

## Mycobiota of juniper *Juniperus x media* with symptoms of dieback in sewage plant facilities area in Poznań

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**Abstract.** The frequency and diversity of fungi in branches, roots and soil was examined in 3–10-years-old diseased *Juniperus x media* trees growing in the surroundings of the sewage treatment facility in Poznań. Symptoms of branch dieback appeared first on the older parts inside the crown and mostly in the lower part of trees subsequently spreading upwards and outwards. Our analyses included extraction of environmental rDNA from branches, roots and soil, amplification of the rDNA with fungi specific primers and sequencing. Fungal taxa from Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota and Zygomycota were detected with a total of 695, 135 and 196 taxa in branches, roots and soil, respectively. Fungal communities included plant pathogens, opportunistic pathogens, epiphytes or endophytes, mycorrhizal taxa, saprotrophs common on organic debris and in soil, human and animal pathogens, entomopathogenic taxa, mycoparasites, white and black yeasts, taxa with antagonistic and medicinal properties and lichenized fungi. The frequency of potential plant pathogens was 2.64–33.12% in branches, 0.88% in roots and 1.29–2.93% in soil. The most common pathogens were species from *Chalara*, *Cytospora*, *Fusarium*, *Ilyonectria*, *Mycosphaerella*, *Setomelanomma* (Ascomycota) and *Armillaria*, *Rhizoctonia* (Basidiomycota) genera. The less frequent pathogens included species from *Leptosphaeria*, *Lophodermium* and *Septoria* genera. In conclusion, oxygen deficiency and the presence of poisonous gases in the air around the sewage plant is likely to have had damaging effects on plants infected or colonized by opportunistic and facultative parasites.

**Keywords:** branches, dieback, fungi, juniper, pathogens

### 1. Introduction

Rust, caused by *Gymnosporangium* spp., is the most common disease of juniper. Juniper shoot dieback can also be caused by *Diaporthe juniperivora* (G.G. Hahn) Rossman & Udayanga (syn. *Phomopsis juniperivora* G.G. Hahn), *Kabatina juniperi* R. Schneid. & Arx, *Pseudocercospora juniperi* (Ellis & Everh.) B. Sutton & Hodges (syn. *Cercospora sequoiae* var. *juniperi* Ellis & Everh) and *Sydowia polyspora* (Bref. & Tavel) E. Müll. (syn. of *Sclerophoma pithyophila* (Corda) Höhn) (Schneider, Arx 1966; Hoffman, and Fliege 1967; Pero, Howard 1969; Bre-ner et al. 1974; Ostrofsky and Peterson 1977; Peterson 1977; Anonymous 2018). Disease symptoms of juniper may also be caused by the genus *Phytophthora* (Green et al. 2012, 2015) and *Botryosphaeria stevensii* Shoemaker (Flynn 1992). The

symptoms of shoot dieback are often similar. *D. juniperivora* and *K. juniperi* cause the dieback of the top parts, usually single shoots in the crowns. *D. juniperivora* symptoms appear in April and May, August and September, and *K. juniperi* symptoms appear earlier, in February and March. Infected and dead needles drop over time. The shoots die. Younger plants (under 5 years old) can also die. *P. juniperi* kills the needles; it first attacks the older, internally placed parts of the shoots and lower branches. From here, it spreads upwards and outwards. The tip of the shoot often remains green and free of infection. Infected needles drop, leading to the formation of gaps, especially inside the crown. *S. polyspora* often attacks the shoots weakened or damaged by frost.

Protection against *D. juniperivora* consists of preventive spraying, performed as soon as the young shoots appear until

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they are completely lignified. Most often, two to three treatments are performed in March and April and one to two treatments in autumn (<https://pnwhandbooks.org/node/2946>). Protection against *K. juniperi* also involves cutting and destroying infected shoot fragments and protecting plants from damage caused by insects and mechanical injuries ([https://wiki.bugwood.org/HPIPM:Kabatina\\_juniperi](https://wiki.bugwood.org/HPIPM:Kabatina_juniperi)).

