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AN OVERVIEW OF THE HISTORY OF THE CHESTNUT BLIGHT EPIDEMIC IN SLOVENIA

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Abstract:
Chestnut blight appeared near the town of Nova Gorica in Slovenia in 1950, but it had most likely been introduced into the country as early as 1940. Quarantine measures were immediately implemented and a special commission was created to fight the disease. The Commission's role was to organize all chestnut blight-related actions, submit new regulations and conduct a broad-based research program. The research work focused on the following areas: finding resistant sweet chestnuts; selecting, introducing and breeding Chinese chestnut; inducing mutations in the seeds of the sweet chestnut; investigating the role of bees in spreading disease; determining the longevity of spores and mycelium in infected bark; disinfecting wood and seeds; preventing new sprouts from infected stumps; and searching for antagonistic microorganisms. All research work and attempts of eradicating or stopping the disease were abandoned in 1969. Hypovirulence of the chestnut blight was presumed to have appeared in Slovenia in the mid-fifties, but was clearly firmly established by the mid-sixties as photographs of harmless superficial infections show.

Key words: chestnut blight, *Cryphonectria parasitica*, history, Slovenia, hypovirulence

PREGLED ZGODOVINE EPIDEMIJE KOSTANJEVEGA RAKA V SLOVENIJI

Povzetek
Kostanjev rak je bil ugotovljen leta 1950 v okolici Nove Gorice, vendar je bil v Slovenijo najverjetneje vnesen že leta 1940. Karantenski ukrepi so bili vpeljani takoj; osrednjo vlogo v boju proti bolezni je dobila posebna komisija, ki je organizirala vsa operativna dela, predlagala nove predpise, njeni člani pa so opravljali tudi obsežen raziskovalni program. Raziskovalno delo je bilo usmerjeno v iskanje odpornih genotipov pravega kostanja; vnos kitajskega kostanja, njegovo selekcijo in žlahtnenje; induciranje mutacij v semenih pravega kostanja; ugotavljanje pomena čebel pri razširjanju bolezni; določanje dolgoživosti trosov in podgobja v okuženi skorji; razkuževanje lesa in semena; preprečevanje odganjanja iz panjev; iskanje antagonističnih mikroorganizmov. Vse raziskovalno delo in poskusi izkoreninjanja ali zaustavitve bolezni so bili opuščeni leta 1969. Hipovirulenca kostanjevega raka je bila v Sloveniji verjetno že v sredini petdesetih let, nedvomno pa v sredini šestdesetih, kar potrjujejo fotografije neškodljivih površinskih okužb.

Ključne besede: kostanjev rak, Cryphonectria parasitica, zgodovina, Slovenija, hipovirulenca

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The chestnut blight epidemic (*Cryphonectria parasitica* (Murrill) Barr, sin. *Endothia parasitica* (Murrill) P.J. & H.W. Anderson) in North America and Europe is an example of a plant disease that man has been unable to stop or suppress despite great effort. The actions undertaken to stop its spread were the result of the knowledge and organizational capabilities of the state at the time when the disease was first discovered. No overview of the work conducted to fight the disease was carried out for what now comprises the territory of Slovenia, but which was then a republic of the former Yugoslavia. Therefore, most of the data on this subject is dispersed among unpublished manuscripts or are only available in Slovenian or Serbo–Croatian, making such data inaccessible to foreign forest pathologists. The actions undertaken, their organization, and research on the disease itself is useful historical information that may give us insight into problems connected with attempts to eradicate new diseases in the future.

2 THE OUTBREAK AND OFFICIAL REACTIONS
IZBRUH IN PRVE URADNE REAKCIJE

The chestnut blight in Slovenia appeared in sweet chestnut trees (*Castanea sativa* Mill.) in 1950 near the town of Nova Gorica (the Panovec forest), about 4 km from the Italian border (KRSTIĆ 1950). The reaction of the relevant state agencies to *C. parasitica* presence was immediate and comprehensive. The Slovenian Ministry of Forestry issued a circular about the disease symptoms and how to suppress it within a week from first identification of the disease (Oct. 27 and Nov. 3, 1950). The federal government also ordered a visit to the site of the outbreak by forest protection experts from all the Yugoslav republics in order to acquaint them with the disease and enable them to perform disease surveys over the entire territory of Yugoslavia the following year (Dec.7 - 8, 1950). The first ordinance concerning chestnut blight mandated the immediate reporting of disease cases to the forestry organization, instant felling of diseased trees as near to the ground as possible, the debarking of the wood and stumps, the burning or burying (0,5 m deep in the ground) of all diseased branches, bark and felling remains at the felling site, as well as a prohibition against transporting chestnut wood out of the infected area

(Odredba o ukrepih..., 1951). A supplement to this ordinance banned the export of sweet chestnut seeds, scions and plants from the diseased area (Odredba o dopolnitvi..., 1951). All the restrictions valid for the sweet chestnut were also put into force for oak wood, plants, scions and seeds after the disease was also found in oaks (Odredba o spremembi..., 1952). In 1952, the federal government banned the rail transport of chestnut and oaks from the infected area. In 1955, an ordinance was issued prohibiting the import of sweet chestnut wood, scions and saplings from other countries (Pravilnik o..., 1955).

In March 1952, the "Permanent Mixed Republic Commission for the Suppression of the Chestnut Canker" (hereafter, "the Commission") was established by the Council for Agriculture and Forestry of Slovenia. The word "mixed" was used to designate the structure of the Commission and its inclusion of various experts, seven in total, from various forestry and agronomy research organizations, forestry organizations, and the republic administration (HOČEVAR 1962). This Commission had a central role in subsequent efforts to fight the disease. Its tasks were:

- to evaluate measures taken against the disease to that time,
- to establish the extent and impact of the disease,
- to submit stringent measures aimed to eliminate the disease,
- to inform the residents of an infected area about the disease, its importance and the damage it produces, and justify measures against the disease,
- to enforce strict quarantine measures with the collaboration of forestry organizations,
- to inform authorities about its work and problems connected with disease suppression,
- to determine necessary funding and
- to cooperate with authorities in other republics of Yugoslavia.

All the actions and new regulations in successive years were proposed by the Commission and its members undertook to carry out a wide-ranging research program.

