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FUNGI OCCURRING ON COLORADO BLUE SPRUCE (*Picea pungens* Engelm.) IN THE CRACOW BOTANIC GARDEN

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Abstract. The aim of the study was to determine the causes of dying down of sprout and needles of Colorado blue spruce (*Picea pungens* Engelm.) and its cultivar 'Glauca' in the park collection of the Botanic Garden of Jagiellonian University in Cracow. For several years, the disease symptoms described in the article have led to the loss of decorative values of these trees. The study was conducted in the years 2002 and 2003 by means of the control of plant healthfulness, phytopathological diagnostics, isolation of microorganisms from tissues exhibiting diseases symptoms, as well as the culture and identification of the isolated fungi. Both, the control of tree healthfulness, and specialized laboratory techniques proved that the symptoms diagnosed in plants had an infectious character, and that the fungi might be the main causing agents. The analysis of the fungal populations isolated from the affected tissues indicates the combined occurrence of fungi in tissues of the studied plants and the domination of the following species: *Alternaria consortiale, Aureobasidium pullulans, Penicillium canescens, Phoma pomorum, Ulocladium consortiale, Zythiostroma pinastri.*

Key words: *Picea pungens*, disease symptoms, fungi

INTRODUCTION

Spruce belongs to the most frequently planted trees in Poland. At the beginning it was our Polish common spruce (*Picea abies* (L.) Karst.) but with time there appeared exotic species of spruce that were brought from other countries and continents and that were new in our climate. One of those was Colorado blue spruce (*P. pungens*), frequently called silver spruce. The most frequently cultivated cultivar is 'Glauca' [Chojnowska 2000, Seneta 1987]. Colorado blue spruce acquired popularity thanks to its appearance and resistance to low temperatures, dry air and soil as well as owing to its high tolerance to air pollution [Bojarczuk 2001, Chojnowska 2000, Seneta 1987]. Be-

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sides, spruce provides raw material for the production of medicines and cosmetics [Bonnenberg 1988, Polakowska 1987, Větvička 1990]. Nevertheless, it is not free from the invasion of pathogenic factors either in growth stands or in nurseries [Cartar and Abrahams 2000; Crane and Hiratsuka 2000; Crane et al. 2000; Halmschlanger et al. 2000a, 2000b; Igoe et al. 1995; Lehtijarvi and Barklund 2000; Łabanowski et al. 2001; Mańka and Mańka 1993; Oberhuber et al. 1999].

The purpose of the studies was to determine the cause of the occurrence of disease symptoms on Colorado blue spruce (*Picea pungens* Engelm.) and its cultivar Glauca (*P. pngens* Engelm. 'Glauca') in the Cracow Botanic Garden of the Jagiellonian University (UJ).

MATERIALS AND METHODS

The studies were conducted in the years 2002–2003 in the area of the Botanic Garden of UJ and in the laboratory of the Chair of Plant Protection at the Horticultural Department of the Agricultural University in Cracow. The study material was made of the trees of Colorado blue spruce (*Picea pungens*) and its cultivar 'Glauca' in growth stands, in the quantity of 9 trees growing in the collection of the Botanic Garden for 35–57 years. Their health state was evaluated through phytopathological diagnostics of the examined plants, considering the types of disease symptoms, their localization and range. An estimation of the trees' healthiness was conducted annually at 3 dates (the middle of V, VV/VIII, and the middle of X). Those studies were carried out on the basis of detailed macro- and microscopic observation of diseased tissues. 40 samples were analyzed, which were the cut off parts of plants with different disease symptoms. During the analysis a detailed description of the changes occurring on the tissues and microscopic preparations were prepared from etiological symptoms. Microscopic observations and a comparison of their results with corresponding mycological studies [Kupriewicz and Uljaniszew 1975, Majewski 1977] led to a direct identification of the causing agents of those changes. The results were put in tables or were converted into percentage values [Czyżewski 1975]. Besides, laboratory studies were conducted on the isolation of infectious factors from the diseased symptoms on standard glucose-potato medium, by means of methods generally accepted in phytopathology [Kiraly et al. 1977]. Only fungi colonies were taken from the populations of isolated microorganisms for further laboratory culture. Next, species were identified within the isolated and grown cultures on the basis of microscopic analysis (measurements of the spores and other morphological elements of fungi) [Biłaj 1977; Domsch et al. 1980; Eliss 1976; Gams 1971; Gerlach and Nirenberg 1982; Gilman 1957; Kiriljenko 1977; Kwaśna et al. 1991; Neergard 1979; Nelson et al. 1983; Pidopkiczko 1977a, 1977b, 1978; Sneh et al. 1994; Sutton 1980].

