INSTITUTE OF FORESTRY • BELGRADE
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SUSTAINABLE FORESTRY **ODRŽIVO ŠUMARSTVO**
COLLECTION OF PAPERS 85-86, 2022 ZBORNIK RADOVA 85-86, 2022

DOI: 10.5937/SustFor2285107R
Original scientific paper

EFFECTS OF DIFFERENT MEDIA ON THE MYCELIAL GROWTH OF *Cryphonectria parasitica* (Murrill) M.E. Barr

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Abstract: *It is a well-known fact that a large number of parasitic and saprophytic fungi grow on sweet chestnut trees. However, the greatest damage is caused by C . parasitica which causes "sweet chestnut blight" and leads to its dieback. Hosts other than Castanea species include the following families: Aceraceae, Betulaceae, Fagaceae, Anacardiaceae, Juglandaceae u Magnoliaceae, where it grows as a saprophyte. The exception, according to some authors, is oak, where it can also occur as a parasite. Mycelial growth of C. parasitica was tested on media made of the bark of sweet chestnut (Castanea sativa), manna ash (Fraxinus ornus), sycamore maple (Acer pseudoplatanus), sessile oak (Quercus petraea), common yew (Taxus baccata), hazel (Corylus avellana), small-leaved linden (Tilia cordata), Norway maple (Acer platanoides) and English walnut (Juglans regia).*

After 28 days, the medium was not completely overgrown only in the variants with sycamore maple and Norway maple bark added to the medium.

Key words: *C. parasitica,* sweet chestnut, bark, mycelia

UTICAJ RAZLIČITIH PODLOGA NA PORAST MICELIJE GLJIVE *Cryphonectria parasitica* (Murrill) M.E. Barr

Izvod: *Poznato je da se na pitomom kestenu razvija veliki broj parazitskih i saprofitskih gljiva. Ipak, najveće štete izaziva C. parasitica koja izaziva "rak kore kestena", dovodeći do njegovog potpunog sušenja. Pored vrsta roda Castanea ostali domaćini gljive*

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C. parasitica pripadaju familijama: Aceraceae, Betulaceae, Fagaceae, Anacardiaceae, Juglandaceae i Magnoliaceae. Na vrstama ovih familija javlja se kao saprofit. Izuzetak, po nekim autorima predstavlja hrast, na kom se može javiti i parazitski. Porast micelije C. parasitica ispitivan je na podlogama napravljenim od kore: pitomog kestena (Castanea sativa), crnog jasena (Fraxinus ornus), javora (Acer pseudoplatanus), hrasta kitnjaka (Quercus petraea), tise (Taxus baccata), leske (Corylus avellana) sitnolisne lipe (Tilia cordata), mleča (Acer platanoides) i oraha (Juglans regia).
Posle 28 dana podloga nije u potpunosti obrasla samo u varijantama kada je podlozi dodavana kora javora i mleča.

Ključne reči: *C. parasitica*, pitomi kesten, kora, micelija

1. INTRODUCTION

Sweet chestnut (*Castanea sativa* Mill.) is a tree species of considerable economic value. Therefore, it has often been the subject of numerous research studies worldwide. It is one of the most useful and economically important forest tree species, with a very high use value in all developmental stages (from saplings to mature stands).

The occurrence of the parasitic fungus *Cryphonectria parasitica* (causing sweet chestnut blight) has markedly decreased the use value of sweet chestnut trees. *C. parasitica* was first recorded in the New York area in 1904 (probably transferred from East Asia) and then quickly spread over the entire distribution area of the American chestnut (*Castanea dentata*). Within 40 years, it practically devastated the American chestnut in North America. More than 3.5 billion American chestnut trees were estimated to be destroyed in the US alone. It was introduced to Europe from North America in 1938, and the European chestnut (*Castanea sativa*) immediately proved very sensitive to the infections caused by this pathogen.

