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Mycobiota of thin roots showing decay of *Fraxinus excelsior* L. young trees

Abstract: The aims of the study were to identify the fungi associated with the decay of thin roots (1–5 mm) of 1, 3 and 15-year-old *Fraxinus excelsior* plants and to estimate the pathogenicity of certain fungi. In total, 400 fungal isolations were performed from which 334 cultures were obtained. Altogether, 26 species of fungi were identified. The following species were most frequently obtained, independently of plant age and origin: *Alternaria alternata, Chaetomium globosum, Cryptosporiopsis* sp., *Fusarium oxysporum, F. solani* and *Cylindrocarpon destructans*. The last three of these were then used in an inoculation test. *F. oxysporum* caused the death of over 80% of 2-month-old seedlings grown in boxes containing perlite and kept in a growth chamber.

Additional key words: symptoms, fungi, pathogenicity.

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Introduction

Significant stem death in European ash trees (1–15 years old) originating from natural and artificial regeneration has been observed in Poland in recent years. Characteristic symptoms visible on the stems are necrotic patches that have developed in the bark at various stem heights. Over 30 species of fungi have been identified from inocula taken from the necrosis occurring on the apical part of stems of plants collected from nurseries and forests (seedlings and natural seedlings). The most numerous are: *Phomopsis controversa, P. scobina, Cytospora ambiens, Discula* sp., *Fusarium lateritium* and *F. solani*. *Diplodia mutila* is also relatively common (Przybył 2001). Moreover, pathogenic *Nectria galligena* was isolated from a sunken area around the leaf scars, wounds and twig stubs of these plants (Przybył, unpublished data).

The aims of the research presented in this paper were firstly to identify the fungi associated with the decay of roots of 1-, 3- and 15-year-old *F. excelsior* plants and secondly to measure the pathogenicity of certain fungi to 2-month-old seedlings of European ash in growth chamber conditions.

Materials and methods

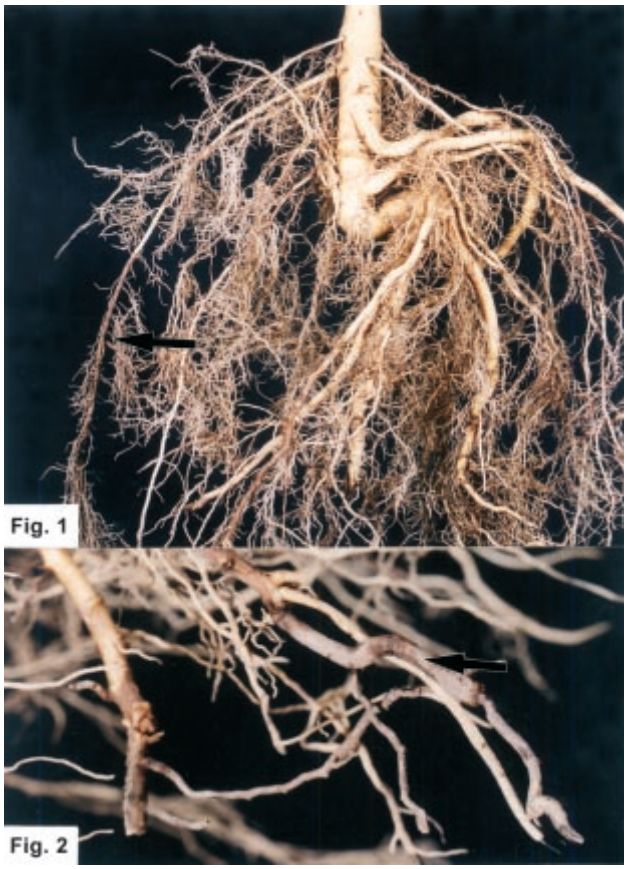
Characterization of the materials

The study examined the root systems of *Fraxinus excelsior* plants aged 1, 3 and 15 years taken from different parts of Poland. The characterization of these is described in Table 1. Large fragments of root systems exhibiting thin roots decay (1–5 mm) were taken from various parts of the root systems of over 20 plants from each site (Figs. 1 and 2). These were then transported inside ice boxes to a laboratory.

Isolation

The sampled roots showing decay (Figs. 1 and 2) were washed in running water and then cut into 5 mm sections and sterilized with 0.5% sublimate (2 min) and 70% alcohol (1 min). Sections (420 in total, 140 taken from each site) were then placed on Potato Dextrose Agar (PDA, Difco).

