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The eucalypt leaf blight pathogen *Kirramyces destructans* discovered in Australia

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Abstract. *Kirramyces destructans* is a serious pathogen causing a leaf, bud and shoot blight disease of *Eucalyptus* species in plantations of the subtropics and tropics of South East Asia. This pathogen was first discovered in Indonesia in 1995 and has subsequently spread to Thailand, China and Vietnam. *Kirramyces destructans* is not known to occur in Australia and has been considered a major biosecurity threat. During the course of the past four years, surveys have been conducted in existing eucalypt trials in tropical Australia. Several *Kirramyces* spp. were detected in these surveys, including isolates with morphological and cultural characteristics resembling those of *K. destructans*. In this study, DNA sequences of three gene regions were used to compare isolates of *Kirramyces* spp. emerging from the surveys and these were compared with those of *K. destructans* and the closely related *K. eucalypti* and *K. viscidus*. Results have shown, for the first time, that *K. destructans* is present in northern Australia (Melville Island, Northern Territory and Derby, Western Australia). The observed sequence variation among a small number of isolates also strongly suggests *K. destructans* is endemic to Northern Australia.

Fig. 1. Leaf blight on 18-month-old *Eucalyptus grandis* caused by *Kirramyces destructans* in eastern Guangdong province, China. Leaf blight caused by *K. destructans* has resulted in the loss of all of the crown except for the recently emerged leaves which were already infected. Photography by B. Dell.

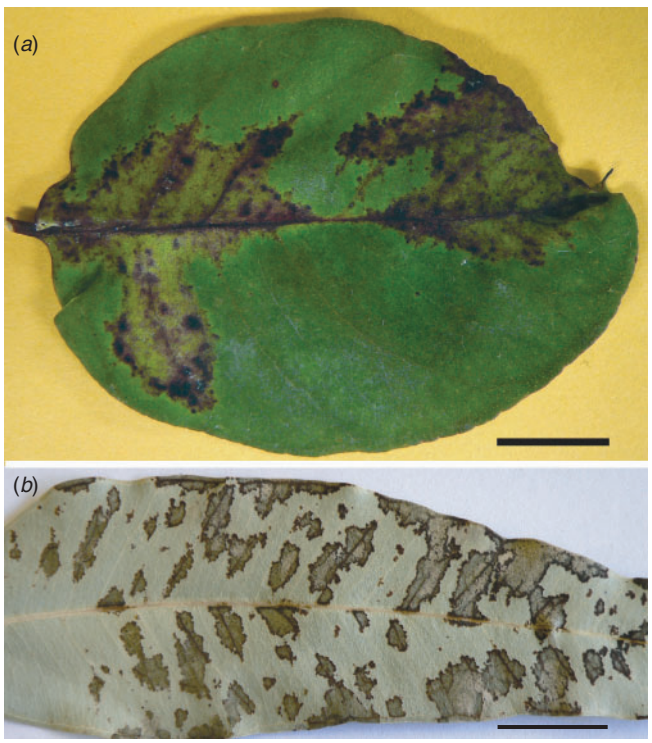

Fig. 2. Symptoms of *K. destructans* on (*a*) juvenile leaves of *Eucalyptus urophylla* × *E. grandis* on Melville Island, Northern Territory and (*b*) adult leaves of an unknown *Eucalyptus* species in Derby, Western Australia. Bars = 1 cm.

