Fungal attacks to root systems and crowns of declining *Fraxinus excelsior*

Remigijus Bakys[1], Rimvis Vasiliauskas[2], Pia Barklund[2], Katarina Ihrmark[2] and Jan Stenlid[2]
[1]Deptartment of Plant Protection, Lithuanian University of Agriculture, LT-4324 Kaunas, Lithuania
[2]Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden
remigijus.bakys@centras.lt

Abstract

The aim of this study was twofold: 1) to investigate the extent of decay in roots and stems of declining ash; 2) to determine fungal species in damaged roots and shoots, and estimate their potential pathogenicity. In central Lithuania, 33 ash trees showing various degree of decline were felled and their root systems excavated. The positive correlation was detected between severity of the dieback and amount of decayed roots, length of decay within the stems and extent of decay over stump cross-section. A total of 150 isolations from root systems (3 samples from 50 root systems: at 0.5 m, 1 m and 1.5 m away from a stem) yielded 96 isolates representing 28 fungal species. Another 195 fungal isolates with 36 identified species were obtained from sound looking, damaged and heavily damaged shoots. *Armillaria cepistipes* was the fungus, most frequently isolated from root samples, whereas *Giberella avenacea*, *Alternaria alternata* and *Epicoccum nigrum* dominated among crown infecting species. Subsequently, 27 fungal species isolated from decayed roots and 18 species from shoots were tested for pathogenicity against 600 one year-old *Fraxinus excelsior* seedlings.

Introduction

The issue of declining European ash (*Fraxinus excelsior* L.) became important since mid-1990s, when this process was initially observed in Poland and Lithuania. Subsequently conducted studies did not reveal any correlation between tree mortality and geographic location of a stand, forest site type, age of a stand, species composition and edaphic factors (Juodvalkis & Vasiliauskas 2002, Przybyl 2002, Lygis *et al.* 2005). Characteristic symptoms of the disease are gradual crown decline due to necrotic patches on shoots and stems.

However, there were certain differences in pathological process of ash decline in different geographic areas. From Lithuania, for example, heavy root and butt rot of dying and dead trees was reported, cause of which was *Armillaria cepistipes* Velen. (Lygis *et al.* 2005). By contrast, in other countries damage to shoots and branches is thought to be of crucial importance for the decline, and no decay of stem bases and roots was observed (Przybyl 2002, Barklund 2005). In order to acquire more knowledge about pathological process in different parts of a tree, during the present study we investigated: 1) the extent of decay in roots and stems of declining ash and its correlation with the severity of the dieback; 2) fungi that invade roots and shoots of diseased trees and their relative pathogenicity.

Materials and methods

The methodology of this study consists of three basic parts: examination and fungal isolation from root systems and crowns, and pathogenicity tests with the isolated fungi.

Root systems were investigated in three 50–100 year-old *F. excelsior* stands located in south western part of Lithuania, Sakiai forestry district. The trees were of four health categories: 1) slight crown damage (dieback of up to 25 % of shoots); 2) moderate crown damage (up to 50 %); 3) severe damage (up to 75 %); 4) crown death (100 %). A total of 33 trees from all four categories were chosen for further investigation. They were situated at least 20 m from each other. The trees were cut down and the extent of decay in stump, stem base and roots (longitudinal and over cross-section) was estimated. For this, the root systems of cut trees were excavated about 40 cm deep at 1m radius from a stem base. Also, the percentage of decayed roots thicker than 2 cm was calculated. For fungal isolations, 150 wood pieces were taken from roots of 50 moderately damaged trees, – one root per tree, 3 wood samples per root (at 0.5 m, 1 m and 1.5 m distance from stem respectively).

Crowns of declining *F. excelsior* were examined in two sites in Sweden, one near Örebro (central Sweden), and another one near Visby (Gotland). The trees with crown dieback symptoms were cut and branch samples were taken. Depending on symptoms at the shoot base, all shoots were divided in three health categories: sound looking, with initial necroses at the shoot base and with advanced necroses. From the shoot bases, altogether 171 wood samples (58 from first, 58 from second and 55 from third health group, respectively) were taken for fungal isolations.

