Unexpected genetic diversity revealed in the *Eucalyptus* canker pathogen *Teratosphaeria gauchensis*

M. N. CORTINAS, I. BARNES, B. D. WINGFIELD and M. J. WINGFIELD

Department of Genetics, Forestry and Agriculture Biotechnology Institute (FABI), University of Pretoria, PBag X20, Hatfield, Pretoria 0028, South Africa.

Corresponding author:

E-mail: brenda.wingfield@fabi.up.ac.za

Tel: +27-12-420-6471

Fax: +27-12-420-3960

Present address:

University of Pretoria

Forestry and Agricultural Biotechnology Institute (FABI)

PBag X20,

Hatfield,

Pretoria 0028

South Africa

Abstract

Teratosphaeria gauchensis causes a serious canker disease on *Eucalyptus* spp. in plantations in South America and Africa. The pathogen is closely related to, but distinct from *T. zuluensis* that causes a similar stem canker disease on *Eucalyptus*. The objective of this study was to use 10 previously developed polymorphic microsatellite markers to study the population diversity of *T. gauchensis,* based on collections of the fungus made in Argentina and Uruguay. The alleles were size -analyzed to determine population genetic parameters of the *T. gauchensis* populations. The results showed that isolates from the two collection sites represent the same population. Overall, the genetic diversity amongst isolates was higher than expected and inconsistent with the notion that the pathogen represents a recent introduction into South America.

Additional keywords: ascomycete, *Eucalyptus* stem canker, forest pathogen, *Teratosphaeria gauchensis*, *Teratosphaeria zuluensis*, microsatellites, population structure.

Teratosphaeria gauchensis (M.N. Cortinas, Crous & M.J. Wingf.) M.J Wingf. & Crous and the related *Teratosphaeria zuluensis* (M.J. Wingf., Crous & T.A. Cout.) M.J. Wingf. & Crous cause a disease known as Coniothyrium canker on *Eucalyptus* spp. *Teratosphaeria zuluensis* was the first of these fungi to be described after it was discovered causing serious damage to the stems of clonally propagated *Eucalyptus grandis* in the Kwa-Zulu Natal province of South Africa (Wingfield *et al.* 1997). The disease spread rapidly in the 1990's and became one of the most serious impediments to *Eucalyptus* plantation forestry in that country (Old *et al*. 2003).

Due to the serious economic impact of Coniothyrium canker on plantations in South Africa, there were various studies undertaken to better understand the relevance and biology of *T. zuluensis* (van Zyl, 1999, van Zyl *et al.* 2002). Some years later, a very similar disease was discovered on *E grandis* clones in Argentina and Uruguay and surprisingly, the causal agent was found to be different to *T. zuluense* (Cortinas *et al*. 2006b). The causal agent of the disease was described as *Teratosphaeria gauchensis*. *Teratosphaeria gauchensis* and *T. zuluensis* are morphologically almost indistinguishable and they give rise to the same symptoms after infection. Thus, the only reliable means to distinguish between the two fungi is via DNA sequence comparisons. Both fungi were initially described as mitotic species and residing in the teleomorph genus *Mycosphaerella* based on phylogenetic inference (Cortinas *et al.* 2006b; Andjic *et al.* 2007) but recent taxonomic re-evaluation has relegated them to anamorphs of *Teratosphaeria* in the Teratosphaeriaceae (Crous *et al.* 2007; Crous *et al.* 2009).

Teratosphaeria gauchensis causes cankers on young branches and tree trunks although it has also been isolated from leaf spots on *E. maidenii* and *E. tereticornis* in Uruguay (Pérez *et al.* 2009a). The typical stem and trunk lesions caused by this fungus are necrotic and have a characteristic dark oval shape (Cortinas *et al.* 2006b). The extent of the lesions varies depending on the susceptibility of the infected trees. Severe infections arise from small cankers that merge to cover large areas of the trunk. Both the soft tissue and wood become malformed resulting in retarded growth and girdling can be observed at the tree tops. Kino pockets are formed as part of the defence response of the trees. Kino that exudes from the cankers can cause the stems to become a black colour. In some cases, diseased trees also produce epicormic shoots alongside the cankers that can cause the terminal parts of the branches and stems to die (Wingfield *et al.*1997; Cortinas *et al.* 2006b).