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Table 1. *Kirramyces* species and isolates examined

Isolate[A]	Name	Host	Origin	Collector	GenBank Accession number		
					ITS	β-tubulin	EF-1α
CMW 22553	*Kirramyces destructans*	*Eucalyptus grandis*	Sumatra, Indonesia	P. A. Barber	DQ632667	DQ632625	DQ632732
CMW 17918	*K. destructans*	*E. grandis*	Sumatra, Indonesia	P. A. Barber	DQ632666	DQ632624	DQ632731
CMW 19832	*K. destructans*	*E. grandis*	Sumatra, Indonesia	P. A. Barber	DQ632665	DQ632623	DQ632730
CMW 17919	*K. destructans*	*E. urophylla*	Guangzhou, China	T. I. Burgess	DQ632701	DQ632622	DQ632729
MUCC 458	*K. destructans*	*E. grandis* × *E. urophylla*	Melville Island, Australia	T. I. Burgess	EU009634	EU009652	EU009643
MUCC 459	*K. destructans*	*E. grandis* × *E. urophylla*	Melville Island, Australia	T. I. Burgess	EU009635	EU009653	EU009644
MUCC 460	*K. destructans*	*E. grandis* × *E. urophylla*	Melville Island, Australia	T. I. Burgess	EU009630	EU009648	EU009639
MUCC 461	*K. destructans*	*E. grandis* × *E. urophylla*	Melville Island, Australia	T. I. Burgess	EU009637	EU009655	EU009646
MUCC 462	*K. destructans*	*E. grandis* × *E. urophylla*	Melville Island, Australia	T. I. Burgess	EU009636	EU009654	EU009645
MUCC 463	*K. destructans*	*E. grandis* × *E. urophylla*	Melville Island, Australia	T. I. Burgess	EU009631	EU009649	EU009640
MUCC 464	*K. destructans*	*E. grandis* × *E. urophylla*	Melville Island, Australia	T. I. Burgess	EU009633	EU009651	EU009642
MUCC 465	*K. destructans*	*E. grandis* × *E. urophylla*	Melville Island, Australia	T. I. Burgess	EU009632	EU009650	EU009641
MUCC 475	*K. destructans*	*Eucalyptus* sp.	Derby, Australia	M. J. Wingfield	EU009629	EU009647	EU009638
MUCC452, CBS 121156	*K. viscidus*	*E. grandis*	Mareeba, Australia	T. I. Burgess	EF031471	EF031483	EF031495
MUCC453, CBS 121157	*K. viscidus*	*E. grandis*	Mareeba, Australia	T. I. Burgess	EF031472	EF031484	EF031496
MUCC456, CBS 121155	*K. viscidus*	*E. grandis*	Mareeba, Australia	T. I. Burgess	EF031475	EF031487	EF031499
CMW 17917	*K. eucalypti*	*E. grandis* × *E. tereticornis*	New South Wales	A. J. Carnegie	DQ632711	DQ632630	DQ632725
CMW 17916	*K. eucalypti*	*E. grandis* × *E. camaldulensis*	Queensland	A. J. Carnegie	DQ632659	DQ632628	DQ632722
CMW 11687	*K. eucalypti*	*E. nitens*	New Zealand	M. Dick	DQ240001	DS890168	DQ235115
MUCC 549	*K. epicoccoides*	*E. grandis* × *E. urophylla*	Melville Island, Australia	T. I. Burgess	EU117049		
MUCC 550	*K. epicoccoides*	*E. grandis* × *E. urophylla*	Melville Island, Australia	T. I. Burgess	EU117050		
MUCC 543	*Kirramyces* sp.	*E. grandis* × *E. urophylla*	Melville Island, Australia	T. I. Burgess	EU009626		
MUCC 544	*Kirramyces* sp.	*Eucalyptus* sp.	Derby, Australia	M. J. Wingfield	EU009628		
MUCC 545	*Kirramyces* sp.	*Eucalyptus* sp.	Derby, Australia	M. J. Wingfield	EU009627		

[A]Designation of isolates and culture collections: CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; CMW = Tree Pathology Cooperative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; MUCC = Murdoch University culture collection, Australia.