From its inception, the Commission's goal was to prevent the disease from spreading to the inner part of the country. The sweet chestnut growing area is divided into two distinct regions of Slovenia: the first is the submediterranean phyto-geographical region in the west of the country and the second is the continental region to the center and east of the country (WRABER 1956). The two regions are divided by an approximately 35 km wide zone (at its narrowest point) of forest with calcareous soils that do not sustain the sweet chestnut. The submediterranean region is comprised of about 2.500 ha of sweet chestnut and the continental part of about 22.500 ha (the area values given represent pure chestnut stands, although the overall area of mixed chestnut forests was about 250.000 ha). The standing wood volume of sweet chestnut was estimated to be more than 1 million m^3, which was the fourth largest standing volume of broadleaves in Slovene forests after beech, oaks and hornbeam.

By 1952, the Commission had proposed the formation of special survey teams, including experienced foresters working in diseased areas who knew the disease symptoms thoroughly and were able to determine the disease. These teams inspected stands of sweet chestnut in the central part of Slovenia twice a year (two 10 days periods: May 20-30 and September 20-30) through the mid-sixties (HOČEVAR 1956a,b, HOČEVAR 1969a).

Four sanitation teams, each comprised of five experienced forestry workers, were created to conduct all prescribed quarantine measures in the infected area (HOČEVAR 1952, ŠEBENIK 1956). The sanitation teams performed the majority of their work in state forests. Private forest owners were rarely allowed to fell diseased trees and then only under the supervision of the sanitation teams (HOČEVAR 1956a,b).

A poster with color drawings of the disease and information about fighting it was printed and 1.500 copies were distributed in 1952 and again in 1956. A popular booklet was issued with a detailed description of the disease and biology of the pathogen, as well as methods to control it and the obligations of forest owners (HOČEVAR 1952). Four thousand copies of the booklet were delivered free of charge. Every year from 1951 to 1959, the Commission organized seminars for foresters working in uninfected areas in order to acquaint them with the symptoms of chestnut blight and with the prescribed quarantine measures (HOČEVAR 1962).

3 THE PROGRESSION OF THE EPIDEMIC
NAPREDOVANJE EPIDEMIJE

After Italy, Spain and Switzerland, Yugoslavia was the fourth country in Europe to be struck by chestnut blight. In the year following its discovery, the disease had already spread to 212 ha of chestnut stands within 9.000 ha of forest area near the Italian border. There were strong indications that the disease had been established well before these larger outbreaks appeared (ŠEBENIK 1951a,b, ŠEBENIK 1956, HOČEVAR 1956a,b). This is based on the appearance of the disease 35 km from the border of Slovenia in Italy near the town of Videm (Udine) as early as 1939 and an increased felling of dead sweet chestnut in the area approximately 10 – 12 years before the outbreak in 1951. However, the lack of a forestry service during the Second World War and afterwards during the Anglo-American military control of the area, until 1948, meant that it was almost impossible to act on these observations. The newly established forestry service, however, ascribed the greater number of dead chestnuts between 1948 and 1950 to ink disease (*Phytophtora cambivora* (Petri) Buism.) (KRSTIĆ 1950, ŠEBENIK 1951b). The final conclusion about the introduction of the chestnut blight into Slovenia has been that the disease was introduced before the Second World War, namely in 1940 (ŠEBENIK 1956, HOČEVAR 1956a,b). In 1951, infections with *C. parasitica* were found on 20 oak trees *Quercus petraea* (Matt.) Liebl. and its hybrids with *Q. robur* L. (KRSTIĆ 1952); they were later also found on *Q. pubescens* Willd. (HOČEVAR 1956a,b).

There were slight differences in area reports, as well as slight differences in the extent of disease progress in subsequent years. The most reliable data were reported by Stana Hočevar, a member of the Commission, and Mithat Uscuplić; these data are combined and quoted here (HOČEVAR 1956a,b, HOČEVAR 1962, HOČEVAR 1969a, USČUPLIĆ 1961, USČUPLIĆ 1962).

An intensive search for the disease in 1951, a year after identification of the fungus, revealed 15 disease foci covering 212 ha. In 1952, thirty newly diseased stands were found and the entire diseased area comprised 610 ha of chestnut forests. The first infection in the district of Sežana was also found in 1952. At this point, the entire county of Nova Gorica was considered to be infected and, therefore, a systematic inventory was no longer conducted in this area. An intensive search for the disease in uninfected areas in the rest of the submediterranean region revealed a total of 53 foci up to 1955, comprising 1.813 ha of chestnut stands. By 1956, the disease had been found in sweet chestnut in previously uninfected areas of the submediterranean region. Thus, by 1956 the entire submediterranean region was considered infected (an equivalent of 2.500 ha of pure chestnut forest).

In 1956, two disease foci in the central part of Slovenia were detected: one in Ljubljana and the other near Sevnica (respectively, about 45 and 90 km from the infected area). The infection in Ljubljana (Cankarjev vrh) comprised only a few trees, which were immediately felled; however, by 1958 the disease had already progressed to 36 ha and by 1962, to 60 ha. The infection near Sevnica (Topolovec hill) was 0,5 ha in size and was successfully exterminated. In 1959, new disease foci were found near Vrhnika (20 km from Ljubljana) and Škofja Loka (25 km from Ljubljana). In 1960, new infections were found near Litija (30 km from Ljubljana) and in 1961 near Celje (70 km from Ljubljana). All these infection foci expanded in the following years and the disease quickly infested all areas of Slovenia containing sweet chestnut trees despite strict quarantine measures.

In 1956, chestnut blight was found near Opatija in Croatia (KIŠPATIĆ 1956) and in 1961 near Cazin and Velika Kladuša in Bosnia, where the disease has already extended to 721 ha and to individual trees at two distant locations. It was later concluded that the disease had probably been introduced 5 – 6 years prior to its detection (USČUPLIĆ 1983).

In Yugoslavia, disease progression was characterized by the absence of a defined disease front and by outbreak points far from existing diseased areas.

