For. Path. 35 (2005) 385–396

Diversity and host association of the tropical tree endophyte *Lasiodiplodia theobromae* revealed using simple sequence repeat markers

By S. Mohali[1,2], T. I. Burgess[1,3] and M. J. Wingfield[1]

[1]Forestry and Agriculture Biotechnology Institute, University of Pretoria, Pretoria, 0002, Republic of South Africa; [2]Facultad de Ciencias Forestales y Ambientales, Laboratorio de Patologia Forestal, Universidad de Los Andes, Merida, Venezuela; [3]Biological Sciences, Murdoch University, Perth, 6150, Australia. E-mail: tburgess@murdoch.edu.au

Summary

Lasiodiplodia theobromae is a cosmopolitan fungus with a worldwide distribution in the tropics and subtropics, where it causes shoot blight and dieback of trees and shrubs and imparts blue stain in timber. In this study, eight simple sequence repeat (SSR) markers were used to evaluate the genetic diversity and gene flow between populations of *L. theobromae*. The relationships between isolates from different hosts were considered using three populations from different tree species in Venezuela (VEN) and the relationships between isolates from different geographical origins included populations from VEN, South Africa (RSA) and Mexico (MEX). A small number of predominant genotypes were encountered in the VEN and RSA populations and thus genotypic diversity was low. There was no evidence of host specificity for isolates of *L. theobromae* and there was very high gene flow between populations from different hosts. Geographical isolation existed between populations of the pathogen from different regions, with unique alleles fixed in the different populations. Gene flow was, however, less restricted between isolates from MEX and the other populations, consistent with MEX as a common source of seed in both VEN and RSA. Genetic analysis suggested predominantly clonal reproduction with some genotypes widely distributed within a region. The broad host range of *L. theobromae* and the lack of evidence for host specialization, coupled with its endophytic nature and the common appearance of symptoms only after harvest, is likely to hinder disease management strategies.

1 Introduction

The fungal pathogen *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. (=*Botryodiplodia theobromae* Pat.) represents the asexual state of *Botryosphaeria rhodina* (Berk. & M.A. Curtis) Arx. It has a worldwide distribution in tropical and subtropical regions and occurs on a very wide range of plants (Punithalingam 1976). Hosts are mainly woody plants including fruit and tree crops such as mango (Sangchote 1991), peach (Britton et al. 1990), avocado (Darvas and Kotze 1987) and *Eucalyptus* spp. (Sharma et al. 1984; Roux et al. 2000, 2001; Apetorgbor et al. 2004). In Venezuela, *L. theobromae* causes shoot blight and dieback of *Pinus caribaea* var. *hondurensis*, *P. oocarpa*, *Azadirachta indica*, *Citrus aurantiifolia*, *C. sinensis*, and *Passiflora edulis* and is also an important agent of blue stain in lumber (Cedeño and Palacios-Pru 1992; Mohali 1993; Cedeño et al. 1995, 1996; Mohali et al. 2002). The greatest disease impact is encountered in eastern Venezuela where areas of *P. caribaea* have been established in plantations. *L. theobromae* is common, causing distension and disruption of the cell walls, weakening the strength and toughness of the Caribbean pine wood, thus reducing its value by up to 50% (Mohali 1993; Cedeño et al. 1996).

Received: 22.02.2005; accepted: 19.05.2005; editor: O. Holdenrieder

Lasiodiplodia theobromae can colonize healthy plant tissue without exhibiting symptoms. MÜLLEN et al. (1991) for example isolated *L. theobromae* from stem cankers on dogwood (*Cornus florida*). Subsequent pathogenicity tests on dogwood stems with and without drought stress, showed that *L. theobromae* could be isolated from all inoculated plants, but cankers developed only on stressed plants (MÜLLEN et al. 1991). Thus, *L. theobromae* can be considered as a latent pathogen capable of endophytic infection such as has been reported for the related fungi *Diplodia pinea* (Desm.) Kickx. on *Pinus* spp. (SMITH et al. 1996; BURGESS et al. 2001a; FLOWERS et al. 2003) and *Botryosphaeria dothidea* (Fr. : Mough.) Ces. & De Not. on *Eucalyptus* spp. (SMITH et al. 1996).

DNA-based markers have been used to recognize and characterize populations, gene flow and evidence of speciation in many fungal pathogens. Simple sequence repeat (SSR) markers represent a class of co-dominant molecular markers consisting of tandem repeat loci, rich in polymorphisms with allele size determined by the addition or deletion of one or more repeats (LEVINSON and GUTMAN 1987). SSR markers have recently been used to examine gene and genotype flow, reproductive mode and speciation in a number of fungi, including *Botryosphaeria* spp. and their anamorphs (BARNES et al. 2001; BURGESS et al. 2001b, 2003, 2004a,b; ZHOU et al. 2002; SLIPPERS et al. 2004).

