Genetic diversity of *Teratosphaeria pseudoeucalypti* in *Eucalyptus* plantations in Australia and Uruguay

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Short title: *Teratosphaeria pseudoeucalypti* in Australia and Uruguay

Abstract

Teratosphaeria pseudoeucalypti is a fungal pathogen that causes a severe leaf blight disease on *Eucalyptus* trees. While presumed to be native to Australia, *T. pseudoeucalypti* has become well established and an important constraint to forestry in South America. The aim of this study was to use microsatellite markers to investigate the genetic diversity of *T. pseudoeucalypti* outbreaks occurring in plantations in two distinct environments. In the New South Wales (NSW) plantation in Australia, a hybrid of two native *Eucalyptus* species was planted outside of its natural environment. In contrast, the plantations in Uruguay were of a non-native *Eucalyptus* species. Sixteen polymorphic microsatellite loci, identified in two genomes of *T. pseudoeucalypti*, were used to genotype 36 individuals from the NSW plantation and 21 were collected from across Uruguay. Genetic diversity in the NSW population was low ($H_{exp} = 0.05$), comprising five genotypes of which one occurred in >80% of individuals. Despite high clonality and one *MAT1-1* isolate among many *MAT1-2* isolates, different genotypes co-occurred on a single tree and the hypothesis of recombination was not rejected. The diversity in the NSW plantation was consistent with that of an introduced pathogen, either from the surrounding native forests, or supporting earlier findings that *T. pseudoeucalypti* has only recently established in NSW. All isolates from Uruguay were clonal, harbouring the *MAT1-1* idiomorph and a genotype distinct from those in Australia. This clonality suggests a single introduction of *T. pseudoeucalypti* into that country.

Keywords: Forestry, leaf blight, microsatellites, pathogen; population genetics

Introduction

Teratosphaeria pseudoeucalypti is one of several closely related Dothideomycete fungi that infects *Eucalyptus* in plantations (Andjic et al. 2019; Burgess and Wingfield 2017; Aylward et al. 2019). This species causes severe leaf blight that initially develops as chlorotic leaf spots that become necrotic (Andjic et al. 2010). Total defoliation is common on susceptible trees. In addition to its aggressive nature, *T. pseudoeucalypti* appears to have an extensive range of *Eucalyptus* hosts. Initially, it was reported primarily from *E. camaldulensis*, *E. grandis*, *E. tereticornis* and hybrids of these trees (Andjic et al. 2010), but a recent study from Uruguay (Simeto et al. 2020) has shown that at least eight different *Eucalyptus* species and their hybrids can be affected.

For many years, most species of *Teratosphaeria* that infect *Eucalyptus* were accommodated in the genus *Kirramyces*, due to the similar morphology of their asexual spores (Walker et al. 1992). These, for example, included foliar pathogens closely related to *T. pseudoeucalypti*, i.e. *T. destructans*, *T. epicoccoides, T. eucalypti* and *T. viscidus* (Crous et al. 2009a; 2009c). Blight caused by these fungi was commonly referred to as Kirramyces leaf blight (Carnegie 2007; Carnegie et al. 2008). Molecular phylogenies, however, discredited anamorph spore morphology as a reliable taxonomic trait for this group (Crous et al. 2009c). As a result, Crous et al. (2009a) transferred the *Kirramyces* pathogens of *Eucalyptus* to the sexual genus *Teratosphaeria*, despite the absence of known sexual states for many species.

Teratosphaeria pseudoeucalypti was first recognised in *Eucalyptus* hybrid plantations in south-eastern Queensland, Australia, in 2005. Based on morphology, it was initially believed to be *T. eucalypti* (Andjic et al. 2019), but the atypically severe nature of the disease outbreaks prompted subsequent molecular systematic studies. Four nuclear barcoding regions confirmed that the leaf blight in tropical and subtropical Queensland was due to a new species, closely related to *T. eucalypti* (Andjic et al. 2010). These authors identified five different haplotypes

of *T. pseudoeucalypti* (referred to as KE8-KE12), with most haplotypes occurring in tropical Far North Queensland. More recently, the pathogen has been detected in plantations in northern New South Wales, where *T. eucalypti* and *T. epicoccoides* had previously been the major disease-causing species (Andjic et al. 2019; Carnegie 2007).

