BALTIC FORESTRY

http://www.balticforestry.mi.lt
ISSN 1392-1355
eISSN 2029-9230

Baltic Forestry 2021 27(1): 563
Category: brief report
https://doi.org/10.46490/BF563

First report of *Brenneria goodwinii* and *Gibbsiella quercinecans* bacteria, detected on weaken oak trees in Poland

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Tkaczyk, M., Celma, L., Ruņģis, D.E. and Bokuma, G. 2021. First report of *Brenneria goodwinii* and *Gibbsiella quercinecans* bacteria, detected on weaken oak trees in Poland. *Baltic Forestry* 27(1): article id 563. (*Brief Report*). https://doi.org/10.46490/BF563.

Received 5 March 2021 **Revised** 26 April 2021 **Accepted** 27 April 2021

Abstract

The decline of oak stands is a phenomenon that has been observed since the beginning of the 20th century in many European countries. It can be caused both with abiotic factors such as drought and fluctuations in groundwater levels, as well as biotic factors such as infestations by insects, fungi and bacteria. Acute Oak Decline (AOD) is an dangerous disease of oak trees which was first observed in the XX century. From the moment the first symptoms were noticed, the disease is able to kill trees in up to 6 years. Two species of bacteria, *Brenneria goodwinii* and *Gibbsiella quercinecans,* are considered as one of the infectious agents of AOD. The decline of *Quercus robur* was reported from a weakened stand from the Chojnów Forest District. Symptomatic exudates were sampled and subjected to laboratory analysis. Using the Real time PCR, bacteria *Brenneria goodwinii* and *Gibbsiella quercinecans* were confirmed in two out of seven collected field samples. This report is the first information on the observation of these bacteria in weakened stands with the participation of *Quercus robur* in Poland.

Keywords: *Quercus robur,* bacteria, *Brenneria goodwinii, Gibbsiella quercinecans*, real time PCR, Acute Oak Decline

Introduction

Mass dying of oak stands in Europe has been observed since the early 20th century (Falck 1918). Native species of oaks reared in Polish forests include English oak (*Quercus robur* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.). In poor habitats, red oak (*Q. rubra* L.) originating from North America (Tokarska-Guzik et al. 2012) was added as an admixture. Those native species are important both from an economic and environmental point of view (Andrzejczyk 2009). In the '80s and '90s of the 20th century, the phenomenon of mass dieback of oaks in Poland were first observed at the area of the Krotoszyn Plateau (Siwecki 1987, Ważny et al. 1991, Woźny and Siwecki 1991). Since then, the situation of oaks in Poland has been deteriorating year by year. Currently, the area of oak dieback in Poland is almost 2,500 ha (Jabłoński 2020). For many years, the cause of that phenomenon was unknown. One of the theories related to that phenomenon relates to the decline disease spiral of Manion (1981). Recently, Acute Oak Decline (AOD), causing rapid deaths of oaks, has been reported in the United Kingdom (Denman and Webber 2009).

According to modern outlooks, the bacterial species *Brenneria goodwinii* and *Gibbsiella quercinecans* have been associated with Acute Oak Decline (Brady et al. 2010, Denman et al. 2012). The symptoms of AOD include the occurrence of dark exudate from stem lesions of infected trees. In heavily affected individuals, deterioration of the crown may be also observed. The progression of the disease is very dynamic, and in some cases mortality of the trees can occur within 4–6 years. The disease mainly affects mature trees of *Q. robur* and *Q. petraea* – over 50 years old and with a diameter over 30 cm (Brady et al. 2010). Larval galleries of *Agrilus biguttatus* are often observed in places where damage occurs, from which *B. goodwinii*, *Rahnella victoriana* and *G. quercinecans* were often isolated (Denman et al. 2014). Therefore, it is believed that the co-occurrence of the beetles and bacteria species leads to the development of AOD. The disease has been reported mainly from the United Kingdom (Denman and Webber 2009),

but in recent years there have been increasing evidences of AOD in other parts of Europe (Denman et al. 2014).

The aim of this research was to determine if oak decline in Poland can be associated with the bacteria *B. goodwinii* and *G. quercinecans* presence and with weakening oaks.

Materials and methods

Weakening of *Q. robur* were observed at the Chojnów Forest District (52°03'43.3"N 20°37'42.4"E) on the trees at the age of approx. 80 years. All the trees grew in the immediate vicinity of the water reservoir and were in one line along the shore. The entire transect was about 100 m long and the distance between the trees was approx. 10 m. Characteristic symptoms such as crown thinning, dead branches and secretions were observed at the same trees. Exudates were present at the southern side of the trunk from 0.5 to 2.0 m above the ground. In addition, it was also observed whether there were any exit holes typical for *Agrilus biguttatus* on the trees. A detailed description of the trees is provided in Table 1. Crowns were assessed according to the five-point ranking proposed by Denman et al. (2014). Seven trees, with clear signs of exudations, were selected for the analysis. Samples were taken on May 2019 with the use of a sterile swab and sent to the State Plant Protection Service of Latvia for further analysis. The samples that were collected in the Chojnów Forest District were analyzed separately for the presence of each bacterium species.