The *Juniperus x media*, aged 3–10 years on the property surrounding the Left Bank Sewage Treatment Facility in Poznań on Serbska Street, showed symptoms of dying needles and shoots. The symptoms occurred on 100 trees, which accounted for 50% of the junipers growing in this area. Needles in the crowns initially yellowed, and then became brown, died and fell. The symptoms occurred first at the base of the shoots and spread to the tops. Neither mycelium nor fruiting bodies of fungi or insect damage were found on the shoots. Discolouration was found with symptoms of moisture on the cross section of the base of the main stem (Figs 1, 2).

The aim of the study was to investigate the causes of the shoot dieback of *Juniperus x media* bushes aged 3–10 years on the property surrounding the Left Bank Sewage Treatment Facility in Poznań.

## 2. Materials and methods

### 2.1. Study material

Dead shoots (1, 2 and 3), roots and soil 1 and 2 were taken from under three *Juniperus x media* bushes with symptoms of dying needles and shoots, growing in the area surrounding the sludge collection tanks in the Left Bank Sewage Treatment Facility in Poznań on Serbska Street. Soil 2 came from a plot treated earlier (in 2017) with Besamid 97 GR.

### 2.2. Molecular analysis

In the environmental laboratory, DNA from sawdust from the shoots or roots and from the soil was isolated using commercially available kits: DNeasy PowerSoil Kit (Qiagen) and Plant Genomic DNA Purification (ThermoScientific). The internal transcribed spacer (ITS) 1/2 rDNA region with fungi-specific primers was then amplified: ITS1 F12 – 5¢ GAA CCW GCG GAR GGA TCA 3¢ and 5.8S – 5¢ CGC TGC GTT CTT CAT CG 3¢ (Schmidt et al. 2013). The reaction mixture consisted of 2.5 µl of DNA, 0.2 µl of each primer, 10.6 µl of deionised water and 12.5 µl of 2 × PCR Mix (A & A Biotechnology). The amplification reaction was performed in a thermocycler. It included initial denaturation (94°C 5 min), 35 cycles, denaturation (94°C 30 s), annealing (56°C 30 s), elongation (72°C 30 s) and final elongation (72°C 7 min). The amplification effect was checked on 1% agarose gel stained with Midori Green



**Figure 1.** Dying juniper needles and branches



**Figure 2.** Discoloration on the cross section of the main stem of juniper

Advance DNA (Genetics). The obtained product was purified and sequenced with SBS Illumina technology at Genomed in Warsaw. The obtained sequences were converted to Fasta format and merged into a single file using PEAR, version 0.9.6, filtered with quality threshold 30 by the FASTX-Toolkit, version 0.0.13 ([http://hannonlab.cshl.edu/fastx\\_toolkit/index.html](http://hannonlab.cshl.edu/fastx_toolkit/index.html)). The sequences were dereplicated and the ITS subregions were selected using the ITSx program, version 1.0.11. Single sequences and <100 bp sequences were deleted. The others, with a similarity of >97%, were grouped. Chimeras were removed using the UNITE UCHIME Reference Data Set, version 6.0 (<https://unite.ut.ee/index.php>). Input sequences were mapped to representative sequences and an Operational Taxonomic Unit (OTU) table was created. The sequences were identified by

comparing them to reference sequences in the UNITE database (<https://unite.ut.ee/>). The frequency of taxa in the assemblage was determined on the basis of the percentage share of a given taxon in the assemblage.

### 2.3. Description of the study area

The study site, that is, the Left Bank Sewage Treatment Facility in Poznań on Serbska Street, is a mechanical–biological treatment facility with enhanced biogenics removal and full processing of the generated sewage sludge. It takes in 50,000 m<sup>3</sup> (50 million litres) of sewage per day.

## 3. Results

The sequencing of environmental rDNA extracted from the dying shoots, roots and soil from under *Juniperus x media* showed the presence of 777 taxa of fungi belonging to Ascomycota (433 taxa), Basidiomycota (257), Chytridiomycota (15), Glomeromycota (16) and Zygomycota (56). The presence of plants (Plantae) and protozoa (Cercozoa, Protista) was also found. There were 685 taxa in the shoots (Ascomycota – 386, Basidiomycota – 232, Chytridiomycota – 3, Glomeromycota – 12 and Zygomycota – 52), 135 in the roots (Ascomycota – 78, Basidiomycota – 43, Chytridiomycota – 2, Zygomycota – 12) and 196 in the soil (Ascomycota – 123, Basidiomycota – 57, Chytridiomycota – 1, Zygomycota – 15). Plant pathogens were found in the fungal communities, including opportunistic and facultative species, epi- and endophytes, mycorrhizal fungi, saprotrophs colonising wood and the organic material in soil, pathogens of humans, animals, insects and nematodes, mycoparasites, yeast-like fungi, species antagonistic to other fungi, species with medicinal properties and components of lichens (Table 1).

