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Plants for planting; indirect evidence for the movement of a serious forest pathogen, *Teratosphaeria destructans*, in Asia

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Abstract

Fungal diseases caused by native pathogens and pathogens introduced with planting stock have a significant impact on exotic plantation forestry in the tropics. *Teratosphaeria destructans* (formerly *Kirramyces destructans*) is a serious pathogen causing leaf, bud and shoot blight diseases of Eucalyptus spp. in plantations in the sub-tropics and tropics of south-east Asia. This pathogen was first discovered in Indonesia in 1995 and has subsequently spread to Thailand, China, Vietnam and East Timor. The biology, ecology and genetics of this important pathogen have not been explored yet. The objective of this study was, thus, to determine the genetic diversity and movement of *T. destructans* throughout south-east Asia using multi-gene phylogenies and microsatellite markers. Out of nine gene regions only two microsatellite markers detected a very low nucleotide polymorphism between isolates; seven other gene regions, ITS, β-tubulin, EF1-α, CHS, ATP6 and two microsatellite loci, reflected genetic uniformity. The two polymorphic molecular markers resolved six haplotypes among isolates from Indonesia and only a single haplotype elsewhere in Asia. The low diversity observed among isolates in the region of the first outbreak is as expected for a small founder population. The spread of a single clone over large distances throughout the region supports the hypothesis of spread via the human-mediated movement of germplasm.

Introduction

Globally, the steady increase in the demand for fibre coupled with deforestation has resulted in a rapid increase in plantation area. This is particularly notable in the southern hemisphere and Asia where productive plantation areas have increased from to 36 to 55 million ha between 1990 and 2005 (FAO 2005). In the southern hemisphere there has been a long tradition of softwood production in plantations (predominantly *Pinus radiata*) (Burgess *et al*. 2001) but in past 20 years eucalypts have become the major source of fibre for pulping (Turnbull 2000). This has seen an expansion of the eucalypt plantation industry into new countries and new regions within countries.

Much of the success of exotic plantations forestry into a new area has been due to the use of fast growing, exotic eucalypts that have left their natural predators behind (Wingfield *et al*. 2001). The introductions or movement of pests and pathogens into such plantations should occur naturally at a rate which is dependent upon their distance to established plantations. If new plantations are

geographically isolated there is no reason why good management could not exclude introduced pests and pathogens in perpetuity. Of course there are cases, such as the eucalypt rust (Glen *et al*. 2007), where pests and pathogens have jumped onto eucalypt plantations from native hosts, usually from within the same plant family, the Myrtaceae (Slippers *et al*. 2005). However, in reality, there tends to be gradual increase in the impact and numbers of new pests and diseases; this is not because they have moved by their own volition, but rather they are moved due to anthropogenic activities. For example, the number of *Teratosphaeria* species isolated in temporal surveys increased with time in Uruguay (Pérez *et al*. 2009), South Africa (Hunter *et al*. 2004) and Western Australia (Jackson *et al*. 2008). Over the past 20 years the Tree pathology Co-operative Program in South Africa has documented the almost annual emergence of a new disease, many of which are known to have originated in Australia, the natural range of most *Eucalyptus* spp. (http://www.fabinet.up.ac.za/tpcp).

The clear movement of pests and diseases between continents and countries illustrates the basic failure of quarantine measures to control such movement either due to lack of resources or unregulated pathways (Brasier 2008). Perhaps the most troubling aspect of the seemingly unfettered pathogen movement is that many of these pests and pathogens may have been moved on germplasm by the same forestry companies whose best interests would have been served by excluding them. It appears that management places a greater importance on improved growth and productivity and underplay or ignore the potential impact of pests and pathogens on this productivity, despite years of evidence to the contrary.

The eucalypt plantation industry in south-east Asia and China is relatively young (Qi 1989). Eucalypt plantations have been established predominantly in Thailand, Indonesia, Vietnam and China with the most rapid recent expansion in China. In this study, we follow the movement of a newly emerged disease, *Teratosphaeria destructans*, an aggressive and often devastating pathogen that causes blight of young leaves, buds and shoots of some *Eucalyptus* spp. This pathogen was first discovered in northern Sumatra, Indonesia, where it caused serious damage to young leaves of *E. grandis* (Wingfield *et al*. 1996). Since then, *T. destructans* has appeared in Thailand, China, Vietnam and East Timor (Burgess *et al*. 2006a; Old et al. 2003a). Disease occurs on *E. camaldulensis* and *E. urophylla*, as well as *E. grandis* and various hybrids between these three species (Burgess *et al*. 2006a; Old *et al*. 2003a; b). *Eucalyptus* spp. are non-native in most of these countries where they have been used to establish plantations for fibre production. The exception is East Timor, where *T. destructans* was found on native *E. urophylla* (Old *et al*. 2003a).

