First Confirmed Report on *Fusarium sporotrichioides* on *Pinus ponderosa* var. *jeffreyi* in Slovakia

**HELENA IVANOVÁ**¹, **Ľudmila HREHOVÁ**² and **PETER PRISTAŠ**²

¹Institute of Forest Ecology of the Slovak Academy of Sciences, Branch for Woody Plants Biology, Nitra, Slovak Republic; ²Institute of Biology and Ecology, Pavol Jozef Šafárik University, Košice, Slovak Republic

Abstract


During 2014–2015, samples from ten 20–25 years old pine (*Pinus ponderosa* var. *jeffreyi*) trees showing wilt symptoms were collected from the Arborétum Mlyňany park. A disease was observed on 20% of the trees. The first symptoms are wilting, stunting, chlorosis, and discolouration of needles, which turned yellow on affected twigs, then red and finally they fell off. Isolations of the pathogen were done from the discoloured tissues of needles (twenty samples from each tree) on Potato Dextrose Agar. Colonies of the fungus (3–4 Petri dishes from each tree) were initially aerial, white or slightly violet, but with age they became red and red pigments were produced in agar. The observed micromorphological characteristics of the fungus, such as presence of simple and proliferating conidiophores with polyphialides, microconidia, macroconidial shape, and chlamydospore presence matched the description of *Fusarium sporotrichioides*. The identity of the fungus was confirmed by phylogenetic analysis of internal transcribed spacer (ITS) sequences. Sequence comparisons placed the fungus to the species *F. sporotrichioides* with similarity of 99.6% at the ITS sequence level.

Keywords: old pine; morphological characteristics; phylogenetic analysis; fungal plant pathogen

*Fusarium sporotrichioides* Sherb. is a cosmopolitan, ecologically widespread fungal plant pathogen, being found across tropical and temperate regions (Domsch *et al.* 1993). The pathogen is responsible for damage to seedlings causing root rot or cankers (Anderson 1986), and needle dieback on mature trees (Karadžić & Milijašević 2008). This fungus is of notable agricultural and economic importance – it is a significant producer of mycotoxins, particularly trichotheccenes, which are known to inhibit protein synthesis in eukaryotes (Leslie & Summerell 2006; Cormick *et al.* 2011).

The aim of this study was to isolate and identify the fungal species associated with the diseased pine species *Pinus ponderosa* Douglas ex C. Lawson, var. *jeffreyi* Balf. ex Vasey, during 2014–2015 and to evaluate the relative tolerance of this species grown in many parks in Slovakia. This study represents the first confirmed report on *F. sporotrichioides* in Slovakia, which is considered as one of the most serious pathogens of *P. ponderosa* var. *jeffreyi* needles.

**MATERIAL AND METHODS**

The *Fusarium* isolates used in this study were obtained from ten 20–25-years old *P. ponderosa* var. *jeffreyi* trees in the geographic location Arborétum Mlyňany SAS (altitude 200 m a.s.l., cool temperate climate, average daily temperature of 10.6°C, average annual atmospheric precipitation of 541 mm). For the isolation of fungi, diseased tissues of needles (twenty samples from each tree) were surface-sterilised (15 min in a commercial

Supported by by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences – VEGA, Grants No. 2/0071/14 and No. 2/0069/14.
solution containing 1.5% sodium hypochlorite) and then rinsed well with sterile distilled water. Small pieces of tissue, cut from the edges of lesions, were placed directly onto PDA medium (Merck, Darmstadt, Germany), and incubated at 23°C for 7 days in a versatile environmental test chamber MLR-351H–Sanyo (Sanyo, Osaka, Japan). Fungi resembling those in the genus Fusarium were transferred to Petri dishes (PD) containing PDA (or Malt extract agar – MA, Agar-agar, Kobe I – AA; Carl Roth GmbH + Co KG, Karlsruhe, Germany). The study of fungal structures was performed with a BX41 clinical light microscope (Olympus, Tokyo, Japan) under 400x and 1000x magnifications. Measurements were done through the medium of QuickPhotomicro 2.2 programme and the morphometric values were compared with previously published data for the taxa (Leslie & Summerell 2006). Fungi were identified to the level of the genus using the taxonomic guide of Barnett and Hunter (1972). Selected isolates of Fusarium were identified to the species level using monographs of Samson et al. (1981) and Gerlach and Nirenberg (1982).

The studied fungus was identified using the phylogenetic analysis of internal transcribed spacer (ITS) sequences. Total DNA was isolated from a fresh mycelium (2 PD) using microwave treatment and subsequent Triton X-100 lysis (Goodwin & Lee 1993). The isolated DNA (10 ng) was used as a substrate in PCR reaction with fungal universal primers ITS1 (5’-TC-CTCAGGTGAACTGCGG-3’) and ITS4 (5’-TC-CTCCCCGCTTATTGATATGC-3’) for amplification of the ITS1-5.8S-ITS2 ribosomal DNA regions (White et al. 1990). PCR amplifications were performed in a Bio-Rad MJ Mini Personal Thermal Cycler (Bio-Rad, Santa Clara, USA) under conditions specified elsewhere (Ivanová et al. 2016). Amplified products were analysed by electrophoresis on 1% agarose gels in TAE buffer (Sambrook et al. 1989). The amplified product was purified from agarose gel using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, USA) and sequenced by the dyeexotermination method with each primer used in the PCR reaction at GATC-Biotech (Konstanz, Germany). DNA sequences were assembled using DNA Baser software (Heracle BioSoft SRL, Arges, Mioveni, Romania) and submitted to the GenBank database under accession No. KU948933. DNA sequences were compared against the GenBank database using the BLASTn algorithm (Altschul et al. 1990). For the analysis of phylogenetic relatedness ITS sequences of related Fusarium spp. were downloaded from the GenBank database, aligned using the MUSCLE algorithm (Edgar 2004) and phylogenetic relationships were constructed using the Neighbour-Joining method available in the MEGA software Version 6 (Tamura et al. 2013). Phylogenetic robustness of observed trees was tested by bootstrap analysis after 1000 replications.

