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Research article

First record of *Biscogniauxia mediterranea* (De Not.) Kuntze on *Quercus rubra* L. in Bulgaria

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Abstract: The endophytic fungus *Biscogniauxia mediterranea,* a causal agent of charcoal canker disease, was established for the first time on red oak (*Quercus rubra*) in Bulgaria. Symptomatic trees were found in 2023–2024 in the field protective forest belts planted in the State Hunting Enterprise Balchik (Sokolovo and Trigortsi villages) and State Forest Enterprise Dobrich (Kozloduytsi Village) in northeastern Bulgaria. Water stress during the last growing seasons seems to reduce red oak trees' resistance and predispose them to be infected with the pathogen *B. mediterranea*. The disease has caused dieback of infected trees and increased their vulnerability to attacks by xylophagous insect pests.

Keywords: *Biscogniauxia mediterranea,* Bulgaria, charcoal canker disease, *Quercus rubra*

Introduction

The endophytic fungus *Biscogniauxia mediterranea* (De Notaris) Kuntze (Xylariaceae, Ascomycota) (syn. *Hypoxylon mediterraneum* (De Not.) Ces. & De Not.), is the causal agent of charcoal canker disease. *B. mediterranea* is one of the most common fungal pathogens involved in oak decline throughout the Mediterranean Region (Ju et al., 1998; Jurc & Ogris, 2006; Henriques, 2015; Yangui et al., 2021), as well as in Africa, New Zealand, and Asia (Mirabolfathy et al., 2011; Henriques, 2015). The pathogen was reported on *Quercus suber* L. (Santos, 2003), *Q. ilex* L. (Collado et al., 2001), *Q. cerris* L., *Q. pubescens* Willd. (Diminić et al., 2019), etc. The fungus was also reported on other plant species including *Castanea* (Spooner, 1986), *Fagus, Lithocarpus, Carpinus, Corylus, Acer, Eucalyptus, Juglans, Pisonia* (Ju et al., 1998), *Fraxinus* (Ragazzi et al., 2012) and *Erica multiflora* (Yangui et al., 2019). The symptoms of the infection are visible as elongated, blackish bark lesions on the trunks and branches.

Typically, *Biscogniauxia* spp. are recognised as endophytes and opportunistic pathogens, attacking individuals exposed to environmental stresses such as drought, fire, or insect defoliation (Spooner, 1986; Vannini et al., 1999; Santos, 2003; Bahmani et al., 2021).

In Bulgaria, *B. mediterranea* was reported for the first time on *Q. cerris* in the northeast in 1992 (Rosnev & Petkov, 1992). Following the prolonged drought period in the 1980-90s, subsequent spread of this pathogen was observed on the water stressed trees (Petkov & Rosnev, 1998; Rosnev & Petkov, 1992, 2003; Rosnev et al., 2006). In 2021, *B. mediterranea* was reported on *Q. suber* and *Q. pubescens* in southwestern Bulgaria (Bencheva & Doychev, 2022).

Recently, symptoms of charcoal canker disease were found on red oak (*Q. rubra* L.) trees, planted in

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Table 1. Characteristics of sample plots with *Biscogniauxia mediterranea* damage on *Quercus rubra*.

State Hunting/ Forest Enterprise	Locality	Date of observation	Latitude, °	Longitude, °	Altitude, m	Age, years
Balchik	Sokolovo	17.05.2023	43.477222	28.111111	231	68
	Trigortsi	23.10.2023 22.12.2023	43.522683	28.190128	200	38
Dobrich	Kozloduytsi	25.07.2023 15.04.2024	43.653611	27.730833	247	40

the field protective forest belts in the territory of the State Hunting Enterprise (SHE) Balchik and State Forest Enterprise (SFE) Dobrich, northeastern Bulgaria. Dark brown exudation appeared from the necrosis. In this region, recent droughts have weakened trees, making them more susceptible to *B. mediterranea* infection and beetle attacks.

This note aims to record charcoal canker disease and dieback of *Quercus rubra* trees caused by *Biscogniauxia mediterranea* in the field protective forest belts of northeastern Bulgaria.