Due to the host range and high impact of disease *T. destructans* was considered a biosecurity threat to Australia's plantations and forests. This prompted us to conduct surveys in the sub-tropics and tropic of northern Australia. In 2005, *Kirramyces* leaf blight was observed in taxa trials in the tropical east of Australia, but the causal agent was found to be a new species, *T. viscidus* (Andjic *et al*. 2007b). However, *T. destructans* was isolated and reported from a taxa trial of non-endemic eucalypts on Melville Island 50 km north of Darwin in the northern territory (Burgess *et al*. 2007). The higher level of nucleotide polymorphism among Australian isolates led to the conclusion that *T. destructans* is endemic to Australia, although cryptic in the native environment (Burgess *et al*. 2007).

The first appearance of the disease in Indonesia was probably the result of an accidental introduction, perhaps on seeds sourced from Australia or East Timor. Regardless of the origin of the pathogen, it has been moved rapidly throughout the region reaching isolated areas very quickly. The only means by which this pathogen could have been dispersed so rapidly is on infected germplasm. For example *T. destructans* had been a serious problem since 2002 in Guangxi nurseries. Disease caused by *T. destructans* was observed in 2003 in central Guangdong Province and on Hainan Dao infecting planting stock originating from Guangxi Province. The production of cuttings from

infested mother stock and poor nursery hygiene resulted in a high proportion of diseased planting stock. Infected material has been, and continues to be, widely distributed across south China, reaching isolated parts of south-west Yunnan in 2003, and Fujian Province in 2006, it is still absent from Guizhou Province (Dell, personal communication).

The aim of this study is to determine if the initial disease outbreak in Indonesia was the result of the introduction of the pathogen into the region and if Indonesia is the source of the subsequent introductions in South-East Asia and China using microsatellite markers and multi-gene phylogenies.

Materials and methods

Fungal isolates and DNA extraction

Eucalyptus leaves infected with *T. destructans* (Fig. 1) were collected from south-east Asian countries including Indonesia, China, Vietnam and Thailand on separate visits between 2003 and 2005 (Fig. 2). *T. destructans* isolation and culture maintenance were performed as in Andjic *et al*. (2007a). The fungal mycelium was harvested and placed in 1.5 ml sterile Eppendorf® tubes. Harvested mycelium was frozen in liquid nitrogen, ground to a fine powder and genomic DNA extracted as described previously (Andjic *et al*. 2007c). All isolates are maintained in the collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. One hundred and five representative isolates, out of a collection of over 200 isolates, from a range of geographical locations and hosts were selected for this study. These included 12 from Thailand, three from Vietnam, 28 from China and 62 from Indonesia (Table 1).