RESULTS AND DISCUSSION

An estimation of the healthiness of the studied trees showed differentiated disease symptoms and their infectious character. Direct microscopic observation of the diseased tissues identified the presence of *Chrysomyxa ledi* and fungi from the genera *Fusarium*

and *Plenodomus* (tab. 1). The studies determining the healthiness of the analyzed trees gave the basis to isolate different disease symptoms in them and to establish their infectious character (tab. 2, photo 1). Those symptoms had varying scopes and localization on plants. They did not develop in the same manner in the analyzed period of time. In the years 2002–2003 those were the symptoms on both one-year-old and two-year-old needles. The causes of this fact can be sought in the course of the weather conditions in that period. The year 2002 had more favourable conditions for the development of microorganisms as compared to the following year. The winter of the turn of 2002 and 2003 was frosty and there was no snow, with the minimum temperature of -20°, which had a negative effect on the development of fungi. The next year was characterized by very small rainfalls and the maximum temperature of over 30°C, which, in turn, together with low air humidity limited the growth of fungi. Hence, no disease symptoms were found on the needles formed in 2003 although they were visible in the previous vegetation season.

The identified disease symptoms lowered the decorative values of spruce in spring and autumn, the most visible sign being the browning and falling of the needles (photo 1). In summer the healthiness of trees was very good. Besides, the studies found considerable occurrence of green spruce aphid and aspidiotus. The preying of the pests marred the trees and made them more susceptible to infection by different infectious factors.

Table 1. Fungi isolated from diseases plants of *P. pungens*
Tabela 1. Grzyby izolowane z chorych roślin *P. pungens*

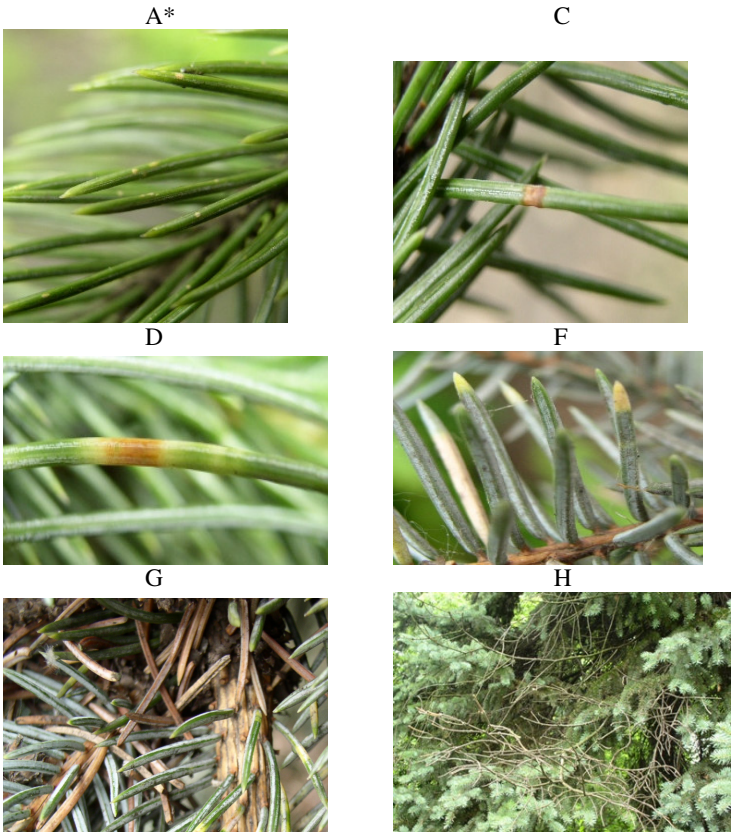
Fungus Grzyb	Identification – Identyfikacja			
	Direct Bezpośrednia	Laboratory – Laboratoryjna		
		Numbers of isolates fungus Liczba izolatów grzyba		
		Ogółem Total	2002	2003
1	*2*	*3*	*4*	*5*
Acremonium butyri (van Beyma) W. Gams		8		8
Acremonium crotocinigenum (Schol-Schwarz) W. Gams		1		1
Acremonium musicola (Speg.) W. Gams		1	1	
Acremonium tubakii W. Gams		37		37
Alternaria consortiale (Thüem.) Hughes		46	2	44
Ampulliferina persimplex Sutton		6		6
Anthostomella conorum (Fuckel) Sacc.		21	8	13
Arthrinium state of Apiospora montagnei Sacc.		9	2	7
Aspergillus niger van Tieghem		3	1	2
Aureobasidium pullulans (de Bary) Arnaud		40		40
Botryodiplodia rubi Syd.		16	5	11
Broomella spp. Sacc.		2		2
Broomella acuta Shoem. & E. Müll		1	1	
Camarosporium passerini Sacc.		6		6
Chaetomidium fimeti (Fuck.) Zopf		4	4	
Chrysomyxa ledi (Alb. et Schw.) de Bary	+			
Coniothyrium sporulosum (W. Gams & Domsch) van der Aa		1	1	
Epicoccum purpurascens Ehrenb ex Schlecht.		1	1	
Fusarium spp. Link	+			

