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Investigation of *Ophiostoma* population infected elms in Poland

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Abstract: Dutch elm disease (DED) still occurs in Poland. Previous studies confirmed occurring *O. ulmi* and two subspecies of *O. novo-ulmi*: subsp. *novo-ulmi* and subsp. *amerciana*. In this study the population of *Ophiostoma* occurred in Poland was investigated. The disease incidence was investigated on elms growing in 39 locations. The pathogen's mycelium was isolated from elm branches and twigs collected from 22 plots. The disease symptoms were noted in 5% to 35% trees. Fungi were identified based on the PCR amplification of the ITS 1/2 rDNA together with phylogenetic analysis of this region. *Ophiostoma novo-ulmi* was the only agent caused DED on *Ulmus glabra*, *U. minor* and *U. laevis*. There were no genetic diversity of *O. novo-ulmi* Polish population in analyzed ITS region. All kind of specific symptoms and disease intensity occurred independently on elm species and host age. *Ulmus minor* was infested most sever among the three elms species.

Keywords: *Ophiostoma novo-ulmi*, Dutch elm disease, *Ulmus*

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Introduction

Dutch elm disease (DED) causes wilting and decay of twigs and branches and in many cases death of native *Ulmus* species in Europe, North America and Asia. The disease is due to two pathogenic fungi *Ohiostoma ulmi* (Buisman) Nannf. and *Ophiostoma novo-ulmi* Brasier (Brasier, 1991; Mańka, 2005). The first notice of this disease in Europe was described in the beginning of XX century. In that time, *O. ulmi* was the agent of the diseases. Nowadays Dutch elm diseases mainly causes *O. novo-ulmi* infecting and killing elms independently of the tree age. This species is much more pathogenic than *O. ulmi* (Brasier, 1996). *Ophiostoma novo-ulmi* was divided into two subspecies *O. novo-ulmi* subsp. *novo-ulmi* and subsp. *americana* (Brasier & Kirk, 2001). Pathogens are transferred from tree to tree by insect vectors. Six species of *Scolytus* were found as a *Ophiostoma* vectors – *Scolytus scolytus* (Fabricius), *S. multistriatus* (Marsham), *S. schevyrewi* Semenov, *S. kirschii* Skalitzky, *S. pygmae-*

us (Fabricius) and *Scolytus laevis* Chapuis (Webber, 1990; Faccoli & Battisti, 1997; Solheim et al., 2011; Jacobi et al., 2013). In Poland both pathogens *O. ulmi* and *O. novo-ulmi* have been found as agents of Dutch elm disease (Guździoł et al., 2004).

The aim of this study was to define the *Ophiostoma* species occurring in Poland nowadays. On the base of symptoms frequency, the disease incidence was described on the three elm species *Ulmus glabra* Huds, *U. laevis* Pall. and *U. minor* Mill.

Materials and methods

The observations were carried out on 39 plots located in pure or mixed elm stands and incidentally along roads or in parks and among fields (Table 1). All elm species (*U. minor*, *U. glabra* and *U. laevis*) occurring in Poland were investigated. The incidence of disease symptoms was also noticed. The dead or dying branches were collected from diseased elms. The cross cutting of each branch revealed a ring of dark brown staining in the outer wood. The samples were collected from 1–3 elms and depended on the number of trees occurred in each localization. In addition three diseased branches, 30 cm long, were cut from each tree. Samples of *U. minor* were collected from 37% of plots, *U. glabra* from 33% and samples of *U. laevis* were cut from 30% of plots. In the laboratory, branches were divided to 1 cm long pieces. Each wood sample was surface disinfected in sodium hypochlorite (2% NaOCl) for 1 minute and rinsed three times in sterile and demineralized water for 10 minutes (Kwaśna & Siwecki, 2002). After drying,

