Pathological evaluation of declining *Fraxinus excelsior* stands of northern Lithuania, with particular reference to population of *Armillaria cepistipes*

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Abstract

Stem bases of 210 *Fraxinus excelsior* trees of three different health categories were sampled by the means of an increment borer in declining ash stands of northern Lithuania. From this number, 15 sound-looking, 132 declining and 63 dead trees from three discrete plots yielded 352 isolates, representing 75 operative taxonomic units (OTU's). *Armillaria cepistipes* was the most common species (86 isolates from 210 wood samples, or 41.0 %), isolated more frequently and consistently than any other potential tree pathogen. It also showed abundant occurrence on a majority of trees in form of mycelial fans and rhizomorphs, from which 64 and 14 respective isolates of the fungus were obtained. Population structure of *A. cepistipes* revealed the presence of 53–93 genets per hectare, some of which extended up to 30–55 m. The present study led to a hypothesis that saprotrophic behaviour of weakly pathogenic *A. cepistipes* has been shifted to aggressive pathogenic by some predisposing factor (-s) (possibly – water stress) after at least 20–30 years of latent presence in the area.

Introduction

Starting in 1996, decline of European ash (*Fraxinus excelsior* L.) has been a permanent and widespread forest health problem in east-European countries (Juodvalkis & Vasiliauskas 2002, Przybyl 2002, Skuodiene *et al.* 2003). In Lithuania, for example, it affected over 30,000 ha of stands, comprising about 60 % of total ash area (Juodvalkis & Vasiliauskas 2002). The decline was especially destructive in the northern parts of the country.

Reasons for the decline remain largely unknown, although preliminary observations suggest that biotic factors and pathogenic fungi in particular, are the likely cause of the disease (Juodvalkis & Vasiliauskas 2002, Przybyl 2002). Judged by external symptoms on the trees, *Armillaria* root rot was among the most probable reasons of mortality in some parts of Lithuania (Juodvalkis & Vasiliauskas 2002). The main aim of the present study was therefore to identify wood-inhabiting fungi that attack stems of *F. excelsior* in declining stands, focusing the attention on populations of possible disease-causing agents.

Materials and methods

Study sites and fieldwork

The study was carried out during the summer 2001 in declining mixed-aged (20–60-year-old) *F. excelsior* stands located in Biržai Forest Enterprise, Buginiai forest district (northern Lithuania). Mapping, numbering, measurement, and sampling of trees was carried out in three discrete permanent sample plots (about 0.15 ha in size each), each consisting of 70 ash trees that represented three different categories of health condition: i) sound looking or healthy; ii) declining; and iii) dead [according to Innes (1990)]. Isolation of fungi was done from a total of 15 sound-looking, 132 declining, and 63 dead standing ash trees. For every tree, we estimated the diameter at breast height, crown density reduction [or defoliation, determined according to Innes (1990)], the presence of disease signs such as tarry spots or dead bark (scales), and the occurrence at the stem base of distinct basal lesions extending from diseased roots, mycelial fans (underneath the bark) and epiphytic rhizomorphs typical to *Armillaria* spp. [according to Morrison *et al.* (1991)].

Isolation and identification of fungi

The sampling of wood for the mycological investigations was performed as described by Lygis *et al.* (2004a). One wood sample per tree was taken by drilling at the root collar with an increment borer and extracting 4–5-cm-long bore cores. Isolation of pure cultures from the woody pieces was made on Petri dishes containing Hagem agar (Stenlid 1985). When available, pieces of *Armillaria* mycelial fans and rhizomorphs were collected; in a laboratory those were surface sterilized and placed on agar plates for isolation of pure cultures. Fungal operative taxonomic units (OTU's) were defined and identified to species or genus level on the basis of sequence similarities of the ribosomal ITS region (e.g. Lygis *et al.* 2004a, b, Vasiliauskas *et al.* 2004).

Intersterility and somatic incompatibility tests with Armillaria

The precise identification of *Armillaria* species was performed by mating tests on agar plates with representatives of known biological species. Those followed the procedures described by Guillaumin *et al.* (1991). Strains were assigned to species by pairing diploid mycelia with haploid tester strains of four European *Armillaria* species, *A. ostoyae*, *A. gallica*, *A. borealis* and *A. cepistipes* (Korhonen 1978). Somatic incompatibility tests were performed on agar plates to distinguish genetically distinct individuals (genets) of *Armillaria* at each plot (Shaw & Roth 1976). The results of the tests were projected on the constructed map (Fig. 2).