Both culture-dependent and -independent studies have suggested that *Teratosphaeria* species are closely associated with their *Eucalyptus* hosts (Kemler et al. 2013; Crous et al. 2009b; Marsberg et al. 2014). Consequently, because *Eucalyptus* trees are native to Australia and some surrounding islands (Rejmánek and Richardson 2011), the native range of *Teratosphaeria* species is also believed to span these areas (e.g. Aylward et al. 2019). Population genetic studies have confirmed that the leaf pathogens *T. epicoccoides* and *T. nubilosa* are native to eastern Australia (Taole et al. 2015; Hunter et al. 2008), a region that is also likely to be the native range of *T. pseudoeucalypti*. All current reports of *T. pseudoeucalypti* from Australia are from plantations and, often, from hybrids of different *Eucalyptus* species; many of these hybrid clones originating from Brazil and South Africa (Crous et al. 2009c). Therefore, these cannot be seen to represent native populations of the pathogen. Such native populations of endemic pathogens would be difficult to obtain, because co-evolution with their hosts makes them "inconsequential in native forests" and often impossible to detect in the natural environment (Burgess and Wingfield 2017).

Outside Australia, *T. pseudoeucalypti* has been reported in only one region of South America. It was almost simultaneously detected in Argentina, Brazil and Uruguay (Cândido et al. 2014; Ramos and Pérez 2015; Soria et al. 2014), specifically in provinces of Argentina and Brazil that share a border with Uruguay. In 2015, an extensive survey of plantations of *E. camaldulensis*, *E. tereticornis* and hybrids of these species confirmed the widespread presence of *T. pseudoeucalypti* in Uruguay (Pérez et al. 2016; Ramírez-Berrutti 2017). Ramírez-Berrutti (2017) showed that all Uruguayan isolates considered had the KE8 haplotype that was also

present in isolates from Australia. This adds credence to the view that the pathogen is likely native to Australia, even though it has never been found in the natural environment.

Genomes recently sequenced for *T. pseudoeucalypti* isolates from both Australia and Uruguay confirmed that the species is heterothallic (Wilken et al. 2020), necessitating clonal reproduction when compatible strains of opposite mating type are absent. Genetic diversity is typically low in introduced pathogen populations (McDonald and Linde 2002), even for sexually reproducing homothallic species, such as *T. nubilosa* (Pérez et al. 2012). The diversity of *T. pseudoeucalypti* introduced into South America is, therefore, expected to be very low. In contrast, it is reasonable to hypothesise that populations of *T. pseudoeucalypti* from natural forests in eastern Australia would have high levels of diversity. However, planted forests of native species could be affected by only small numbers of aggressive genotypes (Burgess and Wingfield 2017) and populations of isolates from such areas would have low genetic diversity. This study arose when two relatively large collections of *T. pseudoeucalypti* isolates became available from *Eucalyptus* plantations in Australia and Uruguay, providing an opportunity to compare the pathogen in these distinct environments. The isolates from Australia were from a plantation in the native range of *Eucalyptus*, raising the question whether this would reflect a native population of the pathogen or whether the outbreak arose from a small number of genetic entities. Microsatellite markers were developed for *T. pseudoeucalypti* and applied to interrogate the genetic diversity of the two collections.

Materials and Methods

Identity and mating types of isolates

Strains of *T. pseudoeucalypti*, previously isolated from the leaves of diseased *Eucalyptus* trees in Uruguay and Australia, were obtained from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, South Africa (Table S1, Online Resource). These included 21 isolates from Uruguay representing a selection of those sampled by Pérez et al. (2016) in 2015 from *E. camaldulensis*, *E. tereticornis* and hybrid *Eucalyptus* trees in 15 of the 19 administrative departments in Uruguay (Fig. 1a). The remaining 36 isolates were collected in 2018 from 16 trees in an *E. grandis* x *E. camaldulensis* plantation in Mallanganee, northern New South Wales (NSW; Fig. 1b). Hierarchical sampling was applied to assess pathogen diversity in this plantation, with 21 isolates derived from 21 different leaves on a single tree and one isolate from a leaf on each of 15 other trees. The Australian *MAT1-2* strain of *T. pseudoeucalypti* (CMW51515) for which a genome sequence is available (Wilken et al. 2020) was previously collected from Miriam Vale, Queensland, during the initial 2005 disease outbreak (Andjic et al. 2010).

Fungal isolates were grown on MEA (Malt Extract Agar; Merck) at 25°C in the dark for approximately 3 weeks. DNA extraction from fresh mycelia followed the protocol described by Aylward et al. (2020). The identity of each individual was verified using the diagnostic microsatellite panel 2 of Havenga et al. (2020a) that allows for the differentiation of known *Teratosphaeria* leaf pathogens of *Eucalyptus*. The mating type of all isolates was determined as previously described (Havenga et al. 2020c). Additionally, the internal transcriber spacer (ITS) region was sequenced for 12 of the isolates, including those that did not match the expected amplicon sizes in the diagnostic panel.

