Contribution to Identify the Causal Agents of Dutch Elm Disease in the Czech Republic

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Abstract

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This study provides new data on Dutch elm disease in the Czech Republic. *Ophiostoma novo-ulmi* is reported for the first time in the area of the Czech Republic, as well as both subspecies ssp. *novo-ulmi* (indigenous in the area of the Ukraine and Moldavia), and ssp. *americana* indigenous in North America. The majority of the recorded strains belonged to *O. n.-u.* ssp. *novo-ulmi,* while *O. n.-u.* ssp. *americana* and hybrids of these two subspecies were found less frequently. On the other hand, *Ophiostoma ulmi* was not found at all in the investigated samples. Identification on the subspecies level was performed by methods of molecular biology, i.e. PCR and RFLP of gene regions *cu* and *col1*.

Keywords: *Ulmus*; *Ophiostoma novo-ulmi*; PCR; RFLP

Dutch elm disease (DED) is the most important disease of elm trees (*Ulmus* spp.) in Europe. The first record of DED in the area of the Czech Republic (former Czechoslovakia), probably caused by *Ophiostoma ulmi* (Buism.) Nannf., was noted by professor Peklo in elm alleys in Prague and Poděbrady in 1932 (Polák 1932). In the following years DED spread mainly in lowlands, especially in flood plain forests along the rivers Morava, Vltava, Ohře and Sázava (Kalandra & Pfeffer 1935). Subsequently, the disease spread throughout the former Czechoslovakia.

The period of the 1960's and 1970's is a turning point in the development of elm decline, when sudden dieback occurred in large parts of crowns or whole trees. In Western Europe the end of the 1960's is considered to be the beginning of aggressive progression of DED. This "aggressive" strain was first detected in Britain at the end of the 1960's (Gibbs & Brasier 1973), or in the Netherlands in 1972 (Jančařík 1999). According to some authors a new aggressive strain was imported on elm logs from Canada to Europe (Jančařík 1999). Brasier (1979) described the causal agent of the DED pandemic as two different subgroups of *Ophiostoma ulmi*. The first group is a less aggressive form which had caused DED from the 1920's to the 1940's. The second group, which succeeded the first one, is substantially more aggressive and has occurred from the end of the 1960's to the present day. This more aggressive

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form was then divided into two races – an Eurasian race (EAN) that probably originated in the area of Moldavia and the Ukraine, and a North American race (NAN) (Brasier & Kirk 2001). Brasier (1991) described this more aggressive form of *O. ulmi* as a new species, *O. novo-ulmi*. These two species differ in DNA characters, morphological features of the mycelium and they cannot spontaneously hybridise. Ten years later Brasier and Kirk (2001) designated races EAN and NAN as subspecies of *O. novo-ulmi* ssp. *novo-ulmi* and *O. novo-ulmi* ssp. *americana*.

In former Czechoslovakia the second period of DED was noticed earlier than in Western Europe. Rapid progression of DED was apparent since the early 1960's in the area of southern Moravia and southern Slovakia, but the record remained unpublished (Černý – personal communication). Although the symptoms of DED are often remarkable in the area of the Czech Republic, the causal agent of the disease has not been identified on the species and subspecies level up to now.

The aim of this paper is to give a first report on the current distribution of *Ophiostoma* species and subspecies causing DED in the Czech Republic.

MATERIALS AND METHODS

Infected elm trees (*Ulmus glabra* Huds., *U. minor* Mill. and *U. laevis* Pall.) were observed and their geographic location (coordinates and altitude) was estimated by GPS. Samples of twigs were cut

