South Africa

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ABSTRACT

Background and Objective: Of the world's top ten phytopathogenic fungi, *Fusarium* is the only group represented by more than one genus causing yield losses on crops and also producing a wide range of mycotoxins in food and feed products which can cause deleterious effects on human and livestock. The study aimed to investigate the pathogenicity of some *Fusarium* species isolated from Gauteng, South Africa on maize. **Materials and Methods:** A complete randomized block design with 26 isolates, belonging to the members of the *Fusarium fujikuroi* species complex (FFSC) was screened in 2017 for their infectivity on maize in Plant Protection Research Institute (PPRI), Pretoria, South Africa. The parameters tested included germination efficiency, plant height, dry weight, and disease severity. The results were analyzed using Duncan's Multiple-Range Test at a 5% level of significance. **Results:** The infection with *F. burgessii* and *F. nygamai* isolates PPRI 21048 and PPRI 21265 produced the highest and lowest disease severity index of 72.89% and 41.33%, respectively. These caused a reduction in plant height by 76.07% and 44.22%, as well as a reduction in dry weight by 58.24% and 20.50%. There was a significant difference ($p < 0.05$) amongst the species and isolates of the same species. A low, yet significant negative correlation was observed both between disease severity and plant height ($r = -0.6463$, $p = 0.0004$) on the one hand and between disease severity and dry weight ($r = -0.5038$, $p = 0.0087$) on the other hand. **Conclusion:** The *Fusarium* species have caused varying degrees of infectivity on maize from a reduction in plant height to a reduction in dry weight with significant differences among the *Fusarium* species and isolates of the same species.

KEYWORDS

Phytopathogenic, pathogenicity, *Fusarium fujikuroi* species complex, disease severity index, yield losses, mycotoxins

INTRODUCTION

The fungal genus *Fusarium* is one of the most economically important fungal genera because of the yield losses it causes on crops due to its plant pathogenic activity. Of the top ten plant-pathogenic fungi, *Fusarium* has two spots while the other eight have one spot each, thus making it one of the most

important fungi affecting a huge diversity of crops worldwide[1]. *Fusarium* produces a wide range of mycotoxins in food and feed products which could cause fatal effects in humans and livestock[2]. Two *Fusarium* species namely *F. graminearum*, which is the causative agent of head blight of wheat, and *F. oxysporum*, which is the causative agent of a range of wilt and stem rot diseases have been listed in the world's top ten most important plant-pathogenic fungi[3]. The American Phytopathology Society's list of diseases of agricultural and horticultural crops shows that 83 of 108 plant species representing 76.85% of the plants surveyed have one or more *Fusarium* diseases affecting their production. *Fusarium* species produce a range of mycotoxins such as trichothecene and fumonisin notably[4]. The former is a protein synthesis inhibitor while the latter causes fatal livestock diseases and is considered potentially carcinogenic for humans[2,5]. Some *Fusarium* species also cause diseases with devastating effects in humans especially those who are immunocompromised[6].

Because of the foregoing, it is critically important for *Fusarium* to have a taxonomy that is stable for easy identification and diagnosis of diseases. This will lead to a proper management of the diseases caused by *Fusarium* species with a greater percentage of confidence. Unfortunately, this has not been the case because of the confusing taxonomy and systematics of *Fusarium*. For example, the genus has witnessed a decline in the number of species in the years between the 1920s and 1950s from a little more than a thousand species to nine species[7]. The application of multi-locus molecular phylogenetics in the past twenty years has revolutionized the studies of *Fusarium* species diversity, evolutionary relationships, and genealogical concordance phylogenetic species recognition[8], with only half of the estimated 300 genealogically exclusive phylogenetic species formally described[7,9].

The role of mycotoxins in plant-pathogen interaction is unclear but some researchers have shown that it plays a role to the benefit of the pathogen enabling it to progress in plant tissues[10,11]. This was demonstrated by Harris *et al.*[11] and Proctor *et al.*[12] in their work on trichothecene–producing fungi in which they showed that some *F. graminearum* strains caused more disease in maize than their trichothecene non-producing mutants that were pathogenic. Although Adams and Hart[13] have reported that deoxynivalenol (DON) was not a pathogenicity and virulence factor for *F. graminearum* in maize, Mesterhazy[10] has reported that there was a significant correlation between the virulence of *F. graminearum* and *F. culmorum* and their DON and nivalenol (NIV) content in wheat grain. The role of fumonisin in pathogenicity, phytotoxicity, and virulence is unclear even though it is phytotoxic in maize seedlings[14,15]. These researchers have further reported the inhibitory effect of fumonisin on root hair development and root growth. In contrast, Van-Asch *et al.*[16] have reported the phytotoxic effect of fumonisin on maize callus in culture with further phytotoxicity symptoms on seedlings being induced when the seedlings were watered with high concentrations of the mycotoxin even in the absence of the pathogen[15]. Fumonisin production by *F. verticillioides* is a necessity for the development of foliar disease symptoms in maize seedlings[17] but may not be essential for pathogenicity to maize seedlings as argued by Desjardins *et al.*[18] Further studies by these researchers have shown that the progeny of a *fum1* positive field strain of *F. verticillioides* with high levels of virulence were found to be associated with fumonisin production. Still, no progeny of a *fum1* negative field strain of *F. verticillioides* were observed to have high levels of virulence. The study aimed to investigate the pathogenicity of some *Fusarium* species isolated from the grassland biome of South Africa on maize.

MATERIALS AND METHODS

Study area: The study was conducted at the University of Johannesburg, South Africa between January, 2017 and December, 2020.