Very little is known regarding the biology of *T. gauchensis*. It is presumed that the fungus exists in a haploid state (Wingfield *et al.* 1997; Crous, 1998; Crous *et al.* 2004; 2006). In nature, only

asexual pycnidia are found on the bark lesions. These structues give rise to mitospores (conidia) that are presumably responsible for short distance dispersal, as is the case for closely related fungi (Feau *et al.* 2005; Milgate *et al.* 2005; Hunter *et al.* 2008). Sexual structures have never been observed in nature nor have they been produced in culture.

The origin of *T. gauchensis* is not known. Its distribution is limited to Uganda and Ethiopia (Gezahgne 2003; Gezahgne *et al.* 2005), Argentina and Uruguay (Gezahgne *et al.* 2004; Cortinas *et al.* 2006b) and Hawaii (Cortinas *et al.* 2004). It is has also never been found on any host other than *Eucalyptus* species, which is an exotic species in all these countries. The current distribution of *T. gauchensis* does not overlap with the distribution of the sibling species *T. zuluensis* (Cortinas *et al.* 2006b). The fact that *Eucalyptus* species are not native to any of the countries where *T. gauchensis* has been found and that *T. gauchensis* has a close phylogenetic relationship to other *Teratosphaeria Eucalyptus*-specific pathogens, it is likely, that this fungus is yet be discovered in the native range of *Eucalyptus*. If that is the case, then one would expect to find fungal populations with low genetic diversity in areas where it has been introduced, which is true for the related *T. nubilosa* (= *M. nubilosa*) (Pérez *et al.* 2009).

The aim of this study was to investigate the population diversity and structure of *T. gauchensis* found on non-native *Eucalyptus* in plantations of Argentina and Uruguay where the associated disease has been particularly serious. To achieve this goal, ten polymorphic microsatellite markers, recently developed for this species (Cortinas *et al.* 2008), were used to calculate estimates of haplotype richness and evenness, haplotypic diversity and genetic differentiation for isolates collected in Argentina and Uruguay.

Sampling and isolations

Necrotic lesions on the bark of infected *Eucalyptus* clones were sampled from plantations in the neighbouring provinces of Entre Ríos, Corrientes and Misiones in Argentina and from two areas (Rivera and Paysandú), in the Northern part of Uruguay. The sampling area covered a range of approximately 450 km in a North-South direction and 300 Km in an East- West direction (Table 1). Samples were collected as part of a disease evaluation project in Uruguay and Argentina between 1999 and 2005. Samples were taken from lesions on the stems of randomly chosen trees approximately 2 m above the ground.

One hundred and thirty one single conidial isolations were made from lesions of 23 trees in Argentina and 43 trees in Urugauy (Table 1) as described previously (Cortinas *et al.* 2006a). These single conidial cultures were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, where they are maintained in long-term storage facilities.

DNA extraction and amplification of microsatellite loci

Single conidial isolates were obtained and DNA from these isolates was extracted as described by Cortinas *et al.* (2006a). Ten pairs of fluorescently labelled primer sets for 10 polymorphic microsatellite loci of *T. gauchensis* (Cortinas *et al.* 2008) were used in this study. The microsatellite loci were originally subscribed to GenBank as "*K. gauchensis*" followed by a number. In this publication they are abbreviated in the tables as "Kg" loci, followed by a number. The microsatellite loci were amplified by Polymerase Chain Reaction (PCR) and the amplified products were size-separated on an ABI 3100 Automated DNA Sequencer (Applied Biosystems, Foster City, USA) using GENSCAN LIZ 500 (-250) (Applied Biosystems) as internal size standard as described in Cortinas *et al.* (2008).