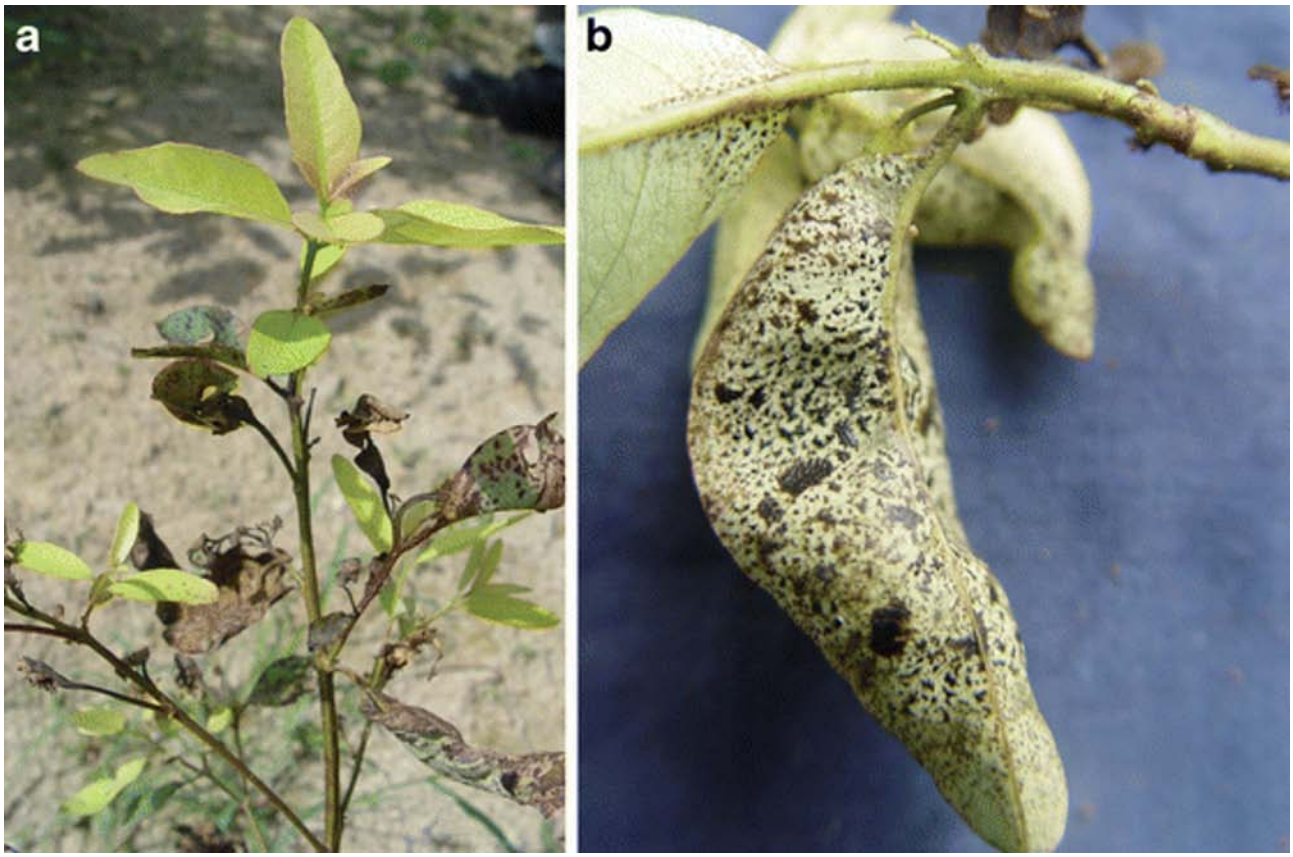

Figure 1. Leaves infected with *Teratosphaeria destructans*; **a** a young *E. grandis* x *E. urophylla* seedling from Indonesia with severe infection, **b** spore mass on the abaxial leaf surface.

