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Root fungal colonisation in *Deschampsia flexuosa*: Effects of pollution and neighbouring trees

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Severe pollution decreased root colonisation by some fungal groups; neighbouring trees decreased root colonisation by dark septate endophytic fungi in highly polluted sites.

Abstract

In industrial barrens adjacent to a nickel-copper smelter at Monchegorsk, the Kola Peninsula, root colonisation in *Deschampsia flexuosa* by arbuscular mycorrhizal (AM)-type of hyphae was lower than in unpolluted forests (60.9 vs. 80.4%), while *Olpidium*-colonisation showed a marginally significant decline, and dark septate endophytic (DSE) hyphal colonisation was not affected. We detected an interactive effect of pollution and a neighbouring tree on DSE hyphal colonisation: at the highly polluted sites, colonisation was lower in *D. flexuosa* growing near trees, whereas at sites with low pollution the presence of the neighbouring tree had no effect on colonisation. High numbers of intracellular DSE sclerotia in the industrial barrens (13.3 vs. 3.4%) may indicate a survial strategy in an unfavourable environment and a dispersal strategy into a more favourable environment. While lower root colonisation by AM fungi has been also earlier reported in graminoids for heavy metal contamination, the results on other ubiquitous fungi colonising *D. flexuosa* roots are more novel.

Keywords: Mycorrhizal colonisation; Dark septate endophytic fungi; Sclerotia; Heavy metals; Kola Peninsula

1. Introduction

In mycorrhizal symbiosis carbon photosynthesized by the host plant and nutrients absorbed by the root-associated fungi are exchanged (Smith and Read, 1997). Different mycorrhizal types are defined by the symbionts forming the association and the morphology of the root structures. In general, ectomycorrhiza is a common association e.g. in boreal and subarctic trees and arbuscular mycorrhiza (AM) in herbaceous plants (Smith and Read, 1997). In addition to mycorrhizal fungi, roots are colonised by numerous other fungi. For example, dark septate endophytes (DSE) are ubiquitous root-associated fungi, possibly symbiotic (Haselwandter and Read, 1982; Jumpponen, 2001), and especially common in stressful environments (Mandyam and Jumpponen, 2005).

Mycorrhizal symbiosis is believed to contribute to the survival of host plants in stressful conditions, including heavy metal contaminated habitats (Van Duin et al., 1991; Wilkinson and Dickinson, 1995). Although a relatively large number of studies have been carried out on mycorrhizal colonisation in the impact zones of large, point pollution sources (e.g. industrial complexes), they are highly biased due to preferential investigation of ectomycorrhizal fungi. We found only eight studies on arbuscular mycorrhiza (AM) and none on dark septate endophytic (DSE) fungi while conducting a meta-analysis

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concerning the impact of point polluters on soil micromycetes in natural conditions, compared with 27 studies on ectomycorrhiza (Ruotsalainen and Kozlov, in press).

Perennial grasses, in particular *Deschampsia flexuosa* (L.) Trin and *D. cespitosa* (L.) Beauv., are relatively tolerant to both heavy metal contamination (Høiland and Øftedal, 1980; Cox and Hutchinson, 1981) and an increased nitrogen input (Aerts and Berendse, 1988; Strengbom et al., 2003). Moreover, although the pH optimum of *D. flexuosa* lies between 5.5–6, this species also grows on more acidic soils (Hackett, 1965). It is thus not surprising that *D. flexuosa* is abundant at polluted sites around the Monchegorsk nickel-copper smelter (Isachenko and Filipova, 1975; Vacek et al., 1999). In this area, diversity of microfungi (Lebedeva, 1993) decreases in forest soils on moving towards the pollution source. Deterioration of the forest soil, leading to losses in soil organic matter and nutrients, is assumedly connected with the adverse effects of heavy metals on soil fungal populations (Polyanskaya et al., 2001). Effects of environmental contamination on AM and other fungi colonising the roots of *D. flexuosa* are almost entirely unknown, except for the contradictory reports on the impact of simulated acid rain on AM colonisation (Dighton, 1988; Malcova et al., 1998). Therefore the first goal of our study was to compare root colonisation of *D. flexuosa* by both AM and DSE fungi in industrial barrens (secondary open habitats that have replaced forests near major pollution sources) and in pristine (unpolluted) forests.

Since *D. flexuosa* benefits from experimentally reduced competition for light (Strengbom et al., 2004), the increased coverage of this species in severely polluted sites may be explained by the higher light availability resulting from forest decline. On the other hand, the field layer vegetation in industrial barrens (mostly ericaceous dwarf shrubs) often benefits from the proximity of trees, because trees mitigate some of the adverse impacts of several abiotic factors (Zvereva and Kozlov, 2004). Therefore the second goal of our study was to compare root colonisation of *D. flexuosa* by both AM and DSE fungi in open and in sheltered microsites.

