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RESEARCH ARTICLE

Monitoring of the health status of *Castanea sativa* in the Belasitsa mountain, southwest Bulgaria

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Abstract
In the period 2017-2023, a survey for the assessment of the phytosanitary condition of sweet chestnut (*Castanea sativa*) was conducted in a permanent sample plot (PSP) Belasitsa, which is part of the European large-scale network for monitoring the health status of forest ecosystems under the International Co-operative Program 'Forests'. The PSP is set in a natural chestnut stand in Belasitsa mountain at an altitude of 643 m. Data collected from the first years of the monitoring determined a slight deterioration in the health status of the chestnut trees caused by an infection with the fungal pathogen *Cryphonectria parasitica*. An improvement in trees' vitality and lack of active necrosis were observed in 2022-2023. On the wounds, initiation of callus along the wounded edges was reported. Currently, the introduction of new and dangerous invasive insect pests *Dryocosmus kuriphilus*, *Corythucha arcuata*, etc., has not been detected. Attacks of both pests could further deteriorate the health status of the sweet chestnut in Belasitsa.

Keywords
Sweet chestnut, monitoring of health status, *Cryphonectria parasitica*, Belasitsa mountain

Introduction

The International Co-operative Program on the Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) has been implemented in Bulgaria since 1986. The program operates under the UNECE Convention on Long-range Transboundary Air Pollution (CLRTAP) (Pavlova, Rosnev, 2006). The main objective of the program is to assess the dynamics in the health status of forest ecosystems in Europe. In Bulgaria, the forest monitoring for assessing the state of conifers (*Pinus sylvestris*, *P. nigra*, *Picea abies*, and *Abies alba*) and broad-leaved (*Quercus* spp., *Fagus sylvatica*, *Carpinus betulus*, *Tilia grandifolia* and *Castanea sativa*) tree species, has been implemented in 160 permanent sample plots. These plots are located on a 16×16 km grid to determine the long-time trend of deterioration or improvement of the health status, the structure and functioning of forest ecosystems, etc.

In 2017, a new permanent sample plot (PSP) was established in a natural sweet chestnut (*Castanea sativa*) forest stand in Belasitsa mountain, where the most representative natural chestnut forests are distributed. Since the end of the last century, a deterioration of chestnut trees was observed because of the progressive spread of the invasive fungal pathogen *Cryphonectria parasitica* (Murrill) Barr. (Petkov, Rosnev, 2000; Georgieva et al., 2011; Georgieva et al., 2013). Over time, the process of dieback of chestnut trees has intensified, with the impact of the disease threatening the chestnut forests throughout the Belasitsa mountain. The observed active necrosis on the stems and branches showed a severe spread of the virulent strain of the pathogen *C. parasitica*.

Despite the considerable resources that have been devoted to research of contributing factors in chestnut decline in Belasitsa mountain, an implementation of biological control by the hypovirulent strain of *C. parasitica* has not been applied (Zlatanov et al., 2013). The transmission of the strain started in 1965 in France and proved to be an effective strategy in promoting rehabilitation of chestnut stands (Grente, 1965; Anagnostakis, 1984; MacDonald, Fulbright, 1991; Heiniger, Rigling, 1994; Robin, Heiniger, 2001). Grente (1965) found that lighter colored and less virulent strains of *C. parasitica* existed. When trees were inoculated, atypical strains were found to be of reduced virulence. After re-introduction of whitish strains, necrotic wounds began to heal in most cases. This phenomenon is called hypovirulence. The whitish or hypovirulent phenotype of the fungus is cytoplasmically controlled and associated with high molecular weight dsRNA. H-strains (hypovirulent) can convert V-strains (virulent) to hypovirulent by dsRNA transfer from hyphal anastomosis. This is the basis of biological control, which is applied to control the disease (Heiniger, Rigling, 1994).

In 2021, EU12 hypovirulent strains of the fungus *C. parasitica* were inoculated for the first time in Bulgaria on infected stems in Belasitsa and Pirin mountains, as an opportunity to apply a biological control of the disease (Filipova, 2021). Following observations of inoculated trees showed visibly healthier trees, and isolations made from healed necrotic wounds in nutrient media showed whitish strains.

The aim of this study was to analyse the data from the monitoring of health status of sweet chestnut trees in a permanent sample plot Belasitsa for the period 2017-2023.

Materials and methods

In 2017, a permanent sample plot of sweet chestnut (at an altitude of 643 m) was established on the territory of the State Forest Enterprise Petrich in order to monitor the health status of the species, as a part of the ICP 'Forests' large-scale network.

In the period 2017-2023, a complex assessment of the defoliation and leaves discoloration of 40 sample trees was carried out in PSP Belasitsa according to the methodology of the ICP 'Forests' (Eichhorn et al., 2016), which allows an analysis of the changes in the health status that have occurred, as well as to assess the impact of individual factors. Five degrees of damage were used to assess the trends in the tree crown condition: 0 – no damage (0–10%); 1 – slightly damaged (>10-25%); 2 – moderately damaged (>25-60%); 3 – severely damaged (>60–99%) and 4 – dead (100%).