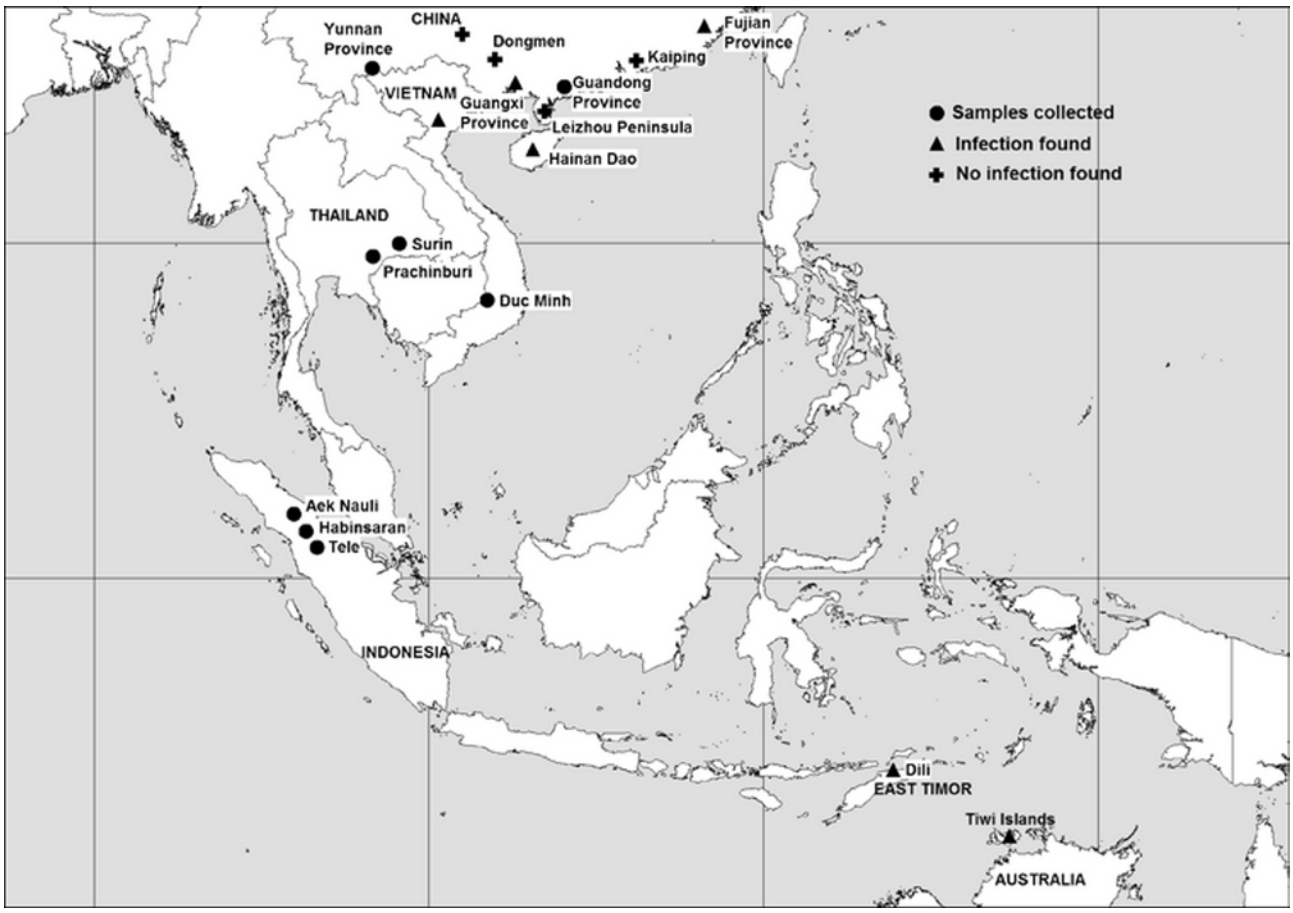

Figure 2. Map showing the localities where leaf material infected with *Teratosphaeria destructans* has been observed and collected.

Development of microsatellite markers

To screen for microsatellites, the Fast Isolation by AFLP of Sequences Containing Repeats (FIASCO) technique (Zane *et al*. 2002) was applied using the procedures described by (Cortinas *et al*. 2006a). DNA was extracted from two Indonesian isolates CMW 19866 and CMW 19832 as well as CMW 19908 from China and pooled. One µg of genomic DNA was digested with *Mse*I (Biolabs, New England) and ligated to the adaptor using highly concentrated T4 DNA ligase 2 000 000 U/ml (Biolabs, New England). The digestion-ligation mixture was incubated overnight at 37°C and the reaction inactivated at 65°C for 20 min. Five µl of the mixture was used for the PCR following the method of Cortinas et al. (2006a). After amplification, the PCR products were hybridised to $(GACA)_5$, $(TCC)_7$, $(CAT)_5$, $(TGC)_4$, $(CA)_{10}$ and $(CT)_{10}$ biotinylated probes. Hybridized DNA complexes were captured on streptavidin magnetic beads (Dynal beads Streptavidin) (DYNAL, Biocompare, Inc. San Francisco, CA, USA) and DNA was separated by washing and denaturation steps. Recovered DNA was precipitated and amplified using the *Mse*I-N primer.

Amplified DNA was cloned into the pGEM®-T Eazy Systems (Promega, Madison, USA) following the manufacturer's instructions. Bacterial clones were selected, amplified, purified and sequenced as described by Cortinas *et al*. (2006a). Primers were developed with the aid of the primer design software PRIMER 3 (Rozen and Skaletsky 2000) available on the Internet at http://frodo.wi.mit.edu. Of the 20 primer pairs tested, six produced single, readily amplifiable bands and these six markers were tested for polymorphism using eight *T. destructans* isolates; CMW19855, CMW19892, CMW19864, CMW19837, CMW19845, CMW19831, CMW19833 and CMW19908.

Table 1. Host and locations of *Teratosphaeria destructans* isolates considered in this study and the GenBank accession numbers for the four nuclear gene regions, ITS, β-tubulin, EF-1α and CHS and the mitochondrial gene region ATP6.