According to Krstić (1950), sweet chestnut blight was first detected in Yugoslavia in 1950 in the Slovenian "Panovac" forest. Its presence in Croatia was first recorded by Halambek in 1955 in the vicinity of Opatija (cited by KRSTIN et al., 2008). In Bosnia and Herzegovina, the blight was first noticed by UŠČUPLIĆ in 1961. According to MIJUŠKOVIĆ (1973), the first signs of chestnut disease in Montenegro were observed by B.Sc. Danilo Stamatović in 1968 in the place known as "Matića Gvozd" at the foot of Rumija, and Mijušković registered it in Boka Kotorska in 1973 (in a chestnut forest above Stolivo). Sweet chestnut bark blight was first recorded in Macedonia in 1974 (PAPAZOV et al., 1974) and in Kosovo and Metohija in 1975 (cited by MARINKOVIĆ and KARADŽIĆ 1985).

C. parasitica was first described in 1906 as *Diaporthe parasitica* Murr. and was renamed *Endothia parasitica* (Murrill) P. J. Anderson & H. W. Anderson in 1912. The taxonomy of the *Endothia* genus was modified by Barr (1978) in his monograph named Diaporthales. Based on the composition and structure of the fruiting body and the appearance of the spores (shape and septation), *Cryphonectria* and *Endothia* were separated (Roane et al., 1986).

Recent taxonomic revisions have restricted the name *Cryphonectria* (*sensu stricto*) only to the following four species: *C. parasitica* (Murr.)

Barr., *C. radicalis* (Schwein.: Fr.) M.E. Barr, *C. macrospora* (Tak. Kobay. & Kaz. Ito) M.E. Barr and *C. nitschkei* (G.H.Otth) M.E. Barr (GRYZENHOUT et al., 2006a, b). Of the four species, only *C. parasitica* is a dangerous plant pathogen.

C. parasitica occurs on all known species of chestnut, causing the most considerable damage to American chestnut *Castanea dentata*. European chestnut *Castanea sativa* is also sensitive, while the Asian species *Castanea crenata* and *Castanea molissima* are more resistant. It grows as a saprophyte on other tree species on which it has been observed. Other hosts of *C. parasitica*, as stated by NASH и STAMBAUGH (1982), TURCHETTI et al. (1991), DALLAVALLE and ZAMBONELLI (1999), and RADOCZ and TARCALI (2005), belong to the families of *Aceraceae, Betulaceae, Corylaceae, Fagaceae, Anacardiaceae, Juglandaceae* and *Magnoliaceae.*

2. MATERIAL AND METHODS

The growth of mycelia was examined on media made from the bark of different tree species. We used the isolate *C. parasitica* (CS3) recovered from the bark of the sweet chestnut growing in the locality of Sobina near Vranje. The bark of sweet chestnut (*Castanea sativa*), manna ash (*Fraxinus ornus),* sycamore maple (*Acer pseudoplatanus*), sessile oak (*Quercus petraea*), common yew (*Taxus baccata*), hazel (*Corylus avellana*), small-leaved linden (*Tilia cordata*), Norway maple (*Acer platanoides*) and walnut (*Juglans regia*) was used. Besides the bark, the medium made from sweet chestnut fruit was also used. The bark and the fruit of sweet chestnut trees were dried to a constant value and ground. The medium was prepared from 10 g of powder ground in this way and 20 g of agar per liter of water. Mycelium growth was measured after 2, 4, 6, 8, 15 and 28 days. The results are expressed as a percentage of mycelial growth relative to the radius of the Petri dish at the time of measurement. We also registered the moment when the fruiting body emerged.

The data relating to the growth of *C. parasitica* mycelia on different media were processed using the STATISTICA 6.0 software package. (StatSoft, Inc).

3. RESULTS AND DISCUSSION

The table below (Table 1) shows the mycelium growth in the study period.

