New Findings and Vegetative Compatibility Groups of *Cryphonectria parasitica* (Murrill) M. E. Barr in the Czech Republic

Libor JANKOVSKÝ, Pavlína HALTOFOVÁ and Dagmar PALOVČÍKOVÁ

Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University in Brno, Brno, Czech Republic

Abstract

Jankovský L., Haltofová P., Palovčíková D. (2010): **New findings and vegetative compatibility groups of** *Cryphonectria parasitica* (Murrill) M. E. Barr **in the Czech Republic**. Plant Protect. Sci., **46**: 28–33.

This study provides new information on the distribution of the chestnut blight agent *Cryphonectria parasitica* and its vegetative compatibility groups (VCGs) in the Czech Republic. This study has revealed the presence of the disease in six localities. The VCG tests showed that each locality was represented by a single distinct VCG, which was different from the others collected in the country. The tests with 31 European testers of *C. parasitica* VCGs (EU-1 to EU-31) had assigned Czech isolates to VCGs EU-1, EU-4, EU-12, EU-13, EU-15, and EU-19. Moreover, the study showed that in the Czech Republic chestnut blight attacks young trees, not exceeding 35 years of age, and that climatic conditions in the country are suitable for *C. parasitica*. As two of the diseased localities were ornamental tree nurseries, one might expect its further spread over the country as a result of plant trade. The origin of infection remains unknown in all the localities where *C. parasitica* was detected.

Keywords: chestnut blight; *Cryphonectria parasitica*; European sweet chestnut; vegetative compatibility groups

Cryphonectria parasitica (Murrill) M. E. Barr (syn. *Endothia parasitica* (Murrill) P. J. Anderson et H. W. Anderson), the causal agent of the chestnut blight, was introduced from Asia to North America about 1902, and to Europe in 1925 (Anonymous, 1950 ex Juhásová, 1999). However, in the Caucasus region the disease has been recorded as early as 1880 in European sweet chestnut (Pridnya *et al.* 1996). In the former Czechoslovakia, for the first time chestnut blight was found in Slovakia, in the locality of Prašice-Duchonka, District of Topoľčany in 1976 (Juhásová 1990, 1991 ex Juhásová 1999). The first record of chestnut blight from the Czech Republic originates from 2002 (Jankovský *et al*. 2004). During 2001–2006, most of the 300 localities of chestnut stands throughout the whole country were checked and the occurrence of *C. parasitica* was confirmed in three other sites (Haltofová *et al*. 2005a, b).

In European populations of fungus, Cortesi Faculty of Forestry and Wood Technology (1998) detected the presence of 31 vegetative compatibility groups (VCGs)[1], and labeled them accord-

[1]Vegetative incompatibility, or somatic incompatibility, inconformity is genetic cellular anti fusion mechanism of the fungal mycelium (Burnett 2003). *C. parasitica* occurs in nature in various VCGs. Robin and Heiniger (2001) ex Cortesi *et al*. (1998) identified and collected 31 European VCGs of *C. parasitica* (EU-1 to EU-31).

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 6215648902.

ingly from EU-1 to EU-31. To date, three of those VCGs, EU-1, EU-12 and EU-15, were found in the Czech Republic (Haltofová 2006). The aim of this study was to update the information on distribution and VCG diversity of *C. parasitica* in the Czech Republic.

MATERIAL AND METHODS

The health condition of the European Sweet Chestnut was studied on the bases of monitoring taken from 2001 (e.g. Haltofová & Jankovský 2003). In the case of occurrence of *C. parasitica* in one tree, only a few pieces of bark were taken with mycelium or visible symptoms of infection of fungi. In the case of occurrence of *C. parasitica* in more trees, samples from trees around the ones without any obvious symptoms were taken as well. Round samples of bark (10 mm in diameter) included several layers of wood. Samples of the symptomatic chestnut bark were rinsed in sterile water, surface-sterilised (96% ethanol 1 min, sterile water, dipping in ethanol), and placed in Petri dishes containing 3% malt extract agar (MA3: malt extract 35 g, peptone for bacteriological reasons 5 g, powdered agar 13 g). Isolated strains were deposited in the collection of the Department of Forest Protection and Wildlife Management at the Mendel University in Brno. The strain from the first finding were given to the Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, strain No. CCM 8354.

