

Cytospora friesii and *Sydowia polyspora* are associated with the sudden dieback of *Abies concolor* in Southern Europe

JELENA LAZAREVIĆ^{1*}, AUDRIUS MENKIS²

¹Biotechnical Faculty, University of Montenegro, Podgorica, Montenegro

²Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden

*Corresponding author: ena.lazarevic@gmail.com

Citation: Lazarević J., Menkis A. (2022): *Cytospora friesii* and *Sydowia polyspora* are associated with the sudden dieback of *Abies concolor* in Southern Europe. *Plant Protect. Sci.*, 58: 258–263.

Abstract: *Abies concolor* was introduced to Europe in the 19th century and commonly planted as an ornamental tree. In 2018, after several very warm and dry vegetation seasons, which has likely caused abiotic stress in the trees, a sudden dieback of *A. concolor* was observed in the city of Arandelovac in Serbia. The external symptoms were the rapid discoloration of needles in a larger part of the crown and eventually the dieback of the trees. The aim of this study was to identify the possible agents of damage by analysing the fungal communities associated with symptomatic needles using high-throughput sequencing as no symptoms of damage were seen on the branches or stems. Symptomatic needles were collected from the lower branches of five standing trees. Half of the needles were surface sterilised and the remaining were left unsterilised. DNA was extracted, amplified using ITS2 rDNA as a marker and sequenced. Quality filtering showed the presence of 6 191 high quality reads, which, following a clustering analysis, was found to represent 79 non-singletons fungal taxa. The most common fungi in both surface sterilised and unsterilised needles were the pathogens *Cytospora friesii* (59.7%) and *Sydowia polyspora* (20.6%). The results demonstrated that *C. friesii* and *S. polyspora* can cause a rapid decline of *A. concolor* trees subjected to abiotic stress.

Keywords: pathogens; phyllosphere; fungal community; urban tree health; abiotic stress

Abies concolor (Gord. & Glend.) Lindl. ex Hildebr., which is native to the mountains of western North America, was introduced to Europe in the middle of the 19th century. Due to the aesthetic appearance, i.e., a dense and conical crown, and long and light-grey needles, it was commonly planted as an ornamental tree in city greeneries in several European countries. It was found to be suitable for planting in more open and drier sites, and also because it tolerates polluted urban areas better as compared to native *A. alba* Mill. or many other introduced ornamental species and cultivars of spruce and fir (Vukićević 1982).

In the summer of 2018, a sudden dieback of ca. 40-year-old *A. concolor* trees was noticed in the city of Arandelovac (Serbia). The trees, which otherwise were healthy-looking in late April, by mid-July showed symptoms of dieback and needle discoloration in a larger part of the crown, and these trees were dead by the next spring (personal observations). Younger trees of *A. concolor* exhibited milder symptoms. The time, when the dieback symptoms were noticed, was preceded by several extremely warm and dry vegetation seasons. However, the vegetation season when the tree dieback was observed, was rainy and warm. Although

<https://doi.org/10.17221/120/2021-PPS>

other tree species in the area were not specifically assessed, they were generally healthy-looking. Knowledge about diseases associated with *A. concolor* in Europe is limited, but it can be expected that pathogens reported from other *Abies* and/or *Picea* species could also endanger *A. concolor*. In North America, Delphinella shoot blight (Chastagner et al. 2017), *Rhizosphaera kalkhoffii* Bubak (Smart et al. 2019) and *Phyllosticta* spp. (Wenner & Merrill 1997) are more specifically connected to *A. concolor*. Recent research on Botryosphaeriaceae complex indicate *A. concolor* being among the hosts (Zlatković et al. 2017), but *A. concolor* was not previously known to have any major disease problems in urban greeneries.

The aim of this study was to identify the possible agents of damage that caused the rapid dieback of *A. concolor* trees. As there were no symptoms of damage on the stems and branches, we analysed the fungi associated with the symptomatic needles using high-throughput sequencing.

MATERIAL AND METHODS

Symptomatic needles of *A. concolor* were taken from ca. 40-year-old, declining trees in the Park of Bukovička banja and from private gardens

in the city of Aranđelovac, central Serbia mid-July 2018. The city park occupies ca. 4 ha area and is on a *Quercetum-Fraxinetum angustifolia* habitat (Lazarević 1978). The climate in the area is moderately dry, i.e., Cfbq- according to the Köppen climate classification (Milovanović et al. 2017). June and July of 2018 were characterised by extremely high temperatures and intensive rains. The year 2017 was characterised by an extraordinary cold winter, but had a very warm vegetation season and long-lasting drought. In the period 2012–2017, four vegetation seasons were dry and very warm, thereby likely subjecting the trees to abiotic stress and predisposing them to infections by fungal pathogens. The climate data is available at <http://www.hidmet.gov.rs/>.