Results

Tree condition and infections by Armillaria

In our investigated stands, about 60 % of ash trees were declining, about 30 % were dead, and only about 10 % looked healthy and were classed as sound-looking. Based on occurrence of mycelial fans characteristic of *Armillaria* underneath the bark (Morrison *et al.* 1991), and the associated distinct typical basal lesions extending from diseased roots, we concluded that 205 out of 210 of the investigated trees (97.6 %) were colonized by *Armillaria* spp. From that number, colonization by the fungus was recorded on 80.0 % of sound-looking, 98.5 % of declining and 100 % of dead ash trees. Moreover, the presence of epiphytic *Armillaria*-like rhizomorphs was recorded at the root collar of every examined tree (above the bark), regardless of tree's condition. No canker or necrotic lesions typical to attacks by other ash pathogenic fungi e.g. *Nectria galligena* or *N. coccinea*, as in Sinclair *et al.* (1987) were observed on the lower part of the stems.

Fungal isolations

Of the 210 wood samples taken, 180 (85.7 %) resulted in fungal growth. A total of 352 isolates were collected and 318 of them (or 90.3 % of the total sample) were identified at least to genus level. They represented 75 distinct OTU's, 60 of which (or 80.0 %) were identified [for reference to the isolated fungal OTU's see Lygis *et al.* (2005)]. *Armillaria* was the most abundant fungus, isolated from 115 trees. Mating tests led to identification of all collected *Armillaria* isolates as *A. cepistipes* Velen. Other 19 OTU's of basidiomycetes were much less common (Lygis *et al.* 2005) and mostly represented widely spread saprotrophic wood decomposers.

Of the 51 isolated OTU's of ascomycetes, several were found quite frequently, although far less often than *A. cepistipes* (Lygis *et al.* 2005). The potential ash pathogens, *Phoma exigua* and *Botryosphaeria stevensii* (anamorph: *Diplodia mutila*) were isolated only in low frequencies irrespectively of tree condition. Other potential ash pathogens included two species of fungi often associated with seedling diseases, *Nectria haematococca* (syn. *Fusarium solani* (Mart.) Sacc.), and *N. radicicola* (syn. *Cylindrocarpon destructans* (Zinssm.) Scholten) (Booth 1971, Domsch & Gams 1972, Sinclair *et al.* 1987). Zygomycetes were isolated at low rates; they were represented only by 4 OTU's (Lygis *et al.* 2005).

Community structure and species richness

The community structure in sound-looking, declining and dead trees differed markedly (Lygis *et al.* 2005). Consequently, Sorensen similarity coefficients (S_s, qualitative) (Krebs 1999) were rather low (Fig. 1). The highest number of OTU's was found in declining (56), followed by dead (36) and sound-looking trees (16). However, this was mainly due to a lower sampling effort in dead and sound-looking trees (Lygis *et al.* 2005). Species accumulation curves (Colwell & Coddington 1994), presented in Figure 1, show that should sampling effort been equal in all three health categories, the differences in species richness between them would be minor. The data indicates also that our sampling efforts did not exhaust the existing diversity of wood-inhabiting fungi.

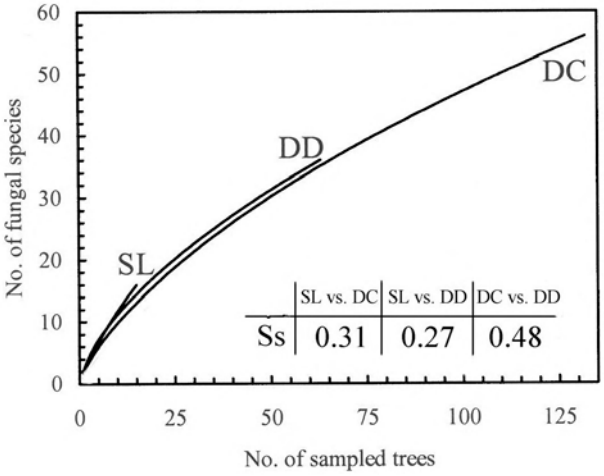

Fig. 1. Increase in species (OTU's) richness in sound-looking (SL), declining (DC), and dead (DD) Fraxinus excelsior *trees as a result of sampling more trees. Species accumulation curves were calculated according to Colwell & Coddington (1994). Qualitative Sorensen similarity coefficients (S_s) are shown between the community structures in SL and DC, between SL and DD, and between DC and DD.*

Population structure of Armillaria cepistipes

Of the 150 isolates of *A. cepistipes*, we identified 8, 13, and 8 genets in three investigated sites respectively (situation on two sites, A and B, is presented in Figure 2), corresponding to 53, 93, and 53 genets per hectare. In all three sites, 11 genets (or 37.9 % of all genets) included only one tree. Genet VII from site A was also found in site B: a forest road built about 20 years ago had seemingly split one large individual into two spatially separated ramets (Fig. 2). Sizes of the genets varied from a single root system to 55 m wide (genet II on the site A, Fig. 2).