Materials and methods

The health status of 120 *Quercus rubra* trees was assessed in three field protective forest belts at the age between 38 and 68 years at the territory of SHE Balchik and SFE Dobrich (Table 1). Sample trees were evaluated by the degree of defoliation and damage extend (Eichhorn et al., 2020). Defoliation of individual trees was assessed in 5% steps, and average values were allocated to good, moderate or severe categories of damage according to the scores of defoliation and presence of dry branches, as follows: good (damage cover ≤25% of tree crown); moderate (damage cover between 25 and 60% of tree crown); severe (damage cover ≥60% of tree crown).

Observations of disease symptoms and collection of materials were performed between May 2023 and April 2024.

The research materials (parts of bark with an approximate length of 20 cm) were collected from 3–5 trees with necrosis and stroma on the bark of the trunks and branches in each field protective forest belts and transported to the laboratory of phytopathology at Forest Research Institute in Sofia.

Bark samples with stroma were surface washed with tap water and sterilised for 1 min in 96% ethanol, subsequently rinsing them once in sterile distilled water. Sterilised materials were placed in Petri dishes on moistened filter paper and incubated at 22±2 °C under artificial light. Isolations of the fungal pathogen *Biscogniauxia mediterranea* were made by placing small pieces of surface-sterilised stroma onto 2% potato-dextrose agar (PDA).

In this study, *B. mediterranea* was identified according to the key to *Biscogniauxia* taxa (Ju & Rogers, 1996).The morphological characteristics of the stroma and perithecia were observed at magnification 40× using an ocular micrometer of Zeiss Stemi 305 equipped with a digital camera DinoEye AM-423X. The shape and size of 100 psc. asci and ascospores were measured with OPTIKA Microscope B810 equipped with a digital camera.

Results

In the period May–July 2023, an extensive decline of red oak (*Quercus rubra*) at the age of 38–40 years was observed in the field protective forest belts created in SHE Balchik (Trigortsi Village) and SFE Dobrich (Kozloduytsi Village), northeastern Bulgaria. It was established an increase in disease severity in the consequence months. Defoliation of trees in both protective belts varied between 70–100% (average 94.5%). The health status of trees in the field protective forest belt near Sokolovo Village (SHE Balchik, 68 years old) was assessed as good. The rate of crown defoliation was evaluated between 10–30% (average 21.2%) at 92.5% of trees, and the rest ones were dying or dead with necrosis bark.

The symptoms on infected tree trunks included lengthwise bark cracks, detached barks, epicormic shoots on stems and withering of the crowns. In July–October 2023, perithecial stromata were observed in the parts of the tree that had lost their bark (Fig. 1A–