The share of potential pathogens in the fungal assemblage was 2.64%–33.11% in the shoots, 0.88% in the roots and 1.29%–2.93% in the soil. The most numerous of the pathogens were species of the genera *Chalara*, *Cytospora*, *Fusarium*, *Ilyonectria*, *Mycosphaerella*, *Pestalotiopsis*, *Setomelanomma* (Ascomycota) as well as *Armillaria*, *Rhizoctonia* and *Thelephora* (Basidiomycota). Less numerous pathogens were from the genera *Cadophora*, *Infundichalara* (syn. *Chalara*), *Leptosphaeria*, *Lophodermium*, *Septoria* and *Sydowia*.

## 4. Discussion

*Juniperus x media* is a cross between *Juniperus chinensis* and *Juniperus sabina*. It is a durable, lushly growing, evergreen variety with a compact and bushy appearance that tolerates cutting and forming well. It is tolerant in terms of the soil in which it grows, resistant to drought, frost and urban pollution, and recommended for parks, naturalistic and home gardens. These

features led to the planting of *Juniperus x media* on the grounds of the sewage treatment facility in Poznań. It grows there on light, sandy and dry soil, in groups as a ground cover plant.

Junipers are susceptible to fungal diseases. The symptoms occurring in the examined junipers were most similar to those caused by infestations of *P. juniperi*. Needles, and single shoots, often in the lower part of the crown, were observed to be dying, and the dieback commenced from the inner part of the shoots and progressed towards the tops, in the centre and perimeter of the trees. However, the mycological analysis performed with the sequencing method using extracted rDNA from the diseased shoots, roots and soil did not show the presence of known juniper pathogens. However, numerous communities of other fungi were found. We assume that many of them may have been part of the biological aerosol suspended over the treatment facility, consisting of microorganisms and their toxins, falling on the juniper branches. Michalak and Pawlas' study (2012) showed that the range of the biological aerosol can impact an area of up to 3 km beyond its origin and the number of fungi in the air reaches 13,000 colony-forming units/m<sup>3</sup>.

Several taxa potentially pathogenic to plants were found. Together, under favourable environmental conditions, they may have caused the observed dieback of the junipers' needles and shoots. Some of them appeared more frequently, for example, the *Chalara* sp. on single shoots appeared with higher frequency. In this study, it was included among the potential pathogens after the experience of the last ash dying epiphytotic caused by *Chalara fraxinea* (Kowalski 2006). The confirmed *Chalara* sp. may be an anamorph of several other plant pathogens, among others *Ceratocystis*, which causes stem dieback in tree crowns (Hunt 1956). *Cytospora* spp. occurred in the roots of the juniper. *Cytospora*, which is a *Valsa* anamorph, is the cause of shoot dieback in deciduous and coniferous trees (Mańka and Mańka 1993; Hudelson 2012;). It usually attacks the lower branches of coniferous trees, from where it spreads towards the top. Infections of the lateral shoots lead to the formation of cancer, whereas infections of the main shoot kill the trees. *Fusarium oxysporum*, *Ilyonectria* spp. and *Pestalotiopsis* spp. are plant pathogens. They attack tree shoots and roots, leading to their dieback (Mańka and Mańka 1993). The first two taxa are common. *Pestalotiopsis verruculosa* that was found was previously isolated from rhododendron (Maharachchikumbura et al. 2012). Another species of the genus *Pestalotiopsis*, that is, *Pestalotiopsis funerea* (Desm.) Steyaert, may cause necrosis of the top shoots of juniper (Orlikowski et al. 2014). The genus *Mycosphaerella* includes over 600 species; it includes numerous plant parasites that cause large economic losses. *Mycosphaerella tumulosa* confirmed by the authors has been isolated thus far from eucalyptus leaves (Carnegie et al. 2007). *Setomelanomma holmii* has thus far been found to cause spruce needles to die (Rossman et al. 2002; Wu et al. 2014; Farr 2019). Among the Basidiomycota, *Armillaria gallica* is a dangerous

