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The occurrence of endophytic fungus *Phomopsis oblonga* on elms in the area of southern Bohemia

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ABSTRACT: The health condition of the population of elms in the region of southern Bohemia was studied from the viewpoint of their decline, the occurrence of Dutch Elm Disease (DED) and the presence of other diseases. Of the total number of 105 elms in total 33 of them were without any symptoms of the disease or other damage. Elms regenerated quite spontaneously in the neighbourhood of mother trees and their increasing population in mixed forests is hopeful. According to macroscopic symptoms, DED was identified in 10 trees but the presence of pathogens *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* was not identified in isolations. A possible reason of this observation is overgrowing the colonies by the *Phomopsis oblonga* mycelium. This fungus was identified in most isolations. Thus, its role requires further research.

Keywords: elm; *Ulmus*; Dutch Elm Disease; *Ophiostoma ulmi*; *Ophiostoma novo-ulmi*; *Phomopsis oblonga*

In 1918, the first symptoms appeared of elm dieback in northern France. However, mechanical damage to trees during the World War I was considered to be the main reason.

The origin of Dutch Elm Disease (DED) is not clear. A hypothesis supposing the origin of DED in eastern Asia and then its importation to Europe during the World War I appears to be most probable. It is indicated by the existence of more or less resistant elms in China, Japan and Korea (Blažek 1970). According to Jančařík (1999) and Kalandra and Pfeffer (1935), Spierenburg links the disease with the attack of large elm beetle *Scolytus scolytus* and with several species of fungi since 1921. The most important of which is considered to be an anamorphic species *Graphium penicillioides* Corda. In 1922, Schwarz determined an anamorphic species *Graphium ulmi* Schw. as the causal agent of elm decline. A relationship with a teleomorph stage *Ceratostomella ulmi* (Schw.) Buism. was demonstrated by Buisman (1934); at present, the causal agent of the disease is ranked among the genus *Ophiostoma* as *O. ulmi* (Buism.) Nannf. Also the anamorphic stage is classed to the genus *Pesotum* as *P. ulmi* (Schw.) Crane et Schoknecht.

The disease quickly spread throughout Europe (with the exception of the Alps and Scandinavian countries). In spite of the endeavour of American plant pathologists and their stressed warning the disease was imported to North America at the end of the 20th of the previous century. Until the World War II, Dutch Elm Disease affected also the Alps and Scandinavia and in 1944 also Canada (Jančařík 1976; Kučera 1991).

In the area of former Czechoslovak Republic, DED occurred most probably at the end of the 20th. In 1920, professor Farský drew attention to elm decline in alleys in Brno. The first occurrence of DED was noted by professor Peklo who found the disease in elm alleys in Prague and Poděbrady (Blažek 1970; Kučera 1991).

The period of the 60s and the 70s is a marked limit in the development of elm decline. Sudden dieback occurred of important parts of crowns or

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whole trees as against previous manifestations. In western Europe, the end of the 60, viz the year 1968 is considered to be the beginning of this aggressive course of DED. Some sources mention that an aggressive strain was imported on elm logs from Canada to Europe being first detected in the Netherlands in 1972 (Jančařík 1999). Brasier (in Wingfield et al. 1993) describes the causal agent of the DED pandemic as two different subgroups of *Ophiostoma ulmi*. The first subgroup is a less aggressive form which caused DED in the 20–40s while the second subgroup is a substantially more aggressive form which is a follow-up of the first form and occurs up to the present day. This newer and more aggressive form was then distinguished to two races, viz a Euroasian (EAN) and a North American race isolate (NAN). In 1991, the more aggressive form was described by Brasier as a separate species *Ophiostoma novo-ulmi* Bras. The new species was differentiated particularly due to different DNA of an aggressive and non-aggressive form, impossibility of their spontaneous hybridization and morphological differences in their mycelium. In 2001, Brasier and Kirk designated races EAN and NAN as subspecies of *Ophiostoma novo-ulmi* ssp. *novo-ulmi* Bras. and *Ophiostoma novo-ulmi* ssp. *americana* Bras. et Kirk.

A fact has not been explained so far that in the region of southern Hungary, the mass decline of elms was observed as early as at the end of the 50s, in southern Slovakia and southern Moravia at the beginning of the 60s. At present, it is not evident if this decline has been induced by *Ophiostoma ulmi* or by *Ophiostoma novo-ulmi*.

The aim of the paper is to assess the present health condition of elms in selected localities of the Czech Republic with respect to the occurrence of the DED causal agents *Ophiostoma ulmi* or *O. novo-ulmi* or perhaps even other fungal pathogens.

