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ENDOPHYTIC FUNGI IN NEEDLES OF *PINUS NIGRA* GROWING UNDER DIFFERENT SITE CONDITIONS

TADEUSZ KOWALSKI & PAWEŁ ZYCH

Abstract: Endophytic fungi in symptomless needles of *Pinus nigra* Arn. were studied in three stands differing in intensity of pollution by industrial emissions. A total 565 colonies of fungi belonging to 40 taxa were isolated from 1728 needle segments. The following fungi occurred most frequently: *Anthostomella formosa* Kirschst., *A. pedemontana* Ferr. & Sacc., *Cenangium ferruginosum* Fr.: Fr., *Cladosporium cladosporioides* (Fresen.) N. F. de Vries, *Cyclaneusma minus* (Butin) DiCosmo, Peredo & Minter, *Lophodermium pinastri* (Schrad. & J. M. Hook) Chev., *L. seditiosum* Minter, Staley & Millar, *Sclerophoma pythiophila* (Corda) Höhn. and *Verticicladium trifidum* Preuss. The frequency of latent infection depended on needle age, needle portion and position in the tree crown. Local site conditions affected the infection frequency as well. There was a distinct reduction in the qualitative and quantitative composition of the endophytic community in a zone heavily polluted by industrial emissions. Endophytes of *Pinus nigra* are compared with a community of fungi infecting symptomless needles of *Pinus sylvestris* L. growing in the vicinity.

Key words: Endophytes, *Pinus nigra*, needles, industrial emissions

Tadeusz Kowalski & Paweł Zych, Department of Forest Pathology, Academy of Agriculture, Al. 29 Listopada 46, PL-31-425, Kraków, Poland

INTRODUCTION

Studies conducted during the last twenty years have shown that latent infection of plants by microorganisms, especially by fungi, is very common, and it may involve all organs of coniferous and broadleaf trees, and also gramineous plants (Carroll *et al*. 1977; Petrini 1986; Carroll 1988; Kowalski & Sadłowski 1993). Such microorganisms are called endophytes (Petrini 1986).

The role of endophytes has not been fully explained. It is thought that they may be beneficial or neutral to plants, becoming harmful under certain conditions (Carroll 1988). Some endophytes are especially harmful in the case of plant weakness or injury caused by biotic or abiotic factors. This may be true of *Lophodermium piceae* (Fuckel) Höhn. in needles of *Picea abies* (L.) H. Karst., and of *Cyclaneusma minus* (Butin) DiCosmo, Peredo & Minter in needles of *Pinus sylvestris* L. (Butin 1986; Kowalski 1993). Some fungi species of the genera *Pezicula* Tul. & C. Tul. and *Phomopsis* (Sacc.) Bubák are harmful in such a way in broadleaf and coniferous trees growing in industrial regions (Kowalski 1999).

In Poland the endophytes of *Pinus sylvestris* needles are well studied in respect to their species composition and in terms of changes occurring under different environmental conditions (Kowalski 1993, 1998; Kowalski & Pożdzik 1993; Kowalski & Stańczykiewicz 2000). The endophytes of *Pinus nigra* Arn. have not been studied in Poland so far. In other countries they have been investigated only locally and on a small scale, for example in France and Slovenia (Carroll *et al*. 1977; Jurc & Jurc 1995; Jurc *et al*. 1996).

This paper describes the results of studies on fungi inhabiting living needles of *P. nigra* growing under different site conditions in southern Poland. The proportion of *P. nigra* in forest stands in this region is quite considerable, especially in converted stands impacted by industrial emissions (Latocha 1976). Under such conditions this pine species is attacked by serious fungal pathogens (Kowalski 1987; Kowalski *et al*. 1998). This study of endophytes, apart from identifying species, should also help answer whether living needles of *P. nigra* can be subjected to symptomless infection by potential fungal pathogens.