Special attention was paid to the occurrence of *Phytophthora* spp. In the case of this, the isolation procedure described by Jung et al. (1996) was used, together with Corn Meal Agar (CMA; corn flour 60 g,



Figs. 1 and 2. Roots showing decay (cross) of 3-year-old plants of *Fraxinus excelsior*

agar 17, distilled water 1000 ml) and V8 agar (vegetable juice 200 ml, $CaCO_3$ 3 g, agar 20 g, distilled water 1000 ml) media (Szkuta et al. 2001).

Isolation frequency was defined as the percentage of isolates of individual species in relation to the total number of isolates obtained from each site.

Pathogenicity tests

The following fungi (considered as putative pathogens) were used in the inoculation test: *Fusarium oxysporum*, *F. solani,* and *Cylindrocarpon destructans*. Three isolates for each species (one for each group of plants) were randomly chosen. Superficial wounds were made to the bases of hypocotyl of 2-month-old seedlings of *Fraxinus excelsior* (8 plants per isolate were used) growing in boxes containing perlite. Discs of 8-day-old cultures grown on MEA (Merck) at 22–24°C were cut from their margins and placed on the wound. Inocula were attached with a parafilm strip. During this experiment, seedlings were kept in a growth chamber at a temperature of 25°C in a 16/8h light/darkness photoperiod, with Photosynthetic Photon Flux Density (PPED) 52 mol. m^{-2} s^{-1}LF 40W, daylight, Polam 380–680 nm and Fluora Osram L36W/77 and fertilized using Hoagland's medium containing 2.5 mM $Ca(NO_3)_2$, 2.5 mM KNO_3, 2.0 mM $MgSO_4$, 1.0 mM KH_2PO_4, 5 ppm $FeSO_4$, 0.5 ppm $MnSO_4$, 0.05 ppm $ZnSO_4$, 0.02 ppm $CuSO_4$, 0.01 ppm H_3BO_3, 0.01 ppm H_2MoO_4 and sequestrene AA (EDTA) (Hoagland et Arnon 1950). Control plants were inoculated with sterile MEA (control 1) and were growing without inoculaton (control 2).

The re-isolation of test fungi was attempted after 4 weeks, using PDA. The sections were taken from roots near the stem base (the point of inoculation) and from the middle and upper parts of the stem of the dead plants.

Results

Isolation

From the 140 root sections (1–5 mm in diameter) taken from each group of plants (1, 3 and 15 years-old), 105, 125 and 104 isolates were obtained respectively (Table 2).

In total, the fungal community was represented by 26 species, although it was only possible to identify 10 species to the genus. The most fungal species were identified in the community obtained from 3-year-old plants (over 20). The fungal community of thin roots of 1-year-old and 15-year-old plants was composed of 13 and 17 species respectively, excluding certain unidentified species from the *Mortierella* and *Penicillium*

Table 1. Disease symptoms found on the investigated *Fraxinus excelsior* L. plants

Age of plant and place of collection	Disease symptoms
1-year-old, nursery, Jędrzejów Forest District, Regional Board State Forest in Radom	Decay of stem and thin roots (1–2 mm)
3-year-old, regeneration by planting, Kościan Forest District, Regional Board State Forest in Poznań	Necrotic apical part of main stem, occurrence of elongated necrotic lesions in bark originating at the bases of killed twigs, decay of thin roots (1–5 mm)
15-year-old, regeneration by planting, Jamy Forest District, Regional Board State Forest in Toruń	Necrotic apical part of main stem, death of twigs, occurrence of necrotic lesions in bark of branches and of main stem, the necrosis associated most often with wounds after the removal of twigs and branches respectively, decay of thin (1–5 mm) and medium-sized roots (c.a. 10 mm), cracks filled with Armillaria rhizomorphs on the surface of medium-sized roots

Table 2. Fungi occurring on roots (2–5 mm in diameter) showing decay of *Fraxinus excelsior* plants

