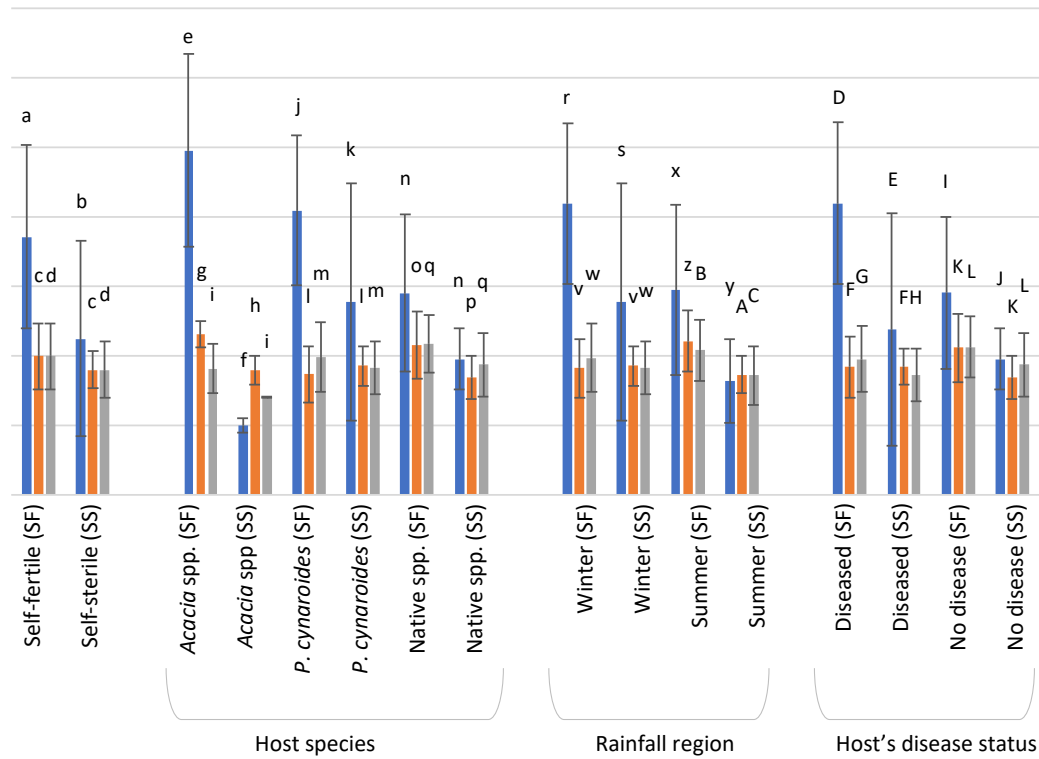


Growth, pathogenicity and sexual fertility of the African tree pathogen *Ceratocystis albifundus*

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SUPPLEMENTARY MATERIAL

Figure S1.	Histograms representing mean mycelial growth at 25°C and 30°C, as well as and mean lesion length for the self-fertile and self-sterile isolates of <i>C. albifundus</i> examined in this study.	p. 2
Table S1.	Geographic origin and host of <i>C. albifundus</i> isolates screened for growth rate and pathogenicity.	p. 3
Table S2.	ANOVA results for seven separate datasets involving the comparison of lesion length data obtained from the pathogenicity study on <i>Acacia mearnsii</i> using the <i>Ceratocystis albifundus</i> isolates examined, as well as comparisons involving pathogenicity and mycelial growth at 25°C and 30°C when isolates are grouped according to their original plant host species (i.e., <i>Acacia</i> species, various native hosts and <i>Protea cynaroides</i>).	p. 5
Table S3.	Results of Student's t-tests for comparisons performed in this study.	p. 6
Table S4.	Results of correlation analyses between the respective continuous and categorical variables examined in this study.	p. 7



Supplementary Figure S1. Histograms representing mean mycelial growth at 25°C (orange) and 30°C (grey), as well as mean lesion length (blue) for the self-fertile (SF) and self-sterile (SS) isolates of *C. albifundus* examined in this study. The isolates are grouped based on their host species, geographic origin, and whether they were isolated from healthy or diseased hosts. Bars represent standard deviation, while the different letters within each set of results indicate significant ($P < 0.05$) differences between or among means (see Supplementary Table S3 for details of statistical analyses).

