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Wide variation in aggressiveness and growth in South African *Fusarium circinatum* isolates with geographical origin as the primary determinant

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***Fusarium circinatum* is a globally important fungal pathogen that causes pitch canker on a wide range of *Pinus* species. In this study, we considered mycelial growth in culture and aggressiveness of a South African population of *F. circinatum* isolates. The specific aim was to determine how these phenotypes are correlated with one another and other isolate characteristics, including mating type, host species and geographic origin of the isolates. The study involved the selection of 102 isolates of *F. circinatum* from five provinces of South Africa based on genotype, mating type and original host from which they were isolated, after which pathogenicity tests were carried out on *Pinus patula* seedlings. Mycelial growth in culture was also determined for all the isolates at three different temperatures. In the pathogenicity tests, most of the isolates produced significant lesions. While seven had low levels of aggressiveness, other isolates tested were as or more aggressive than a standard set of isolates routinely used for resistance/tolerance screening. Most *F. circinatum* isolates grew best at 25 °C, followed by growth at 20 °C and 30 °C. A notable correlation between aggressiveness and the geographical origin was identified. Moreover, the study highlighted a moderately positive relationship between the growth rate of *F. circinatum* isolates and their reported aggressiveness. The extensive data gathered on the aggressiveness of *F. circinatum* will offer a broad range of isolates for future comparative studies using other genotypes of *F. circinatum* and will also aid in the consideration of *F. circinatum* isolates used for tolerance screening studies.**

Key words*:* pitch canker, pathogenicity, mycelial growth, *Pinus*

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Introduction

The pitch canker fungus *Fusarium circinatum* is an important pathogen of *Pinus* species in many countries around the world (Wingfield et al. 2008; Drenkhan et al. 2020). In natural stands and plantations, infections typically manifest as cankers associated with resin-impregnated wood (Hepting and Roth 1946). In seedlings, the fungus causes serious root and root collar disease, which can be an important constraint to the propagation of *Pinus* species (Wingfield et al. 2008; Drenkhan et al. 2020). In South Africa, for example, *F. circinatum* severely impacts seedling production in commercial nurseries, while the deployment of apparently healthy but infected plants further hampers plantation establishment (Viljoen 1994; Mitchell 2005). In the case of *Pinus patula*, the most widely cultivated *Pinus* species in South Africa, about a quarter of all seedlings succumb to *F. circinatum*-associated root and root collar disease within the first year of being planted in the field (Crous 2005; Mitchell et al. 2012a).

The first occurrence of *F. circinatum* in South Africa was in 1990 on *P. patula* seedlings in a nursery in Mpumalanga (Viljoen 1994, Viljoen et al. 1997). The pathogen subsequently spread to nurseries in most pine-growing areas of the country and to many different *Pinus* species (Steenkamp et al. 2014; Santana et al. 2016; Fru et al. 2017). *F. circinatum* remained a nursery and plantation establishment problem for more than a decade and was only reported as a causal agent of pitch canker on mature plantation trees in 2007 (Coutinho et al. 2007). The disease is now established in plantations in various parts of the country, where it mainly affects susceptible species, including *P. greggii*, *P. radiata* and *P. patula* (Wingfield et al. 2008; Steenkamp et al. 2014; Santana et al. 2016; Fru et al. 2017; Fru et al. 2019).

To minimise the impact of *F. circinatum*-associated disease in the plantation forestry setting, tolerant or resistant planting stock is developed through *Pinus* breeding programmes (Vivas et al. 2011; Nel et al. 2014). These efforts exploit the

natural variation in susceptibility to *F. circinatum* that exists within and between *Pinus* species (Schmale III and Gordon 2003; Aegerter and Gordon 2006; Roux et al. 2007). In South Africa, screening of *Pinus* planting stock for tolerance to *F. circinatum* relies mainly on the use of three widely used aggressive strains, previously isolated from diseased *Pinus* tissue in the country (Roux et al. 2007; Mitchell et al. 2012b; Mitchell et al. 2014, Nel et al. 2014). The South African *Pinus* breeding programmes would benefit substantially from expanding the set of *F. circinatum* strains used for resistance screening, given the pathogen's high genetic diversity (Drenkhan et al. 2020).