Dried samples of infected needles of P. ponderosa var. jeffreyi are deposited at the herbarium, specimen R 5377, a pure culture of the fungus F. sporotrichioides (leg. K. Pastirčáková, det. H. Ivanová, P. Pristaš, Arboretum Mlyňany, 2015) can be obtained from the culture collection of the Institute of Forest Ecology of the SAS, Branch for Woody Plant Biology in Nitra.

**RESULTS AND DISCUSSION**

In our study we confirm the first report on *F. sporotrichioides* in Slovakia, as one of the most serious pathogens of *P. ponderosa* var. *jeffreyi* needles. It is a fast-growing fungus, usually able to grow up to 8–9 cm in diameter within four days. Optimal growth temperature ranges from 22.5°C to 27.5°C and the minimum humidity level required for vegetative growth is 88% (Domsch & Gams 1979; Gagkaeva 2008). Colonies of the fungus (3–4 PD from each tree) were initially aerial, white or slightly violet, later pinkish to brownish red. Growth of the mycelium, which is usually able to grow up to 6–7 cm in diameter within 5 days after inoculation, depends on the used medium (Figure 1). This fast-growing fungal mycelium obtained from *Pinus* needles was identified as *F. sporotrichioides* based on phylogeny, cultural and morphological features. The presence of typical abundant macroconidia with 1–3 septa with dimensions of 23(29)37 × 4–5(6) µm and with 4 septa of Fusarium with dimensions of 40-43 × 4–5 µm

![Figure 1. Comparison of growth rates of mycelium F. sporotrichioides on different medium](image-url)
was observed on damaged needles. Macroconidia
formed on hyphae and on sporodochia were falcate
with apical curved, pointed cell and basal notched
or foot-shaped cell. One-celled ovoid, oval to pyriform, spindle-
shaped or pip-shaped microconidia of *Fusarium sporotrichioides*
on damaged needles of *Pinus ponderosa* var. *jeffreyi
were 12 × 3.6 (9–20 × 2.5–5.0) µm, 3-septum conidia
30 × 5 (18–29 × 3–5.5 µm), and 4–5-septum conidia
36 × 5.4 (30–40 × 4–6) µm. According to ReFAI et al. (2015) colonies of *F. sporotrichioides* produce the
profuse white to pale red mycelium. Macroconidia
are abundant in orange sporodochia, falcate to lunate,
3–5 septate, apical cell curved and tapering, basal
cell poorly developed. Microconidia produced from
mono- or polyphialides are pyriform and 0–1 septate.
Chlamydospores are abundant. According to ELLIS and
ELLIS (1997) macroconidia in this culture are slightly
curved, falcate with 3–5 septa. Apical cell is curved
and pointed, basal cell is notched or foot-shaped. The
fungus is compared with taxonomically related spe-
cies, which show the presence of polyphialides that
produce pyriform as well as fusiform microconidia and
blastosporas (CHELkowski et al. 1989). An important
feature for their distinction from other fusaria is nu-
merous brown and globose chlamydospores, which are
thick-walled, filled with lipid-like material serving to
carry the fungus over winter in soil when no suitable
host is available. The chlamydospores may be borne
singly, in pairs, in clumps, or in chains, and the outer
wall may be smooth or rough (SAMSON et al. 1981).
Amplification and the sequence comparisons of
the ITS region have become a common approach to
the molecular identification of fungi (NILsson et al.
2008). ITS sequence of the R5377 isolate was obtained
and compared against GenBank database. The compar-
isons placed the isolate clearly to the *F. sporotrichioides*
group. ITS sequence comparisons showed similarities of 546/548 nucleotides to *F. sporotrichioides*,
545/548 nucleotides to *F. armeniacum*, and 502/548
nucleotides to *F. circinatum* ITS sequences. Similar
intraspecies ITS sequence similarities (99.3–99.8%)
were observed e.g. for the isolates of *F. culmorum*.
(Mishra et al. 2000). Multiple sequence alignment (Figure 3) confirmed that the R5377 isolate belongs to the species *F. sporotrichioides*.

During an investigation on the mycoflora of pine trees in park greenery we isolated on *Pinus ponderosa* var. **jeffreyi** the fungus with distinctive characteristics which was identified – on the basis of light-microscope morphological studies and by phylogenetic analysis – as a new recorded fungus *F. sporotrichioides* occurring in Slovakia.

**References**


Accepted after corrections: 2016–07–07

Published online: 2016–08–12