Supplementary Table S1. Geographic origin and host of *C. albifundus* isolates screened for growth rate and pathogenicity.

Isolate Number ^a	Fertility status ^b	Host species	Province	Reference or Collector
CMW17620	SF	<i>Terminalia sericea</i>	Limpopo	J Roux (FABI)
CMW17628	SS	<i>Faurea saligna</i>	Gauteng	Heath (2009)
CMW21146	SS	<i>Vachellia grandicornuta</i>	Limpopo	Heath (2009)
CMW21150	SS	<i>Senegalia nigrescens</i>	Limpopo	Heath (2009)
CMW21473	SF	Nitulid beetle	Gauteng	Heath (2009)
CMW22302	SF	<i>Brachypephus</i> beetle	Gauteng	Heath (2009)
CMW23823	SF	<i>Acacia mearnsii</i>	Mpumalanga	Heath (2009)
CMW23839	SF	<i>Terminalia sericea</i>	Limpopo	Heath (2009)
CMW37312	SF	<i>Terminalia sericea</i>	Mpumalanga	Mbenoun et al. (2014)
CMW37313	SF	<i>Combretum zeyheri</i>	Mpumalanga	Mbenoun et al. (2014)
CMW37949	SF	<i>Combretum apiculatum</i>	Mpumalanga	Mbenoun et al. (2014)
CMW40625	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW4075	SF	<i>Acacia mearnsii</i>	Kwa Zulu Natal	Roux et al. (2001)
CMW4084	SF	<i>Acacia mearnsii</i>	Kwa Zulu Natal	Roux et al. (2001)
CMW4090	SF	<i>Acacia mearnsii</i>	Kwa Zulu Natal	Roux et al. (2001)
CMW41550	SS	<i>Lannea schweinfurthii</i>	Mpumalanga	DH Lee (FABI)
CMW41551	SF	<i>Lannea schweinfurthii</i>	Mpumalanga	DH Lee (FABI)
CMW41552	SF	<i>Lannea schweinfurthii</i>	Mpumalanga	DH Lee (FABI)
CMW41580	SF	<i>Terminalia sericea</i>	Mpumalanga	Lee et al. (2016)
CMW41601	SF	<i>Terminalia sericea</i>	Mpumalanga	AC Misse (FABI)
CMW42100	SF	Nitidulid beetle	Western Cape	AC Misse (FABI)
CMW42102	SF	Staphilinid beetle	Western Cape	AC Misse (FABI)
CMW42103	SF	<i>Acacia melanoxylon</i>	Western Cape	AC Misse (FABI)
CMW42104	SF	<i>Acacia melanoxylon</i>	Western Cape	AC Misse (FABI)
CMW42117	SF	<i>Acacia melanoxylon</i>	Western Cape	AC Misse (FABI)
CMW42118	SF	<i>Terminalia sericea</i>	Mpumalanga	Lee et al. (2016)
CMW42119	SF	<i>Terminalia sericea</i>	Mpumalanga	Lee et al. (2016)
CMW42120	SF	<i>Terminalia sericea</i>	Mpumalanga	Lee et al. (2016)
CMW42123	SF	<i>Terminalia sericea</i>	Mpumalanga	Lee et al. (2016)
CMW42408	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42409	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42415	SS	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42416	SS	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42417	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42419	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42420	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42423	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42425	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42428	SS	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42429	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42432	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42435	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42436	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42437	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42438	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42442	SS	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42444	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42445	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)

CMW42446	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42447	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42450	SS	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42474	SS	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42476	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42477	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW42478	SS	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW43681	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW43682	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)
CMW43683	SF	<i>Protea cynaroides</i>	Western Cape	Lee et al. (2016)

^a All isolates are available from the CMW culture collection of the Forestry and Agricultural Biotechnology Institute (FABI) of the University of Pretoria.