MATERIAL AND METHODS

Within field studies, elm trees were mapped in the region of southern Bohemia. Mensurational characteristics of examined stems were determined and their present health condition was recorded and documented.

In trees with symptoms of decline samples were taken to be processed in a laboratory. If possible, samples of twigs were taken at the dividing line of living and dying or dead parts of trees.

The samples were used for the cultivation of fungal pathogens on a nutrient medium. From each of the samples disks were separated and surface-sterilized using a standard procedure in 7% solution of sodium hypochlorite and subsequently 96% ethanol. Before a transfer to malt agar medium (MEA) on Petri dishes, samples were let surface dry on a sterile gauze. The cultivated cultures were reinoculated for the subsequent determination on newly prepared Petri dishes with agar or for more durable conservation on oblique agar in glass test tubes.

RESULTS AND DISCUSSION

Within studies carried out in the region of southern Bohemia, elms were examined in 105 localities. The attack of shoots by an ascomycetous fungus *Phomopsis oblonga* (Desm.) Hoehn., namely in 18 elm trees, was most frequent. The second most frequent damage are false leaf galls caused by an aphid *Eriosoma ulmi* L., namely in 17 cases. Discoloration of conductive tissues occurred in 14 elms. In total, 33 elms of the total number of 105 elms were quite sound (Fig. 1).

External symptoms of DED were evident only in 10 elms (Fig. 1). In no isolate obtained by a method placed small pieces of wood direct on nutrient medium either *Ophiostoma ulmi* or *O. novo-ulmi* were found. An endophytic fungus *Phomopsis oblonga* (53% isolations) is the most frequent species determined in isolations. Fungi of the genus *Fusarium* were also frequent (15% isolations) (Fig. 2).

Even in one isolate *Ophiostoma ulmi* or *Ophiostoma novo-ulmi* were not found. This finding is not probable as compared with all literature sources (Jančařík 1999; Blažek 1970; Kučera 1991; Potoček et al. 1986, etc.). The spread of DED is evidently affected by the infection of weakened trees by a fungus *Phomopsis oblonga* which was identified in 18 samples from 34 isolations. In the course of isolations, the fungus overgrowths the mycelium of ophiostomatoid fungi. Gibbs et al. (1994), Jančařík (1999), Jassim et al. (1990), Ken-

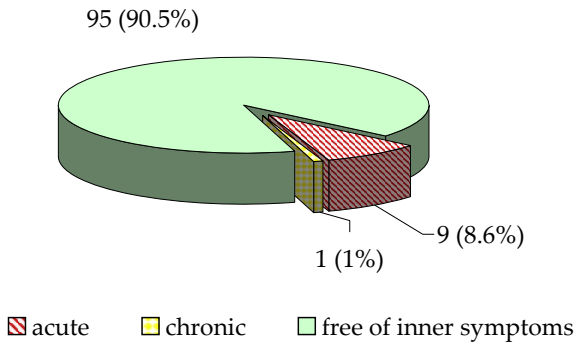

Fig. 1. The proportion of elms with macroscopic symptoms of Dutch Elm Disease

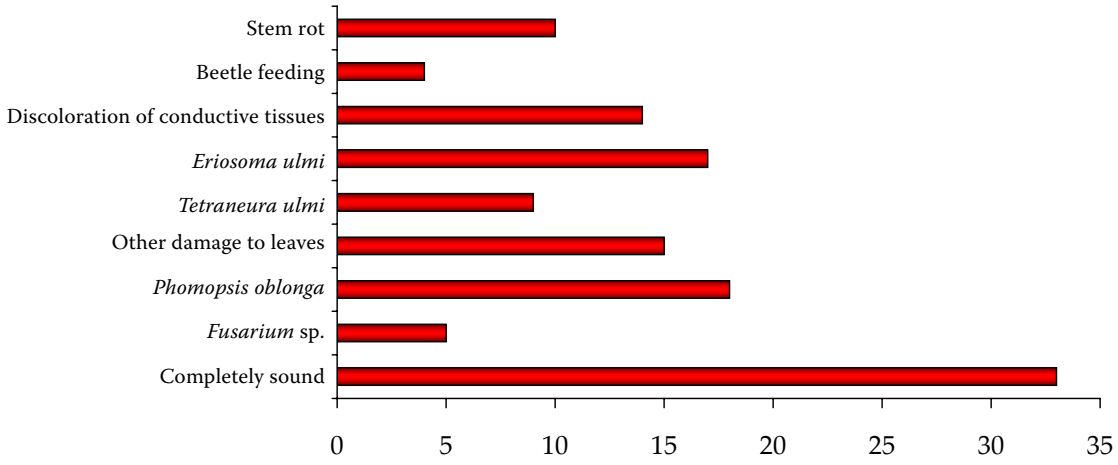

Fig. 2. The frequency of symptoms and the presence of pathogens on elms. Numeric data express the number of trees on which the fact was found