1	2	3	4	5
Fusarium camptoceras Wollenw. et Reinking		20		20
Fusarium moniliforme Sheld var. *lactis* (Pir. et Rib.) Bilai		5	5	
Fusarium sporotrichioides Sherb. (syn. *Fusarium sporotrichiella var. sporotrichioides* (Sherb.) Bilai)		2	2	
Gonatobotrys simplex Corda		1		1
Gonytrichum macrocladum (Sacc.) Hughes		2	2	
Hansfordia pulvinata (Berk. & Curtis) Hughes		1	1	
Moniliella spp. Stolk et Dakin		1		1
Nodulisporium gregarium (Berk. & Curt.) Meyer		1	1	
Penicillium canescens Sopp		22	14	8
Pestalotiopsis funerea (Desm.) Steyaert		4	4	
Phoma eupyryna Sacc.		1	1	
Phoma pomorum Thüm.		17		17
Phoma ribes-grossulariae Petrak.		4		4
Phoma thujana Thuem.		3	3	
Phyllosticta limbalis Pers.		8		8
Pithomyces chartarum (Berk. & Curt.) M. B. Ellis		3		3
Plenodomus spp. Preuss	+			
Plenodomus lingam (Tode ex Schw.) Desm.		5	1	4
Plenodomus melilotis Markova-Letova.		2		2
Pseudophacidium picea E. Müller		1	1	
Rhinocephalum spp. Kamyschko		1		1
Rhizosphaera kalkhoffii Bubák		11	3	8
Sagenomella diversispora (van Beyma) W. Gams		1	1	
Sclerophoma mali (Brun.) Syd.		1		1
Sclerophoma pruni Died.		8	5	3
Scytalidium spp. Pesante		6		6
Scytalidium lignicola Pesante		6	4	2
Seiridium intermedium (Sacc.) Sutton		3		3
Septoria antirrhini Robak		3		3
Sordaria fimicola (Rob.) Ces. & de Not.		1		1
Spilodochium vernoniae Syd.		3		3
Sporotrix schenckii Hektoen & Perkins		1	1	
Stemphylium state of *Pleospora herbarum* (Pers. ex Fr.) Rabenh.		1		1
Thanatephorus cucumeris (Frank) Donk		1		1
Trichosporiella cerebriformis (de Vries & Klein-Natrop) W. Gams		5		5
Trichurus terrophilus Swifth & Povah		1		1
Ulocladium consortiale (Thüm.) Simmons		11		11
Zythiostroma pinastri (Karst.) Höhn.		30		30