^b Fertility status was scored based on the presence of absence of mating type gene MAT1-2-1, where isolates containing this gene were regarded as self-fertile (SF) and those lacking it as self-sterile (SS).

Supplementary Table S2. ANOVA^a results for seven separate datasets involving the comparison of lesion length data obtained from the pathogenicity study on *Acacia mearnsii* using the *Ceratocystis albifundus* isolates examined, as well as the comparisons involving pathogenicity and mycelial growth at 25°C and 30°C when isolates are grouped according to their original plant host species (i.e., *Acacia* species, various native hosts and *Protea cynaroides*).

Source of variance	Degrees of freedom	Sum of Squares	Mean Squares	F -value	P-value ^b
Lesion length					
Between-treatments	58	1.3 x 10 ⁷	564419	21.3452	1.58 x 10⁻⁸⁴
Within-treatments	351	9.3 x 10 ³	26442	-	-
<i>Including all isolates, both self-fertile and self-sterile</i>					
Host species vs. Growth at 25°C					
Between-treatments	2	598	299	4.1220	0.0221
Within-treatments	51	3708	73	-	-
Host species vs. Growth at 30°C					
Between-treatments	2	330	165	1.8839	0.1620
Within-treatments	51	4471	88	-	-
Host species vs. Lesion length					
Between-treatments	2	5917	2758	3.6989	0.0316
Within-treatments	51	40790	799	-	-
<i>Including only the self-sterile isolates</i>					
Host species vs. Growth at 25°C					
Between-treatments	2	821	410	5.7588	0.0066
Within-treatments	38	2708	71	-	-
Host species vs. Growth at 30°C					
Between-treatments	2	272	136	0.6545	0.2050
Within-treatments	38	3128	82	-	-
Host species vs. Lesion length					
Between-treatments	2	7972	3986	7.8591	0.0014
Within-treatments	38	19274	507	-	-

^a Conducted using the online statistical tools available at <https://www.socscistatistics.com/tests/biserial/default.aspx> and <https://acetabulum.dk/anova.html>.

^b P-values that were significant at the 95% confidence level are indicated in bold.

^c To determine if the lesion length of the isolates differ significantly from control, all isolates and the control were included.

Supplementary Table S3. Results of Student's *t*-tests^a for comparisons performed in this study. The first set (A) involve those involving the lesion lengths obtained from a pathogenicity study on *Acacia mearnsii* and mycelial growth at 25°C and 30°C for the *Ceratocystis albifundus* isolates examined and grouped according to their reproductive status, diseases status of their original host and geographic origin. The second set (B) of comparisons involved those between the self-fertile and self-sterile isolates.

