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FUNGI COLONIZING SHOOTS OF COMMON YEW (*TAXUS BACCATA* L.) IN THE JAGIELLONIAN UNIVERSITY BOTANIC GARDEN IN CRACOW

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S u m m a r y

The aim of the investigations was determination the reasons of dying down of needles and branches of common yew (*Taxus baccata* L.) in the park collection of Botanic Garden of Jagiellonian University in Cracow. The disease changes of infectious nature were observed on the examined plants' parts. The changes'appearance, its location and extent were described in details. At first the spots were brown colour with distinct hem. Then the needle and branch tissues were dying down. From end of May to July mass falling down of infesteted needles was observed. Mycological analysis of diseased tissues showed 34 fungi species. To the dominant species belonged: *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Fusarium* spp., *Pestalotiopsis guepinii*, *Phomopsis juniperivora* and *Zythiostroma pinastri.* Common yew pathogens: *Phomopsis juniperivora* and *Sclerophoma pityophila* were found among the isolated fungi.

Key words: common yew, *Taxus baccata*, needles, branches, fungi, disease symptoms

INTRODUCTION

Man has long planted gardens in which, *inter alia*, different trees and shrub species are found. In urbanised areas parks, among others, are such gardens. This greenery performs a comprehensive role as the aesthetic or environment-forming factor. They are also attributed with an advantageous effect on climatic conditions, dust retention, absorption of noxious gases, enrichment of air with oxygen, etc. (Ł u k a s i e w i c z , 1989). One of our native conifer species, recommended for use in urban plantings, is common yew (*Taxus baccata* L.), due to its resistance to adverse conditions existing in the urban environment (Ł u k a s i e w i c z , 1989). This is a species which has been long appreciated, but relatively rarely found in urban plantings (Grzywacz, 2001). These plants, growing in urban green areas for many years, are subjected to the effect of different adverse abiotic factors. Hence, they become more susceptible to infection by pathogens. There are a small number of studies on infectious diseases of yew, including fungal diseases (S i w e c k i , 1975). In phytopathological literature, the paper of K a p u ś c i ń s k i (1947) is one of the most frequently cited; the author mentions in it 21 fungi species occurring on yew needles and shoots. To date, the problem of healthiness of yew has been addressed in Poland mostly in the yew tree reserves in Wierzchlas and Rokita (M a ń k a et al. 1968, 1985; M a ń k a 1993). The authors identify the culprits of the dieback of yew seedlings – *Cylindrocarpon radicicola* (M a ń k a et al. 1968) and *C. destructans* (M a ń k a 1993); as well as the culprits of yew wood rot, i.e. *Phellinus hartigii* and *Ph. pini* (M a ń k a et al. 1985). But the problem of yew needle dieback is only addressed by M a ń k a (1992) who mentions the following fungi among the culprits of this disease: *Macrophoma taxi*, *Phomopsis taxi* and *Coniothyrium fuckelii*.

The aim of the study was to diagnose the causes of dieback of common yew needles and branches in the plantings of the Cracow Botanic Garden and to identify fungi species isolated from diseased tissues.

MATERIALS AND METHODS

The studies were conducted in the years 2005-2007 in the Jagiellonian University Botanical Garden in Cracow where the dieback of needles and branches of common yew (*Taxus baccata* L.) had been observed for over a dozen years. The investigated material comprised common yew individuals in the amount of 21 trees aged between 50 and 60 years. The studies covered one-, two- and three-year-old living needles with local infection spots as well as two- and three-year-old completely browned needles, still attached to the branch. Branches on which infection spots occurred and completely dead branches (1-, 2- and 3-year-old) were also subjected to the analysis. During the vegetation period, the most frequently occurring pathological changes (colour,

shape, structure of infected tissues, presence of etiological symptoms) in the abovementioned organs were recorded and described. 10 pieces from each year group of needles were sampled from all the yew tree individuals. The same procedure was applied to the analysed branches. In the laboratory, 20 sections, 5-10 mm long, from each batch were excised separately in order to isolate fungi from tissues of the needles and branches affected by pathological symptoms or completely dead. These sections were rinsed in running water, subsequently they were surface disinfected in 75-percent ethanol for 1 minute and then rinsed again in sterile distilled water. After drying, the material was placed on potato dextrose agar medium (PDA) and incubated at a temperature of about 23ºC. The species in the grown colonies of fungi, after their separation and obtaining single spore cultures on the same growth medium, were identified using species identification keys and monographs (Gams 1971; Domsch et al. 1980; Sutton, 1980; Ellis and Ellis, 1985; Kwaśna et al. 1991). The identification of fungi from the genus *Penicillium* was made based on the cultures grown on Czapek-Dox medium and malt medium (Ramirez, 1982). Isolates were made from a total of 200 needles and 160 branches sections. The results of fungi species identification were tabulated, taking into account the number of the obtained cultures and their percentage shares in the total population of the isolates. In addition, symptomatologic data were also tabulated, indicating symptom locations on needles and branches, as well as data documenting the colonization of diseased tissues by the isolated and identified fungi.