A useful *F. circinatum* trait that could be considered in *Pinus* breeding programmes is strain aggressiveness (i.e. the quantitative component of pathogenicity) (Pariaud et al. 2009). Indeed, the aggressiveness of *F. circinatum* has been a topic of interest since its description, using lesion length caused by artificial inoculation with the fungus as an indicator of host susceptibility (Schmale III and Gordon 2003). Several studies worldwide have reported *F. circinatum* to be aggressive on many *Pinus* species (Drenkhan et al. 2020), although the level of variation depends on the host and other environmental factors (Amaral et al. 2019). In South Africa, however, the aggressiveness of only a few isolates has been tested (Coutinho et al. 2007; Herron et al. 2015; Fru et al. 2017; Herron et al. 2020), and relatively little is known regarding the virulence and pathogenicity of *F. circinatum*.

For certain fungal pathogens, strain aggressiveness is strongly correlated with traits that can easily be diagnosed in the laboratory. For example, mating type and growth in culture can be used as a proxy for the pathogen's aggressiveness, where faster-growing isolates or isolates having a particular mating type are more aggressive to their host plants (Alvarez-Perez et al. 2010; Lee et al. 2015; Zhan et al. 2016). Whether such a correlation between strain aggressiveness and other traits is also found in *F. circinatum* is unknown. Numerous studies agree that *F. circinatum* grows optimally at warmer temperatures such as between 20 and 25 °C (Inman et al. 2008; Berbegal et al. 2013; Mullett et al. 2017; Elvira-Recuenco et al. 2021) and that this is not affected by the geographic origins of the isolates or their genotypes. A tentative correlation between mating type and strain aggressiveness in *F. circinatum* from Spain has also been suggested (Pérez-Sierra et al. 2007). In South Africa, however, little is known regarding the growth profiles of the population of *F. circinatum* in culture and how they might be influenced by temperature. Also, the possible link between strain aggressiveness and mating type has not been evaluated for South African isolates of *F. circinatum*.

The primary aim of this study was therefore to compare the relative aggressiveness of the South African *F. circinatum* by making use of a collection of isolates that are representative of local populations of the fungus. These representatives were defined as isolates with different microsatellite-based genotypes and originating from different *Pinus* hosts and geographic locations. A secondary aim was to determine whether strain aggressiveness is correlated with mating type, location and growth in culture. Broadly, these questions sought to fine-tune resistance screening methodologies to the benefit of *Pinus* breeding programmes in South Africa.

Materials and methods

Fungal isolates

A total of 102 *F. circinatum* isolates were used in this study (Table 1). Of these, 99 were from previous studies (Santana et al. 2016; Fru et al. 2017; Fru et al. 2019; Fru et al. 2023) and were selected to span the diversity of *F. circinatum* in South Africa in terms of genotype (vegetative compatibility and/or microsatellite-based) and mating type, as well as the location, original host plant species and type of plant tissue from which they had been isolated. In terms of host species, 50 of the isolates were obtained from *P. patula*, 38 from *P. greggii* and 14 from *P. radiata* spanning various locations in the Limpopo, Mpumalanga, KwaZulu-Natal, Eastern Cape and Western Cape provinces. While most of the isolates came from the diseased tissues of plantation trees of varying ages, 36 isolates came from nursery seedlings displaying root or root collar disease. The remaining three *F. circinatum* isolates (CMWF1217, also known as FCC3577; CMWF1218, also known as FCC3578; and CMWF1219, also known as FCC3579) were those that are routinely used to screen *Pinus* seedlings in South Africa for tolerance to infection (Porter et al. 2009; Mitchell et al. 2014; Nel et al. 2014). Isolates were routinely grown on half-strength potato dextrose agar (1/2 PDA; Sigma-Aldrich ™) at 25 °C. All isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

The identity and mating type of each isolate was confirmed using routine DNA-based methods. For this purpose, a portion of the gene encoding transcription elongation factor-1α (TEF-1α) was sequenced as described previously (Fru et al. 2017) and compared against verified *F. circinatum* sequences in the nucleotide database of the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST) (Johnson et al. 2008). The mating type of the isolates was confirmed using whole genome sequence information of the isolates (unpublished data). For isolates where genome sequences were not available, mating type was determined using the PCR-based method described by Steenkamp et al. (2000).

Growth in culture

Mycelial growth of all *F. circinatum* isolates was assessed at 20 °C, 25 °C and 30 °C. These temperatures were selected based on the study by De Vos et al. (2011). Mycelial plugs (6 mm in diameter) were cut from seven-day-old cultures using a sterile cork borer and placed onto 1/2 PDA medium (with mycelium facing the medium). Each isolate was inoculated onto ten 1/2 PDA-containing Petri dishes, and these were incubated for seven days in the dark. Following incubation, colony diameter was recorded by taking the average of two measurements (in millimetres) perpendicular to each other. The average mycelial growth of the *F. circinatum* isolates at the three temperatures tested was compared to identify the optimal growth temperature for the isolates.