Table 1. Origin and identification of *Ophiostoma novo-ulmi* isolates in the CR

Isolate number	Locality name	Coordinates (WGS 84)	Altitude (m)	Isolator name	Species	Subspecies according to gene region	
						col1	*cu*
MUAF 930	Jilmová skála III	N 48°57.456' E 13°47.713'	1073	Dvořák M.		*novo-ulmi*	*novo-ulmi*
MUAF 931	Radešov	N 49°09.193' E 13°30.819'	555	Dvořák M.		*americana*	*americana*
MUAF 848	Brno, Lužánky I	N 49°12.374' E 16°36.580'	214	Dvořák M.		*novo-ulmi*	*novo-ulmi*
MUAF 849	Valtice, Rendes-vous	N 48°44.944' E 16°47.668'	208	Dvořák M.		*novo-ulmi*	*novo-ulmi*
MUAF 932	Brno, Lužánky II	N 49°12.376' E 16°36.744'	215	Dvořák M.		*novo-ulmi*	*novo-ulmi*
MUAF 933	Strážovice	N 49°00.960' E 17°02.230'	310	Dvořák M.		*americana*	*novo-ulmi*
MUAF 934	Lanžhot	N 48°42.300' E 16°57.953'	233	Dvořák M.		*americana*	*novo-ulmi*
MUAF 935	Sádek I	N 49°42.697' E 16°13.244'	560	Dvořák M.		*novo-ulmi*	*novo-ulmi*
MUAF 936	Sádek II	N 49°42.860' E 16°12.983'	555	Dvořák M.		*novo-ulmi*	*novo-ulmi*
MUAF 937	Bedrč I	N 49°48.490' E 14°42.955'	314	Dvořák M.		*novo-ulmi*	*novo-ulmi*
MUAF 938	Řetenice I	N 49°07.834' E 13°36.757'	881	Dvořák M.	*O. novo-ulmi*	*novo-ulmi*	*novo-ulmi*
MUAF 939	Vrabcov	N 49°13.282' E 13°31.022'	483	Dvořák M.		*novo-ulmi*	*novo-ulmi*
MUAF 940	Divišov	N 49°12.316' E 13°30.511'	502	Dvořák M.		*americana*	*novo-ulmi*
MUAF 941	Nové Městečko	N 49°11.144' E 13°29.836'	517	Dvořák M.		*novo-ulmi*	*novo-ulmi*
MUAF 942	Rejštejn	N 49°07.921' E 13°30.015'	581	Dvořák M.		*americana*	*novo-ulmi*
MUAF 943	Brno, MUAF	N 49°12.629' E 16°36.884'	244	Dvořák M.		*novo-ulmi*	*novo-ulmi*
CPPF 332	Kančí obora I	N 48°46.102' E 16°51.495'	165	Novotný D.		*novo-ulmi*	*americana*
CPPF 331	Kančí obora II	N 48°46.217' E 16°51.502'	165	Novotný D.		*novo-ulmi*	*novo-ulmi*
CPPF 333	Průhonice	N 50°00.023' E 14°33.663'	305	Černý K.		*novo-ulmi*	*novo-ulmi*
CPPF 334	Hrádek u Sušice	N 49°16.789' E 13°28.568'	513	Černý K.		*americana*	*novo-ulmi*
CPPF 277	Kančí obora III	N 48°46.173' E 16°51.311'	165	Kolařík M.		*novo-ulmi*	*novo-ulmi*

MUAF – Culture collection of Mendel University of Agriculture and Forestry in Brno, Faculty of Forestry and Wood Technology, Department of Forest Protection and Game Management, Brno

CPPF – Collection of Phytopathogenic Fungi, Crop Research Institute, Prague-Ruzyně

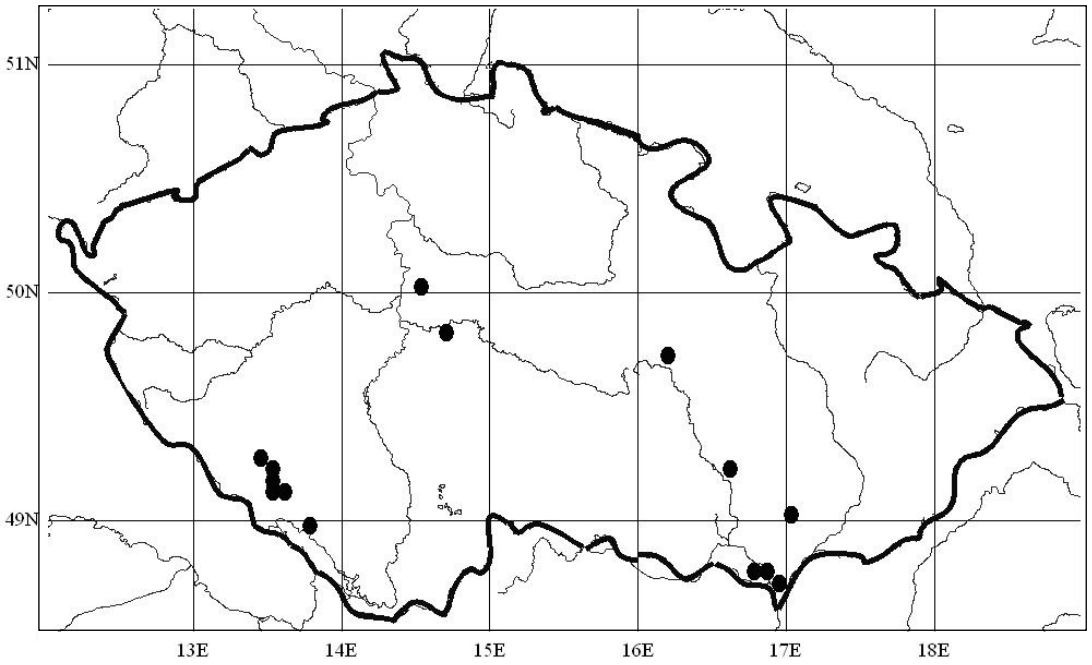

Figure 1. Geographic locations where in the Czech Republic the examined *Ophiostoma novo-ulmi* isolates originated