Table 1. Localization of investigated plots

Plot no	Location	GPS guidance	Site type
1	Miękinia	N51° 11' 25.6049" E16° 44' 10.3042"	Alluvial forest
2	Babki	N52° 14' 25.4191" E17° 5' 29.5212"	Park
3	Miękinia	N51° 11' 25.6049" E16° 44' 44.4036"	Alluvial forest
4	Durowo	N52° 50' 23.561" E17° 12' 47.237"	Along road
5	Szczecinek	N53° 42' 14.923" E16° 39' 55.321"	Deciduous forest
6	Choszczno	N53° 13' 19.891" E15° 25' 2.6"	Deciduous forest
7	Kolbudy	N54° 16' 34.214" E18° 25' 12.323"	Deciduous forest
8	Oleśnica	N51° 15' 28.353" E17° 25' 33.471"	Deciduous forest
9	Piaski	N52° 5' 34.7516" E16° 59' 16.8787"	Alluvial forest
10	Legnica	N51° 17' 32.064" E16° 13' 37.492"	Deciduous forest
11	Wałbrzych	N50° 44' 7.171" E16° 17' 57.786"	Deciduous forest
12	Wałbrzych	N50° 46' 18.38" E16° 17' 3.774"	Park
13	Ośno Lubuskie	N52° 28' 43.556" E14° 52' 45.847"	Deciduous forest
14	Oborniki Śląskie	N51° 23' 44.1853" E16° 59' 32.2047"	Deciduous forest
15	Grotniki	N51° 53' 57.449" E19° 19' 30.813"	Deciduous forest
16	Grotniki	N51° 53' 37.619" E19° 17' 32.16"	Deciduous forest
17	Piaski	N51° 7' 20.8048" E22° 50' 38.9283"	Deciduous forest
18	Złoczew	N51° 26' 51.01" E18° 40' 34.845"	Deciduous forest
19	Rudy	N50° 11' 22.1586" E18° 27' 17.2998"	Deciduous forest
20	Babki	N52° 20' 40.7489" E17° 4' 15.9815"	Along road
21	Rudy	N50° 10' 39.3661" E18° 30' 0.5494"	Deciduous forest
22	Żmigród	N51° 28' 31.7328" E16° 55' 3.5065"	Alluvial forest
23	Sulechów	N52° 2' 19.7075" E15° 33' 1.1647"	Alluvial forest
24	Rudka	N52° 9' 12.7654" E21° 49' 39.4923"	Among fields
25	Rudka	N52° 11' 7.9888" E21° 53' 17.0216"	Deciduous forest
26	Białowieża	N52° 42' 34.6814" E23° 49' 33.7793"	Alluvial forest
27	Czarna Białostocka	N53° 19' 27.3738" E23° 17' 10.8472"	Deciduous forest
28	Zwierzyniec	N50° 36' 50.544" E22° 58' 30.432"	Deciduous forest
29	Chojnów	N51° 18' 53.6627" E15° 58' 58.2294"	Along road
30	Chojnów	N51° 19' 23.7885" E15° 54' 39.9133"	Among fileds
31	Dobieszyn	N51° 37' 18.5189" E21° 9' 45.1868"	Deciduous forest
32	Grójec	N51° 51' 44.1218" E20° 53' 50.5916"	Along road
33	Chojnów	N51° 18' 53.6627" E15° 58' 58.2294"	Among fields
34	Kwidzyń	N53° 43' 1.009" E18° 47' 57.319"	Deciduous forest
35	Srokowo	N54° 14' 52.426" E21° 34' 14.003"	Deciduous forest
36	Świdnik	N51° 15' 7.931" E22° 43' 4.094"	Deciduous forest
37	Dukla	N49° 32' 1.074" E21° 40' 28.006"	Deciduous forest
38	Ustroń	N49° 43' 44.264" E18° 50' 1.657"	Deciduous forest
39	Łopuchówko	N52° 28' 46.5673" E16° 49' 32.5159"	Along road

the wood pieces were divided for 4–5 pieces and put on SNA (glucose, 0.2 g, sucrose 0.2 g, KH_2PO_4, 1 g, KNO_3, 1 g, $MgSO_4 \times 7\ H_2O$, 0.5 g, agar, 15 g, streptomycin, 0.001 g, distilled water, 1 l) (Nirenberg, 1976) and PDA (potato-dextrose agar) medium in Petri dishes. After 3–5 days of incubation in the dark and in 25°C temperature wood pieces were investigated for a presence of conidial stage of *Ophiostoma* fungi with the aid of micromorphological features of colonies.