4 MEASURES AND RESEARCH UNDERTAKEN TO SUPRESS THE DISEASE
UKREPI IN RAZISKOVALNO DELO ZA ZATIRANJE BOLEZNI

4.1 THE SEARCH FOR RESISTANT SWEET CHESTNUT TREES
ISKANJE ODPORNIH GENOTIPOV PRAVIH KOSTANJEV

In the early sixties, forestry organizations in the infected submediterranean area started to search for disease resistant sweet chestnut trees (HOČEVAR 1969a). By 1965, 40 healthy trees, detected in an area that had been infected for 15 years, had been registered. Eight of the most promising specimens were selected and each was inoculated with eight different isolates of *C. parasitica* showing variable pathogenicity. After four years, the effect of the disease on the inoculated trees was varied: two trees had died, two were surviving and "their regeneration was stronger than the infectious capabilities of the fungus," while the results for the remaining trees were inconclusive. HLIŠČ and HOČEVAR (1969) concluded that there was only a very small probability of finding a resistant domestic sweet chestnut.

A study to determine whether sweet chestnut trees from the rest of Yugoslavia might contain resistant varieties had been carried out during the 1950's with no success. Saplings grown from 42 kg of seed collected in Macedonia, Serbia, Croatia and Bosnia and Herzegovina, as well as saplings brought from Bosnia (100 saplings), Serbia (100 saplings), and Macedonia (200 saplings) were collected in a forest nursery in the infected area. All the saplings were inoculated with a spore suspension and mycelium of *C. parasitica* in 1954 when they were 3,5 - 4 m high with a 3 cm dbh. All of these trees died during the year following the inoculation (HOČEVAR 1969a).

4.2 DISINFECTION OF WOOD AND SEEDS
RAZKUŽEVANJE LESA IN SEMENA

It was forbidden to transport infected chestnut wood from sanitation felling out of the infected area due to quarantine measures. The wood was exported to Italy, mostly to the tannin producing factory in Čedad (Cividale del Friuli). This export was stopped in 1955 and the Commission proposed disinfecting the wood prior to transport to tannin extraction factories in undiseased areas in Yugoslavia. At the beginning of the fifties, there were three tannin extraction factories in Slovenia (in Sevnica, Šoštanj and Majšperk) and four in Croatia (in Belišće, Sisak, Zagreb and in Djurdenovac). They used about 126.000 tons of sweet chestnut wood (302.000 scm) per year (SUČIĆ 1956).

In 1956, the federal authority allowed the tannin producition factory in Sevnica (in the central part of Slovenia) to import sweet chestnut wood from an infected area on condition that all the wood be debarked and disinfected for 5 minutes in a 5 % solution of 40 % formaldehyde. The regulation was based on experiments by Janežič, which were only published later (JANEŽIČ 1964). Railroad wagons were also disinfected with formaldehyde after the transport of the disinfected chestnut wood. At railroad stations in diseased areas, special concrete reservoirs for the disinfection of chestnut wood were built. Disinfection was controlled by special quarantine supervisors. The possibility to sell sweet chestnut wood from infected areas enabled the Commission to intensify the felling of diseased trees. After the introduction of these measures, the annual felling of sweet chestnut was raised to 8.000 m^3 in 1956 and to an average of 3.000 – 4.000 m^3 in subsequent years. Before these measures were introduced, sanitation felling over the entire infected submediterranean area totaled approximately 8.000 m^3 between 1950 and 1955 (HOČEVAR 1962).

The Commission had been very concerned since the outset of the disease with the possibility of the disease being spread by infected sweet chestnut seeds. In the submediterranean region yearly production of sweet chestnut seeds was approximately 450 t, about 200 t of which was the top grade "marroni" edible chestnut. There was a "black market" for the marroni type chestnut seed from disease free areas (Ljubljana, Kranj, Jesenice, and Celje, as well as Reka and Zagreb in Croatia), so the seed was smuggled in despite the ban (HOČEVAR 1956a,b). Janežič's experiments from 1953 found that in diseased stands 1 kg of chestnuts (about 150 seeds) can carry 5.632 – 87.240 conidia and 912 – 3.240 ascospores (JANEŽIČ 1964). Disinfecting the seeds requires approximately 30 minutes of immersion in 0,5 % solution of 40 % formaldehyde.

By 1953, the Flores company from Šempeter near Nova Gorica had built a reservoir for disinfecting seeds and received permission to sell the seeds. One member of the Commission was personally responsible for supervising seed disinfection and certifying the seeds to be sold. As the Commission did not intend to create a monopoly, they allowed two other firms to built disinfection reservoirs and collect seeds from private forest owners. In spite of these measures, the "black market" was still active and, in 1954, the Commission ordered disinfection reservoirs built wherever seeds were purchased from private forest owners and at railroad stations. The police were brought in to inspect produce and vegetable trucks, because seeds were often smuggled under vegetables. Announcements on metal signs containing information on businesses where seeds could be bought and sold, as well as quarantine measures against chestnut blight, were put up at road crossings and in villages, mostly near the police stations.

4.3 LIFETIME OF SPORES AND MYCELIUM IN INFECTED BARK
DOLGOŽIVOST TROSOV IN PODGOBJA V OKUŽENI SKORJI

Research was conducted to determine whether burying infected branches and other felling remains poses a threat to the forest in terms of disease retention and spread. It was shown that the fungus was able to produce viable spores in bark and in infected felling remains for more than one year after burial. If the material was buried in soil, the conidiomata were not able to produce spores after 6 – 12 months and the mycelium in the bark died out in 12 to 15 months. Burying was the preferred disposal method when the conditions were such that material could not be burnt due to the high risk of forest fires; the recommended burying depth was 15 cm (HOČEVAR 1956a,b, KRSTIĆ / HOČEVAR 1958b).

4.4 PREVENTION OF NEW GROWTH FROM STUMPS
PREPREČEVANJE ODGANJANJA IZ PANJA

The production of sprouts from stumps of sweet chestnut was abundant after sanitation felling operations. These new sprouts were considered to be the most important source of new infections in sweet chestnut forests due to their large quantity and their greater susceptibility to disease than slower growing older trees. Therefore, the Commission made every effort to eliminate new growth from stumps in infected forests. It was obligatory to peel the bark from stumps and cover the stumps with soil. Fire was also used to prevent stumps from sprouting in the forests; all felling remains were piled onto the stumps and burned. Dynamite was used to raze stumps more than 1 m wide; bulldozers were also used to uproot stumps. Many chemical substances were tested for use in killing the stumps and new growth. In 1959, arboricides with 2,4 D and 2,4,5 T as active ingredients (a.i.) were introduced on a wider scale. These were used in forests in concentrations of 4-6 % a.i. in diesel oil to kill stumps and 1-2 % a.i. in diesel oil for suppressing 2 and 3 year old new growth sprouts (HOČEVAR 1956a,b, HOČEVAR 1962, KRSTIĆ / HOČEVAR 1958a,b).