Aggressiveness of isolates

The aggressiveness of all the isolates was tested on six-month-old *P. patula* seedlings. This was achieved by growing

Table 1: Mating type, *Pinus* host and geographic origin in South Africa of the *Fusarium circinatum* isolates used in this study

Isolate number[1]	Mating type	Geographic origin[2]	*Pinus* species	References
CMWF1807, CMWF1806	MAT1-2	Demagtenberg (KZN)	*P. greggii*	Fru et al. (2017)
CMWF2602 (553F)	MAT1-1	Tweefontein (KZN)	*P. greggii*	Fru et al. (2023)
CMWF2598 (507F), CMWF2600 (535F), CMWF2597 (495F), CMWF2599 (514F)	MAT1-2	Tweefontein (KZN)	*P. greggii*	Fru et al. (2023)
CMWF2591 (184F), CMWF2592 (192F), CMWF2590 (109F), CMWF2584 (25F), CMWF2589 (89F)	MAT1-1	Tweefontein (KZN)	*P. greggii*	Fru et al. (2023)
CMWF2586 (33F), CMWF2594 (336F), CMWF2588 (87F), CMWF2587 (61F), CMWF2585 (18F)	MAT1-2	Tweefontein (KZN)	*P. greggii*	Fru et al. (2023)
CMWF2617 (742F), CMWF2613 (728F)	MAT1-1	Martindale (L)	*P. patula*	Fru et al. (2019)
CMWF2618 (747F), CMWF2620 (757F), CMWF2616 (740F), CMWF2619 (753F)	MAT1-2	Martindale (L)	*P. patula*	Fru et al. (2019)
CMWF2615 (714F), CMWF2614 (719F), CMWF2612 (713F)	MAT1-1	Soutpansberg (L)	*P. patula*	Fru et al. (2019)
CMWF2604 (583F), CMWF2595 (447F), CMWF2596 (460F)	MAT1-1	Soutpansberg (L)	*P. patula*	Fru et al. (2019)
CMWF2605 (584F)	MAT1-2	Soutpansberg (L)	*P. patula*	Fru et al. (2019)
CMWF2607 (590F)	MAT1-1	Soutpansberg (L)	*P. patula*	Fru et al. (2019)
CMWF2606 (585F), CMWF2609 (702F), CMWF2608 (701F)	MAT1-2	Soutpansberg (L)	*P. patula*	Fru et al. (2019)
CMWF2610 (703F)	MAT1-1	Soutpansberg (L)	*P. patula*	Fru et al. (2019)
CMWF2603 (582F)	MAT1-2	Soutpansberg (L)	*P. patula*	Fru et al. (2019)
CMWF2630 (854F)	MAT1-1	Rooihoogte (M)	*P. patula*	Fru et al. (2019)
CMWF2629 (851F), CMWF2628 (848F)	MAT1-2	Rooihoogte (M)	*P. patula*	Fru et al. (2023)
CMWF2626 (788F), CMWF2623 (779F)	MAT1-2	Sabie (M)	*P. patula*	Fru et al. (2023)
CMWF2624 (783F), CMWF2627 (801F), CMWF2625 (784F), CMWF2621 (766F)	MAT1-1	Sabie (M)	*P. patula*	Fru et al. (2023)
CMWF2581 (3DC), CMWF2582 (5DC)	MAT1-1	Ngodwana (M)	*P. patula*	Fru et al. (2023)
CMWF2583 (9DC)	MAT1-2	Ngodwana (M)	*P. patula*	Fru et al. (2023)
CMWF35 (FCC124), CMWF23 (FCC514), CMWF19 (FCC542), CMWF15 (FCC479), CMWF11 (FCC341), CMWF36 (FCC13), CMWF54 (FCC521)	MAT1-1	Ngodwana (M)	*P. patula*	Santana et al. (2016)
CMWF61 (FCC1034), CMWF51 (FCC1052), CMWF34 (FCC52), CMWF76 (FCC560), CMWF41 (FCC493), CMWF49 (FCC321), CMWF24 (FCC1035)	MAT1-2	Ngodwana (M)	*P. patula*	Santana et al. (2016)
CMWF6 (FCC62)	MAT1-1	Sabie (M)	*P. greggii*	Santana et al. (2016)
CMWF22 (FCC477), CMWF29 (FCC65), CMWF56 (FCC51)	MAT1-2	Sabie (M)	*P. patula*	Santana et al. (2016)
CMWF1219 (FCC3579), CMWF1217 (FCC3577)	MAT1-1	Ngodwana (M)	*P. patula*	Porter et al. (2009), Mitchell et al. (2014)
CMWF1218 (FCC3578)	MAT1-2	Ngodwana (M)	*P. patula*	Mitchell et al. (2014)
CMWF568 (L53A4), CMWF541 (O6), CMWF590 (KS51), CMWF515 (B1-8), CMWF535 (CBH19)	MAT1-1	George (WC)	*P. radiata*	Santana et al. (2016)
CMWF534 (CBH11), CMWF577 (BG-2)	MAT1-2	George (WC)	*P. radiata*	Santana et al. (2016)
CMWF573 (B2H6), CMWF660 (11-2)	MAT1-1	Tokai (WC)	*P. radiata*	Santana et al. (2016)
CMWF636 (20-2i), CMWF588 (KS49), CMWF594 (KS22)	MAT1-1	Karatara (WC)	*P. radiata*	Santana et al. (2016)
CMWF674 (KS17), CMWF597 (KS40)	MAT1-2	Karatara (WC)	*P. radiata*	Santana et al. (2016)
CMWF2640 (UG17), CMWF2641 (UG18)	MAT1-1	Ugie (EC)	*P. greggii*	Fru et al. (2017)
CMWF2643 (UG27), CMWF2639 (UG10), CMWF2642 (UG23), CMWF2644 (UG32)	MAT1-2	Ugie (EC)	*P. greggii*	Santana et al. (2016)
CMWF2650 (8.1.2), CMWF2657 (17.5), CMWF2645 (3.1.4), CMWF2651 (8.1.6), CMWF2656 (14.1), CMWF2655 (13.8), CMWF2649 (8.1.1), CMWF2646 (4.1.1)	MAT1-1	Maclear (EC)	*P. greggii*	Santana et al. (2016)
CMWF2653 (12.2), CMWF2654 (12.3), CMWF2647 (6.1.1), CMWF2648 (6.2.1), CMWF2652 (8.2.1)	MAT1-2	Maclear (EC)	*P. greggii*	Santana et al. (2016)