The cultures was cultivated on liquid medium (10 g NaCl, 10 g tryptone, 5 g yeast extract, distilled water) for two weeks. The mycelium was harvested with a strainer, lyophilized and ground to a fine powder in liquid nitrogen. Total genomic DNA was extracted from mycelium using the Bead-Beat Micro Gravity kit (A&A BIOTECHNOLOGY, Gdynia). PCR amplification of the ITS 1/2 rDNA was done with DNA diluted (10^{-2}) in deionized water. Primers used were ITS1-F (5'CTT GGT CAT TTA GAG GAA GTA A) (Gardes & Bruns, 1993) as well as ITS4 (5' TCCTCCGCTTATTGATATGC 3') (White et al., 1990). Each 25 μl PCR mixture consisted of 0.2 μmol L^{-1} of each primer, 0.25 U of Taq polymerase (MBI Fermentas, St. Leon-Rot, Germany), buffer (10 mmol L^{-1} Tris-HCl pH 8.8, 50 mmol L^{-1} KCl, 0.08% Nonidet P-40, 0.1 mg ml–1 BSA, 1.5 mmol L^{-1} $MgCl_2$), 0.2 mmol L^{-1} deoxyribonucleoside triphosphates (dNTPs) and 2 μl of DNA. PCR conditions included an initial denaturation step at 94°C for 10 min, followed by 30 cycles of 94°C for 30 s, 42°C for 1 min and 72°C for 2 min. This was followed by a final extension of 72°C for 10 min. The PCR products were checked by electrophoresis of 5 μl of the product in a 1% agarose gel containing ethidium bromide (0.5 mg ml^{-1}). PCR products were purified using the MinElute PCR purification kit (Qiagen, Crawley, UK). The amplified fragments were sequenced at the Centre of DNA Studies in Poznań, Poland. Sequences were identified by comparison with reference sequences in the NCBI GenBank.

Sequences were obtained after sequencing together with referenced sequences and sequences of *O. ulmi*, *O. himal-ulmi* Brasier & M.D. Mehrotra, *O. piceae* (Münch) Syd. & P. Syd. In addition sequence of *Diaporthe eres* Nitschke were used to outgroup. This species was chose as a outgroup, because it was isolated from branches previously infected by *Ophiostoma*. The aligment of 484pz length was generated automatically with the aid of Clustal X version 1.8 (Thompson et al., 1997). The identical sequences were removed by manual operation and remained one which was representative. Next the phylogenetic analysis was carried out on the base of neighbor-joning method. To confirm reliability of tree clusters, the Bootstrap was used with 1000 replications. The level of phylogenetic analysis was accepted on > 60%. The analysis was carried out with the aid of MEGA 5.2 program (Tamura et al., 2011).

Results

The symptoms of DED were observed on every investigated plots. Disease appeared on all elm species and occurred with different intensity. Both rapid and slow disease course was noticed. In the rapid course of DED leaves wilted, dried out rapidly, turn dull green, died and remain attached to the twigs for some weeks. In slow case wilting leaves curl, turn yellow and brown and fall of after death. In particular on the same crowns of mature *U. laevis* both disease courses were observed in the same time. *Ulmus minor* was the most suffered species by DED and *U. laevis* was the less diseased. The disease incidence on plots differ from 5% to 35% of all elms specimens. In particular tree the simple branches were infested, but in the severe crown infestation was observed, even up to 70% of dead branches. Such DED intensity was observed independently of elm species and plot localization. Moreover in all cases the dying process was noted independently of elm species and tree age.

Isolates of *Ophiostoma* were collected from twigs and branches of elms from 19 locations. In rest (52%) samples the mycelium of others fungi was isolated from twigs and branches with DED symptoms. Alive pathogen's mycelium was noted in 55% of branch samples. *Ophiostoma* was isolated from all elm species, in addition from *U. laevis* the pathogen's mycelium was obtained from 6 locations (12 isolates), from *U. minor* from 9 locations (18 isolates) and from *U. glabra* the mycelium was isolates from 6 locations (11 isolates). On two localities Sulechów (23) and Łopuchówko (39) *U. minor* and *U. laevis* were infested by pathogen in the same time. On the base of molecular analysis all isolates belonged to the only species – *Ophiostoma novo-ulmi* (Fig. 1, Table 2). All isolates produced white mycelium and morphologically were identical. The *Sporothrix* conidiophores appeared abundantly on aerial hyphae especially growing on the SNA medium.

