

First report of *Neofusicoccum parvum* causing canker and die-back of *Eucalyptus* in Spain

Eugenia Iturrutxa · Bernard Slippers · Nebai Mesanza · Michael J. Wingfield

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Abstract One of the most common groups of fungi causing canker on eucalypts is the Botryosphaeriaceae. A large number of species have been reported from this host in recent years. The *Neofusicoccum parvum*/*Neofusicoccum ribis* species complex includes some of the most aggressive members of Botryosphaeriaceae. Cankers on stems are commonly sunken and elongated and infected tissue may be darkly pigmented. Bark cracking and kino exudation are often present. In this study *Neofusicoccum parvum* has been first reported as the main cause of canker disease in *Eucalyptus globulus* in North Spain.

Keywords Eucalyptus canker · *Neofusicoccum parvum* · Botryosphaeriaceae

A canker disease outbreak was observed for the first time on *Eucalyptus globulus* in North Spain, Vizcaya, during sampling in July 2009. Symptoms included dieback of shoots and branches, lesion and canker formation on the stems and brown and red exudates on stems and branches with copious exudation of kino. *Eucalyptus globulus* was established in the area in 1957 (Muro

1975). The expansion of fast growing eucalypt plantations was stimulated by the increase of demands from the rapidly growing pulp industry. As in other parts of the world, pest and pathogens are rapidly emerging as one of the greatest threats to such plantation forestry based on non-native species (Wingfield 2003; Old et al. 2003; Old and Davison 2000). The most well-known disease of eucalypts in Spain is leaf spot caused by *Mycosphaerella* species. Various species of *Mycosphaerella* have been found on *Eucalyptus* in plantations (Crous et al. 2006; Otero et al. 2004; De Blas et al. 2009). There is, however, little known about other diseases on this host and clearly a significant need exist to expand the knowledge on eucalyptus health in Spain. Diseased samples of *E. globulus* were cultivated on oatmeal agar (OA) and incubated at 25°C. Fungal isolates developed abundant, aerial mycelium that became dark grey after 2–3 days and formed black, globular pycnidia after 2 weeks. Conidia were hyaline, aseptate, not becoming septate or darker with age, thin walled and fusiform and measured 18.9–23×4–4.9 µm. Furthermore, the ITS rDNA locus was amplified and sequenced using the ITS1 and ITS4 primers, and compared to sequences in GenBank using BLAST. Based on the symptoms, cultural morphology, conidial characters and sequencing (GenBank Accession numbers JN119283 and JN119284; 100% identity to the ex-type isolate CMW9081) the fungus was identified as *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips. The isolates used in this study are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa and in Agricultural Institute of Neiker, in the Basque Country, Spain, culture collection numbers CMW37773 and CMW36774.

To confirm pathogenicity, an inoculation experiment was conducted. Two mm diameter actively growing mycelium plugs of *N. parvum* were applied to the same size bark

E. Iturrutxa (✉) · N. Mesanza
Forest Health Neiker tecnalia,
Granja Modelo de Arkaute 46 Post,
Vitoria Gasteiz 01080 Álava, Spain
e-mail: eiturrutxa@neiker.net

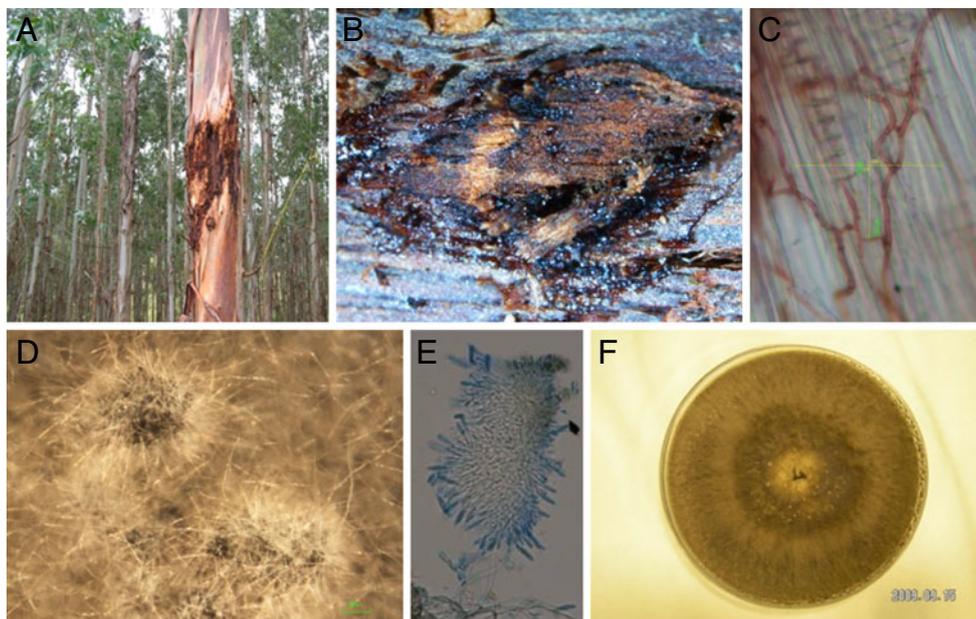
B. Slippers · M. J. Wingfield
Department of Genetics, Forestry and Agricultural Biotechnology
Institute (FABI), University of Pretoria,
Pretoria 0002, South Africa

wounds on the middle point of the stems of seedlings of *E. globulus*. Control plants were inoculated with sterile PDA plugs. The inoculation was carried out under controlled conditions in the greenhouse (temperature $22 \pm 3^\circ\text{C}$, relative humidity $65 \pm 5\%$). After 3 weeks, all the inoculated seedlings showed dark vascular stem tissue, with a size lesion $4,3 \pm 1,9$ cm. *N. parvum* was reisolated from all the inoculated tissues. No symptoms were visible in the control seedlings and no fungus was isolated from them.

Neofusicoccum parvum has previously been reported as causing canker symptoms of *Eucalyptus* species in many parts of the world, including Australia, Chile, China,

Ethiopia, Indonesia, South Africa, Uganda, Uruguay, Venezuela (Rodas et al. 2009). Previous *Botryosphaeria dothidea* (Moug. Ex Fr.) Ces. & De Not. and *Neofusicoccum ribis* Grossenb & Dugg. was thought to be common on *Eucalyptus*, but DNA sequence comparisons have now shown that many of the early identification were possibly incorrect and that these species are rare on *Eucalyptus*. Earlier reports of these fungi probably represent species such as *N. parvum* and others (Slippers et al. 2004a, b; Burgess et al. 2005; Rodas et al. 2009).

To the best of our knowledge, this is the first report of *N. parvum* causing eucalypt canker in Spain.



A: *Eucalyptus globulus* plantation, showing advanced stem canker. B: Exudation of kino on stem with severe canker. C: Micellial invasion of *Eucalyptus* tissues. D: Fruiting bodies of *Neofusicoccum parvum* growing on oatmeal agar. E: Conidia and conidiophores. F: Appearance of culture on oatmeal agar

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