Fungal species	Frequency (%) on plants		
	1-year-old	3-year-old	15-year-old
Acremonium sp.	–	1.6	–
Alternaria alternata (Fr.) Keissler	20.8	8.8	6.7
Botrytis cinerea Pers.	0.9	0.8	–
Chaetomium globosum Kunze: Stend.	2.9	20.0	–
Chalara sp.	0.9	–	–
Chloridium preussii W. Gams et Hol.-Tech.	0.9	–	–
Chloridium virescens (Pers.: Pers.) W. Gams	–	0.8	2.9
Cladosporium herbarum (Pers.) Link: Gray	–	1.6	–
Cladosporium macrocarpum Preuss	–	0.8	–
Cryptosporiopsis sp.	1.9	10.4	8.6
Cryptosporiopsis radicicola Kowalski et Barnik	–	1.6	3.8
Cylindrocarpon candidum (Link) Wollenw.	–	–	1.9
Cylindrocarpon destructans (Zins.) Scholten	4.8	7.2	26.9
Cylindrocarpon didymum (Hartig) Wollenw.	–	–	1.9
Fusarium sp.	2.6	–	1.9
Fusarium chlamydosporum Wollenw. et Reink.	–	0.8	–
Fusarium oxysporum Schlecht.	27.3	4.0	4.8
Fusarium sambucinum Fuckel	–	0.8	–
Fusarium solani (Mart.) Sacc.	21.9	1.6	3.8
Gliocladium virens Mill., Gidd. et Foster	–	0.8	1.9
Humicola sp.	–	0.8	–
Mortierella spp.	7.6	5.6	3.8
Mortierella isabelina Oudem.	–	0.8	–
Mortierella minutissima van Tiegh.	–	0.8	2.9
Mortierella ramanniana (Mõller) Linnem	–	0.8	–
Mortierella simplex van Tiegh.	–	6.4	–
Mortierella vinaceae Dixon – Stewart	2.8	1.6	–
Mycelium radicis atrovirens Melin	–	2.4	5.8
Penicillium spp.	1.9	5.6	2.9
Penicillium canescens Sopp	–	2.4	–
Phialophora sp.	–	0.8	1.9
Phoma sp.	–	–	4.8
Phomopsis scobina v. Höhn	–	2.4	1.9
Phytophthora sp.	0.9	–	–
Zygorrhynchus moelleri Vuill.	–	4.0	–
Trichoderma koningii Oudem.	–	1.6	5.8
Nonsporulating	1.9	3.2	2.9
Number of isolates (100%)	105	125	104
Number of inocula	140	140	140

genera. The most frequently occurring (over 10 isolates) were *Alternaria alternata* (1-year-old; 20.8%, 3-year-old; 8.8%, 15-year-old; 6.7%), *Chaetomium globosum* and *Cryptosporiopsis* sp. (3-year-old; 20.0% and 10.4%, respectively), *Fusarium oxysporum* and *F. solani* (1-year-old; 27.3 and 21.9%, respectively) and *Cylindrocarpon destructans* (15-year-old; 26.9%). The fungi with the lowest frequency (1–2 isolates) were: *Chloridium preussii, Cryptosporiopsis* sp., *Phytophthora* (1-year-old plants), *Botrytis cinerea* (1- and 3-year-old plants), *Ch. virescens, Cladosporium macrocarpum, Cryptosporiopsis radicicola, Fusarium chlamydosporum, F. sambucinum, F. solani, Humicola* sp., *Gliocladium virens, Mortierella isabelina, M. minutissima, M. ramanniana*, and *Phialophora* sp. (3-year-old-plants). The fungi most rarely isolated from roots of 15-year-old plants were: *Cylindrocarpon candidum, C. didymum, Fusarium* sp., *Gliocladium virens, Phialophora* sp. and *Phomopsis scobina.*

The following fungi were common on the roots of plants independently of their origin and age: *Alternaria alternata*, *Cryptosporiopsis* sp., *Cylindrocarpon destructans*, *Fusarium oxysporum* and *F. solani*.

Pathogenicity tests

For the three isolates of *Fusarium oxysporum*, the average spot lengths were 18.1, 18.9 and 19.4 mm 7 days after inoculation. A wilting of leaves connected with their yellowing and browning was observed after 2 and 3 weeks. 4 weeks after inoculation 83% of the seedlings had died (Figs. 3 and 4). In the case of *Cylindrocarpon destructans* and *Fusarium solani*, only a brown discoloration (ca 3–5 mm) around the inoculation point was observed. No symptoms were found on the control plants.