In the annual assessment, damage on the leaves, branches and stems of trees as a result of abiotic, biotic or anthropogenic factors was reported.

Results and discussion

In 2017, active necrotic wounds were found on the trunks and branches of all sample trees. The fungal pathogen *Cryphonectria parasitica* was identified as a causer of the mass dieback and deterioration of the health in the sample plot. The fungus had destroyed the phloem and xylem of the cambial tissue, resulting in necrosis (Fig. 1A). Over the necroses, orange-yellow stroma of the fungus appeared, in which two types of fruiting structures were identified: perithecia (sexual structures producing ascospores) and pycnidia (asexual structures producing pycnidiospores) (Fig. 1B). As a result of this activity, pale-white mycelial fans appeared in the inner bark.

The monitoring carried out in 2017 showed that all sample trees were with deteriorated health status due to the development of the virulent pathogenic fungus *Cryphonectria parasitica*. Out of all trees, 82.5% were moderately damaged with 30-60% defoliation (Fig. 2). The results of the monitoring in 2018 confirmed the process of deterioration of the health of the chestnut trees, as a result of the pathogen development. Compared to 2017, 20% of moderately damaged trees become severely damaged, with defoliation of 70-90%. In the following years, the health status continued to deteriorate, as *C. parasitica* was detected on all surveyed trees, with the proportion of severely damaged trees varying from 20% (2019) to 30% (2020), but no completely dead tree was reported. In the sample plot, single egg masses of *Lymantria dispar* L.) (Lepidoptera: Erebidae) were found on the stems of the trees, but no defoliation by the pest was recorded. The leaves of individual trees were severely damaged by the miner moth *Phyllonorycter messaniella* (Zeller) (Lepidoptera: Gracillariidae).

The tendency for the deterioration of the health status of the sweet chestnut was maintained in 2021. Necrosis on the stems and branches was found in trees. Among them, 82.5% were moderately damaged, and 15.0% - severely damaged. One tree (2.5%) was completely killed by the pathogen. An improvement of tree vitality and lack of active necrosis were observed in 2022-2023. On the wounds, initiation of callus along the wounded edges was noted (Fig. 3). It was reported that 85% of the trees were characterised as moderately damaged, with defoliation of the crowns 30-60%, and 10-15% - severely damaged, with defoliation 70-80%. No completely dead trees were reported.

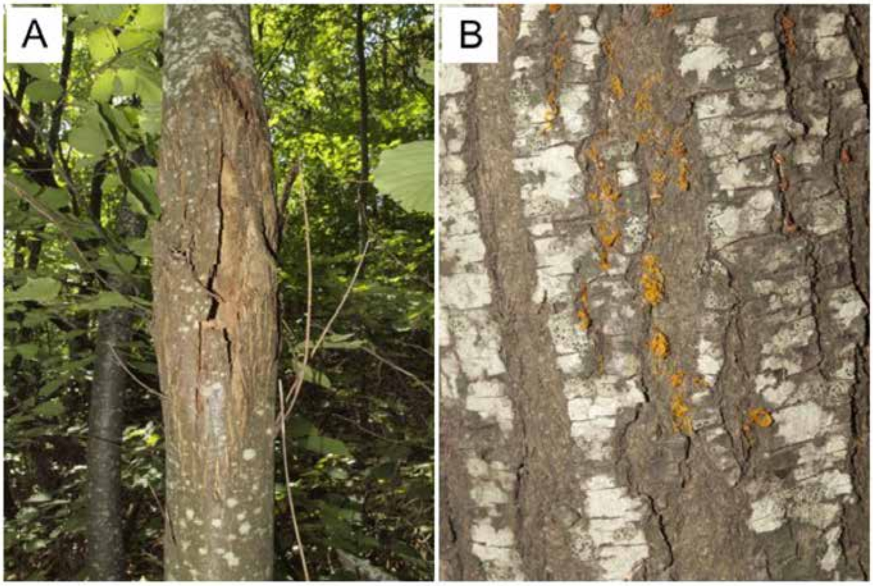

Figure 1. Symptoms of the disease caused by *Cryphonectria parasitica*: A – occurrence of a necrotic wound on the stem; B – development of orange stroma with sporocarps

