Australasian Plant Pathol. (2011) 40:407–415
DOI 10.1007/s13313-011-0051-0

High population diversity and increasing importance of the *Eucalyptus* stem canker pathogen, *Teratosphaeria zuluensis*, in South China

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Received: 7 December 2010 / Accepted: 6 April 2011 / Published online: 21 May 2011

Abstract Coniothyrium stem canker, caused by *Teratosphaeria zuluensis*, is one of the most important diseases of plantation-grown *Eucalyptus* trees in tropical and subtropical areas of the world. Previous research on the population structure of *T. zuluensis* in China, Malawi and South Africa has suggested that *T. zuluensis* in these countries had independent origins, with the highest genetic diversity found in a population from South China. In this study, the genetic diversity of three *T. zuluensis* populations from different regions in South China was determined using ten microsatellite markers. Results showed that more than one genotype of *T. zuluensis* can occur on a single tree in all three populations and that a moderate to high genetic diversity exists within the populations. Population differentiation was evident between populations, and in one population there was evidence for a low level of genetic recombination. Comparisons among the three populations of *T. zuluensis* from South China suggest that they originated independently of each other.

Keywords Coniothyrium canker · Genetic diversity · Microsatellites · Plantation health

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Introduction

Coniothyrium canker is a serious stem and branch disease of *Eucalyptus* trees (Wingfield et al. 1996). Two different species, *Teratosphaeria zuluensis* (M.J. Wingf., Crous & T. A. Cout.) M.J. Wingf. & Crous and *T. gauchensis* (M.-N. Cortinas, Crous & M.J. Wingf.) M.J. Wingf. & Crous are known to cause the disease and they have also undergone substantial taxonomic re-evaluation since their discovery (Cortinas et al. 2006c; Andjic et al. 2007; Crous et al. 2007, 2009). The disease is typically characterized by small necrotic lesions on the branches and stems which coalesce to form large cankers resulting in deformed branches and stems (Wingfield et al. 1996). These large cankers reduce wood quality and can lead to stunting of growth and tree death (Wingfield et al. 1996; Roux et al. 2002; Van Zyl et al. 2002a; Old et al. 2003; Cortinas et al. 2006b).

Coniothyrium canker caused by *T. zuluensis* was first discovered in the mid 1980's in South Africa (Wingfield et al. 1996). Since then, the disease has been reported from China (Cortinas et al. 2006b), Malawi (Roux et al. 2005; Cortinas et al. 2006c), Mexico (Roux et al. 2002), Thailand (Van Zyl et al. 2002b), Vietnam (Gezahgne et al. 2003; Old et al. 2003) and Zambia (Chungu et al. 2010).

Teratosphaeria zuluensis was first described as a species of *Coniothyrium* (Wingfield et al. 1996). DNA sequence comparison of isolates from Ethiopia, Mexico, South Africa,

Thailand and Uganda, however, showed that the fungus is most closely related to species in the Teratosphaeriaceae (Gezahgne et al. 2005; Cortinas et al. 2006c; Crous et al. 2009). Cortinas et al. (2006c) also discovered the existence of a second species, *T. gauchensis*, causing Coniothyrium canker in Argentina (Gezahgne et al. 2003), Ethiopia (Gezahgne et al. 2005), Hawaii (Cortinas et al. 2004), Uganda (Gezahgne et al. 2005) and Uruguay (Cortinas et al. 2006c).