The macro- and microscopic identification of the analyzed samples in both years of studies showed that the plant material contained parasite *Chrysomyxa ledi*, causing brown rust, and fungi from the genera *Fusarium* and *Plenodomus.* Fungi were found in the population of microorganisms obtained as a result of the laboratory mycological analysis of the diseased tissues. The microbiological and mycological analysis of the isolated population identified 54 fungi species and a complex colonization of the tissues showing the identified disease symptoms was determined. The most frequent fungi genera included *Acremonium, Anthostomella, Aureobasidium, Fusarium, Penicillium, Phoma* and *Zythiostroma.* The majority of the identified fungi participated in causing more than 2 of different types of disease symptoms in plants and those were the following species: *Acremonium butyri, Alternaria consortiale, Aureobasidium pullulans, Penicillium canescens, Phoma pomorum, Phyllostica limbalis, Plenodomus lingam,*

Rhizosphaera kalkhoffii, Scytalidium spp., *Trichosporiella cerebriformis, Ulocladium consortiale, Zythiostroma pinastri.*

During the culture of fungi meant for the identification on the standard glucose-potato medium it was found out that the time of growth and development of fungi was much longer than that quoted in mycological studies. It was, respectively for the following fungi: *Acremonium tubakii, Anthostomella conorum* and *Botryodiplodia rubi* – 2 months, *Fusarium moniliforme* var. *lactis* and *F. sporotrichioides* – from 2–3 months, *Moniliella* spp. – 2.5 months, *Hansfordia pulvinata* and the fungi from genus *Phoma* – 3 months, and *Zythiostroma pinastri* – more than 1 month.



Phot. 1. Diseases symptoms types diagnosed in *P. pungens.* (letters: A, C, D, F, G, H – see table 2)
Fot. 1. Typy objawów chorobowych zdiagnozowanych u *P. pungens*. (litery: A, C, D, F, G, H – patrz tabela 2)

The qualitative and quantitative structure of fungi populations isolated in the laboratory pointed to a big proportion of fungi isolated from the needles and sprouts (61.25%), much lower from needles (24%) and from sprouts (14.75%). Besides, it should be

Table 2. Participate fungi in emergence pathological symptoms in the *P. pungens*
Tabela 2. Udział grzybów w powstawaniu zmian chorobowych *na P. pungens*

Fungus Grzyb	Types of pathological symptoms – Typ objawu chorobowego																	
	A		B		C		D		E		F		G		H		I	
	Year – Rok																	
	02	03	02	03	02	03	02	03	02	03	02	03	02	03	02	03	02	03
Acremonium butyri		X				X						X		X				
Acremonium crotocinigenum						X												
Acremonium musicola																		
Acremonium tubakii	X													X		X		X
Alternaria consortiale		X		X		X							X	X	X	X		X
Ampulliferina persimplex												X		X		X		
Anthostomella conorum															X	X	X	
Arthrinium state of Apiospora montagnei						X			X					X	X	X		X
Aspergillus niger		X		X														
Aureobasidium pullulans		X		X		X		X				X		X		X		X
Botryodiplodia rubi		X	X			X							X					
Broomella spp.						X								X				
Broomella acuta							X											
Camarosporium passerini				X										X				
Chaetomidium fimeti					X		X											
Chrysomyxa ledi	X	X																
Coniothyrium sporulosum							X											
Epicoccum purpurascens													X					
Fusarium spp.							X											
Fusarium camptoceras																X		X
Fusarium moniliforme var. *lactis*													X		X			
Fusarium sporotrichioides															X			
Gonatobotrys simplex				X														
Gonytrichum macrocladum																	X	
Hansfordia pulvinata			X															
Moniliella spp.														X				
Nodulisporium gregarium							X											
Penicillium canescens			X		X	X	X	X				X	X	X		X		
Pestalotiopsis funerea															X			
Phoma eupyryna									X									
Phoma pomorum		X				X						X		X		X		