MATERIALS AND METHODS

Study material was collected in September and October 1998 in three pure stands of *Pinus nigra* growing under different conditions of pollution from industrial emissions, and threatened by fungal diseases to different degrees (Table 1). Two branches without disease symptoms were cut from each of 12 trees at site A and 6 trees at site B and 6 trees at site C, growing in the centers of the stands. One branch originated from the upper crown (third whorl from the top), and the other from the lower crown (second living whorl from the bottom). In the laboratory, 3 needles without macroscopic disease symptoms were taken from each first-order shoot formed in 1998 and 1997. In this way 144 1-year-old and 144 2-year-old needles were obtained from 48 branches. Surface disinfection was done by immersing the needles in 96% ethyl alcohol for 1 min and then in 4% NaOCl for 3 min, followed by rinsing in 96% ethyl alcohol for 0.5 min. After this treatment the needles were dried on sterile filter paper. Each needle was cut into 6 segments which were placed in Petri dishes on 2% malt agar medium. In total, isolations from 1728 needle segments were made. Incubation took place in the dark at room temperature. The colonies of fungi growing out of the needles were identified. The frequencies of occurrence of individual species of fungi were expressed as percents of colonized needles. For the most frequent species this frequency was computed for different crown portions, needle ages, and needle segments.

RESULTS

A total 565 colonies of fungi from 40 taxa were isolated from symptomless needles of *Pinus nigra*. Only 3 species formed ascospores *in vitro* (*Anthostomella formosa, A. pedemontana, Pezicula eucrita*). Three species were basidiomycetes and the remaining fungi were anamorphs of Ascomycota or fungi of Deuteromycota (Table 2). The *P. nigra* needles were most frequently colonized by *Cenangium ferruginosum* (54.9% of needles), *Anthostomella formosa* (25.3%), *Lophodermium pinastri* (5.9%), *Anthostomella pedemontana* (3.8%), *Cladosporium cladosporioides* (2.8%), *Sclerophoma pythiophila* (2.8%), *Lophodermium seditiosum* (2.1%) and *Verticicladium trifidum* (1.4%). The remaining fungi colonized no more than 1% of the needles (Table 2). Besides fungi, bacteria were isolated from 9.7% of the needles (Table 2).

The quantitative participation and species diversity of mycobiota in the needles depended on the place of occurrence of *P. nigra*. There were 23 species of fungi found from site A, 24 species from site B, and 7 species from site C (Table 2). *Anthostomella formosa* was almost seven times more frequent from site A than from site B, and over three times more frequent than from site C.

Table 1. Characteristics of sampled *Pinus nigra* stands.

Stand	Stand location Forest District Forest Range compartment	Zone of pollution with industrial emissions, location	Forest site type	Stand age (years)	Occurrence of pathogenic fungi
Site A	Miechów Goszcza, 71	I – low emission concentration (25 km north east from the Sendzimir Steelworks in Kraków)	uppland broadleaf forest	16	*Cenangium ferruginosum* *Dothistroma septospora*
Site B	Świerklaniec Repecko, 58	II – medium emission concentration (12 km west from Zink and Lead Smelting Works in Miasteczko Śląskie)	fresh coniferous forest	24	*Cenangium ferruginosum* *Gremmeniella abietina* *Crumenulopsis sororia*
Site C	Świerklaniec Imielów, 203	III – high emission concentration (1.2 km east from Zink and Lead Smelting Works in Miasteczko Śląskie)	fresh coniferous forest	26	*Cenangium ferruginosum* *Gremmeniella abietina*

Table 2. Number and frequency (%) of living symptomless needles of *Pinus nigra* colonized by fungi.