Kirramyces destructans is an aggressive pathogen first reported causing disease on 1–3-year-old *E. grandis* in Sumatra, Indonesia (Wingfield *et al*. 1996). Since then it has been detected in Thailand, China and Vietnam. In these countries it has been found on *E. grandis* as well as *E. camaldulensis* and *E. urophylla* and various hybrids between the three species (Old *et al*. 2003*a*, 2003*b*; Barber 2004; Burgess *et al*. 2006). *Kirramyces destructans* has also been reported from native *E. urophylla* in East Timor (Old *et al*. 2003*a*).

Symptoms of infection by *K. destructans* include distortion of infected leaves and blight of young leaves, buds and shoots. The pathogen causes severe defoliation of juvenile leaves on trees in plantations (Fig. 1) and infection of young tissue on clonal mother plants in production nurseries can seriously affect productivity. The pathogen has never been found in Australia, where most *Eucalyptus* spp. are native, but its discovery in East Timor, where *E. urophylla* occurs naturally (Old *et al*. 2003*a*), suggested this country might represent the area of origin of *K. destructans*. As such, *K. destructans* could have moved into South East Asia on infected germplasm from the substantial collections of *E. urophylla* from Timor. Due to the devastating impact that *K. destructans* could have on eucalypt plantations and native forests in Australia, this pathogen has been listed on the Plant Biosecurity

Watch List for Australia (http://www.daff.gov.au, verified 12 September 2007).

During the course of the last four years, we have been studying the population diversity and distribution of *K. destructans* in Asia and the biosecurity threat this pathogen might pose to eucalypt plantations and forests in Australia. As part of this project, surveys have been conducted in Northern Australia, using existing trials of non-endemic eucalypts as sentinel plantings and by evaluating these trials for disease caused by *Kirramyces* spp. A new species, *Kirramyces viscidus*, which is closely related to *K. destructans*, was discovered in a taxa trial in Northern Queensland (Andjic *et al*. 2007*b*).

Juvenile eucalypt leaves with symptoms resembling those of *K. destructans* (Fig. 2*a*) were collected in July 2006 from a clonal taxa trial on Melville Island, 50 km off the coast from Darwin, Northern Territory, Australia. The trees had been severely damaged by cyclone Ingrid in March 2006 and they were coppiced approximately 2 months later. The abaxial surfaces of the leaves were covered with pycnidia, exuding conidia resembling those of various species including *K. destructans*, *K. eucalypti* and *K. epicoccoides*. Adult leaves were also collected from a mature tree of an unknown *Eucalyptus* sp. at the Kimberly Entrance caravan park in Derby, Western Australia (Fig. 2*b*). Although the symptoms on these leaves were not typical of *K. destructans*, this material was studied because the conidia were similar to those of the pathogen.

Isolations from conidia taken from leaves of trees on Melville Island and those from Derby were made as described previously (Andjic *et al*. 2007*b*). Cultural characteristics of several isolates were the same as those of *K. destructans*, with white to pink colonies producing black spore masses on the upper surface and olive-green to black at the centres on the reverse sides of the plates. All isolates have been maintained in the culture collection of Murdoch University (MUCC) (Table 1).

Genomic DNA was extracted from cultures as described previously (Andjic *et al*. 2007*c*). Initially, the second internal transcribed spacer and part of 5.8 S region of the rDNA (ITS2) was amplified and sequenced for all *Kirramyces* isolates. For those isolates with sequence data similar or identical to *K. destructans*, the β-tubulin (BT) and translation elongation factor 1α (EF-1α) gene regions were amplified and sequenced as previously described (Andjic *et al*. 2007*c*). Parsimony analyses were performed on the combined datasets in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2003) and Bayesian analysis was made using MrBayes (Ronquist Heuelsenbeck 2003) following the methods described by (Andjic *et al*. 2007*c*). Sequence data for isolates collected from northern Australia were compared with those for *K. destructans* from Asia and the closely related species *K. eucalypti* and *K. viscidus* (Andjic *et al*. 2007*a*, 2007*b*).