Fungal isolates and plant material: A collection of 26 *Fusarium fujikuroi* species complex (FFSC) isolates used in this study was sourced from the Plant Protection Research Institute (PPRI) of the Agricultural Research Council (ARC), Pretoria, South Africa. All the isolates *F. nygamai* (12), *F. bacteroides* (10), and

Table 1: *Fusarium* strains analyzed in this study indicate species and strain

Fusarium species	Strain[ac]
Fusarium nygamai	PPRI 19184[ac]
Fusarium nygamai	PPRI 19196[ac]
Fusarium nygamai	PPRI 19219[ac]
Fusarium nygamai	PPRI 20533[ac]
Fusarium nygamai	PPRI 20740[ac]
Fusarium nygamai	PPRI 21056[ac]
Fusarium nygamai	PPRI 21098[ac]
Fusarium nygamai	PPRI 21255[ac]
Fusarium nygamai	PPRI 21265[ac]
Fusarium nygamai	PPRI 21294[ac]
Fusarium nygamai	PPRI 21308[ac]
Fusarium nygamai	PPRI 21573[ac]
Fusarium bacteroides	PPRI 19149[ac]
Fusarium bacteroides	PPRI 19231[ac]
Fusarium bacteroides	PPRI 21033[ac]
Fusarium bacteroides	PPRI 21055[ac]
Fusarium bacteroides	PPRI 21071[ac]
Fusarium bacteroides	PPRI 21253[ac]
Fusarium bacteroides	PPRI 21272[ac]
Fusarium bacteroides	PPRI 21300[ac]
Fusarium bacteroides	PPRI 21306[ac]
Fusarium bacteroides	PPRI 22756[ac]
Fusarium burgessii	PPRI 21048[ac]
Fusarium burgessii	PPRI 21079[ac]
Fusarium burgessii	PPRI 21097[ac]
Fusarium burgessii	PPRI 21263[ac]

a: Plant Protection Research Institute (PPRI), Agricultural Research Council (RC), Pretoria, South Africa and c: Originated from willem pretorius nature reserve, free state, South Africa

F. burgessii (4) originated from the soils of the Willem Pretorius Nature Reserve, Free State Province, South Africa (Table 1). The isolates were tested in greenhouse trials for pathogenicity on commercial seeds of maize (cultivar "PAN 8816").

Inoculum preparation: The method of Nel *et al.*[19] was used to prepare the inoculum with slight modifications. The *Fusarium* isolates germinated from a single macroconidium were sub-cultured and grown on PDA under cool-white and near-ultra violet fluorescent lights at 25±°C for 7 days, after which the mycelia and conidia were harvested from the cultures by adding 20 mL of sterile distilled water and gently scraping the surface with a sterile Drigalski spatula into a sterile 100 mL beaker. The harvested mycelia and conidia were used to inoculate 250 g sterilized millet seeds in a 500 mL sterile Erlenmeyer flask. The flask was covered with cotton wool, wrapped with foil paper, and then incubated in the dark at 24°C for 24 days. The infested millet seeds were ground to powder using a blender (Salton 450W, Model: SB400E) and added to steam-sterilized soil at the rate of 22 g 1000/g soil. The inoculated soil was mixed and dispensed in 12.5 cm diameter plastic pots to produce a 2.2% inoculum concentration in the soil. The soil consisted of a mixture of potting and compost soil in a ratio of 1:1.

***In vivo* pathogenicity assay:** The greenhouse trials were carried out using three biological repeats (bioreps) with 2 week intervals between them. The trials consisted of three inoculated 12.5 cm plastic pots per isolate and each pot was planted with 5 seeds and was watered three times a week for 4 weeks. The experiments were laid out in a complete randomized design consisting of 26 treatments and three replications. The control consisted of three 12.5 cm plastic pots dispensed with ground un-inoculated sterilized millet seeds mixed with steam-sterilized soil at a 2.2% inoculum concentration in the soil.

Disease symptoms were visually assessed and rated on a scale of 0-5[2], with slight modifications in which 5 = Dead seedlings; 4 = 76-100% roots showing symptoms, and 3.1-$\geq$5 cm necrotic lesion size on leaf; 3 = 51-75% roots showing symptoms and 1.1-3.0 cm necrotic lesion size on leaf; 2 = 26-50% roots showing symptoms and 0.1-1.0 cm necrotic lesion size on leaf; 1 = 1-25% roots showing

symptoms; 0 = healthy plant with no symptoms. Disease severity, seed germination, plant height as well as dry weight were measured. Data were converted to a disease severity index score using the formula reported by Liu *et al.*[20]. The disease severity index was calculated from the rating by the following formula:

$$\text{Disease severity index (DI)} = \frac{\sum(\text{Rating number} \times \text{Number of plants in rating})}{\text{Total number of plants} \times \text{Highest rating}} \times 100$$

Microscopy

Killing and fixation: The Glycol Methacrylate (GMA) method[21] was used to prepare the root samples. Ten root samples were collected from the greenhouse at the end of the pathogenicity trials. Root pieces 2-4 cm long were cut and surface sterilized using 0.1% Sodium Hypochlorite (NaOCl), and rinsed three times with distilled water. The samples were then fixed in FAA fixative solution (10 mL of 40% formaldehyde, 5 mL of 99% glacial acetic acid, 50 mL of 99.9% ethanol, and 35 mL of sterile distilled water) for 48 hrs.

Dehydration: The samples were dehydrated by processing in a graded series of ethanol twice for 6 hrs in each of 70 and 100%. This was followed by processing in 100% n-propanol twice for 6 hrs and final processing in 100% n-butanol for 6 hrs.

Infiltration and embedding: The root samples were covered with GMA using the first infiltration GMA and placed in a dark cupboard for 24 hrs. The second and third infiltration GMA was used to replace the first infiltration GMA and placed in a dark cupboard for 24 hrs. The root samples were embedded in GMA in labeled gelatine capsules and placed in an oven ('Brickovens' - New York, USA) set at 60°C for 24 hrs.