Fungi	Site A		Site B		Site C		Total	
	number	(%)	number	(%)	number	(%)	number	(%)
Anthostomella formosa Kirschst.	59	(41.0)	5	(6.9)	9	(12.5)	73	(25.3)
Anthostomella pedemontana Ferr. & Sacc.	5	(3.5)	6	(8.3)			11	(3.8)
Aspergillus sp.			1	(1.4)			1	(0.3)
Aureobasidium pullulans (de Bary) G. Arnaud			1	(1.4)			1	(0.3)
Basidiomycetes sp. 1	1	(0.7)					1	(0.3)
Basidiomycetes sp. 2	1	(0.7)					1	(0.3)
Cenangium ferruginosum Fr.: Fr.	90	(62.5)	39	(54.2)	29	(40.3)	158	(54.9)
Cladosporium cladosporioides (Fresen.) N. F. de Vries	2	(1.4)	4	(5.6)	2	(2.8)	8	(2.8)
Cladosporium herbarum Pers.: Fr.	1	(0.7)	1	(1.4)			2	(0.7)
Crumenulopsis pinicola (Rebent.) J. W. Groves			1	(1.4)			1	(0.3)
Cyclaneusma minus (Butin) DiCosmo, Peredo & Minter			6	(8.3)			6	(2.1)
Dothistroma septospora (Dorog.) M. Morelet	3	(2.1)					3	(1.0)
Epicoccum nigrum Link	2	(1.4)					2	(0.7)
Geniculosporium cf. *serpens* Chesters & Greenh.	2	(1.4)					2	(0.7)
Hypoxylon sp.	1	(0.7)					1	(0.3)
Lecytophora hoffmannii (van Beyma) W. Gams & McGinnis	1	(0.7)					1	(0.3)
Lecytophora sp.	1	(0.7)					1	(0.3)
Lophodermium pinastri (Schrad. & J. M. Hook) Chev.	11	(7.6)	6	(8.3)			17	(5.9)
Lophodermium seditiosum Minter, Staley & Millar	5	(3.5)			1	(1.4)	6	(2.1)
Melanconium sp.			1	(1.4)			1	(0.3)
Mollisia cinerea (Batsch *ex* Merat) P. Karst.			1	(1.4)			1	(0.3)
Nodulisporium sp.	1	(0.7)					1	(0.3)
Pezicula eucrita (P. Karst.) P. Karst.			2	(2.8)			2	(0.7)
Phialophora sp. 1			2	(2.8)			2	(0.7)
Phialophora sp. 2	1	(0.7)					1	0.3)
Phialophora fastigiata (Lagerb. & Melin) Conant			1	(1.4)			1	(0.3)
Rhizoctonia sp.			1	(1.4)			1	(0.3)
Sclerophoma pythiophila (Corda) Höhn.	4	(2.8)	3	(4.2)	1	(1.4)	8	(2.8)
Scoleconectria cucurbitula (Tode: Fr.) Booth			1	1.4)			1	(0.3)
Sirodothis sp.			1	(1.4)	1	(1.4)	2	(0.7)
Sphaeropsis sapinea (Fr.: Fr.) Dyko & B. Sutton	1	(0.7)					1	(0.3)
Sporormiella intermedia (Fr.) Rehm	1	(0.7)					1	(0.3)
Verticicladium trifidum Preuss	1	(0.7)	3	(4.2)			4	(1.4)
Xylaria hypoxylon (L.) Grev.	1	(0.7)					1	(0.3)
Non-sporulating fungi (6 species)	2	(1.4)	3	(4.2)	2	(2.8)	7	(2.4)
Bacteria	21	(14.6)	6	(8.3)	1	(1.4)	28	(9.7)
Number of tested needles	144		72		72		288	
Number of tested needle segments	864		432		432		1728	
Percentage of segments colonized by microorganisms	43.8		30.8		15.0		33.3	

Table 3. Frequency of colonization of living needles of *P. nigra* depending on their age and position in the tree crown.

Fungi	Index of distribution of fungi in needles*	Colonized needles (%)		Colonized needles (%)	
		lower crown	upper crown	1-year-old	2-year-old
Anthostomella formosa	0.28	27.8	22.9	27.8	22.9
Anthostomella pedemontana	0.18	2.8	4.9	4.2	3.5
Cenangium ferruginosum	0.34	66.0	43.8	35.4	74.3
Cladosporium cladosporioides	0.17	2.1	3.5	4.2	1.4
Cyclaneusma minus	0.17	4.2	0.0	1.4	2.8
Lophodermium pinastri	0.18	9.7	2.1	3.5	8.3
Lophodermium seditiosum	0.22	4.2	0.0	2.8	1.4
Sclerophoma pythiophila	0.19	3.5	2.1	3.5	2.1
Bacteria	0.21	9.7	9.7	8.3	11.1
Percentage of colonized needle segments		42.1	24.6	25.9	40.7