Table 1. *Media and mycelial growth of C. parasitica*

Medium	X ± SE	X ± SE	X ± SE	X ± SE	X ± SE	X ± SE
	2nd day	4th day	6th day	8th day	15th day	28th day
Oak	7.6±0.68 d	26.7±1.66 e	42.8±1.60 de	63.8±1.76 e	100.0±0.00 e	100.0±0.00 b
Manna ash	4.5±0.40c	10.0±0.90 c	22.8±1.48 c	36.5±1.58 c	83.8±1.41 c	100.0±0.00 b
Sycamore maple	0.0±0.00a	0.0±0.00 a	0.0±0.00 a	1.6±0.30 a	7.6±0.68 a	14.7±0.68 a
Chestnut bark	10.9±0.45e	33.9±0.68 f	54.2±0.68 f	75.2±0.52 fg	100.0±0.00 e	100.0±0.00 b
Chestnut fruit	6.7±0.45 d	21.2±1.63 d	44.6±1.84 e	71.3±1.81 f	100.0±0.00 e	100.0±0.00 b
Hazel	11.1±0.42e	34.4±1.12 f	57.3±1.26 f	80.0±1.40 g	100.0±0.00 e	100.0±0.00 b
Linden	7.4±0.46 d	24.9±1.12 de	44.0±1.57 e	73.6±1.85 f	100.0±0.00 e	100.0±0.00 b
Norway maple	2.6±0.16b	5.1±0.31 b	8.9±0.44 b	10.5±0.55 b	15.6±1.12 b	15.6±1.12 a
Walnut	6.7±0.39 d	25.8±1.04 de	44.9±1.71 e	56.8±1.17 d	90.2±0.92 d	100.0±0.00 b
Yew	6.7±0.46 d	22.0±1.21 de	36.9±1.20 d	51.6±1.14 d	90.4±0.94 d	100.0±0.00 b
F	133.08	219.88	374.82	448.34	1772.32	6990.42
P	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

After two days, no mycelial growth was observed on the medium with the addition of sycamore maple, while the highest growth was recorded on the media with the addition of hazel bark and sweet chestnut bark.

After four days, there was still no mycelial growth of *C. parasitica* on the medium with the addition of sycamore maple bark, while the medium with the addition of hazel bark and chestnut bark had the highest growth.

On the sixth day, the greatest increase was still achieved on the bark of hazel and sweet sweet chestnut. Mycelia still did not grow on the sycamore maple, while on the Norway maple it reached 8.9% of the Petri dish radius.

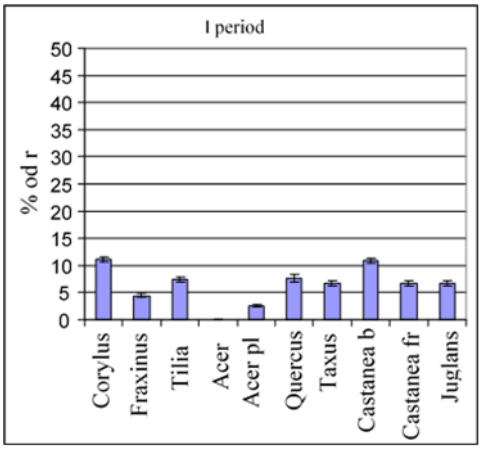
On the eighth day, the mycelium of *C. parasitica* started to grow on the medium with the addition of sycamore maple bark. The highest increase in mycelial growth was recorded on the medium with the addition of hazel bark and sweet chestnut bark. We also noted considerable growth on the medium with the addition of linden bark (73.6%).

On the fifteenth day, Petri dishes were completely filled on the media with the addition of sessile oak, sweet chestnut, hazel, and linden bark, as well as sweet chestnut fruit. The lowest growth was recorded on the medium with the addition of sycamore maple (7.6%), followed by Norway maple (15.6%), and manna ash (83.8%). On the media with the addition of walnut and yew bark, the increase was approximately the same and amounted to 90%.

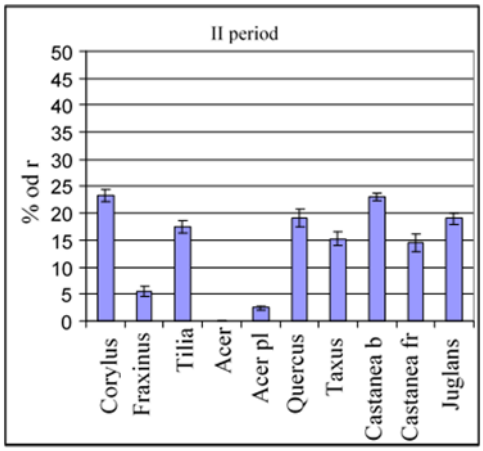
After 28 days, the only media that were not completely grown in mycelia were the ones with the addition of sycamore maple and Norway maple bark.