[1] All isolates are available in the CMWF collection at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Alternative strain numbers are indicated in brackets

[2] Provinces: EC = Eastern Cape, KZN = KwaZulu-Natal, L = Limpopo, M = Mpumalanga, WC = Western Cape

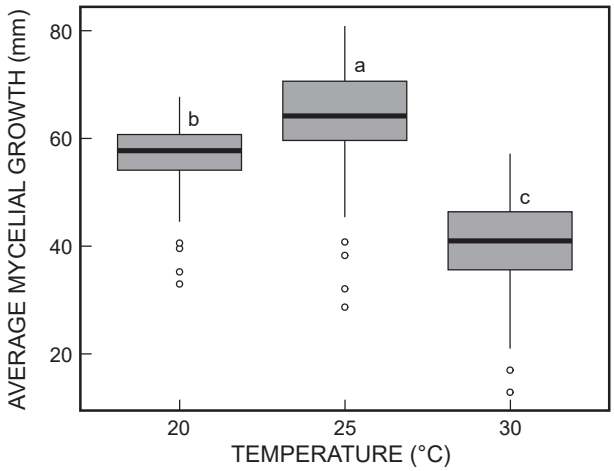

Figure 1: Box and whisker plot of the distribution of *F. circinatum* growth at three temperatures (20 °C, 25 °C and 30 °C)

the isolates on 1/2 PDA medium for seven days in the dark at 25 °C. Using 15% glycerol (v/v), spores were dislodged from the surface of the cultures and quantified using a hemocytometer. For each isolate, the concentration of the spore suspensions was adjusted to 5 x 10^4 spores/ml using sterile distilled water. Seedling tip inoculations were subsequently conducted by applying 10 µl of the spore suspension onto a wound made by excising the plant tips with sterile scissors (Porter et al. 2009). For each isolate, 20 seedlings were inoculated, and the entire trial was repeated once. For the control treatments, seedlings were inoculated with 10 µl of 15% glycerol. Inoculated seedlings were kept in a plant growth tunnel for three weeks, after which lesion lengths were measured.

