CZECH MYCOL. 56 (1–2), 2004

The first record of Cryphonectria parasitica in the Czech Republic

LIBOR JANKOVSKÝ[1], PAVLÍNA HALTOFOVÁ[1], GABRIELA JUHÁSOVÁ[2], MAREK KOBZA[2], KATARÍNA ADAMČÍKOVÁ[2] and DAGMAR PALOVČÍKOVÁ[1]

[1] Mendel University of Agriculture and Forestry,
Faculty of Forestry and Wood Technology, Department of Forest Protection and Game Management, Zemědělská 3, 613 00 Brno, Czech Republic
e-mail: jankov@mendelu.cz
[2] Institute of Forest Ecology, Slovak Academy of Sciences, Branch of Woody Plants Biology, Akademická 2, 949 01 Nitra, Slovak Republic

Jankovský L., Haltofová P., Juhásová G., Kobza M., Adamčíková K. and Palovčíková D. (2004): The first record of Cryphonectria parasitica in the Czech Republic. – Czech Mycol. 56: 45–51

The causal agent of chestnut blight *Cryphonectria parasitica* (Murrill) M. E. Barr is a quarantine pest that has been recorded for the first time on the territory of the Czech Republic. *Cryphonectria parasitica* was observed in a sweet chestnut in the town of Uherský Brod. Infected tree was imported as a two-year-old seedling from Bratislava (Slovakia), 25 years ago. The isolate of *Cryphonectria parasitica* has been compatible with European vc type 13 (EU 13). *Castanea sativa* Mill. occurs in more than 293 localities in the Czech Republic. Its state of health was checked in 232 localities.

Key words: *Castanea sativa*, chestnut blight, Czech Republic, *Cryphonectria parasitica*, quarantine pest

Jankovský L., Haltofová P., Juhásová G., Kobza M., Adamčíková K. a Palovčíková D. (2004): První nález Cryphonectria parasitica v České republice. – Czech Mycol. 56: 45–51

Původce rakoviny kůry kaštanovníku *Cryphonectria parasitica* (Murrill) M. E. Barr byl v ČR zjištěn poprvé. Infikovaný kaštanovník v Uherském Brodě byl dovezen ze Slovenska, konkrétně z Bratislavy, před 25 lety jako dvouletá sazenice. Izolát *Cryphonectria parasitica* byl kompatibilní s evropským kmenem EU 13. Kaštanovník jedlý se vyskytuje v ČR na více než 293 lokalitách. Kontrola zdravotního stavu byla provedena na 232 z nich.

INTRODUCTION

Chestnut blight fungus *Cryphonectria parasitica* (Murrill) M. E. Barr (syn. *Endothia parasitica* (Murrill) P. J. Anderson et H. W. Anderson) is one of the most serious threats to sweet chestnut. The disease comes from Asia where it occurs on local species of chestnut such as *Castanea crenata* Sieb. et Zucc., *C. mollissima* Bl., *C. seguinii* Dode, *C. henryi* (Skan) Rehder et Wilson, and on representatives of the genus *Castanopsis* (D. Don) Spach. Its unintentional introduction to the American continent is dated back to 1902 or 1904. The infection spread rapidly throughout the

North-American continent. On the European *Castanea sativa*, however, the disease was noticed in about 1880, in the region of the Caucasus (Pridnya et al. 1996). In the region of Western Europe the first data come from Belgium and England in 1925 (Anonymus 1950 ex Juhásová 1999). In France, it was identified on a chestnut tree of Asian origin in 1936 (Darpoux 1948). In 1938, the chestnut blight was detected near Genoa in Italy (Biraghi 1946 ex Juhásová 1999). Damage to the European chestnut *Castanea sativa* has not reached such an extent as the damage to the American chestnut *Castanea dentata*. One of the factors in slowing down the progress of infection in Europe is the simultaneous occurrence of hypovirulent strains of the fungus (Heiniger and Stadler 1991, Seemann and Unger 1993).

In the region of the former Czechoslovakia, chestnut blight was identified at the locality of Prašice – Duchonka, district Topoľčany, Slovakia in 1976 (Juhásová 1999), about 60 km from the Czech border. In the region of the Czech Republic, the disease had not been observed until 2002.

The objective of our research was to assess the state of health of chestnuts with the aim to exclude or confirm the occurrence of chestnut blight fungus (*Cryphonectria parasitica*) in the Czech Republic.