Symptoms first appeared on the lower branches and gradually spread upwards. These symptoms included browning and the premature fall off of the needles. The needles, which were two or three years-old, often showed brown bands across the needles and browning of the tips (Figure 1). No symptoms were on the twigs, branches or stems. The trees, which were growing in more open areas, died earlier as compared to the trees growing in denser urban plantations.

Up to 25 symptomatic, but still living, two-three-year-old needles were collected from the lower

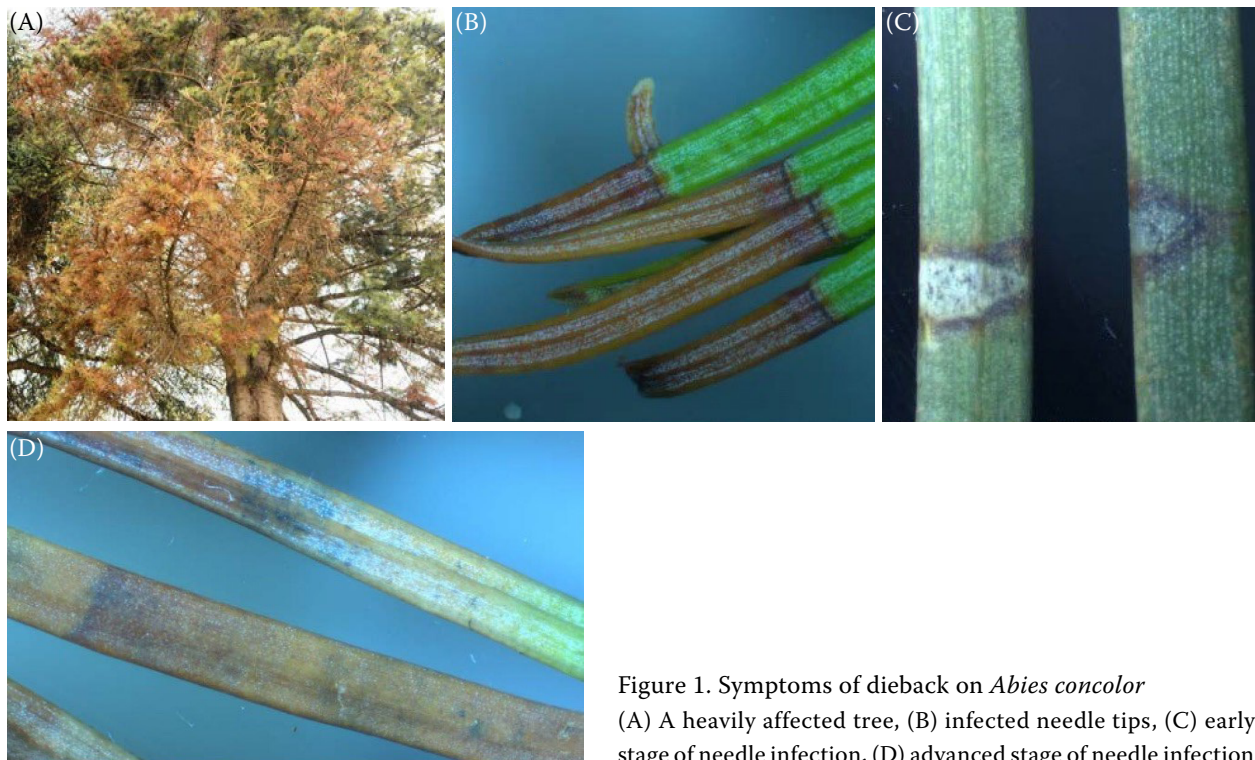


Figure 1. Symptoms of dieback on *Abies concolor* (A) A heavily affected tree, (B) infected needle tips, (C) early stage of needle infection, (D) advanced stage of needle infection

<https://doi.org/10.17221/120/2021-PPS>

branches of each of five standing trees. The needles of each tree were analysed separately. Among these, one tree showed discoloration and dieback of the needles only on the lower branches, three trees showed similar symptoms up to the middle part of the crown, and one tree had these symptoms over the whole crown (Figure 1). Before isolation of the DNA, half of the collected needles were surface sterilised while the remaining were unsterilised.