The origin of *T. zuluensis* is unknown. Due to the fact that the pathogen appeared in countries that had imported *Eucalyptus* germplasm from South Africa, it was suggested that South Africa might be the origin of the pathogen (Wingfield et al. 1996). However, a recent study comparing populations of *T. zuluensis* from China, Malawi and South Africa, found a higher genetic diversity for isolates collected in China and Malawi than in South Africa (Cortinas et al. 2010). This was unexpected as *Eucalyptus* spp. are exotic in the three countries, and plantation establishment using *Eucalyptus* spp. in China and Malawi is a more recent practice (60 and 50 years, respectively) compared to that in South Africa where *Eucalyptus* have been planted for more than 200 years (Nkaonja 1982; Geldenhuys 1997; Qi 2002). The study also found high numbers of private alleles in all three populations and significant population differentiation between them. Cortinas et al. (2010) thus concluded that *T. zuluensis* in China, Malawi and South Africa originated independently, through multiple introductions from a source that is yet to be discovered.

Coniothyrium canker caused by *T. zuluensis* on *Eucalyptus* in China appears to be spreading with the disease appearing in increasing numbers of plantations in South China. In China, the pathogen was first reported on *E. urophylla* trees in GuangDong Province in 2006 (Cortinas et al. 2006b). At that stage, the disease was only found in relatively limited number of regions of GuangDong Province. Two years later, the disease was reported from many additional areas in the GuangDong and GuangXi Provinces of South China (Zhou et al. 2008).

The aim of this study was to extend surveys for Coniothyrium stem canker in South China and to obtain an improved understanding of its distribution and impact in the region. An additional aim was to collect populations of *T. zuluensis* in greater numbers than has previously been possible to further investigate the genetic diversity and population structure of this pathogen in China.

Materials and methods

Sampling and isolation

Eucalyptus plantations were surveyed in three regions of South China between August and November 2008, and samples collected from trees showing symptoms of Coniothyrium canker. Trees included *E. grandis* and its hybrid clones with *E. urophylla* (Table 1). A collection of samples from these infected trees was made from one region in the GuangXi Province (Region One) and two regions in the GuangDong Province (Region Two and Three) (Table 1, Fig. 1). In Region One, collections were made from two plantations approximately 90 Km apart. In Region Two, samples were collected from one plantation and two parks, which are approximately 30 Km apart from the plantation to the parks. In Region Three, collections were made from two plantations approximately 60 Km apart. Regions One and Two are ~270 Km apart, Regions Two and Three ~350 Km apart and Region Three ~500 Km from Region One (Fig. 1). The collections of isolates from the three regions are treated as Population One, Two and Three, respectively.

Samples for population diversity studies were collected from trees showing typical symptoms of Coniothyrium stem canker. Representative samples were collected from the main stems of trees (two to three-year-old) at approximately 2 m above ground level. The samples were collected from *Eucalyptus* plantations using the same method described by Cortinas et al. (2010) where five to ten diseased trees at the centre of a plantation were selected as the central group for the collection. Samples were then collected from trees randomly selected in the plantations around the central group of trees, extending outwards. For the two parks in Region Two, samples were collected from randomly chosen *Eucalyptus* trees with disease symptoms (Table 1).

Sampling consisted of collecting two to three bark pieces bearing single lesions from the stems of each tree. Bark pieces were incubated in moist chambers to induce the production of spores from the fruiting bodies. Spore drops taken from each bark sample were transferred to 2% malt extract agar (MEA) (20 g malt extract, 20 g agar, 1 L water: Biolab, Midrand, South Africa). Single hyphal tip isolations were made later following the method described by Chungu (2010). An isolate of *T. zuluensis* (identity confirmed in this study) from *E. grandis* that was collected in GuangXi Province in 2006 was also included in this study (Table 1). Isolates were grown on 2% MEA at 25°C for 30 days prior to DNA extraction.