An earlier study, using SSR markers developed for *L. theobromae*, suggested relationships among isolates were more closely linked to host than to geographical origin (BURGESS et al. 2003). That study was focussed largely on the development of appropriate markers to study populations of the pathogen and it included only nine isolates. The aim of the present study was to consider the relationships between host and geographical origin of isolates of *L. theobromae* in greater detail and with a considerably more robust collection of isolates. The study initially emerged from an interest in the fungus in Venezuela, where it causes serious problems on forestry crops. Thus, relatively large populations of *L. theobromae* isolates were available from Venezuela (VEN) and these could be compared with those available from South Africa (RSA) and Mexico (MEX).

2 Materials and methods

2.1 Fungal isolates

Three *L. theobromae* subpopulations (total 84 isolates) were randomly collected in 2003 from *P. caribaea* var. *hondurensis*, *E. urophylla* and *Acacia mangium* at three locations in VEN (Table 1). The isolates were made from asymptomatic plant tissue as well as from trees exhibiting blue stain, dieback and from entirely dead trees. In addition, two populations of *L. theobromae* were used for comparative purposes. These included 70

Table 1. **Source of *Lasiodiplodia theobromae* isolates from Venezuela, Mexico and South Africa**

Country	Location	Cultivar	Origin of seed	No. of isolates	Collector
Venezuela	Falcon state	*Pinus caribaea* var. *hondurensis*	Guatemala	30	S. Mohali
	Portuguesa and Cojedes state	*Eucalyptus urophylla*	Brasil	29	S. Mohali
	Portuguesa and Cojedes state	*Acacia mangium*	Indonesia	25	S. Mohali
Mexico	San Cristóbal	*Pinus pseudostrobus*	Mexico	23	M. Wingfield
South Africa	Kwa Zulu Natal and Mpumulunga	*Pinus elliotti*	unknown	70	W. de Beer

isolates randomly collected from blue-stained *P. elliotti* lumber in RSA and 23 isolates obtained from *P. pseudostrobus* seed cones collected near San Cristobal, MEX (Table 1). Each of these isolates was selected to originate from a different tree, growing in the same area.

For primary isolations, the plant tissue samples were surface sterilized, rinsed and placed on 2% malt extract agar at 25°C. To induce sporulation, isolates were transferred onto water agar supplemented with sterilized pine needles and incubated for 3–6 weeks at 25°C under near-ultraviolet and cool-white fluorescent light. Isolates were derived from single conidia and maintained in the collection (CMW) of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, RSA.

2.2 DNA extraction and SSR-PCR

Fungal cultures were grown on half strength potato dextrose agar (Difco, Becton Dickinson, Cockeysville, MD, USA) in Petri dishes. Mycelium was scraped from the surface of 7-day-old cultures and freeze-dried. DNA was extracted from the dried mycelium following the protocol of Barnes et al. (2001). SSR-PCR was performed on all isolates with eight fluorescent-labelled markers, specifically designed to amplify polymorphic regions in *L. theobromae* as described previously (Burgess et al. 2003).

Labelled SSR-PCR products were separated on an ABI Prism 377 DNA sequencer and allele size was estimated by comparing the mobility of the SSR products to that of the TAMRA internal size standard (PE Applied Biosystems, Foster City, CA, USA) as determined by genescan 2.1 analysis software (PE Applied Biosystems) in conjunction with genotyper 2 (PE Applied Biosystems). A reference sample was run on every gel to ensure reproducibility.

2.3 Gene and genotypic diversity

For each of the loci, individual alleles were assigned a different letter. Each isolate was assigned a haplotype based on the data matrix of eight multistate characters (one for each locus) (e.g. AABDCGDD). The frequency of each allele at each locus for entire and clone-corrected populations was calculated, and allele diversity determined using the program popgene (Yeh et al. 1999) and the equation $H = 1 - \Sigma x_k^2$, where x_k is the frequency of the kth allele (Nei 1973) (haplotypes are considered only once in clone corrected populations). Chi-square tests for differences in allele frequencies at each locus were performed for clone-corrected populations (Chen and McDonald 1996).

Genotypic diversity (G) was estimated using the equation $G = 1/\Sigma p_i^2$, where p_i is the observed frequency of the ith phenotype (Stoddart and Taylor 1988). To compare G between populations, the maximum percentage of genotypic diversity was obtained using the equation $\hat{G} = G/N \times 100$, where N is the sample size.

2.4 Population differentiation

Population differentiation (G_{ST}) as measured by theta (Weir 1996) was calculated between all pairs of clone-corrected populations in multilocus v. 1.3 (Agapow and Burt 2001). The statistical significance were determined by comparing the observed G_{ST} value to that of 1000 randomized data sets in which individuals were randomized among populations. The number of migrants (M) that must be exchanged between populations for each generation, to give the observed G_{ST} value, was calculated using the equation $M = [(1/\theta) - 1]/2$ (Cockerham and Weir 1993).