Thirty-eight isolates were analysed from Argentina. These included 10 isolates from the Entre Rios province, 17 from the Corrientes province, eight from the Misiones of Argentina and three from undefined source within these provinces. Ninety-three isolates were obtained and analysed from Uruguay including 33 from the Paysandú department and 60 from the Rivera department (Table 1). GENEMAPPER, version 3.0 (Applied Biosystems) software package was used to carry out the fragment size analysis. Based on size differences of the amplicons produced for each locus, different alleles were identified. For further analyses, each allele was designated by their size in nucleotides or by a letter of the alphabet.

The gene diversity (*H*), genotypic diversity (*G*), richness and evenness, population differentiation and trecombination analyses using linkage disequilibrium (LD) and Index of Association (I_A) were performed as in Cortinas *et al.* 2008.

Allele and genetic diversity

Forty- three different alleles were recovered for the 131 isolates of the *T. gauchensis* collected and analysed. Individually, 31 different alleles were recovered from the Argentinean samples and 35 from the Uruguayan population (Table 2). The number of alleles at individual loci, for both populations, ranged from two to eight. Private alleles were observed in both populations; five from Argentina and nine from Uruguay. The majority of these private alleles were present with frequencies higher than 3%. No monomorphic loci were observed.

The gene diversity (H) calculated for *T. gauchensis* was 0.43 in Argentina and 0.42 in Uruguay (Table 2). Ninety-one different genotypes were identified across the two *T. gauchensis* populations (Table 2). One genotype was found to be shared between the Argentinean and Uruguayan populations. The number of repeated genotypes was 26.3% for the Argentinean population and 33.3% for the Uruguayan population. The maximum genotypic (haplotype) diversity was similar for Uruguay ($\hat{G}$ = 50%) and Argentina ($\hat{G}$ = 54%) (Table 2). The *t* test ($P < 0.05$) showed no significant differences between the genotypic diversities of the Argentinean and Uruguayan populations.

The heterogeneity within the populations (relative richness and evenness) values obtained were S= 3.29 and V'= 0.965 for Argentina and S= 3.96 and V'= 0.967 for Uruguay, very similar for both populations. Both had regression values = 1 and similar slopes (β= 2.64 for Argentina and β= 2.43 for Uruguay). Together, these results showed moderate to high haplotype heterogeneity and a high level of evenness (groups of clones of similar membership size). The majority of repeated haplotypes in Argentina and Uruguay formed groups of two individuals.

Population differentiation and assignment tests

The allelic frequencies across populations were compared by calculating the differences in allelic frequencies per locus and between pairwise populations (Table 3). The analysis of the loci showed that the frequencies of the alleles between the populations of Argentina and Uruguay were only significantly different at one (Locus 6) of 10 loci. The theta value of 0.011 ($P< 0.05$) indicated no differentiation among the Argentinean and Uruguayan populations.

No admixture patterns were detected using STRUCTURE as clusters were not detected. The assignment diagrams showed that the majority of individuals assigned to all different K groups in similar proportions in the tested range between K=1 to K=10.

Recombination analyses

In *T. gauchensis,* low LD was found using two-locus pairwise analyses: zero out of 45 comparisons in the Argentinean population and four of 45 comparisons in the Uruguayan population showed linkage disequilibrium (Table 4). The results obtained from the multilocus Index of Association (I_A) analyses were comparable to the LD results calculated using the pairwise method (Table 4). The observed values of I_A in *T. gauchensis* fell within the randomized distribution of allelic frequencies suggesting that recombination could be occurring in both *T. gauchensis* populations.

Discussion

Teratosphaeria gauchensis is a pathogen of growing importance to a rapidly expanding *Eucalyptus* plantation industry in South America. This study provides the first consideration of its genetic diversity and thus, long term durability of resistance in intensively propagated planting stock. As such, populations of *T. gauchensis* from Argentina and Uruguay showed a genetic structure that is very different to one expected for a recently introduced pathogen. These populations contained moderate to high level of genetic variation, homogeneous distribution of haplotypes, no differentiation between populations and indications that recombination is occurring.