The research results related to the growth rate of *C. parasitica* mycelia in different periods are shown in the graphs below (1-6). Period 1 refers to the mycelial growth from the beginning of the experiment to the measurement conducted on the second day. Period 2 shows the growth from the second to the fourth day, Period 3 from the fourth to the sixth, Period 4 from the sixth to the

eighth, Period 5 from the eighth to the fifteenth, and Period 6 from the fifteenth to the twenty-eighth day. Results are shown as a percentage of the Petri dish radius.



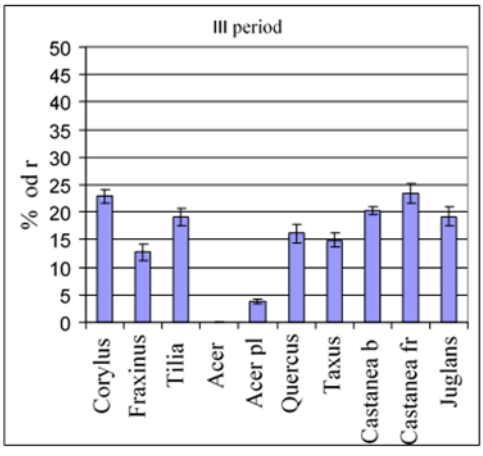
Graph 1. *Mycelial growth (Period 1)*



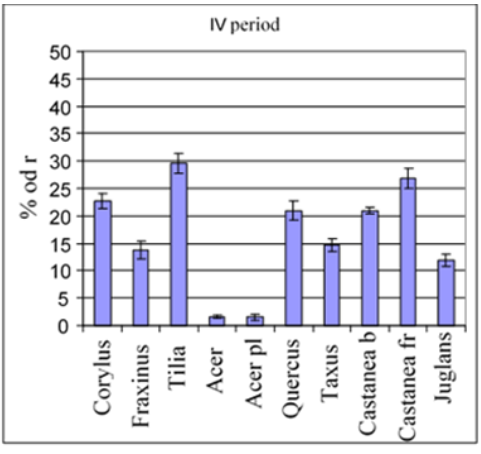
Graph 2. *Mycelial growth (Period 2)*

The highest mycelial growth increase in the first period was recorded on the medium with hazel bark, while it was slightly lower on the medium with sweet chestnut bark. In this period, the mycelium began to grow on all media, except for the medium with the addition of sycamore maple bark.

In the second period, the highest increase in growth was again noted on the medium with the addition of hazel bark and the medium with the addition of sweet chestnut bark. In this period, the mycelium was still not growing on the medium with the addition of sycamore maple bark.



Graph 3. *Mycelial growth (Period 3)*

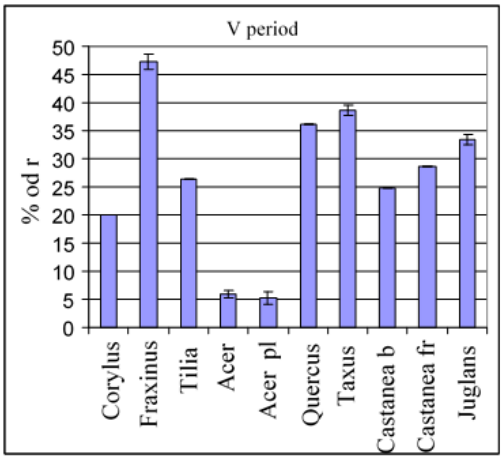


Graph 4. *Mycelial growth (Period 4)*

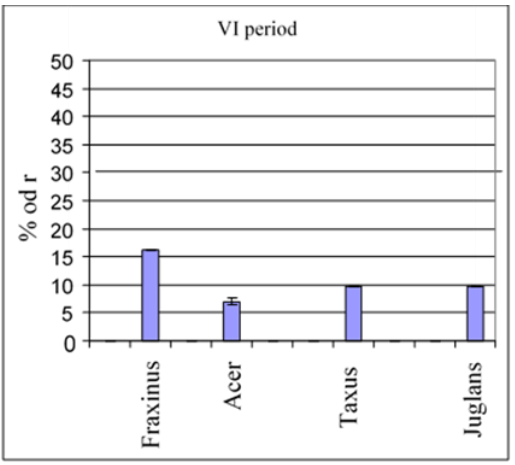
In the third period, the mycelium had the greatest growth on the medium with the addition of sweet chestnut fruit, followed by the medium with the addition of hazel bark. Even in this period, the mycelium did not start growing on the

medium with the addition of sycamore maple bark. Significant mycelial growth was achieved on the media with the addition of chestnut, linden and walnut bark.