4.5 INTRODUCTION OF CHINESE CHESTNUT, ITS SELECTION AND BREEDING
UVAJANJE KITAJSKEGA KOSTANJA, NJEGOVA SELEKCIJA IN KRIŽANJE

Based on data from North America, where *C. parasitica* had heavily damaged *Castanea dentata* (Marsh.) Borkh., the experts were convinced that the sweet chestnut could not survive in infected areas. Therefore, they decided to introduce other species of chestnuts resistant to chestnut blight. The Chinese chestnut (*Castanea mollissima* Blume) was chosen to directly substitute for *C. sativa* in forests and in fruit production, as well as for hybridization with the sweet chestnut to obtain resistant hybrids. They contacted the U. S. Department of Agriculture, Bureau of Plant Industry, Soils and Agricultural Engineering, Division of Plant Exploration and Introduction - Inspection House, Washington D. C. and the following amounts of selected lines of Chinese chestnut (*C. mollissima*) seeds were obtained from Dr. Filippe Gravatt, Chico, Beltsville, Maryland: in 1952 – 2,26 kg seeds of FP-1533; in 1953 – 2,26 kg FP-1586 A; in 1954 – 0,45 kg seeds of FP-1628-D and 0,45 kg FP-1628-H; in 1954 – 1,36 kg GBL A 2098206, 1,8 kg GBI A 2098277, 0,7 kg FP-1628-B, 0,45 kg FP-1628-H and 0,1 kg FP-1628-D.

The saplings produced from these seeds were planted in the Panovec forest area (Dep. 17, 18 and 12), where the disease had first been observed and felling of the diseased sweet chestnut had been most intensive. By 1969, some of these trees were already more than 7 m high but many had been damaged by deer and the winter cold. Four-year old saplings of Chinese chestnut were also provided by Dr. Pavari and were sent from an Italian nursery near Bagni di Lucca, Tuscany in 1954. Some of the above seeds were obtained by KRSTIĆ (1955). In 1954, he carried out hybridization of selected lines of Chinese chestnut in Glenn Dale, Maryland, U.S.A. The seedlings from these experiments were planted in the collection of the Forestry Research Institute of Serbia in Belgrade.

In 1957, the forestry authority of Slovenia bought 1.000 kg of Chinese chestnut seeds from a plantation in Grassonville, Maryland, U.S.A. Foresters succeeded in raising 60.000 saplings, 37.000 of which were planted in the spring of 1958, the rest in the autumn of 1958 and spring 1959, on 85 research plots at all important sweet chestnut growing areas in Slovenia. Seventy-one ha of Chinese chestnut plantations were divided into 10 areas. After four years in plantations, the condition of *C. mollissima* trees was not satisfactory. The trees did not grow as quickly as sweet chestnut trees, they were damaged by cold winters (most of them did not shed their leaves in autumn and branches were often broken by heavy snow; the ends of the branches were also dying due to cold), their shape was bush-like rather than treelike, and they were heavily damaged by deer grazing (HOČEVAR 1962, HLIŠČ / HOČEVAR 1969).

Inoculation of Chinese chestnut trees with *C. parasitica* was successful. All inoculated trees with a DBH of less than 4 cm died and the survival of the larger trees depended on their overall condition. In trees on suitable sites and with good care (e.g. weed removal, nutrients supply, etc.), chestnut blight infections were limited to slowly expanding cankers that were not lethal to the tree or branches. HOČEVAR (1969b) concluded that the susceptibility of Chinese chestnut to infection with chestnut blight is about the same as the susceptibility of sweet chestnut, but the Chinese chestnut reaction is superior in terms of disease resistance. On suitable sites, it can withstand the disease indefinitely in spite of being infected.

In 1956, the sweet chestnut was crossed with pollen from the Chinese chestnut. Chinese chestnut pollen was obtained from Italy and mother trees were selected from among trees that had best withstood the epidemic up to that time in the most heavily affected areas. (HOČEVAR 1969b). Despite successful growth, many of the saplings were destroyed by deer and only 42 had survived up to 1959. In 1962, the survivors were planted on an experimental plot in Dolsko near Ljubljana.

Extensive research on the seed quality of the Chinese chestnut took place from 1965 to 1968 on two experimental plantations planted in 1954 (Panovec I, line FP-1533, and Osek, 4 year old saplings from Dr. Pavari, Lucca, Tuscany, Italy). Nearly all the trees bore male flowers and more than half the trees bore seeds. Researchers selected six forest type trees and three fruit type Chinese chestnut trees from Panovec I and 10 trees from

Osek. Some types of fruit trees had "marroni" seeds with an average weight of more than 15 g per seed. (HLIŠČ / HOČEVAR 1969).

4.6 INDUCED MUTATIONS IN SWEET CHESTNUT SEEDS
INDUCIRANE MUTACIJE V SEMENU PRAVEGA KOSTANJA

In 1959, Janežič (JANEŽIČ 1969) began to irradiate the seeds of the sweet chestnut with gamma rays from ^{60}Co to induce mutations. It was hoped that the mutations induced in the seeds would produce sweet chestnut trees resistant to disease. In the first set of experiments, he defined the doses of radiation that would induce mutations but not kill the seeds. The germination capacity of seeds was dependant on the radiation dose received: 5.000 gray and 10.000 gray - 100% seeds dead, 2.500 gray - 50% seeds germinated, 1.000 gray - 60 to 75 % seeds germinated, and 500 gray - no lowered germination. These results were obtained using marroni type seeds (large seeds), but for smaller sweet chestnut seeds (average size) an absorbed dose of 500 gray lowered the germination capacity by 20-35 %.

