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THE MOST FREQUENT SWEET CHESTNUT DISEASES IN VRANJE AREA

Zlatan Radulović[1]

Abstract: Sweet chestnut (*Castanea sativa* Mill.) is one of the most endangered tree species. Its survival in our country does not depend on the climate as much as on the sensitivity to fungus *Cryphonectria parasitica* (Murrill) Barr which causes "chestnut blight". Besides this fungus, grave damages to Sweet chestnut are also caused by species from *Phytophthora* genus causing "chestnut ink disease". This paper states the fungi which appear most frequently on chestnut in two localities in the vicinity of Vranje. The total of 13 fungal species was reported. The most significant of them is *Cryphonectria parasitica,* which is present in both localities. In both localities the I_{zs} is extremely high (2.93 and 2.5), which points to the fact that the tree damage is great. The most significant species reported on leaves is *Mycosphaerella maculiformis*, and on the fruits *Stromatinia pseudotuberosa* is of the highest significance.

Key words: Sweet chestnut, *Cryphonectria parasitica*, *Phytophthora* spp., health condition index

NAJČEŠĆE BOLESTI PITOMOG KESTENA NA PODRUČJU VRANJA

Izvod: Pitomi kesten (*Castanea sativa* Mill.) spada u najugroženije vrste drveća. Njegov opstanak kod nas ne zavisi toloko ni od klime koliko od osetljivosti prema gljivi *Cryphonectria parasitica* (Murrill.) Barr. koja izaziva "rak kore kestena". Pored ove gljive velike štete na pitomom kestenu izazivaju i vrste iz roda *Phytophthora* koje izazivaju "mastiljavu bolest" kestena. U radu su navedene gljive koje se najčešće javljaju na kestenu na dva lokalitete u okolini Vranja. Ukupno je zabeleženo prisustvo 13 vrsta gljiva. Najveći značaj ima vrsta *Cryphonectria parasitica*, koja je prisutna na oba lokaliteta. I_{ZS} na oba lokaliteta je veoma visok (2,93 i 2,5) što ukazuje da je stepen oštećenja stabala veliki.

[1] Zlatan Radulović, M. Sc, Institute of Forestry, Belgrade, Serbia
Translation: Marija Stojanović

Najznačajnija vrsta konstatovana na lišću je *Mycosphaerella maculiformis*, a na plodovima najveći značaj ima *Stromatinia pseudotuberosa*.

Ključne reči: pitomi kesten, *Cryphonectria parasitica*, *Phytophthora* spp., indeks zdravstvenog stanja

1 INTRODUCTION

Sweet chestnut (*Castanea sativa* Mill.) is a European species belonging to the *Fagaceae* family and *Castanea* genus. Thirteen species from this genus have been described so far. Besides the Sweet chestnut significant species in America are: *Castanea dentata* (Marsh.) Borkh, in Japan: *Castanea crenata* Siebold & Zucc., and in China: *Castanea mollissima* Blume.

The chestnut in Serbia is, according to Glišić's research (1975), present in Vršački Breg, a few localities in Fruška Gora, in Gučevo, Kostajnik near Kučevo, near Prijepolje (Hisardžik Village), near Prokuplje (Čukovac Village), near Čačak (Trnava Village), near Niš (in Gorica and Seličevica), in localities near Vranje and Kruševac, and between Peć and Prizren, where the number of it is highest. Since Sweet chestnut is economically very valuable tree species, it is the subject of many researches all over the world. In almost every neighbouring country and most Mediterranean countries there are intensive activities aimed at Sweet chestnut natural population research and genetic resources protection, as well as at the possibilities of the range expansion, which is required by the industries of these countries.

In Serbia, as well as in all parts of Europe, the Sweet chestnut is one of the most endangered tree species. Its survival in our country does not depend so much on the climate but rather on sensitivity to fungus *Cryphonectria parasitica* (Murrill) Barr which causes "chestnut blight" and species from genus *Phytophthora*, agents of chestnut "ink disease".