Statistical analysis

All statistical analyses were performed using R software version 4.3.0 (R Core Team 2021), with Rstudio version 1.4.1717 (RStudio Team 2016) serving as the graphical user interface. To assess whether the data obtained for aggressiveness of *F. circinatum* isolates tested in the two trials could be combined, a Levene's test was used to test the equality of variances. Furthermore, a multivariate analysis of variance (MANOVA) was used to determine the relationship between these two traits (mycelial growth at 25 °C and lesion length) and the isolate metadata (mating type, original host and geographic origin). Where *P*-values were significant ($p < 0.05$), Tukey HSD post-hoc tests were performed. Additionally, regression analysis was used to explore any possible correlation between the growth of *F. circinatum* at 25 °C and the aggressiveness of the isolates.

Results

Fungal isolates

Analysis of TEF-1α sequences confirmed that all 102 isolates were *F. circinatum* (Table 1), of which 98 represented distinct microsatellite-based genotypes reported previously (Santana et al. 2016; Fru et al. 2017; Fru et al. 2019; Fru et al. 2023). Microsatellite data were not available for the three isolates

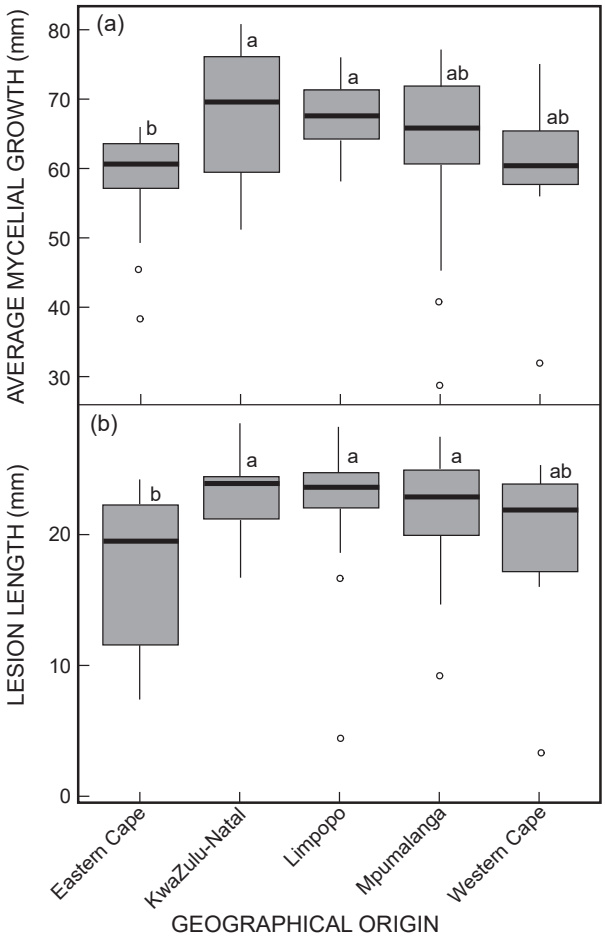

Figure 2: Box and whisker plots of the distribution of (a) *F. circinatum* growth at 25 °C and (b) the lesion lengths, obtained from the pathogenicity assay, when isolates were grouped according to their geographical origins

routinely used for screening (CMWF1217, CMWF1218 and CMWF1219) and one isolate (CMWF674) from the Western Cape province. Nevertheless, of the 102 isolates included, 53 had the MAT1-1 and 49 isolates the MAT1-2 idiomorphs.

Growth in culture

The mycelial growth in culture of *F. circinatum* on average was notably faster at 25 °C, with an overall mean colony diameter of 63.98 ± 9.39 mm, compared to growth at 20 °C (56.80 ± 6.31 mm) and 30 °C (40.35 ± 7.75 mm) as depicted in Figure 1. Specifically, when comparing growth at 20 °C and 25 °C, only 81 of the isolates grew significantly faster ($p < 0.05$) at 25 °C, while 14 isolates showed no significant differences in mycelial growth at 20 °C and 25 °C ($p > 0.05$).

Examination of mycelial growth at 25 °C among the isolates tested found that isolate CMWF2584 displayed the highest growth, while isolate CMWF2628 exhibited the least mycelial growth (Supplementary information, tables S1–S3). The ten isolates with the highest mycelial growth were from KwaZulu-Natal (CMWF2584, CMWF1806, CMWF2587, CMWF2589 and CMWF 2602), Mpumalanga (CMWF11, CMWF23 and CMWF56) and Limpopo (CMWF2610 and CMWF2612).