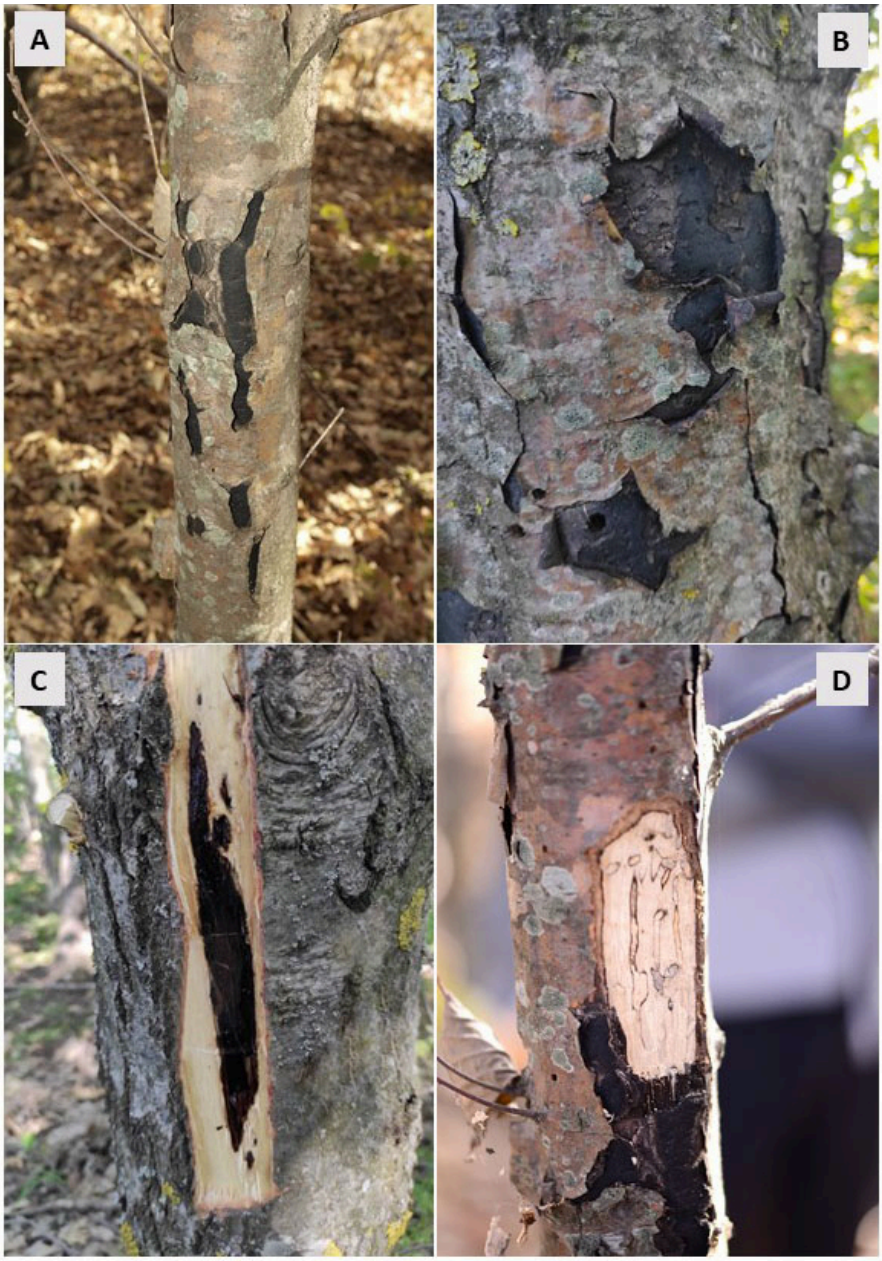

Fig. 1. *Biscogniauxia mediterranea* in *Quercus rubra:* A; B) carbonaceous stroma on the bark; C) brown discolouration of tissue under the stroma; D) black hyphae of the fungus grow in the wood.

B). The damage was expressed in the death of trees and branches.

A high percentage of infection was detected in the spring of 2024 in the field protective forest belts in Trigortsi and Kozloduytsi villages. The symptoms in the beginning of the growing season were the appearance of dark brown wet spots on the red oak bark. Under the necrosis, brown discolouration and wet exudate were visible (Fig. 1C). Wood white coloration was noticed. Hyphae of the fungus stained the wood black as they grew, causing black lines in the sapwood (Fig. 1D).

The length of the stroma varied between 3.0–15.0 cm and 2.5–8.0 cm wide, up to 1.0 mm thick, carbonaceous, mature surface black, shiny, with coarsely papillate ostioles, the margin was raised (Fig. 2A–B). Asci were cylindrical, short stipitate at the spore-bearing parts 83–110 × 8–10 µm, containing eight ascospores. Ascospores were ellipsoid, dark brown, 14.2–21.5 × 6.5–8.5 µm (Fig. 2C–D). Fungal

