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SHORT COMMUNICATION

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Pathogenicity of *Sirococcus tsugae* on major coniferous tree species of Belgian forest

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Abstract

To evaluate the potential impact of *Sirococcus tsugae* on Belgian coniferous forest, a pathogenicity test was conducted through wound inoculation of two-year-old seedlings of different tree species. *Picea abies*, *Pseudotsuga menziesii*, *Pinus sylvestris*, *Larix x eurolepsis* as well as *Tsuga heterophylla* and *Cedrus atlantica* were inoculated with an isolate of *S. tsugae* collected from the only known infected site in Belgium. The tested strain proved to be highly pathogenic towards seedlings not only of two known host species of the disease (*T. heterophylla* and *C. atlantica*) but also of *L. x eurolepsis*. This result highlights the risk that a possible emergence of *S. tsugae* might represent for some important forest tree species, including species currently not known as hosts of the disease. However, as the two main coniferous tree species in Belgium (*P. abies* and *P. sylvestris*) did not appear sensitive to *S. tsugae*, the potential impact of the pathogen on the Belgian forest is limited.

KEYWORDS
artificial inoculation, *Cedrus atlantica*, host range, invasive pathogen, necrosis length, shoot tip blight, *Tsuga heterophylla*

1 | INTRODUCTION

Sirococcus tsugae is an aerial fungal pathogen known to cause shoot tip blight in several coniferous species, Atlas cedar (*Cedrus atlantica* (Endl.) Carr.) being the most affected in Europe. In spring, diseased trees show characteristic light brown to pink discoloration of needles, which dry out and fall prematurely. Affected branches also display depressed and purplish zones in the bark; removing the outer bark in these zones reveals extended necroses of the phloem. Dieback of the affected shoots is a consequence of the disease, which in some cases can affect several branches of the same tree (EPPO, 2019).

The pathogen originates from North America, where it has been found to affect a broad spectrum of species in the *Cedrus* and *Tsuga* genera (Rossman et al., 2008). In Europe, the first symptoms related to *S. tsugae* were reported in 2013 on Atlas cedar in England (Pérez-Sierra et al., 2015). Since then, the number of cases has increased significantly and *S. tsugae* has been recorded for a range of locations and hosts in the United Kingdom. Atlas cedar is the main affected species, but the pathogen's presence on several hemlocks (*Tsuga heterophylla* (Ref.) Sarg. and *Tsuga mertensiana* (Bong.) Carr.) has also been reported. Several cases have also been locally detected on *C. atlantica* in Germany (Butin et al., 2015) and in Belgium (Schmitz et al., 2018). *Sirococcus tsugae* was added to the EPPO Alert List in 2015 but removed in 2019, although much uncertainty remains concerning its distribution and potential impact in Europe (EPPO, 2019). In Belgium, following the first report of the disease in 2018, a survey was conducted in 23 forest plantations of *C. atlantica* but did not lead to any additional detection of the pathogen (Pirronitto et al., 2020).

Atlas cedar is at present poorly represented in Belgian forests, but its drought resistance makes it a promising species in the context of climate change and for foresters in search of diversification (Pirronitto et al., 2020). The supply of seedlings for plantations is, however, highly dependent on imports, with the associated risk of introducing diseases.

TABLE 1 Occurrence of necroses induced on stems of two-year-old seedlings of different coniferous tree species four weeks after artificial inoculation with *Sirococcus tsugae* (strain 5609)

Tree species	Number of control seedlings	Number of seedlings inoculated with *S. tsugae*	Number of inoculated seedlings showing necrosis	Re-isolation frequency (%)
Cedrus atlantica	10	10	10	100
Tsuga heterophylla	10	10	10	100
Larix x *eurolepsis*	10	10	10	90
Pseudotsuga menziesii	10 (6)[a]	10 (7)[a]	7	100
Picea abies	10	10	2	100
Pinus sylvestris	10	10	0	-

[a]The number in brackets displays the number of surviving seedlings after transplanting

Considering the possible extension of the forest area covered by Atlas cedar and the phytosanitary risk represented by *S. tsugae*, the present study aimed at evaluating the susceptibility of the most common coniferous species of Belgian forest to this fungal pathogen. The results will also help to predict the risk that the potential emergence of *S. tsugae* may represent for coniferous forest in Western Europe.