Isolate no.[1]	Host	Location	Year	Collector	GenBank accession no.				
					ITS	ß-tubulin	EF-1α	CHS	ATP6
CMW 19855	*E.g* x *E.u*[2]	Tele, Indonesia	2003	PA Barber	EU019888	EU019880	EF686485	EF686308	EF686254
CMW 19892	*E. grandis*	Aek Nauli, Indonesia	2004	P Dolok Saribu			EF686486	EF686309	EF686255
CMW 19854	*E.g* x *E.u*[2]	Tele, Indonesia	2003	PA Barber			EF686487	EF686310	EF686256
CMW 19864	*E.g* x *E.u*[2]	Aek Nauli, Indonesia	2004	P Dolok Saribu			EF686481	EF686304	EF686250
CMW 19886	*E. grandis*	Aek Nauli, Indonesia	2004	P Dolok Saribu	EU019889	EU019881	EF686490	EF686313	EF686259
CMW 19887	*E. grandis*	Aek Nauli, Indonesia	2004	P Dolok Saribu			EF686491	EF686314	EF686260
CMW 19860	*E.g* x *E.u*[2]	Habinsaran, Indonesia	2003	PA Barber			EF686488	EF686311	EF686257
CMW 19851	*E.g* x *E.u*[2]	Habinsaran, Indonesia	2003	PA Barber	EU019890	EU019882	EF686497	EF686320	EF686266
CMW 19850	*E.g* x *E.u*[2]	Habinsaran, Indonesia	2003	PA Barber	EU019895	EU019887	EF686495	EF686318	EF686264
CMW 19835	*E.g* x *E.u*[2]	Tele, Indonesia	2003	PA Barber			EF686489	EF686312	EF686258
CMW 19852	*E. grandis*	Aek Nauli, Indonesia	2003	PA Barber			EF686482	EF686305	EF686251
CMW 19837	*E.g* x *E.u*[2]	Tele, Indonesia	2003	PA Barber	EU019892	EU019884	EF686483	EF686306	EF686252
CMW 19853	*E. grandis*	Aek Nauli, Indonesia	2003	PA Barber			EF686484	EF686307	EF686253
CMW 19842	*E.g* x *E.u*[2]	Tele, Indonesia	2003	PA Barber	EU019893	EU019885	EF686492	EF686315	EF686261
CMW 19845	*E.g* x *E.u*[2]	Tele, Indonesia	2003	PA Barber			EF686493	EF686316	EF686262
CMW 19891	*E. grandis*	Aek Nauli, Indonesia	2004	P Dolok Saribu	EU019894	EU019886	EF686494	EF686317	EF686263
CMW 19832	*E.g* x *E.u*[2]	Tele, Indonesia	2003	PA Barber			EF686496	EF686319	EF686265
CMW 19831	*E.g* x *E.u*[2]	Tele, Indonesia	2003	PA Barber			EF686499	EF686322	EF686268
CMW 19834	*E.g* x *E.u*[2]	Tele, Indonesia	2003	PA Barber			EF686500	EF686323	EF686269
CMW 13705	*E. camaldulensis*	Tatoom, Thailand	2003	MJ Wingfield	EF686502	EF686271	EF686460	EF686280	EF686226
CMW 13337	*E. camaldulensis*	Tatoom, Thailand	2003	MJ Wingfield	EF031469	EF031481	EF031493	EF686281	EF686227
CMW 13709	*E. camaldulensis*	Tatoom, Thailand	2003	MJ Wingfield	EF686503	EF686272	EF686462	EF686282	EF686228
CMW 13330	*E. camaldulensis*	Tatoom, Thailand	2003	MJ Wingfield	EF686504	EF686273	EF686463	EF686283	EF686229
CMW 13710	*E. camaldulensis*	Tatoom, Thailand	2003	MJ Wingfield	EF686505	EF686274	EF686464	EF686284	EF686230
CMW 16136	*Eucalyptus* sp.	Prachinburi, Thailand	2003	MJ Wingfield	EF686506	EF686275	EF686465	EF686285	EF686231
CMW 16124	*Eucalyptus* sp.	Prachinburi, Thailand	2003	MJ Wingfield	EF686507	EF686276	EF686466	EF686286	EF686232
CMW 16123	*E. camaldulensis*	Prachinburi, Thailand	2003	MJ Wingfield	EF031468	EF031480	EF031492	EF686287	EF686233
CMW 16120	*Eucalyptus* sp.	Prachinburi, Thailand	2003	MJ Wingfield	EF031470	EF031482	EF031494	EF686288	EF686234
CMW 16138	*Eucalyptus* sp.	Prachinburi, Thailand	2003	MJ Wingfield	EF686508	EF686277	EF686469	EF686289	EF686235
CMW 16137	*Eucalyptus* sp.	Prachinburi, Thailand	2003	MJ Wingfield	EF686509	EF686278	EF686470	EF686290	EF686236
CMW 16126	*Eucalyptus* sp.	Prachinburi, Thailand	2003	MJ Wingfield	EF686510	EF686279	EF686471	EF686291	EF686237
CMW 15089	*E. camaldulensis*	MinhDuc, S-E Vietnam	2005	TI Burgess	EF031465	EF031477	EF031489	EF686292	EF686238
CMW 15090	*E. camaldulensis*	MinhDuc, S-E Vietnam	2005	TI Burgess	EF031466	EF031478	EF031490	EF686293	EF686239
CMW 15092	*E. camaldulensis*	MinhDuc, S-E Vietnam	2005	TI Burgess	EF031467	EF031479	EF031491	EF686294	EF686240
CMW 19934	*E. urophylla*	Guangdong, China	2005	TI Burgess	EU046370	EU046366	EF686472	EF686295	EF686241
CMW 19914	*E. urophylla*	Guangdong, China	2005	TI Burgess	EU046368	EU046364	EF686473	EF686296	EF686242
CMW 19911	*E. urophylla*	Guangdong, China	2005	TI Burgess	EU046369	EU046365	EF686474	EF686297	EF686243
CMW 19922	*E. urophylla*	Guangdong, China	2005	TI Burgess			EF686475	EF686298	EF686244
CMW 19933	*E. urophylla*	Guangdong, China	2005	TI Burgess	EU046371	EU046367	EF686478	EF686300	EF686247
CMW 19921	*E. urophylla*	Guangdong, China	2005	TI Burgess			EF686501	EF686324	EF686270
CMW 19909	*E. urophylla*	Yunnan, China,	2004	B Dell	EF031464	EF031476	EF031488	EF686300	EF686246
CMW 19910	*E. urophylla*	Yunnan, China	2004	B Dell	DQ632701	DQ632622	DQ632729	EF686302	EF686248
CMW 19908	*E. urophylla*	Yunnan, China	2004	B Dell	EF686511		EF686480	EF686303	EF686249