DNA extraction and isolate identification

Actively growing mycelium was scraped from the agar surface using a sterile scalpel and transferred to 1.5 mL Eppendorf tubes. DNA was extracted from the mycelium using a modified version of Raeder and Broda's (1985) phenol/chloroform method as described by Myburg et al. (1999). Samples were treated with 3 μL RNase (1 mg/mL)

Table 1 Origin and hosts of *T. zuluensis* isolates used in this study

Region	Origin	Host	No. Isolates	No. Trees	No. Genotypes	Collectors[a]	Collection Date	GenBank accession no[b]
Region One	ChongZuo & QinZhou, GuangXi Province	*E. grandis*, *Eucalyptus* clone/ species	21	12	20	JR, XDZ, MJW & SFC	Nov. 2006, Aug. 2008	DQ239961, DQ239963 & DQ239965
Region Two	ZhanJiang, GuangDong Province	*E. urophylla* × *E. grandis* clone	38	21	36	SFC & GXZ	Sept. to Oct. 2008	DQ239961, DQ239963 & DQ239965
Region Three	ZhaoQing & YunFu, GuangDong Province	*E. urophylla* × *E. grandis* clone	38	24	17	JR, XDZ & SFC	Aug. 2008	DQ239961, DQ239963 & DQ239965

[a] *JR* Jolanda Roux, *XDZ* XuDong Zhou, *MJW* Michael J. Wingfield, *SFC* ShuaiFei Chen, *GXZ* GuiXiang Zhao

[b] ITS sequences produced in this study for each region are identical to those with GenBank numbers presented

and left overnight at room temperature to degrade the RNA. DNA was separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide and visualised under ultraviolet (UV) light.

To confirm the identity of the isolates collected, DNA sequence data were derived for all isolates. The internal transcribed spacer (ITS) regions, ITS1 and ITS2, and the 5.8S gene of the ribosomal DNA (rDNA) operon, were amplified using the primers ITS1 (5′ TCCGTAGGTGAACCTGCGG 3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC 3′) (White et al. 1990). PCR and sequencing reactions were performed as described in Cortinas et al. (2006b, c). Sequence data for the isolates collected in this study were compared with those in GenBank (http://blast.ncbi.nlm.nih.gov/) using a Blast search, after which they were compared more closely with sequences (GenBank No. DQ240207, DQ240214) of the authentic strains (epitypes CMW17321, CMW17322) of *T. zuluensis* (Cortinas et al. 2006c).

Simple sequence repeat (SSR)—PCR and gene scan analyses

For all *T. zuluensis* isolates, polymorphic loci were amplified using ten pairs of fluorescently labelled species-specific primers (Cortinas et al. 2006a, 2010) (Table 2). PCR reactions and conditions were performed as described in Cortinas et al. (2006a, 2010). Amplified DNA was separated by electrophoresis on a 2% agarose gel, stained with ethidium bromide and visualised under UV light.

PCR products were size-separated on an ABI PRISM™ 3100 Automated DNA Sequencer (Applied Biosystems, Foster City, USA) together with the internal size standard GENSCAN LIZ 500 (−250) (Applied Biosystems, Warrington, UK). Alleles were determined using the software GENEMAPPER version 3.0 (Applied Biosystems, Foster City, USA), and based on the size of each amplicon. Alleles for each locus were assigned an alphabetical character and together provided a multilocus genotype for each isolate. Isolates with the same multilocus genotype at each region were considered clones.

The total number of alleles, private alleles at each locus as well as the allelic frequency of each population was calculated using the program POPGENE version 1.31 (Yeh et al. 1999). The allelic richness of each population was calculated using FSTAT version 2.9.3.2 (FSTAT for windows, version 2.9.3.2) (Goudet 2001).

Genotypic diversity versus number of loci

In order to ascertain whether the sample size for each of the three regions and the number of loci used in this study was sufficiently large to support statistical significance of genotypic diversity for *T. zuluensis*, a plot for mean