2.5 Mode of reproduction

Index of association (I_A) was used to measure multilocus linkage disequilibrium for each clone-corrected population (Maynard Smith et al. 1993). The tests were performed on a data matrix of eight multistate characters using the program multilocus V1.3. The distribution under the null hypothesis of recombination was estimated by 1000 randomly recombining data sets and compared with the observed data.

3 Results

3.1 Segregation of SSR alleles

The SSR markers produced 63 alleles across the eight loci examined (Table 2). There were 47 alleles among the populations from VEN, 28 alleles in the Mexican population and 34 alleles in the South African population (Table 2). Of the 63 alleles, 17 (27%) were present in all regions and a further 12 (19%) were present in two of the three populations. Thirty-four alleles (54%) were unique to specific populations of *L. theobromae* (Table 2). There were unique alleles in the Venezuelan population at seven loci (21 alleles in total), in the Mexican population at three loci (3 alleles in total) and in the South African population at six loci (10 alleles in total) (Table 2).

3.2 Gene and genotype diversity

The mean gene diversity (H) for all eight loci across all populations of *L. theobromae* was 0.665 for clone-corrected populations. The gene diversity among hosts from VEN was 0.63 for *Pinus*, 0.67 for *Eucalyptus* and 0.51 for *Acacia* (Table 3). The distribution among geographical regions was 0.70 for VEN, 0.54 for MEX and 0.49 for RSA (Table 5). Values for RSA and MEX were lower than the total mean gene diversity, indicating greater between-population than within-population diversity. Diversity for VEN was similar to the total diversity indicating that all observed diversity is reflected in VEN population.

The genotypic diversity for the Venezuelan subpopulations was moderate to low (Table 2) as each of these populations had a single dominant haplotype (data not shown). Genotypic diversity for the combined VEN population was also low, again due to the predominance of a single haplotype (Table 2). Genotypic diversity in RSA was also low with only 23 haplotypes among 70 isolates. Diversity in MEX was higher because, although there were fewer alleles, a single dominant haplotype was not observed (Table 2).

3.3 Population differentiation and gene flow

Contingency chi-square test indicated no significant differences ($p < 0.05$) in allele frequencies at any loci for the Venezuelan populations of *L. theobromae* from *Pinus*, *Eucalyptus* and *Acacia* (Table 3). This is reflected in the lack of population differentiation and very high gene flow between the different populations (Table 4). Therefore, all three Venezuelan populations were pooled.

The results of the chi-square test indicate significant differences ($p < 0.05$) in allele frequency between the populations from the three different countries at six of the eight loci (Table 5). Gene flow (number of migrants) between countries was restricted, especially between RSA and VEN (Table 6). Although θ values indicate significant population differentiation, gene flow was less restricted between MEX and RSA and MEX and VEN, than between RSA and VEN.

3.4 Mode of reproduction

The index of association (I_A) of the observed data differed significantly from the values obtained for the recombined data set for all the individual *L. theobromae* populations (Fig. 1).

4 Discussion

In this study, we have considered for the first time, the population structure of the common, generally tropical pathogen *L. theobromae*. In terms of geographical distribution, this is a relatively poorly understood fungus. Whilst it was first described in South America (Patouillard and De Lagerheim 1892), its very wide host range and geographical distribution suggests it has been actively moved between countries and its true origin is

Table 2. **Allele size (bp) and frequency at eight loci (LAS1–8) for *Lasiodiplodia theobromae* populations collected from Venezuela (VEN), Mexico (MEX) and South Africa (RSA)**

Locus	Allele	VEN	MEX	RSA
LAS1	352	0.643	0.391	–
	355	–	–	0.014
	358	–	0.131	–
	360	–	0.217	0.071
	361	0.274	0.217	0.886
	364	0.012	–	–
	367	0.036	–	–
	369	–	–	0.014
	370	0.036	0.044	0.014
LAS2	312	0.060	–	–
	313	0.095	–	–
	314	–	0.044	–
	316	0.560	0.522	0.872
	317	0.274	0.391	0.114
	320	0.012	0.043	0.014
LAS3	326	–	–	0.014
	329	0.012	–	–
	330	0.155	–	–
	334	0.036	–	–
	336	0.262	0.348	0.871
	343	–	–	0.029
	348	–	–	0.029
	352	0.012	0.609	0.043
	354	0.466	–	–
	355	0.036	–	–
	Null	–	0.043	0.014
LAS4	248	–	–	0.029
	251	0.012	0.044	0.043
	254	0.095	–	0.014
	255	0.993	0.956	0.900
	258	–	–	0.014
LAS5	383	0.024	0.522	–
	385	0.500	–	–
	387	0.143	0.435	–
	388	0.060	–	0.771
	389	0.202	0.043	0.200
	400	0.071		0.029