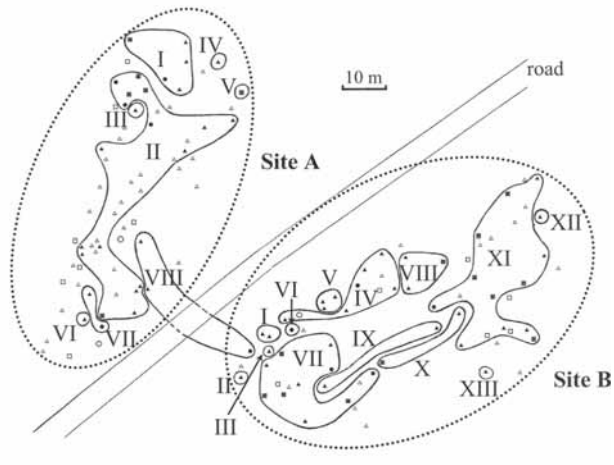

Fig. 2. Distribution of Armillaria cepistipes *genets in two infested sites (A and B) of* Fraxinus excelsior *in northern Lithuania. The small symbols, circles, squares, and triangles, label sound-looking, declining, and dead* F. excelsior *trees, respectively. Black symbols indicate the trees from which* A. cepistipes *has been isolated, while the open ones the trees from which* A. cepistipes *has not been isolated. Limits of genets are encircled by the solid line.*

Discussion

In this study, *A. cepistipes* was found to be the dominant fungus in all tree health categories, as it was most commonly observed and isolated from stem bases of sound-looking, declining and dead trees. This is to a certain extent surprising, since *Fraxinus* seems to be an uncommon host to *Armillaria* (e.g. Sokolov 1964). Moreover, *A. cepistipes* is generally considered to be a weak pathogen, only capable of slow infection of roots of healthy trees (Rishbeth 1982, Guillaumin *et al.* 1985, 1989, Gregory *et al.* 1991, Prospero *et al.* 2004). In our study sites, active decay caused by *A. cepistipes* was consistently recorded on 80.0 % of sound-looking, 98.5 % of declining and 100 % of dead trees, thus the fungus undoubtedly contributed to and accelerated the decline of investigated stands.

On the other hand, it is unlikely that the attacks by *A. cepistipes* were the primary cause of the decline. It is generally accepted that *Armillaria* spp. are opportunistic pathogens able to invade hosts weakened by certain stress factors (Wargo 1977, Singh 1983, Entry *et al.* 1986). However *A. cepistipes* is known to produce abundant rhizomorph networks on the roots of living trees, this characteristic giving it a competitive advantage in a pathogenic colonisation should the tree become stressed or in saprobic colonisation once the host dies (Rishbeth 1985, Redfern & Filip 1991). Increased frequency of dry years and lowered level of a ground water are among the abiotic stress factors that could be involved in ash decline in our geographic area (Juodvalkis & Vasiliauskas 2002, Skuodiene *et al.* 2003), while fungal infection to crowns might be an important biotic factor.

The revealed extensive territorial clonality of *A. cepistipes* (62.1 % of all genets detected colonized more than one host tree) indicates that the fungus was present on the diseased sites for many years before the decline started. According to mycelial growth rates for *Armillaria* in north-temperate forests (Shaw & Roth 1976, Rishbeth 1988, 1991, Smith *et al.* 1992, Legrand *et al.* 1996), the age of the largest *A. cepistipes* genets on our study sites were estimated to be at least 20 years. The forest road that split genet VIII between the sites A and B was also built 20 years ago (Fig. 2). We hypothesize that latent saprotrophic behaviour of *A. cepistipes* has been shifted to the pathogenic by some predisposing factor (-s) after 20–30 years of its presence in the stands, this leading to decline of *F. excelsior*.

Other basidiomycetes isolated during the present work are commonly fruiting on dead wood in northern European forests and are generally considered to have a saprophytic behavior (Lygis *et al.* 2005). It was surprising to find *Bjerkandera adusta* (isolated from intact wood) and *Trametes hirsuta* (isolated from a fresh necrosis) in sound-looking stems of ash, and their possible impact on ash decline cannot be excluded. Even less is known about the role played by the now isolated numerous microfungi (Lygis *et al.* 2005) in the pathological process.

An interesting finding of the present work was also the detection of principally different fungal communities in trees of different health condition growing within the same forest stand (Lygis *et al.* 2005). Although equal sampling effort provided us with rather similar number of OTU's in sound-looking, declining and dead trees (Fig. 1), the shift in the fungal community structure was considerable (Lygis *et al.* 2005), showing that stems of sound-looking, declining and dead ash are inhabited predominantly by different species of fungi. As in our previous study (Lygis *et al.* 2004b), we hypothesize that fungal species in wood of living trees likely change along with changes in tree condition.

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