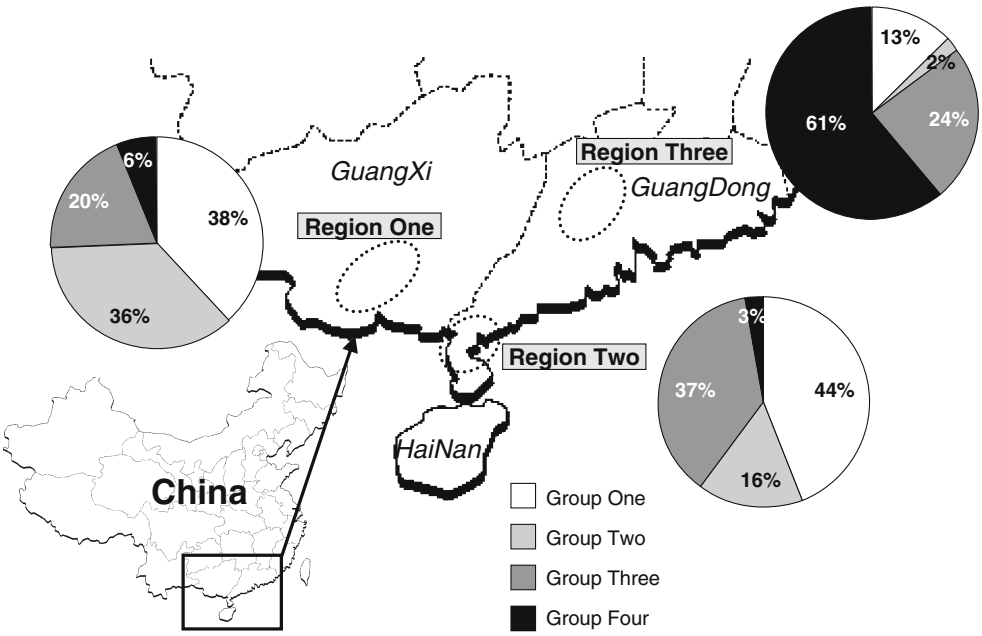

Fig. 1 Map showing the position of the sampling regions for this study in South China. Samples from Region One were collected in the province of GuangXi and those from Regions Two and Three were collected in the province of GuangDong. The assignment of individuals (from the three regions) based on their allele frequencies into one of four groups, as determined with STRUCTURE, is represented as percentages in the pie charts

genotypic diversity against the number of loci was obtained. This was done using MULTILOCUS version 1.3 (Agapow and Burt 2001) and made it possible to statistically define the populations for further analyses.

Gene and genotypic diversity

The gene diversity for each clone-corrected population was determined using the program POPGENE version 1.31 (Yeh et al. 1999) based on the equation $H = 1 - \Sigma x_k^2$, where x_k is the frequency of the kth allele (Nei 1973). Genotypic diversity (G) was estimated by using the equation $G = 1/\Sigma p_i^2$, where p_i is the observed frequency of the ith genotype in each of the non clone-corrected populations (Stoddart and Taylor 1988). The maximum percentage of genotypic diversity ($\hat{G}$), obtained from the equation $\hat{G}=G/N\times 100\%$ (where N is the population size), was used to compare the genotypic diversities between populations (Chen et al. 1994). The significance of differences in genotypic diversity between populations was calculated using a t-test (Chen et al. 1994). For each of the three populations, the different observations regarding the number of genotypes obtained from the variable number of isolates per tree were documented.

Population differentiation and assignment

In order to detect the differences in allele frequencies at individual loci between clone-corrected populations of *T. zuluensis*, the program POPGENE version 1.31 (Yeh et al. 1999) was used. The significance of differences in allele frequencies was determined with Chi-square tests (Workman and Niswander 1970).

Population differentiation was calculated using theta (θ) (Weir 1996), which is a modification of F_{ST} (Wright 1978). Theta (θ) values were measured using MULTILOCUS version 1.3 (Agapow and Burt 2001). The observed value was compared to that of 1 000 randomizations in which individuals were randomized across populations. The significance of θ was evaluated by the P value.