[1] CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa

[2] E.g x E.u = E. grandis x E. urophylla hybrid

PCR amplification and sequencing

This study included amplification of the complete internal transcribed spacer region 2 (ITS2), part of the β-tubulin gene region (βT), part of the elongation factor 1α gene (EF-1α), part of the chitin synthase 1 gene (CHS), part of the ATPase gene (ATP-6) and the microsatellite markers VA-1, VA-2, VA-6, VA-13, VA-15 and VA-18. Primers used to amplify these regions and repeat motif of microsatellites are listed in Table 2. The PCR reaction mixture (25 µl), PCR conditions and visualisation of products were as described by (Cortinas *et al*. 2006a) except for the ATP-6 region, which was amplified using the following conditions: initial denaturation of 7 min at 94°C, followed by 40 cycles of 45 s at 94°C, 45 s at 45°C, 2 min at 65°C and a final elongation step of 10 min at 68°C. Where amplifications failed, the magnesium concentration was increased to 4 mM, and the primer concentration were increased to 0.9 pmol and the following PCR conditions were used: 7 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 45°C, 2 min at 72°C and a final elongation step of 10 min at 72°C. The PCR products were purified with the Ultrabind®DNA purification kit (MO BIO Laboratories, Solana Beach, CA) following the manufacturer's instructions. Amplicons were sequenced as described previously (Burgess et al. 2005).

Table 2. Primer sets and annealing temperature (AT) used to amplify different regions within *Teratosphaeria destructans*.

Region	Oligos	Oligo Sequence (5'-3')	Amplicon size (bp)	Number of repeats	AT (°C)	Reference
ITS	ITS-3	GTATCGATGAAGAACGCAGC	250		55	(White et al. 1990)
	ITS-4	TCCTCCGCTTATTGATATGC				
β-tubulin	Bt-2a	GGTAACCAAATCGGTGCTGCTTTC	680		45 55	(Glass and Donaldson 1995)
	Bt-2b	ACCCTCAGTGTAGTGACCCTTGGC				
EF-1α	EF1-728F	CATCGAGAAGTTCGAGAAGG	350		45-55	(Carbone and Kohn 1999)
	EF1-986R	TACTTGAAGGAACCCTTACC				
CHS	CHS-79F	TGTGGGCAAGGATGCTTGGAAGAAG	300		55	(Carbone and Kohn 1999)
	CHS-354R	TGGAAGAACCATCTGTGAGAGTTG				
ATP6	ATP6-1	ATTAATTSWCCWTTAGAWCAATT	600		45	(Kretzer and Bruns 1999)
	ATP6-2	TAATTCTANWGCATCTTTAATRTA				
VA-1	VA-1F	CAGAGATCGCAGCAGTACAG	267	$(TGG)_8$	55	This study
	VA-1R	CAGTTGGAGGCAAGGACAAG				
VA-2	VA-2F	CTGCGATTCTGGAAGCTTCG	296	$(TG)_{8-12}$	53	This study
	VA-2R	GGCAATGATCTCAATGCGGTC				
VA-6	VA-6F	CTACTTCCTAAGTACCTAAGCC	281	$(TA)_4$	55	This study
	VA-6R	CTAAGCTCTTAGAAGAGCTCG				
VA-13	VA-13F	GTACAGGAACCAGACTTCCTAC	294	$(CCA)_8$	53	This study
	VA-13R	GATGCGCCTCACTTCTATCC				
VA-15	VA-15F	CAGGTGATTCGACACAATGC	321	$(CA)_7$	45	This study
	VA-15R	GATGAGTCCTGAGTAAGTTGTGG				
VA-18	VA-18F	CGATGAAGTTGACGATAGGC	383	$(CCA)_5$	53	This study
	VA-18R	CATGCGCCACGCACGACCAGG				

Results

DNA sequence comparisons

Five gene regions were compared for sequence variation in *T. destructans*. Previous studies (Andjic *et al*. 2007a; b; c; Burgess *et al*. 2007) have shown no sequence variation in ITS and βT gene regions between T. destructans isolates from Indonesia and China (Table 1). Thus, only the 12 isolates from Thailand and three isolates from Vietnam were sequenced and compared with sequences from Indonesia and China. Direct sequencing of PCR amplicons of the ATP6, CHS and

EF-1α gene regions from all 43 isolates showed no sequence variation.