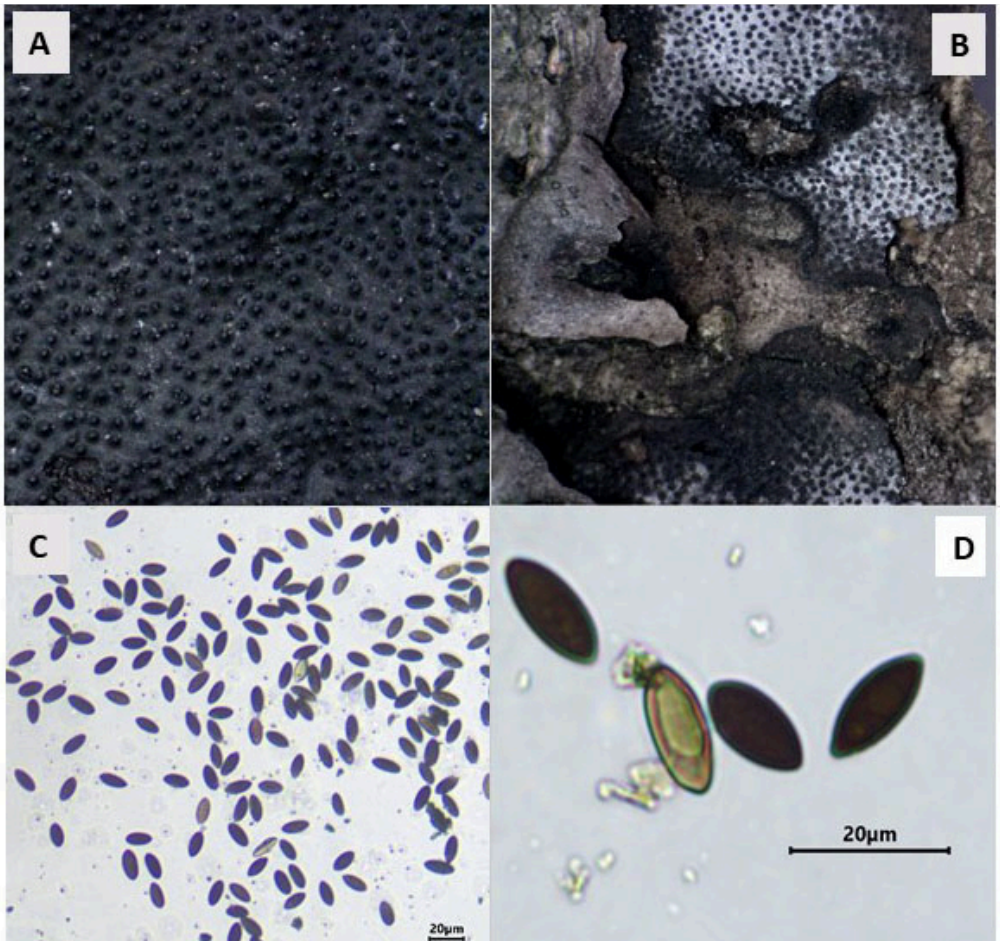

Fig. 2. *Biscogniauxia mediterranea* in *Quercus rubra:* A; B) surface of the carbonaceous stroma; C; D) immature and mature ascospores.

mycelium colonies obtained from the stroma developed for 5–7 days on PDA placed at 22–24 °C. Colonies grew fast, revealed whitish colour firstly, and then elevated, dark grey mycelium.

The symptoms of charcoal canker disease, morphological characteristics of the stroma, and the size and shape of ascospores confirmed that the causal agent of the disease was the ascomycete fungus *Biscogniauxia mediterranea*.

Additionally, spores of *Diplodia* sp. were detected in individual samples. *Armillaria* root and butt diseases and saprotrophic fungus *Schizophyllum commune* Fr. were observed on dead trees.

Discussion

The study is the first report of *Biscogniauxia mediterranea* causing extensive dieback of red oak (*Q. rubra*) in Bulgaria. The pathogen was isolated and identified as the main causal agent of charcoal canker disease. In recent years, the disease has developed rapidly and caused severe damage and dead on infected trees planted as main or secondary tree species in the field protective forest belts in northeastern Bulgaria. The system of protective forest belts were created as reclamation facilities in the largest agricultural area in the country (Georgiev, 1960). To fulfil their main purpose, the planted tree species in the belts have to be in good physiological and healthy condition (Dodev et al., 2023a, b).

The fungus *B. mediterranea* was reported on *Q. cerris* in northeast part of the country (Rosnev & Petkov, 1992) and on *Q. suber* and *Q. pubescens* in southwestern Bulgaria (Bencheva & Doychev, 2022). In southern European and Middle East countries, *B. mediterranea* is an economically important pathogen of several species of oak, especially *Q. cerris* in Italy

(Vannini et al., 1996) and *Q. suber* in Portugal (Henriques et al., 2014) as well as North Africa (Yangui et al., 2021). The fungus could be spread both by means of aerial dispersion of ascospores (Henriques et al., 2014), as well as by insect vectors (Inácio et al., 2011; Martín et al., 2005; Bencheva & Doychev, 2022).