MATERIAL AND METHODS

The distribution and state of health of *Castanea sativa* Mill. in the Czech Republic (CR) was monitored in 232 localities (about 600 trees) out of 293 well-known (Haltofová and Jankovský 2003, 2004). This is about 80 % of the Czech population of chestnuts older than 20 years. Included were different plantings in villages or in forest stands where individual examinations could not be carried out in each of the trees. The tree position was surveyed using GPS for further processing in a GIS.

Voucher specimens are deposited in the herbarium of the Department of Forest Protection, Faculty of Forestry and Wood Technology, Mendel University of Agriculture and Forestry Brno (BRNL).

Isolation of strains

The strain from the first find of *Cryphonectria parasitica* in the CR was used for study. Samples of bark were washed in sterile water, surface-sterilised (96 % ethanol 1 min., sterile water, dipping in ethanol), placed on 3 % (weight) malt extract agar (MEA 3) and incubated at room temperature.

Growth of the isolated strain was tested on 3 % malt extract agar (MA3) at five different temperatures (10, 15, 20, 25 and 30 °C). Mycelium of the tested strains

was transferred to Petri dishes in three replicates per temperature. The cultures were cultivated in the dark.

Beginning with the fourth day after inoculation, the growth of the strains was observed regularly every day. The aspect of colonies was recorded using a digital camera, and pictures were further analysed using a system of image analyses in which the surface of the colonies was measured. The period of fructification was also monitored.

The isolated strain has been deposited in the Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno, CR under no. CCM 8354.

Determination of vegetative compatibility with EU vc types

The vegetative compatibility (hereafter vc) test for *C. parasitica* was performed according to Cortesi, Milgroom and Bisiach (1996). We used PDAg (Potato dextrose agar green) medium described by Powell (1995): 24 g Difco potato dextrose broth, 2 g yeast extract, 7 g malt extract, 0.8 g tannic acid, 2 mg biotin, 2 mg thiamin, 100 mg methionine, 20 g agar per litre of distilled water and the pH indicator bromocresol green 50 $mg.l^{-1}$. Instead of Difco potato dextrose broth we used broth prepared as follows. 200 g skinned potatoes were cooked in one litre of distilled water for one hour. Then they were percolated through gauze. The broth was filled up to one litre with distilled water, and 20 g glucose was added.

Mycelial plugs were removed from the margin of actively growing colonies of *C. parasitica* from 2 % malt agar. Pairs of plugs were placed in contact with the mycelial surfaces on the medium. Each of six pairs of plugs were placed in each of the Petri dishes (9 cm in diameter) with 20 ml of PDAg per plate, about 5 mm from the edge of the dish. The dishes were incubated at 24 °C in the dark and then scored after 5–6 days of cultivation (Cortesi, Milgroom and Bisiach 1996). Each vc test was replicated in a different dish. Incompatible reactions exhibited a demarcation, a dark discoloration in the reaction zone between the colonies in agar, as viewed on the bottom of a Petri dish. Compatible reactions were distinguished by merged colonies with no detectable line (Powell 1995). The isolate of *C. parasitica* CCM 8354 was compared with European testers (EU) of *C. parasitica*. Vc types were labelled with the acronym EU, followed by progressing numbers and may constitute the base for a common European nomenclature. EU testers were established to compare the distribution of vc types of *C. parasitica* in Europe. The original European testers of *C. parasitica* are deposited in ATCC (American Type Culture Collection, www.ATCC.org).

RESULTS

From the total number of assessed trees the causal agent of chestnut blight *Cryphonectria parasitica* was observed only on single, 27 years old tree which was imported as a two-year-old plant from Slovakia, namely from Bratislava, 25 years ago. Localisation: Uherský Brod (eastern Moravia), Za Humny st., co-ordinates: 49° 01' 33" N, 17° 39' 11" E, in a private garden; 27-year-old tree; height 5 m, girth 95 cm (measured at the ground), date: 19^{th} July 2002, rev. 16^{th} October 2002, leg. Pavlína Haltofová, det. Haltofová, Jankovský, Palovčíková, rev. Juhásová. The infected chestnut tree was conspicuous by a fissured longitudinally scaling of the bark and orange-coloured pycnidia of the fungus. Under the bark, a characteristic fan-shaped mycelium was found. In addition to *Cryphonectria parasitica*, fruit bodies of other fungi such as *Coryne sarcoides* (Jacq.) Tul. et C. Tul. and *Stereum hirsutum* (Willd.: Fr.) Pers. were identified in the attacked tree. According to the decision of the State Phytopathological Administration, the infected chestnut tree was felled and removed in March 2003. The isolate of *Cryphonectria parasitica* has been compatible with European vc type 13 (EU 13).