In the three subsequent years, they collected large numbers of seeds from all chestnut growing areas in Slovenia (1960 - 330 kg, 1961 - 270 kg, 1962 - 170 kg). Half of these seeds were irradiated with a dose of 500 gray, the other half were not irradiated. Every year they irradiated marroni type seeds with 1.000 gray and with different combinations of smaller irradiation doses. When the stems of saplings grown from these seeds were 2 – 2,5 cm wide at ground level they were inoculated with pycnospores or mycelium of *C. parasitica*. As pycnospore inoculations were not effective enough for successful infections (90% were negative), they began to use inoculations with mycelium from culture in 1965 (90 % were successful). The inoculation of each tree was repeated every year from 1966 to 1968, once or twice per year, but in these inoculations, bark from sweet chestnut with pycnidial stromata was used.

The results of this experiment showed that about 30% of saplings showed slow, stunted growth and had a bushy form. Such undesirable trees were not inoculated. In some saplings of marroni chestnuts, successful inoculations produced bark necrosis that later healed (3 saplings). In some saplings, the entire crowns and trunks died, but sprouts from the root collar were healthy and vigorous. The most important outcome was the 28 saplings produced from the "average seed," which were positive for infection, but in which the mycelium of the fungus did not kill the cambium and was growing only in the outer layers of the bark. However, there remained hundreds of trees from which results could not be obtained because they were too thin for inoculations. The author had hoped to continue the experiment but financing was halted in 1969.

4.7 IMPORTANCE OF BEES IN DISEASE DISSEMINATION
POMEN ČEBEL PRI PRENAŠANJU BOLEZNI

The Commission banned the transport of bees to and from infected area between June 1 and August 1 (Odredba o dopolnitvi..., 1954) on the assumption that bees can act as vectors of the disease. After six years of fungal isolation attempts from bees visiting infected chestnuts in bloom (all of which were negative), as well as microscopic examination of the water obtained by rinsing the bees and analysis of pollen from bee hives, JANEŽIČ (1964) concluded that bees are not plausible carriers of the disease and the ban was ended.

4.8 ANTAGONISTIC MICROORGANISMS
ANTAGONISTIČNI MIKROORGANIZMI

In 1952, Krstić and Hočevar began to search for microorganisms that could be used as inhibitors of *C. parasitica* in sweet chestnut bark. They isolated 35 microorganisms from bark, wood and stumps of both healthy and diseased chestnut trees. Four of these (*Penicillium rubrum*, *P. lilacinum*, *Verticillium* sp. and *Bacillus subtilis*) showed an inhibitory effect on the growth of mycelium of *C. parasitica* in dual laboratory cultures. The most promising results were obtained in field trials with *P. rubrum*, which decreased the natural infection rate of *C. parasitica* through bark incisions by five times compared to the control (KRSTIČ / HOČEVAR 1959). Mass cultivation of *P. rubrum* was performed in sawdust and it was introduced into two research plots. *P. rubrum* was regarded as the most successful microorganism as it was able to persist in artificial wounds in sweet chestnut. No conclusive results were given regarding the use of microorganisms as inhibitors of chestnut blight, although the author did note that while the disease had not invaded sweet chestnut on experimental plots, it had invaded nearby forests (HOČEVAR 1969a).

5 HYPOVIRULENCE OF CHESTNUT BLIGHT
HIPOVIRULENCA KOSTANJEVEGA RAKA

The transmissible hypovirulence of *C. parasitica* is regarded as the most promising phenomenon for the survival of chestnuts susceptible to this parasite (ANAGNOSTAKIS 1987, HEINIGER / RIGLING 1994). Throughout this text review attention has been focused on any indicators that could enable us to establish the dates of any abnormal (hypovirulent) infection description in sweet chestnut in Slovenia.

In the concluding remarks of his article about indirect measures against chestnut blight, KRSTIĆ (1955) stated that some sweet chestnut trees in Panovec forest had a higher level of disease resistance than others. He personally observed necroses on chestnut bark that were healing. In his opinion, trees with higher disease resistance were, after "residual mycelium growth of *E. parasitica,*" spontaneously forming callous tissue and healing. He concluded that in such situations the resistance of the chestnut had to be proven by artificial inoculations with the fungus. We believe that his description fits what is today called a "healing canker." Healing cankers are the result of the conversion of a virulent fungal individual into hypovirulent one after a hypovirus transfer or of the infections produced by a hypovirulent strain directly.

HOČEVAR (1969a) pointed out that she systematically searched for the most pathogenic isolates of *C. parasitica* and that inoculations to find resistant trees were performed with only the most aggressive isolates. This clearly demonstrates the existence of less virulent isolates, which were excluded from further experiments. She also mentioned her experiments on the effect of high temperatures on pathogen virulence (HOČEVAR 1962). She found that the change in virulence after high temperature treatment was temporary and that the virulence could become even higher after two successive inoculations of sweet chestnut.

She provided two black and white photographs of abnormal infections in her manuscript with the following description: "New growths of sweet chestnut in Stara Gora near Gorica (100 m a.s.l.) recovering from chestnut blight" (HOČEVAR 1969a). Fig. 1 is reproduction from her manuscript and it clearly represents superficial infections produced by hypovirulent strains of *C. parasitica.*