drick (2001), O'Callaghan et al. (1984), Webber (1981), and Webber et al. (1984) mention accordingly that phloem attacked by *Phomopsis oblonga* is then non-attractive for the reproduction of the DED vector coming from the genus *Scolytus.* Thus, it would result in the inhibition of DED through the decrease of trophic and reproduction possibilities of the disease vector the population of which would decline due to the fact.

The presence of *Phomopsis oblonga* was tested by Webber and Gibbs (1984) in various parts of England. In northern and western England, more than 40% samples were infected and in some parts even over 80% of otherwise sound trees free of any symptoms of DED. He reached a view that *Phomopsis oblonga* colonized most frequently the outer bark of wych elm *Ulmus glabra*. In case of DED infection, its mycelium spreads to phloem parts where lesions occur, viz most frequently at a contact with xylem affected by *Ophiostoma* spp., by the typical blackening of vascular bundles. These lesions of elongated form are sometimes surrounded with black lines bordering variously coloured phloem. In the Czech Republic, *Phomopsis oblonga* often colonizes the bark of *Ulmus glabra.* During infection, it comes also to the region of phloem where creates minute necrotic lesions at a contact with vascular bundles attacked by the mycelium of *Ophiostoma* spp.

It is possible to confirm that through the colonization of *Phomopsis oblonga* the bark and phloem of elms become non-attractive for the invasion and reproduction of DED vectors, namely *Scolytus* spp. Thus, the beetles do not spread conidia of *Ophiostoma* spp. to other living trees (Gibbs 1994; Jančařík 1999; Jassim et al. 1990; Kendrick 2001; O'Callaghan et al. 1984; Webber 1981; Webber, Gibbs 1984).

From the methodical point of view it is possible to admit that at the inoculation of MEA directly with small pieces of wood the overgrowth occurs of the ophiostomatoid fungi mycelium by other fungi if these fungi are not early isolated from growing colonies. The method of moist chamber described by Potoček et al. (1986) appears to be an alternative. From the twig sample anamorphous fruiting bodies of the pathogen would be grown first in the moist chamber. Subsequently, the fruiting bodies would be inoculated on an agar medium.

According to macroscopic characters, of the total number of 105 localities 10 of them are attacked by DED. It is of interest to observe the spread of DED in relation to altitude. The most critical localities along a road from Rejštejn to Sušice including tens of declining elms are situated at an altitude above 550 m, namely in the inverse valley of the Otava river. The locality of Řetenice occurs an altitude of 880 m, the Jilmová skála locality and DED suspicious localities in the Novohradské hory Mts. even at an altitude about 1000 m. Thus, an assumption has been proved that *Ulmus laevis* is more resistant to infection than *Ulmus glabra.* Through the assessment of macroscopic characters DED would be confirmed only in *Ulmus glabra*.

CONCLUSION

It is possible to state that at present the health condition of elms in southern Bohemia is stabilized from the viewpoint of symptoms of decline. Of the total number of 105 registered elms 33 of them are free of symptoms of the disease or other damage.

Elms regenerate in the vicinity of mother trees their proportion being stable in mixed stands of younger age classes. According to macroscopic symptoms DED was identified in 10 elms of the total number of 105 examined trees. In laboratory isolations by the method placed small pieces of wood direct on nutrient medium, *Phomopsis oblonga* was the most frequent fungus proved in 18 declining elms.

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Výskyt endofyta *Phomopsis oblonga* na jilmech v jižních Čechách

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ABSTRAKT: Zkoumali jsme zdravotní stav populace jilmů v oblasti jižních Čech z hlediska jejich chřadnutí, výskytu projevů grafiózy jilmů a přítomnosti dalších chorob. Z celkového počtu 105 šetřených jilmů bylo celkem 33 bez jakýchkoliv symptomů onemocnění nebo jiného poškození. Jilmy se celkem živelně zmlazují v okolí svých mateřských stromů a jejich zastoupení ve smíšených lesích dává tedy naději na pozvolný růst. Podle makroskopických symptomů byla grafióza jilmů identifikována u 10 jilmů. Štěpinkovou metodou se nepodařilo prokázat přítomnost *O. ulmi* ani *O. novo-ulmi.* Možnou příčinou je přerůstání izolátů houbou *Phomopsis oblonga*, která byla zaznamenána v největším počtu vzorků. Její úloha vyžaduje další studium.