Vegetative compatibility tests. VCG tests for all Czech isolates were carried out on malt extract agar (MA3) with 31 European testers (EU-1 to EU-31) (Cortesi *et al.* 1998). Pieces of agar approx. 3 × 3 mm in size, containing mycelium were cut from the periphery of actively growing colonies (max. 7 days-old), and in each test six pairs of them were placed in 90 mm Petri dish. The cut pieces were arranged in individual pairs in 5 mm distance from each other, and were grown at 24°C, and after one week the results were evaluated. The pure cultures isolated from the same locality were tested reciprocally in the first phase. As a single VCG was found in each locality, they were confronted with the representatives of the known European VCGs EU-1–EU-31 in all combinations. Vegetative compatibility was assessed according to the barrage-merging response (Anagnostakis 1977).

RESULTS AND DISCUSSION

In the Czech Republic, *C. parasitica* was observed for the first time on sweet chestnut in the town of Uherský Brod (Jankovský *et al*. 2004). Its VCG was identified as EU-13, and the strain was deposited at CCM 8354. The infected tree was imported as a two-year-old seedling from Bratislava (Slovakia) in 1977. However, the origin of the infection has remained unknown.

Subsequently, in May 2004 and January 2006, blight-infected chestnuts were found in Kuřim near Brno in two localities. The VCGs in each of the localities were different, and were assigned to European VCGs EU-1 and EU-12, respectively. The chestnut from the first locality originated from former Yugoslavia (probably Croatia), from where young trees were imported in 1975. In this locality also one stump of a chestnut was observed in 2004, which was dead for several years, probably since around 1995 for unknown reasons, because any visible reasons were observed and as well isolation from the bark was negative. VCG was identified as EU-1 in this locality. In 2006, it was found some other solitary growing chestnut tree, planted in public garden, around 400 m distance from first locality. In spite of detailed screening in 2004 it was not found, due not typical shrub growth. Its VCG was identified as EU-12, different from precedent finding in 2004. The chestnut tree from the second place in this time has the same origin as in the case of first finding. Other chestnut trees in this time or in their vicinity were not observed.

Moreover, the occurrence of chestnut blight was registered in June 2004 in samples from a nursery at Moravský Písek. The infected trees were chestnuts and red oaks *Quercus rubra,* planted in shelterbelts (Haltofová & Jankovský 2004). The trees were grown from nuts imported for consumption from Italy in the 70's. The origin of the infection has remained unclear. Out of eight examined shelterbelts, most of the blight cankers were found in one of them, and only very sporadically in two others. Presence of *C. parasitica* on seedlings and fruits was not recorded. Twenty pure cultures of *C. parasitica* were isolated from 195 samples of bark from chestnuts and oaks and all of them were identified as VCG EU-15. According to morphological features, as a e.g. abundant fructification, morphology of colony etc., all of the isolated strains have a significance of virulent strains.

In the Czech Republic, the locality of *C. parasitica* at the highest altitude 434 m a.s.l. is Unín near Tišnov. Here, the disease was found on two chestnuts estimated to be 8 years-old planted in a private garden in 2006. Its VCG was identified as EU-19. Infected plants were grown from nuts collected in Bystřice na Pernštejnem (49°30'49''N, 16°15'22''E). However, no symptoms of chestnut blight were observed at the original place of nut collection, thus the origin of the infection in Unín has also remained unknown. Both trees were planted simultaneously, and there were no old chestnuts in the vicinity. The closest locality to Unín with *C. parastica* is Kurim about 20 km away, and there are no chestnuts in the vicinity closer than 10 km.

Yet another find of *C. parasitica* is in Žatčany near Brno, where infected chestnuts were planted again in shelterbelts of a former nursery of ornamental trees about 20 years ago. Only one strain has been isolated from this locality, and assigned to VCG EU-4. Also in this locality the origin of infection is not known. Cancers were observed on the stem bases only and they were covered by litter commonly.