Table 2: MANOVA results of growth at 25 °C of *F. circinatum* in response to the host species, geographic origin and mating type of isolates used in this study

Effect	Degrees of freedom	*F* statistic	*p*-value
Isolate	101	76.02	2×10^{-16}
Host	4	1.23	0.30
Geographic origin	4	3.58	9.1×10^{-3}
Mating type	1	0.51	0.48

Table 3: MANOVA results of aggressiveness of *F. circinatum* in response to the host species, location and mating type of isolates used in this study

Effect	Degrees of freedom	*F* statistic	*p*-value
Isolate	101	27.09	2×10^{-16}
Host	4	1.43	0.23
Geographic origin	4	4.91	1.2×10^{-3}
Mating type	1	1.73	0.19

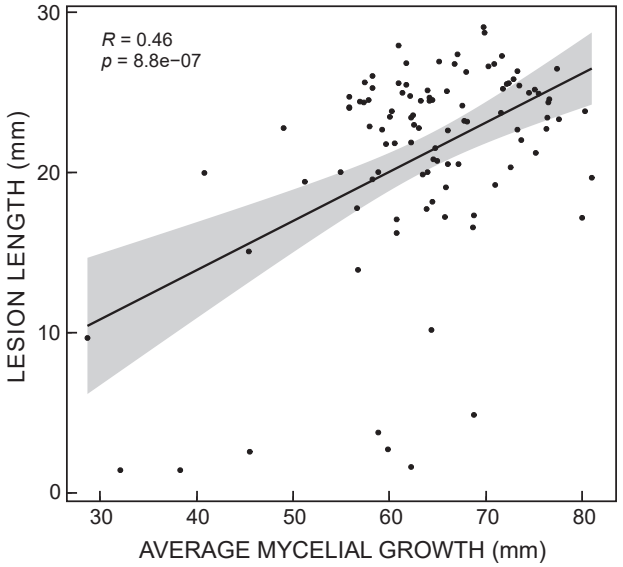

Figure 3: Results of Pearson's correlation analysis between average mycelial growth of South African *F. circinatum* isolates at 25 °C and their corresponding lesion lengths caused on P. patula seedlings. The shaded area indicates the 99% confidence interval. The correlation significance (*p*) and Pearson's correlation coefficient (*R*) are indicated

Conversely, isolates with the lowest mycelial growth were from Mpumalanga (CMWF2626, CMWF1219, CMWF1218 and CMWF51), KwaZulu-Natal (CMWF2590), Eastern Cape (CMWF2652, CMWF2648 and CMWF2651) and Western Cape (CMWF674 and CMWF636).

The MANOVA results (Table 2) revealed that neither isolate mating type nor the original host appeared to affect growth in culture at 25 °C. However, variation in growth at 25 °C among the *F. circinatum* isolates ($p = 2 \times 10^{-16}$) and across the geographic origin of the tested isolates ($p = 9.1 \times 10^{-3}$) was observed. Notably, isolates from the Eastern Cape displayed significantly lower overall growth compared to those from Limpopo and KwaZulu-Natal, based on the average growth data from the five geographic origins (Figure 2a). Additionally, there was no significant difference in the average mycelial growth at 25 °C between isolates from Mpumalanga and Western Cape compared to those from Eastern Cape, Limpopo or KwaZulu-Natal.

Aggressiveness of isolates

Inoculation with all 102 isolates resulted in lesions associated with the wounded *P. patula* tips, while no lesions were observed on the plants inoculated as controls. The recorded lesion lengths varied significantly (Table 3), although there was minimal variation among most of the isolates (Supplementary table S4). The majority of isolates produced lesions longer than 5 mm, with the majority resulting in lesions between 22 mm and 24 mm long (Supplementary table S4). Only seven isolates (CMWF2615, CMWF674, CMWF594, CMWF2654, CMWF2648, CMWF2652 and CMWF2646) produced small lesions that were less than 5 mm in length (Supplementary table S4). This was significantly smaller than mean lesion length of all the isolates, which was 20.71 mm.