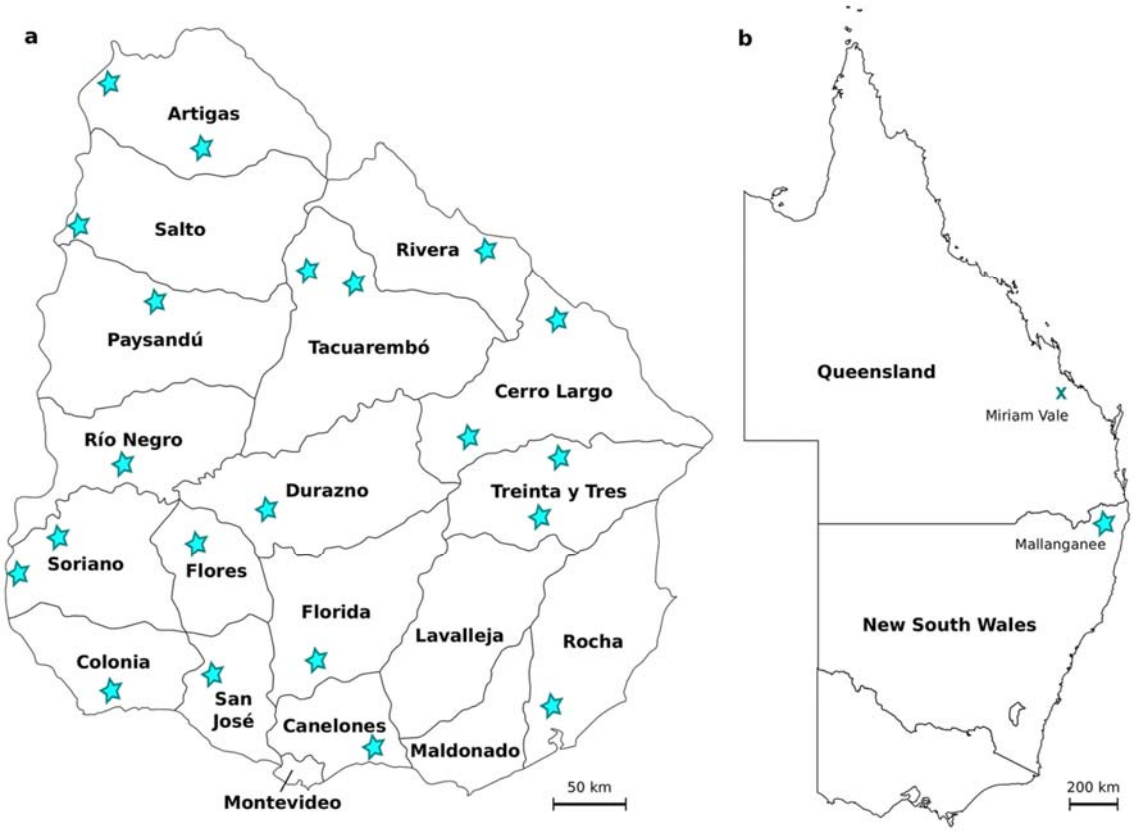

Figure 1 Sampling locations of *Teratosphaeria pseudoeucalypti* in (a) Uruguay and (b) New South Wales, Australia. In b, the origin of the Australian genome isolate is indicated with an X. Map outlines provided by Vemaps.com.

Primers and PCR amplification with the Ampliqon Taq DNA Polymerase Master Mix RED (Biomol, Germany) followed methods previously described for *Teratosphaeria* species (Havenga et al. 2020c). Sanger sequencing was performed at the Central Analytical Facilities (CAF), Stellenbosch University, South Africa. A maximum likelihood phylogeny was calculated on the NGPhylogeny.fr platform (Lemoine et al. 2019) using MAFFT 7.407_1 (Katoh and Standley 2013), BMGE 1.12_1 (Criscuolo and Gribaldo 2010) and PhyML+SMS 1.8.1_1 (Lefort et al. 2017), applying 1000 bootstrap replicates.

Microsatellite marker development and amplification

The genomes of two *T. pseudoeucalypti* isolates, one from Uruguay (isolate CMW49161) and one from Australia (isolate CMW51515; Wilken et al. 2020), were used to identify polymorphic microsatellite loci following the protocol described by Engelbrecht et al. (2017). Tandem Repeats Finder 4.09 (Highnam et al. 2013) identified simple repeats in the CMW49161 genome (GenBank accession JABBMY000000000.1). The raw reads of the CMW51515 genome (SRA accession PRJNA625190; SRX9428925), trimmed with Trimmomatic v0.39 (Bolger et al. 2014), were aligned to the CMW49161 genome using Bowtie 2.4.1 (Langmead and Salzberg 2012) with the "--local" option. Microsatellite regions polymorphic between the two genomes were identified with RepeatSeq v0.8.2 (Highnam et al. 2013).