Sectioning: Section (5 μm thick) was done using the microtome. Blocks were prepared in GMA in gelatine capsules and thin sections 5 μm thick were cut with the help of a microtome (SORVALL "PORTER-BLUM" Ultra Microtome MT-1 Connecticut, USA). At least 10 slides were prepared for each sample.

Staining and mounting: The slides were placed in saturated 2, 4-dinitrophenylhydrazine (DNPH) solution for 30 min and then washed 5 times in tap water. Slides were placed in 1% periodic acid solution for 10 min and washed 3 times in tap water. The slides were dissolved in Schiff's reagent for 30 min and followed by 31 min washes with tap water. The slides were dried on the slide warmer, stained with 0.05% toluidine blue for 3 min, and followed by three 1 min washes with tap water until the water was more or less colorless. The slides were dried overnight on the slide warmer. After drying the slides, they were mounted with an entellan synthetic resin, covered with cover slips, and viewed on the microscope. Observations were made using an OLYMPUS microscope (CX41RF, Tokyo, Japan) coupled to a camera (OLYMPUS Stream Essentials 2.2.3 Build 17023).

Statistical analysis: Data from the pathogenicity assay were subjected to Analysis of Variance (ANOVA) using the program Minitab 16 (Minitab Inc. Pennsylvania, USA). Disease index percentages were arcsine-transformed before ANOVA. Mean values in each treatment were compared by the Least Significant Difference (LSD) and Duncan's New Multiple Range (DNMR) test at a 5% ($p = 0.05$) level of significance. Pearson's linear correlation coefficient was used to evaluate the relationship between the disease severity index and the disease parameters

RESULTS

All the 26 *Fusarium fujikuroi* species complex (FFSC) isolates under investigation in this study originated from the Willem Pretorius Nature Reserve (Table 1) and were able to infect maize and produce characteristic disease symptoms such as disease severity index (Fig. 1), correlation of disease severity with plant height (Fig. 2), zero as well as low germination, root rots (Fig. 3), tip and blade necrotic lesions (Fig. 4), reduced/stunted aerial and root growth (Fig. 5), and reduced plant biomass (dry weight). The levels of aggressiveness differed between species and between isolates of the same species.
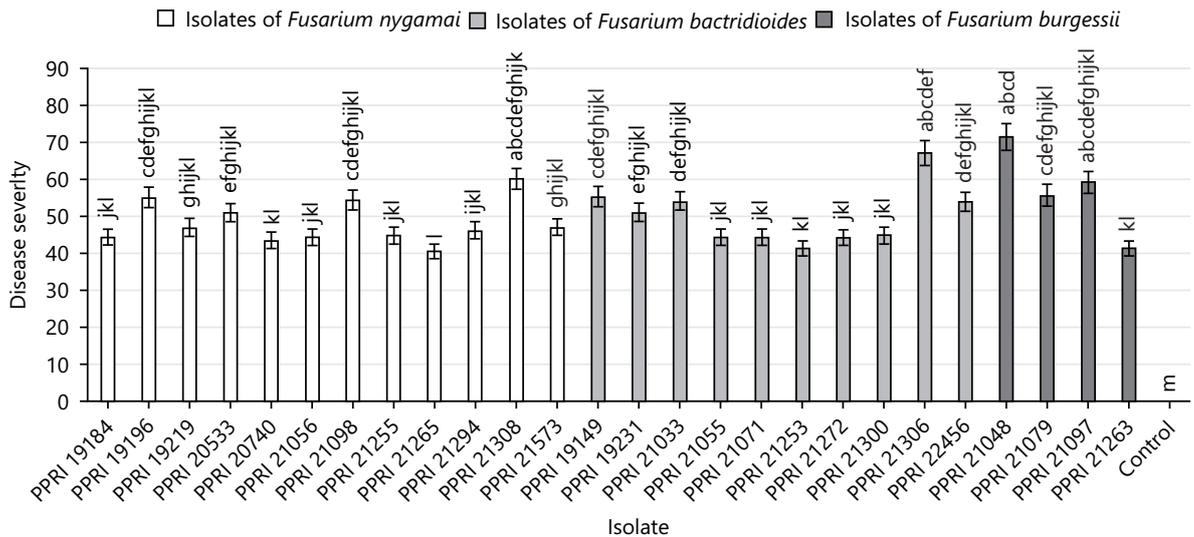
Fig 1: Effect of *Fusarium* species isolated from the grassland biome of South Africa on disease severity of maize

Means followed by the same letter(s) are not significant at a 5% level of significance (Duncan's Multiple Range Test)
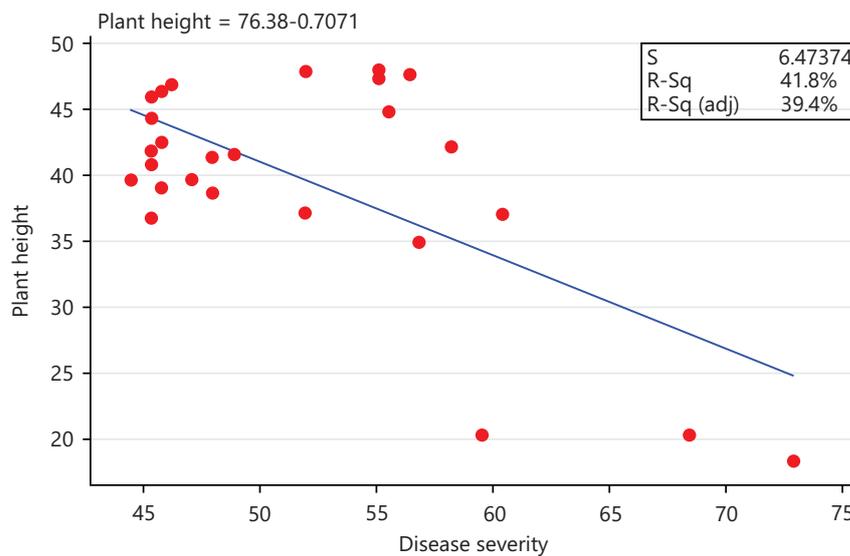

