

## First report of the pitch canker fungus *Fusarium circinatum* affecting *Pinus taeda* seedlings in Uruguay

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**Abstract.** In recent years *Pinus* spp. plantations have become an important resource that is continuously increasing in Uruguay as well as in other countries. Fungal diseases in nurseries are common, and among them, *Fusarium circinatum* (the pitch canker fungus) was found associated with *Pinus taeda* seedling, representing the first record of this pathogen in Uruguay.

The pitch canker fungus *Fusarium circinatum* (Nirenberg and O'Donnell 1998) is a destructive pathogen that affects several *Pinus* species. Although the main symptom most frequently associated with this pathogen is the presence of large resinous cankers on the main trunk and lateral branches of trees, it can also be associated with roots, shoots, cones and seedlings (Barnard and Blakeslee 1980; Viljoen *et al.* 1994). This disease has been detected in south-eastern USA (Kuhlman *et al.* 1982), Mexico (Guerra-Santos 1999), South Africa (Viljoen *et al.* 1994), Chile (Wingfield *et al.* 2002), Spain (Pérez-Sierra *et al.* 2007) and other countries (Wingfield *et al.* 2008).

In Uruguay, forest plantation activity has increased over the last 20 years and the main exotic species planted are *Eucalyptus* spp. and *Pinus* spp. (Petraglia and Dell'Acqua 2006).

Nursery diseases are common and most of them are related to fungal pathogens. Recently, a symptom associated with *Pinus taeda* seedlings was observed. Needles of affected seedlings showed different stages of chlorosis ranging from pale green to reddish-brown discolouration. Stem and collar rot with bark cracks were detected and in some of them fungal sporodochia were observed (Fig. 1).

For the isolation of fungi associated with the stem lesions, segments of stems were placed in a moist chamber to stimulate mycelial development and also segments of affected tissues were surface disinfected (80% ethanol for 1 min and 2% sodium hypochlorite solution for 2 min) rinsed with sterile distilled water and placed in Petri dishes containing potato dextrose agar (PDA). From colonies belonging to *Fusarium* sp. single-conidium transfers were made to obtain pure cultures on PDA and Spezieller Nährstoffarmer agar (SNA) media allowing the identification to species level (Leslie and Summerrell 2006). Micromorphological characteristics, such as the presence of simple and proliferating conidiophores with polyphialides, microconidia in false heads, macroconidial shape, absence of chlamydospores and the typical sterile coiled hyphae (Fig. 2), led to the identification of *Fusarium circinatum* (Nirenberg and O'Donnell 1998; Britz *et al.* 2002). Molecular methods

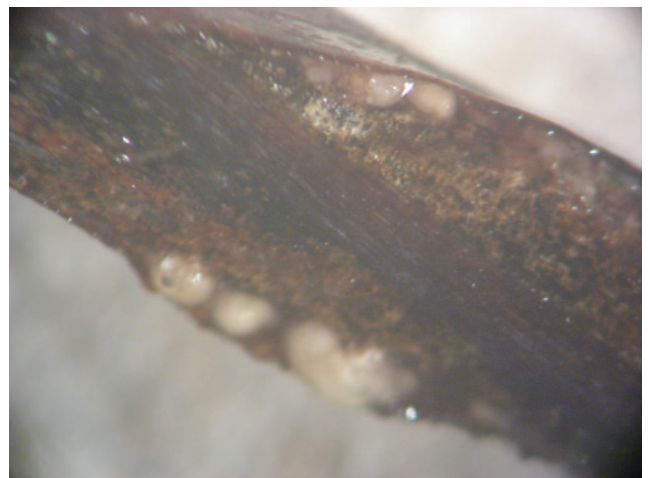


Fig. 1. Stem bark crack showing fungal sporodochia (×30).

were also performed to verify the identification. DNA was obtained from fresh aerial mycelia by extraction with cetyltrimethylammonium bromide (CTAB), followed by organic extraction and isopropanol precipitation of the DNA using the method described by Lee and Taylor (1990). The intergenic spacer (IGS) region was amplified by the polymerase chain reaction (PCR) using the CIRC1A and CIRC4A species-specific primers (Schweigkofler *et al.* 2004). The amplicons were separated by electrophoresis in agarose gel (2%), stained with ethidium bromide (10 µg/µL), and visualised and photographed under ultraviolet light. The amplicon corresponded to the 360-bp fragment in accordance with those predicted for *F. circinatum*.

This is the first report of the presence of this pathogen in nurseries of *Pinus taeda* in Uruguay. This exotic pathogen was probably introduced with seeds since in field plantations symptoms have not yet been observed. On the other hand, a significant volume of *Pinus* timbers is not frequently imported.



Fig. 2. Coiled hypha and macroconidia of *Fusarium circinatum* ( $\times 400$ ).

Although the incidence in nurseries is low, the control and eradication of affected seedlings is very important in order to avoid the expansion of the pathogen into the field. Since other *Fusarium* species are very frequently found affecting several crops in Uruguay due to the cool and humid climatic conditions that favour the development of these fungi, monitoring of this disease has become necessary.

On the other hand, although some practices for disease control may include seedling destruction, application of chemical products or biological control agents (Mitchell *et al.* 2004), the best way to reduce the disease incidence seems to be the selection of plant-resistant genotypes to *F. circinatum*.

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