The isolation of the total DNA was completed using the cetyltrimethylammonium bromide (CTAB) method, after freeze drying and homogenising the needles. Amplification of the DNA using ITS2 rDNA as a marker, the PacBio amplicon sequencing and bioinformatics followed the method in a study by Lazarević and Menkis (2020). The fungal taxa were taxonomically identified using the GenBank database and the Blastn algorithm. The following criteria were used for the taxonomic identification:

sequence coverage > 80%; 94–97% similarity to the genus level and \geq 98% similarity to the species level. Sequences deviating from these criteria were identified only to a high taxonomic rank and were given unique names as shown in Table 1. Representative sequences of the fungal non-singletons are available from the GenBank under accession numbers MZ983658-MZ983736.

RESULTS AND DISCUSSION

The high-throughput sequencing and quality filtering resulted in 6 191 high-quality reads, which, following the clustering at 98% similarity, showed the presence of 79 non-singleton fungal taxa. The singletons and non-fungal taxa were excluded. The detected fungi were 74.7% Ascomycota and 25.3% Basidiomycota. Identification was successful for 82.9% of fungal taxa to at least

Table 1. Relative abundance of the 20 most common fungal taxa associated with the surface-sterilised and surface-unsterilised needles of declining *Abies concolor* in Serbia

Fungal taxon	Phylum	GenBank reference	Sequence length (bp)	Compared (bp)	Sequence similarity (%)	Needles (%)		
						unsterilised	sterilised	all
<i>Cytospora friesii</i>	A	KM100568	255	255/255	100	61.4	45.2	59.7
<i>Sydowia polyspora</i>	A	MN186854	256	256/256	100	18.1	42.2	20.6
<i>Aureobasidium pullulans</i>	A	MT645930	249	249/249	100	3.2	2.2	3.1
<i>Parafenestella austriaca</i>	A	NR165542	251	250/251	99	2.3	1.6	2.2
<i>Botrytis cinerea</i>	A	MT573470	240	240/240	100	1.6	–	1.5
<i>Neocatenulostroma germanicum</i>	A	MK622897	242	242/242	100	1.4	0.5	1.3
<i>Alternaria alternata</i>	A	MW776186	253	253/253	100	1.3	–	1.2
<i>Variabilispora flava</i>	A	NR_165906	244	238/244	98	1.3	–	1.2
<i>Cladosporium cladosporoides</i>	A	MN186740	243	243/243	100	0.9	1.2	0.9
<i>Dothideales</i> sp. 4235_265	A	JX535090	256	256/256	100	1.0	–	0.9
<i>Paracamarosporium fagi</i>	A	MN244221	247	247/247	100	0.6	0.3	0.6
<i>Jattaea discreta</i>	A	NR_166038	256	252/256	98	0.6	0.7	0.6
<i>Filobasidium wieringae</i>	B	MN128850	329	329/329	100	0.5	0.5	0.5
<i>Pseudeurotiaceae</i> sp. 4235_61	A	LR865258	240	240/240	100	0.4	–	0.3
<i>Tremella moriformis</i>	B	AF042426	284	284/284	100	0.4	0.2	0.3
<i>Thyronectria aquifolii</i>	A	MH855538	255	251/255	98	0.0	2.9	0.3
<i>Pezizomycotina</i> sp. 4235_240	A	JF449825	249	246/249	99	0.3	–	0.3
<i>Dothideomycetes</i> sp. 4235_427	A	KP991575	256	250/257	97	0.3	–	0.3
<i>Vishniacozyma carnescens</i>	B	MT595884	234	234/234	100	0.3	–	0.3
<i>Pucciniomycotina</i> sp. 4235_512	B	NR_111685	320	277/329	84	0.3	–	0.3
All of 20 taxa	–	–	–	–	–	96.3	97.6	96.4

A – Ascomycota; B – Basidiomycota

the genus level [Table S1 in electronic supplementary material (for the supplementary material see the electronic version)], and those represented 78.8% of all the fungal sequences. Information on the 20 most common fungal taxa representing 96.4% of all the high-quality fungal sequences is listed in Table 1.

