Coniothyrium stem canker of Eucalyptus, new to Argentina and Vietnam

Alemu Gezahgne*, J. Roux*, P.Q. Thu and M.J. Wingfield

During routine disease surveys of Eucalyptus species in Argentina and Vietnam, symptoms of a damaging stem canker disease were observed. This study identifies the causal agent of the disease as Coniothyrium zuluense. This is the first report of Coniothyrium stem canker from Argentina and Vietnam.

Introduction

Eucalyptus species have been planted as exotics in several tropical and subtropical countries. It is estimated that plantations of Eucalyptus species cover approximately 10 million hectares of land worldwide.1 Several root, stem and leaf diseases have been identified in these plantations. These diseases result in considerable loss of yield and profit to forestry industries.

Stem canker, caused by Coniothyrium zuluense Wingfield, Crous and Coutinho, was first described from South Africa and is considered to be a serious threat to Eucalyptus forestry around the world.2 Coniothyrium stem canker is characterized by small, discrete necrotic lesions/spots on young green bark. These lesions may coalesce to form larger patches and cracks (Fig. 1A). The disease also leads to abundant kino exudation and the formation of kino pockets in the xylem of infected trees (Fig. 1B). This makes the wood unsuitable for construction and as sawn timber. In severe cases infection leads to the stunting of trees and the die-back of branches.3 Initially it was believed that C. zuluense is native to South Africa. Recent studies have, however, shown that the pathogen also occurs in Thailand4 and Mexico.4

Surveys of Eucalyptus diseases have recently been conducted in Vietnam and Argentina. Disease symptoms similar to those of Coniothyrium stem canker were observed on E. urophylla S.T. Blake in Vietnam and on E. grandis Hill-Maiden in Argentina. This study was carried out to determine the identity of the causal agent of the stem canker in the two countries.

Methods

External disease symptoms were used to select infected trees for sampling. Segments of plant parts with discrete lesions were incubated in moist chambers at 25°C to induce sporulation. Masses of spores exuding from pycnidia were transferred to Petri dishes containing 2% malt extract agar (MEA). Spores and pycnidia were also examined microscopically to establish a
preliminary identification. All isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria.

DNA was isolated from pure cultures of five representative isolates and the Internal Transcribed Spacer (ITS) regions and 5.8S gene of the ribosomal DNA (rDNA) as well as the β-tubulin genes were sequenced. Sequences of Vietnamese and Argentinian isolates were compared with those of authentic C. zuluense isolates obtained from South Africa (CMW11220, CMW11221), Thailand (CMW5232, CMW5235) and Mexico (CMW11230, CMW11231). Sequences of the ITS rDNA and the β-tubulin gene were combined and aligned by the insertion of gaps, where needed. The phylogenetic relationships of the isolates were determined using a heuristic search with parsimony. Mycosphaerella molleriana (Thumb.) Lindau, and M. nubilosa ( Cooke) Hansf. were used as outgroups to root the trees.

Results

Based on disease symptoms observed on the infected trees, the disease was tentatively identified as Coniothyrium canker. The disease was common in Argentinian plantations, but not so in Vietnam. In total 52 isolates were examined from Argentina and two from Vietnam. Identification based on symptoms was further supported by microscopic and cultural characteristics of the isolates obtained from the necrotic lesions. Isolates showed typical light brown amero-conidia, 4–6 × 2–3 μm in size. On MEA all isolates showed the typical slow growth and dark/olivaceous colony colour typical of C. zuluense.

Blast searches of the ITS sequence data, for the Vietnamese and Argentinian isolates, in the GenBank database [National Center for Biotechnology Information (NCBI), National Institutes of Health, Bethesda, MA (http://www.ncbi.nlm.nih.gov.BLAST)] showed that the sequences had a high homology with sequences of C. zuluense. Analysis of the combined data sets generated six trees, with only minor differences in the internal arrangement of the cladines. The consensus phylogenetic tree (Fig. 2) had a consistency index (CI) of 0.924 and retention index (RI) value of 0.847. The tree contained two sub-clades. The Vietnam isolates resided in a clade including authentic C. zuluense isolates from South Africa, Thailand and Mexico (80% bootstrap support), whereas the isolates from Argentina resided in a separate clade with bootstrap value of 99%. The two sub-clades, however, grouped together as C. zuluense (100% bootstrap), separate from other species (Fig. 2).

Conclusions

Our study confirmed the presence of Coniothyrium stem canker in Argentina and Vietnam. This is the first report of C. zuluense and the disease it causes on Eucalyptus species in these two countries. Coniothyrium stem canker is a serious disease in countries with subtropical climates and that have high annual rainfall. Infection by this pathogen reduces growth, wood quality and causes death of tree tops. Thus, the occurrence of C. zuluense in Argentina and Vietnam should be of great concern to the development of Eucalyptus plantations in these countries. This study provides added evidence that this important fungus has a wider geographical distribution than was initially believed.

We thank the members of the Tree Pathology Co-operative Programme and the THRIP initiative of the Department of Trade and Industry for financial support. We acknowledge ACIAR for a grant that made collections in Vietnam possible, and William Davidson, formerly of CAPSA, Argentina, for providing assistance in the collection of specimens from Argentina.

Fig. 1. Symptoms of Coniothyrium stem canker on Eucalyptus grandis. A, Discrete lesions on stem of infected tree. B, multiple kino pockets in wood.

Fig. 2. Phylogenetic tree of Coniothyrium species generated from the combined ITS and β-tubulin sequences. Bootstrap values are shown at each branch.

---

Received 9 June. Accepted 7 October 2003.