All obtained cultures are likely to represent virulent strains, at least according to their morphological characteristics and pycnidia production. Virulent strains start to grow after two days with white mycelium and after 4–7 days the mycelium fructification starts. On the surface of the colony a lot of round orange pycnidia (1–2 mm) are produced. Inside many of conidia were observable (Jankovský *et al.* 2003). Hypovirulent strain fructification within the lab condition starts after one to two months and the amount of pycnidia is from 1 to 15 (Juhásová 1999). So far, in the Czech Republic, no healed *C. parasitica* canker has been observed and no hypovirulent strain of the fungus has been ever isolated. Most of cankers occurred at the basal parts of the trees, especially in Unin and Tesany, where most of the cankers expended even underneath leaf litter and below the ground. Treatments of the disease using biological control with hypovirulent isolates were not allowed due the phytosanitary law. Fol-

Table 1. Findings of *Cryphonectria parasitica* in the Czech Republic (arranged by chronological order)

	Locality	Coordinates	Altitude (m a.s.l)	Year of finding	VCG	Origin of trees	References
1.	Uherský Brod; trees planted in 1977	49°01'33''N 17°39'11''E	242	July, 19 2002	EU-13	Slovakia in 1977	Jankovský *et al.* (2006)
2.	Kuřim I; trees planted approx. in 1976	49°18'31''N 16°32'15''E	322	May, 27 2004	EU-1	former Yugoslavia in 1975	Haltofová (2006)
3.	Moravský Písek, forest nursery Kladíkov; *Castanea sativa* and *Quercus rubra*; trees approx. 25–30 years old	49°00'07''N 17°19'34''E	178	July 2004	EU-15	nuts imported from Italy in the end of 1970s	Haltofová (2006)
4.	Unín by Tišnov; approx. 8 years-old chestnut	49°22'57''N 16°29'05''E	434	January, 6 2006	EU-19	nuts collected in Bystřice nad Pernštejnem – no infection in this area	not published
5.	Kuřim II, public park	49°18'29''N 16°31'55''E	304	January, 16 2006	EU-12	not exactly known, bushy tree old app. 25 years	not published
6.	Žatčany by Brno, area of former nursery, windbreaks, age ca. 20 years	49°5'0.895''N 16°43'46.718''E	213	May 2006	EU-4	not known	not published Found by State Phytosanitary Administration of the CR
7.	Hovorčovice, ca. 20 km SE from Prague	50°10'37.038''N 14°30'48.238''E	240	May 2008	–	Bottegone, Pistoia, Italy	imported siblings in spring 2008

lowing the decision of the State Phytosanitary Administration of the Czech Republic, all infected trees were destroyed.

This paper reports the occurrence of six European VCGs of *C. parasitica*, EU-1, EU-4, EU-15, EU-12, EU-13 and EU-19 in the Czech Republic. Yet, only a single different VCG has been found at each of the localities. EU-1 and EU-13 are the main frequent *C. parasitica* VCGs found in Europe (Table 2). In Slovakia, eight different VCGs of the fungus had been detected so far, EU-13 and EU-12 being the most common (Juhásová & Bernadovičová 2001; Juhásová *et al.* 2004). In Hungary, 18 *C. parasitica* VCGs were identified, four of which, EU-1, EU-6, EU-12 and EU-13 were dominating, while EU-15 was found only in one locality (Radócz 2001). Simultaneously, all the Romanian and Ukrainian isolates were assigned to the VCG EU-12, implying a very low diversity of the fungus in these geographic areas (Radócz 2001). According to Cortesi *et al.* (1998), VCGs EU-1 and EU-5 are primarily spreading in northern Italy and Switzerland. EU-1 has been found in the Galicia, north-west of Spain (Aguín *et al*. 2004). VCG EU-12 has been reported to be dominating in southern and eastern Europe (but not in Turkey), whereas in western and north-western parts of the continent VCG EU-2 has been found to be the most common (Robin & Heiniger 2001). In present studies, VCGs EU-1, EU-12 and EU-13 were detected in three localities in the Czech Republic, and those VCGs are quite frequent also in