To test for structure in the isolates, the model-based Bayesian clustering method in STRUCTURE version 2.2 (Pritchard et al. 2000; Falush et al. 2003) was used. This method uses the allelic frequencies at each locus of each isolate and excludes any prior information about location. To test for the optimal number of groups, K, the model of admixture ancestry and independent allele frequency was used. Twenty iterations with K set from 1 to 10 were simulated with 300 000 runs each with a burn-in set at 30 000 runs. The likelihood values were plotted against the ln likelihood and delta likelihood values to determine the K with higher likelihood and lower standard deviation (Evanno et al. 2005). For the assignment of individuals into the optimal K obtained above, a second analysis was conducted by setting the runs at 1 000 000 with a burn-in of 100 000.

Mode of reproduction

In order to consider the reproduction mode for each of the clone-corrected populations, the Index of Association (I_A) using the program MULTILOCUS version 1.3, was calculated (Maynard Smith et al. 1993; Agapow and Burt 2001). The I_A tests the likelihood that two different individuals sharing the same allele at one locus will share the same allele at another locus. By comparing the observed data with the expected data of 1 000 randomly recombining

Table 2 Diversity indices of three populations of *T. zuluensis* from China obtained using ten microsatellite loci

Locus	Allele size	Population one	Population two	Population three
Czulu1	150	0.250	0.111	
	152	0.700	0.722	1.000
	156	0.050	0.167	
Czulu2	178	0.500	0.195	0.294
	186	0.500	0.472	0.294
	188		0.333	0.412
Czulu3	163	0.050		0.177
	172	0.950	1.000	0.823
Czulu6	322	0.250		
	325	0.400	0.194	0.118
	331	0.250	0.389	0.882
	334	0.100	0.417	
Czulu7	209	0.150		
	213	0.150	0.222	0.294
	221	0.550	0.584	0.412
	224	0.150	0.194	0.294
Kzulu5	234			0.059
	236		0.028	
	246	0.100	0.139	
	254	0.050		0.294
	256	0.500	0.667	0.471
	258	0.050		
	260	0.200	0.083	0.059
	270	0.100	0.083	0.117
Kzulu10	335	0.050	0.417	0.118
	365	0.250	0.139	
	385	0.050	0.083	0.882
	414	0.650	0.167	
	426		0.194	
Kzulu12	254			0.118
	260	0.050	0.028	
	268			0.470
	288	0.050	0.028	
	296	0.300		
	298	0.500	0.638	0.294
	304	0.100	0.306	0.118
Kzulu13	121	0.050		0.588
	123	0.350	0.139	
	127	0.350	0.389	0.118
	131		0.028	
	138	0.150	0.333	0.176
	152	0.050	0.083	0.118
	154	0.050	0.028	
Kzulu14	254	0.050		0.529
	256	0.350	0.139	0.059
	260	0.350	0.361	0.118
	264		0.028	
	270	0.150	0.333	0.176
	282	0.050	0.111	0.118
	284	0.050	0.028	
Ni[a]		21	38	38
Ng[b]		20	36	17
Na[c]		42	39	31
Npa[d]		4	4	3
AR[e]		4.17	3.70	3.10
H[f]		0.57	0.55	0.46
Npl[g]		10	9	9
G[h]		19.17	34.38	10.78
$\hat{G}$[i]		91%	90%	28%

[a] *Ni* Number of isolates (non clone-corrected)

[b] *Ng* Number of genotypes

[c] *Na* Number of alleles

[d] *Npa* Number of private alleles

[e] *AR* Allelic Richness

[f] *H* Gene Diversity (Nei 1973)

[g] *Npl* Number of polymorphic loci

[h] *G* Genotypic Diversity (Stoddart and Taylor 1988)

[i] $\hat{G}=G/N\%$ Percentage maximum diversity of genotype

datasets, recombination in each of the three populations could be determined (Taylor et al. 1999). If the observed data remain within the distribution range of the recombined data, the population is likely to be undergoing recombination. Otherwise, the population is most likely clonal and not undergoing recombination.