The symptoms of the disease (discolouration of the woody tissues, dieback and stem canker, progressing to the appearance of characteristic black carbonaceous stromata on dead tissues) identified in our study and characteristics of the causal agent's reproductive structures, were compared with other studies on the etiology of the disease and pathogen's host range (Spooner, 1986; Vannini et al., 1996; Henriques et al., 2014; Henriques, 2015, etc.). Based on the literature, it was established that *B. mediterranea* is wide distributed, causing the charcoal disease on *Quercus* species in the Mediterranean Region (Sinclair et al., 1987). It is considered as an opportunistic fungus, unable to cause damage on trees of normal vitality, colonised weakened, stressed or injured trees.

Recently, a morphologically closely to *B. mediterranea* new species – *B. rosacearum* has been described for the first time as causer of charcoal disease on plum, pear and quince trees in South Italy (Raimondo et al., 2016). The pathogen *B. rosacearum* has also been reported as a serious treat for the grapevine stands in Iran (Bahmani et al., 2021) and on the strawberry trees in Tunisia, northern Africa (Yangui et al., 2024). Despite the similar morphological features of the two species, *B. rosacearum* has ascospores that are nearly twice as smaller (8.0–11.2 × 4.0–6.3 μm) (Raimondo et al., 2016) than those of *B. mediterranea* (15.5–21.0 × 7.0–10.0 μm) (Ju & Rogers, 1996). Additionally, the identification of the species could be confirmed with DNA analyses.

In Bulgaria, *Q. rubra* has been used for afforestation. The plantations are characterised by intensive growth. Long-term studies on the health status of the species in the region of Shumen (northeastern Bulgaria) showed that at a young age (up to 25–30 years), the trees were in good condition (Rosnev et al., 2010). At the age of 40–50 years, cambium necrosis and slime flux from parts above the diseased areas were formed on the stems. Identification of the fungal pathogen *Pezicula cinnamomea* (Dc.: Fr.) Sacc. was reported in the stand.

The fungus *B. mediterranea* occurs as an endophyte in oak tissues but becomes pathogenic when the oaks are weakened by prolonged abiotic or biotic stress (Henriques et al., 2014).

The spread of *B. mediterranea* on *Q. rubra* in northeastern Bulgaria most likely was due to the physiological weakling of trees because of the long period of lack of precipitations during the last vegetation periods. Recent climate changes towards a warmer and drier conditions in the region of northeastern Bulgaria, and consequent increase of water stress of host species, could be the main reason of spreading *B. mediterranea* on red oak trees planted in the field protective forest belts. This region has the scarcest and most uneven precipitation, which is why it was defined as the most vulnerable to climate change in the country (Raev et al., 2011). Symptoms of charcoal canker disease were also detected on the stems of *Q. cerris* in the protective forest belts, but the degree of damage is low. An expected future trend is increasing in spread and damage caused by *B. mediterranea* in *Q. cerris* stands in northeastern Bulgaria.

In Bulgaria, Bencheva & Doychev (2022) reported eight coleopteran and lepidopteran saproxylophagous or xylomycetophagous species as potential vectors of *Biscogniauxia mediterranea* on *Quercus suber,* causing wounds or transporting the inoculum. In this regard, seven longhorn beetle taxa (Coleoptera: Cerambycidae) were found in trophyc associations with *Quercus rubra* in Borisova Gradina Park in Sofia: *Prionus coriarius* (Linnaeus, 1758), *Pachytodes erraticus* (Dalman, 1817), *Cerambyx scopolii scopolii* Fuessly, 1775, *Phymatodes testaceus* (Linnaeus, 1758), *Ropalopus femoratus* (Linnaeus, 1758), *Leiopus nebulosus nebulosus* (Linnaeus, 1758), *Mesosa curculionoides* (Linnaeus, 1760) (Doychev et al., 2018).

Conclusions

The recent climate changes towards warmer and drier conditions in the region of northeastern Bulgaria, and the subsequent increase in water stress on the trees, are undoubtedly the main reason for the spread of *Biscogniauxia mediterranea* on *Quercus rubra* trees in the field protective forest belts. In the future, an increase in the distribution and damage caused by *B. mediterranea* should be expected not only in the field protective forest belts, but also in the oak stands and plantations in the region.