The moderate to high levels of genetic diversity found in the *T. gauchensis* populations from South America were unexpected as the disease was only discovered in Argentina and Uruguay in the last two decades. Because of its occurrence on *Eucalyptus* trees, it was thought to have been introduced into these countries relatively recently, probably from the native range of *Eucalyptus*. Thus, a low genetic diversity and a small number of predominant haplotypes (clones) were expected in the populations of *T. gauchensis*. This would be similar to a number of other closely related *Eucalyptus* pathogens recently reported in Uruguay (Balmelli *et al.* 2004; Pérez *et al.* 2009). For example, the *Eucalyptus* leaf blotch pathogen *T. nubilosa,* was found to be clonal, which suggests a recent, localized introduction in the area (Pérez *et al.* 2009).

The levels of genetic diversity of *T. gauchensis* found in this study were comparable with the genetic diversities of other phylogenetically related *Mycosphaerella* and *Teratosphaeria* species from their native ranges. These species include *M. musicola* (Hayden *et al.* 2003b; 2005; Zandjanakou-Tachin *et al.* 2009), *M. fijiensis* (Carlier *et al.* 1996; Hayden *et al.* 2003a) and *T. nubilosa* (Hunter *et al.* 2008; 2009). Interestingly, with the exception of *T. gauchensis*, all these species have well characterized sexual states that would promote their genetic diversity.

Results of this study showed evidence of recombination in the studied *T. gauchensis* population from Argentina. This result was unexpected as sexual structures have never been found in the field for this fungus. Nonetheless, there is precedence for finding evidence of recombination in apparently asexual fungi (Taylor *et al.* 1999; Zhou *et al.* 2007). From this study we can conclude that *T. gauchensis* in all likelihood has a mixed mode of reproduction and has asexual and sexual reproductive structures similar to the most closely related *Mycosphaerella* spp. (Cortinas *et al*. in press; Crous *et al.* 2004; 2006; Hunter *et al.* 2008; Pérez *et al.* 2010).

Population genetic analyses showed that the two collections of isolates from Argentina and Uruguay can be considered as part of the same genetic pool, rather than two separate and unrelated populations. Thus, the differentiation tests showed weak to no differentiation between the two *T. gauchensis* populations. These results were further supported by the assignment tests whereby the individuals from Argentina and Uruguay, regardless of the number of clusters tested, were separated in equal proportions among clusters, indicating a lack of population structure for the isolates (Pritchard *et al.* 2000).

Analyses of *T. gauchensis* isolates from Argentina and Uruguay are not compatible with the hypothesis that this is a recently introduced pathogen. One possible explanation for this result is that the fungus originated in Australasia where *Eucalyptus* is native, as in the case of *T. nubilosa* (Hunter *et al.* 2008; 2009). This would be consistent with recent well documented examples of new *Eucalyptus* pathogens first being described from plantations outside the native range of *Eucalyptus* and later being discovered in Australia (Wingfield *et al.* 1996; Burgess *et al.* 2007). An alternative interpretation is that the pathogen has undergone a host shift from native Myrtaceae in Argentina and Uruguay. There are a growing number of *Eucalyptus* pathogens that have undergone host jumps (Slippers *et al.* 2005) from native Myrtaceae and Melastomataceae (Myrtales) in countries where *Eucalyptus* spp. have been planted as exotics (Wingfield 2003; Wingfield *et al.* 2008; Glen *et al.* 2007) Many of these examples

are from South America including Uruguay (Pérez 2008). The most recent examples are *Quambalaria eucalypti* (Pérez *et al.*2008), *Neofusicoccum eucalyptorum* (Pérez *et al.* 2009b), *Puccinia psidii* (Pérez *et al.*2010a, in press) and members of Botryosphaeriaceae (Pérez *et al.*2010b). It would not be unusual for *T. gauchensis* to have behaved in a similar fashion.

Acknowledgements

We are grateful for the assistance from forestry companies in Uruguay and Argentina and express our gratitude to Sophie Arnaud-Haond for her comments and help using GENCLONE 2.O. We also acknowledge the National Research Foundation (NRF), members of the Tree Protection Co-operative Program (TPCP), the THRIP initiative of the Department of Trade and Industry and the DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB), University of Pretoria, South Africa for financial support.