The fourth period saw the greatest mycelial growth on the medium with the addition of linden bark, followed by sweet chestnut fruit, hazel and oak. It was in this period that the mycelium began to grow on the medium with the addition of sycamore maple bark.



Graph 5. *Mycelial growth (Period 5)*

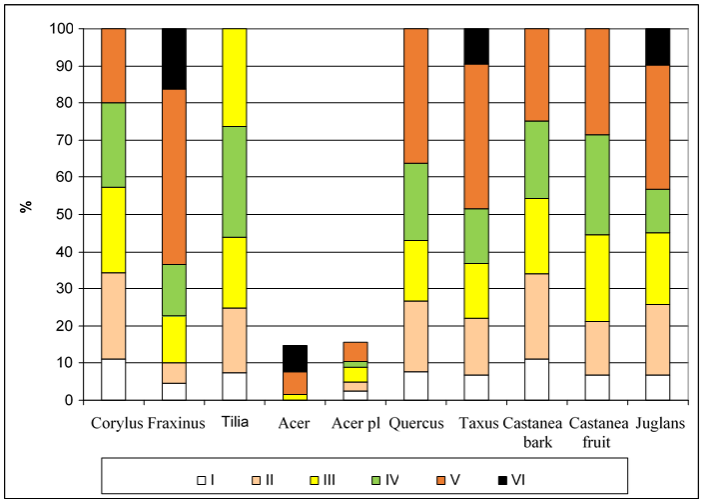


Graph 6. *Mycelial growth (Period 6)*

In the fifth period, the greatest growth was on the medium with the addition of manna ash bark, followed by yew, oak and walnut bark. The lowest growth was observed on the Norway maple and sycamore maple media.

After the fifth period (on the fifteenth day), the mycelia filled the Petri dishes on the media with the addition of hazel, linden, oak, sweet chestnut bark, as well as chestnut fruit. In the sixth period, mycelial growth on the medium with the addition of Norway maple bark stopped (it was the same as in the previous measurement). In this period, mycelial growth continued on the media with the addition of ash, yew, walnut and sycamore maple.

The graph below (Graph 7) shows the mycelial growth for each investigated variant by period.



Graph 7. *Mycelial growth for each tested variant by period*

Regarding the variant with the addition of hazel bark, the growth of mycelia was roughly equal in all periods (20-23.9%). A slightly smaller growth was observed only in the first period (11.06%). The variant with the addition of manna ash bark had low growth in all periods, except for Period 5 (with approximately half of the total growth, 47.31%). Regarding the variant with the addition of linden bark, the most significant growth was attained in Period 4 (29.65%) and the lowest in Period 1 (7.41%) period.

Regarding the variant with the addition of sycamore maple bark, the mycelium had not begun to grow until Period 4, while the growth was the same in Periods 5 and 6. The variant with the addition of Norway maple had low growth in the first four periods, while it was the highest (3.88%) in Period 5 and then stopped in Period 6. Regarding the variant with the addition of oak bark, the weak growth in the first period was followed by three fairly even periods, and the highest growth was in Period 5 (36.24%). In the case of the variant with the addition of yew bark, the highest growth was recorded in Period 5 (36.71%), and the lowest was in Periods 1 (6.91%) and 6 (9.65%). The variant with the addition of chestnut bark had the most invariable mycelial growth across periods (from 20.31-24.63%). It was the lowest in Period 1 (10.90%). The variant with the addition of chestnut fruit had the lowest growth in the first period (6.69%), and with each subsequent period, mycelial growth kept increasing slightly (from 14.49% in Period 2 to 28.67% in Period 5). The variant with the addition of walnut bark had the highest growth in Period 5 (33.41%), and the lowest in Period 1 (6.71%).