Table 2. **(Continued)**

Locus	Allele	VEN	MEX	RSA
LAS6	454	–	–	0.029
	459	0.036	–	–
	463	0.262	0.435	0.828
	465	0.024	0.043	0.029
	468	0.476	0.478	0.100
	488	0.071	–	–
	490	0.048	–	–
	492	0.060	–	–
	496	0.024	–	–
	504	–	0.044	0.014
LAS7	180	0.012	–	–
	182	0.024	–	–
	183	0.643	0.522	–
	192	0.274	0.478	0.986
	195	0.036	–	–
	199	–	–	0.014
	201	0.012	–	–
LAS8	372	–	–	0.029
	376	0.012	0.087	0.428
	377	0.083	–	0.114
	380	0.012	0.261	0.400
	381	0.012	0.043	–
	382	0.190	–	–
	384	0.012	–	–
	385	0.679	0.565	0.029
	392	–	0.044	–
N		84	23	70
No. alleles		47	28	34
No. unique alleles		21	3	10
N(g)		24	11	23
G		4.76	5.05	5.09
$\hat{G}$ (%)		5.66	21.94	7.27

N, number of isolates; *N*(g), number of haplotypes; *G*, genotypic diversity (Stoddart and Taylor 1988); $\hat{G} = G/N\%$ = percentage maximum diversity; Null = primers that failed to amplify a product probably indicate a mutation in the primer-binding site.

unknown. Populations of isolates considered in this study were specifically from forest tree crops and the results should be interpreted within the context of the relatively narrow focus of the study.

One of the interesting results of this study was the high gene flow between populations from the three host types considered. These hosts are from three very different families, including conifers and hardwood trees and results show clearly that host of origin of isolates plays no role in partitioning of the pathogen haplotypes. The study also included isolates from three geographically isolated countries and there was a barrier to gene flow between them.

Many species of *Botryosphaeria*, including *L. theobromae*, are known to have a cosmopolitan distribution with wide host ranges (Barr 1972; Punithalingam 1976; von Arx 1987). Thus, the association of *L. theobromae* with three different hosts in Venezuela was not unexpected. However, the lack of host specificity is surprising, with the same haplotypes found on all three host species. In the study of Burgess et al. (2003), only nine isolates of

Table 3. **Gene diversity (*H*) and contingency chi-square tests for differences in allele frequencies for the eight polymorphic SSR loci across clone-corrected populations of *Lasiodiplodia theobromae* from Venezuela collected from *Pinus caribaea*, *Eucalyptus urophylla* and *Acacia mangium*. χ^2 values were not significant**

	Gene diversity (*H*)				
Locus	*Pinus*	*Eucalyptus*	*Acacia*	χ^2	d.f.
LAS1	0.62	0.58	0.57	10.9	8
LAS2	0.54	0.72	0.49	13.1	8
LAS3	0.80	0.66	0.49	20.4	12
LAS4	0.34	0.42	0.24	3.0	4
LAS5	0.80	0.74	0.69	7.8	10
LAS6	0.78	0.84	0.61	15.5	14
LAS7	0.62	0.74	0.49	12.1	10
LAS8	0.56	0.70	0.49	14.2	12
N(g)	10	10	7		
Mean	0.63	0.67	0.51		

Table 4. **Pairwise comparisons of population differentiation, G_{ST} (above the diagonal) and number of migrants, *M* (below the diagonal) among clone corrected populations of *Lasiodiplodia theobromae* from Venezuela collected from *Pinus caribaea*, *Eucalyptus urophylla* and *Acacia mangium*. There was no significant differentiation between populations**

	Pinus	*Eucalyptus*	*Acacia*
Pinus	–	0.020	0.005
Eucalyptus	24.5	–	0.065
Acacia	99.5	7.19	–

Table 5. **Gene diversity (*H*) and contingency chi-square tests for differences in allele frequencies for the eight polymorphic SSR loci across clone corrected populations of *Lasiodiplodia theobromae* from Venezuela (VEN) Mexico (MEX) and South Africa (RSA)**

	Gene diversity (*H*)				
Locus	VEN	MEX	RSA	χ^2	d.f.
LAS1	0.68	0.76	0.43	45.7***	16
LAS2	0.71	0.61	0.48	19.7*	10
LAS3	0.79	0.58	0.59	43.2**	20
LAS4	0.39	0.16	0.49	10.6	8
LAS5	0.82	0.51	0.57	27.6**	10
LAS6	0.73	0.55	0.58	23.6	18
LAS7	0.69	0.40	0.08	26.3**	12
LAS8	0.67	0.74	0.75	39.0***	16
N(g)	24	11	23		
Mean	0.70	0.54	0.49		

For χ^2 values asterisks indicate level of significance (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$).

L. theobromae were considered, however, those from *Eucalyptus* spp. and *Pinus* spp. grouped separately and host specificity was suggested. All three host species in Venezuela are non-native in that country and the lack of specificity might be associated with this fact. If it is assumed that *L. theobromae* is also non-native in Venezuela, there may have been limited

Table 6. **Pairwise comparisons of population differentiation, G_{ST} (above the diagonal) and number of migrants, M (below the diagonal) among clone-corrected populations of *Lasiodiplodia theobromae* from Venezuela (VEN) Mexico (MEX) and South Africa (RSA)**

	VEN	MEX	RSA
VEN	–	0.077*	0.152***
MEX	5.99	–	0.087**
RSA	2.79	5.24	–

For G_{ST} values, asterisks represent level of significance (***p < 0.001, **p < 0.01, *p < 0.05).