Table 2. Occurrence VCG of *C. parasitica* in Europe

Country	Dominant vcg	Subdominant VCG	References
Bosnia-Herzegovina	EU-1, EU-2, EU-12, EU-13	EU-4–EU-31	Trestic *et al*. (2001)
Croatia	EU-1 (42.9%), EU-2 (21%), EU-12 (14.2%)		Krstin *et al*. (2008)
Czech Republic	–	EU-1,EU-13,EU-15, EU-4, EU-12, EU-19	Haltofová (2006), Unpublished data
France	EU-1, EU-5, EU-33, EU-72	EU-2, EU-66, EU-14	Trestic *et al*. (2001)
Italy	EU-2, EU-1, EU-5	–	Cortesi *et al*. (1998, 1996)
Hungary	EU-1, EU-6, EU-12, EU-13	EU-2-5, EU-9, EU-11, EU-14-17, EU-21, EU-22, EU-28, EU-29	Radócz (2001)
Mecedonia	EU-12	EU-1, EU-2, EU-10, EU-22	Sotirovski *et al*. (2004)
Germany	EU-2	–	Robin and Heiniger (2001)
Austria	EU-2	–	Robin and Heiniger (2001)
Romania	EU-12	–	Radócz (2001)
Greece	EU-12 (88%)	EU-2 (6%), EU-10 (3%), EU-1 (2%)	Perlerou and Diamandis (2006)
Slovakia	EU-12, EU-13	EU-2, EU-5, EU-8, EU-14–EU17, EU-25	Juhásová *et al*. (2004), Adamčíková *et al*. (2006)
Spain	EU-2	EU-1, EU-5, EU-12	Trestic *et al*. (2001)
Spain NW	EU-1, EU-66, E1, E2, E3, E4		Montenegro *et al*. (2008)
Switzerland	EU-1, EU-2, EU-5	–	Robin and Heiniger (2001)
Portugal	EU-11 (80.2%)	EU-12 (7.1%) and EU-66 (6.6%), 2 Vc types not be assigned to a known European Vc type	Bragança *et al*. (2005, 2006)
Turkey	EU-1	EU-12	Çeliker and Onoğur (2001), Gurer *et al*. (1998)
Ukraine	EU-12	–	Radócz (2001)

Europe. By contrast, other two VCGs found in the country, EU-4 and EU-19, are very rare, and are known only from Bosnia-Herzegovina (Trestic *et al*. 2001).

Other studies have demonstrated low diversity of *C. parasitica* VCGs in areas where the disease has been recently introduced (Robin & Heiniger 2001; Sotirovski *et al*. 2004; Perlerou & Diamandis 2006), which differs much from the current observations in the Czech Republic, presented in this paper. On the contrary, high VCG diversity of the fungus has been reported from areas where chestnut blight has been established for a longer time (Cortesi *et al*. 1996; Robin *et al*. 2001). Therefore, modes of spread of *C. parasitica* to Czech Republic are unclear. Moreover, all infected trees are restricted to southern Moravia only, despite the fact that whole area of the country was investigated.

CONCLUSIONS

In the Czech Republic, chestnut blight is found on young trees, not exceeding 35 years of age and climatic conditions in the Czech Republic are suitable for *C. parasitica*. As two of the localities where the disease was found were ornamental tree nurseries, one might expect its further spread over the country as a result of plant trade. The origin of infection remained unknown in all the localities where *C. parasitica* was detected.

Originator of chestnut blight *Cryphonectria parasitica* was found on seven localities. The test showed that each locality was represented by a single distinct VCG, which was different from the others collected in the country. The tests with 31 European testers of *C. parasitica* VCGs (EU-1 to EU-31) had assigned Czech isolates to VCGs EU-1, EU-4, EU-12, EU-13, EU-15, and EU-19. The last occurrence was not yet tested. The obtained cultures for morphological characters and pycnidia production are referred to the virulent isolates. Any healed canker was not yet observed and no hypovirulent culture of *C. parasitica* has been found in any locality.

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Received for publication September 23, 2009
Accepted after corrections January 13, 2010

Corresponding author:

Doc. Dr. Ing. Libor Jankovský, Mendelova univerzita v Brně, Lesnická a dřevařská fakulta, Ústav ochrany lesů a myslivosti, Zemědělská 1, 613 00 Brno, Česká republika
tel.: + 420 545 134 116, fax: + 420 545 134 529, e-mail: jankov@mendelu.cz