MONITORING ON APPEARANCE AND SPREAD OF HARMFUL INVASIVE PATHOGENS AND PESTS IN BELASITSA MOUNTAIN

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Abstract

Belasitsa Mountain is located on the territory of Southwest Bulgaria, Northern Greece and Northern Macedonia. The Bulgarian part of the mountain includes most of its northern slopes and ridge territories, characterized by variety of vegetation zones and plant communities. At lower altitudes, *Quercus* spp., are the most distributed, but gradually replaced by the sweet chestnut forests (*Castanea sativa*). Chestnuts are distributed between 400-1300 m a.s.l., forming the largest natural locality in the country. Along the river valleys in the mountain, natural population of *Platanus orientalis* occurs. Since 2018, a system for monitoring of two pathogens (*Cryphonectria parasitica* on *Castanea sativa* and *Ceratocystis fimbriata* on *Platanus orientalis*) and three invasive pests (*Corythucha arcuata* on different oak species and chestnut, *C. ciliata* on *P. orientalis* and *Dryocosmus kuriphilus* on chestnut forests) has been implemented. Until now, a high density of *C. ciliata* was established on *P. orientalis* causing significant damages on host trees. *C. arcuata* has spread in Bulgaria since 2013. Over the next few years, the species penetrated many regions of the country by affecting predominantly oak forests, but in 2017, it was registered for first time on the chestnut trees. In Bulgaria, *D. kuriphilus* has not been found yet, but as the pest was established in Greece in 2014, natural spreading of the species to Belasitsa Mt. could be expected. The possible penetration of this pest could increase the rate of damaged stands that had already been severely attacked by the fungal pathogen *C. parasitica.*

Key words: *invasive pests, monitoring, Belasitsa Mt., Bulgaria*

Introduction

Belasitsa Mountain is located on the territory of three countries: Bulgaria, Northern Greece and Northern Macedonia. The mountain is about 60 km long and 7 to 9 km wide. Its main massif is oriented in east-western direction. The climate is characterized by strong Mediterranean influence: precipitation is at its highest in the autumn and winter, and at its lowest in the summer. Bulgarian part of the mountain includes most of its northern slopes and ridge territories. It is characterized by variety of vegetation zones and plant communities. *Castanea sativa* Mill. population located on the northern slopes of Bulgarian part of Belasitsa Mt., at attitudes from 500-600 up to 900-1000 m a.s.l. This population is the largest and most valuable one at the Balkan Peninsula. It is also among the few well preserved autochthonous populations of this species in Europe. Most investigations on natural *C. sativa* forests in Europe and at the Balkan Peninsula in particular, reveal progressive degradation of this species, even in nature protected territories. The area occupied by the *C. sativa* forests on the northern slopes of Bulgarian part of Belasitsa Mountain is around 1400 ha. Natural stands cover an area of about 650 ha. More than 90% of them are older than 100 years of age, and around 75% are older than 140. Most natural stands are in poor health condition: with dieback in the crowns.

Pests and fungal pathogens are a part of the natural biocenoses complex inhabiting different types of ecosystems. Their development in plants causes destructive processes that have negative influence on the vitality and productivity of the trees. During the last years,

introduced pests and pathogens have turned into growing threat for the natural plant species disturbing the biodiversity and ecological dynamics in forest ecosystems.
The aim of study was to determine penetration of invasive pathogens and insect pests threatening tree vegetation in Belasitsa Mountain.

Materials and methods

In 2018, a network of 14 sample plots (SPs) was established in Bulgarian part of Belasitsa Mt. in outskirts of six settlements (Table 1). Three SPs were selected in *Platanus orientalis* L. stands for monitoring of *Corythucha ciliata* (Say, 1832) (Hemiptera: Tingidae) and *Ceratocystis fimbriata* Ellis & Halsted *f.sp. platani* (J.M. Walter) Engelb. & T.C. Harr (Microascales: Ceratocystidaceae) and the remaining ones – predominately in *Castanea sativa* and *Quercus petraea* Liebl. stands for monitoring of *Cryphonectria parasitica* (Murrill) Barr. (Diaporthales: Valsaceae), *Corythucha arcuata* (Say, 1832) (Hemiptera: Tingidae) and *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae).

Table 1. Main characteristics of sample plots in Belasitsa Mt.

№	Locality	Geographical coordinates		Altitude (m)	Stand age	Stand composition*
		Latitude (N)	Longitude (E)			
1	Petrich town	41.382000	23.204890	313	70	P.o.; C.s.; Q.p.
2	Petrich town	41.367282	23.199985	739	65	C.s.; F.s.
3	Petrich town	41.365502	23.196742	832	50	C.s.; F.s.
4	Belasitsa vill.	41.356295	23.104209	664	75	C.s.; Q.p.
5	Kolarovo vill.	41.361280	23.108780	459	80	P.o.; C.s.; Q.p.
6	Kolarovo vill.	41.360740	23.110676	505	65	C.s.; Q.p.
7	Kolarovo vill.	41.359445	23.095323	650	70	C.s.; Q.p.
8	Samuilovo vill.	41.371220	23.08514	316	65	P.o.; C.s.
9	Samuilovo vill.	41.364934	23.090233	481	60	C.s.; Q.p.
10	Samuilovo vill.	41.364357	23.083757	523	65	C.s.; Q.p.
11	Yavornitsa vill.	41.359567	23.045905	589	70	C.s.; Q.p.
12	Yavornitsa vill.	41.358411	23.052411	614	70	C.s.; Q.p.; F.s.
13	Kluch vill.	41.357716	23.019078	559	60	C.s.; Q.p.
14	Kluch vill.	41.356752	23.059601	663	65	C.s.; F.s.

