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RESEARCH ARTICLE

Distribution of *Biscogniauxia mediterranea* and its potential insect vectors on *Quercus suber* in Southwestern Bulgaria

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Abstract

This is the first study in Bulgaria on the distribution of *Biscogniauxia mediterranea* in cork oak plantations (*Quercus suber*). Symptoms of the disease (presence of exudate and wounds with stroma) caused by the fungus have been found on stems and branches of cork oak in Maleshevska Mountain. Harvesting of cork does not visibly affect the phytosanitary status of the trees. A significantly higher risk of parasitic fungus development occurs in coppice-managed crops where it causes the death of young trees. The associations of ten insect species with the *Quercus suber* are new for Bulgaria. Eight of them are possible vectors of the fungus.

Keywords

Biscogniauxia mediterranea, Quercus suber, insect vectors

Introduction

Various biotic and abiotic factors and the excessive use of forest resources are recognized as major causes of cork oak forests decline. In some areas, excessive grazing is also important. Stress for trees occurs after sudden shifts in periods of insufficient and excess moisture in the soil or extreme winter temperature drops. Repetition of such stressful conditions can lead to a chronic decline that includes bark beetle infestations and fungi infections damaging stems and roots (Kim et al., 2017). Under such conditions canker diseases caused by pathogens of genera *Botryosphaeria*, *Sphaeropsis*, *Cytospora* and *Biscogniauxia* (Desprez-Loustau et al., 2006) evolve broadly. Since 1980, some of these species have already been associated with the etiology of cork oak decline in many countries (Moricca et al., 2016). Such is the *Biscogniauxia mediterranea* (De Not.) Kuntze fungus, known as an opportunistic parasite, endophytically developing in all above-ground parts of trees. The increase in its influence is associated with the extremely dry and hot years of this period (Desprez-Loustau et al., 2006; La Porta et al., 2008).

The life of *B. mediterranea* in cork oak involves an initial latent endophytic phase, followed by parasitic and saprophytic phases. Physiological changes in the host under the effect of stress factors, particularly water stress, facilitate the colonization of tissues by the pathogen and its transition from asymptomatic endophytic to parasitic phase (Jiménez et al., 2005; Desprez-Loustau et al., 2006; Vannini et al., 2009). *B. mediterranea* is necrotrophic pathogen whose infections cause extensive necrosis of the inner bark and the xylem, accompanied by brown-black exudation from the outer bark. It forms a specific black stroma on the infected tissues, which appear on the surface after the bark bursts. In this saprophytic phase (on dead tissues), *B. mediterranea* produces perithecia with a large number of ascospores spread through air, water, insects and humans though the major factor for releasing the predominantly airborne ascospores are precipitations (Henriques et al., 2014). The fungus also forms conidia (Jiménez et al., 2005; Henriques et al., 2015). Main symptoms of the disease are the coal black stromata on dead stems or branches and brown exudate from the injured bark (La Porta et al., 2008).

B. mediterranea is described as a secondary pathogen on cork oak in Portugal in 1931 on dying trees, but the disease is already affecting more and more young plantations (Henriques et al., 2014), expanding its range and hosts. The favourable climatic changes turn it into an invasive pathogen. The disease has been reported in Africa, Europe, Central America, the United States, Asia, Australia over a wide range of hosts and is already a serious problem not only on *Quercus suber* L., but also on *Q. cerris* L., *Q. frainetto* Ten., *Q. pubescens* Willd., *Q. ilex* L., *Carpinus betulus* L., *Corylus avellana* L., *Fagus sylvatica* L., *Fraxinus excelsior* L. (Desprez-Loustau et al., 2006; La Porta et al., 2008; Jurc et al., 2005; Ragazzi et al., 2012).

In Bulgaria there is high prevalence of B. *mediterranea* on *Q. cerris* in windbreaks (Petrov et al., 2002), pure and mixed oak plantations of Central and Eastern Balkan Mountains (Rossnev et al., 2010).