The dessication of the American chestnut (*Castanea dentata* (Marsh.) Borkh.), caused by fungus *Cryphonectria parasitica* (Murrill) Barr is one of the greatest botanical catastrophes in history of the mankind. The first trees infected by this fungus (former name *Endohtia parasitica* (Murr.) & And.) were registered in New York zoo in 1904. More than 3.5 trillions of American chestnut trees had been destroyed by 1950, which endangered its survival as well (Rittenour, 2005).

In Europe, *C. parasitica* was first registered in Italy in 1938, whence it spread to the most European countries. According to the reports by Robin and Heiniger (2001), its presence has not been reported only in the Netherlands and Great Britain.

2 MATERIAL AND METHOD

In Sobina locality chestnut is present in the belt between 600 m and 800m above sea level, at S and SE exposures. The age of the observed chestnut trees is between 40 and 100 years. In Muhovac locality it is present in the belt at altitudes above 800m, at NE and E exposures. The tree age is between 40 and 70 years.

Sweet chestnut health condition was studied in the localities Sobina and Muhovac in the vicinity of Vranje. The total of 197 trees was inspected and on every tree visible disease symptoms or the presence of fruiting bodies were reported. In cases where the

cause could not be determined macroscopically samples were taken for laboratory analysis. The isolation was performed on Malt-agar (MEA) and Potato-dextrose agar (PDA). The fungi were identified by keys of Dennis (1978), Sutton (1980), Courtecuisse (1999) and Carmichael et al. (1980).

The method used for evaluation of damages caused by fungus *Cryphonectria parasitica* was described by Juhásová et al. (2004). The trees were assessed against the following criteria:

0 - healthy trees without symptoms;
1 - smaller and yellow leaves;
2 - dry brown leaves and the beginning of canker wound formation (change of bark colour);
3 - dry brown leaves, open canker wounds on the tree;
4 - more than 2/3 of tree crown with dried branches,
5 - almost completely dead trees with numerous large canker wounds.

The damage degree was expressed by health condition index (I_{zs}) obtained by the formula:

$$I_{ZS} = \frac{1n1+2n2+3n3+4n4+5n5}{n}$$

In formula the symbols stand for:
n – total number of assessed trees,
n1…n5 – the number of trees in the appropriate category of injure

The intensity of other disease occurrences was determined against the following criteria:

1 – intensity of occurrence of disease agent or pest is weak (up to 10% of diseased plants);
2 – intensity of occurrence of disease agent or pest is medium (10 – 25% of diseased plants),
3 – intensity of occurrence disease agent or pest is strong (25 – 60% of diseased plants),
4 – intensity of occurrence of disease agent or pest is extremely strong (60 – 90% of diseased plants).

3 RESULTS

The damaged degree expressed by $I_{zs,}$ in the examined localities is presented in the following table:

Table 1- The results of the assessment of the damages caused by fungus C. parasitica

Locality	Number of assessed trees	Number of damaged trees (per categories)						
		0	1	2	3	4	5	(I_{ZS})
Sobina	129	12	10	30	28	21	28	2.93
Muhovac	68	9	11	13	15	12	8	2.5
Total	197	21	21	43	43	33	36	

In Sobina locality 129 trees were assessed and I_{ZS} is 2.93. In locality Muhovac 68 trees were assessed and I_{ZS} is 2.5.

On examined trees besides *C. parasitica* the presence of another 12 fungi species was reported. In table 2 give index fungi species reported, intensity of occurrence and locality:

Table 2- Fungi reported on the Sweet chestnut

Fungi species	Plant organ	Intensity of occurrence	Locality	
			Sobina	Muhovac
Mycosphaerella maculiformis (Pers.) Schroet.	leaf	3	+	+
Cytospora intermedia Sacc.	branches and stem	1	+	+
Vuilleminia camedens (Nees.) Maire.	branches and stem	1	+	-
Schizophyllum communae Fr.	branches and stem	1	+	+
Trametes hirsuta (Wulfen.) Pilát	branches and stem	1	+	+
Armillaria sp.	root	1	+	+
Phytophthora sp.?	root	1	+	-
Stromatinia pseudotuberosa Rehm.	fruit	1	-	+
Trichothecium roseum Link.	fruit	1	+	+
Alternaria tenuis Nees.	fruit	1	-	+
Penicillium sp.	fruit	1	+	+
Aspergillus sp.	fruit	1	+	-

The species *Mycosphaerella maculiformis* was reported on leaves on approximately 25-60% of trees (intensity of occurrence 3). However, the attack intensity on leaves is very low.