RESULTS AND DISCUSSION

The analysis of the healthiness of yew demonstrated the presence of infection spots both on 1-, 2-year-old and 3-year-old needles. The intensification of spot occurrence was different, depending on the age of the needles. On 1-year-old needles, spots occurred sporadically, whereas on 2-year-old needles their most frequent occurrence was found. In the case of 3-year-old needles, their complete browning and dieback were observed. The infection changes on the needles were initially observed as brown-coloured spots, and then as chocolate-coloured to dark brown spots, with a clear darker rim. They were first visible in the form of oval spots, expanding with time to the whole surface of the needles, in the final effect leading to their complete dieback. Similar pathological changes were also observed on the branches (2- and 3-year-old, sporadically on 1-year-old). These were dark brown necrotic streaks which were often located at the base of the needles. Subsequently, these streaks expanded along the branches as well as on their circumference, leading to their dieback together with the needles located above the place of spot occurrence. In the period from the end of May to July, the mass falling of dead needles was observed, mainly 3-year-old needles, but also younger ones, which had died as a result of the death of the branch.

The mycological analysis showed the colonization of diseased common yew needles and branches by a complex of fungi.

From the diseased common yew tissues (needles and branches), 1085 cultures were obtained belonging to 34 species (Tab. 1, 2, 3). The following fungi occurred most frequently in the community of the fungi isolated from the investigated material: *Phomopsis juniperivora*, *Alternaria alternata*, *Colletotrichum gloeosporioides* and *Zythiostroma pinastri*.

Colletotrichum gloeosporioides and *A. alternata* were isolated most frequently from 1- and 2-year-old needles affected by the described pathological symptoms, whereas *P. juniperivora* and *S. pityophila* were isolated less frequently. Species such as *Z. pinastri*, *P. juniperivora*, *A. alternata*, *Pestalotiopsis guepinii* and *C. gloeosporioides* predominated in the isolates from the browned needles.

Phomopsis juniperivora, *Z. pinastri* and *C. gloeosporioides* were isolated most numerously from the branchs which showed spot disease and necrosis, whereas *P. juniperivora*, *A. alternata* and *Fusarium* spp. were isolated most frequently from dead branches.

In 2005, 323 fungi cultures belonging to 20 species were isolated (Tab. 1). *Alternaria alternata, C. gloeosporioides, P. juniperivora* and *Z. pinastri* were predominant among the obtained isolates. Fungi from the genus *Fusarium – F. avenaceum* and *F. sacchari* also occurred in great numbers, but the latter species was noted sporadically. *Pestalotiopsis guepinii* was also isolated frequently from dead tissues. In the population of the isolated fungi, saprotrophs, i.e. *Chaetomium bostrychodes* and sporadically *Penicillium expansum*, were also observed.

In 2006 a larger number of fungi colonies were obtained – 366 belonging to 19 species (Tab. 2). Similarly to the previous year, the same fungi species were predominant among them. In these isolates, fungi from the genus *Fusarium* (*F. avenaceum, F. sacchari, F. sporotrichioides*) occurred. More cultures of *Pe. guepinii* were isolated from dead yew needles compared to the previous year, likewise from living needles – the species *S. pityophila*. But another saprotrophic species was identified, i.e. *Epicoccum purpurascens*.

The largest number of fungi colonies was isolated in 2007, notably 396, among which 21 species were identified (Tab. 3). Just like in the previous years, the same fungi species predominated. But a smaller number of *Pe. guepinii* isolates was found, compared to the previous years, and in turn *S. pityophila* occurred only on living yew needles. In that year of study, the presence of 2 species from the genera *Phoma – Ph. exigua* and

Table 1
Fungi isolated from diseased needles and branches of common yew in 2005.