Results

Sampling and isolate identification

Numerous lesions were found on the stems of the infected trees, and in GuangDong Province, all the trees in the plantations surveyed were affected. Bark samples showing symptoms typical of Coniothyrium canker were collected from a total of 89 trees in the three regions sampled (Table 1, Fig. 1). One to four isolates were obtained from each tree with a total of 238 isolates resembling species of *Teratosphaeria*. Blast results of the ITS gene sequences of the isolates from China showed that 97 of them represented *T. zuluensis* (Table 1). Most of the remaining isolates represented different species in the Mycosphaerellaceae and Teratosphaeriaceae and their identities will be characterised. Of the isolates confirmed as *T. zuluensis*, twenty-one originated from 12 trees in Region One, 38 isolates from

21 trees in Region Two and 38 isolates from 24 trees in Region Three (Table 1, Fig. 1).

Polymorphic microsatellite loci

For the 97 isolates of *T. zuluensis*, a total of 50 different alleles were amplified by the ten species-specific polymorphic microsatellite markers (Table 2). Forty-two different alleles were observed in the population from Region One, 39 from Region Two and 31 from Region Three. There were two to eight alleles per locus. Private alleles were observed in all three populations. In total, 11 private alleles were identified, four each of which were detected in Regions One and Two, and three in Region Three. The allelic richness was highest in the population from Region One (4.17) followed by those from Regions Two (3.70) and Three (3.10). Locus Czulu3 was monomorphic in the population from Region Two and locus Czulu1 was monomorphic for the population from Region Three (Table 2). No monomorphic loci were detected in the population from Region One (Table 2).

Genotypic diversity versus number of loci

The plot for mean genotypic diversity against the number of loci for isolates from the three regions showed that a plateau of genotypic diversity had been reached using the set of ten microsatellite markers. This suggested that for each of the three regions, the genotypic diversity calculated for the isolates was sufficient to characterize the populations. When isolates from individual plantations or parks were considered, statistical support was not obtained in all cases. Populations for further analyses were, therefore, based on isolates from regions and not from individual plantations or parks.

Gene and genotypic diversity

The gene diversity levels in the three *T. zuluensis* populations were moderate to high. Values were H=0.57 for Population One, 0.55 for Population Two and 0.46 for Population Three (Table 2). The levels of clonality for each population were 4.8%, 5.3% and 55.3%, respectively (Table 2). Seventy-two genotypes were identified across all the Chinese *T. zuluensis* isolates, with 20 different genotypes in Population One, 36 in Population Two and 17 in Population Three. Only one genotype was shared between any of the populations and this was between Population One and Two (Table 2). The maximum genotypic diversity was $\hat{G}$= 91% for Population One, 90% for Population Two and 28% for Population Three (Table 2). There was no significant difference (P=0.033) for the genotypic diversity between Populations One and Two, while the genotypic diversities between Populations One and Three, as well as Populations Two and Three were significant (P= 1.61, P=1.67, respectively).

In all three populations, there were situations where more than one genotype of *T. zuluensis* was present on a single *Eucalyptus* tree. The greatest number of genotypes obtained from a single tree was in Population Two with four genotypes obtained from four isolates from different cankers on a single tree. In Population Three, one genotype was identified among six isolates from four trees.

Population differentiation and assignment

Significant differences in allelic frequencies were found between loci for the populations in the pairwise comparisons. The majority of these differences were detected between Populations One and Three, and between Populations Two and Three (Table 3).

Results showed that all three populations were significantly different from each other (P<0.001) based on calculations of theta (θ). The largest differentiation was found between Populations One and Three (θ=0.207) (Table 4).