On the branches and stem in both localities the presence of species *Cytospora intermedia* Sacc., *Vuilleminia camedens* (Nees.) Maire, *Schizophyllum communae* Fr., *Trametes hirsuta* (Wulfen.) Pilát and *Cryphonectria parasitica* (Murrill.) Barr. was reported. The intensity of occurrence of other fungi is low (up to 10% of diseased plants), except for species *Cryphonectria parasitica*.

On the roots of 5 trees the presence of *Armillaria* sp. Was reported and on 2 trees symptoms typical for *Phytophthora* genus were determined .By the subsequent laboratory analysis the presence of species from *Phytophthora* genus was not proved.

By the laboratory analyses conducted on fruits the following species were reported: *Stromatinia pseudotuberosa* Rehm., *Penicillium* sp., *Aspergillus* sp., *Trichothecium roseum* Link. and *Alternaria tenuis* Nees.

4 DISCCUSSION

C. parasitica has the greatest significance for the survival of Sweet chestnut of all the reported fungi. It is very frequent in the investigated localities. The I_{zs} in both localities is extremely high (2.93 and 2.5), which points to the fact that the damage degree of trees is very high. In locality Sobina on 12 trees out 129 investigated no disease symp-

toms were observed, and the large number of trees is in the desiccation phase. In locality Muhovac the situation is a little better. Assessing the damages caused by *C. parasitica* in Slovakia Juhásová et al. (2004) report the I_{zs} values during 1999, ranging from 1.11 to 2.36. The I_{zs} values in the next year ranged from 1.11 to 3.50. The highest values (2.36 and 3.50) were reported only in one case (an orchard), while I_{zs} values in other cases were lower than 2.

In Europe, *C. parasitica* was first registered in Italy in 1938, whence it spread to the most European countries. According to the reports by Robin and Heiniger (2001) its presence was not reported only in the Netherlands and Great Britain. The chestnut blight in Former Yugoslavia, according to Krstić (1950), was recorded for the first time in "Panovac" forest in Slovenia in 1950, and in Serbia, according to Marinković and Karadžić (1985), its presence was first recorded in area of Kosovo and Metohija in 1975.

In the beginning the pathogenicity of this fungus was expressed in Europe, almost to the same degree as in America. However, Biranghi discovered in 1951 that 85% of the infected trees seemed "surprisingly healthy". By the subsequent research Grente obtained atypical isolates from canker wounds of these trees in 1964, white-colored and of reduced virulence. He called this phenomenon hypo-virulence.

During 1969 it was proved that hypo-virulence is caused by the presence of double ribonucleic acid (dsRNK), which is transferred by conidia but not by ascospores. Transferring by contact of hyphae (anastomosis) is the most frequent but it is conditioned by vegetative compatibility. In 1992 it was proved that dsRNK is of viral origin.

Insertion of hypo-virulent fungus forms in infected chestnut stands is used as a biologic method of fight. This has been the only acceptable method in fighting against *C. parasitica* so far. For the successful application of this method it is necessary to determine the number of vegetative compatibility types (vc) of fungus present in infected chestnut stands. If the number of vegetative compatibility types (vc) is lower, the method is more successful. The number (vc) types is conditioned by sexual reproduction of different fungal genotypes. The lower number of dominant (vc) fungal types in Europe is conditioned by a rare occurrence of perithecia, which enabled a more successful hypo-virulence application than in America where the number of (vc) types is much higher. Giving the outline of the previous researches, Robin and Heiniger (2001) report that in Europe the presence of 40 (vc) types were determined, by which EU-2 is dominant in the west and north-west and EU-12 in the south and east of Europe.