Pure cultures of fungi were isolated from about 4 x 0.5 cm wood pieces taken from roots, and 2 x 0.5 cm pieces of wood and bark taken from shoots. The pieces were cut out, sterilized in open fire and plated on Petri dishes containing Hagem agar. All samples were incubated at room temperature for two weeks. All obtained fungal pure cultures were grouped depending on mycelial morphology. The representatives of each groups, were selected for molecular identification by ITS sequencing (White *et al.* 1990), similarly as in our previous study (Vasiliauskas *et al.* 2005). Sequence results were checked against available databases – NCBI BLAST database (Altshul *et al.* 1997), and database of the Dept. of Forest Mycology and Pathology at the Swedish University of Agricultural Sciences.

A total of 27 fungal species, isolated from decayed roots and 18 species, isolated from shoots were tested for pathogenicity against 600 one year-old *F. excelsior* seedlings planted under bare root conditions. Pieces of wood 1×1×5 mm in size, autoclaved and pre-colonized with

respective strain, were used as an inocula. Sterile wood pieces were used as control. They were attached with a tape to a 1×5 mm size wound made respectively at the base or at the shoot of a tree. The results will be evaluated after two vegetation seasons.

Results and discussion

The amount of decayed roots varied from 10 to 30 % in trees with slight crown damage, from 20 to 70 % in trees with moderate crown damage, from 30 to 90 % in trees with severe crown damage, and from 80 to 100 % in dead trees. The corresponding values for length of decay in a butt of a stem were 0.1–0.4 m, 0.2–1.5 m, 0.4–1.6 m and 0.4–2.5 m. For extent of decay over stem cross-section the corresponding values for the health categories were 10–20 %, 5–60 %, 30–60 %, and 70–100 %. As a result, there were positive correlations between severity of the dieback and amount of decayed roots (rS = 0.86), length of decay in a butt of a stem (r_S = 0.57), and extent of decay over stump cross-section (r_S = 0.87).

The isolations from roots yielded 96 fungal strains representing 24 species. Mainly the same species of fungi were isolated from roots at different distances from the stem (0.5, 1 and 1.5 m), as in comparisons between the communities Sorensen indices of quantitative similarity (Magurran 1988) were high (S_N = 0.84–0.96). However, general species richness was relatively high and species accumulation curve was not asymptotic, indicating that increased sampling effort in obtained roots would reveal additional species of fungi.

The dominating basidiomycete was *Armillaria* spp. In addition, some other wood-decomposing basidiomycetes, as *Coprinus disseminatus* and *Pholiota carbonaria* were also present. Characteristic ascomycetes were *Nectria* spp., *Xylaria* sp. and *Scytalidium lignicola*. Although mating tests with the isolates of *Armillaria* spp. were not performed in the present study, we suspect species to be *A. cepistipes*, as this species was reported to invade stem bases of declining *F. excelsior* in other parts of Lithuania (Lygis *et al.* 2005). On the other hand, the cited study also demonstrated that the fungus is not the primary cause of *F. excelsior* decline, as its genotypes on examined sites was large and several decades old, when the decline there has been recorded only few years previously (Lygis *et al.* 2005). Moreover, *A. cepistipes* is known as weak opportunistic pathogen, invading trees under stress, weakened by some other factor (Entry *et al.* 1986). Moreover, during earlier extensive field observations sporocarps of the fungus on *Fraxinus* had not been observed (Sokolov 1964), indicating that this tree species is somehow unusual host.

The isolations from shoot bases yielded 195 fungal strains representing 36 species. Mainly the same species of fungi were isolated from crown samples collected at different localities (Örebrö and Visby), as in comparisons between the communities Sorensen indice of quantitative similarity (Magurran 1988) was high (S_N = 0.89). However, general species richness was relatively high and species accumulation curves from both localities were not asymptotic, indicating that increased sampling effort in crowns would reveal additional species of fungi.

Species most commonly isolated were asco- and deuteromycetes: *Alternaria alternata*, *Fusarium* spp., *Epiccocum nigrum*, *Lewia* sp., *Botryosphaeria stevensii*, *Phomopsis* sp., *Phoma glomerata Cladosporium* sp., *Cytospora* spp. and many others. Occasionally, in shoots we recorded the presence of wood decay basidiomycetes – *Coprinus* sp., *Pharenochaete* spp., and one unidentified basidiomycete. As in our work, many similar or related asco- and deuteromycetes were detected in crowns and stems of declining *F. excelsior* during the recent studies in Poland and Lithuania (Przybyl 2002; Lygis *et al.* 2005; Kowalski & Lukomska 2005). However, the question of which of those are primarily responsible for the dieback of crowns, to date remains largely unclear, and we look forward towards the evaluation of the pathogenicity tests.

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