Structure analyses showed that the number of groups (K) obtained with the highest likelihood (ln and delta K) and lowest standard deviation was equal to four. Assignment of individuals into these groups resulted in the majority of

Table 3 Pairwise Chi-square comparisons of allelic frequencies among three Chinese *T. zuluensis* populations

Pairs of populations		Locus										Number of significantly different loci[a]
		Czulu1	Czulu2	Czulu3	Czulu6	Czulu7	Kzulu5	Kzulu10	Kzulu12	Kzulu13	Kzulu14	
Population One and Two	Chi^2	2.95	4.82	1.83	16.01*	5.91	6.34	19.88*	13.92*	7.07	7.36	3 out of 10
	df	2	2	1	3	3	6	4	4	6	6	
Population One and Three	Chi^2	6.09*	7.00*	1.52	15.46*	4.68	8.50	30.54*	19.55*	18.35*	14.87*	7 out of 10
	df	2	2	1	3	3	6	3	6	5	5	
Population Two and Three	Chi^2	5.82	1.59	6.73*	12.62*	1.39	16.51*	33.43*	26.38*	28.44*	24.08*	7 out of 10
	df	2	2	1	2	2	6	4	5	6	6	

[a] In the pairwise comparison, the total number of loci where the frequency differed significantly from each other (*P<0.05), is shown in the last column

Table 4 Pairwise comparisons of population differentiation (θ, above the diagonal) among three Chinese *T. zuluensis* populations

Teratosphaeria zuluensis	Population one	Population two	Population three
Population One	—	0.058***	0.207***
Population Two		—	0.183***
Population Three			—

For θ, asterisks represent the level of significance (***P<0.001)

isolates from Region One (Population One) being assigned to Group One (38%) and Two (36%). Most of the isolates of Region Two (Population Two) were assigned to Group One (44%) and Three (37%) and more than half (61%) of the isolates of Region Three (Population Three) were assigned to Group Four (Fig. 1).

Mode of reproduction

The I_A values of the observed data for Populations One and Three fell outside the distribution range for a recombining population (P<0.001) (Table 5), indicating that no recombination is evident in these populations. There was marginal evidence for recombination in Population Two (P=0.011) (Table 5).

Discussion

In this study, the genetic diversity and structure of three geographically different populations of *T. zuluensis* from *Eucalyptus* plantations in South China were analyzed using ten microsatellite markers. The results showed that multiple genotypes of the pathogen exist on a single infected tree. In all three populations, the genetic diversity of the pathogen was relatively high and private alleles were present. A significant amount of population differentiation was observed between the different populations, suggesting that the three *T. zuluensis* populations in China most likely originated independently of each other.

The genetic diversity of two populations considered in this study and that of a previous Chinese population analysed by Cortinas et al. (2010) were the highest for any population of *T. zuluensis* studied thus far. This includes that of a population from South Africa, which was previously hypothesised to represent a possible origin of this pathogen (Wingfield et al. 1996). This, together with marginal evidence for recombination in Chinese populations from *Eucalyptus* in the current and a previous study (Cortinas et al. 2010), supports the view that *T. zuluensis* is not native to South Africa as has previously been suggested (Cortinas et al. 2010).

Coniothyrium canker has only ever been reported on *Eucalyptus* spp. grown in plantations outside Australia. It is possible that this pathogen is native to Australia but has never been found in that country. This might be due to the fact that the intensively managed and highly selected *Eucalyptus* clones planted in China enable the expression of a disease by a pathogen that is unable to produce symptoms on *Eucalyptus* in their native range in Australia. Alternatively, the pathogen is present in Australia but is rare and has not yet been detected in plantations or native forests.

The collection of different genotypes of *T. zuluensis* from the same *Eucalyptus* tree was unexpected. This has not been observed for populations of the pathogen studied from South Africa or Malawi (Cortinas et al. 2010). However, a similar observation has been made for the closely related pathogens *T. nubilosa* (Cooke) Crous & U. Braun in South Africa (Hunter et al. 2008; Pérez et al. 2010), and *Mycosphaerella populorum* G.E. Thomps. from North-eastern America, where more than 90% of the genetic diversity was distributed within a single tree (Feau et al. 2005). The high level of genotypic diversity for isolates collected on the same tree reflects a diverse population in the area, with multiple and independent infections occurring on individual trees.