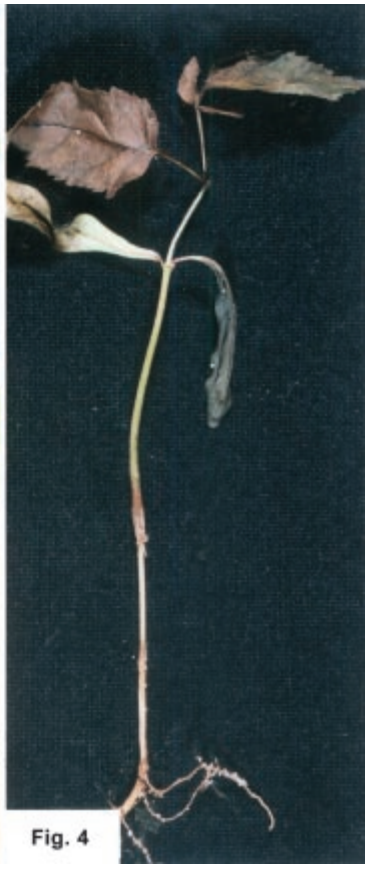
These fungi were consistently re-isolated; *F. oxysporum* from roots near the base stem and from the middle part of stem, *F. solani* and *C. destructans* from the brown spot at the point of inoculation.

Discussion

The following fungi were isolated from roots of European ash (1–5 mm in diameter) showing decay independently of their origin and age: *Alternaria alternata*, *Cryptosporiopsis* sp., *Cylindrocarpon destructans* (teleomorfa: *Nectria radicicola* Gerlach et Nilsson), *Fusarium solani* and *F. oxysporum*. These species are universally regarded as soil-borne fungi. The majority of soil-borne fungi (e.g. some *Fusarium* spp.) commonly invade necrotic tissue in soil but certain ones can be pathogenic.

Fusarium oxysporum has been identified as a pathogen in nurseries of both coniferous and broadleaved seedlings (Mańka 1997). Also, its pathogenicity to some species of *Quercus* was demonstrated by Gallego et al. (1999). In opinion of these authors, *F. oxysporum* caused similar disease symptoms to *Phytophthora cinnamomi* Rands on *Q. ilex* seedlings. According to the results of the present study, *Fusarium oxysporum* was able to kill off 2-month-old seedlings of *F. excelsior* in growth chamber conditions, whereas *C. destructans* and *F. solani* cause only brown discoloration at the point of inoculation. *F. oxysporum* can be responsible for the decay of roots and can be involved together with other factors (other pathogenic fungi, droughts, frost) in the dying process of European ash trees. It should be added that *F. oxysporum* together with *C. destructans* have also been isolated from seeds without disease symptoms collected from ash trees growing in some Forest District areas (Przybył, unpublished data).

Cylindrocarpon has a worldwide distribution. Host range varies between species within the genus; e.g. *C.*



Figs. 3 and 4. Symptoms on *F. excelsior* seedlings inoculated with *Fusarium oxysporum*; Fig. 3 – 2 weeks after inoculation, Fig. 4 – 4 weeks after inoculation

cylindroides occurs mainly on conifers, whereas *C. candidum* (isolated also from ash roots), *C. heteronema* and *C. willkommii* occur on broadleaved trees. In the case of *C. destructans,* both conifers and broadleaved trees can be inhibited (Perrin and Sampangi 1986, Ocamb and Juzwik 1995, Mańka 1997, Kowalski and Nowik 1998, Przybył 1999), however differences in pathogenicity between strains on certain hosts have also been reported (Dahm and Strzelczyk 1987, Singleton et al. 1993). The reactions of plants to *C. destructans* and *F. solani* suggest the occurrence of a hypersensitive reaction rather than disease symptoms.

The one isolate of fungus belonging to *Phytophthora* sp. was obtained only from the roots of 1-year-old plants. Due to its very low frequency of occurrence, its pathogenicity was not studied. Some *Phytophthora* spp. are aquatic saprophytes, but most species are pathogens of a great diversity of seed plants (Singleton et al. 1993, Szkuta et al. 2001). According to some authors, the destructiveness of *Phytophthora* spp. in forests depends on many other biotic and abiotic factors (Hansen and Delatour 1999, Vettraino et al. 2002). More studies should be done in Poland on *Phytophthora* spp. and their influence not only on the decline of seedlings growing in nurseries but more particularly on the decline of mature trees growing in various forest-type sites. Isolate morphologically similar to ash isolate of *Phytophthora* has been obtained in Poland from declining *Alnus glutinosa* trees (over 20 years old) in Celestynów Forest District (Przybył 2001, report sent to the Regional Board State Forest in Warsaw).

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