Primers were designed for candidate loci using Primer3Plus (Untergasser et al. 2007). Microsatellite regions were selected for primer design only if they did not occur within predicted coding regions, the regions flanking the repeat did not contain polymorphisms, the microsatellite had at least six repeat units and the total repeat length did not exceed 100 bp. Primers were chosen to have a melting temperature of approximately 60°C and produce amplicons of varying lengths, between 100 and 500 bp, to facilitate multiplexing. Amplification of each primer pair was tested in 20 µl reactions containing 10 µl Ampliqon Taq Master Mix, 0.4 µM of each primer and approximately 100 ng DNA. PCR conditions were 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 45 s and a final extension at 72°C for 10 min. To verify amplification of the correct locus as well as the quality of the microsatellite region, the amplicons were sequenced in three *T. pseudoeucalypti* strains (CMW52432, CMW52478 and CMW52494).

For multiplexing and capillary electrophoresis, reverse primers labelled with 5' fluorescent dyes were obtained from Inqaba Biotechnical Industries (Pretoria, South Africa). The

Table 1 Polymorphic microsatellite markers developed for *Teratosphaeria pseudoeucalypti*

	Locus	Primer	Repeat motif	Forward primer (5'-3')	Reverse primer (5'-3')	Dye	GenBank Accession	Primer concentration (nmol)	Alleles			
									Number	Size range (bp)	H_{exp}[d]	E_5[e]
Panel 1	**LocA**	Tpse11	CAACCG	GGAGAGATGCCAAGCCTCTA	CTGAGAAGGAGCGAGCTTTG	6-FAM	MZ297306	17.5	2	199-211	0.47	0.93
	LocB	Tpse5	AT	CAGTCTTCCAGGGTTCCAAG	GGGAGATGGAGAGGGAAGTC	6-FAM	MZ297307	20	2	375-381	0.47	0.93
	LocC	Tpse17	CAT	ATCTGACGTCTGCCTCACG	GATGTTGTCCGGAGCAGTTG	6-FAM	MZ297308	17.5	3 (s)	462-474	0.53	0.87
	LocD	Tpse2	ATGA	AGTGAGGTGGTGGTTTGAGG	GCAGCAGGTAGACAGGAAGG	TET	MZ297309	15	3	348-360	0.51	0.84
	LocE*	Tpse18	AGA	CGTCAATCCACCTCGTGAC	GGGCGGTGATTTGATTGC	HEX	MZ297310	10	2	273-276	0.48	0.94
	LocF	Tpse19	GTT	TCCACTTCTGGACCCTTGAC	CCAGCACGAGGGAAGTAGTC	HEX	MZ297311	20	3	436-442	0.51	0.84
Panel 2	**LocG**	Tpse12	GAT	GAAGGCTGGTCTCGAATGAG	GCCCAGCGACAAGACTGTAT	6-FAM	MZ297312	17.5	2	128-131	0.47	0.93
	LocH	Tpse8	GTGTGG	CCTTGAACTCGGAGACTTGC	CCACACTGTCCACATCCAAG	6-FAM	MZ297313	12.5	2	352-358	0.47	0.93
	LocI	Tpse4	TAGG	CGTCCATTCATTAGCACACC	CCCTCCCCCTTTGAACTCTA	6-FAM	MZ297314	12.5	2	471-475	0.47	0.93
	LocJ	Tpse6	GTC	AGACAAACCGACGTCCACTC	GAAGACCCTTCCATCCTTCC	TET	MZ297315	8	3	368-374	0.49	0.86
	LocK	Tpse1	GT	GTCGTCAAGTGGGAGTTTCC	GATTTCCAGTCGCCTCTACG	HEX	MZ297316	12.5	3	212-218	0.53	0.84
	LocL	Tpse14	TGAC	CCCGAATGAAGAGACAGAGG	GGACTTGGCTAAGCAAGCAG	HEX	MZ297317	10	2	456-460	0.47	0.93
Panel 3	**LocM**	Tpse3	GA	TCCTAGACCACGGAAACTGC	GGGTTGGCTGCTCTGACTAC	6-FAM	MZ297318	12.5	3	118-124	0.55	0.84
	LocN	Tpse20	AATT	AGGCTTCTTGGACGACAGAG	GAAGGGAATGCGTCGGTACT	6-FAM	MZ297319	32.5	2	383-391	0.47	0.93
	LocO*	Tpse10	TG	GTTGACGAGGTGGGCAAAG	CACGACTCAGACCAACGAGA	6-FAM	MZ297320	15	3	435-449	0.51	0.84
	LocP	Tpse13	GAC	TCTCTGGCAACGTCTGTGTC	TTCCGACCCTTAGTCTGCAC	TET	MZ297321	12.5	3	376-385	0.49	0.86
	LocQ	Tpse9	ATC	TGAGTCAGGGACACTGCAAC	GCGATACATACTGCCCCCTA	HEX	MZ297322	10	2	332-335	0.47	0.93
	LocR	Tpse7	CCTCA	CCCAGCTAAGGATGGATCTG	CAGGCTTGTAGCGACAACAG	HEX	MZ297323	15	2 (s)	437-452	0.48	0.94