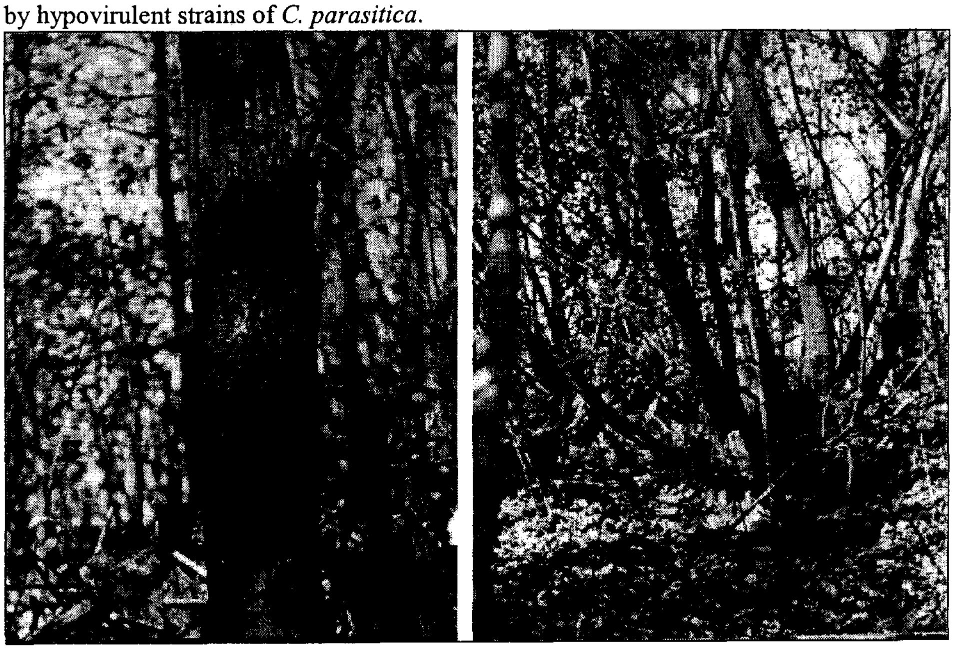

Figure 1: Photographs of sweet chestnut shoots from HOČEVAR (1969a) designated as "recovering from chestnut blight". In our opinion these infections are typical superficial infections produced by hypovirulent strains of *C. parasitica*

Slika 1: Sliki poganjkov pravega kostanja, ki jih je HOČEVAR (1969a) opisala kot "prebolevajo bolezen"; po našem mnenju so to tipične površinske okužbe, ki jih povzročajo hipovirulentni sevi C. parasitica

JANEŽIČ (1969) tried to find resistant mutants of sweet chestnut and made hundreds of inoculations. In one experiment, he describes three and in another experiment 28 healing necroses of bark, which we believe to be healing abnormal cankers.

An article by BIRAGHI (1953), who attributed the resistance of chestnut to chestnut blight to the repeated coppicing of sweet chestnut, was of considerable interest to chestnut blight researchers in Slovenia. Biraghi's findings have been often cited as an interesting biological phenomenon with an unclear biological basis. Slovenian researchers searched intensively for resistance in the sweet chestnut, but the observed variable pathogenicity in the fungus did not provoke their interest. They stopped their research work in 1969 when the nature of transmissible hypovirulence was discovered.

At the beginning of the eighties, widespread healing of chestnut blight cankers and superficial, non-harmful bark infections were noticed in the submediterranean region of Slovenia. Information about transmissible hypovirulence and its possible use was presented to foresters (JURC 1985). Isolations of the fungus showed a different morphology of the isolates in culture (white or poorly colored, producing less pycnidia). In 1989, thirteen isolates of typical hypovirulent *C. parasitica* were inoculated in three experimental plots in central Slovenia to test their pathogenicity. The areas of necroses were measured periodically and it was shown that the white isolates differed in their pathogenicity, ranging from completely harmless to intermediate pathogenicity (JURC 1997). Seven inoculations of hypovirulent strains around virulent infections were also performed. All seven trials succeeded and virulent infections began to heal although vegetative compatibility tests among virulent and hypovirulent isolates were not performed. In experiments of chestnut blight pathogenicity, HALAMBEK (1988, 1991) used two strains of *C. parasitica* from Slovenia that had been isolated from abnormal, healing chestnut blight cankers. She proved their intermediate pathogenicity.

During the nineties, a wide range of activities was carried out to inform active forestry workers in the Forestry Service of Slovenia about the nature of hypovirulence and the possibilities for its use. Active foresters from all regions with sweet chestnut attended one-day seminars, where changes in the management of sweet chestnut were proposed. Up to that time, sweet chestnut was regarded as a doomed tree species that had no future due to chestnut blight. New management strategies, based on an article by TURCHETTI (1982), represented a major change in attitude: sweet chestnut was again regarded as a viable tree species. However, care clearly had to be taken to maintain levels of abnormal (hypovirulent) infections in large numbers in the stand and to help the trees with these hypovirulent infections via maintenance measures and via an intensification of fellings of virulent infections.

The dsRNA of hypoviruses in isolates from abnormal chestnut blight infections from Slovenia was proved by TURCHETTI and MARESI (2000).

6 CONCLUSIONS
ZAKLJUČKI

The fight against chestnut blight has undoubtedly been the most extensive action undertaken by Slovene forestry in the field of forest pathology. The execution of quarantine measures in forests was appropriate and effective due to the well-organized and conscientious forestry service. The research work performed in Slovenia after the chestnut blight outbreak has been intensive and comprehensive. The quarantine measures implemented were based on the results of research in both Slovenia and abroad. A wide variety of experiments was also carried out in an attempt to find a way to save the sweet chestnut. All systematic attempts to suppress the disease wound down in the sixties after measures to stop the spread of the disease in Yugoslavia had failed. In 1969, research work on chestnut blight was halted and was not taken up again until the end of the nineties.

Among the citizens and foresters from the infected areas, there are still vivid memories of the rigorous quarantine measures, as well as a number of anecdotes and tragic stories from that era. Sometimes the police were involved in enforcing quarantine measures. In other cases, the inhabitants of the village Ukanje (Goriška Brda) got together to protect their valuable marroni trees, which had been grafted and cared for centuries. These trees were not felled and many remain alive even today. Among private forest owners, there is still folk wisdom from the days of the chestnut blight progression like: "If you cut sweet chestnut repeatedly, it will become resistant to chestnut blight." Marjan Šebenik, a member of the Commission, told the story of driving his motorcycle all night to bring Chinese chestnut pollen back from the forest nursery Lucca in Tuscany, Italy, in good enough condition for pollination (personal observations and communications with Marjan Šebenik Jr. 2002).

From the written materials of discussions at conferences, one can feel the enthusiastic spirit of determination and hope to save sweet chestnut from the chestnut blight. Perhaps such enthusiasm was also the result of the political changes of the post-WWII era, in which the socialist system, in synch with the nation's new found freedom and will to renew the country after the war, wanted to prove its superiority, both organizationally and scientifically.