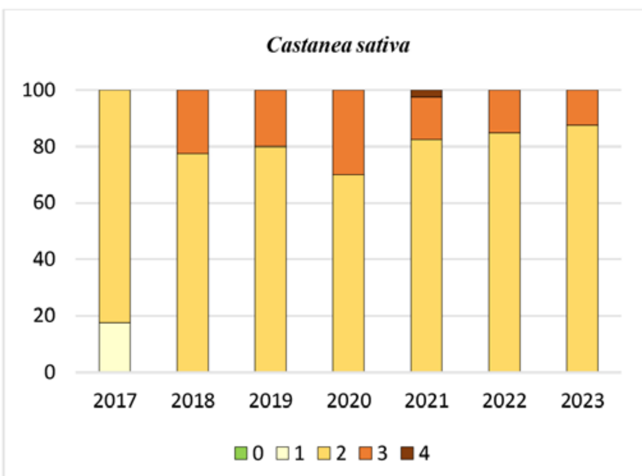

Figure 2. Distribution of chestnut trees in PSP 0153 Belasitsa according to the degree of damage (2017-2023)

In Europe, hypovirulent strains ensure long-term biological control of chestnut trees (Anagnostakis, 1987). The introduction of such strains into chestnut stands has been carried out in Bulgaria's neighbouring countries Greece, Turkey and North Macedonia. Currently, an improvement in the health status of the chestnut forest stands and orchards in these countries is being observed. In Bulgaria, the first attempt

Figure 3. Closed necrosis on chestnut stems in PSP Belasitsa (2023)

for biological control to *C. parasitica* by an artificial inoculation with a hypovirulent strain EU-12 was carried out in 2021 on infected trees in three sample plots in Belasitsa and Pirin mountains (Filipova, 2021).

The presence of an extensive callus layer around the wounds in the sweet chestnut population in Belasitsa mountain indicates that the hypovirulent strain of *C. parasitica* most probably entered in our country through the natural spread from North Macedonia or Greece, where EU-12 hypovirulent strain was inoculated some years ago (Sotirovski et al., 2011; Diamandis et al., 2015).

Undoubtedly, one of the most effective methods for controlling this economically important disease is the biological method based on the spread of the hypovirulent strains of the pathogen *C. parasitica*. This method exploits the low pathogenicity of the hypovirulent strains that are able to associate with virulent ones and suppress their pathogenicity. However, it is successful only if there is vegetative genetic compatibility between the virulent and hypovirulent strains, which will facilitate the establishment of an anastomosis between them, and the exchange of cytoplasmic material. For this reason, the efficacy of hypovirulence as a means of biological control against the disease depends mainly on the number of vegetative compatibility groups existing in the populations of the pathogen. In Belasitsa mountain the most common strain of the pathogen is EU-12, but strains EU-2, EU-10 have also been spread (Filipova, 2021).

Early detection of pest and disease outbreaks and rapid response to prevent the introduction and spread of invasive species are critical to maintaining the health and productivity of European forests (EEA, 2023). The introduction of new invasive pests and pathogens leads to deterioration of the vitality and functioning of forest ecosystems. A real threat for chestnut stands in Belasitsa mountain are two new invasive pests - the widespread oak lace bug (*Corythucha arcuata* Say) and the chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu, which is expected to be introduced into the country from Greece. The quarantine pest *Dryocosmus kuriphilus* causes severe damage to species of the genus *Castanea*. It is widely distributed in China, Korea and Japan, and in 2002 it appeared for the first time in Italy. The pest has spread rapidly to several European countries, probably due to the trade of chestnut plants. Despite measures taken to prevent the further spread of this pest in other countries, *D. kuriphilus* was detected in Greece in 2014 (Michaelakis et al., 2016). In Bulgaria, the pest has not been detected, yet.

The oak lace bug *Corythucha arcuata* is native to North America, widely distributed in the USA and southern Canada. It is an invasive species recorded for the first time in Europe in Italy (Bernardinelli, Zandigiacomo, 2000) and Switzerland (Forster et al., 2005). *C. arcuata* is distributed in a large part of Turkey (Mutun et al., 2009), from where it most likely entered Bulgaria in 2013 in Simeonovgrad and reached Plovdiv (Dobreva et al., 2013). In the next years, the species spread into many regions of the country, mainly affecting oak forests (Georgiev et al., 2017), but in 2017 it was registered for the first time in Bulgaria on the sweet chestnut (*Castanea sativa*) in the region of Pazardzhik (Simov et al., 2018).

Conclusions

Over a period of seven years, a slight deterioration in the health status of sweet chestnut trees (*Castanea sativa*) has been reported in the PSP Belasitsa. In the first years of monitoring, an increase in the number of trees damaged by the fungal pathogen *Cryphonectria parasitica* was reported. An improvement of trees vitality and lack of active necrosis were observed in 2022-2023. On the old wounds, initiation of callus along the wounded edges was reported.

The most effective method of controlling the economically important chestnut blight disease is the biological method based on the hypovirulence of the pathogen *C. parasitica*. In 2021, for the first time in Bulgaria, a hypovirulent strain of the fungus was inoculated as an opportunity to control the disease. The results show an improvement in the health status of the chestnut forests in Belasitsa mountain.

Currently, the penetration of new and dangerous invasive insect pests (*Dryocosmus kuriphilus*, *Corythucha arcuata*, etc.) which could further deteriorate the health status of the sweet chestnut in Belasitsa mountain, has not been detected.

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