References

Agapow PM, Burt A (2001) Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes* **1**, 101–2.

Andjic V, Barber PA, Carnegie AJ, Hardy GEStJ, Wingfield MJ, Burgess TI (2007) A morphological and phylogenetic reassessment of the genus *Phaeophleospora* and the resurrection of the genus *Kirramyces*. *Mycological Research* **111**, 1184-1198.

Arnaud-Haond S, Belknir K (2007) GENCLONE 1.0: a new program to analyse genetics data on clonal organisms. *Molecular Ecology Notes* **7**, 15–17.

Arnaud-Haond S, Duarte CM, Alberto F, Serrão (2007) Standardizing methods to address clonality in population studies. *Molecular Ecology* **16**, 5115–5139.

Balmelli G, Marroni V, Altier N, Garcia R (2004) Potencial del mejoramiento genético para el manejo de enfermedades en *Eucalyptus globulus*. Serie Técnica INIA Tacuarembó, Uruguay **143**, 1–43.

Burgess TI, Andjic V, Wingfield MJ, Hardy GEStJ (2007) The eucalypt leaf blight pathogen *Kirramyces destructans* discovered in Australia. *Australasian Plant Disease Notes* **2**, 141–144.

Carlier J, Lebrun MH, Zapater MF, Dubois C, Mourichon X (1996) Genetic structure of the global population of banana black streak fungus, *Mycosphaerella fijiensis*. *Molecular Ecology* **5**, 499–510.

Chen RS, Boeger JM, McDonald BA (1994) Genetic stability in a population of a plant pathogenic fungus over time. *Molecular Ecology* **3**, 209–18.

Cortinas MN, Barnes I, Wingfield MJ Wingfield BD (2010) Genetic diversity in the *Eucalyptus* stem pathogen *Teratosphaeria zuluensis.Australasian Plant Pathology* (in press).

Cortinas MN, Burgess T, Dell B, Xu D, Crous PW, Wingfield BD, Wingfield MJ (2006a) First record of *Colletogloeopsis zuluense* comb. nov., causing stem canker of *Eucalyptus* in China. *Mycological Research* **110,** 229–236.

Cortinas MN, Crous PW, Wingfield BD, Wingfield MJ (2006b) Multilocus gene phylogenies and phenotypic characters distinguish two species within the *Colletogloeopsis zuluensis* complex associated with *Eucalyptus* stem cankers. *Studies in Mycology* **55**, 135–148.

Cortinas MN, Koch N, Thane J, Wingfield BD, Wingfield MJ (2004) First record of the *Eucalyptus* stem canker pathogen, *Coniothyrium zuluense* from Hawaii. *Australasian Plant Pathology* **33**, 309–312.

Cortinas MN, Wingfield BD, Wingfield MJ (2008) Microsatellite markers for the *Eucalyptus* stem canker fungal pathogen *Kirramyces gauchensis*. *Molecular Ecology Resources* **8**, 590–592.

Crous PW (1998) *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of *Eucalyptus*. *Mycologia Memoir* **21**, 1–170.

Crous PW, Braun U, Groenewald JZ (2007) *Mycosphaerella* is polyphyletic. *Studies in Mycology,* **58**: 1–32.

Crous PW, Groenewald JZ, Mansilla JP, Hunter GC, Wingfield MJ (2004) Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. *Studies in Mycology* **50**, 195–214.

Crous PW, Groenewald JZ, Summerell BA, Wingfield BD, Wingfield MJ (2009) Co-occurring species of *Teratosphaeria* on *Eucalyptus*. *Persoonia* **22**, 38-48.

Crous PW, Wingfield MJ, Mansilla JP, Alfenas AC, Groenewald JZ (2006) Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus.* II. *Studies in Mycology* **55**, 99–131.

Falush D, Stephens M, Pritchard JK (2003) Inference of Population Structure Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. *Genetics* **164**, 1567–1587.