Among the isolates tested, most exhibited higher aggressiveness compared to the three isolates used for routine screening of *Pinus* planting stock. Notably, among the latter, isolate CMWF1217 displayed the highest level of aggressiveness, while isolate CMWF1218 showed the least aggressiveness. Some of the isolates with the highest lesion lengths included CMWF1807 from *P. greggii* in KwaZulu-Natal, CMWF2617 from *P. patula* in Limpopo, and CMWF2624 and CMWF2627 from *P. patula* in Mpumalanga (Supplementary table 4). Although there were significant differences ($p < 0.05$) between isolates with the largest and smallest lesion lengths, the majority of the lesion lengths of the *F. circinatum* isolates did not vary largely (see Tukey HSD rankings in Supplementary table S4).

The MANOVA results further showed that neither mating type nor the isolates' original host influenced their aggressiveness (Table 3). However, there was a significant impact of the isolates themselves ($p = 2 \times 10^{-16}$) and geographic origin on the observed lesion lengths ($p = 1.2 \times 10^{-3}$) (Table 3). Isolates from KwaZulu-Natal, Limpopo and Mpumalanga exhibited higher average lesion lengths compared to isolates from Eastern Cape. The average lesion lengths of isolates from Western Cape did not differ from those reported for isolates from the other four geographic locations (Figure 2b). A moderate positive correlation between the mycelial growth at 25 °C and lesion length was observed (Figure 3) as supported by an *R*-value of 0.46.

Discussion

This study is the first to explore the aggressiveness among a large collection of South African *F. circinatum* isolates. Although various strategies are typically employed to control this pathogen (Wingfield et al. 2008; Martín-García et al.

2019; Vainio et al. 2019), breeding for resistant *Pinus* planting stock remains the most important long-term solution (Vivas et al. 2011; Mitchell et al. 2012a). Our findings showed that most of the isolates tested were more aggressive than or equally aggressive as the standard set of three isolates used for routine screening of planting stock (Coutinho et al. 2007; Porter et al. 2009; Herron et al. 2020). This is an important finding because those three isolates, originally obtained from a commercial nursery in the Mpumalanga province (Porter et al. 2009), are widely used by South African forestry to identify *Pinus* families, clones and breeding material needed to sustain their operations. The aggressiveness of *F. circinatum* to *P. patula* seedlings varied minimally among the 102 isolates tested. In only a few cases, the isolates produced small and non-significantly larger ($p > 0.05$) lesions than those observed for the control treatments. Generally, the lesion lengths observed were in the same range as those reported in previous studies on South African isolates (Schmale III and Gordon 2003; Coutinho et al. 2007; Herron et al. 2020). This was particularly evident for the three screening isolates, for which lesion lengths were comparable to previous studies considering their relative aggressiveness (Coutinho et al. 2007; Herron et al. 2020). However, for five other isolates that were also included in previous studies, our results differed in producing either smaller (Fru et al. 2019) or longer lesions (Roux et al. 2007). These differences could be due to the differences in environmental effects such as the duration for which the seedlings were maintained before and after infection, the time of year when inoculations were conducted, and the different *Pinus* hosts used. Indeed, such variation in the use of different host species and the duration of infection trials complicates direct comparisons between studies.

Results of this study showed that neither mating type nor original host correlated with *F. circinatum* aggressiveness. This contrasts with other fungal pathogens, where mating type positively correlates with relative aggressiveness (Alvarez-Perez et al. 2010; Xu et al. 2017; Yong et al. 2020). For *F. circinatum*, Pérez-Sierra et al. (2007) used a collection of strains from northern Spain to demonstrate that MAT1-1 isolates were generally more aggressive than MAT1-2 isolates. A later study also showed that MAT1-2 isolates grew more slowly than MAT1-1 isolates at 25 °C (Mullett et al. 2017). These patterns were, however, likely influenced by the population dynamics of the pathogen (Mullett et al. 2017). For example, only a few asexually reproducing genotypes of *F. circinatum* occur in this region (Iturritxa et al. 2011; Berbegal et al. 2013). Our findings are thus similar to those reported for a worldwide collection of *F. circinatum* isolates (Mullett et al. 2017), where no apparent correlation was observed between the lesion lengths caused by particular isolates and their mating type. Practically, this means that preliminary estimates of strain aggressiveness cannot be obtained from this trait, and assays using *P. patula* seedlings remain the only reliable method to score this phenotype effectively.