Species of fungi	Number of isolates (%)						
	Needles			Branches			Total
	With infection spots	Dead	Total	With infection spots	Dead	Total	
Acremonium apii (M. A. Sm. & Ramsey) W. Gams					1 (0.84)	1 (0.57)	1 (0.31)
Acremonium butyri (van Beyma) W. Gams		3 (3.16)	3 (2.04)				3 (0.93)
Acremonium falciforme (Carrión) W. Gams		2 (2.11)	2 (1.36)		4 (3.36)	4 (2.27)	6 (1.86)
Alternaria alternata (Fr.) Keissl.	12 (23.08)	21 (22.11)	33 (22.45)	5 (8.77)	18 (15.13)	23 (13.07)	56 17.34)
Anthostomella conorum (Fuckel) Sacc.		1 (1.05)	1 (0.68)				1 (0.31)
Aspergillus flavus Link		2 (2.11)	2 (1.36)				2 (0.62)
Aspergillus niger van Tiegh.	1 (1.92)	1 (1.05)	2 (1.36)		1 (0.84)	1 (0.57)	3 (0.93)
Chaetomium bostrychodes Zopf					15 (161)	15 (8.52)	15 (4.64)
Cladosporium macrocarpum Preuss		2 (2.11)	2 (1.36)				2 (0.62)
Colletotrichum gloeosporioides (Penz.) Penz. & Sacc.	26 (50.00)	4 (4.21)	30 (20.41)	10 (17.54)	4 (3.36)	14 (7.95)	44 (13.62)
Cytospora taxi Sacc.					4 (3.36)	4 (2.27)	4 (1.24)
Fusarium avenaceum (Fr.) Sacc.				4 (7.02)	11 (9.24)	15 (8.58)	15 (4.64)
Fusarium sacchari (Butler & Hafiz Khan) W. Gams					3 (2.52)	3 (1.70)	3 (0.93)
Penicillium expansum Link		2 (2.11)	2 (1.36)				2 (0.62)
Pestalotiopsis guepinii (Desm.) Steyaert		9 (9.47)	9 (6.12)		8 (6.72)	8 (4.55)	17 (5.26)
Phomopsis juniperivora Hahn	6 (11.54)	21 (22.11)	27 (18.37)	19 (33.33)	26 (21.85)	45 (25.57)	72 (22.29)
Sclerophoma pityophila (Corda) Höhn.	2 (3.85)	2 (2.11)	4 (2.72)	4 (7.02)	2 (1.68)	6 (3.41)	10 (3.10)
Zythiostroma pinastri (Karst.) Höhn.	1 (1.92)	23 (24.21)	24 (16.33)	15 (26.32)	13 (10.92)	28 (15.91)	52 (16.10)
Fungi not sporulating (2 species)	4 (7.69)	2 (2.11)	6 (4.08)		9 (7.56)	9 (5.11)	15 (4.64)
Total	52 (100)	95 (100)	147 (100)	57 (100)	119 (100)	176 (100)	323 (100)

Table 2
Fungi isolated from diseased needles and branches of common yew in 2006.

Species of fungi	Number of isolates (%)						
	Needles			Branches			Total
	With infection spots	Dead	Total	With infection spots	Dead	Total	
Alternaria alternata (Fr.) Keissl.	18 (25.35)	11 (10.19)	29 (16.20)	12 (14.46)	19 (18.27)	31 (16.58)	60 (16.39)
Anthostomella conorum (Fuckel) Sacc.		2 (1.85)	2 (1.12)		2 (1.92)	2 (1.07)	4 (1.09)
Aspergillus niger van Tiegh.		1 (0.93)	1 (0.56)				1 (0.27)
Colletotrichum gloeosporioides (Penz.) Penz. & Sacc.	23 (32.39)	14 (12.96)	37 (20.67)	13 (15.66)	7 (6.73)	20 (10.70)	57 (15.57)
Epicoccum purpurascens Ehrenb.		4 (3.70)	4 (2.23)		4 (3.85)	4 (2.14)	8 (2.19)
Fusarium avenaceum (Fr.) Sacc.				10 (12.05)	15 (14.42)	25 (13.37)	25 (6.83)
Fusarium sacchari (Butler & Hafiz Khan) W. Gams	2 (2.82)	3 (2.78)	5 (2.79)		14 (13.46)	14 (7.49)	19 (5.19)
Fusarium sporotrichioides Sherb.		4 (3.70)	4 (2.23)				4 (1.09)
Hansfordia spp. Hughes	2 (2.82)		2 (1.12)				2 (0.55)
Mucor hiemalis Wehmer	2 (2.82)		2 (1.12)				2 (0.55)
Pestalotiopsis guepinii (Desm.) Steyaert		24 (22.22)	24 (13.41)		3 (2.88)	3 (1.60)	27 (7.38)
Phomopsis juniperivora Hahn	9 (12.68)	14 (12.96)	23 (12.85)	29 (34.94)	29 (27.88)	58 (31.02)	81 (22.13)
Phyllosticta spp. Pers.		2 (1.85)	2 (1.12)				2 (0.55)
Sclerophoma pityophila (Corda) Höhn.	7 (9.86)		7 (3.91)	1 (1.20)		1 (0.53)	8 (0.19)
Sporotrix spp. Hektone & Perkins	4 (5.63)		4 (2.23)				4 (1.09)
Ulocladium botrytis Preuss					5 (4.81)	5 (2.67)	5 (1.37)
Zythiostroma pinastri (Karst.) Höhn.	4 (5.63)	21 (19.44)	25 (13.97)	18 (21.69)		18 (9.63)	43 (11.75)
Fungi not sporulating (2 species)		8 (7.41)	8 (4.47)		6 (5.77)	6 (3.21)	14 (3.83)
Total	71 (100)	108 (100)	179 (100)	83 (100)	104 (100)	187 (100)	366 (100)