In ITS region there was detected a very low genetic diversity. Only two isolates had simple nucleotides changes. In isolate 39.3 at position 158 the adenine was altered by cytosine and in isolate 39.4 at position 260 the adenine was altered by thymine and at position 360 guanine was altered by cytosine. The rest isolates were identical. The isolate 24.1 was chosen as a representative sequence. All analyzed sequences were located in one cluster with *O. novo-ulmi* sequences. Other *Ophiostoma* isolates were located outside the *O. novo-ulmi* cluster. *Diaporthe eres* was made as an external group (Fig. 2).

Branches, inside which the *O. novo-ulmi* was not active, were colonized by other fungi. Partially there were mitosporic fungi from genus *Penicillium* or *Trichoderma,* but in many cases the weak pathogens, which cause the bark blight or branches decline occurred.

Fig. 1. Geographical distribution of *Ophiostoma novo-ulmi* isolates

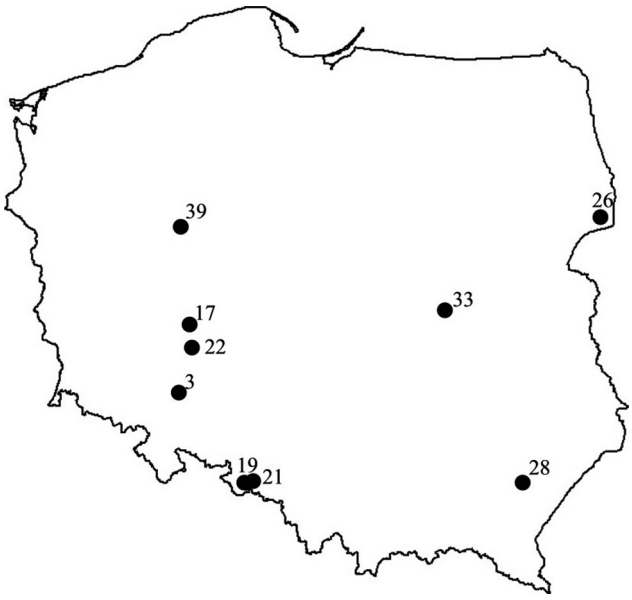

Fig. 3. Geographical distribution of other fungi isolated from elm branches

Seven species were identified *Gibberella avenacea* R.J. Cook, *Diaporthe eres*, *Diaporthe melonis* Beraha & M.J. O'Brien, *Aureobasidium pullulans* (de Bary & Löwenthal) G. Arnaud, *Nectria nigrescens* Cooke, *Gibberella baccata* (Wallr.) Sacc., *Fusarium sambucinum* Fuckel. The *Diaporthe eres* was the most often species isolated from branches (35%) (Fig. 3, Table 3).

Branches of *U. laevis* were colonized the most frequently by other fungi (64%) in comparison to other elm species (18% of each).