The studies of Petkov and Rosnev (2014) on the health status of *Q. suber* in plantations created under different habitat conditions indicate that with the usual droughts in Southwestern Bulgaria, plantations on deep and fresh soils are in better condition, while the cork oak on shallow and poor soils is in poor condition. Symptoms of damage (wet spots, brown colouring) caused by *B. mediterranea* have been observed on stems, especially in the lower and basal parts, but stroma and fruiting bodies have not been found.

Zíbarová & Kout (2017) have determined B. *mediterranea* on specimens in the oak herbarium from the Eastern Balkan Mountains, but there is no information about its finding on cork oak.

This paper reports the results of studies on the distribution of *B. mediterranea* in *Q. suber* plantations and its potential insect vectors in Southwestern Bulgaria.

Materials and methods

The first cork oak afforestation processes in Bulgaria were carried out in 1954 in the Kresna region in Southwestern Bulgaria (Petrov, 1994). This survey was conducted in 2018-2020 on the territory of the "Strumyani" State Forestry where the majority of forests are located on the eastern slopes of the Maleshevska Mountain. The cork oak plantations encompass 27.4 ha or 0.1% of the total afforested area of the holding. They are aged 10 to 40 with the predominant age being 30-40 years (Forest management plan, 2010).

The cork oak plantations in Maleshevska Mountain are planted at 4×2 m in the Lower hilly-plain belt of oaks (0-800 m above sea level), with an average annual temperature of 10.5 °C, average absolute minimum and maximum temperature of -17 °C and 34.5 °C. The annual rainfall amount ranges from 605 to 780 mm. The vegetation period duration is between 7 and 8 months. Months of maximum rainfall amounts are November and December and in August is the minimum.

Three plantations, created after terracing the terrain, on leached cinnamon soils were selected for the study, and according to the Forest management plan (2010) were in good condition.

Plantations were examined using the routine method and temporary sample plots of 30 trees each were set for evaluating the condition of each tree by degree of defoliation, colour changes in crowns, presence of damage (wounds with exudate and stroma) and number of lesions on stems and branches, chest height stem diameters were also measured. Symptomatic plant samples for laboratory analysis were collected. To determine the size of ascospores in the temporary microscopic preparations 30 of them were measured in each.

Cuts of dead branches and stems of the cork oak were also collected for insects rearing in the laboratory of entomology in University of Forestry, Sofia.

Results and Discussion

The results of the survey on cork oak in the assigned temporary sample plots are summarized in Table 1, including some data on the plantations.

The plantation in plot 2 which is located at an altitude of 450 m on the upper border of the optimum for development of cork oak was in the best phytosanitary state.

Table 1. Results of the pathological survey of cork oak plantations in Southwestern Bulgaria

Plot №		1	2	3
Forest unit		247 k	248 b	257 g
Altitude, m a.s.l.		300	450	300
Exposure		E	SW	S
Slope, °		27	26	22
Age, yr		38	38	33
d_{av}, cm		15.7	19.4	11.0
R %	defoliation	40.0	15.0	52.5
	discoloration	42.5	20.8	55.0
Dead trees	number	7	0	13
	%	23.3	0	43.3
Number of trees with wounds	total	23	6	21
	stems	19	6	20
	branches	17	3	16
	with exudate	4	4	7
Average number of wounds per tree	total	8	2	6
	stems	5	2	4
	branches	6	2	3
Cork harvested	number of trees	27	28	8
	%	90	93	27
Coppicing origin	number of trees	3	5	15
	%	10	17	50

The average chest height stem diameter in it was the highest (19.4 cm). The summarized assessment of crown defoliation is 15% (0-35%), which defines it as a healthy one. The slightly higher discoloration of the crowns is mainly due to the flowering during the survey period. There were no dead trees in this plantation; stromata were detected on the stems and branches of 2 trees only, and wounds with brown black exudate – on 4 stems.