Table 3
Fungi isolated from diseased needles and branches of common yew in 2007.

Species of fungi	Number of isolates (%)						
	Needles			Branches			Total
	With infection spots	Dead	Total	With infection spots	Dead	Total	
Alternaria alternata (Fr.) Keissl.	12 (17.39)	19 (17.76)	31 (17.61)	3 (4.00)	28 (19.31)	31 (14.09)	62 (15.66)
Anthostomella conorum (Fuckel) Sacc.		1 (0.93)	1 (0.57)		2 (1.38)	2 (0.91)	3 (0.76)
Aspergillus flavus Link					2 (1.38)	2 (0.91)	2 (0.51)
Aspergillus niger van Tiegh.	2 (2.90)		2 (1.14)		1 (0.69)	1 (0.45)	3 (0.76)
Cladosporium macrocarpum Preuss		3 (2.80)	3 (1.70)				3 (0.76)
Colletotrichum gloeosporioides (Penz.) Penz. & Sacc.	27 (39.13)	9 (8.41)	36 (20.45)	18 (24.00)	8 (5.52)	26 (11.82)	62 (15.66)
Epicoccum purpurascens Ehrenb.		13 (12.15)	13 (7.39)	2 (2.67)	5 (3.45)	7 (3.18)	20 (5.05)
Fusarium avenaceum (Fr.) Sacc.	2 (2.90)		2 (1.14)	9 (12.00)	10 (6.90)	19 (8.64)	21 (5.30)
Fusarium sacchari (Butler & Hafiz Khan) W. Gams	5 (7.25)	7 (6.54)	12 (6.82)		10 (6.90)	10 (4.55)	22 (5.56)
Mucor hiemalis Wehmer	3 (4.35)		3 (1.70)	2 (2.67)		2 (0.91)	5 (1.26)
Penicillium nigricans Bainier ex Thom.		2 (1.87)	2 (1.14)				2 (0.51)
Penicillium tardum Thom.					2 (1.38)	2 (0.91)	2 (0.51)
Pestalotiopsis guepinii (Desm.) Steyaert		2 (1.87)	2 (1.14)		10 (6.90)	10 (4.55)	12 (0.03)
Phoma exigua Desm.		11 (10.28)	11 (6.25)		2 (1.38)	2 (0.91)	13 (3.28)
Phoma pomorum Thüm.	2 (2.90)	4 (3.74)	6 (3.41)		19 (13.10)	19 (9.64)	25 (6.31)
Phomopsis juniperivora Hahn	2 (2.90)	17 (15.89)	19 (10.80)	30 (40.00)	26 (17.93)	56 (25.45)	75 (18.94)
Sclerophoma pityophila (Corda) Höhn.	7 (10.14)		7 (3.98)				7 (1.77)
Trichoderma viride Pers.		3 (2.80)	3 (1.70)	2 (2.67)	2 (1.38)	4 (1.82)	7 (1.77)
Ulocladium botrytis Preuss		2 (1.87)	2 (1.14)		10 (6.90)	10 (4.55)	12 (3.03)
Zythiostroma pinastri (Karst.) Höhn.	7 (10.14)	11 (10.28)	18 (10.23)	9 (12.00)	8 (5.52)	17 (7.73)	35 (8.84)
Fungi not sporulating (1 species)		3 (2.80)	3 (1.70)				3 (0.76)
Total	69 (100)	107 (100)	176 (100)	75 (100)	145 (100)	220 (100)	396 (100)

Ph. pomorum, was found for the first time which were isolated mainly from dead yew needles and branches. But saprotrophs, i.e. *E. purpurascens* as well as *Penicillium nigricans, P. tardum* and *Trichoderma viride*, were isolated more frequently than before.