* P.o. – *Platanus orientalis*; C.s. – *Castanea sativa*; Q.p. – *Quercus petraea*; F.s. – *Fagus sylvatica*

Defoliations caused by biotic and abiotic stresses were assessed according to the European methodology of the UNECE International ICP Forests Program (Eichhorn et al., 2010).
The studies were carried out by periodical observations and collection of biological material (leaf, twigs, bark and branches) for laboratory testing at the Forest Research Institute, Sofia. Isolation of the fungi into pure cultures on PDA (Difco) nutrient media was done. The colonized fungi were further identified by combination of colony macroscopic (colour, morphology and growth rate) and microscopic characteristics (spore shape, size, colour and morphology).
During the growing seasons, samples of leaves and shoots were collected regarding the presence/absence of studied species and the intensity of the attack (expressed as percentage of injured leaves). The attack intensity was assessed by the degree of foliage fading, as a deviation from the normal colour of the host tree leaves. Larvae of insect pests were placed in entomological boxes and photo eclectors to obtain adult emergence. Adult insects, nymphs and eggs were also collected for species identification.

Results and discussion

In this study, only *Cryphonectria parasitica* was established on *Castanea sativa*, and *Corythucha ciliata* – on *Platanus orientalis* L. The remaining invasive insect pests, *Corythucha arcuata* and *Dryocosmus kuriphilus*, as well as the pathogen *Ceratocystis fimbriata f.sp. platani* were not detected in Belasitsa Mt. Distinct decline in crown condition was observed in sampled chestnut trees.

The assessing of the distribution of *C. parasitica* carried out in this study shows that from the sites of primary infection, the disease has been spread across the whole mountain, between 400 and 800 m a.s.l., affecting 85% of the surveyed trees. Only 15% of chestnut trees were classified as healthy (defoliation of up to 25%). In the areas, located at higher altitude (between 700 and 900 m a.s.l.), the rate of infected by *C. parasitica* trees were only 10%. The effect of trees decline varied from partial crown dieback to completely death trees (18%). In individual SPs, chestnut blight incidence ranged from 18% to 100% of the trees in the SPs, and mortality caused by the fungus was between 2% and 80%. The results obtained indicated that chestnut blight had spread into most chestnut stands of Belasitsa Mt., the most affected stands between 400-800 m. a.s.l.

The pathogen *Cryphonectria parasitica* was found to be widely distributed in all studied SPs in in Belasitsa Mt. It causes chestnut blight disease and is considered to be the most destructive pathogen on both American (*Castanea dentata* (Marsh.) Borkh.) and European chestnut (*C. sativa*) (Anagnostakis, 1987). The first documented observation of *C. parasitica* in Bulgaria was on chestnut trees in pure and mixed stands on the northern slopes of Belasitsa Mt. and on the Eastern Rhodopes mountain in 1993 (Petkov, Rossnev, 2000). The pathogen is detected in both largest localities of chestnut in Bulgaria – in Belasitsa Mt. and West Balkan Range, and causes serious damages on stems and branches (Georgieva et al., 2013). *C. parasitica* attacks bark tissues producing cankers that can develop as sunken regions due to tissue collapse, damage to vascular tissues produces wilts and diebacks. A high infection rate and crown defoliation of the chestnut trees manifest that chestnut blight disease was a major stress factor and likely an important driver of chestnut decline. Epidemic of disease reduces the abundance of a dominant native species, resulting in a change in landscape structure. The infected dead branches with wilted, yellow or brown leaves which remain hanging on the branches, producing a so-called flag. The stems developing numerous epicormic shoots below the necrotic areas. A parasitic fungus causing necrotic lesions (cankers) on the bark of chestnut stems and branches. The pathogen attacks all the aerial parts of the tree: the main stem, branches and young suckers; infecting the tree via wounds in the bark and forming sunken canker due to necrosis and collapse of bark tissue (Hebard et al., 1984). The buff-brown mycelia of the fungus invade and destroys the inner bark (i.e. phloem, vascular cambium and xylem) resulting in the death of host tissue distal to the point of infection. Thus, the disease leads to the loss of an important part of the chestnut production and the progressive death of the tree.