Defoliation of the cork oak in plot 1 and plot 3 was respectively 40% and 52.5% (0-100%), the higher values coming as a result of the higher number of dead trees, respectively 23.3% and 43.3%. In some still vital trees, a dark brown to black exudate (Fig.1a) is observed, usually in the lower part of the stem. Flat coal-black perithecial stroma (Figure 1b, c) of the fungus B. *mediterranea* were found on dead branches and stems with cracked bark. The stromata dimensions vary and on some dried-out trees they merge and exceed 1 m in length. With the appearance of cracks (wounds) on the stems begins a change in the crowns – leaves become smaller, yellowish, and after drying-out instead of falling down they remain on the tree longer.

Wounds were found on 23 of the 30 trees surveyed in plot 1. The largest average number of wounds found on one tree is also determined here. Wounds were found on 21 of the trees surveyed in plot 3. This plantation has the smallest average diameter (11 cm) and the largest number of trees of coppice origin (50%) which have been infected at a younger age before cork production had taken place. In this plot, symptoms of the same disease were found on *Quercus pubescens*, sporadically found in the plantation.

Cork harvesting has been carried out in all three surveyed crops, but no relationship to the deterioration of the phytosanitary status has been established despite the conclusion of Oliveira and Costa (2012) that it increases the sensitivity of *Quercus suber* to drought and pests. Cork has been harvested on 93% of the stems in plot 2, where no damages of *B. mediterranea* were found. It was found only in a saprophytic phase, on dead branches. The greatest damages were observed in the coppicing plantation of plot 3, in which the fungus causes the destruction of young trees, from which cork has not yet been harvested.

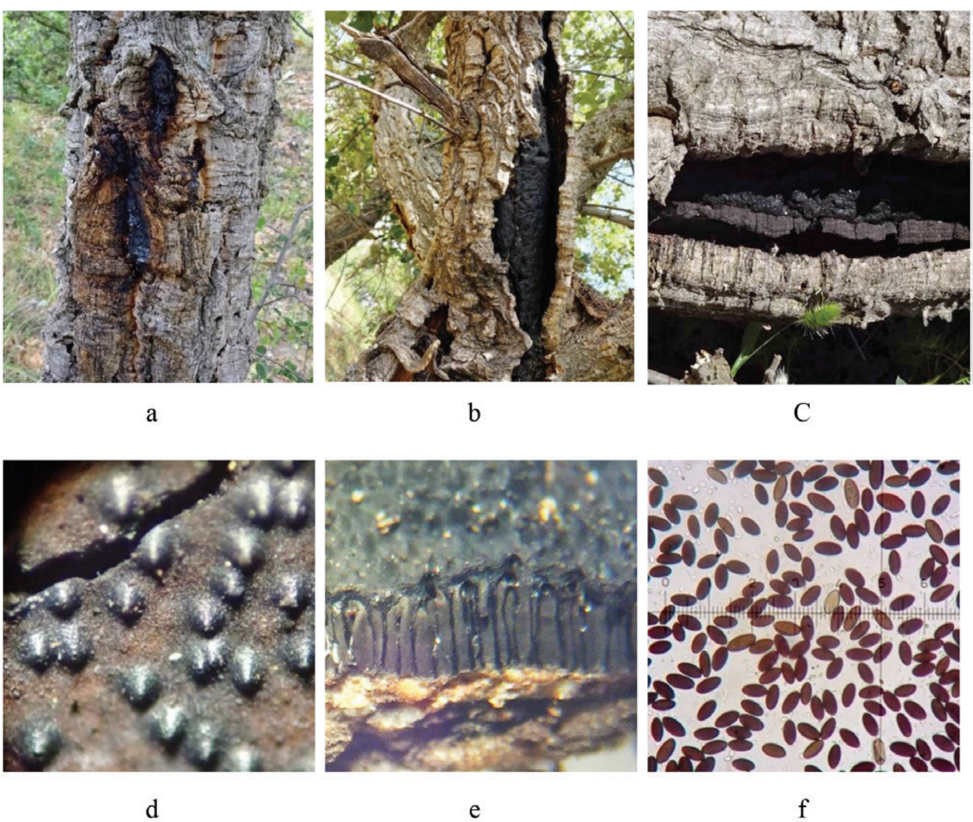

Figure 1. Wounds on the cork oak stems – with exudate (a) and stromata (b, c); perithecia (d, e) and ascospores (f).