Besides *C. parasitica*, large damages on chestnut are also caused by the species belonging to *Plythophthora* genus. In Sobina locality the symptoms typical for species of this genus were reported (ink colour beneath the bark) on two trees. By the subsequent laboratory analyses the presence of species from *Plythophthora* genus was not proved. *Phytophthora cinnamomi* Rand. and *Phytophthora cambivora* (Petri.) Buis, which cause the chestnut ink disease, are the most frequent on chestnut. Prior to the epidemic of *Cryphonectria parasitica* fungus, in the 1930s, the ink disease represented the main threat to survival of Sweet chestnut.

In the period after 1995, in a few European countries an increase of chestnut desiccation, which was caused by *Plythophthora* genus species, was reported. By the subsequent researches besides species *P. cinnamomi* and *P. cambivora* from the soil on which the chestnut dessicated species *P. cactorum*, *P. citricola*, *P. megasperma*, *P. cryptogea* and *P. syringae* were isolated. In the Balkans, according to Milev and Sotirovski (2007) only two species *P. cambivora* and *P. cactorum* were reported on chestnut, while the most

pathogenic species *P. cinnamomi* are present only in countries which are located to the north. Portela et al. (1999) report that the interaction of several factors predisposed chestnut to the attack by the species of this genus. As the most significant factors, he reports a low fertility and bad soil aeration, as well as butt end and root injuries, caused by extracting of stumps and soil cultivation.

Considerable damages to chestnut are caused by the species from *Armillaria* genus. However, their presence in the both localities was reported on only 5 trees. Of the fungi reported on the leaves the most significant is *Mycosphaerella maculiformis*. It is frequently present in the both localities, but small foliages have been infected so far. If the conditions for its development become favorable in the foreseeable future, significant damages might be expected as well.

The physiologically weakened trees will simultaneously lose assimilation organs owning to the influence of *C. parasitica,* which will inevitably accelerate their desiccation. *M. maculiformis* causes leaf spots on many broadleaf species, but is the most frequent on Sweet chestnut. The fungus survives through the winter on fallen leaves in perithecium stage. In spring ascospores are released, which initiate the primary infections. In the infected place small characteristic polygonal necrosis with darker edges appear. In case of a stronger attack necrosis group and cover the whole leaf. On the area covered in necroses in the summer an imperfect stage of this fungus is formed, which is described as *Cylindrosporium castaneicolum*. The conidiae at this stage have, according to Haltofová (2003), 2-4 partitions, and their measures are 39-58 µm x 2.3-3.3 µm. Sometimes picnidia are also formed, i.e. the second imperfect stage of development of this fungus called *Phyllosticta maculiformis*. Both conidia and picnospores form the secondary infections during summer.

Of all species reported on the fruits in chestnut groves, *Stromatinia pseudotuberosa* has the greatest significance. Although it most frequently appears on acorns, it causes significant damages to chestnut fruits as well, causing the mummification of them. The fruits can become infected while they are on trees but most fruits get infected upon falling from the tree. In the first phase of the attack yellow spots are formed on the fruit with thinner rims which gradually become larger. The cracks with grey fungal mycelia appear later. In the end the whole fruit blackens and becomes mummified. Next autumn, apothecia begin to grow on such a fruit. In them asci with ascospores 8-10 µm x 5-6 µm are formed (Lazarev, 2001).

Other species (*Penicillium* sp., *Aspergillus* sp., *T. roseum* and *A. tenuis*) cause damages due to inappropriate collecting and seed transport or unsuitable storage.

5 CONCLUSION

Based on the research of Sweet chestnut health condition conducted in localities Sobina and Muhovac in the vicinity of Vranje the following conclusions might be drawn:

- Out of 197 Sweet chestnut trees inspected only on 21 there were no visible exterior signs of disease.

- The total of 13 fungi species was reported and the largest number was reported on branches, stem and fruits, 5 on each.

