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First report of *Seiridium cardinale* on *Cupressus sempervirens* in Serbia

Ivan Milenković[1,2], Zlatan Radulović[3], Dragan Karadžić[1]*

[1]*Faculty of Forestry, University of Belgrade, Belgrade, Serbia*
[2]*Phytophthora Research Centre, Mendel University in Brno, Brno, Czech Republic*
[3]*Institute of Forestry, Belgrade, Serbia*

**Corresponding author: ivan.milenkovic@sfb.bg.ac.rs*

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Abstract: While monitoring the health of different ornamental and shade trees in Serbia, symptoms indicative of cypress canker disease were observed in young *Cupressus sempervirens* trees in the Belgrade urban area. Symptoms included tree mortality (specimens were recorded with a change in needle colour, branch decline or longitudinal bark cankers on the stems with resin exudates) and the appearance of acervuli fruiting bodies on the bark and cones. Using light microscopy, cross sections of fruiting bodies on the cankered bark and cones were examined and numerous fusoid, six-cell conidia were recorded with four inner coloured cells and two hyaline cells at the ends. Based on the unique combination of the morphological features and the infected host, this pathogen was identified as *Seiridium cardinale*. This is the first report of *S. cardinale* on the common cypress in Serbia. Possible introduction pathways and the implications of the findings are discussed.

Keywords: common cypress; cypress canker; *Coryneum cardinale*; ornamental plantings; nursery infestation

The common cypress (*Cupressus sempervirens* L.) is widespread throughout the Mediterranean region, as a native or naturalised species. The species has great ornamental value and is widely used in decorative plantings worldwide (Danti & Della Rocca 2017). The common cypress does not grow naturally in Serbia (Jovanović 1971); the closest localities where this species naturally occurs are the Greek islands, such as Crete, Karpathos, Kos, Kalymnos, Rhodes and Samos, and in Turkey (Farjon & Filer 2013). However, the common cypress and other members of the Cupressaceae family have often been planted in urban areas in Serbia (Milijašević 2003); there is also significant domestic nursery production of these tree species and importation from nurseries abroad.

The main pathogen of the common cypress, which significantly affects its natural populations and plantings worldwide, is the fungus *Seiridium cardinale* (Wagener) Sutton & Gibson, known to be a causal agent of cypress canker disease (Danti & Della Rocca 2017; Bonthond et al. 2018). Since recent taxonomic revisions have been made, this fungus now belongs to the Sporocadaceae family (Jaklitsch et al. 2016; Bonthond et al. 2018). According to Danti and Della Rocca (2017), the regions most

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affected by cypress canker disease are California and the Mediterranean countries. In addition to the Mediterranean area, where cypress canker epidemics have seriously threatened the landscape and caused huge economic losses for over 60 years (Della Rocca et al. 2011, 2013), the disease has been recorded in England, Ireland, Northern Ireland and Germany (https://gd.eppo.int/taxon/SEIRCA/distribution). Besides species from the *Cupressus* genus, *S. cardinale* can seriously affect *Chamaecyparis*, *Cryptomeria*, *Juniperus*, *Thuja* and ×*Cupressocyparis* species (Danti & Della Rocca 2017).

During studies on the decline of various ornamental plant species in urban areas in Serbia, dieback symptoms were noticed in late summer 2014 in planted common cypress trees in one public garden in Belgrade. Symptoms indicative of cypress canker disease, with branch decline and a change in the needle colour (Figure 1G) were recently recorded in another private garden in urban Belgrade.

This study aimed to determine the cause of the observed dieback of young common cypress trees in urban areas in Serbia and to clarify the presence of cypress canker disease.

MATERIAL AND METHODS

Study area and disease symptoms. The study was performed in one public garden located in urban Belgrade (44°47'8.8"N; 20°24'56.1"E). The symptoms observed in a single planted common cypress tree in this garden were recorded in September 2014. The symptoms included dead tree with discoloured needles (Figure 1A), longitudinal bark cankers on the stems with resin exudates (Figures 1B and 1C) and with the appearance of fruiting bodies on the bark (Figure 1D) and cones (Figures 1E and 1F). The tree was approximately 10 years old, while the average diameter at breast height was 10–12 cm. Similar symptoms (Figure 1G) were recorded in the spring 2019 in a private garden in Belgrade (44°46'51"N; 20°25'12"E), but we did not have access to closely inspect and sample this symptomatic cypress tree. The public garden is located approximately 1.2 km from the former private one.

Sampling and isolation. Three portions of the cankered bark were cut for sampling, including two from the trunk and one from the branch, and cones from the declining tree were collected. After undergoing surface sterilisation (70% ethanol for 1 min) in the laboratory, the bark samples were thoroughly washed in sterilised distilled water (SDW) and dried on sterilised filter paper. After removing the outer bark to expose the underlying inner bark affected by the necrotic process, the fragments were taken from the upper and lower areas at the boundaries between necrotic and healthy tissues. Fragments 4–5 mm in size were cut with a scalpel, then sterilised in 96% ethanol and burned over an open flame and plated on malt extract agar [MEA; 18 g/L of Malt Extract (MERCK, Germany) and 18 g/L of agar-agar (Torlak, Serbia)]. In total, 15 fragments from three cankers were plated. The Petri dishes were then incubated at 20–22 °C in the dark and checked for emerging hyphae at 48-hour intervals under a binocular microscope (Olympus SZ7, Japan) at 20× magnification. The first hyphae growing out were immediately transferred onto fresh MEA media using a sterilised mycological needle.

