



First report of *Sirococcus tsugae* causing shoot blight on *Cedrus atlantica* in Belgium

S. Schmitz*, A. Charlier and A. Chandelier

Walloon Agricultural Research Centre, Rue de Liroux 4, 5030 Gembloux, Belgium

*E-mail: s.schmitz@cra.wallonie.be

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In June 2018, a group of about ten Atlas cedar (*Cedrus atlantica*) was found showing severe shoot blight symptoms. Affected trees presented a light brown to pink discolouration of the needles (Fig. 1) as well as premature defoliation (Fig. 2). A closer examination of the branches revealed necrosis of phloem tissues (Fig. 3). The trees, located in a forest environment in the province of Luxembourg (Belgium), were all displaying symptoms. Their diameter at breast height ranged from 45 to 55 cm.

Isolation attempts were made from the margin of necrotic tissues. Transverse sections of shoots were surface disinfected for 60 seconds in a sodium hypochlorite solution (1.25% active chlorine). Disinfected segments were debarked, plated onto potato dextrose agar (Difco, USA) and incubated at 20°C in the dark. A fungus forming a white to light grey aerial mycelium consistently grew out of the plates after about one week. Colonies became darker in the centre as the fungus produced dark immersed pycnidia (Fig. 4). The fusiform, hyaline and one-septate conidia measured 7-12 (9.7) × 2-3 (2.5) µm (n=50) and were characteristic of the genus *Sirococcus* (Fig. 5).

Considering the different *Sirococcus* species affecting conifers (Rossman *et al.*, 2008) and the recent outbreak of *Sirococcus tsugae* on cedars in Europe (Butin *et al.*, 2015; Pérez-Sierra *et al.*, 2015), DNA was extracted from the pure culture to further assess the identity of the species. PCR was carried out with the *S. tsugae* specific primers SirTf/SirTr2 (Smith & Stanosz, 2008) and an amplicon of the expected size (350 bp) was detected. In addition, ITS sequencing was performed using the ITS1/ITS4 primer pair. The nucleotide sequence was submitted to GenBank (Accession no. MH828347). BLAST analysis showed 100% identity with available *S. tsugae* sequences.

Pathogenicity was studied in a quarantine facility using excised shoots of Atlas cedar of about 30 cm long. Twelve shoots were wounded and agar plugs from the actively growing front of a one-week-old colony of the isolated *S. tsugae* strain (no. 5185 in the fungi collection of the Walloon Agricultural Research Centre) were inserted. Twelve other shoots were inoculated with a sterile agar plug and served as negative controls. The excised shoots were placed separately in flasks containing a small amount of sterile tap water and maintained at 22°C under daylight. After four weeks, all the shoots inoculated with strain 5185 displayed extended necrosis around the inoculation point. *Sirococcus tsugae* was consistently re-isolated from all lesions, thereby completing Koch's postulates. Control

shoots remained asymptomatic.

Native to North America, *S. tsugae* was introduced in Europe during the last decade and detected at several locations in Germany and in the United Kingdom. To our knowledge, this is the first report in Belgium of this organism which was added to the EPPO Alert List in 2015. *Cedrus atlantica* is regarded as a promising tree species under predicted climate change conditions (Courbet *et al.*, 2012) and emerging diseases affecting this tree species warrant particular attention. The distribution of *S. tsugae* within Belgian forests and nurseries as well as on amenity trees therefore requires further investigation. Surveys should also consider other host plants of the disease such as other *Cedrus* or *Tsuga* species.

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Figure 1



Figure 2



Figure 3



Figure 4



Figure 5

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