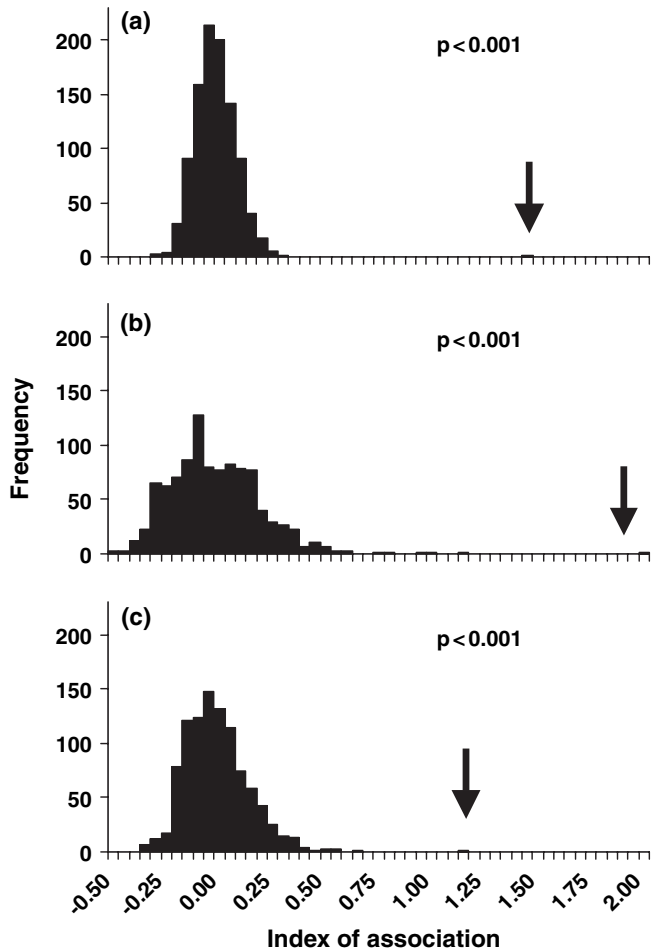

Fig. 1. Histograms of the frequency distribution representing multilocus disequilibrium estimate I_A for 1000 randomized data sets. (a) Venezuela, (b) Mexico and (c) South Africa. Results were compared with the observed data set (arrows)

introductions and selection pressure, coupled with the lack of niche competition, could have forced the same genotypes onto the different hosts. This has been observed for the mycorrhizal fungus, *Pisolithus*, in the non-native environment (Dell et al. 2002). *Pisolithus* spp. exhibit host specificity but, for example, a pine-specific isolate will develop superficial mycorrhizae on *Eucalyptus* spp. in the absence of *Eucalyptus*-specific isolates (Dell et al. 2002). In order to determine whether a similar situation exists with *L. theobromae* in Venezuela, pathogenicity trials using the same fungal genotypes on different host species will be required.

While there appears to be no host specificity for *L. theobromae*, at least on the plants considered in this study, there was a clear restriction to gene flow between geographically isolated regions. The lowest level of gene flow was between populations from Venezuela and South Africa. However, whilst still somewhat limited, there was evidence of some gene flow between the population from Mexico and those from both Venezuela and South Africa. Across all loci, only three alleles were unique to Mexico, compared with 21 unique alleles in Venezuela and 10 for South Africa. Mexico is a common source of *Pinus* seed in many subtropical countries maintaining plantations of non-native *Pinus* spp. (Burgess and Wingfield 2001). *Lasiodiplodia theobromae* is well known to occur on *Pinus* seed (Cilliers 1993) and Mexican isolates used in this study were also from seed collected in a native pine stand. It thus seems likely that this fungus has been distributed with seed to many subtropical pine growing regions including South Africa and Venezuela.

This observed linkage of alleles between different loci in all populations suggests a predominantly clonal mode of reproduction for the fungus. This 'clonal' mode of reproduction can be either because of asexual reproduction or homothallic sexual reproduction (selfing) (Coppin et al. 1997; Turgeon 1998). However, pseudothecia (sexual structures) of *L. theobromae* are seldom seen in the nature. Despite repeated collections, we have failed to connect isolates of *L. theobromae* from *Acacia*, *Pinus* and *Eucalyptus* to sexual structures on these hosts. On these hosts and at the sites studied, the fungus appears to exist in a predominantly asexual form and we were not surprised to find association of alleles at unlinked loci and a clonal genetic structure. Similarly, Burgess et al. (2004b) found no evidence of recombination among populations of the related pine endophyte *Diplodia pinea*, and the same genotypes were found across continents. *Diplodia pinea* is the predominant pine endophyte in temperate regions (Burgess and Wingfield 2001, 2002; Burgess et al. 2001a) and this niche appears to be replaced by *L. theobromae* in tropical and subtropical regions (Burgess and Wingfield 2002). *L. theobromae* appears to be similar to *D. pinea* with single genotypes found over large distances.