Feau N, Hamelin RC, Vamdecasteele C, Stanosz GR, Bernier L (2005) Genetic structure of *Mycosphaerella populorum* (Anamorph *Septoria musiva*) populations in North-Central and Northeastern North America. *Phytopathology* **95**, 608–616.

Gezahgne A, Cortinas MN, Wingfield MJ, Roux J (2005) Characterisation of the *Coniothyrium* stem canker pathogen on *Eucalyptus camaldulensis* in Ethiopia. *Australasian Plant Pathology* **34**, 1–6.

Gezahgne A, Roux J, Thu PQ, Wingfield MJ (2004) Coniothyrium stem canker of *Eucalyptus*, new to Argentina and Vietnam. *South African Journal of Science* **99**, 587–588.

Gezahgne A (2003) Diseases of exotic plantations *Eucalyptus* and *Pinus* species in Ethiopia. *South African Journal of Science* **99**, 29–33.

Glen M, Smith AH, Langrell SRH, Mohammed CL (2007) Development of nested polymerase chain reaction detection of *Mycosphaerella* spp. and its application to the study of leaf disease in *Eucalyptus* plantations. *Phytopathology* **97,** 132–144.

Hayden HL, Carlier J, Aitken EAB (2003a) Genetic structure of *Mycosphaerella fijiensis* populations from Australia, Papua New Guinea and the Pacific Islands. *Plant Pathology* **52,** 703–712.

Hayden HL, Carlier J, Aitken EAB (2003b) Population differentiation in the banana leaf spot pathogen *Mycosphaerella musicola*, examined at a global scale. *Plant Pathology* **52**, 713–9.

Hayden HL, Carlier J, Aitken EAB (2005) The genetic structure of Australian populations of *Mycosphaerella musicola* suggests restricted gene flow at the continental scale. *Phytopathology* **5,** 489–498.

Hunter GC, van der Merwe NA, Burgess TI, Carnegie AJ, Wingfield BD, Crous PW, Wingfield MJ (2008) Global movement and population biology of *Mycosphaerella nubilosa* infecting leaves of cold-tolerant *Eucalyptus globulus* and *E. nitens*. *Plant Pathology* **57**, 235–242.

Hunter GC, Crous PW, Carnegie AJ, Wingfield MJ (2009) *Teratosphaeria nubilosa*, a serious leaf disease pathogen of *Eucalyptus* spp. in native and introduced areas. *Molecular Plant Pathology* **10,** 1–14.

Milgate AW, Vaillancourt RE, Mohammed C, Powell M, Potts BM (2005) Genetic structure of a *mycosphaerella cryptica* population. *Australasian Plant Pathology* **34**, 345–354.

Nei M, 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences USA* **70**, 3321–3.

Old KM, Wingfield MJ, Yuan ZQ (2003) *A manual of diseases of eucalypts in South-East Asia*. Center for International Forestry Research, Bogor, Indonesia.

Pérez C (2008) Relationship between pathogens of *Eucalyptus* and native Myrtaceae in Uruguay. Doctoral Thesis. University of Minnesota. Saint Paul. 133 p.

Pérez CA, Altier N, Simeto S, Wingfield MJ, Blanchette RA (2010a) *Puccinia psidii* on cultivated *Eucalyptus* and native Myrtaceae in Uruguay. *Mycological Progress*, in press.

Pérez CA, de Beer ZW, Altier NA, Wingfield MJ, Blanchette RA (2008) Discovery of the eucalypt pathogen *Quambalaria eucalypti* infecting a non-*Eucalyptus* host in Uruguay. *Australasian Plant Pathology* **37,** 600–604.

Pérez CA, Wingfield MJ, Altier NA, Blanchette RA (2009a) Mycosphaerellaceae and Teratosphaeriaceae associated with *Eucalyptus* leaf diseases and stem cankers in Uruguay. *Forest Pathology* **39**, 349–360.

Pérez CA, Wingfield MJ, Slippers B, Altier NA, Blanchette RA (2009b) *Neofusicoccum eucalyptorum*, a *Eucalyptus* pathogen, on native Myrtaceae in Uruguay. *Plant Pathology* **58,** 964–970.