Table 2. Origin of *Ophiostoma* isolates

Plot no.	Location	Host	Isolates no.	Molecular identification	Probability %	No of referring sequence at NCBI
1	Miękinia	*Ulmus minor*	1	*Ophiostoma novo-ulmi*	100	KJ677112
2	Babki	*Ulmus glabra*	1	*Ophiostoma novo-ulmi*	100	KJ677112
9	Piaski	*Ulmus minor*	1	*Ophiostoma novo-ulmi*	100	KJ677112
14	Oborniki Śląskie	*Ulmus minor*	1	*Ophiostoma novo-ulmi*	100	KJ677112
19	Rudy	*Ulmus glabra*	1	*Ophiostoma novo-ulmi*	100	KJ677112
20	Babki	*Ulmus glabra*	4	*Ophiostoma novo-ulmi*	100	KJ677112
21	Rudy	*Ulmus laevis*	1	*Ophiostoma novo-ulmi*	100	KJ677112
22	Żmigród	*Ulmus minor*	1	*Ophiostoma novo-ulmi*	99	KJ677112
23	Sulechów	*Ulmus laevis*	1	*Ophiostoma novo-ulmi*	100	KJ677112
		Ulmus minor	1	*Ophiostoma novo-ulmi*	99	KJ677112
24	Rudka	*Ulmus minor*	2	*Ophiostoma novo-ulmi*	100	KJ677112
			2	*Ophiostoma novo-ulmi*	100	KJ677112
25	Rudka	*Ulmus laevis*	1	*Ophiostoma novo-ulmi*	100	KJ677112
26	Białowieża	*Ulmus glabra*	2	*Ophiostoma novo-ulmi*	100	KJ677112
27	Czarna Białostocka	*Ulmus glabra*	2	*Ophiostoma novo-ulmi*	100	KJ677112
28	Zwierzyniec	*Ulmus glabra*	1	*Ophiostoma novo-ulmi*	100	KJ677112
29	Chojnów	*Ulmus minor*	3	*Ophiostoma novo-ulmi*	100	KJ677112
30	Chojnów	*Ulmus minor*	3	*Ophiostoma novo-ulmi*	99	KJ677112
31	Dobieszyn	*Ulmus laevis*	3	*Ophiostoma novo-ulmi*	100	KJ677112
32	Grójec	*Ulmus laevis*	2	*Ophiostoma novo-ulmi*	100	KJ677112
39	Łopuchówko	*Ulmus laevis*	4	*Ophiostoma novo-ulmi*	100	KJ677112
		Ulmus minor	3	*Ophiostoma novo-ulmi*	100	KJ677112
		Ulmus minor	2	*Ophiostoma novo-ulmi*	99	KJ677112
		Ulmus minor	2	*Ophiostoma novo-ulmi*	99	KJ677112

Table 3. Fungi isolated from elm branches

Plot no.	Location	Host	Isolates no.	Molecular identification	Probability %	No of referring sequence at NCBI
3	Miękinia	*Ulmus minor*	1	*Gibberella avenacea*	99	EU255805
17	Piaski	*Ulmus glabra*	1	*Gibberella avenacea*	99	EU255805
19	Rudy	*Ulmus glabra*	1	*Diaporthe eres*	100	KJ210530
			1	*Gibberella avenacea*	100	JQ765664
21	Rudy	*Ulmus laevis*	2	*Diaporthe eres*	99	KC343073
22	Żmigród	*Ulmus minor*	1	*Fusarium sambucinum*	99	KM231813
26	Białowieża	*Ulmus glabra*	1	*Diaporthe eres*	100	KC343073
28	Zwierzyniec	*Ulmus laevis*	1	*Diaporthe eres*	99	KJ210518
33	Chojnów	*Ulmus minor*	1	*Diaporthe melonis*	99	JN032733
			1	*Diaporthe eres*	99	JQ765658
			1	*Aureobasidium pullulans*	99	JF440584
39	Łopuchówko	*Ulmus laevis*	3	*Gibberella avenacea*	99	EU255805
			2	*Diaporthe eres*	99	KC343073
			2	*Diaporthe melonis*	99	JN032733
			1	*Nectria nigrescens*	99	HQ897812
			2	*Gibberella baccata*	99	FN547471