Laboratory microscopic analysis of the collected plant samples with exudate did not reveal the presence of fruiting bodies and sporulation.

Microscopic observations of the samples with stromata proved presence of the fungus B. *mediterranea*. In black stromata of 1-1.5 mm thickness a large number of cylindrical perithecia (Fig. 1d, e) were set, formed in groups of common ostioles. The ascuses of the fungus are cylindrical, 122.8 (94.5-143.1) × 6.8 (5.4-10.8) µm in size and containing eight ascospores each. The asospores are brownish-black, elliptical, with narrowed ends and a longitudinal slit on one side (Figure 1f). Their dimensions are 17.6 (13.5-21.6) × 7.7 (6.8-9.5) µm. These dimensions exceed those indicated by Ragazzi et al. (2012) for *B. mediterranea* on *Fraxinus excelsior* in Italy (14.6-20 × 6.99.1 µm), but are similar to those of Zíbarová & Kout (2017) for *Quercus cerris* in Bulgaria, *Corylus avellana* and *Fagus sylvatica* in the Czech Republic (14-21 × 6.5-8.5 µm); Fournier, Magni (2004) for *Carpinus betula*, *Corylus avellana* and *Fagus sylvatica* in France (14-19 × 7-9 µm); Jurc & Ogris (2005) for *Quercus cerris* in Slovenia (16 (13-19.5) × 7.5 (6-9) µm). Molecular analyses are required for more precise determination of the relationships between different origins of the fungus.

Insects are the main biotic vectors, not only transporting the inoculum but also causing wounds that act as an infection point (Jiménez et al., 2005; Henriques et al., 2014). Several insects have been identified as *B. mediterranea* vectors. Martín et al. (2005) associates the presence of *Cerambyx* spp. with the incidence of the disease in *Q. suber* in southwestern Spain. Investigations of Inácio et al. (2011) prove the transport of the *B. mediterranea* propagules by ambrosia beetle *Platypus cylindrus* in Portuguese cork oaks stands. A similar link could be sought in the area we investigate because of the existence of damages from different xylophagous insects.

During 2018-2020, ten saproxylic insect species belonging to three orders and eight families were found or reared from *Quercus suber* in two localities as follows:

1. Maleshevska planina Mt., 1.3 km SW of Mikrevo Vill., 310 m a.s.l.; 41˚36'50.9"N; 23˚11'03.5"E.

Coleoptera, Buprestidae

Acmaeodera crinita Spinola, 1838 – alive adult in wood of dead branch with diameter of 8 cm, 12 May 2018;

Acmaeodera ottomana (Frivaldszky, 1837) – dead adult in wood of fallen branch with diameter of 9 cm, 12 May 2018;

Chrysobothris leonhardi Obenberger, 1916 – 3 ♂♂, 1♀ reared from dead branch with diameter of 7 cm, sample collected 28 October 2018, dead adults – 12 December 2019;

Coleoptera, Bostrichidae

Lichenophanes varius (Illiger, 1801) – 3 adults reared from parts of dead standing stem with diameter of 10 cm, sample collected – 23 March 2019, emergence – 21 May 2019 (two adults) and 15 June 2019;

Coleoptera, Cerambycidae

Callimus angulatus (Schrank, 1789) – adult reared from part of dead standing stem with diameter of 10 cm, sample collected – 23 March 2019, emergence – 28 March 2019;

Coleoptera, Malachiidae

Anthocomus fasciatus (Linnaeus, 1758) – adult reared from part of dead standing stem, sample collected – 08 April 2018, emergence – 17 April 2018;