Phoma ribes-grossulariae										X						
Phoma thujana											X					
Phyllosticta limbalis		X			X							X				X
Pithomyces chartarum		X		X												
Plenodomus spp.	X															
Plenodomus lingam	X				X		X									X
Plenodomus melilotis		X		X												
Pseudophacidium picea															X	
Rhinocephalum spp.				X												
Rhizosphaera kalkhoffii				X						X	X	X				
Sagenomella diversispora	X															
Sclerophoma mali		X														
Sclerophoma pruni	X			X		X					X					
Scytalidium spp.		X			X							X				
Scytalidium lignicola										X	X	X				
Seiridium intermedium																X
Septoria antirrhini							X									
Sordaria fimicola														X		
Spilodochium vernoniae														X		X
Sporotrix schenckii	X															
Stemphylium state of *Pleospora herbarum*										X						
Thanatephorus cucumeris												X				
Trichosporiella cerebriformis		X										X		X		
Trichurus terrophilus										X						
Ulocladium consortiale		X		X		X						X		X		
Zythiostroma pinastri		X		X		X				X		X		X		X

A – on needles two-year old and older – small spots, oval-shaped, light yellow – na igłach dwuletnich i starszych – plamy drobne, okrągławe, barwy jasnożółtej

B – on needles two-year old and older – small spots, smear-like, light- or dark-bronze coloured – na igłach dwuletnich i starszych – plamy drobne, smugowate, barwy jasno- lub ciemnobrunatnej

C – on needles two-year old and older– small, bronze ribbon-like patterns in the central and base needle regions – na igłach dwuletnich i starszych – drobne brunatne otaśmienia w części centralnej i nasadowej igły

D – on needles two-year old and older – small, ribbon-like patterns coloured originally in gray-bronze, than in rusty-bronze, edged with a broader light-green band; later, the spots become less apparent, gray-bronze – na igłach dwuletnich i starszych – drobne, otaśmienia, początkowo szarobrunatne później rdzawobrunatne obwiedzione szerszą jasnozielona obwódką, później plamy są mniej wyraźne szarobrunatne

E – on one-year old needles – oval spots, coloured from bronze to black – na jednorocznych igłach – plamy okrągławe, barwy brunatnej do czarnej

F – yellowing of needle endings – żółknięcie końców igieł

G – whole needles turning bronze, in particular at the end of the vegetative season, as well as the falling of died-down needles at the end of the vegetative season (two-year old and older) – brunatnienie całych igieł, szczególnie pod koniec sezonu wegetacyjnego, oraz opadanie zamarłych igieł pod koniec sezonu wegetacyjnego (2-letnie i starsze)

H – dying down of branches, single offshoots or whole shoots – zamieranie gałęzi, poszczególnych odgałęzień lub całych pędów

I – resin-covered thickenings on shoots – ożywicowane zgrubienia na pędach

added that the isolates obtained from different plant parts commonly included the following species: *Acremonium tubakii, Altenaria consortiale, Ampulliferina persimplex, Arthrinium* state of *Apiospora montagnei, Aureobasidium pullulans, Fusarium moniliforme var. lactis, Penicillium canescens, Phoma pomorum, Phyllostica limbalis, Trichosporiella cerebriformis, Ulocladium consortiale, Zythiostroma pinastri.* On the other hand, the second year of the mycological analysis showed differences in the colonization of plants *Picea pungens* and *P. pungens* 'Glauca' by fungi. It was found out that the species observed on *P. pungens* included *Acremonium crotocinigenum, Aspergillus niger, Sordaria fimicola,* while the following were found exclusively on *P. pungens* 'Glauca': *Botryodiplodia rubi, Bromella* spp., *Gonatobotrys simplex, Moniliella* spp., *Phoma ribes-grossulariae, Penodomus melilotis, Rhinecephalum* spp., *Rhizosphaera kalkhoffii, Sclerophoma mali, Septoria antirrhini, Seiridium intermedium, Stemphylium* state of *Pleospora herbarum, Thanatephorus cucumeris, Trichurus terrophilus.* The other species of fungi occur simultaneously on the studies species of spruce and its cultivar.