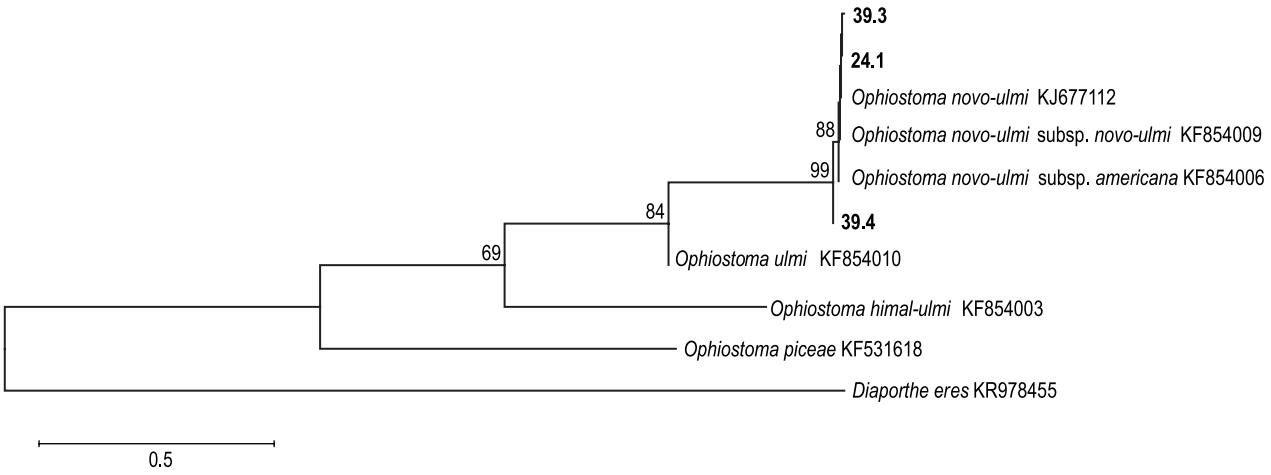

Fig. 2. Phylogenetic tree of *Ophiostoma novo-ulmi* isolates

Discussion

Ten years ago the investigation of DED in Poland confirmed occurrence of *O. ulmi* and two subspecies of *O. novo-ulmi*: subsp. *novo-ulmi* and subsp. *americana* (Guździoł et al., 2004). In addition the authors found the occurrence of isolates called as fax-waxy, described earlier by Brasier et al. (1998). Przybył et al. (2006) showed that isolated of *O. novo-ulmi* and "fax-waxy" were more pathogenic then *O. ulmi*. Brasier & Kirk (2010) found among 20 isolates collected from the Baltic Ports region of Poland in 1980 three exhibited the introgression of subsp. *americana* DNA.

Our study confirmed that *O. novo-ulmi* is the only *Ophiostoma* species isolated from diseased elms. Bartnik et al. (2015) also found that only *O. novo-ulmi* infested studied elms in four stands localized in Carpathians (SE Poland). Only 30% of elms didn't show a disease symptoms. Authors found that DED might play a significant role in promotion the appearance of a habitat suitable for a rare insect Rosalia longicorn (*Rosalia alpina* L.) within the species' range. In our study the symptoms intensity of infected elms differ from 5% to 70% of crown reduction, because of disease development. In addition only in 55% of investigated branches the alive mycelium of *O. novo-ulmi* was found, what prove that the infestation of these elms took place at least two years earlier. In the rest cases the DED agent was replaced by the other fungi.

In this study there were no possibility to identified isolates to the subspecies level on the base of analyzing region and referring sequences deposit. Sequences were deposited in NCBI data base for this region as a subspecies. There were a few sequences, and it