The blast results using the sequences of the ITS gene region showed that some of the obtained isolates were not those of *T. zuluensis*, but reside within the Mycosphaerellaceae or Teratosphaeriaceae. Nothing is known regarding these fungi but species in these families are common on

Table 5 Observed Index of Association (I_A) and range of I_A values obtained for the three Chinese *T. zuluensis* populations, after 1 000 randomizations

Populations	Observed I_A	Range of obtained I_A values	Obs. I_A within randomized the data range. (i.e. evidence for recombination)	P value
Population One	0.867	–0.329 – 0.431	No	P<0.001
Population Two	0.198	–0.224 – 0.260	Yes	P=0.011
Population Three	0.831	–0.364 – 0.550	No	P<0.001

Eucalyptus and are probably endophytes able to sporulate on moribund tissue. Further investigations will be needed to clarify the role of these fungi and whether they might contribute to disease development.

The genetic diversities obtained for isolates of *T. zuluensis* from the three different geographic regions correlate with the history of *Eucalyptus* plantation development in these regions of South China. *Eucalyptus* trees have a longer history of planting in Region One (GuangXi Province) and Region Two (GuangDong Province) than in Region Three also in GuangDong Province (Qi 2002). Similarly, the genetic diversity of the populations in Regions One and Two was higher than that in Region Three. In the early stage of *Eucalyptus* development in China, *Eucalyptus* trees were generally established from seeds, most of which were imported from Australia (Qi 2002). It has been suggested that species of Teratosphaeriaceae and Mycosphaerellaceae infecting *Eucalyptus* have been moved between countries on seeds (Keane et al. 2000; Zhan et al. 2003; Burgess et al. 2007; Hunter et al. 2008; Pérez et al. 2009). It is thus possible that *T. zuluensis* was introduced into China on infected *Eucalyptus* seeds. The pathogen would then have spread to different regions of China over time, leading to the genetic diversity for its populations in different regions of China being different, and correlating with the history of *Eucalyptus* plantation development.

In order to manage the disease caused by *T. zuluensis*, the introduction and exchange of different genotypes of this fungus to new regions needs to be prevented. This is especially important to reduce the development of new and possibly more virulent genotypes. Significant evidence for the movement of *T. zuluensis* between the regions sampled in this study was observed. This was reflected in the presence of a shared genotype in Regions One and Two, as well as by results of the allele frequencies and assignment analyses. Isolates from Regions One and Two were more similar to each other than to those from Region Three. These results reflect the frequent trade and exchange of *Eucalyptus* breeding material that occur between regions that are in close proximity as suggested by Wingfield et al. (2008).

It is clear that *T. zuluensis* is becoming increasingly important in Chinese *Eucalyptus* plantations. Compared to previous studies (Cortinas et al. 2006b, 2010), the distribution of Coniothyrium canker and associated *T. zuluensis* on *Eucalyptus* spp. and clones in China continue to increase. Although no girdling cankers were observed on trees in this study and tree death was not a concern, *T. zuluensis* results in lesions in the wood and this is especially important in *Eucalyptus* sawn timber products (Wingfield et al. 1996; Old et al. 2003). Selection and breeding programmes focusing on developing *Eucalyptus* genotypes tolerant to infection by *T. zuluensis* will be important in the future.

Acknowledgements This study was initiated through a bilateral agreement between South Africa and China, and funded through projects 30771732, 2007DFA31190 and 2008B050100014. We also appreciate the financial and logistical support of the members of Tree Protection Co-operative Programme (TPCP). We are grateful to Mr Albe Van Der Merwe for advice and discussion, and Chinese colleagues at the XiJiang Forestry Bureau, JinGui Nursery and DongMen Forestry Farm, XinTao Mou and GuiXiang Zhao for their valuable assistance in the field.

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