- Out of all fungi species reported *C. parasitica* has the greatest significance for Sweet chestnut survival. It is very frequent in the investigated locations. The I_{zs} in the both localities is extremely high (2.93 and 2.5), which points to the fact that degree of tree damage is high. In Sobina locality on 12 trees out 129 inspected no disease symptoms

were observed, and the large number of trees is in the desiccation phase. In locality Muhovac the situation is a little better.

- Besides *C. parasitica*, large damages on chestnut are caused by the species genera *Plythophthora* and *Armillaria* but their presence in these localities is not significant for now.

- The species *Mycosphaerella maculiformis* was reported on leaves on approximately 25-60% of trees (intensity of occurrence 3). However, the attack intensity on leaves is extremely low. It can become a more significant danger if the attack intensity increases. In that case, the physiologically weakened trees will due to the *C. parasitica* influence simultaneously lose the assimilation organs, which will inevitably accelerate the desiccation of them.

- Out of all species reported on fruits, *Stromatinia pseudotuberosa*. has the greatest significance. Although it appears most frequently on acorns, it causes significant damages to chestnut fruits as well, causing the mummification of them. Other species (*Penicillium* sp., *Aspergillus* sp., *T. roseum* and *A. tenuis*) can cause damages due to inappropriate collecting and seed transport or unsuitable storage.

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THE MOST FREQUENT SWEET CHESTNUT DISEASES IN VRANJE AREA

Zlatan Radulović

Summary

In localities Sobina and Muhovac in the vicinity of Vranje a total of 13 fungi species was reported. Out of all species reported, *Cryphonectria parasitica,* which causes "chestnut blight" has the greatest significance for the survival of Sweet chestnut. Its presence is extremely high, which can be concluded from the values of I_{ZS} (2.5 and 2.93).

Besides *C.parasitica*, large damages on chestnut are caused by species from genera *Plythophthora* and *Armillaria* but their presence in these localities is not significant for now.

The species *Mycosphaerella maculiformis* was reported on leaves. It can become a significant danger if the attack intensity increases. In that case, the physiologically weakened trees will due to the *C. parasitica* influence simultaneously lose assimilation organs, which will inevitably cause the dessication of them.

Out of all species reported on fruits, *Stromatinia pseudotuberosa* has the greatest significance. Although it appears most frequently on acorns, it causes significant damages to chestnut fruits, causing the mummification of them. Other species (*Penicillium* sp., *Aspergillus* sp., *T. roseum* and *A. tenuis*) can cause damages due to inappropriate collecting and seed transport or unsuitable storage.

NAJČEŠĆE BOLESTI PITOMOG KESTENA NA PODRUČJU VRANJA

Zlatan Radulović

Rezime

Na lokalitetima Sobina i Muhovac u okolini Vranja konstatovano je prisustvo 13 vrsta gljiva. Od svih konstatovanih vrsta najveći značaj za opstanak pitomog kestena ima *Cryphonectria parasitica*, koja izaziva "rak kore kestena". Njeno prisustvo je veoma veliko što se vidi iz visokih vrednosti I_{ZS} (2,5 i 2,93).

Pored *C. parasitica* na kestenu velike štete izazivaju i vrste rodova *Phytophthora* i *Armillaria* ali njihovo prisustvo za sada na ovim lokalitetima nije veliko.

Na lišću je prisutna *Mycosphaerella maculiformis*. Ona može predstavljati značajniju opasnost ako se intenzitet napada poveća. U tom slučaju fiziološki slaba stabla usled

dejstva *C. parasitica* ostaće istovremeno bez asimilacionih organa što će neminovno ubrzati njihovo sušenje.

Od vrsta konstatovanih na plodovima najveći značaj ima *Stromatinia pseudotuberosa*. Iako se najčešće javlja na plodovima hrasta značajne štete pričinjava i plodovima kestena izazivajući njihovu mumifikaciju. Ostale vrste (*Penicillium* sp., *Aspergillus* sp., *T. roseum* i *A. tenuis*) mogu izazvati značajnije štete samo ako se plodovi nepravilno sakupljaju i transportuju, ili nepravilno skladište.

Reviewer: Prof. Dragan Karadžić, Ph.D, Fakulty of Forestry, Belgrade