Fig. 2: Correlation of disease severity (%) with plant height (cm) of maize

A low, yet significant negative correlation between disease severity and plant height of maize was observed (r = - 0.6463, p = 0.0004)

In vivo pathogenicity assay

Disease severity: The highest maize disease severity among the three *Fusarium* species under investigation manifested by root rots symptoms (Fig. 3), as w ell as leaf tip and leaf blade necrotic lesions (Fig. 4) was observed in *F. burgessii* isolate PPRI 21048 (72.89%), followed by *F. bacteroides* and *F. nygamai* isolates PPRI 21306 and PPRI 21308 (68.44% and 61.33%), respectively (Fig. 1). These isolates were responsible for the r eduction in height of the plants (Fig. 5). The lowest disease severity among the three tested *Fusarium* species was observed in *F. nygamai* isolate PPRI 21265 (41.33%), followed by *F. bacteroides* isolate PPRI 21253 and *F. burgessii* isolate PPRI 21263 (42.22%) (Fig. 1). Further disease severity in maize included isolates PPRI 19196 of *F. nygamai* (56.11%); PPRI 19149 and PPRI 21033 of *F. bacteroides* (56.44% and 55.11%, respectively) and PPRI 21097 of *F. burgessii* (60.44%) (Fig. 1). There was a low, yet significant negative correlation between disease severity and plant height of maize (Fig. 2) (r = - 0.6463, p = 0.0004).
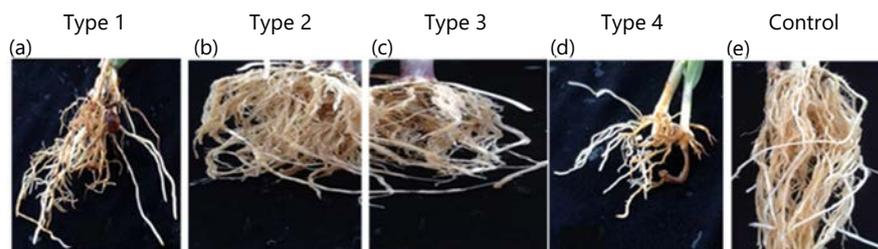
Fig. 3: Root rots symptoms caused by three *Fusarium* species on maize @28 DAI, (a) Type 1: Root rots symptoms on maize caused by *Fusarium nygamai* with up to 25% of the roots infected, (b) Type 2: Root rots symptoms on maize caused by *F. bacteroides* with up to 50% of the roots infected, (c) Type 3: Root rots symptoms on maize caused by *F. burgessii* with up to 75% of the roots infected, (d) Type 4: Root rots symptoms on maize caused by *F. nygamai* with >75% of the roots infected and (e) Control: No root rots symptoms
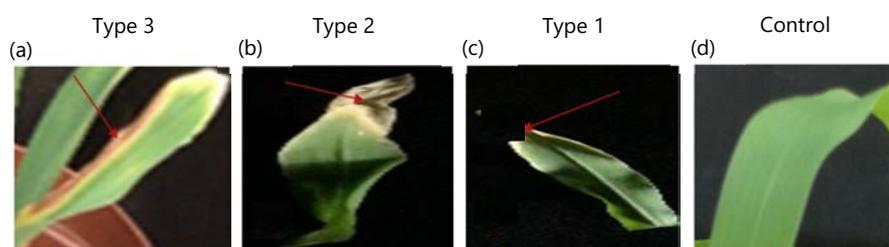

Fig. 4: Leaf tip and blade necrotic lesion symptoms caused by three *Fusarium* species @28 DAI, (a) Type 1: Leaf tip and blade necrotic lesion symptoms caused by *F. nygamai* with 0.1-1.0 cm necrotic lesion size, (b) Type 2: Leaf tip and blade necrotic lesion symptoms caused by *F. bacteroides with* 1.1-3.0 cm necrotic lesion size, (c) Type 3: Leaf tip and blade necrotic lesion symptoms caused by *F. burgessii with* 3.1 - >5 cm necrotic lesion size and (d) Control: No leaf tip or blade necrotic symptoms

Plant height: The isolates caused a reduction in both aerial growth and root growth (Fig. 3-5). The highest plant height was observed in *F. bacteroides* isolate PPRI 21033, (48.0267 cm), followed by the same *F. bacteroides* isolate PPRI 19231 (47.9333 cm) in second position, while *F. bacteroides* isolates PPRI 19149 and PPRI 22756 (47.6767 cm and 47.44 cm) emerged in the third and fourth positions, respectively (Fig. 6). These isolates caused the least damage thereby producing the tallest maize hosts. The lowest maize plant height was observed in *F. burgessii* isolate PPRI 21048 (18.2523 cm), followed by *F. bacteroides* isolate PPRI 21306 (20.2733 cm) in the second lowest position while *F. nygamai* isolates PPRI 21308 (20.3633 cm) emerged in the third lowest position (Fig. 6). These isolates caused more damage thereby producing the shortest hosts (Fig. 6). Infection of maize with *F. nygamai* isolates PPRI 21308, PPRI 21056 and PPRI 20533 produced plant heights of 20.36 cm, 36.79 cm and 37.12 cm which represent 26.7%, 48.23% and 48.66%, respectively, when compared with the control (76.28 cm). Similarly, infection with *F. bacteroides* isolates PPRI 21306 and PPRI 21272 produced plant heights of 20.27 cm and 40.73 cm which represent 26.57% and 53.39%, respectively, while maize plants infected with *F. burgessii* PPRI 21048 and PPRI 21079 produced heights of 18.25 cm and 34.98 cm which represent 23.92% and 45.86%, respectively (Fig. 6). There was a low, yet significant negative correlation between maize plant height and disease severity (Fig. 2) (r = -0.6463, p = 0.0004).

