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Air Pollution and Fungal Endophytes in Needles of Austrian Pine

By

MAJA JURC[1)], DUŠAN JURC[1)], NADA GOGALA[2)] & PRIMOŽ SIMONČIČ[1)]

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Summary

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Species composition of endophytic fungi in healthy needles of Austrian pine (*Pinus nigra* Arn.) was investigated at eight locations in Slovenia. Results from October 1994 and January 1995 isolations were compared with analyses of macronutrients, sulphur and lead content of the needles. About 80 species of microfungi were revealed. The pollution load of all measured elements was low and the environmental factors are very diverse. At the site with the highest amount of lead in needles the frequency of isolated fungi was the lowest. Ecological factors have the most pronounced effects on species composition and on frequency of colonisation.

Introduction

Austrian pine is restricted in Slovenia to small, scattered natural stands but it is planted and sown on more than 20 000 ha as allochtone tree species on Karst. Its vitality and health status differ from place to place and depends mostly on the quality of the growing site.Three years research of endophytic fungi in the needles of Austrian pine was conducted on eight locations. As collecting sites of needles are situated in areas with different air pollution loads we supposed that the analyses of certain pollutants in needles could provide an insight into the reasons for the great diversity in fungal populations in needles from different locations.

There are indications that endophytic species composition and the frequency of colonisation are significantly dependent from the vitality of the host and from the degree of air pollution being negatively correlated with the degree of air pollution. Simulated acid rain treatment reduced the number of isolated

[1)] Slovenian Forestry Institute, Večna pot 2, P.O.Box 523-X, 1001 Ljubljana, SLO

[2)] Biology Department of Biotechnical Faculty, University of Ljubljana, 1000 Ljubljana, SLO

endophytes in birch leaves by approximately 25% (HELANDER & al. 1993). SIEBER 1989 reported that air pollutants were suspected of being the possible cause of changes in endophyte populations of Norway spruce and white fir.

Material and Methods

One to eight years old, healthy looking needles were collected in October 1994 and January 1995 from the lower branches of tree crowns at eight locations which differ in many ecological parameters: Kobjeglava (1Ay, dry, understory tree), Vipava (2Ao, near main road, edge tree), Veliko Trebeljevo (3Ao, shallow soil, solitary tree), Benko (4Ao, shallow soil, solitary tree), Krnica (5Ao, sunny, windy), Smolnik (1No, 1100 m a.s.l., windy), Dolina Krvavice (2Ny, wet, shadow), Konjska dolina (6Ay and 6Ao, shallow soil) (abbreviations: A- artificial stand, N-natural stand, y-young tree, o-old tree). Fungal endophytes were isolated from the base, middle and the tip segments of the needles using the methods described by JURC & JURC 1995. Altogether 2016 segments of needles were analysed. Macronutrients (N, P, K, Ca, Mg), total sulphur and Pb content in needles were determined by routine laboratory methodology.

Results

Species composition and isolation frequency of fungal endophytes from all locations in October 1994 and January 1995 were (most frequent are underlined): *Acremonium kiliense* Grütz 7x (isolation frequency: 7x), *Alternaria alternata* (Fr.) Keissler (5x), *Anthostomella formosa* Kirschst. (3x), *Apospheria* sp.1 (12x), *Apospheria* sp.2 (17x), *Aureobasidium pullulans* (deBary)Arn. *var. melanigenum* Hermanides-Nijhof (2x), Basidiomycotina: (27x), <u>*Cenangium ferruginosum* Fr.</u> (106x), *Ceratocystis fimbriata* Ellis&Halst (2x), *Cladosporium cladosporioides* (Fresen.)deVries (22x), *Cladosporium tenuissimum* Cooke (2x), *Coniosporium olivaceum* Link ex Fr. (2x), *Coniothyrium* sp.1 (3x), *Coniothyrium* sp.2 (9x), <u>*Cyclaneusma minus* (Butin)DiCosmo, Peredo & Minter</u> (32x), <u>*Cyclaneusma niveum* (Pers.:Fr.)DiCosmo, Peredo&Minter</u> (193x), <u>*Cyclaneusma* sp.</u> (16x), *Cylindrocladium scoparium* Morg. (3x), *Cystodendron* sp. (2x), *Exophiala* sp.1 (3x), *Exophiala* sp.2 (4x), *Geotrichum* sp. (10x), *Haplosporella* sp. (8x), *Hendersonula pini* (Fr.)Dyko & Sutton (9x), <u>*Hormonema dematioides* Lagerberg & Melin</u> (32x), *Humicola grisea* Traaen (9x), *Hypoxylon serpens* (Pers. ex Fr.) Kickx (6x), *Hypoxylon* sp. (8x), *Kabatina* sp. (4x), *Lichenoconium boreale* (Karst.)Petrak & Syd. (1x), <u>*Lophodermium conigenum* (Brunaud) Hilitz</u> (50x), *Monilia cinerea* (7x), *Nectria* sp. (*Aquifolii* grup) (2x), *Penicillium* sp.2 (9x), *Pestalotiopsis funerea* (Desm.)Steyaert (5x), *Phacidium lacearum* Fr. (2x), <u>*Phialophora hoffmannii* (Beyma)Schol-Schwarz</u> (67x), *Phialophora bubakii* (Laxa) Schol-Schwarz (4x), *Phialophora hoffmannii* grup (18x), *Phoma eupyrena* Sacc. (2x), *Phoma* sp.1 (15x), *Phoma* sp.2 (3x), <u>*Phomopsis* sp.1</u> (36x), *Phomopsis* state (*Diaporthe* cf. *eres* group) (6x), *Ramichloridium pini* de Hoog & Rahman (2x), *Rhizosphaera kalkoffii* Bub. (22x), *Trimmatostroma betulinum* (Corda) Hughes (2x), *Tryblidiopycnis pinastri* Höhn. (3x), *Xylaria* sp. (5x). sterile mycelium (102x).