Microsatellite markers

Six of the 20 primer pairs amplified a microsatellite-containing region for all tested representative isolates of *T. destructans*. Four of the primer pairs (VA-1, VA-6, VA-13 and VA-15) were monomorphic among the tested isolates (Table 3)1. Two of the primer pairs (VA-2 containing a TG repeat and VA-18 containing a CCA repeat) showed a low level of polymorphism (Table 3) and were amplified and sequenced for the 43 selected isolates. In addition VA-13, which appeared to be monomorphic, was also sequenced as it contained a long CCA repeat.

Table 3. GenBank Accession numbers for the six microsatellite loci amplified for 8 representative isolates of *Teratosphaeria destructans*. For the polymorphic loci VA-2 and VA-18, the allele for each isolate is given next to the GenBank accession no.

Isolate no.[1]	Location	GenBank accession no.					
		VA-1	VA-2	VA-6	VA-13	VA-15	VA-18
CMW 19855	Indonesia	EU620606	EF686353 (C)	EU620611	EF686398	EU620619	EF686443 (A)
CMW 19892	Indonesia	EU620603	EF686354 (A)	EU620612	EF686399	EU620620	EF686444 (B)
CMW 19864	Indonesia	EU620604	EF686349 (B)	EU620613	EF686394	EU620621	EF686439 (A)
CMW 19837	Indonesia	EU620601	EF686351 (A)	EU620610	EF686396	EU620618	EF686441 (A)
CMW 19845	Indonesia	EU620605	EF686361 (C)	EU620614	EF686406	EU620622	EF686451 (A)
CMW 19831	Indonesia	EU620608	EF686367 (A)	EU620609	EF686412	EU620517	EF686457 (A)
CMW 19933	China	EU620602	EF686346 (A)	EU620615	EF686391	EU620623	EF686436 (A)
CMW 19908	China	EU620607	EF686348 (A)	EU620616	EF686393	EU620624	EF686438 (A)

[1] Designation of isolates and culture collections: CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa

The locus amplified by VA-13 was monomorphic among all 43 isolates (GenBank Accession no. EF686370-EF686414). However, the loci amplified by VA-2 and VA-18 showed low nucleotide polymorphism (EF686325-EF686368 and EF686415-EF686458 respectively). The alleles were coded by the number of repeats (A–C). Three alleles (A–C) were observed for microsatellite marker VA-2, and two alleles (A–B) were observed for microsatellite marker VA-18 among the 20 isolates from Indonesia. For both markers, the predominant allele (A) was the only allele in isolates from China, Thailand and Vietnam (Table 4).

The microsatellite markers VA-2 and VA-18 were then amplified and sequenced for an additional 62 isolates (43 from Indonesia and 19 from China). There were six possible haplotype profiles for the two polymorphic loci; AA, AB, BA, BB, CA and CB (Table 4). All six haplotypes were found among the Indonesian isolates. The most common haplotype was AA, followed by AB, BA and CA with the least common being BB and CB. Only haplotype AA was found among isolates from China, Thailand and Vietnam (Table 4).

Since the genetic diversity among the sampled isolates was extremely low the phylogenetic analysis was omitted as it would not make much sense.

Discussion

After an extensive molecular study, the only sequence variation observed among isolates of *T. destructans* from south-east Asia and China was in two microsatellite loci. The polymorphism resulted in the detection of six haplotypes in Indonesia where *T. destructans* was first reported in

1996. Since 1996, *T. destructans* has been reported in Thailand, Vietnam and most recently China, with only a single haplotype being found in all these countries. This data provides clear evidence that the fungus is a newly introduced pathogen to Thailand, Vietnam and China.

Table 4. Distribution of *Teratosphaeria destructans* haplotypes between regions as determined from the multilocus profile generated from the two polymorphic microsatellite loci (VA-2 and VA-18).