Dry weight: The highest dry weight among the three *Fusarium* species under investigation was observed in *F. nygamai* isolate PPRI 21265 (4.0837 g), followed by *F. bacteroides* isolate PPRI 21253 (3.819 g) in the second position while *F. nygamai* and *F. burgessii* isolate PPRI 19184 and PPRI 21263 (3.4823 and

Fig. 5: Stunted aerial and stunted root growth symptoms caused by *F. nygamai* (top), *F. bacteroides* (middle) and *F. burgessii* (bottom) on maize@28 DAI, (a) Stunted aerial growth caused by *F. nygamai,* (b) Stunted root growth caused by *F. nygamai*, (c) Stunted aerial growth caused by *F. bacteroides,* (d) Stunted root growth caused by *F. bacteroides*, (e) Stunted aerial growth caused by *F. burgessii* and (f) Stunted root growth caused by F. burgessii
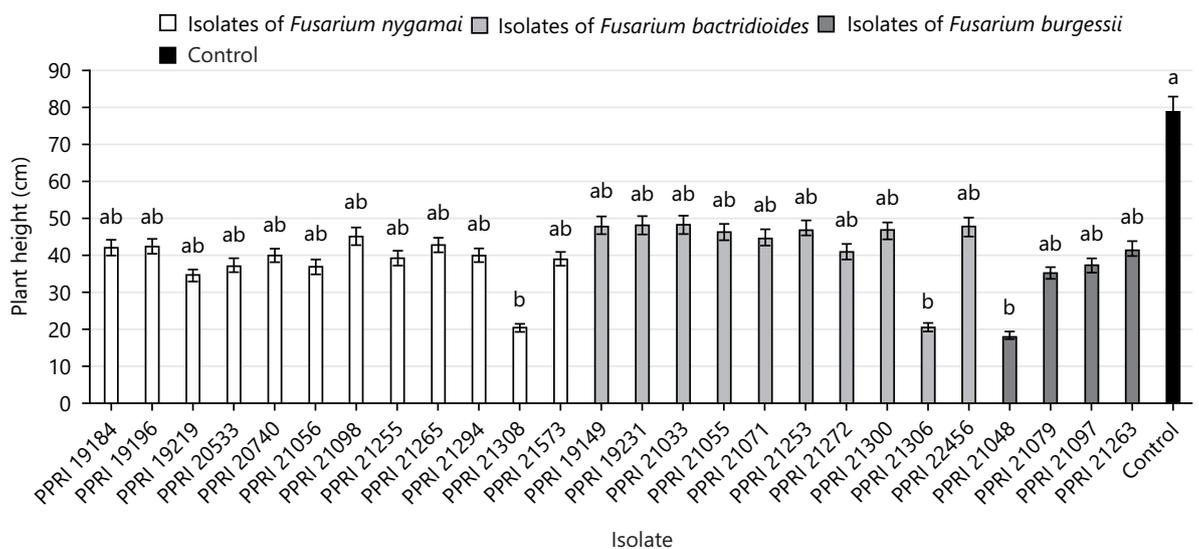

Fig 6: Effect of *Fusarium* species isolated from the grassland biome of South Africa on plant height of maize

Means followed by the same letter(s) are not significant at 5% level of significance (Duncan's Multiple Range Test)

3.4247 g) occupied the third and fourth positions, respectively (Fig. 7). These isolates caused least damage to their maize plant hosts. The lowest maize dry weight was observed in *F. burgessii* isolate PPRI 21048 (2.145 g), followed by *F. bacteroides* isolates PPRI 21306 and PPRI 21071 (2.3337 g and 2.4537 g) in the second and third lowest positions, respectively (Fig. 7). These isolates caused more damage to their maize plant host. Infection of maize plants with *Fusarium nygamai* isolates PPRI 21308, PPRI 21255, PPRI 21056, PPRI 21573 and PPRI 19196 produced reduced dry weights of 2.706 g, 2.7733 g, 2.816 g, 2.871 g and 2.9557 g, which represent 52.40%, 53.71%, 54.53%, 55.60%, and 57.24%, respectively, when compared to the control (5.1637 g) (Fig. 7). Similarly, *F. bacteroides* isolates PPRI 21306 and PPRI 21071 infection of maize plant hosts produced reduced dry weights of 2.3337 g and 2.4536 g which represent 45.19% and
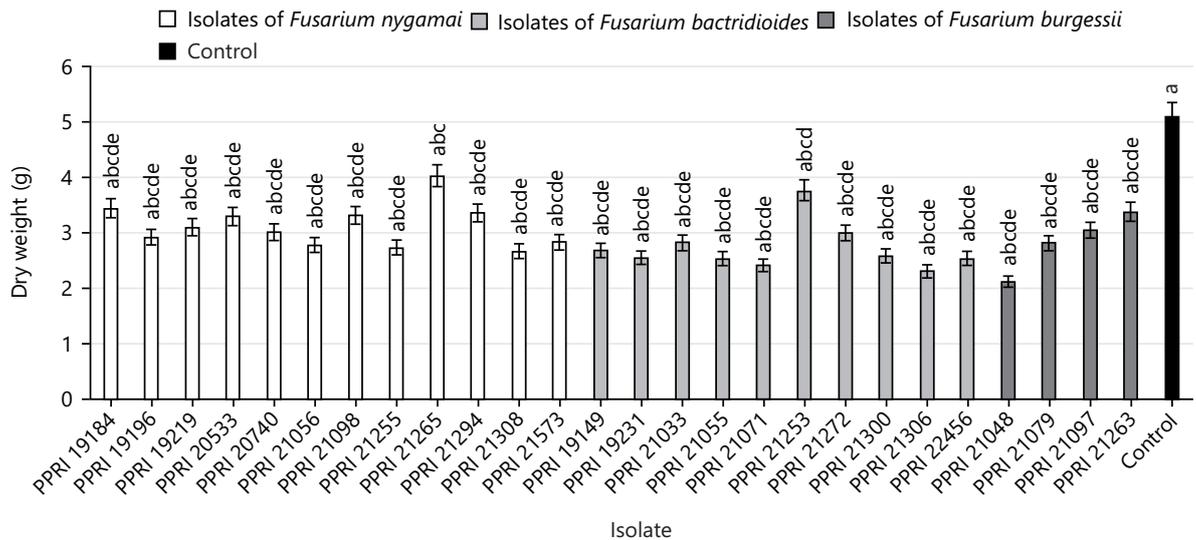
Fig 7: Effect of *Fusarium* species isolated from the grassland biome of South Africa on dry weight of maize