Pérez CA, Wingfield MJ, Slippers B, Altier, NA, Blanchette RA (2010b) Endophytic and canker-associated Botryosphaeriaceae occurring on non-native *Eucalyptus* and native Myrtaceae trees in Uruguay. *Fungal Diversity* **41,** 53–69.

Pérez G, Hunter GC, Slippers B, Pérez C, Wingfield BD, Wingfield MJ (2009) *Teratosphaeria* (*Mycosphaerella) nubilosa*, the causal agent of Mycosphaerella leaf disease (MLD), recently introduced into Uruguay. *European Journal of Plant Pathology* **125**, 109–118.

Pérez G, Slippers B, Wingfield BD, Hunter GC, Wingfield MJ (2010) Micro- and macrospatial scale analyses illustrates mixed mating strategies and extensive geneflow in populations of an invasive haploid pathogen. *Molecular Ecology* **19,** 1801–1813.

Slippers B, Stenlid J, Wingfield MJ (2005) Emerging pathogens: fungal host jumps following anthropogenic introduction. *Trends in Ecology and Evolution* **20,** 420–421.

Stoddart JA, Taylor JF (1988) Genotype diversity: Estimation and prediction in samples. *Genetics* **118**, 705–711.

Taylor JW, Jacobson DJ, Fisher MC (1999) The evolution of asexual fungi: reproduction, speciation and classification. *Annual Review of Phytopathology* **37,** 197–246.

Van Zyl LM (1999) Factors associated with Coniothyrium Canker of *Eucalyptus* in South Africa. PhD thesis, University of the Orange Free State, Bloemfontein.

Van Zyl LM, Coutinho TA, Wingfield MJ (2002) Morphological, cultural and pathogenic characteristics of *Coniothyrium zuluense* isolates from different plantation regions in South Africa. *Mycopathologia* **155,** 149–153.

Weir BS (1996) *Genetic Data Analysis II*. Sinauer Associates, Sunderland, MA.

Wingfield MJ, (2003) Increasing threat of diseases to exotic plantation forests in the southern hemisphere: Lessons from *Cryphonectria* canker. *Australasian Plant Pathology* **32,** 133–139.

Wingfield MJ, Crous PW, Boden D (1996) *Kirramyces destructans sp. nov., a serious leaf pathogen of Eucalyptus in Indonesia. South African Journal of Botany* **62**, 325–327.

Wingfield MJ, Crous PW, Couthinho TA (1997) A serious new canker disease of *Eucalyptus* in South Africa caused by a new species of *Coniothyrium. Mycopathologia* **136,** 139-145.

Wingfield MJ, Slippers B, Hurley BP, Coutinho TA, Wingfield BD, Roux J (2008) Eucalypt pests and diseases: growing threats to plantation productivity *Southern Forests: a Journal of Forest Science* **70,** 139–144.

Workman PL, Niswander JD (1970) Population studies in South-western Indian tribes. II. Local genetic differentiation in the Papago. *American Journal of Human Genetics* **22**, 24–49.

Wright S (1978) *Evolution and the Genetics of Populations*, *vol.4: Variability Within and Among Natural Populations*. Chicago, USA: University of Chicago Press.

Yeh FC, Yang RC, Boyle T (1999) *POPGENE*. Version 1.31. *Microsoft Windows based freeware for population genetic analysis*. University of Alberta, Alberta.

Zandjanakou-Tachin, M,Vroh-Bi I, Ojiambo PS, Tenkouano A, Gumedzoe YM, Bandyopadhyay R (2009) Identification and genetic diversity of *Mycosphaerella* species on banana and plantain in Nigeria. *Plant Pathology* **58**, 536–546.

Zhou X, Burgess TI , De Beer WZ, Lieutier F, Yart A, Carnegie A, Portales JM, Wingfield BD, Wingfield MJ (2007) High intercontinental migration rates and population admixture in the sapstain fungus *Ophiostoma ips*. *Molecular Ecology* **16**, 89–99.

Table 1. List of *T. gauchensis* isolates included in this population study.