Generally, fungi undergoing sexual reproduction exhibit greater genotypic diversity than those reproducing asexually (Milgroom 1996). In our study, low genotypic diversity was observed in populations from Venezuela and South Africa, arising from the predominance of single haplotypes. In both cases the area from which the samples were collected was greater than 100 km^2, indicating haplotype flow across a region. Although the limited genetic diversity suggests this, the scope of this study was insufficient to be able to say that *L. theobromae* has been introduced into Venezuela and South Africa. The isolates from Mexico originated from native trees in an undisturbed area. The higher genetic diversity among isolates suggests that this population might be native. Confirmation of this fact would require larger numbers of isolates collected in a more structured fashion from a wider diversity of sites.

Lasiodiplodia theobromae is an important pathogen on many tree crops, tempting speculation of host-specific groups as is, for example, found with the root pathogen *Fusarium oxysporum* (Gordon and Martyn 1997). Our study has shown no evidence for host specificity, and demonstrated very high gene flow between populations of isolates from different hosts. Reproduction was predominantly clonal with some haplotypes widely distributed with a region. This was observed for a purported native population

(Mexico) and probable introduced populations (South Africa, Venezuela). The broad host range of *L. theobromae* and lack of host specialization, coupled with its endophytic nature and the appearance of symptoms such as blue stain only after harvest, are likely to hinder efforts to manage this pathogen.

Acknowledgements

We thank the National Research Foundation, members of the Tree Protection Co-operative Programme (TPCP) the THRIP initiative of the Department of Trade and Industry, South Africa and the University of Los Andes, Facultad de Ciencias Forestales y Ambientales, Mérida, Venezuela for financial support. This included support from the latter organization for the graduate studies of the first author. We are also grateful to Dr Mauricio Marin for his advice and assistance in the laboratory and to Mr Wilhelm de Beer and Ms Busi Tshabalala who provided isolates used in this study.

Résumé

Diversité et association d'hôte de Lasiodiplodia theobromae, endophyte d'arbres tropicaux, étudiées à l'aide de marqueurs SSR

Lasiodiplodia theobromae est un champignon cosmopolite, présent dans les zones tropicales et subtropicales du monde entier, à l'origine de flétrissements de pousses et de dépérissements d'arbres et arbustes, et entraînant un bleuissement du bois. Dans cette étude, huit marqueurs SSR ont été utilisés pour évaluer la diversité génétique et les flux de gènes entre populations de *L. theobromae.* Les relations entre isolats de différents hôtes ont été étudiées à partir de trois populations provenant d'arbres de différentes espèces au Vénézuela, et les relations entre isolats de différentes origines géographiques à partir de populations du Vénézuela, d'Afrique du Sud et du Mexique. Un petit nombre de génotypes dominants a été trouvé pour les populations du Vénézuela et d 'Afrique du Sud et la diversité génétique est donc faible. Aucune spécificité d'hôte n'a pu être mise en évidence entre isolats de *L. theobromae* et il existe un très fort flux de gènes entre populations d'hôtes différents. Un isolement géographique entre populations de l'agent pathogène de différentes régions a été montré, avec des allèles particuliers fixés dans les différentes populations. Les flux de gènes sont toutefois moins limités entre les populations du Mexique et les autres populations, en accord avec le fait que le Mexique est une source de graines à la fois pour le Vénézuela et l'Afrique du Sud. L'analyse génétique suggère une prédominance de la reproduction clonale, avec quelques génotypes largement répartis dans chaque région. La large gamme d'hôte de *L. theobromae*, l'absence apparente de spécialisation parasitaire, associées à la nature endophyte du champignon et l'apparition souvent tardive des symptômes, seulement après récolte, risquent de poser problème pour le développement de méthodes de gestion de la maladie.

Zusammenfassung

Diversität und Wirtsspezifität des tropischen Baumendophyten Lasiodiplodia theobromae, nachgewiesen mit SSR-Markern