was difficult to compare, because there were no similar in whole its length. The differences between subspecies were based on occurrence additional nucleotide at the beginning or at the end of sequence. There were no differences in the shared part 670 pz of these sequences and in addition these deposited sequences were not confirmed in paper or any morphological data of *Ophiostoma* subspecies cultures. There were no genetic diversity of Polish population in analyzed ITS region. Two isolates among 45 differed from the others but only in one or two nucleotides in DNA. Both were isolated from the same plot. On the base of culture morphology all our isolates were similar and we did not isolate a waxy cultures as Guździoł et al. (2004) or Brasier et al. (1998). Similarly Santini et al. (2005) found that Italian elms were infected by both subspecies of *O. novo-ulmi – novo-ulmi* and *americana* and they didn't notice *O. ulmi*. Kirisits et al. (2001) noticed that *O. ulmi* disappeared from area of Austria. Most of the isolates, which they analyzed belonged to *O. novo-ulmi* and only few were described as a hybrid between *O. ulmi* and *O. novo-ulmi*. The hybrid between *O. novo-ulmi* subspecies occurrence was also suggested by Santini et al. (2005). In Spain *O. ulmi* as well as *O. novo-ulmi* subsp. *novo-ulmi* and subsp. *americana* were found on elms, moreover the occurrence of hybrids between species were confirmed (Solla et al., 2008). In other studies the genetic analysis recognized fragment of *O. novo-ulmi* subsp. *americana* DNA built in *O. novo-ulmi* subsp. *novo-ulmi*, what might have suggested that there was an hybrid between these subspecies (Konrad et al., 2002; Brasier & Kirk, 2010). Brasier & Kirk (2010) created a hypothesis of hybrid, that nowadays spread in Europe and could infest the elms as well as the parental pathogens. Elm resources in Poland were investigated on the base of forest service inventory lately (Napierała-Filipiak et al., 2014). They have noticed the increase of forest area dominated by elms since 1970's, the last inventory data (Głaz, 1986). Only in Sudety Mountains the decrease of elm population is visible. Data showed by authors might suggested the collapse of DED pandemic development. But in this study on many investigated stands and places with small elm groups both the diseased and healthy trees were observed. The DED agent is still present and common on the area of a whole country. Our observations confirm the previous data from Poland (Mańka et al., 1978) that *U. minor* is the most sever damaged by DED and the less *U. laevis*. Although in our study the number of isolates received from *U. leavis* was the highest, however, field studies suggest that this species prevails in the elm resources in Poland (Danielewicz, 2008; Filipiak & Napierała-Filipiak, 2015; Napierała-Filipiak et al., 2016). Data provided by Peterken & Mountford (1998) or Oheimb & Brunet (2007) showed that DED was able to decrease the elm population but very rarely cause the total elimination of this tree species. They observed the enhanced position of elm in stand, where the population decreased in the past. At the other hand there was a notice of decrease of DED on *U. glabra* in the north part of Norway and at the same time appearing the young generation of wych elm (Solheim et al., 2011). Other fungi species that were isolated from branches with DED symptoms belonged to the pathogen, weak pathogen, saprotrophs or even endotrophs. They colonized alive, dead or weekend tissue and alter *O. novo-ulmi*. For example a polyphagus species *D. eres* was described as pathogenic to more than 300 woody plant species, including *Populus* spp. *Carpinus* spp., *Magnolia* spp., *Prunus persica* (L.) Batsch, *Rubus* sp., *Juglans cinerea* L. and *Vitis vinifera* L. (Anagnostakis, 2007; Thomidis & Michailides, 2009; Vrandečić et al., 2011; Kaliterna, et al., 2012). The fungi that colonized elm twigs and branches after *O. novo-ulmi* disappearance have been noted in some papers in Polish. Different species of *Diaporthe*, *Nectria* and some species of *Fusarium* were found in dead or dying trees of several deciduous species. Thirty one fungal species were found in dead oak branches. Among them *Gibberella avenacea* and *Aureobasidium pullulans* were identified (Kwaśna & Siwecki 2002). Buttin and Kowalski (1986) investigated fungi that colonized dead twigs during natural pruning of branches of *Acer pseudoplatanus* L., *Alnus glutinosa* (L.) Gaertn., *Betula pendula* Roth, *Carpinus betulus* L. and *Fraxinus excelsior* L. *Diaporthe* spp., *Fusarium* sp. and *Nectria* spp. had been isolated from maple, birch, hornbeam and ash dead branches. Among several species of Ascomycota, Basidiomycota and Fungi imperfecti *D. acerina* (Peck) Sacc., *D. carpini* (Pers.) Fuckel, *Fusarium stilboides* Wollenw. (current name *Gibberella stilboides* W.L. Gordon ex C. Booth), *N. cinnabarina* (Tode) Fr. and *N, episphaeria* (Tode) Fr. (current name *Dialonectria episphaeria* (Tode) Cooke) were identifies. In particularly *D. carpini* was the most frequent species in hornbeam wood of dead twigs. Moreover *Nectria radicicola* Gerlach & L. Nilsson (current name *Ilyonectria radicicola* (Gerlach & L. Nilsson) P. Chaverri & Salgado) was also one of species colonizing diseased or dead young *Alnus inacana* (L.) Moench in industrial area in southern Poland (Domański & Kowalski, 1984) and in dead wood of oaks both in branches and trunks *Fusarium solani* (Mart.) Sacc. was one of several species that occupied necrotic tissue (Kowalski, 1991, 1996).

In conclusion the *Ophiostioma novo-ulmi* is the main agent of DED in Poland, and it seems that in spite of common pathogen occurrence in the country the existence of elms populations are not endangered. The population of *O. novo-ulmi* doesn't differ genetically. *Ulmus minor* is the most sensitive species to DED among elms occurring in Poland.

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