Country	Province /Department	Host	Collection Period	Collectors	Number of trees	Number of isolates
Argentina	Total 3 provinces	*E. grandis*	2001/2003/2004	MJ Wingfield/	23	38
	Entre Ríos		2003, 2004	MN Cortinas	12	26
	Corrientes		2001		1	1
	Misiones		2001		7	8
	Undefined within the 3 provinces		1999		3	3
Uruguay	Total 2 departments	*E. grandis*	1999/2001/2005	MJ Wingfield/	43	93
	Paysandú		1999, 2001, 2005	MN Cortinas	23	33
	Rivera		1999, 2001, 2005		20	60

Table 2. Allelic frequencies and other diversity indices of the clone-corrected populations from Argentina and Uruguay at 10 *T. gauchensis* microstellite loci.

Loci	Alleles	Argentina	Uruguay
Kg-1	A	0.036	0.064
	B	0.179	0.302
	C	0.536	0.508
	D	0.179	0.079
	E	0.036	0.016
	F		0.016
	G		0.016
	H	0.036	
Kg-2	A	0.643	0.429
	B	0.215	0.427
	C	0.143	0.127
	D		0.016
Kg-3	A	0.500	0.508
	B	0.036	
	C	0.429	0.429
	D	0.036	0.032
	E		0.032
Kg-4	A	0.964	0.968
	B	0.036	
	C		0.016
	D		0.016
Kg-5	A	0.679	0.740
	B	0.286	0.222
	C	0.036	0.032
Kg-6	A	0.607	0.571
	B	0.143	0.397
	C		0.032
	D	0.250	
Kg-7	A	0.679	0.6825
	B	0.286	0.2857
	C	0.036	0.0317
Kg-8	A	0.071	
	B	0.036	
	C	0.893	0.984
	D		0.016
Kg-9	A	0.464	0.333
	B	0.536	0.667
Kg-10	A	0.679	0.571
	B	0.321	0.381
	C		0.032
	D		0.016
N		38	93
Nc		28	63
Na		31	35
Number of private alleles		5	9
H		0.43	0.42
Number of different genotypes (haplotypes)		28	63
G		20.41	46.29

$\hat{G}$	54%	50%
S	3.29	3.96
V’	0.963	0.967
β paramter	2.64	2.43

N= Number of isolates (non clone-corrected)

Nc= Number of haplotypes in the clone-corrected populations

Na= Observed number of alleles

H = Gene Diversity according to Nei (1973)

G = Genotypic Diversity (Stoddart and Taylor, 1988)

$\hat{G}$ = G/N% = percent maximum diversity

S= Shannon–Weiner index

V’= Evenness index derived from Shannon-Weiner (V’)

β parameter: Pareto distribution parameter

Table 3. Pairwise Chi-square comparisons of allelic frequencies between *T. gauchensis* populations of Argentina and Uruguay.

Locus/clone corrected populations		Kg-1	Kg-2	Kg-3	Kg-4	Kg-5	Kg-6	Kg-7	Kg-8	Kg-9	Kg-10
Argentina and Uruguay	Chi^2	6.63	4.73	3.15	3.13	0.45	20.60*	0.009	7.36	1.42	1.89
	df	7	3	4	3	2	3	2	3	1	3

*significant Chi-square values ($P < 0.05$)

Table 4. Two-locus linkage disequilibrium analysis (LD) expressed as the number of loci with significant differences over the total pairwise loci comparisons, range of I_A values after 1000 randomisations and observed Index of Association (I_A). In the last column recombination is indicated as a 'yes' based on the observation that the observed I_A value falls within the randomized dataset values.

	LD between pairs of loci	Range of obtained I_A values after 1000 randomizations	Obs. I_A	Obs. I_A within the randomized data range. (i.e. evidence for recombination)
Argentina	0/45	-0.0005- 0.33	0.22	Yes
Uruguay	4/45	-0.0066- 0.13	0.08*	Yes
All	4/45	-0.00015- 0.15	0.13	Yes

*significant $p<0.05$