from different parts of dying crowns, and samples infested by the beetles *Scolytus multistriatus* Mrsh. or *S. scolytus* Fabr. were also taken. Pieces without bark about 1 cm long were placed on plates with 3% malt extract agar (MEA) with addition of cycloheximide and incubated at room temperature. The beetles or sawdust particles from beetle galleries were aseptically placed on 2% MEA and incubated at room temperature. Typical fibrous-striate mycelium of *Ophiostoma ulmi* s. l. was subsequently inoculated onto a new plate with MEA and after a week it was prepared for DNA isolation. For this step a PowerSoil DNA Kit (Mo Bio) or DNeasy Plant Mini Kit (Qiagen) was used. The species and subspecies were distinguished by Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) of two gene regions: the cerato-ulmin gene (*cu*) (for details see Pipe *et al*. 1997) and gene *col1* that encodes colony type (according to Konrad *et al*. 2002). The PCR-reactions were performed with DyNAzyme II (Finnzymes) DNA-polymerase according to the papers cited above. Amplified fragments of the two genes were cleaved by restriction endonucleases, *Hph* I (Fermentas) for gene *cu*, and *Bfa* I (New England Biolabs) for gene *col1* according to the manufacturers' instructions. Gene *col1* was purified before cleavage using NucleoSpin Extract II (Macherey-Nagel). RFLP fragments were visualised on 3% agarose gel and evaluated according to Konrad *et al*. (2002) and Pipe *et al*. (1997).

The investigated strains were deposited in the culture collection at Mendel University of Agriculture and Forestry in Brno, Faculty of Forestry and Wood Technology, Department of Forest Protection and Game Management, Brno, Czech Republic, and in the Collection of Phytopathogenic Fungi, Crop Research Institute, Prague-Ruzyně, Czech Republic. DNA sequences of *cu* and *col1* genes of selected strains were submitted to GenBank database (accession numbers EU006078–EU006087).

RESULTS AND DISCUSSION

Twenty-one strains of *O. ulmi* s. l. were isolated. Geographic locations (coordinates and altitude) where the samples originated are presented in Table 1 and Figure 1. They came from East, South, Central and West Bohemia and South Moravia. All isolated strains were identified as *O. novo-ulmi*. Both subspecies *O. novo-ulmi* ssp. *novo-ulmi* and *O. novo-ulmi* ssp. *americana* were found as well as their hybrids. The complete absence in the sample of *O. ulmi* confirms the results of many other authors (Gremmen *et al*. 1976; Brasier 1983; Hoegger *et al*. 1996), who suppose that in Europe *O. novo-ulmi* had replaced *O. ulmi* by the end of the 1970's.

Fourteen isolates were *O. novo-ulmi* ssp. *novo-ulmi*, one isolate belonged to ssp. *americana,* and six isolates were identified as hybrids between the subspecies. In five of these hybrid strains the RFLP patterns of gene *cu* corresponded to ssp. *novo-ulmi,* and those of gene *col1* to ssp. *americana*. One isolate appeared to be the inverse hybrid of subspecies, that means patterns of gene *cu* indicated ssp. *americana,* and those of gene *col1* ssp. *novo-ulmi* (Table 1).

Geographical distances between trees infected by different subspecies or hybrids can be remarkably small. In West Bohemia, many infected elms occur along the Otava River south of the town Sušice. From this ca 15 km long segment of bank forest five samples were collected (No. 931, 939, 940, 941 and 942). The used methods revealed that two of the resulting isolates belong to ssp. *novo-ulmi,* one belongs to ssp. *americana,* and the remaining two are hybrids of the subspecies. The situation is similar in the floodplain forest Kančí obora near Břeclav (samples No. 331, 332 and 277).

The occurrence of hybrids between *O. novo-ulmi* ssp. *americana* and ssp. *novo-ulmi* confirms earlier results of Konrad *et al*. (2002) who found only three subspecies hybrids among 20 Austrian isolates. Konrad *et al*. (2002) mentioned some hybrid isolated in Germany already in 1980. Hoegger *et al*. (1996) also identified one Swiss isolate as a hybrid of subspecies, because the genetic pattern acquired by RAPD markers did not correspond with fertility tests. However, to assign subspecies to isolates mainly on the basis of analyses of the *cu* and *col1* regions may not be sufficient. Both genes are situated on chromosome IV, but hybridisation at another chromosome could not be excluded. It will be necessary to include other nuclear genes from different chromosomes, as well as mitochondrial genes or the whole genome (AFLP), to obtain reliable results on the occurrence and frequency of hybrids (Konrad *et al*. 2002). The presence of hybrids within *Ophiostoma novo-ulmi* supports the presumptions of Konrad *et al*. (2002) that hybrids may acquire resistance against mycoviruses which could then have considerable influence on the epidemiology of DED. This may be the explanation for the occurrence of hybrids in the areas of the currently more expanded DED.

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