2 | MATERIALS AND METHODS

2.1 | Isolation

Samples were collected from the site where *S. tsugae* was first detected in Belgium (Tellin, province of Luxembourg), which is so far the only site known to be infected by the disease in the country. Branches showing dieback symptoms were selected in the field for isolation in the laboratory. The edge of the lesion between necrotic and visually healthy tissue was excised and surface-disinfected for 60 s in a sodium hypochlorite solution (1.25% active chlorine). The disinfected segments were debarked, plated onto potato dextrose agar (PDA, Difco, USA) and incubated at 22°C in the dark. After one week, white to light grey colonies developing in the plates and showing morphological characteristics of *S. tsugae* were subcultured.

DNA was extracted from the pure culture, and the internal transcribed spacer (ITS) region was amplified using *S. tsugae*-specific primers SirTf/SirTr2 to further assess its identity (Smith & Stanosz, 2008). The freshly obtained pure culture of *S. tsugae* (strain 5609 in the fungus collection of the Walloon Agricultural Research Centre) was used for the pathogenicity test.

2.2 | Plant material

Two-year-old seedlings of six different coniferous tree species were obtained from forest tree nurseries in February 2020 and directly potted in a growing substrate. Plants were then placed in a quarantine facility (22°C, ambient daylight) and watered regularly. These seedlings belonged to four major coniferous species of Belgian forests (*Picea abies* (L.) Karst, *Pseudotsuga menziesii* (Mirb.) Franco., *Pinus sylvestris* L. and *Larix* x *eurolepsis* A. Henry) and two species already known to be the host species of *S. tsugae* (*C. atlantica* and *T. heterophylla*).

2.3 | Pathogenicity test

Two weeks after potting, pathogenicity was tested in the quarantine facility. Twenty seedlings of each of the six test species were used: ten seedlings were inoculated with mycelium plugs from the actively growing front of a 12-day-old colony of *S. tsugae* (strain 5609) grown on PDA, while ten others served as negative controls. The stem of each seedling was surface-disinfected with 70% ethanol, and a U-shaped incision was made in the bark with a sterilized scalpel about 10 cm above the root collar. The flap of bark was raised up, and the mycelial plug was placed on each wound with mycelium facing the cambium. The inoculation site was sealed with parafilm. The controls were inoculated with sterile PDA plugs.

The test was assessed after four weeks when blight symptoms had developed on 90% of the inoculated seedlings of the two known host species of *S. tsugae* (*C. atlantica* and *T. heterophylla*). All seedlings were cautiously debarked in the area around the inoculation point, and the length of necrotic lesions was measured with a precise ruler. When necroses were present, re-isolation was attempted using the procedure described for isolation from field samples. As previously, the method of Smith and Stanosz (2008) was followed to confirm the specific identity of the re-isolated *Sirococcus* strains.

2.4 | Statistical analyses

Data on lesion length induced on seedlings of different tree species inoculated with *S. tsugae* were subjected to analysis of variance (ANOVA) in RStudio (R Foundation, Vienna, Austria). Following the ANOVA, significant differences between tree species were further evaluated by means of a post hoc Tukey test.

3 | RESULTS

3.1 | Occurrence of necroses and re-isolation attempts

Four weeks after inoculation, necrotic lesions had developed on all inoculated seedlings of *C. atlantica* and *T. heterophylla* (Table 1), as expected considering the known host range of *S. tsugae*. Necroses were also recorded on all inoculated *L.* x *eurolepsis* and *P. menziesii* seedlings. However, for the latter tree species, desiccation symptoms linked to transplantation failure appeared on some seedlings shortly after the beginning of the test. Despite the related mortality (30 and 40% of inoculated and control seedlings, respectively), necrotic lesions could be observed on all remaining inoculated seedlings. In contrast, necroses rarely occurred on inoculated *P. abies* (only on two seedlings out of ten) and symptoms never developed for *P. sylvestris*. No necrosis formed on the control seedlings of any of the tree species.