The survival of sweet chestnut has been threatened in the entire territory of Serbia and unfortunately, its area has been constantly decreasing from year to year. According to the research by GLIŠIĆ (1975), chestnut is autochthonous (of relict origin) but rare in Serbia today. It grows in Vojvodina – on Vršački Breg and a few localities on Fruška Gora, in central Serbia on Gučevo, Kostajnik near Krupanj, Hisardžik near Prijepolje, localities near Vranje, Gorica and Saličevica near Niš. In Kosovo and Metohija, there are several chestnut sites near Kosovo Polje, Peć,

Dečani, Đakovica, Prizren, Žar Planina, etc. The present-day fragmented sites or individual trees of sweet chestnut are the remnants of the former stronger presence of chestnut forests in our area. Hence it is necessary to protect and rehabilitate the existing natural chestnut sites and then gradually expand the area to sites optimal for its cultivation in Serbia. At the same time, due to the spread of the fungus *C. parasitica*, it is necessary to take care of other tree species that grow with sweet chestnuts in communities.

4. CONCLUSIONS

After 28 days of investigating the growth rate of *C. parasitica* mycelia on media made from the bark of different tree species, the following results were obtained:

- After two days, no mycelial growth was observed on the medium with the addition of sycamore maple bark, while the media with the addition of hazel bark and chestnut bark had the highest growth recorded.
- After four days, there was still no mycelial growth of *C. parasitica* on the medium with the addition of sycamore maple bark, while the media with the addition of hazel and chestnut bark recorded the highest growth.
- After six days, the greatest growth increase was still on the hazel and chestnut bark; the maple medium still had no growth, while 8.9% of the Petri dish radius was covered in the hazel medium.
- After eight days, the mycelium of *C. parasitica* began to grow on the medium with the addition of maple bark, while it had the highest growth increase on the medium with the addition of hazel bark and chestnut bark. We noted a large increase on the medium with the addition of linden bark (73.6%).
- On the fifteenth day, the Petri dishes were completely filled on the media with the addition of oak, chestnut, hazel and linden bark, as well as chestnut fruit. The lowest growth was recorded on the medium with the addition of sycamore maple (7.6%), followed by Norway maple (15.6%), and manna ash (83.8%). On the media with the addition of walnut and yew bark, the increase was approximately 90%.
- After 28 days, the media not completely overgrown were only in the variants in which sycamore maple and Norway maple bark were added.
- The largest number of pycnidia were formed on the media with the addition of sweet chestnut and sessile oak bark (formed in concentric circles), and chestnut fruit (formed over the entire surface of the medium).

The obtained results indicate that some tree species that grow together with sweet chestnuts can be infected with *C. parasitica* and become a potential source of further infection. However, due to the small number of pycnidia formed on these media, the dispersion of ascospores is very small, so the infectious potential is low. Based on this research and bearing in mind that regarding the natural conditions in

our country, this fungus has only been found on sessile oak trees, the highest risk when afforesting with sweet chestnut occurs in the localities where sessile oak trees are already present.