Characteristics of the strain behaviour in a culture

At the beginning, *Cryphonectria parasitica* forms a white mycelium on MA3. Later on, orange fruit bodies are formed. The temperature optimum for the process was 25 °C (Fig. 2). The mycelium, however, fully colonised the surface of the Petri dishes in the course of 3 weeks even at a temperature ranging between 15 and 25 °C. At a temperature of 30 °C, the growth soon stopped. However, the mycelium continued to grow even at a temperature of 10 °C. The culture anamorph fructified first at a temperature of 20 °C, viz. 9 days after inoculation. At a temperature of 25 °C fruit bodies were formed beginning with the 10th day, and one day later the formation of fruit bodies was noticed in cultures cultivated at a temperature of 15 °C. At marginal temperatures of 30 and 10 °C, the formation of fruit bodies was not detected in the course of 22 days.

DISCUSSION AND CONCLUSION

Although the spread of the chestnut blight causal agent by wind is quite common, the occurrence of the disease in an absolutely isolated tree is rather interesting. No more chestnut trees were found in the vicinity. The character of branching of the chestnut near the ground surface could indicate that the infection had taken place long ago. Thus, it is not possible to exclude that the infection was

Fig. 1. The localisation of the find of *Cryphonectria parasitica* (star point). The points indicate the sites with trees of *Castanea sativa* checked from 2001 to 2003.

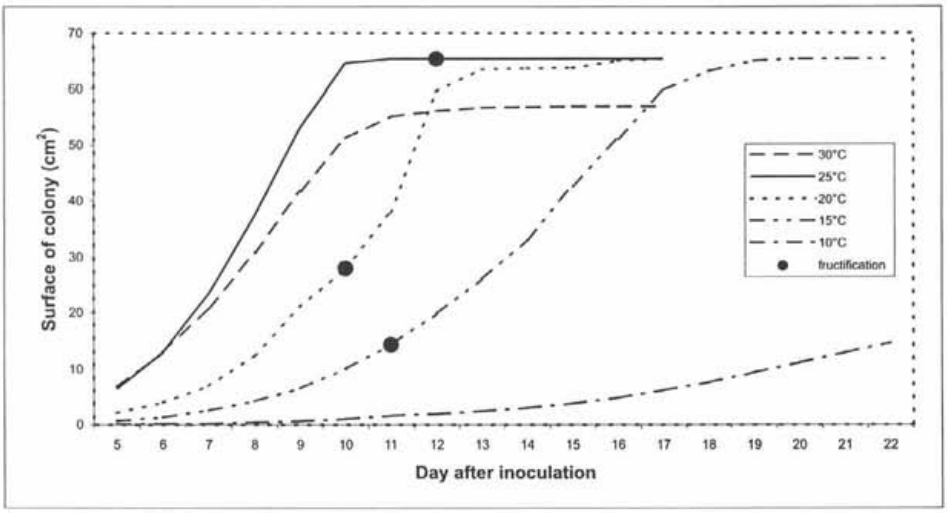

Fig. 2. Growth curves of *Cryphonectria parasitica*, strain no. CCM 8354 at various temperatures.

already transported with a two-year-old plant 25 years ago, i.e. at the time when the first data on the disease were known from Slovakia (1976; Juhásová 1999). The disease may occur in other, unknown and unchecked localities close to the border with Slovakia.

The vegetative compatible group EU 13 (Allemann et al. 1999) has been detected in Slovakia in all three sub-regions of the distribution of *Castanea sativa* where *C. parasitica* was recorded, viz. at 9 localities. In the Malé Karpaty Mts. sub-region, which is geographically the nearest one to the Czech Republic, 44.5 % of examined isolates of *C. parasitica* belonged to the vc group EU 13. The infected tree plant came from Bratislava. However, it is of interest that in Bratislava (Koliba) this vc group has not been identified. On the other hand, in Bratislava – Rača, the vc group includes 28.6 % of the tested isolates. Just in the vicinity of Bratislava (in the Malé Karpaty Mts.), there are localities where the EU 13 group includes even more than 90 % of the tested isolates (Pezinok 96.1 %, Limbach 94.2 %, Griňava 91.1 %). At another localities in Slovakia, the vc group is less numerous. In the region of the Inovec-Tríbeč Mts., it has been recorded at two localities (Lipovník and Radošina), thus representing 13.6 % of the tested isolates. In the third sub-region (Štiavnica-Krupina) which is the most distant from the borderline with the Czech Republic, the EU 13 group includes an even lower number of isolates (5.6 %) (Juhásová and Bernadovičová 2001).