K. epicoccoides and three undescribed *Kirramyces* spp. were found among isolates from Melville Island and Derby (Table 1). Nine isolates with an ITS2 profile similar to that of *K. destructans* were retained for further analysis. In both parsimony and Bayesian analyses, *K. eucalypti* was distant from *K. destructans* and *K. viscidus*, forming a clade with 100% bootstrap support (parsimony analysis) and a posterior probability of 1.0 (Bayesian) (Fig. 3). Although related to

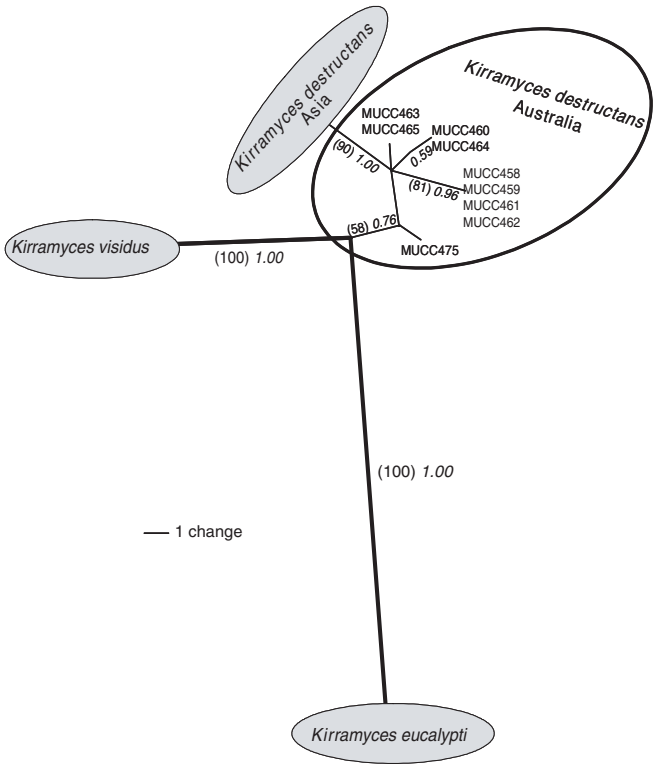

Fig. 3. Unrooted phylogram of one of the 51 most parsimonious tree of 46 steps obtained from combined ITS, BT and EF-1α sequence data. The numbers next to the branches represent bootstrap support (in brackets) and the posterior probabilities of the branch nodes based on Bayesian analysis. *Kirramyces eucalypti*, *K. viscidus* and *K. destructans* all represent strong supported terminal nodes.

K. destructans, isolates of *K. viscidus* resided in a clade discrete from those of *K. destructans* (Fig. 3).

Isolates of *K. destructans* from Asia grouped together, but were most closely related to those from Melville Island and the single isolate from Derby. There was only 1 bp difference in BT and EF-1α sequences between the *K. destructans* isolates from Asia and those from Australia. ITS2 sequence data showed the greatest amount of variation with up to 4 bp difference between Asian isolates and some of the Australian isolates. Thus, from ~1000 bp of sequence, the maximum difference among isolates was 6 bp. This is within the normal limits of intraspecies variation and justifies the identification of the Australian isolates as *K. destructans*.

The observed symptoms on leaves, conidial morphology, culture characteristics and multilocus sequence data lead us to conclude *K. destructans* is present in Australia. Variability amongst isolates from Melville Island suggests *K. destructans* is endemic to the region. This information has been provided to Biosecurity Australia and we believe it is appropriate to remove *K. destructans* from the Biosecurity Australia Watch List for eucalypt pathogens. Although, regular surveys have been conducted in northern Queensland and *K. viscidus*, *K. epicoccoides*, *K. eucalypti* and several, as yet, undescribed *Kirramyces* spp. have been isolated (unpubl. data), we have not irrefutably detected *K. destructans* in this region. Due to

the potential impact of this pathogen on eucalypt plantations in tropical Australia, it is essential that monitoring in this region is maintained.

Acknowledgements

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