Haplotype	INDO	CHN	THA	VTN	Total
AA	23	28	12	3	66
AB	18	0	0	0	18
BA	9	0	0	0	9
BB	2	0	0	0	2
CA	7	0	0	0	7
CB	3	0	0	0	3
Total	62	28	12	3	105

It was expected that the microsatellite loci would show more sequence variation compared with housekeeping genes such as ITS and βT, as microsatellites are highly variable with exceptionally high mutation rates (Jarne and Lagoda 1996; Kimmel *et al*. 1996; Levinson and Gutman 1987). Newly established populations or introduced populations would be expected to have low gene and allelic diversity, while well-established populations would have higher diversity especially in their centres of origin (Dutech *et al*. 2007; McDonald and Linde 2002). If several isolates within a species are sequenced, a low level of nucleotide substitution in housekeeping genes is commonly observed among most species in an anamorph stage of *Teratosphaeria* eg. (Andjic *et al*. 2010; Andjic *et al*. 2007c; Burgess *et al*. 2006b; Cortinas *et al*. 2006b; Hunter *et al*. 2006; Jackson *et al*. 2008). No sequence variation was found in four genomic genes and one mitochondrial gene (over 2,000 bp of sequence) for isolates of *T. destructans* from Asia. In addition, four of six microsatellite loci were monomorphic, while two loci exhibited very low polymorphism among isolates from Indonesia. Low diversity and evidence of founder effects have been observed in introduced populations of many eucalypt pathogens, probably reflecting the recent expansion of the industry world-wide (Hunter *et al*. 2008; Nakabonge *et al*. 2008). This is in contrast with high diversity as often observed in well established pathogens of agricultural crops (Blanke and McDonald 2005; Groenewald *et al*. 2008; Stukenbrock *et al*. 2006). Thus, *T. destructans* in Indonesia has the structure of a recently established pathogen and its movement throughout Asia has probably been clonal.

The leaf and shoot blight disease caused by *T. destructans* was first observed in the Lake Toba area of north Sumatra in 1995 (Wingfield *et al*. 1996). Thus, this is a relatively newly recognised pathogen of *Eucalyptus*. After its first appearance in northern Sumatra, it was found in southern Sumatra and thereafter in Thailand in 2000, Vietnam and East Timor in 2002 (Old *et al*. 2003b) and in China in 2003 (Burgess *et al*. 2006a). Because of the intensity of production of *Eucalyptus*, it is unlikely *T. destructans* would have been in these areas for long without being detected. Thus, the

sequential appearance in countries of the region over a short period of time strongly suggests anthropogenic movement. The molecular data supports the anthropogenic movement of *T. destructans* from Indonesia via infected germplasm or mother plants, most likely linked to the rapidly growing eucalypt plantation industry. A similar founder effect was observed in the genetic structure of *Mycosphaerella fijiensis* in the Latin America-Caribbean region and Africa, and was attributed to the movement of infected plant material by anthropogenic activities such as food wrapping with infected leaves (Rivas *et al*. 2004).

In China, it is most likely that the organism has been disseminated on infected cuttings from mother stock plants. It is less likely the pathogen has been distributed by spores in air moving from Vietnam into China, as the more exposed eucalypt nurseries in coastal regions of south China (i.e. in Hainan Dao, Leizhou Peninsula and Zhangjiang) were free of this pathogen in 2003/4 (B. Dell unpublished data). Seed contaminated with spores could have been inadvertently introduced into Guangxi during the China-Australia Eucalypt Afforestation Technical Cooperation Project (ACIAR) (1981–1989) near Dongmen. However, this seems unlikely as no symptoms of *T. destructans* were reported in ACIAR field trials on plantation eucalypts in south China carried out in the 1990's. One of these trials, near Kaiping in Guandong Province, was monitored annually for two rotations from 1992 and no defoliation due to leaf disease had occurred (Daping Xu pers. comm.). Thus, in China, it is likely *T. destructans* was introduced on cuttings used in the establishment of clonal nurseries.

Variation within the microsatellite loci of the *T. destructans* isolates from Indonesia indicates recent mutations or a smaller level of genetic variation in the founder population. This variability within the pathogen could lead to more pathogenic strains. We believe that while *T. destructans* has already been moved around Asia, care should now be taken not to move new genotypes throughout the region. In addition, forestry companies in the tropics and sub-tropics of South America, Africa and Australia should import germplasm, including seed from Asia, with extreme care.

In conclusion, this study provides a classic example of the anthropogenic movement of a pathogen on infected germplasm. The world-wide nursery trade (plants for planting), both legal and illegal, is considered the major pathway for the movement of pathogens around the world (Brasier 2008). Whilst legislation in most countries restricts plant movement and requires certification and chemical treatment, many plant pathogens can exist asymptomatically and escape detection during inspections. Any industry wishing to protect its investment should enforce the utmost precautions when obtaining germplasm from other countries or regions.

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