Lasiodiplodia theobromae ist ein kosmopolitischer Pilz, der in den Tropen und Subtropen weit verbreitet ist und dort Trieb- und Zweigsterben an Bäumen und Sträuchern sowie Bläue verursacht. In der vorliegenden Untersuchung wurde die genetische Diversität und der Genfluss zwischen Populationen von *L. theobromae* anhand von acht SSR-Markern untersucht. Die Verwandtschaft von Isolaten aus verschiedenen Wirten wurden an drei Populationen von verschiedenen Baumarten aus Venezuela untersucht, die Vergleiche zwischen verschiedenen geographischen Herkünften umfassten Populationen aus Venezuela, Südafrika und Mexiko. In Venezuela und Südafrika wurden nur wenige sehr häufige Genotypen gefunden, die genotypische Diversität war somit gering. Es ergaben sich keine Hinweise auf eine Wirtsspezifität, der Genfluss zwischen Populationen von verschiedenen Wirten war hoch. Eine geographische Isolation zwischen den Populationen aus verschiedenen Gebieten wurde nachgewiesen, in den verschiedenen Populationen waren spezifische Allele fixiert. Der Genfluss war jedoch zwischen den Isolaten aus Mexiko und den anderen Populationen weniger eingeschränkt. Dieser Befund ist dadurch erklärbar, dass Saatgut in Venezuela und Südafrika häufig aus Mexiko importiert wird. Aus der genetischen Analyse lässt sich eine vorwiegend klonale Reproduktion ableiten, bei der einige Genotypen innerhalb einer Region weit verbreitet auftreten. Das breite Wirtsspektrum von *L. theobromae*, das Fehlen von Hinweisen auf eine Wirtsspezialisierung, das endophytische Verhalten und das häufige Auftreten von Symptomen erst nach der Ernte erschweren die Entwicklung von Managementstrategien gegen diesen Pilz.

References

Agapow, P.-M.; Burt, A., 2001: Indices of multilocus linkage disequilibrium. Mol. Ecol. Notes **1,** 101–102.

Apetorgbor, M. M.; Darkwa, N. A.; Frimpong, O.; Agyeman, V. K., 2004: Biodeteriorating agents associated with three tropical timber species. For. Ecol. Manag. **195,** 311–323.

von Arx, J. A., 1987: Plant Pathogenic Fungi. Berlin, Germany: J. Cramer, 288. pp.

Barnes, I.; Gaur, A.; Burgess, T.; Roux, J.; Wingfield, B. D.; Wingfield, M. J., 2001: Microsatellite markers and DNA sequence reflect relationships between isolates of the vascular wilt and canker pathogen *Ceratocystis fimbriata*. Mol. Plant Pathol. **2,** 319–327.

Barr, M. E., 1972: Preliminary studies on Dothideales in temperate North America. Contrib. Univ. Mich. Herbarium **9,** 523–638.

Britton, K. O.; Hendrix, F. F.; Pusey, P. L.; Okie, W. R.; Reilly, C. C.; Daniell, J. W., 1990: Evaluating the reaction of peach cultivars to infection by three *Botryosphaeria* species. Hort. Sci. **25,** 468–470.

Burgess, T.; Wingfield, M. J., 2001: Exotic pine forestry in the Southern Hemisphere: a brief history of establishment and quarantine practices. S. Afr. For. J. **192,** 79–84.

Burgess, T.; Wingfield, M. J., 2002: Quarantine is important in restricting the spread of exotic seed-borne tree pathogens in the southern hemisphere. Int. For. Rev. **4,** 56–65.

Burgess, T.; Wingfield, B. D.; Wingfield, M. J., 2001a: Comparison of genotypic diversity in native and introduced populations of *Sphaeropsis sapinea* isolated from *Pinus radiata*. Mycol. Res. **105,** 1331–1339.

Burgess, T.; Wingfield, M. J.; Wingfield, B. D., 2001b: Simple sequence repeat (SSR) markers distinguish between morphotypes of *Sphaeropsis sapinea*. Appl. Environ. Microbiol. **67,** 354–362.

Burgess, T.; Wingfield, M. J.; Wingfield, B. D., 2003: Development and characterization of microsatellite loci for the tropical tree pathogen *Botryosphaeria rhodina*. Mol. Ecol. Notes **3,** 91–94.

Burgess, T.; Gordon, T. R.; Wingfield, M. J.; Wingfield, B. D., 2004a: Geographic isolation of *Diplodia scrobiculata* and its association with native *Pinus radiata*. Mycol. Res. **108,** 1399–1406.

Burgess, T.; Wingfield, B. D.; Wingfield, M. J., 2004b: Global distribution of *Diplodia pinea* genotypes as revealed using SSR markers. Aust. Plant Pathol. **33,** 513–519.

Cedeño, L.; Palacios-Pru, E., 1992: Identificación de *Botryodiplodia theobromae* como la causa de las lesiones y gomosis en cítricos. Fitopat. Venez. **5,** 10–13.

Cedeño, L.; Carrero, C.; Mohali, S.; Palacios-Pru, E.; Quintero, K., 1995: Muerte regresiva en parchita causada por *Lasiodiplodia theobromae* en Venezuela. Fitopat. Venez. **8,** 11–14.

Cedeño, L.; Mohali, S.; Palacios-Pru, E., 1996: Ultrastructure of *Lasiodiplodia theobromae* causal agent of Caribbean pine blue stain in Venezuela. Interciencia **21,** 264–271.

Chen, R. S.; McDonald, B. A., 1996: Sexual reproduction plays a major role in the genetic structure of populations of the fungus *Mycosphaerella graminicola*. Genetics **142,** 1119–1126.

Cilliers, A., 1993: A review of *Lasiodiplodia theobromae* with particular reference to its occurrence on coniferous seeds. S. Afr. For. J. **166,** 47–52.