The species *Phomopsis juniperivora*, which is recognised as a pathogen of this plant (Siwecki, 1975; Grzywacz, 2001; Łabanowski et al. 2001), was isolated both from yew needles and branches. The obtained results confirm the presence of this fungus both in shoots and needles of common yew. This species is primarily the culprit of spot disease and the dieback of shoots (Siwecki, 1975; Grzywacz 2001), and its presence in diseased needles suggests that it may also be the culprit of their spot disease, and subsequently it may transfer from them to the branches (Łabanowski et al. 2001).

In the studies, the presence of the fungus *Sclerophoma pityophila* was found, which is known as a pathogen of pine needles causing in them the disease called autumn needle cast (Mańka et al. 1979, 1982). In the studies of the present paper's author, this fungus was more frequently isolated from yew needles, which may indicate its participation in the development of pathological changes in them. Grzywacz (2001) mentions 11 other fungi species among the culprits of needle cast of yew, whereas *S. pityophila* is attributed only with the ability to infect young yew shoots.

The species *Colletotrichum gloeosporioides* was isolated most frequently from living needles affected by pathological changes. It may be the culprit of the described pathological changes on needles typical for anthracnose occurring on many plants. Fu et al. (2003) reports the pathogenicity of this species for *Taxus brevifolia* and *T. mairei* seedlings in Taiwan.

From the tissues of common yew, the following fungi from the genus *Fusarium* were isolated: *F. avenaceum, F. sacchari* and *F. sporotrichioides*. They primarily develop as saprotrophs, which is consistent with the results obtained in these studies, since they occurred most frequently on dead tissues. Pathogenic forms, capable of causing infection in favourable conditions, may also occur among them. Werner and co-authors (2003) report the occurrence of *F. oxysporum* and its harmfulness to yew.

In the mycological analysis, the occurrence of the species *Pestalotiopsis guepinii* on dead needles and branches was also demonstrated. Many authors informed earlier about another species from the genus *Pestalotiopsis*, i.e. *P. funerea*, which is pathogenic for yew (Kapuściński, 1947; Siwecki, 1975; Grzywacz, 2001; Kozłowska et al. 2002). The presence of *P. guepinii* only in dead tissues may confirm the suggestions that it occurs as a saprotroph in the places of primary damage of needles or branches by other factors (Grzywacz, 2001).

In turn *Zythiostroma pinastri*, isolated quite frequently from shoots, may colonize them without causing any symptoms as an endophyte or participate in the production of pathological changes in them. As reported by Sutton (1980), this species has already been noted on yew shoots. *Cytospora taxi* was also isolated from dead branches; as reported by Kapuściński (1947), it lives on debarked yew branches.

Alternaria alternata was frequently isolated both from living and dead organs affected by pathological changes. In accordance with Przybył and Bojarczuk (1991), this species is recognised as a saprotroph of common yew. According to others, it is termed as the weakness pathogen of plants, since among many isolates there may be found those which show pathogenic abilities in relation to weakened plants on which they occur (Kopacki and Wagner, 2005).

The appearance of certain pathogenic species among the isolated fungi may intensify, in particular under the influence of adverse atmospheric conditions, the presence of industrial emissions or the relevant age of tress, needles and branches (Kowalski, 1987; Grzywacz, 2001).

The obtained results justify the expediency of the continuation of further studies on healthiness of common yew by determining the pathogenicity of identified fungi, and subsequently the possibility of protection against them.

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Grzyby zasiedlające pędy cisa pospolitego (*Taxus baccata* L.) w Ogrodzie Botanicznym UJ w Krakowie

Streszczenie

Badania miały na celu określenie przyczyn zamierania igieł i gałązek cisa pospolitego (*Taxus baccata* L.) w kolekcji Ogrodu Botanicznego UJ w Krakowie. Na badanych częściach roślin występowały zmiany chorobowe o charakterze infekcyjnym. Opisywano szczegółowo ich wygląd, zlokalizowanie i zasięg. Początkowo były to plamy brązowawo zabarwione z wyraźną obwódką. Następnie tkanki igieł i gałązek zamierały. Obserwowano też masowe opadanie porażonych igieł w okresie od końca maja do lipca. Przeprowadzona analiza mikologiczna chorych tkanek wykazała w nich obecność 34 gatunków grzybów, wśród których dominowały: *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Fusarium* spp., *Pestalotiopsis guepinii*, *Phomopsis juniperivora* oraz *Zythiostroma pinastri*. W populacji wyizolowanych grzybów stwierdzono obecność patogenów cisa pospolitego, tj.: *Phomopsis juniperivora* i *Sclerophoma pityophila*.

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