During re-isolation attempts, strains presenting the morphological characteristics of the genus *Sirococcus* were isolated from all the inoculated seedlings with necrotic lesions, except for one *L.* x *eurolepsis* seedling. DNA extraction and PCR performed on these isolates (Smith & Stanosz, 2008) confirmed that they all belonged to the species *S. tsugae*.

3.2 | Length of necroses

Regarding the length of the necroses induced by *S. tsugae*, analysis of variance showed significant differences between tree species ($p < .001$) (Figure 1). The largest necroses developed on seedlings of *C. atlantica*, *T. heterophylla* and *L.* x *eurolepsis*. Within this group, the length of the lesions induced on *L.* x *eurolepsis* was not significantly different from those developing on *C. atlantica* ($p = .39$) and *T. heterophylla* ($p = .65$), which were already known to be hosts of *S. tsugae*. Large necroses also formed on *P. menziesii*, although they were significantly smaller than those developing on *T. heterophylla* and *C. atlantica* ($p < .01$). The rare necroses observed on inoculated *P. abies* were significantly smaller than those induced on all other tree species except for *P. sylvestris*, which was not symptomatic.

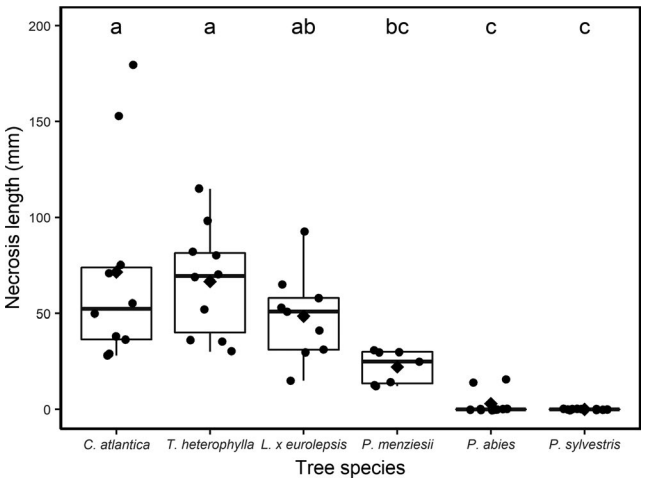

FIGURE 1 Length of necroses induced on stems of two-year-old seedlings of different coniferous tree species four weeks after artificial inoculation with *Sirococcustsugae* (strain 5609). Variables sharing the same letter were not significantly different at $p \leq .05$ according to Tukey's (HSD) test following ANOVA

4 | DISCUSSION AND CONCLUSION

In this study, in agreement with several surveys conducted in North America and Europe (Pérez-Sierra et al., 2015; Rossman et al., 2008), *S. tsugae* proved to be pathogenic to *C. atlantica* and *T. heterophylla*. Two-year-old seedlings of *L.* x *eurolepsis* also appeared to be very sensitive to *S. tsugae* and, compared with *T. heterophylla* and *C. atlantica*, presented a similar sensitivity in terms of occurrence and length of induced necroses. However, given the fairly aggressive inoculation method used in this study, these results should be considered with caution and also need to be confirmed with different strains of *S. tsugae*. As a complement to this indicative screening test conducted on various coniferous tree species, additional pathogenicity tests could therefore be performed i) focusing on *L.* x *eurolepsis* and on other larches of European forest such as *Larix decidua* Mill. and *Larix kaempferi* (Lambert) Carr., and ii) with strains of *S. tsugae* of various origins.

Inoculation had almost no impact on seedlings of the two main coniferous species of Belgian forest, *P. abies* and *P. sylvestris*. Symptoms never occurred on seedlings of *P. sylvestris* inoculated with *S. tsugae*, and necroses induced on *P. abies* were rare and of limited extent. Therefore, in the event that favourable environmental conditions lead to the spread of *S. tsugae* in Belgium, the results of this study suggest that the impact on Belgian coniferous forest should be limited given its tree species composition.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/efp.12689.

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