[a] Asterisks indicate loci excluded from the analyses due to significant linkage disequilibrium

[b] 5' fluorescent labels on reverse primers

[c] Loci in which the allele found in Uruguay was also present in (shared with) the Australian population are indicated with (s)

[d] Nei's unbiased gene diversity ($H_{exp} = [n/n-1][1-\Sigma p_i^2]$; Nei 1978)

[e] E_5 evenness index ($E_5 = [1/\lambda -1]/[e^H-1]$; Grünwald et al. 2003, Ludwig et al. 1988)

hypothesis of random mating in NSW was assessed with $\bar{r}_d$ and 999 permutations, using both the full dataset and a clone-corrected dataset comprised of only the unique MLGs in each subpopulation. A minimum spanning network (MSN) was constructed with Bruvo's distance (Bruvo et al. 2004).

Results

Identity and mating types of isolates

A profile identical to that described for *T. pseudoeucalypti* in the Havenga et al. (2020a) diagnostic panel was obtained for the three isolates with sequenced genomes, the remaining 19 isolates from Uruguay and 34 of the 36 NSW isolates (Table S2, Online Resource). Two of the loci (I and J) did not amplify in the NSW isolates CMW52444 and CMW52460. The ITS phylogeny, however, confirmed that these isolates, as well as eight randomly chosen isolates, resolved with the strains from which the genomes have been sequenced and the ex-type isolate of *T. pseudoeucalypti* (CBS124577) with strong support (Fig. S1). The four barcoding regions used by Andjic et al. (2010) to describe nuclear haplotypes were not sequenced in this study. However, analysis of these sequences in the *T. pseudoeucalypti* genomes revealed that all three have the KE8 haplotype. All 21 isolates from Uruguay had the *MAT1-1* idiomorph (Table S1, Online Resource). In contrast, both mating types were detected in the NSW population. However, the *MAT1-2* idiomorph was found in 35 isolates, whereas *MAT1-1* was represented by a single isolate (CMW52460).

Microsatellite marker development and amplification

A comparison of the genomes of the Australian isolate CMW51515 and isolate CMW49161 from Uruguay identified 294 polymorphic microsatellite (2-6 bp repeats) regions.

Microsatellites composed of tri-nucleotide repeat motifs were most common (74.5%) and more than half (57.2%) of the microsatellites had 8-12 repeat units. Primers were designed for 20 candidate loci that met the necessary criteria and 18 of these amplified consistently, with their polymorphism confined to the microsatellite region (Table 1).

Two or three alleles were obtained per locus (Table 1), with eight of the loci (A, B, G, H, I, L, N and Q) being monomorphic within the two countries. Nei's gene diversity (H_{exp}) ranged between 0.47-0.55. Higher gene diversity was associated with a lower evenness (E_5 range=0.84-0.94). Analysis of pairwise linkage disequilibrium necessitated removal of two loci (E and O), however, their exclusion did not affect the number of unique multilocus genotypes (MLGs) obtained. The genotype accumulation curve (Fig. S2), calculated by randomly sampling *n* loci 1000 times (Kamvar et al. 2015), reached a plateau at 12 loci, showing that the remaining 16 loci were more than sufficient to describe the genetic diversity of these isolates.

Diversity and relatedness of *T. pseudoeucalypti* genotypes

Isolates from Australia

The 36 isolates collected in the NSW plantation represented five different microsatellite genotypes (Fig. 2; Table S1, Online Resource). Genotype MLG5 and MLG6 occurred in isolates collected from both the single tree that was sampled intensively as well as from the 15 different trees. MLG6 was, however, dominant at both levels, comprising 19/21 (90.4%) single tree isolates and 11/15 (73.3%) of the isolates from different trees in the plantation. The remaining three genotypes (MLG2-4), that also included the only *MAT1-1* isolate (MLG3) in NSW, were represented by a single isolate each. The Australian strain (CMW51515) for which the genome had been sequenced also had a unique genotype (MLG1), but was excluded from further analyses, because it did not reside in the NSW population.

Diversity and evenness within isolates from NSW was low because of the dominance of genotype MLG6 and Nei's diversity index predicted only a 4.7% chance of randomly sampling two different genotypes. It was, therefore, significant that multiple genotypes were detected on a single *E. grandis* x *E. camaldulensis* tree. Genetic diversity was slightly higher in the genotypes obtained for isolates from the 15 different trees, compared to those from the single tree (Fig. S3). Six alleles were detected only in the 15 trees and approximately 4.0 genotypes could be expected from them, compared to the 2.4 expected from the single tree (Table 2). Population differentiation between these two sampling levels was, however, not significant (ϕ=0.06, P=0.20; Table 3).