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Summary

The occurrence of the parasitic fungus Cryphonectria *parasitica* (causing bark blight) has significantly reduced the use value of sweet chestnut trees. *C. parasitica* was first observed in the New York area in 1904 (probably introduced from East Asia), and then quickly spread over the entire distribution area of the American chestnut (*Castanea dentata)*. Fungus *C. parasitica* occurs on all known species of chestnut, but it causes the greatest damage to the American chestnut *Castanea dentata*. The European chestnut *Castanea sativa* is also sensitive, while the Asian species *Castanea crenata* and *Castanea molissima* are more resistant. It occurs as a saprophyte on other tree species, with the exception of oak, where it develops as a parasite. Other hosts of the fungus *C. parasitica* belong to the families of *Aceraceae, Betulaceae, Fagaceae, Anacardiaceae, Juglandaceae* and *Magnoliaceae*. Mycelial growth of *C. parasitica* was examined on media made from the bark of different tree species. We used the isolate *C. parasitica* (CS3) recovered from the bark of the sweet chestnut growing in the locality of Sobina near Vranje. The bark of sweet chestnut (*Castanea sativa*), manna ash (*Fraxinus ornus)*, sycamore maple (*Acer pseudoplatanus*), sessile oak (*Quercus petraea*), common yew (*Taxus baccata*), hazel (*Corylus avellana*), small-leaved linden (*Tilia cordata*), Norway maple (*Acer platanoides*) and English walnut (*Juglans regia*). Besides the bark, we used a medium made from the fruit of the sweet chestnut trees. Statistically significant differences in the growth of mycelia On the media made from the bark of different tree species were determined between the experimental groups after 15 days. By that time, the Petri dishes had been completely filled on the media with the addition of oak, chestnut, hazel, linden and bark, and chestnut fruit. The lowest growth was recorded on the medium with the addition of sycamore maple (7.6%), followed by Norway maple (15.6%), and manna ash (83.8%). The media with the addition of walnut and yew bark had the same growth rate that amounted to about 90%. After 28 days, the media was not completely overgrown only in the variants with the addition of sycamore maple and Norway maple bark. On the medium with the addition of sycamore maple bark, the mycelium hadn't begun to grow until after eight days and had the slowest growth (14.7%). Based on this research and bearing in mind that regarding the natural conditions in our country, this fungus has only been found on sessile oak trees, the highest risk when afforesting with sweet chestnut occurs in the localities where sessile oak trees are already present.

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Rezime

Zbog pojave parazitne gljive *Cryphonectria parasitica* (izaziva rak kore) upotrebna vrednost kestena je znatno umanjena. *C. parasitica* prvi put je primećena u okolini Njujorka 1904. godine (verovatno prenešena iz istočne Azije), a zatim se brzo proširila preko celog areala rasprostranjenja američkog kestena (*Castanea dentata*). Gljiva *C. parasitica* javlja se na svim poznatim vrstama kestena, ali najveće štete pričinjava na američkom kestenu *Castanea dentata*. Osetljiv je i evropski kesten *Castanea sativa*, dok su azijske vrste *Castanea crenata* i *Castanea molissima* otpornije. Na ostalim vrstama drveća na kojima je konstatovana javlja se kao saprofit, izuzev hrasta na kome se razvija parazitski. Ostali domaćini gljive *C. parasitica* pripadaju familijama: *Aceraceae, Betulaceae, Corylaceae, Fagaceae, Anacardiaceae, Juglandaceae* i *Magnoliaceae.* Porast micelije *C. parasitica* ispitivan je na podlogama napravljenim od kore različitih vrsta drveća. Korišćen je izolat *C. parasitica* (CS3) dobijen iz kore pitomog kestena sa stabla na lokalitetu Sobina kod Vranja. U ovim ispitivanjima je korišćena kora: pitomog kestena (*Castanea sativa*), crnog jasena (*Fraxinus ornus*), javora (*Acer pseudoplatanus*), hrasta kitnjaka (*Quercus petraea*), tise (*Taxus baccata*), leske (*Corylus avellana*) sitnolisne lipe (*Tilia cordata*), mleča (*Acer platanoides*) i oraha (*Juglans regia*). Pored kore korišćena je i podloga napravljena od ploda pitomog kestena. Na veštačkim podlogama napravnjenim od kore različitih vrsta drveća posle 15 dana utvrđeno je postojanje statistički značajnih razlika među ekperimentalnim grupama u porastu micelije. U ovom periodu Petri posude su skroz popunjene na podlogama sa dodatkom kore hrasta, kestena, leske, lipe i ploda kestena. Najmanji porast je zabeležen na podlozi sa dodatkom javora (7,6%), potom mleča (15,6%), pa jasena (83,8%). Na podlogama sa dodatkom kore oraha i tise porast je isti, i iznosi oko 90%. Posle 28 dana podloga nije u potpunosti obrasla samo u varijantama kada je podlozi dodavana kora javora i mleča. Na podlozi sa dodatkom kore javora micelija počinje sa rastom tek posle osam dana i na ovoj podlozi ima najmanji porast (14,7%). Na osnovu ovih istraživanja, a imajući u vidu činjenicu da je ova gljiva kod nas do sada u prirodnim uslovima konstatovana samo na kitnjaku, najveći rizik pri pošumljavanju pitomim kestenom pretstavljaju lokaliteti na kojima je već prisutan kitnjak.