The most common fungi in the symptomatic needles of *A. concolor* were the pathogens *Cytospora friesii* (Duby) Sacc. (59.7% of all the high-quality fungal sequences) and *Sydowia polyspora* (Bref. & Tavel) E. Mull. (20.6%) (Table 1). *Cytospora friesii* [syn. *Valsa friesii* (Duby) Fuckel] was more often detected in the surface unsterilised needles (61.4%) than in the surface sterilised needles (45.2%), while *S. polyspora* was more often detected in the surface sterilised needles (42.2%) than in the unsterilised ones (18.1%) (Table 1). *Cytospora* species are known as common pathogens associated with stem canker diseases of woody plants. These fungi have a worldwide distribution and a broad host range (Pan et al. 2021). Several *Cytospora* species are known to be associated with conifers (Norphanphoun et al. 2017). The pathogenic behaviour of *C. friesii* was tested on 16 different conifer species using inoculation tests, which resulted in cankers and tree dieback in *Abies balsamea* and four different *Picea* species (Smerils 1971). Previously, *C. friesii* was found among the frequent fungi associated with senescent needles of *A. concolor* (Milijašević 1996), but whether it could cause the dieback of *A. concolor* remained obscure. *In vitro* studies showed that the preferred conditions of spore germination of *C. friesii* were a high relative humidity and temperature between 15 and 35 °C. The optimum temperature for the spore germination was 30 °C and the optimal temperature for the growth of the fungal mycelia was 26 °C (Milijašević 1996). This demonstrates that *C. friesii* can be favoured by a warmer climate. *Sydowia polyspora* has a wide geographical distribution and is common in Europe. *Sydowia* symptoms usually include needle necrosis and shoot dieback. It is often detected on trees subjected to abiotic stress or following insect attacks (Muñoz-Adalia et al. 2017). The host range includes the genera *Thuja*, *Abies*, *Tsuga*, *Larix*, *Picea* and *Pinus* (Talگو et al. 2010). It was also found on needles of high-altitude pines in the Balkan region (Lazarević & Menkis 2020), showing that it has adapted to a broad host range and different environmental conditions.

In the present study, the remaining 77 fungal taxa were relatively rare and constituted 19.7% of all the high-quality fungal sequences. Among these fungi, there were several pathogens including *Neocatenulostroma germanicum* (Crous & U. Braun) Quaedvl. & Crous (1.3%) (Table 1). *Neocatenulostroma germanicum* is a recently described fungus causing needle blight on pines in Lithuania, Ukraine and Poland (Markovskaja et al. 2016; Behnke-Borowczyk et al. 2019), but was also detected on needles of *Pinus heldreichii* H. Christ in Montenegro (Lazarević & Menkis 2020). The occurrence of *N. germanicum* on *Abies* was not known before, thereby the present study provides new information on its host range. *Botrytis cinerea* Pers. (1.5%) and *Alternaria alternata* (Fr.) Keissl. (1.2%), which are known as generalist fungal pathogens, were also detected at low abundances.

Several fungi in the study likely occurred as saprotrophs or endophytes, including *Parafenestella austriaca* Jaklitsch & Voglmayr (2.2%). Species from the genus *Parafenestella* are known to be associated with *Cytospora* on hardwoods (Jaklitsch & Voglmayr 2020). *Jattaea discreta* (Berl.) Reblova (0.6%) is known as a species with a saprotrophic lifestyle, appearing on decaying wood with canker symptoms (Réblová 2011). *Paracamarosporium fagi* Crous & R.K. Schumach (2015) (0.6 %) is a recently described fungus, which has a restricted geographical distribution and limited host range (Hyde et al. 2020). *Aureobasidium pululans* (de Bary & Lowenthal) G. Arnaud (3.1%), *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (0.9%) and *Filobasidium wieringae* (A. Fonseca, Scorzetti & Fell) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout (0.5%) are widespread and common fungi in environmental samples (Wijayawardene et al. 2020). As the knowledge on fungal communities associated with symptomatic needles of *A. concolor* is scarce, the results of the present study provide new insights into the host specificity and possible effects on a tree's health.

In summary, *C. friesii* and *S. polyspora* were found to dominate in the symptomatic needles, showing that these fungi can be a great threat to *A. concolor* trees subjected to abiotic stress. The ongoing climate change, which causes high summer temperatures and droughts, together with these pathogenic fungi could endanger *A. concolor* and possibly other *Abies* species on larger territo-

<https://doi.org/10.17221/120/2021-PPS>

ries in Europe, resulting in significant losses in city greeneries and forest stands.

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Received: September 6, 2021

Accepted: March 7, 2022

Published online: May 9, 2022