Fungal growth has been established as a proxy for predicting the pathogenicity of certain fungal pathogens(Belisario et al. 2008; Brasier and Kirk 2010; Lee et al. (2015). In a study by Lee et al. (2015), it was demonstrated that mycelial growth patterns and the fertility status of *Ceratocystis albifundus* are positively linked to its pathogenicity. While the relationship between growth and aggressiveness of *F. circinatum* was found to be moderately associated in this study, it suggests that the growth of *F. circinatum* influences its aggressiveness minimally. Therefore, using the mycelial growth of *F. circinatum* alone as an indicator of its aggressiveness may be informative but not definitive.

A significant finding revealed in this study is that the *F. circinatum* isolates from the different regions vary in their aggressiveness and growth. Notably, isolates from regions with summer rainfall exhibited an average faster growth (i.e. KwaZulu-Natal and Limpopo) and higher aggressiveness (i.e. KwaZulu-Natal, Limpopo and Mpumalanga) than those from the winter rainfall region. An interesting exception to note was the average growth of isolates from Western Cape, which showed no significant differences to the average growth and aggressiveness of those from the summer rainfall region. Currently, the observed genetic diversity in South Africa is attributed to multiple introductions of *F. circinatum* (Fru et al. 2023). Considering that isolates were selected based on the genetic diversity across South Africa (Santana et al. 2016; Fru et al. 2017; Fru et al. 2019; Fru et al. 2023), this might suggest that genetic variation could also impact the observed differences. Our study thus provides valuable insights into the role of geographical origin in shaping the growth and aggressiveness of *F. circinatum* isolates in South Africa. However, for a comprehensive understanding of how geographical origin impacts growth and aggressiveness in the South African population, future studies should include a balanced representation of isolates from various regions to directly compare the influence of geographical origin on the average growth and aggressiveness levels.

The 102 *F. circinatum* isolates used in this study varied widely in their growth at 25 °C, 20 °C and 30 °C. Nevertheless, most isolates grew most rapidly at 25 °C, which is consistent with the fact that it is regarded as the optimal temperature for the growth of *F. circinatum* (Leslie and Summerell 2006; Inman et al. 2008; De Vos et al. 2011; Elvira-Recuenco et al. 2021). Although mycelial growth rate has not been studied extensively in South African isolates, some isolates (CMWF35, CMWF23 and CMWF674) were previously included in a growth study considering a global collection of isolates (Mullett et al. 2017). Their growth patterns were generally the same, with isolates CMWF35 and CMWF23 growing faster at 25 °C in the present study and in that of Mullett et al. (2017). Isolate CMWF674, however, grew somewhat slower in our study. This could have been due to differences in the growth media used in the two studies. Nevertheless, isolates displaying more rapid growth at 30 °C might be better adapted to growth in warmer regions (Mullett et al. 2017), which is an aspect of the pathogen's biology deserving further research.

Conclusions

Overall, the diversity in growth and aggressiveness observed among the 102 isolates in this study is consistent with what is known regarding the population biology of *F. circinatum* in South Africa. The results of various detailed population genetic analyses have shown that the fungus is highly diverse, resulting from numerous independent introductions

into the country and in particular regions (Steenkamp et al. 2014; Santana et al. 2016; Fru et al. 2017; Fru et al. 2019). Also, despite its predominantly asexual reproduction, gene flow across populations is frequent and mostly associated with human activity, irrespective of the *Pinus* host or tissue type affected (Santana et al. 2016; Fru et al. 2017; Fru et al. 2019; Fru et al. 2023). It was thus not surprising that the aggressiveness and growth data resulting from this study lacked obvious patterns associated with the original *Pinus* host from which the isolates were obtained or their mating type.

In summary, this study highlights the need for careful consideration of the *F. circinatum* isolates used in screening trials where planting stock or breeding material is selected. Ideally, the pathogen strains should be representative of the population diversity of *F. circinatum* in the region where the plants will be deployed. Our results also highlight the need for continued efforts to restrict the movement of the pathogen, both locally and across greater distances. This should limit the occurrence of conditions conducive to mutation and/or sexual reproduction, which could lead to new *F. circinatum* genotypes that are more aggressive and/or fitter for survival in a plantation forestry environment (Steenkamp et al. 2014; Fru et al. 2023).

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Author contributions

BS Swalarsk-Parry: conceptualisation, methodology (infection and growth trials and statistical analysis), data curation, writing of the original draft, review and editing, visualisation.

L de Vos, QC Santana, MA van der Nest, BD Wingfield, MJ Wingfield, ET Steenkamp: conceptualisation, methodology (infection and growth trials and statistical analysis), review and editing, supervision.

FF Fru: methodology (statistical analysis), review and editing.

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