Coleoptera, Zopheridae

Colobicus hirtus (Rossi, 1790) – 3 adults reared from parts of dead standing stem with diameter of 7 cm, sample collected – 23 March 2019, emergence – 23 March 2019, 11 June 2019 and 23 July 2019;

Lepidoptera, Tineidae

Neurothaumasia ankerella (Mann, 1867) – adult reared from fallen branch with diameter of 4 cm, sample collected – 08 April 2018, emergence – 20 June 2018;

Hymenoptera, Stephanidae

Stephanus serrator (Fabricius, 1798) – 1♂, 1♀ reared from parts of dead standing stem with diameter of 7.5 cm, sample collected – 23 March 2019, emergence – 23 April 2019 (♀) and 05 March 2020 (♂);

2. Ograzhden Mt., 1.5 km NW of Parvomay Vill., 250 m a.s.l.; 41˚25'01.1"N; 23˚07'44.0"E

Coleoptera, Curculionidae

Xyleborinus saxesenii (Ratzeburg, 1837) – adult (♀) with larvae in gallery of dead standing stem wood, 24 March 2019.

The associations of all ten reared insect species with the *Quercus suber* are new for Bulgaria. Many of them are saproxylophagous – the representatives of Cerambycidae, Bostrichidae, Buprestidae and Tineidae. *Xyleborinus saxesenii* is xylomycetophagous. *Colobicus hirtus* was reported as predator (Horion, 1961) but some *Colobicus* species were also associated with fungi (Benick, 1952). These 8 species, as well as cerambycids *Alocerus moesiacus* (Frivaldszky von Frivald, 1837), *Cerambyx cerdo* (Linnaeus, 1758) and *Aegomorphus krueperi* (Kraatz, 1859), recorded from the same locality in Maleshevska planina Mt. (Doychev et al., 2018) could be regarded as potential vectors of xylotrophic fungi on *Quercus suber* including *Biscogniauxia mediterranea*.

Larvae of *Anthocomus fasciatus* are predators on various subcortically living insects and *Stephanus serrator* is a larval parasitoid of some Cerambycidae, Buprestidae, Siricidae and solitary bees also. From Bulgaria *S. serrator* was reported as a parasitoid of the longhorned beetle *Saperda similis* (Georgiev et al., 2004) but our findings cannot be related to a specific host.

Other than *B. mediterranea* fungi identified on dead cork oak wood in the investigated plantations were *Diatrype stigma*, *Libertella quercina*, *Peniophora quercina*, *Pycnoporus cinnabarinus*, *Hapalopilus nidulans*, *Polyporus alveolaris*, *Auricularia mesenterica*, *Stereum hirsutum* and *Steccherinum ochraceum*, the latter two being developed independently or together with the *B. mediterranea* stroma. These fungi do not represent a threat for the living trees in the plantations.

Conclusions

The health status of cork oak in Maleshevska Mountain has been compromised. Symptoms (presence of exudate and wounds with stroma) of the disease caused by *Biscogniauxia mediterranea* were found on stems and branches, but exudate was observed only on stems. The presence of stroma on dead branches indicates a saprophytic form of the fungus, providing a source of infection for trees, mainly after water stress periods. The sudden contrasting changes in weather conditions in recent years, including insufficient and excessively heavy rainfalls and extreme temperature values, create conditions for the transition of *B. mediterranea* into a parasitic phase.

It is not possible to make an explicit relation between the disease rate and the altitude because the differences are within 150 m and the plantation located near the upper borderline of the cork oak areal proved to be in the finest health condition out of the three examined. The finding of stroma on single trees in the Ograzhden Mountain shows that the disease spreads from north to south.

Cork harvesting does not visibly affect the phytosanitary status of the trees. However, it is considered that it increases the sensitivity of *Quercus suber* to the impact of abiotic and biotic factors.

Significantly higher risk of parasitic development of the fungus occurs in coppice-managed plantations where it affects young trees and causes their death.

Eight insect species identified on cork oak in Southwestern Bulgaria are possible vectors of the *Biscogniauxia mediterranea*.

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