**Table 1.** Frequency of the potential pathogens in communities of fungi on *Juniperus x media*

Taxon	Branch 1	Branch 2	Branch 3	Roots	Soil 1	Soil 2
<i>Cadophora orchidicola</i> (Sigler & Currah) M.J. Day & Currah.	0.021	0.15	0.249	0	0.015	0
<i>Cadophora spadicea</i> Travadon. D.P. Lawr. Roon.-Lath. Gubler. W.F. Wilcox. Rolsh. & K. Baumgartner	0	0	0	0	0.229	0
<i>Chalara</i> sp.	5.062	0.148	0.005	0	0	0
<i>Colpoma quercinum</i> (Pers.) Wallr.	0.119	0.069	0	0	0	0
<i>Cytospora mougeotii</i> Lév. + <i>Cytospora</i> sp.	0.004	0	0	0	1.732	0
<i>Diplodia scrobiculata</i> J. de Wet. Slippers & M.J. Wingf.	0	0	0.034	0.049	0	0
<i>Fusarium oxysporum</i> Schlecht.	0	0.205	0.005	0.042	0	0
Heliotales	0.358	1.097	0.999	0.072	0.302	0
Herpotrichiellaceae	0.062	0.142	0	0.019	0.011	0
Hypocreales	0.004	1.713	0.426	0.110	0.072	0
<i>Ilyonectria mors-panacis</i> (A.A. Hildebr.) A. Cabral & Crous + <i>I. robusta</i> (A.A. Hildebr.) A. Cabral & Crous	0.054	0.229	0.705	0.017	0	0
<i>Infundichalara microchona</i> (W. Gams) Réblová & W. Gams	0.325	0.038	0.108	0.040	0	0
<i>Leptosphaeria</i> sp.	0.004	0.009	0.055	0	0.011	0
<i>Lophodermium pinastri</i> (Schrad.) Chevall.	0.025	0.006	0.003	0.232	0	1.299
<i>Mycosphaerella tumulosa</i> Carnegie & Beilharz + Mycosphaerellaceae	5.589	0	0	0	0	0
Mycosphaerellaceae	0.111	0.003	0	0	0.047	0
Nectriaceae	0.078	0.113	0.005	0.086	0.047	0
<i>Pestalotiopsis verruculosa</i> Maharachch. & K.D. Hyde	0.309	0.188	0	0	0	0
Pleosporales	1.099	0.052	0.017	0.133	0.074	0
<i>Septoria lepidii</i> Desm.	0.029	0.130	0	0	0	0
<i>Setomelanomma holmii</i> M. Morelet	3.214	0.289	0	0	0	0
<i>Sydowia polyspora</i> (Bref. & Tavel) E. Müll.	0.008	0	0	0.004	0.171	0
Venturiaceae	0	0.171	0	0.002	0.006	0
Venturiales	0.140	0.006	0	0	0	0
Xylariaceae	1.045	0.003	0	0	0	0
<b>Ascomycota</b>	<b>17.660</b>	<b>4.761</b>	<b>2.611</b>	<b>0.806</b>	<b>2.717</b>	<b>1.299</b>
<i>Aecidium</i> sp.	0	0.020	0	0.078	0.160	0
<i>Armillaria gallica</i> Marxm. & Romagn.	0.971	0.020	0	0	0	0
Ceratobasidiaceae	0.268	0.043	0.026	0	0.009	0
<i>Rhizoctonia</i> sp.	14.200	0.969	0.003	0	0	0
<i>Thelephora terrestris</i> Ehrh.+ Thelephoraceae	0.016	2.333	0	0	0.045	
<b>Basidiomycota</b>	<b>15.455</b>	<b>3.385</b>	<b>0.029</b>	<b>0.078</b>	<b>0.214</b>	<b>0</b>
<b>Ascomycota + Basidiomycota</b>	<b>33.115</b>	<b>8.146</b>	<b>2.640</b>	<b>0.884</b>	<b>2.931</b>	<b>1.299</b>

tree parasite that causes honey fungus rot, which manifests itself as gradual crown dying. *Rhizoctonia* spp. are soil fungi that cause serious diseases of many plants. They can damage the roots and the base of shoots. The authors' observations show that they can also attack the shoots in crowns, locally and very intensively, and juniper belongs to the group of plants most frequently attacked (Baysal-Gürel et al. 2018). Negative relationships also occur between juniper (especially, the young ones) and *Thelephora terrestris* (Belomesyatseva 2002). However, the family Thelephoraceae listed in Table 1 includes eight genera, with numerous saprotrophic species found on dead wood after shoot dieback.