Klíčová slova: jilm; *Ulmus*, grafióza jilmů; *Ophiostoma ulmi*; *Ophiostoma novo-ulmi*; *Phomopsis oblonga*

V roce 1922 určila Schwarzová jako původce chřadnutí jilmů anamorfní druh *Graphium ulmi* Schw. Podle místa prvního nálezu se choroba, kterou tato houba v cévních svazcích způsobuje, nazývá holandská nemoc jilmů (Dutch Elm Disease). V ČR je ovšem běžnější název grafióza jilmů, odvozený od onoho původního názvu anamorfního stadia původce [dnes *Pesotum ulmi* (Schw.) Crane et Schoknecht]. Teleomorfní stadium bylo nejprve objeveno Buismannovou (1934) a pojmenováno *Ceratostomella ulmi* (Schw.) Buism. V současné době je nejužívanější název *Ophiostoma ulmi* (Buism.) Nannf. Na území bývalé Československé republiky se grafióza jilmů s největší pravděpodobností objevila koncem dvacátých let a téměř zlikvidovala populace našich jilmů – postupně od nižších nadmořských výšek až do hor. Výrazným předělem ve vývoji chřadnutí jilmů jsou šedesátá a sedmdesátá léta, kdy proti dřívějším projevům docházelo k náhlému odumírání významných částí buď koruny, nebo celé dřeviny. V roce 1991 popsal agresivnější formu Brasier jako samostatný druh *Ophiostoma novo-ulmi* Bras. a v roce 2001 označili Brassier a Kirk její geografické rasy EAN (euroasijská) a NAN (severoamerická) jako poddruhy *Ophiostoma novo-ulmi* ssp. *novo-ulmi* Bras. a *Ophiostoma novo-ulmi* ssp. *americana* Bras. et Kirk.

Dosud nevysvětlená je skutečnost, že v oblasti jižního Maďarska bylo masové chřadnutí jilmů pozorováno již koncem padesátých let, na jižním Slovensku a jižní Moravě na počátku šedesátých let. V současnosti není zřejmé, zda toto chřadnutí bylo vyvoláno druhem *Ophiostoma ulmi* nebo již druhem *Ophiostoma novo-ulmi*.

Cílem příspěvku je zhodnotit současný zdravotní stav jilmů na vybraných lokalitách České republiky právě s ohledem na výskyt původců grafiózy jilmů *Ophiostoma ulmi*, resp. *O. novo-ulmi*, případně dalších houbových patogenů, kteří mají na jejich šíření vliv.

Výsledky šetření byly překvapivé. Vnějším příznakům grafiózy jilmů odpovídalo pouze 10 jilmů. Většina z grafiotických jilmů byla situována ve vyšších nadmořských výškách, někdy až kolem 1 000 m n. m. V žádném izolátu získaném štěpinkovou metodou nebyla zachycena ani *Ophiostoma ulmi*, ani *O. novo-ulmi*. Z hlediska metodického je možné připustit, že při štěpinkové metodě dochází k přerůstání mycelia ophiostomatálních hub dalšími houbami, pokud nejsou tyto houby včas odizolovány z narůstajících kolonií. Nejčastějším determinovaným druhem v izolacích byla endofytická houba *Phomopsis oblonga* (53 % izolací), která má zřejmě vliv i na šíření grafiotické infekce.

Phomopsis oblonga nejčastěji kolonizuje kůru jilmu horského (*Ulmus glabra*). V případě nákazy grafiózou jilmu se pak rozšiřuje mycelium do lýkové části, kde vznikají léze, a to nejčastěji na kontaktu s xylémem napadeným houbami *Ophiostoma* spp., typickým černáním cévních svazků. Vzhledem k šíření grafiózy je podstatné, že lýko napadené *Phomopsis oblonga* je dále neatraktivní pro přenašeče grafiózy jilmů z rodu *Scolytus*, kteří pod kůrou jilmů obvykle zakládají nové generace. Jedná se tedy o přirozenou bioregulaci grafiózy prostřednictvím snížení potravních a rozmnožovacích příležitostí jejího vektora.

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