Cockerham, C. C.; Weir, B. S., 1993: Estimation of gene flow from F-statistics. Evolution **47,** 855–863.

Coppin, E.; Debuchy, R.; Arnaise, S.; Pichard, M., 1997: Mating types and sexual development in filamentous ascomycetes. Microbiol. Mol. Biol. Rev. **61,** 411–428.

Darvas, J. M.; Kotze, J. M., 1987: Fungi associated with pre- and postharvest diseases of avocado fruit at Westfalia Estate, South Africa. Phytophylactica **19,** 83–85.

Dell, B.; Malajczuk, N.; Dunstan, W. A., 2002: Persistence of some Australian *Pisolithus* species introduced to eucalypt plantations in China. For. Ecol. Manag. **19,** 271–281.

Flowers, E.; Hartman, J.; Vaillancourt, L., 2003: Detection of latent *Sphaeropsis sapinea* infections in Austrian pine tissues using nested-polymerase chain reaction. Phytopathology **93,** 1471–1477.

Gordon, T. R.; Martyn, R. D., 1997: The evolutionary biology of *Fusarium oxysporum*. Ann. Rev. Phytopathol. **35,** 111–128.

Levinson, G.; Gutman, G. A., 1987: Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol. Biol. Evol. **4,** 203–221.

Maynard Smith, J.; Smith, N. H.; O'Rourke, M.; Spratt, B. G., 1993: How clonal are bacteria? Proc. Natl. Acad. Sci. U. S. A. **90,** 4384–4388.

Milgroom, M. G., 1996: Recombination and multilocus structure of fungal populations. Ann. Rev. Phytopathol. **34,** 457–477.

Mohali, S., 1993: Estudio histologico de Madera de pino caribe con manchado azul causado por *Botryodiplodia theobromae*. Fitopat. Venez. **6,** 14–17.

Mohali, S.; Encinas, O.; Mora, N., 2002: Manchado azul en madera de *Pinus oocarpa* y *Azadirachta indica* en Venezuela. Fitopat. Venez. **15,** 30–32.

Müllen, J. M.; Gilliam, C. H.; Hagen, A. K.; Morgan Jones, G. 1991: *Lasiodiplodia theobromae* cancer of dogwood, a disease influenced by drought stress or cultivar selection. Plant Dis. **75,** 886–889.

Nei, M., 1973: Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U. S. A. **70,** 3321–3323.

Patouillard, N.; De Lagerheim, G., 1892: Champignons de l'equateur (Pugillus II). Bull. Soc. Mycol. Fr. **8,** 113–140.

Punithalingam, E., 1976: *Botryodiplodia theobromae*. CMI Descriptions of Pathogenic Fungi and Bacteria. No. 519. Kew, Surrey, UK: Commonwealth Mycological Institute.

Roux, J.; Coutinho, T. A.; Wingfield, M. J.; Bouillet, J. P., 2000: Diseases of plantation *Eucalyptus* in the Republic of Congo. S. Afr. J. Sci **96,** 454–456.

Roux, J.; Coutinho, T. A.; Byabashaija, D. M.; Wingfield, M. J., 2001: Diseases of plantation *Eucalyptus* in Uganda. S. Afr. J. Sci. **97,** 16–18.

Sangchote, S., 1991: *Botryodiplodia* stem end rot of mango and its control. Acta Hort. **291,** 293–303.

Sharma, J. K.; Mohanan, C.; Maria Florence, E. J., 1984: A new stem canker disease of *Eucalyptus* caused by *Botryodiplodia theobromae* in India. Trans. Br. Mycol. Soc. **83,** 162–163.

Slippers, B.; Burgess, T.; Wingfield, B. D.; Crous, P. W.; Coutinho, T. A.; Wingfield, M. J., 2004: Development of SSR markers for *Botryosphaeria* spp. with *Fusicoccum* anamorphs. Mol. Ecol. Notes **4,** 675–677.

Smith, H.; Wingfield, M. J.; Crous, P. W.; Coutinho, T. A., 1996: *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. S. Afr. J. Bot. **62,** 86–88.

Stoddart, J. A.; Taylor, J. F., 1988: Genotypic diversity: estimation and prediction in samples. Genetics **118,** 705–711.

Turgeon, B. G., 1998: Application of mating type gene technology to problems in fungal biology. Ann. Rev. Phytopathol. **36,** 115–137.

Weir, B. S., 1996: Genetic Data Analysis II. Sunderland, MA: Sinauer Associates.

Yeh, F. C.; Yang, R.-C.; Boyle, T., 1999: POPGENE, Version 1.31. Microsoft windows-based freeware for population genetic analysis. University of Alberta, Alberta: http://www.ualberta.ca/~fyeh/

Zhou, X.-D.; Burgess, T.; de Beer, Z. W.; Wingfield, B. D.; Wingfield, M. J., 2002: Development of polymorphic microsatellite markers for the tree pathogen and sapstain agent, *Ophiostoma ips*. Mol. Ecol. Notes **2,** 309–312.