Among the less numerous fungi, *Cadophora orchidicola* is considered an endophyte. However, other *Cadophora* species have the ability to decompose wood and are considered to be pathogens of herbaceous and woody plants (Travadon et al. 2014). *Infundichalara microchona* was present on the shoots and roots of juniper. Although the fungus is classified as an endophyte of coniferous species (Réblová et al. 2011), it can be assumed (author, not published) that in favourable conditions, it may be involved in plant infestations, similarly as *Leptosphaeria* sp., which may cause shoot dieback and loss of juniper needles (e.g. in the USA, not published). *Lophodermium pinastri* is a needle pathogen. Another species of *Lophodermium*, *Lophodermium juniperinum* (Fr.) De Not., may cause juniper needlecast (Hou et al. 2005). The observed *Septoria lepidia* is known as an herbaceous plant pathogen (Berner et al. 2015). This fungus had not been observed previously in Poland (Woźcańska 2013). Among 200 other *Septoria* species occurring in Poland, many cause spotting and necrosis of the herbaceous parts of plants. The infection of juniper by a fungal community similar to that found at the sewage treatment facility in Poznań was previously observed by Nadziakiewicz et al. (2018).

The classification of many fungi to taxa higher than the species or genus, for example, to a family or order – Ceratobasidiaceae, Helotiales, Hypocreales, Nectriaceae, Pleosporales, Thelephoraceae, Xylariaceae (Table 1) – does not facilitate the diagnosis of juniper shoot dieback. These taxa include large groups of similar individuals classified in phylogenetic taxonomy on the basis of common origin. It is very likely that they include obvious or unobvious and hitherto unknown juniper pathogens.

Despite the hermetic sealing of the Left Bank Sewage Treatment Facility on Serbska Street in Poznań, a problem with odour emissions from air purification installations has existed since 2010. Odours are generated by the natural biodegradation processes of biomass (plant and animal), for example, decomposition of proteins. The decomposition products are multicomponent mixtures of air pollutants (odorants and odourless compounds). These are mixtures of chemical compounds with different odour and degrading properties. Contaminated air contains less oxygen and large amounts of carbon dioxide,

nitrogen compounds (ammonia, amines, diamines), sulphur (hydrogen sulphide, thiols, mercaptans, sulfides, organosulfur compounds), oxygen (alcohols, aldehydes, ketones, esters, fatty acids), methane and hydrogen. The tested junipers were growing around the sediment storage tanks. They were exposed to the influence of aerosol and odours containing the compounds noted above. The lack of oxygen inhibits energy processes in the cells. Additionally, these gases are harmful to plants. The oxidation of ammonia to hydronium ions acidifies the environment and inhibits cell respiration (Vines and Wedding 1960). Hydrogen sulphide interferes with the release of energy in the cells and, like ammonia, inhibits cell respiration by blocking oxygen transport. Methane inhibits plant growth (Arif and Verstraete 1995). Other gases present in the air also have a harmful effect on the plants. Weakened plants are more susceptible to infection by the numerous opportunistic and facultative pathogens found in the genera *Chalara*, *Fusarium*, *Ilyonectria*, *Mycosphaerella*, *Pestalotiopsis*, *Setomelanomma* (Ascomycota) and *Armillaria*, *Rhizoctonia* (Basidiomycota) as well as other genera from the higher systematic units listed in Table 1.

## 5. Conclusions

No typical pathogens of juniper were found in the area of the assessed sewage treatment facility in Poznań. Nevertheless, opportunistic and facultative pathogens were found, which are, however, a threat to weakened plants. The primary cause was the exposure of plants to the harmful biological aerosol. The more frequently found fungi can be considered as indicators of poor tree vitality in industrialised regions.

## Conflicts of interest

The authors declare the lack of potential conflicts of interest.

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## Authors' contributions

J.B.-B. – methods, molecular analysis and elaboration, statistical elaboration; H.K. – literature review, manuscript preparation, corrections; W.Sz. – concept, technical assistance with the literature review; J.Z. – concept, photographs