The results obtained by the authors of the present paper partly correspond to the findings in literature on infectious diseases of spruce in growth stands [Magan and Smith 1996, Manter and Livingston 1996, Werner and Frużyńska-Jóźwiak 1999, Werner et al. 2001]. Considerable convergence of the disease symptoms and the results of phytopathological analysis of plants with the studies by Werner and Frużyńska-Jóźwiak [1999] and Werner et al. [2001] was found. Fungi from the genera *Altenaria, Epicoccum, Fusarium* and *Pestalotiopsis* were also isolated from the infected needles and sprouts. Besides, the occurrence of species *Rhizosphere kalkhoffii* on Colorado blue spruce at different periods of its growth was confirmed after Magan and Smith [1996] and Manter and Livingston [1996], and the occurrence of *Chrysomyxa ledi* was confirmed after Wescott [1971]. The remaining isolated and identified fungi seem not to have been recorded on spruce in this type of stands. At the same time, the studies observed an inseparable effect of the environmental conditions and the preying of pests on the growth of the trees' susceptibility to the effect of infectious factors.

CONCLUSIONS

1. The macroscopic, microscopic and mycological analyses of the healthiness of Colorado blue spruce *Picea pungens* and its cultivar 'Glauca', growing in the collection of the Botanic Garden of UJ, showed that fungi were the agents causing the disease symptoms.

2. The most frequent in the population of isolated and next identified fungi were *Acremonium tubakii, Altenaria consortiale, Anthostomella conorum, Arthrinium* stae of *Apiospora montagnei, Aureobasidium pullulans, Botryodiplodia rubi, Fusarium camptoceras, F. moniliforme* var. *lactis, Penicillium canescens, Phoma pomorum, Rhizosphaera kalkhoffii, Ulocladium consortiale, Zythiostroma pinastri.*

3. The analysis of the isolated fungi colonizing the diseased tissues of the studied parts in respect of the qualitative and quantitative composition pointed to the domination of such species as *Altenaria consortiale, Aureobasidium pullulans, Penicillium canescens, Phoma pomorum, Ulocladium consortiale* and *Zythiostroma pinastri.*

4. Laboratory studies isolating and identifying the fungi proved that time of the culture meant for identification on glucose-potato medium was long and it was, correspondingly, for the fungi: *Acremonium tubakii, Anthostomella conorum* and *Botryodiplodia rubi* - 2 months, *Fusarium moniliforme* var. *lactis* and *F. sporotrichioides* – from 2-3 months, *Moniliella*. spp. – 2.5 months, *Hansfordia pulvinata* and fungi from the genus *Phoma* – 3 months, *Zythiostroma pinastri* – more than 1 month.

5. Healthiness of the studied trees was shaped by fungi and the habitat conditions (weather, coexisting plants, traces of pests' feeding).

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GRZYBY WYSTĘPUJĄCE NA ŚWIERKU KŁUJĄCYM (*Picea pungens* Engelm.) W KRAKOWSKIM OGRODZIE BOTANICZNYM

Streszczenie. Badania miały na celu określenie przyczyn zamierania pędów i igieł świerka kłującego (*Picea pungens* Engelm.) i jego odmiany 'Glauca' w nasadzeniach stałych w kolekcji Ogrodu Botanicznego UJ w Krakowie. Wymienione objawy chorobowe przyczyniały się już od kilku lat do utraty walorów dekoracyjnych u tych drzew. W latach 2002 i 2003 przeprowadzono badania w oparciu o ocenę zdrowotności roślin, diagnostykę fitopatologiczną, wyosobnienie mikroorganizmów z tkanek z objawami chorobowymi oraz hodowlę i identyfikację wyosobnionych grzybów. Zarówno określanie zdrowotności drzew jak i specjalne techniki laboratoryjne dowiodły, że zdiagnozowane u roślin objawy mają charakter infekcyjny, a grzyby mogą być ich głównymi sprawcami. Analiza populacji grzybów wyosobnionych z chorych tkanek wskazała na ich kompleksowe występowanie w tkankach badanych roślin, jak też na dominację takich gatunków jak *Alternaria consortiale, Aureobasidium pullulans, Penicillium canescens, Phoma pomorum, Ulocladium consortiale, Zythiostroma pinastri*.

Słowa kluczowe: *Picea pungens*, objawy chorobowe, grzyby

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