* index = number of segments from which a given taxon was isolated/number of needles from which a given taxon was isolated

Cenangium ferruginosum and *Lophodermium seditiosum* occurred more frequently from site A than from the other sites, and *Anthostomella formosa*, *Cladosporium cladosporioides*, *Lophodermium pinastri*, *Sclerophoma pythiophila*, and *Verticicladium trifidum* were more frequent from site B. No species was more frequent from site C than from the other two sites. Some species occurred only locally; for example, *Cyclaneusma minus* occurred only from site B, and *Dothistroma septospora* only from site A (Table 2).

Individual species of fungi were characterized by different degrees of distribution in the needles. The needles were most intensively colonized by *Anthostomella formosa* and *Cenangium ferruginosum*; *Cladosporium cladosporioides* and *Cyclaneusma minus* had the most limited distribution (Table 3). In general, microorganisms were most abundant in the apical portions of the needles, and least abundant at the needle base (Fig. 1). The needle colonization frequency depended on the needle's position in the tree crown. Most of the fungal species were more frequent in the lower part of the crown. In the case of *Lophodermium pinastri*, needles from the lower crown were over four times more frequently colonized than needles from the upper crown. *Cyclaneusma minus* and *Lophodermium seditiosum* were not isolated from the needles of the upper crown at all. On the other hand, such fungi as *Anthostomella pedemontana* and *Cladosporium cladosporioides* were isolated slightly more frequently from needles of the upper crown.

All the more frequent species of fungi occurred in 1- and 2-year-old needles (Table 3). There were differences in frequencies of occurrence depending on the age of the needles. Some species such

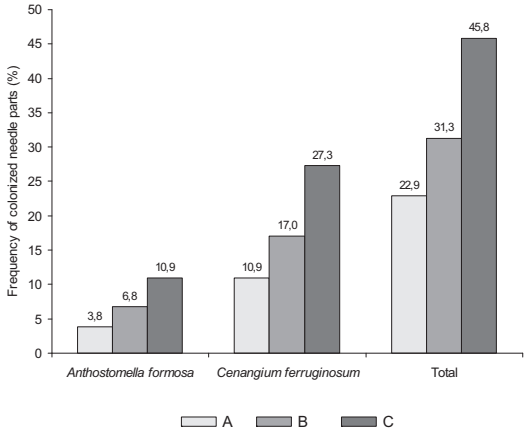

Fig. 1. Frequency of colonization of different parts of *Pinus nigra* needles by two most frequent species of fungi (A – base, B – central part, C – top).

as *Anthostomella formosa* tended to colonize young needles more frequently. Species such as *Cenangium ferruginosum* and *Lophodermium pinastri* were isolated more than twice as frequently from 2-year-old needles than from 1-year-old needles.

DISCUSSION

This study demonstrated considerable species diversity of fungi potentially able to produce infection of living needles of *Pinus nigra*. The range of diversity was similar to the species spectrum found in southern Poland in needles of *Abies alba* Mill. and *Picea abies*, which numbered 34 and 44 species, respectively (Kowalski & Ryś 1996), and slightly poorer than the spectrum found in *Pinus sylvestris* needles, numbering 50–68 species (Kowalski & Poździk 1993; Kowalski & Stańczykiewicz 2000). In spite of the high species diversity, only a few species infected the *P. nigra* needles very frequently. This is a common finding for most communities of endophytes isolated from various plant organs (Petrini 1986; Sieber *et al*. 1999). Most often the isolated fungi represent groups with a varied host range. *Cladosporium cladosporioides* is a cosmopolitan fungus, *Sclerophoma pythiophila* and *Anthostomella formosa* are characteristic of coniferous trees and shrubs, and the occurrence of *Cenangium ferruginosum*, *Cyclaneusma minus* and *Lophodermium* spp. is generally limited to *Pinus* spp. In terms of species composition, the endophytes in *P. nigra* needles are not significantly different from the endophyte assemblages in *P. sylvestris* needles. All the more frequent species found in *P. nigra* are also the most frequent in *P. sylvestris*. Species of fungi such as *Anthostomella formosa*, *Cyclaneusma minus*, *Lophodermium seditiosum*, *L. pinastri* and *Sclerophoma pythiophila* were twice as frequent in *P. sylvestris* as in *P. nigra* (Kowalski & Poździk 1993; Kowalski & Stańczykiewicz 2000).