There were a considerable number of people involved in the fight against chestnut blight in Slovenia but four of them played an exceptional role. Dr. Mihajlo Krstić, professor of Forest Pathology, Faculty of Forestry, Belgrade University, Serbia, was the first to put forward a strategy of disease eradication. He was familiar with up-to-date chestnut blight research and practices abroad and in the U.S.A. in particular, where he had been a visiting researcher. His contribution to the efforts to fight the disease has not yet been fully assessed. Stana Hočevar from the Slovenian Forestry Institute, Ljubljana, was devoted to the fight against chestnut blight for nearly 20 years and was responsible for key organizational and research work carried out in this field in Slovenia. Dr. Franc Janežič was a professor of plant protection in the Agronomy Department, Biotechnical Faculty, University of Ljubljana. His practical research work has had an impact on current methods of treating and understanding chestnut blight (e.g. disinfection methods for wood and seeds of the sweet chestnut, disproving the hypothesis that bees are vectors of chestnut blight spores). Marjan Šebenik has acted as a bridge between Slovenia and contemporary Italian research into the chestnut blight through his continuous contacts with Dr. Biraghi and others. He has also been responsible for organizing fieldwork in the Nova Gorica region where much of the chestnut blight activity and research has been centered.

No comprehensive evaluation of the organization, its activities or research work has been attempted here. In view of today's understanding of transmissible hypovirulence effects, the attempt to bring about a prompt and total extermination of diseased sweet chestnut trees in infected areas in the 1950's in Slovenia was clearly wrong. Even when the disease progression was at its height, the hypovirulence phenomenon was already beginning to take place. Ironically, however, the strictly enforced quarantine measures against the disease most likely also slowed the spread of hypovirulence - the only biotic suppression method that has made the sweet chestnut in Slovenia viable again.

7 POVZETEK

V Sloveniji so kostanjevega raka ugotovili na pravem kostanju leta 1950 pri Novi Gorici (gozd Panovec), 4 km od meje z Italijo. Že en teden po determinaciji je slovensko ministrstvo za gozdarstvo izdalo okrožnico o simptomih bolezni in o zatiranju. Januarja 1951 je bila objavljena uredba, ki je predpisovala takojšnjo prijavo bolezni na gozdarsko raziskovalno organizacijo; takojšen posek okuženega drevja čim bliže tal; lupljenje debla in panja; sežig vseh okuženih vej, lubja in sečnih ostankov; prepoved prevoza kostanjevega lesa, iz okuženega območja. V februarju 1951 je bilo objavljeno dopolnilo k uredbi, s katerim je bil prepovedan prevoz kostanjevih semen, cepičev in sadik. Ker so kostanjevega raka našli tudi na hrastih, so bile vse omejitve, ki so veljale za les, rastline, cepiče in semena pravega kostanja, leta 1952 uveljavljene tudi za hraste.

Marca 1952 je bila ustanovljena Mešana republiška komisija za zatiranje kostanjevega raka (v nadaljevanju Komisija). Imela je široka pooblastila, njeni člani so izvajali tudi obsežen raziskovalni program. Od ustanovitve naprej je bil glavni namen Komisije preprečiti širitev kostanjevega raka iz submediteranskega dela, ki je obsegal približno 2.500 ha sestojev pravega kostanja, v kontinentalni del areala; le-ta je obsegal približno 22.500 ha kostanjevih sestojev (površina vseh gozdov s prisotnim kostanjem je sicer obsegala približno 250.000 ha).

V prvem letu po odkritju (leta 1951) je bila bolezen razširjena že na 212 ha kostanjevih sestojev na površini 9.000 ha gozda. Domnevali so, da je bil kostanjev rak prisoten v Sloveniji že leta 1940. Razlogi za to domnevo so bili: v Vidmu, 34 km od meje s Slovenijo, je bil kostanjev rak ugotovljen že leta 1939; lastniki gozdov so poročali o povečanem sušenju pravega kostanja že 10 do 12 let pred ugotovitvijo kostanjevega raka pri nas; gozdarska služba na tem območju ni obstajala zaradi druge svetovne vojne in anglo-ameriške vojaške cone. Ustanovljena je bila šele leta 1948, vendar je sušenje pravega kostanja pripisovala črnilovki (Phytophtora cambivora). Leta 1952 so odkrili trideset novih okuženih sestojev v goriškem in enega v sežanskem okraju; celotna okužena površina je obsegala 610 ha čistih kostanjevih gozdov. Do leta 1955 so našli še 53 žarišč bolezni; celotna okužena površina je takrat obsegala 1.813 ha. V letu 1956 so našli kostanjevega raka še v ostalih predelih submediteranskega areala pravega kostanja in tako so celotno območje obravnavali kot okuženo. Leta 1956 so odkrili kostanjevega *raka v notranjosti Slovenije: na Cankarjevem vrhu v Ljubljani in na Topolovcu pri Sevnici. Na Cankarjevem vrhu je bilo okuženih nekaj dreves, ki so jih takoj posekali, vendar se je okužba širila in je leta 1958 obsegala 36 ha, leta 1962 pa že 60 ha. Okužba pri Sevnici je obsegala 0,5 ha in je bila uspešno zatrta. Leta 1959 so našli kostanjevega raka pri Vrhniki in Škofji Loki, leta 1960 pri Litiji in leta 1961 v bližini Celja. Vsa žarišča bolezni so se naglo širila in bolezen je nato kljub strogemu izvajanju karantenskih ukrepov hitro zavzela celoten areal pravega kostanja v Sloveniji.*

Kostanjev rak v Jugoslaviji ni napredoval v enotni liniji, ampak se je značilno pojavljal v točkovnih izbruhih bolezni daleč od okuženih območij.

V okuženem območju so gozdarji v začetku 60-tih let iskali prave kostanje, ki so bili še zdravi. Leta 1965 je bilo registriranih 40 dreves; osem najobetavnejših so okužili s kostanjevim rakom. Dve drevesi sta se posušili, dve sta preživeli, pri ostalih pa rezultat ni bil jasen; raziskovalci so zaključili, da obstaja le majhna verjetnost za obstoj odpornih osebkov pravega kostanja. Poleg tega so gojili sadike iz drugih republik in jih nato okuževali s kostanjevim rakom. Vse sadike so se posušile.