Means followed by the same letter(s) are not significant at a 5% level of significance (Duncan's Multiple Range Test)
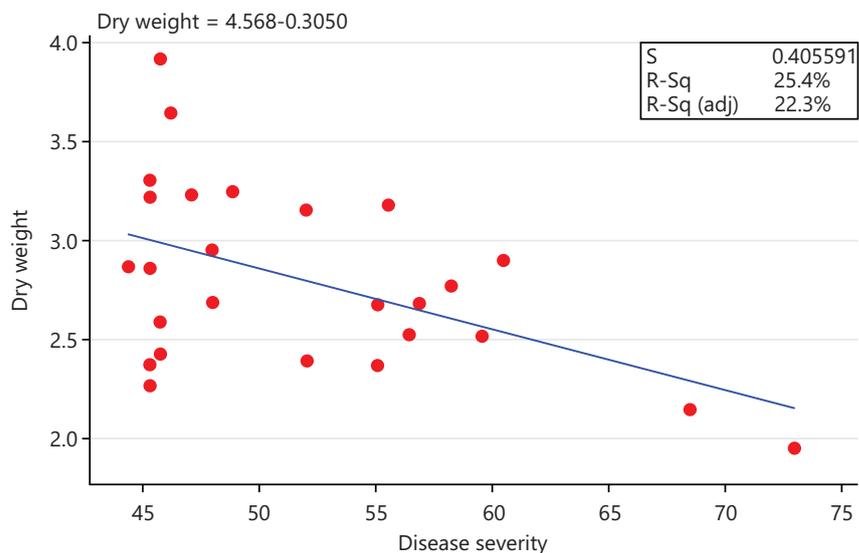

Fig. 8: Correlation of disease severity with dry weight of maize

A low, yet significant negative correlation was observed between disease severity and dry weight (r = -0.5038, p = 0.0087)

47.52%, respectively, whereas infection with *F. burgessii* isolates PPRI 21048 and PPRI 21079 resulted in a reduction in dry weights of 2.145 g and 2.8607 g, which represent 41.54% and 55.40%, respectively, when compared with the control. There was no significant difference (p>0.05) between isolates of the same species. A low, yet significant negative correlation was observed between disease severity and dry weight (Fig. 8) (r = -0.5038, p = 0.0087).

Germination efficiency: The highest maize seed germination efficiency among the three *Fusarium* species under investigation was observed in *F. bacteroides* isolates PPRI 21071 and PPRI 21033 (4.7778 and 4.6667) in the first and second positions, respectively, while four *F. bacteroides* isolates PPRI 19231, PPRI 21055, PPRI 21300 and PPRI 22756 tied in the third position with 4.4444 (Fig. 9). Infection of maize with *F. nygamai* isolates PPRI 20533, PPRI 21294 and PPRI 21573 produced reduced seed germination of 2.3333, 2.7778 and 3.1111, which represent 46.67%, 55.56% and 62.22%, respectively, when compared to the control (5.0000) (Fig. 9). Infection of maize with *F. bacteroides* isolates PPRI 21272 and PPRI 21253
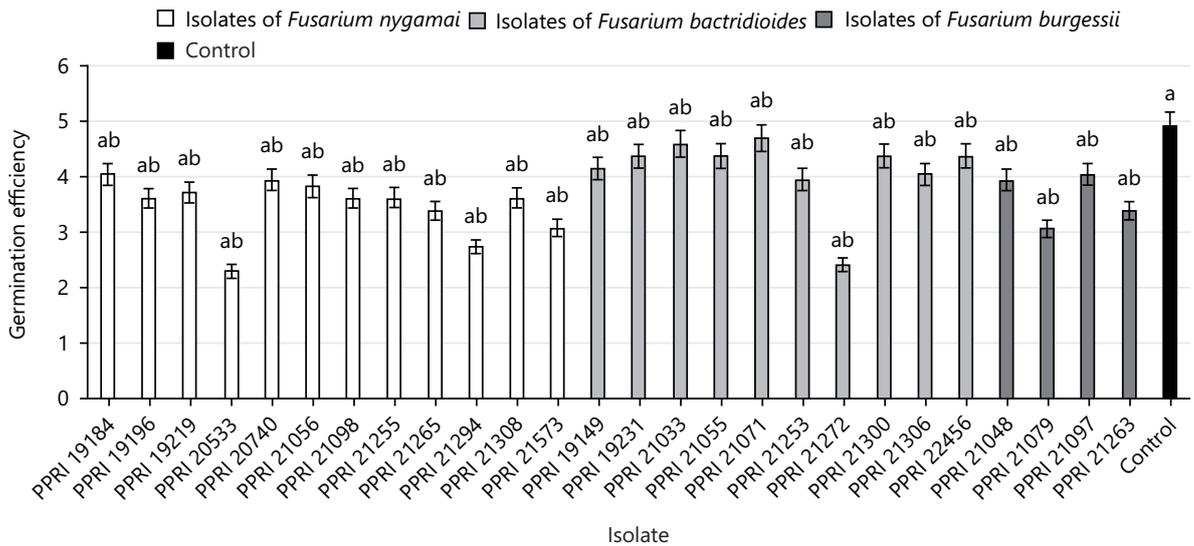
Fig 9: Effect of *Fusarium* spp isolated from the grassland biome of South Africa on germination efficiency of maize