The $\bar{r}_d$ index rejected the hypothesis of random recombination in NSW (P<0.01; Table 2). However, within the two NSW levels, $\bar{r}_d$ only rejected the hypothesis for the full dataset of the 15 different trees, which was the level in which the only *MAT1-1* isolate was detected. For the full dataset of the single tree and for both clone-corrected levels, $\bar{r}_d$ was not significantly

Table 2 Diversity and recombination statistics for *Teratosphaeria pseudoeucalypti* in Uruguay and at the different population levels in Australia

		Alleles			Diversity of multilocus genotypes (MLGs)[j]						LD[k] - full dataset		LD[k] - clone-corrected	
Population	**Isolates**	**Na ± SE[a]**	**Np[b]**	**H_{exp}[c]**	**MLGs[d]**	**eMLG ± SE[e]**	**H'[f]**	**G[g]**	**Corrected λ[h]**	**E_5[i]**	**rbarD[l]**	**P-val**	**rbarD[l]**	**P-val**
Uruguay	21	1.00 ± 0.00	14		1									
NSW	36	1.47 ± 0.61	22	0.0466	5	5 ± 0.00	0.658	1,42	0.305	0.453	0.405	0.001	0.291	0.001
Single tree	21	1.13 ± 0.33	0	0.0173	3	2.43 ± 0.62	0.381	1,21	0.186	0.464	0.648	0.088	-0.500	1.000
15 trees	15	1.50 ± 0.61	6	0.0875	4	4.00 ± 0.00	0.857	1,77	0.467	0.569	0.427	0.001	0.094	0.177

[a] Average number of alleles (Na) and standard error (SE) per locus

[b] Total number of private alleles (Np)

[c] Nei's unbiased gene diversity ($H_{exp} = [n/n-1][1-\Sigma p_i^2]$; Nei 1978)

[d] Total number of multilocus genotypes (MLGs)

[e] Expected number of multilocus genotypes based on rarefaction

[f] Shannon's Index of MLG diversity ($H' = -\Sigma^s[p_i \times ln(p_i)]$; Shannon and Weaver 1949)

[g] Stoddart and Taylor's Index of MLG diversity ($G = 1/\Sigma p_i^2$; Stoddart and Taylor 1988)

[h] Simpson's Index (λ; Simpson 1949) corrected for sample size ($\lambda \times N/[N-1]$)

[i] E_5 evenness index ($E_5 = [1/\lambda - 1]/[e^H - 1]$; Grünwald et al. 2003, Ludwig et al. 1988)

[j] The 95% confidence intervals for the sample-size corrected estimates of H', G, λ and E_5 are shown in Fig. S3

[k] Linkage Disequilibrium (LD)

[l] The standardised Index of Association (Agapow and Burt 2001)

Table 3 Analysis of Molecular Variance (AMOVA) based on the lowest sampling hierarchies in Australia and Uruguay

				Variance			
	Df	**Sum Sq**	**Mean Sq**	**Sigma (σ)**	**%of total**	**Phi (ϕ)**	**P-value**
Between Australia and Uruguay	1	412,59	412,59	15,51	96,91	0,97	0.331
Between single and different trees in Australia	1	0,99	0,99	0,03	0,19	0,06	0.202
Within countries and levels	54	25,12	0,47	0,47	2,91	0,97	0.001
Total	56	438,7	7,83	16,01	100	-	-

different from zero, suggesting that though clonal reproduction appears prevalent, recombination may be possible.