In this study the percentage of individual fungi in the species composition depended on the position of the needles in the tree crown. This should be explained mainly by the distance from the sites of substratum storage where these fungi live as saprophytes and produce ample fruitbodies. Such species as *Cyclaneusma minus*, *Lophodermium pinastri*, *L. seditiosum* produce them mainly on needles in the litter, so there is a greater probability of infection in the lower than in the upper crown. *Cenangium ferruginosum* and *Sclerophoma pythiophila* also produce ample fruitbodies on dead branches in the tree crown. In the case of shoot diseases their inoculum is abundant in the upper crown, while in healthy stands it is only present in the lower crown on branches dying due to lack of light.

The percentages of fungi depended on needle age. Many species of fungi more easily infect old needles; this is connected with erosion of wax substances (Sieber-Canavesi & Sieber 1987). This is not the rule, however. For example, *A. formosa* infected living 1-year-old needles more frequently than 2-year-old ones; the same was also observed in *P. sylvestris* (Kowalski & Stańczykiewicz 2000). In different needle portions there may be different conditions favorable or unfavorable to needle infection. In *P. nigra* the apical needle sections were the most susceptible to symptomless infection (Fig. 1). The situation was similar in *Pinus mugo* Turra (Sieber *et al*. 1999) and *Picea abies* (Kowalski & Ryś 1996), while the reverse was observed in *Abies alba* (Kowalski & Ryś 1996). In *Pinus sylvestris* some species of fungi more frequently infected needle bases (*Cenangium ferruginosum*, *Sclerophoma pythiophila*), and some needle apices (*Anthostomella formosa*, *Cyclaneusma minus*).

Industrial pollution can affect endophytic assemblages in *P. nigra* needles. The needle infection frequency decreased as air pollution increased. Moreover, in a zone of heavy pollution (site C) the species composition of endophyte assemblages was very little diversified. The situation observed here was similar to those observed in spruce and birch (Barklund & Rowe 1983; Solheim & Aamlid 1992; Helander *et al*. 1993), and also in some species of fungi (*C. minus*, *L. seditiosum*) infecting needles of *P. sylvestris* in industrial regions (Kowalski & Stańczykiewicz 2000). Industrial emissions can directly affect the development of fungi, and also indirectly through changes

in the physiological processes of trees (Huttunen *et al*. 1983; Zobel & Nighswander 1991).

Site A differed from the remaining two sites in having an ample reservoir of inoculum of *Dothistroma septospora*, a dangerous pathogenic fungus causing red band disease (Kowalski & Jankowiak 1998; Kowalski *et al*. 1998). This species was isolated from symptomless needles only sporadically. This means that it produces visible disease symptoms very quickly after infection, and such needles were not collected during this study. No needles were infected by fungi such as *Crumenulopsis sororia* (P. Karst.) J. W. Groves and *Gremmeniella abietina* (Lag.) M. Morelet, which are pathogens of shoots and stems in some sites (Table 1). The situation was different in the case of *Cenangium ferruginosum*. It produced fructifications on dead shoots of *P. nigra* at all investigated sites, and it was the species most frequently isolated from living symptomless needles. The situation was identical in *P. sylvestris* (Kowalski & Poździk 1993; Kowalski 1998; Kowalski & Stańczykiewicz 2000) and other pine species (Sieber *et al*. 1999; Hata & Futai 1996).

The species of fungi most frequently isolated from symptomless needles of *P. nigra* in southern Poland were no different from the most frequent species in populations obtained from *P. nigra* needles in Slovenia (Jurc & Jurc 1995, Jurc *et al*. 1996). This means that *P. nigra* needles are subject to latent infection by a specific community of fungi, and individual species differ mainly in infection frequency, depending on local conditions.

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