The sycamore lace bug, *Corythucha ciliata* was found at high density in the three studied SPs (Petrich, Kolarovo and Samuilovo) in Belasitsa Mt. Additional surveys showed that the species was penetrated in *Platanus orientalis* stands along the rivers and streams in the mountain. It is naturally distributed in USA and south parts of Canada. In Europe the species was found for first time in Italy in 1964 and later in other countries (France, Switzerland, Croatia, Greece, etc.). In Bulgaria the pest was found for first time in 1989 in Sofia (Josifov, 1990). Currently, it is distributed all over the country. The species is trophycally related to plane trees (sycamore) (*Platanus* spp.). In urban environment in Bulgaria it frequently feeds on *Platanus x acerifolia* (a hybrid between *Platanus occidentalis* and *P. orientalis*). It has penetrated into all natural localities of *P. orientalis* in the country. In Europe, *C. ciliata* usually develops two generations per year, but in some south regions with Mediterranean

climate it has three. The female lay eggs around the nerves on the lower leaf's surface. The larvae and adults feed in groups on the lower surface and the colonies could reach scores and hundreds of specimens. The generation develops within 45 days. The second generation appears at the end of August/beginning of September. It winters as imago in bark crevices of thick branches and stems. At the places where larvae and imago were feeding, small white spots appear. Later the spots grow, and this leads to leaf depigmentation and yellowing. In case of massive attack, the leaves dry and fall prematurely at the end of summer. In combination with other stress factors the massive development of *C. ciliata* during some consecutive years could lead to ruin of the attacked trees. It should be noted that the damages caused by the pest are most significant in urban conditions (Georgiev et al., 2017).

The oak lace bug, *Corythucha arcuata*, is naturally distributed in North America. In Europe the species was found for first time in Italy in 2000 (Bernardinelli, Zandigiacomo, 2000). In 2002 was reported for Switzerland (Forster et al., 2005), and in 2003 for Turkey (Mutun, 2003). In Bulgaria it was found a new species for the Balkan Peninsula in 2012 in the region of Plovdiv and Simeonovgrad (Dobreva et al., 2013). Later, invasion of the oak lace bug (*C. arcuata*) was reported in it in Hungary (Csóka et al., 2013), Croatia (Hrašovec et al., 2013), Romania (Tomescu et al., 2018) and other countries. In its natural area, *C. arcuata* feeds mainly on *Quercus* spp., and rarely representatives of other genera (*Castanea, Acer, Pyrus, Malus, Rosa*). In Europe, it was found on *Quercus petraea, Q. robur, Q. pubescens, Q. cerris, Q. rubra, Rubus idaeus, R. ulmifolius, Castanea sativa* and *Rosa canina* (Dobreva et al., 2013). *C. arcuata* biology and ecology is similar to that of Sycamore lace bug (*C. ciliata*). The feeding of larvae and adult colonies causes depigmentation, aging and premature leaf falling. Recently, the species was found on 8 oak species in high number nearly all over Bulgaria, on *Castanea sativ*a in Plovdiv region and on 5 occasional tree and shrub hosts (Simov et al., 2018). In case of penetration in Belasitsa Mt., *C. arcuata* could turn into a danger for chestnut stands due to progressive degradation and the poor health condition of the species.

The quarantine pest *Dryocosmus kuriphilus* causes severe damage to species of *Castanea* genus. It is widely spread in China, Korea and Japan, and in 2002, it first appeared in Italy. After the first penetration of the pest in Europe, it has spread rapidly in several other European countries, probably due to the trade in chestnut plant material. Despite the measures taken to prevent further spread of this pest in other countries, it was first obtained in Greece in 2014 (Michaelakis et al., 2016). However, studies show that the pest is still very limited in Greece. In Bulgaria, the species is not found at present, but its possible penetration at Belasitsa Mt. will increase the damage caused by *Cryphonectria parasitica*, which has caused significant losses in chestnut forests over the last 20 years.

The pathogen *Ceratocystis fimbriata* is an introduced fungal pathogen from North America to South Europe, distributed on species from genus *Platanus*, causing destructive tracheomicotic disease. Since 2004, the disease has been detected in southwest parts of Greece, where it caused dead of natural plantations and urban trees (Tsopelas, Angelopoulos, 2004). *Platanus* species are the only hosts for pathogen, with highest sensibility being *P. acerifolia*, often used for planting in urban environments in the countries with moderate climate. In transversal cut of the stem blue-black coloration growing radially to the periphery, with fusiform patterns could be observed. The dead cambial tissue provokes appearance of dark longitudinal spots on the bark surface. These spots are very important characteristics for diagnostics even if the infection has penetrated through the roots. Infection with the pathogen *Ceratocystis platani* f.sp. *platani* develops in the places of injuries on the bark or root, stem or branches, timber during pruning or other mechanical damages. After fungus spores appear on the plant tissue fast distribution starts in the xylem tissue along the stem and radially to its core. The fungus

could overwinter at temperatures up to -17°C, but does not develop under 10°C and over 45°C.

Conclusion

Finally, it should be noted that monitoring of invasive fungal pathogens and insect pests threatening forest vegetation in Belasitsa Mt. should continue to take adequate measures in case of penetration of highly aggressive species. This is mainly true for species whose health status is severely degraded, such as chestnut (*Castanea sativa*), as well as another species of high conservation significance, the eastern plane (*Platanus orientalis*).

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