EU 13 was one of the four most frequent vc types in the Carpathian basin (Radócz 2001). In north Italy (Corniglio), 20 % of the samples consisted of vc type EU 13. This vc type was found several times in Bregalia in Switzerland (Bazzigher, Kanzler and Kübler 1981). EU 13 was identified as one of the dominant vc types in Austria (Robin and Heiniger 2001). This vc type is rare in the remaining part of Europe.

The presented occurrence of *Cryphonectria parasitica* is the first documented record of the pathogenic fungus on the territory of the Czech Republic. It is necessary to emphasise that it is so far the only case at more than 232 localities visited. This is roughly about 80 % of the total population of chestnut trees in the CR.

From the epidemiological poit of view, however, new focuses cannot be excluded when their source can only be represented by chestnut trees imported from abroad. With respect to the patchy character of the chestnut distribution in the CR the probability of a rapid spread and of potential economic damage is minimal. However, according to scenarios connected with global climate change it is necessary to consider potential disturbances of natural geographical barriers preventing the spread of some diseases and thus further progress of chestnut blight northwards cannot be eliminated.

In the Czech Republic, chestnuts suffer particularly from non-specific decline at some localities. Numerous factors, both abiotic and biotic, participate in the decline.

ACKNOWLEDGEMENT

We would like to express our warm thanks to Prof. Dr. Ottmar Holdenrieder for his comments. The authors are grateful for support by the Czech Republic Ministry of Education (project no. MSM434100005).

REFERENCES

ALLEMANN C., HOEGGER P., HEINIGER U. and RIGLING D. (1999): Genetic variation of Cryphonectria hypoviruses (CHV1) in Europe, assessed using restriction fragment length polymorphism (RFLP) markers. – Mol. Ecol. 8: 843–854.

BAZZIGHER G., KANZLER E. and KÜBLER T. (1981): Irreversible Pathogenitätsverminderung bei Endothia parasitica durch übertragbare Hypovirulenz. – Eur. J. For. Pat. 11: 358–369.

CORTESI P., MILGROOM M. G. and BISIACH M. (1996): Distribution and diversity of vegetative compatibility types in subpopulations of Cryphonectria parasitica in Italy. – Mycol. Res. 100: 1087–1093.

DARPOUX H. (1948): Chestnut canker caused by Endothia parasitica. – Rev. Tech. Prot. Veg. I, 4, p. 307–330.

HALTOFOVÁ P. and JANKOVSKÝ L. (2003): Distribution of sweet chestnut Castanea sativa Mill. in the Czech Republic I. – Jour. For. Sc. 49(6): 259–272.

HALTOFOVÁ P. and JANKOVSKÝ L. (2004): Rozšíření kaštanovníku setého Castanea sativa Mill. v České republice. – Les. Pr. 83 (1): 12–13.

HEINIGER U. and STADLER B. (1991): Kastanienrindenkrebs auf der Alpennordseite. – Schweiz. Z. Forstwes. 141: 383–388.

JUHÁSOVÁ G. (1999): Hubové choroby gaštana jedlého (Castanea sativa Mill.). – 190 p. Bratislava.

JUHÁSOVÁ G. and BERNADOVIČOVÁ S. (2001): Cryphonectria parasitica (Murr.) Barr and Phytophthora spp. in chestnut (Castanea sativa Mill.) in Slovakia. – For. Snow Landsc. Res. 76(3): 373–377.

POWELL W. A. (1995): Vegetative incompatibility and mycelial death of Cryphonectria parasitica detected with a pH indicator. – Mycologia 87: 738–741.

PRIDNYA M. V., CHERPAKOV V. V. and PAILLET F. L. (1996): Ecology and pathology of European chestnut (Castanea sativa) in the deciduous forests of the Caucasus Mountains in southern Russia. – Bull. Torrey Bot. Club 123(3): 213–222.

RADÓCZ L. (2001): Study of subpopulations of the chestnut blight (Cryphonectria parasitica) fungus in the Carpathian basin. – For. Snow Landsc. Res. 76: 368–372.

ROBIN C. and HEINIGER U. (2001): Chestnut blight in Europe: Diversity of Cryphonectria parasitica, hypovirulence and biocontrol. – For. Snow Landsc. Res. 76: 361–367.

SEEMANN D. and UNGER J. G. (1993): Rindenkrebs der Esskastanie in der Bundesrepublik Deutschland. – Nachr. Dtsch. Planzenschutzd. 45: 120–122.