2. Materials and methods

2.1. Study area and study sites

The study was conducted in a lowland forest zone on the Kola Peninsula (NW Russia), north of the Arctic Circle. Four study sites (Table 1) were located within 41 km of the Severonikel nickel-copper smelter complex at Monchegorsk (67° 55′ N, 32° 48′ E). This smelter is one of the largest European sources of aerial pollution, consisting predominantly of sulphur dioxide and heavy metals (Pearce, 1994), and the only major polluter in the central part of the Kola Peninsula. In 1990 it emitted 2.33×10^8 kg of sulphur dioxide, 2.71×10^6 kg of nickel and 1.81×10^6 kg of copper. Since then, however, the emissions have steadily declined to levels of 0.44×10^8 kg of sulphur dioxide, 1.21×10^6 kg of nickel and 0.83×10^6 kg of copper in 2001 (Barcan, 2002).

Although the smelter was built in 1937, large-scale smelting did not begin until 1946–1947, and forest damage became visible around the smelter already in the early 1950s (Kozlov and Barcan, 2000). The vast quantities of sulphur and heavy metals emitted during the last 60 years have caused widespread destruction of the soil and vegetation (Rigina and Kozlov, 1999). The total area influenced by aerial pollution recently exceeded 10,000 km^2, with industrial barrens covering tens of square kilometres (Kryuchkov, 1993).

The unpolluted (pristine) sites were selected in forests dominated by either Norway spruce (*Picea abies* (L.) Karst., 33.6 km S of the smelter) or Scots pine (*Pinus sylvestris* L., 40.9 km SSW of the smelter). Two heavily polluted sites represent industrial barrens that have developed from pine-dominated forest (4.7 km NW of the smelter) and spruce-dominated forest (8 km S of the smelter). Only a few conifers still remain in these sites, which are now covered by sparsely growing mountain birch, *Betula pubescens* subsp. *czerepanovii* (Orlova) Hämet-Ahti, goat willows (*Salix caprea* L.), patches of ericaceous dwarf shrubs (*Vaccinium* spp., *Empetrum nigrum* subsp. *hermaphroditum* (Hagerup) Böcher) and grasses (*Deschampsia* spp., *Festuca* spp.) scattered over the bare ground. For a detailed description of the pollution gradient see Rigina and Kozlov (1999).

2.2. Sampling and processing

AM and DSE colonisation in the roots of four subarctic meadow herbs was earlier reported to be relatively constant throughout the growing season or decrease towards the end of the season (Ruotsalainen et al., 2002). We therefore sampled *D. flexuosa* only once, in the beginning of June, 2003, when tuft leaves of the grass had developed but no flowering shoots had emerged. The sampled plants were thus in comparable phenological stages.

At each of four study sites (two unpolluted and two heavily polluted) we have selected five sampling plots (some 20–50 m apart) in such a way, that each plot included *D. flexuosa* growing: (a) in an open microsite (no trees within 5 m distance); (b) within 1.5 m from a mountain birch (height 1.5–5 m); and (c) within 1.5 m from a goat willow (height 2–3 m). One plant was collected from each of the three microsites. The roots were cleaned of soil, and a root sample consisting of young and healthy-looking roots was preserved in 50% ethanol. The aboveground plant parts were dried (+60 °C, 48 h). Current year leaves were separated from previous year shoots and homogenized in a ball mill (Retsch MM 301, Retsch GmbH&Co., Haan, Germany). Foliar carbon and total nitrogen were determined in 36 samples by the dynamic flash combustion technique (EA 1110 Elemental Analyzers, CE Instruments, Milan, Italy), shoot material in the remaining samples being too low to allow reliable C and N analyses.

Table 1
General information on the study sites (mean ± S.E.)

Stress level	Distance from polluter, km	Location			Stand basal area, m^2/ha	Field layer vegetation cover, %	Foliar metal concentrations, µg g^{-1}	
		Latitude, N	Longitude, E	Altitude, m a.s.l.			Ni	Cu
H	8.0	67°50′54″	32°48′08″	225	<0.3	3.9 ± 1.78	172.7 ± 23.2	43.9 ± 7.4
L	40.9	67°34′38″	32°32′54″	140	5.0 ± 1.2	29.1 ± 3.6	16.7 ± 1.0	6.4 ± 0.4
H	4.7	67°58′01″	32°49′57″	135	<0.3	11.3 ± 4.2	145.5 ± 21.0	79.0 ± 0.6
L	33.6	67°38′20″	32°45′00″	160	1.7 ± 0.7	32.1 ± 6.6	24.1 ± 0.4	10.3 ± 0.6

Environmental data collected in 2004. Stress levels: H = high, L = low. Vegetation cover: 10 plots of 1 × 1 m size. Concentrations of Ni and Cu: measured in leaves of three mountain birches at each site; indicative of pollutant deposition on leaf surface (consult Kozlov et al., 2000 for explanation). Stand basal area: relascope, 3 replicates.