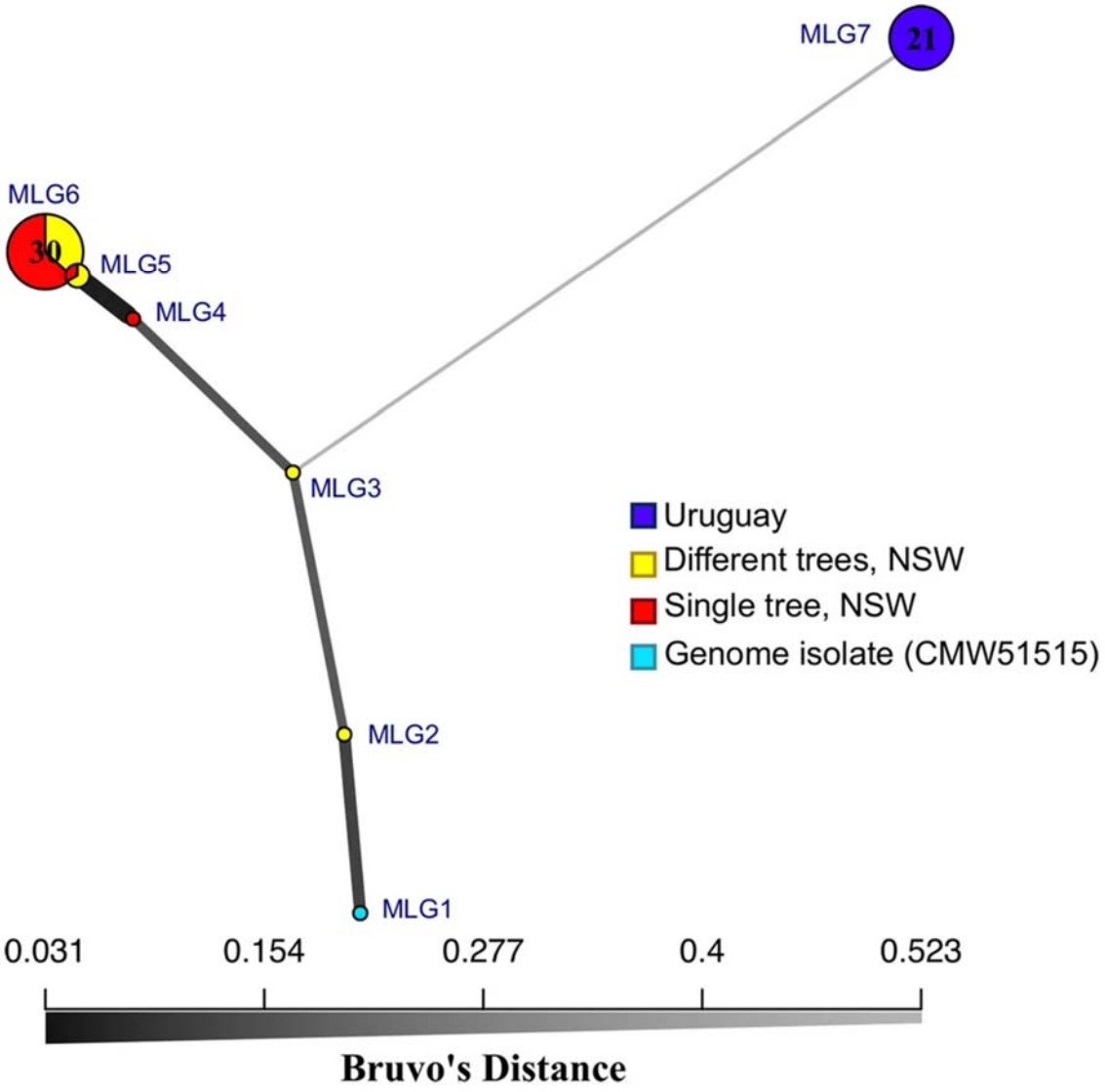

Figure 2 Minimum spanning network (MSN) showing the distribution and identity of the *Teratosphaeria pseudoeucalypti* genotypes in Australia and Uruguay. Each node (circle) represents one of the seven microsatellite multilocus genotypes (MLGs) and the size of the node is proportional to the number of individuals with that MLG. Nodes are coloured according to their sampling locality. Line thickness and intensity decreases with increasing genetic distance. NSW = New South Wales.

Isolates from Uruguay

All 21 of the isolates from Uruguay were of a single genotype (MLG7). The alleles found at 14 loci were unique to Uruguay (private), indicating that this genotype was distinct from the Australian genotypes (Fig. 2; Table 2). The genetic distance between isolates from Uruguay and Australia was further illustrated by the AMOVA that observed 96.9% genetic variance and almost maximal differentiation (ϕ=0.97) between the two countries (Table 3). This strong differentiation was, however, only significant when comparing the lowest population hierarchies, which included Uruguay as a locality (ϕ=0.97; P=0.001).

Discussion

This study confirmed that a number of genotypes of *T. pseudoeucalypti* are associated with the aggressive outbreak of leaf blight in a *Eucalyptus* plantation in NSW, Australia. These isolates had low genetic diversity and one genotype dominated the population. This supports the hypothesis that the outbreak was caused by a population of *T. pseudoeucalypti* isolates not representing a natural situation. Samples collected in Uruguay were of a single *T. pseudoeucalypti* genotype, typical of a recently introduced pathogen. A large genetic distance separated the Uruguay genotype from the Australian genotypes identified in this study. The clonal nature of the Uruguay population suggests a single introduction of *T. pseudoeucalypti,* most likely from Australia, but not linked to the NSW collection considered in this study.