Morphological characterisation. To examine the fruiting bodies on the bark and cones, cross sections were made using a sterile razor blade and the thin layers were observed in a drop of SDW under a light microscope (CETI®MAGNUM-T/Trinocular Microscope, UK) at 200× and 400× magnification. Fifty conidia from ten acervuli were randomly selected and measured using a camera (Si3000®; Fisher Scientific, UK) and XliCap® (Xl Imaging Ltd, UK) imaging software. The colony shape patterns of pure cultures were determined on the MEA media after incubation at 20 °C in the dark for four weeks.

The data obtained were compared with the species description provided in Sutton (1980) and Bonthond et al. (2018). The pathogen was identified based on the unique combination of the affected host, the presence of bark cankers with fruiting bodies and the shape and size of the conidia.

RESULTS AND DISCUSSION

The acervuli were black, emerging, peridermal to subepidermal (Figure 1D) and slightly lighter in colour in the conidiogenous region (Figure 1H). The acervuli averaged 579.0 ± 37.69 × 368.1 ± 34.76 µm, ranging from 560.8–665.6 × 280.3–450.4 µm. The conidiophores were hyaline, branched, cylindrical, septate and mixed with hyphae. The conidia

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Figure 1. *Seiridium cardinale* on young Mediterranean cypress trees in Serbia
(A–C) Symptoms recorded on a young tree in urban Belgrade; (D) fruiting bodies on the bark; (E–F) fruiting bodies on the cones; (G) declining branches on cypress trees in the second locality; (H) a cross section through acervuli and a mass of spores; (I) fusoid six-cell conidia with four dark internal and two light cells at the edges and very short appendages; scale bar: H = 100 µm; I = 10 µm

were smooth-walled, broad-fusoid, five septate, six-celled, with four inner light brown, and two outer hyaline and conical cells (Figure 1I). The conidia were observed to have very short, almost invisible appendages (Figure 1I). The conidia averaged 25.3 ± 0.37 × 9.1 ± 0.07 µm, ranging from 19.4–31.9 × 8.0–10.0 µm.

The isolation tests confirmed that all the plated pieces were positive and yielded fungal colonies. The colonies were medium fast growing and filled

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a 90 mm Petri dish after four weeks of incubation at 20 °C in the dark. The colonies cultivated on the MEA were dark ochre around the centre and had a characteristic olive greenish ring in the middle, whereas the growing edges were white. The mycelium was aerial, cottony or slightly fluffy.

All the observed morphological characteristics and recorded symptoms in the common cypress indicated an infection with the pathogenic fungus *Seiridium cardinale*.

This paper is the first to report the presence of *S. cardinale* on *C. sempervirens* in Serbia. The European and Mediterranean Plant Protection Organization (EPPO) website contains incorrect information that cypress canker has been present in Serbia since 2003 (https://gd.eppo.int/taxon/SEIRCA/distribution/RS); Milijašević (2003) is cited as the source of this record. However, Milijašević clearly shows that *S. cardinale* was recorded on common cypress trees in plantations and gardens in Montenegro, not in Serbia. This confusion probably originated from the name of the country, as, during that period, Serbia and Montenegro were part of one country, Yugoslavia, which was renamed Serbia and Montenegro in 2003 until the final splitting of the countries in 2006. It is, therefore, likely that the findings applicable to Montenegro were incorrectly assigned to Serbia as well. In subsequent studies conducted in various natural and urban areas in Serbia, the disease has not been recorded.

In this study, symptoms were recorded in infected *C. sempervirens* trees, including the dieback of the tree and branches and longitudinal bark cankers, which are indicative signs of the presence of the disease (Danti & Della Rocca 2017). Canker development is almost always followed by intense resin flow and we also recorded this phenomenon on the infected tree (Figures 1B and 1C). In studies by Danti and Della Rocca (2017) and Bonthond et al. (2018), it was shown that *S. cardinale* infections were triggered by various bark injuries, such as mechanical, insect or frost damage in which the spores or mycelia of this pathogen can start a new infection. In some urban areas, stress factors are even more pronounced, particularly where the common cypress is a non-native species as is the case in Serbia. Therefore, it is most likely that the combination of hot summers and very cold winters, as is characteristic of the climate in Serbia, may have been a factor in the damage occurring to the *C. sempervirens* bark and subsequently to the emergence of *S. cardinale* infections.

It was not possible to trace the origin of the plant and to clarify whether it originated from some local nursery or was brought in from elsewhere. Thus, it was not possible to determine whether the planted tree was introduced from a local nursery or from some foreign nursery already infected, or if the inoculum arrived from some other nearby source. It is most likely that this pathogenic fungus was introduced to Serbia together with plants for horticulture or with some other planting material. It is of significant interest for the national plant protection service in Serbia to clarify the path of introduction of this aggressive invasive pathogen, and detailed nursery monitoring can help solve this problem.

The presence of *S. cardinale* poses a high risk to common cypress plants in many urban areas in Serbia. There is also a high degree of risk for the nursery industry as the spreading of the disease may threaten the production of other susceptible plants, including *Chamaecyparis*, *Cryptomeria*, *Juniperus*, *Thuja* and ×*Cupressocyparis* genera (Danti & Della Rocca 2017). The disease may also spread in uninfected urban areas via plants for planting. It is urgently required to continue monitoring the presence of the disease within nurseries and other urban areas in Serbia.

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