Groups compared	Treatment	<i>t</i> -value ^b	P-value ^c
<i>Set A: All isolates (self-fertile isolates only) vs defined groups</i>			
Host's disease status (<i>symptoms or no symptoms</i>)	Growth at 25°C	3.1358 (-3.1761)	0.0136 (0.0014)
	Growth at 30°C	-1.2607 (-0.8335)	0.1063 (0.2046)
	Lesion length	1.8349 (2.01422)	0.0359 (0.02514)
Geographic origin (<i>winter or summer rainfall region</i>)	Growth at 25°C	2.2311 (-2.9508)	0.0149 (0.0026)
	Growth at 30°C	0.1570 (0.8332)	0.3412 (0.2047)
	Lesion length	-3.9670 (3.8853)	0.0001 (0.0002)
<i>Set B: Self-fertile vs self-sterile isolates for each defined group</i>			
Host's disease status (<i>symptoms</i>)	Growth at 25°C	0.8310	0.2064
	Growth at 30°C	-0.8833	0.1922
	Lesion length	-3.6014	0.0006
Host's disease status (<i>no symptoms</i>)	Growth at 25°C	-2.2549	0.0168
	Growth at 30°C	-0.1171	0.4539
	Lesion length	-1.6262	0.0588
Geographic origin (<i>winter rainfall region</i>)	Growth at 25°C	-0.0392	0.4845
	Growth at 30°C	-2.0650	0.0236
	Lesion length	-2.6172	0.0066
Geographic origin (<i>summer rainfall region</i>)	Growth at 25°C	-0.0392	0.4845
	Growth at 30°C	-2.0650	0.0236
	Lesion length	-2.6172	0.0066
Host species (<i>non-native host</i>)	Growth at 25°C	-3.195	0.0121
	Growth at 30°C	-1.5741	0.0882
	Lesion length	-3.7911	0.0064
Host species (<i>commercial Protea</i>)	Growth at 25°C	-0.7274	0.2366
	Growth at 30°C	-0.7215	0.2379
	Lesion length	-2.4135	0.0114
Host species (<i>native host</i>)	Growth at 25°C	-1.803	0.0452
	Growth at 30°C	-1.2279	0.1186
	Lesion length	-1.5774	0.0671

^a Conducted using the online statistical tools available at <https://www.socscistatistics.com/tests/biserial/default.aspx>.

^b The *t*-values in brackets were obtained when all self-sterile isolates were excluded from the analyses.

^c The P-values in brackets were obtained when all self-sterile isolates were excluded from the analyses. Those significant at the 95% confidence level are indicated in bold.

Supplementary Table S4. Results of correlation analyses^a between the respective continuous and categorical variables examined in this study.

Variable 1	Variable 2	Coefficient ^b	P-value ^c
<i>Continues variable vs. Continues variable</i>			
Mycelial growth at 25°C	Mycelial growth at 30°C	0.6291 (0.6484)	< 0.00001 (00001)
Lesion length	Mycelial growth at 25°C	0.1229 (-0.0449)	0.3669 (0.7745)
	Mycelial growth at 30°C	0.1622 (0.0815)	0.2323 (0.6034)
<i>Continues variable vs. Categorical variable</i>			
Lesion length	Fertility status	0.4674	0.00028
	Original host's disease status	0.2524 (0.3171)	0.0497 (0.0299)
	Geographic origin	0.46272 (0.5084)	0.0003 (0.0005)
Mycelial growth at 25°C	Fertility status	0.2952	0.0272
	Original host's disease status	-0.3646 (-0.4121)	0.0042 (0.0044)
	Geographic origin	-0.0696 (-0.1221)	0.6101 (0.4354)
Mycelial growth at 30°C	Fertility status	0.0517	0.7051
	Original host's disease status	-0.1179 -0.0700)	0.3654 (0.6402)
	Geographic origin	-0.0809 (-0.1584)	0.5535 (0.3101)
<i>Categorical variable vs. Categorical variable</i>			
Fertility status	Original host's disease status	0.2369	0.0712
	Geographic origin	0.0773	0.5628

^a Conducted using the online statistical tools available at <https://www.socscistatistics.com/tests/biserial/default.aspx>.

Point biserial correlation analysis using comparisons between continuous growth or pathogenicity data and their associated categorical data for fertility or laccase production. Phi Coefficient analysis of the binary data involving reproductive status (i.e., self-fertile or sterile), host's disease status, and pathogenicity against *A. mearnsii*.

^b Coefficient $r_{(Pearson)}$ for Pearson's product-moment correlation analysis, coefficient $r_{(pbc)}$ for the Point Biserial correlation analysis and Φ for Phi coefficient analysis. Values in brackets were obtained when all self-sterile isolates were excluded from the analyses.

^c The P-values in brackets were obtained when all self-sterile isolates were excluded from the analyses. Those significant at the 95% confidence level are indicated in bold.