The most frequently isolated fungi were: *C. niveum* (9,6%), *C. ferruginosum* (5.3%), *P. hoffmannii* (3.3%), *L. conigenum* (2.5%), *Phomopsis* sp. (1.8%), *C. minus* (1.6%) and *H. dematioides* (1.6%). These fungi can be regarded as typical endophytes for Austrian pine because their presence was consistent at all collecting sites and over the whole period of investigation. Other fungi were isolated rarely, irregularly or only at certain locations.

Frequency of isolations of fungal endophytes was different in different sampling locations (Tab. 1)

Tab. 1. Frequency of isolations of fungal endophytes

Sampling locations	1Ay	2Ao	3Ao	4Ao	5Ao	1No	2Ny	6Ay	6Ao	Total
Segments isolated	192	144	240	264	240	264	192	192	288	2016
Total infections	95	37	118	102	195	90	101	76	127	941
% of infections	49.5	25.7	49.2	38.6	81.2	34.1	52.6	39.6	44.1	46.7

Analyses of macronutrients and sulphur showed only slight changes in composition (Tab. 2)

Tab. 2. Content of macronutrients, sulphur and lead in current year needles (1994)

sampling location	N (mg/g)	P (mg/g)	K (mg/g)	Ca (mg/g)	Mg (g/kg)	S (mg/g)	Pb (µg/g)	N/P	N/S
Vipava - 2Ao	13.9	1.40	5.09	3.67	1.36	1.16	6.1	10.6	12.0
Vel. Trebeljevo - 3Ao	12.2	1.18	3.04	7.17	2.49	1.23		10.3	9.8
Benko - 4Ao	14.8	1.52	4.26	3.60	1.80	1.18		9.7	12.7
Krnica - 5Ao	11.4	1.25	4.82	2.72	1.48	1.07		9.1	10.3
Smolenc - 1No	14.2	1.83	5.14	2.60	1.27	0.96	3.6	7.7	14.6
Dolina Krvavice - 2Ny	11.7	1.02	3.89	2.06	1.93	1.23		11.4	9.5
Konjska dol. - 6Ay	10.1	1.32	3.15	1.72	1.33	1.01		7.6	9.9
Konjska dol. - 6Ao	12.1	1.54	5.04	2.06	1.93	0.96		7.9	12.5

Contents of nitrogen in needles were not sufficient specially at locations 6Ay, 5Ao and 2Ny (young tree). The content of phosphorus was not sufficient at locations 3Ao and 5Ao where it was slightly <1.3 mg/g dw below which a tree is considered to be deficient in P. Values of N/P and N/S ratios are in the optimum range. Potassium contents were low in two samples (3Ao and 6Ay), while needle samples from all other locations were satisfactoraly supplied with K (about 5.0 mg/g dw). Contents of calcium were in the optimum class if the classification of HUETTL 1992 is used, but were rather high in the sample from 3Ao and low in the samples from 6Ay, 6Ao; 1No and 5Ao if the modified classification of Gussone was used (GUSSONE 1964). Sulfur contents in needles were in the lowest class (class 1) after KALAN & al. 1990 and these values were not considered to be possible causes of damage. The highest amount of sulfur was found in samples from 3Ao which lies in pollution zone of Ljubljana and Trbovlje. Lead content was rather low in both measured samples.

Discussion

The lowest frequency of colonisation (25.7%) was detected in needles from the location 2Ao where lead content is the highest. But it attains only class 2 (of five) after KNABE 1984 where the possibility of lead damage is expected (analyses were made on one year's needles of Norway spruce). More analyses are needed to evaluate the possible impact of lead on endophyte populations in needles of Austrian pine. Species composition in needles of different sampling locations were not recognizably grouped, due to the pollution load of all the measured elements because the concentrations were relatively low and the environmental factors very diverse. Ecological differencies of two natural growing sites of Austrian pine were very pronounced (1No being windy and high above sea level, 2Ny being wet and shady) and it seems that these differences have the greatest influences on frequency of colonisation and species composition of fungal endophytes. SAESTAD & JENSSEN 1993 suggested that the difficulties of measuring the effect of air pollution on forest mycota can be partly overcome by considering the effects of atmospheric pollution on fungal communities instead of the effects on single species. This approach was used in the presented work, but the knowledge of endophytic populations and the factors affecting their composition are scarce. In such an unexplored field of investigations as fungal endophytes are, it is probably also very difficult (as with macrofungi) to deduce the mechanisms causing changes in fungal biota without testing the mechanisms by experimental manipulation.

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