Interestingly, the low genetic diversity and evenness of the *T. pseudoeucalypti* population in NSW resembled that of introduced pathogen populations (McDonald and Linde 2002). Only five genotypes were detected in this collection, one of which comprised > 80% of the isolates. The low number of genotypes is comparable to the more recent reports of *T. destructans* in South East Asia (Havenga et al. 2020b), *T. nubilosa* populations outside of eastern Australia

(Hunter et al. 2008; Pérez et al. 2009a) and *T. epicoccoides* in Western Australia, the least diverse population of that cosmopolitan pathogen (Taole et al. 2015). In contrast, native populations of *T. epicoccoides* and *T. nubilosa* in eastern Australia are characterised by many different genotypes that each occur only once or twice and by the presence of almost all alleles identified in the global population (Taole et al. 2015; Hunter et al. 2008; Pérez et al. 2012). The low diversity of *T. pseudoeucalypti* indicates recent colonisation of the NSW plantation, with an apparently small number of genotypes, presumably with high levels of aggressiveness, becoming dominant. This could be the result of *T. pseudoeucalypti* moving from the natural forest into plantations or may indicate the recent arrival of *T. pseudoeucalypti* in NSW, as suggested by Andjic et al. (2019).

The fact that the NSW population included only a single *MAT1-1* isolate and the large proportion (>80%) of clones of the pathogen, makes sexual recombination in the NSW population unlikely. This is not surprising, since the sexual state of *T. pseudoeucalypti* is unknown, as is the case for most other *Teratosphaeria* pathogens (Aylward et al. 2020; Havenga et al. 2020c). Furthermore, clonal reproduction commonly dominates even in indigenous populations of *T. epicoccoides* (Taole et al. 2015). The Index of Association for the NSW *T. pseudoeucalypti* population, however, did not disregard the possibility of recombination. If *T. pseudoeucalypti* does undergo sexual reproduction, it may follow a strategy similar to that of *Aspergillus* species (Dyer and O'Gorman 2012), where well-adapted individuals reproduce clonally and recombine only when conditions are unfavourable (Ni et al. 2011) and then only if they find a compatible partner. In their native environments, recombination may, therefore, be a rare event that becomes even scarcer or absent in introduced populations of heterothallic *Teratosphaeria* species.

Teratosphaeria pseudoeucalypti isolates collected from numerous plantations across Uruguay were clonal. This clonality and widespread nature is similar to the situation of *T. nubilosa*, the

other important *Teratosphaeria* leaf pathogen present in Uruguay. In 2007, a single genotype of *T. nubilosa* was found to be the cause of defoliation in Uruguay (Pérez et al. 2009a) and additional genotypes of the pathogen have yet to be found in that country (Simeto et al. 2020). *Teratosphaeria nubilosa* likely spread from Uruguay into Rio Grande do Sul, Brazil, or *vice versa* (Pérez et al. 2009b), and this could have occurred naturally via windborne ascospores (Park and Keane 1982). For *T. pseudoeucalypti*, however, only pycnidia are encountered in plantations (Andjic et al. 2010) and these would move over short distances by rain-splash dispersal. In contrast, long distance spread would likely have occurred through the movement of *Eucalyptus* germplasm, potentially including plant material and seed (Wingfield et al. 2008).

The genotype of *T. pseudoeucalypti* found in Uruguay is different to any other recovered from Australia. Consequently, the source population of the introduction into South America remains unknown. It is typical for populations of *Teratosphaeria* pathogens in different countries to be almost completely differentiated (Burgess and Wingfield 2017). This is the case for the stem canker pathogens *T. gauchensis* (Jimu et al. 2016) and *T. zuluensis* (Chen et al. 2011; Cortinas et al. 2010), implying multiple independent introductions from undetermined sources (Aylward et al. 2020). The leaf pathogens *T. epicoccoides* (Taole et al. 2015), *T. destructans* (Havenga et al. 2020b) and even the exclusively sexually reproducing *T. nubilosa* (Hunter et al. 2008; Pérez et al. 2012) also have strong population structure. Ultimately, collections from yet unidentified native populations of *T. pseudoeucalypti* may be the only means to clarify the source of the introduction into Uruguay.

Conclusions

The recent emergence of *T. pseudoeucalypti* in Uruguay plantations represents a serious threat to plantations of *Eucalyptus* trees. The results of the present study also show that a small number of *T. pseudoeucalypti* genotypes can cause significant damage to plantations of these trees in their native range. Since its description from collections in Queensland (Andjic et al. 2010), *T. pseudoeucalypti* has been detected further south in eastern Australia (Andjic et al. 2019) and appears to have become well-established in South America (Soria et al. 2014; Pérez et al. 2016; Cândido et al. 2014; Ramos and Pérez 2015). The movement of *T. pseudoeucalypti* outside of its presumed native range is concerning, especially since it increases the likelihood of further spread (Lombaert et al. 2010; Wingfield et al. 2015). Such spread is exemplified by the steady movement of the closely related pathogen, *T. destructans*, across South East Asia (Havenga et al. 2020b; in press) and into South Africa (Greyling et al. 2016). Planted forests worldwide are experiencing an ever increasing burden of pests and diseases (Wingfield et al. 2015), emphasising the importance of managing known risks, such as *T. pseudoeucalypti*, carefully.

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