Means followed by the same letter(s) are not significant at a 5% level of significance (Duncan's Multiple Range Test)
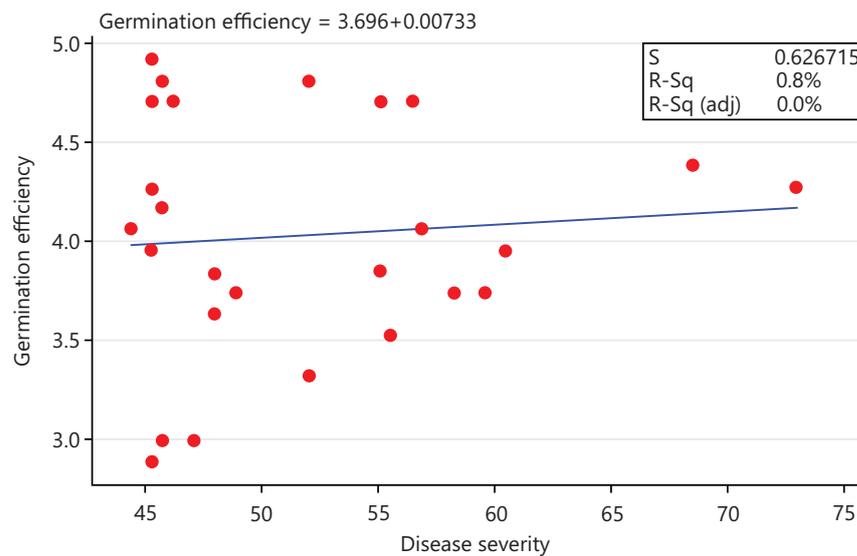

Fig. 10: Correlation of disease severity with germination of maize

A low, yet not significant positive correlation was observed between the maize germination efficiency and disease severity (r = 0.0904, p = 0.6606)

produced reduced seed germination of 2.4444 and 4.0000, which represent 48.89% and 80%, while maize plants infected with *F. burgessii* isolates PPRI 21079 and 21263 produced seed germination of 3.1111 and 3.4444, which represent 62.22% and 68.89%, respectively (Fig. 9). There was no significant difference (p>0.05) between the isolates of the same species. A low, yet not significant positive correlation was observed between the maize germination efficiency and disease severity (Fig. 10) (r = 0.0904, p = 0.6606).

Microscopy: At 28 DAI the test isolates had demonstrated different levels of colonization of the different root tissues of the host. *Fusarum nygamai* hyphae localized colonization in the cells of the cortex only (Fig. 11a), whereas *F. bacteroides* has already invaded and digested most of the cells of the cortex creating gaps within the cortex (Fig. 11b). *Fusarum bacteroides* was restricted to the cortex and was not able to penetrate the endodermal layer to colonize the central cylinder (Fig. 11b). *Fusarum burgessii* at this
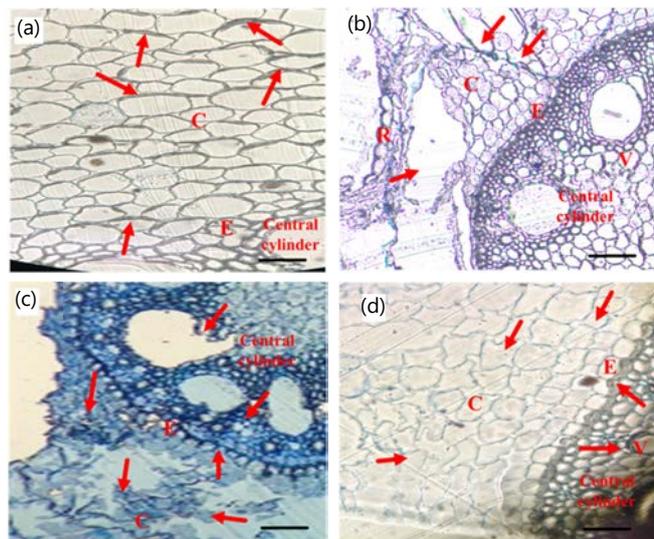
Fig. 11: Cross-section colonization of maize tissues by hyphae of three *Fusarium* species @28 DAI,
(a) Extensive colonization of cells of the cortex by *F. nygamai* hyphae (arrows) at 28 DAI,
(b) Infection with *F. bacteroides*, (c) Infection with *F. burgessii* and (d) Part of the control root

stage had not only colonized and digested most of the cells of the cortex but had also penetrated the endodermal thick-walled layer and entered the central cylinder (Fig. 11c). *Fusarum burgessii* had massively destroyed the cortical cells and created gaps leaving the remnant of cells within the cortex (Fig. 11c). The maize control root tissues were intact and completely free of colonization by hyphae (Fig. 11d).

Fusarum bacteroides has caused digestion of the cells of the cortex leaving some gaps and remnants of undigested cells in the cortex (arrows) at 28 DAI. The central cylinder was also colonized by hyphae. Infection with *F. burgessii* caused massive digestion and degradation of the cells of the cortex leaving gaps and remnants of cortical cells in the cortex (arrows). The inner thick wall of the endodermal cells (E) was reduced to a thin layer. The cells of the central cylinder were also invaded (arrows).