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References

Bahmani Z., Abdollahzadeh J., Amini J., Evidente A. 2021 *Biscogniauxia rosacearum* the charcoal canker agent as a pathogen associated with grapevine trunk diseases in Zagros region of Iran. Scientific Reports 11: e14098. https://doi.org/10.1038/s41598-021-93630-w

Bencheva S., Doychev D. 2022 Distribution of *Biscogniauxia mediterranea* and its potential insect vectors on *Quercus suber* in Southwestern Bulgaria. Silva Balcanica 23 (1): 57–65. https://doi.org/10.3897/silvabalcanica.22.e89314

Dodev Y., Georgiev G., Belilov S., Ivanov V., Georgieva M., Madzhov S., Georgieva L. 2023a Creation and management of field protective forest belts in northeastern Bulgaria – history, problems and gained experience. Ecologia Balkanica 15 (2): 154–161.

Dodev Y., Georgiev G., Georgieva M., Ivanov V., Belilov S., Madzhov S., Georgieva L. 2023b Health status of the field protective forest belts in Dobrudzha – results from the monitoring carried out in 2022. Silva Balcanica 24 (3): 17–26. https://doi.org/10.3897/silvabalcanica.24.e116284

Doychev D., Topalov P., Zaemdzhikova G., Sakalian V., Georgiev G. 2018 Additions to xylophagous longhorn beetles (Coleoptera: Cerambycidae) host plants in Bulgaria. Silva Balcanica 19 (2): 47–54.

Eichhorn J., Roskams P., Potočić N., Timmermann V., Ferretti M., Mues V., Szepesi A., Durrant D., Seletković I., Schröck H.-W., Nevalainen S., Bussotti F., Garcia P., Wulff S. 2020 Part IV: Visual assessment of crown condition and damaging agents. In: UNECE ICP Forests Programme Coordinating Centre. Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests. Thünen Institute of Forest Ecosystems, Eberswalde, Germany, Annex. https://www.icp-forests.org/pdf/manual/2020/

Georgiev G. 1960 Field protective forest belts in the country. Varna State Publishing House, Varna, 206 pp. (In Bulgarian)

Henriques J. 2015 Charcoal canker (*Biscogniauxia mediterranea*) in cork oak decline in Portugal. PhD Thesis, University of Lisbon, 140 pp.

Henriques J., Barrento M.J., Bonifácio L., Gomes A.A., Lima A., Sousa E. 2014 Factors affecting the dispersion of *Biscogniauxia mediterranea* in Portuguese cork oak stands. Silva Lusitana 22 (1): 83–97.

Ju Y.-M., Rogers J.D. 2001 New and interesting *Biscogniauxia* taxa, with a key to the world species. Mycological Research 105 (9): 1123–1133. https://doi.org/10.1016/S0953-7562(08)61976-0

Ju Y.-M., Rogers J.D., Martin F.S., Granmo A. 1998 The genus *Biscogniauxia*. Mycotaxon 66: 1–98.

Jurc D, Ogris N. 2006 First reported outbreak of charcoal disease caused by *Biscogniauxia mediterranea* on Turkey oak in Slovenia. Plant Pathology 55: 299. https://doi.org/10.1111/j.1365-3059.2005.01297.x

Mirabolfathy M., Groenewald J.Z., Crous P.W. 2011 The occurrence of charcoal disease caused by *Biscogniauxia mediterranea* on chestnut-leaved oak (*Quercus castaneifolia*) in the Golestan Forest of Iran. Plant Diseases 95: e876. https://doi.org/10.1094/PDIS-03-11-0153

Petkov P., Rosnev B. 1994 Fungi causing damage to red oak (*Quercus rubra* L.) in northeastern Bulgaria. In: Tsankov G. (ed.) Proceeding of the National Conference on Forest Protection and Monitoring of Forest Ecosystems, 30–31 March 1994, Sofia, PSSA Publish Ltd, Sofia, 85–91. (In Bulgarian)

Petkov P., Rosnev B. 1998 Pathological problems in the oak forests of Bulgaria. In: Stoykov H. (ed.) Proceedings of the Jubilee Scientific Conference with International Participation 70-th Anniversary of the Forest Research Institute, 6–7 October 1998, Sofia, Advertising Agency Iris – L. Zhelyazkova, Sofia, Vol. 2: 240–246. (In Bulgarian)