Karantenski ukrepi so prepovedovali prevoz kostanjevega lesa iz okuženega območja. Do leta 1955 so les iz sanitarne sečnje prodajali tovarni tanina v Čedadu, leta 1956 pa je zvezna uprava za gozdarstvo dovolila prevoz okuženega lesa na neokuženo območje – s pogojem, da je bil les obeljen in razkužen za pet minut v 5 % raztopini 40 % formaldehida. Po prevozu so s formaldehidom razkužili tudi železniške vagone. S tem ukrepom je komisija leta 1956 povečala sečnjo okuženih kostanjev na 8.000 m^3, v naslednjih letih pa je bila sečnja med 3.000 in 4.000 m^3. Med letoma 1950 in 1955 so v celotnem okuženem območju posekali 8.000 m^3 okuženega kostanja.

Od vdora bolezni je Komisija posvečala največjo pozornost možnosti prenosa kostanjevega raka s kostanjevim semenom. V okuženem submediteranskem delu je bila letna proizvodnja semena povprečno okoli 450 t, od tega je bilo 200 ton maronov. Kljub karanteni so seme tihotapili v mesta v notranjosti Slovenije in na Hrvaško. Ugotovili so, da lahko 1 kg semena iz okuženega sestoja na svoji površini nosi 5.632 - 87.240 konidijev in 912 - 3.240 askospor. Komisija je odredila razkuževanje semena za 30 minut v 0,5 % raztopini 40 % formaldehida. Leta 1954 so na vseh odkupnih mestih za kostanjevo seme *in na železniških postajah zgradili rezervarje za dezinfekcijo semena. Policija je poostrila nadzor nad tovornjaki za prevoz zelenjave, s katerimi so tihotapili kostanjevo seme.*

V kolikor okuženih sečnih ostankov niso mogli sežgati zaradi požarne nevarnosti, so tak material zakopali v tla. Ugotovili so, da gliva odmre in ni sposobna oblikovati trose na zakopanem lubju po 6 - 12 mesecih, podgobje pa je odmrlo po 12 - 15 mesecih.

Poganjki iz panjev okuženih dreves so bolj dovzetni za bolezen kot odraslo drevje. Zato je Komisija naredila vse, da bi bilo teh poganjkov čim manj. Obvezno je bilo lupljenje panjev in njihovo prekrivanje s prstjo. Pogosto so vse sečne ostanke zložili na panj in jih zažgali. V nekaterih primerih, ko so bili panji širši kot 1m, so jih odstranili z dinamitom ali z buldožerjem. Preizkušali so uporabo herbicidov za preprečevanje odganjanja iz panjev in uničevanje poganjkov. Po letu 1959 so intenzivno uporabljali arboricide, ki so imeli aktivni substanci 2,4 D in 2,4,6 T.

Na podlagi izkušenj iz ZDA je Komisija domnevala, da v okuženem območju pravi kostanj ne bo preživel. Zato so pričeli širok program uvajanja odpornejšega kitajskega kostanja in križanja le-tega s pravim kostanjem. Od leta 1952 do 1955 so iz ZDA poskusno uvozili manjše količine izbranih linij kitajskega kostanja, leta 1957 pa so uvozili 1000 kg semena. Vzgojili so 60.000 sadik, ki so jih posadili v desetih skupinah poskusnih ploskev v skupni površini 71 ha. Po štirih letih so ocenili, da razvoj te vrste ni ustrezen: kitajski kostanj je rastel počasneje kot pravi kostanj; drevesca je poškodoval sneg, saj večinoma niso odvrgla listja; oblika rasti je bila grmasta; sadike je močno poškodovala srnjad. Ugotovili so tudi, da je kitajski kostanj enako dovzeten za bolezen, vendar močno prekaša pravi kostanj v odpornosti na kostanjevega raka. Na ustreznih rastiščih uspeva kljub močni okuženosti s kostanjevim rakom. Med posajenimi kitajskimi kostanji so našli osebke, ki so bili ustrezni za gojenje plodov, saj je seme tehtalo več kot 15 g.

Od leta 1959 do 1969 so potekali poskusi obsevanja semena pravega kostanja z gama žarki iz ^{60}Co, da bi pridobili mutante, ki bi morda bili odporni na kostanjevega raka. Pridobljene sadike so okužili s kostanjevim rakom in preverjali njihovo odpornost nanj. 30 % sadik je bilo zakrnelih in neuporabnih; med preostalimi so do leta 1969 našli 3 *drevesca iz semen maronov in 28 drevesc iz povprečno velikega semena, ki so zaraščali s kostanjevim rakom okužene rane.*

Komisija je leta 1954 prepovedala prevoz čebel v okuženo območje in iz njega zaradi predpostavke, da čebele verjetno prenašajo trose kostanjevega raka. Po šestih letih neuspelih poskusov izolacije trosov iz čebel in iz peloda so prepoved preklicali.

Iskali so antagonistične mikroorganizme, ki bi jih lahko uporabili za zatiranje kostanjevega raka. Najučinkovitejša je bila gliva Penicillium rubrum, ki se je uspela obdržati v ranah na pravem kostanju v naravi in je zmanjšala okužbo umetno narejenih ran na skorji pravega kostanja za petkrat v primerjavi s kontrolo. Do praktične uporabe izsledkov ni prišlo.

V pregledu objavljenih prispevkov je bila posebna pozornost namenjena vsakemu podatku, ki bi nakazoval, kdaj se je v Sloveniji pojavila hipovirulenca kostanjevega raka. Prvi jasni opis zaraščajočih ran je iz leta 1955 v Panovcu; domnevamo, da opisuje rane, ki so se pričele zaraščati zaradi prenosa hipovirusa v virulentno obliko kostanjevega raka. V letu 1969 sta bili v elaboratu objavljeni dve sliki, ki prikazujeta tipične površinske okužbe s hipovirulentnimi sevi (slika 1). Zaraščajoče okužbe, ki so jih ugotovili na sadikah iz obsevanega semena v 60-tih letih prejšnjega stoletja, so po našem mnenju tudi nastale zaradi konverzije virulentne oblike kostanjevega raka v hipovirulentno, ali zaradi okužbe s hipovirulentno glivo.

Množična razširjenost hipovirulence kostanjevega raka je bila pri nas ugotovljena v sredini 80-tih let, gozdarska stroka pa je pričela uporabljati spremenjene koncepte gojenja pravega kostanja ob koncu 90-tih let. Virusna dsRNA v izolatih iz Slovenije je bila dokazana leta 2000.

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