Part of the control root is shown devoid of hyphae in the cortex (C), the endodermis (E), and the central cylinder which are all intact. The thick-walled endodermis (E) separates the cortex from the central cylinder. V, vessels; R, rhizodermis; a, b, c, and d are cross sections of maize roots infected with *F. nygamai*, *F. bacteroides*, *F. burgessii,* and the control, respectively. Scale bars = 50 μm.

DISCUSSION

Many *Fusarium* species have been associated with grain crops in South Africa[22]. Those that affect maize include *F. verticillioides* (Sacc.) Nirenberg; *F. subglutinans* (Wollenweber & Reinking) Nelson, Toussan and Marasas. Infection with F. burgessii (PPRI 21048) and F. nygamai (PPRI 21265) resulted in the highest (72.89%) and lowest (41.33%) disease severity indices, reducing plant height by 76.07% and 44.22% and dry weight by 58.24% and 20.50%, respectively. Significant differences ($p < 0.05$) were observed among species and isolates, with a negative correlation between disease severity and both plant height ($r = -0.6463$, $p = 0.0004$) and dry weight ($r = -0.5038$, $p = 0.0087$).

The recovery of more isolates of *F. nygamai* (46.15%) than the isolates of *F. bacteroides* (38.46%) and *F. burgessii* (15.38%) in the roots coupled with the fact that *F. nygamai* limited its infection to the epidermal and cortical cells, in this study, refutes the report of Leyva-Madrigal *et al.*[23] in which they showed the ability of *F. nygamai* to colonize the vascular tissues among two other *Fusarium* pathogens of cereal crops that only limited their infection to the epidermis and adjacent cortical cells.

The isolates of *F. bacteroides* and *F. burgessii* have only been associated with root rot and leaf tip and blade necrosis in maize in South Africa in this study. The plants infected with the two latter *Fusarium* species developed the same symptomatology as the plants infected with *F. nygamai* (root rot, leaf tip, and blade necrotic lesion, and reduced aerial and root growth) but at varying degrees of infectivity. In this study, the isolates PPRI 21048 of *F. burgessii*, PPRI 21306 of *F. bacteroides* and PPRI 21308 of *F. nygamai* which produced the highest disease severity also produced the lowest plant height and plant biomass (dry weight) which was consistent with the report of Leyva-Madrigal *et al.*[23].

The mean seed germination showed that there was no significant difference (p>0.05) between species and among isolates of the same species (Fig. 9). No correlation was observed between the disease severity and seed germination (Fig. 10) (r = 0.0904, p = 0.6606),

The low, yet significant negative correlation observed in this study between disease severity and plant height (Fig. 2) (r = -0.6463, P = 0.0004), was consistent with the report of Leyva-Madrigal *et al.*[23] where they observed a low, significant negative correlation between plant height of maize and the disease severity in a mixed infection trials of maize with *F. verticillioides*, *F. nygamai*, *F. thapsinum* and *F. andiyazi* in Mexico.

Infection with *F. burgessii* isolate PPRI 21048 produced the most reduced dry weight (biomass). A significant negative correlation was observed between disease severity and dry weight (Fig. 8) (r = -0.5038, p = 0.0087). This refutes the report of Leyva-Madrigal *et al.*[23] in which they observed no correlation between disease severity and shoot and root biomass in maize mixed infection trials. *Fusarium nygamai* infection was limited to the cortex, whereas *F. bacteroides* and *F. burgessii* had access to the central cylinder confirming previous observations of Elzein *et al.*[24]. Cell wall degrading enzymes (CWDEs) such as xylanases, and proteases and polygalacturonases are secreted by *Fusarium* species during the penetration and colonization of host roots[25]. The ability of *F. nygamai* to colonize and digest cortical cells but not the endodermal cells and the central cylinder could be attributed to the fact that the enzymes required were not expressed at the point of the infection cycle or that the enzymes required were absent as can be evidenced by the report of Ruiz-Roldan *et al.*[26] where they demonstrated the differential expression of two genes *xyl3* and *xyl2* which encode xylanases in tomatoes infected with *F. oxysporum* f. sp. *lycopersici*. The former gene was expressed throughout the disease cycle while the latter gene was expressed only during the final stages of the disease cycle, which is an indication that genes are differentially expressed during a disease cycle.

To the best of our knowledge, this is the first report of the infectivity of these three *Fusarium* species in the grassland biome of South Africa. Pathogenicity of *F. nygamai* on sorghum in South Africa has been reported earlier[22] but there is little or no accompanying data regarding the pathogenicity of *F. bacteroides* and *F. burgessii* on grain crops in South Africa[22].

Fusarims burgessii has shown more aggressiveness followed by *F. bacteroides* and *F. nygamai* in that order in this study. The low aggressiveness of *F. nygamai* compared with its infectivity in the report of Beukes *et al.*[22] could be attributed to the differences in host preferences[27,28]. Further pathogenicity trials on other grain crops are suggested to enable robust accompanying data on the pathogenicity of the members of the FFSC on grain crops in South Africa.

CONCLUSION

Fusarium species exhibited varying degrees of pathogenicity on maize in Gauteng, South Africa, causing significant reductions in plant height and dry weight. Among the species studied, *F. burgessii* caused the most severe disease. This is the first report of the pathogenicity of these Fusarium species on maize in this

region, highlighting the need for further studies on their impact and pathogenicity on diverse grain crops. Such investigations are essential to address food security challenges and improve maize production in the area.

SIGNIFICANCE STATEMENT

This study highlights the significant impact of *Fusarium* species, particularly *F. burgessii*, on maize production in Gauteng, South Africa. These pathogens not only cause severe yield losses but also produce mycotoxins that threaten food safety and security. As the first report of these species' pathogenicity on maize in the region, it underscores the need for further research to develop effective management strategies and support sustainable maize production.

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