Raev I., Zhelev P., Grozeva M., Markov I., Velichkov I., Zhianski M., Georgiev G., Miteva S., Aleksandrov V. 2011 Programme of measures for adaptation of the forests in Republic of Bulgaria and mitigation the negative impact of climate change on them. EU, Interreg IV C, Executive Forestry Agency, Sofia, 194 pp. (In Bulgarian)

Raimondo M.L., Lops F., Carlucci A. 2016 Charcoal canker of pear, plum, and quince trees caused by *Biscogniauxia rosacearum* sp. nov. in Southern Italy. Plant Disease 100 (9): 1813–1822.

Rosnev B., Petkov P. 1992 On the pathological causes of deterioration of health status of Turkey oak (*Quercus cerris* L.) in Bulgaria. In: Tsankov G. (ed.) Proceedings of the National Conference on Forest Protection, 24 March 1992, Sofia, Publishing House Phenomen, Sofia, 45–52. (In Bulgarian)

Rosnev B., Petkov P. 2003 Some results of the studies on the drying of *Q. cerris* in northeastern Bulgaria. In: Rosnev B. (ed.) Proceedings of the International Scientific Conference 75 years Forest Research Institute, 1–5 October 2003, Sofia, Prof. Marin Drinov Academic Publishing House, Sofia, Vol. 2: 191–196. (In Bulgarian)

Rosnev B., Petkov P., Georgieva M. 2010 Investigations on the health status of tree species from genus *Quercus* (*Q. rubra, Q. petraea, Q. frainetto, Q. cerris*) in Middle and East Balkan Range. Nauka za gorata [Forest science] 3: 3–16. (In Bulgarian)

Rosnev B., Mirchev P., Petkov P., Georgiev G., Tsakov H., Stoikov, H., Petrov Y., Naidenov Y., Hristov H., Matova M., Georgieva M., Kirilova M. 2006 Status of Turkish oak stands in Bulgaria and measures for its improvement. Silvika, Sofia, 120 pp. (In Bulgarian)

Santos M.N.S. 2003 Contribuição para o conhecimento das relações *Quercus suber Biscogniauxia mediterranea* (syn. *Hypoxylon mediterraneum*). Silva Lusitana 11: 21–29.

Spooner B.M. 1986 New or rare British microfungi from Esher Common, Surrey. Transactions of the British Mycological Society 86 (3): 401–408. https://doi.org/10.1016/S0007-1536(86)80184-X

Vannini A., Valentini R. 1994 Influence of water relations on *Quercus cerris–Hypoxylon mediterraneum* interaction: a model of drought-induced susceptibility to a weakness parasite. Tree Physiology 14 (2): 129–139. https://doi.org/10.1093/treephys/14.2.129

Vannini A., Mazzaglia A., Anselmi N. 1999 Use of random amplified polymorphic DNA (RAPD) for detection of genetic variation and proof of the heterothallic mating system in *Hypoxylon mediterraneum*. European Journal of Plant Pathology 29: 209–218.

Yangui I., Boutiti M.Z., Messaoud C., Ben Jamaa M.L., Vannini A., Vettraino A.M. 2019 First report of *Biscogniauxia mediterranea* causing canker on *Erica multiflora* L. in Tunisia. Journal of Plant Pathology 101: 1273–1273. https://doi.org/10.1007/s42161-019-00339-x

Yangui I., Jamaa Ben M.L., Boutiti Z.M. Vettraino A.M., Vannini A., Messaoud C. 2021 Occurrence of *Biscogniauxia mediterranea* in cork oak stands in Tunisia. Phytoparasitica 49: 131–141. https://doi.org/10.1007/s12600-020-00872-x

Yangui I., Hlaiem S., Khadraoui H., Messaoud C., Ben Jamaa M.L., Ezzine O. 2024 *Biscogniauxia rosacearum:* A newly identified pathogen of strawberry tree (*Arbutus unedo* L.) in North Africa. Forest Pathology 54 (2): e12862. https://doi.org/10.1111/efp.12862