Screening tests for *B. goodwinii* and *G. quercinecans* were adapted from the Forest Research in the United Kingdom (DEFRA 2016). The swabs were processed according to Crampton et al. (2020). Real time PCR assays were performed using a Rotor-Gene Q thermocycler (Qiagen, Hilden, Germany) according to a modified protocol of Crampton et al. (2020) as follows: for *G. quercinecans* assay, primer pair GQ gyrB qPCR F / GQ gyrB qPCR (Pettifor et al. 2020) were used. Real-time PCR reactions were carried out with 18 µL master mix (consisting of 0.15 µM forward primer, 0.15 µM reverse primer and 1x PCR master mix (5x HOT FIREPol EvaGreen qPCR Mix Plus (no ROX) and molecular grade water) and 2 µL of bacterial cell suspension. Real-time PCR thermal conditions included: an initial denaturation step 95°C for 12 min followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 62°C for 30 s. The fluorescence was measured at the end of each cycle. Immediately after PCR melting curve analysis was done. In this process, the amplicon DNA was heated gradually from around 50°C up to around 95°C. Specific melt peaks were identified with a reference to the positive amplification control.

For the *B. goodwinii* assay, the primer pair BG-F/BG-R and hydrolysis probe BG-P (Hunter et al. 2013) were used. Real time PCR reactions were carried out with 19 µl master mix, consisting of 0.6 µM forward primer, 0.6 µM reverse primer, 0.3 µM probe and 1x PCR buffer (Rotor gene probe PCR kit) and 1 µL of bacterial cell suspension. Real time PCR thermal conditions included: an initial denaturation step 95°C for 3 min followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 63°C for 40 s. The fluorescence was measured at the end of each cycle. Real time PCR cycle threshold (ct) values higher than 35 were considered as negative.

To confirm the results of the screening test, real time products were proceeded for sequencing analysis. DNA from the analyzed samples (bacterial cell suspension) was extracted with the use of *DNeasy Plant Mini Kit* (Qiagen, Hilden, Germany) according to the manufacture protocol. DNA was amplified with the use of the same real time PCR assays used in screening assays. PCR products were sent to an independent company for sequencing (Limited liability company "SIA GENERA"). Acquired sequencing results were analyzed with the use of *Geneious Prime* 11.0.5 bioinformatics software platform (Biomatters 2018). Consensus sequences were compared with the NCBI database (NCBI 2020) using the Basic Local Alignment Search Tool for nucleotides (BLASTn) (Lobo 2008).

Results

Real time PCR results indicated that two out of seven samples were positive to both AOD related bacteria *G. quercinecans* (ct value 32.81) and *B. goodwinii* (ct value 32.34). The other five samples were negative for both assays (Table 1).

The consensus sequence obtained from the PCR product from the *B. goodwinii* real time PCR assay (GenBank Acc. No. MN947628) was 86 nt in length and had 99% identity with *B. goodwinii* sequences (KY231179.1) in the NCBI database.

The consensus sequence obtained from the PCR product from the *G. quercinecans* real time PCR assay (GenBank Acc. No. MN947629) was 96 nt in length and had 99% identity with *G. quercinecans* sequences (GenBank Acc. No. CP014136.1) in the NCBI database.

A total of seven individuals with dieback symptoms (bark exudate, crown deterioration) were analyzed, and the presence of bacteria *G. quercinecans* and *B. goodwinii* was confirmed in the exudates of two individuals.

Discussion

Our study has demonstrated the presence of bacteria (*B. goodwinii* and *G. quercinecans*) in samples collected from the exudates in bark cracks of oak trees with observed AOD declining symptoms. Seven samples in total were proceeded to analysis and two of them were identified as *B. goodwinii* and *G. quercinecans* bacteria species.

According to the study results of Denman et al. (2016), bacteria and fungi were isolated from oak trees with AOD symptoms and healthy-looking oaks in the UK

Table 1. Real time PCR result, characteristics and conditions of trees

Tree No.	DBH [cm]	Estimated hight [m]	Crown condition and ranking (1–5)	Presence of D-shaped exit holes	*Brenneria goodwinii*	*Gibbsiella quercinecans*
1	40	26	Crown transparency, moderate tree (3)	Yes	-	-
2	26	24	Good condition (4)	No	-	-
3	30	22	Crown transparency, moderate poor tree (2)	Yes	-	-
4	28	23	Moderate tree (3)	No	+	+
5	32	25	Moderate tree (3)	No	-	-
6	22	23	Crown transparency, moderate poor tree (2)	Yes	+*	+
7	46	27	Crown transparency, very poor tree (1)	No	-	-

Note: "+" – positive for bacteria, "-" – negative for bacteria, * – positive but bacteria at a low concentration.

and on differences in fungal communities were found suggesting that fungi might be not associated with AOD development. Results of bacteria community analysis showed that constantly *B. goodwinii*, *G. quercinecans* and many other bacteria species (particularly *Enterobacteriaceae* sp.) were isolated from AOD symptomatic oak bleedings, but also the ultra-low level of *B. goodwinii* can be detected in healthy oak (Denman et al. 2018). It was suggested that AOD development has polymicrobial cause since none of the bacteria were dominating. At the same time, the difference in fungal communities was not observed, leading to a conclusion that fungi are not associated with AOD development (Denman et al. 2016).

The abiotic factors such as frost or drought and biotic factors may also contribute to tree decline and cause the same symptoms as AOD (Denman et al. 2014). When discussing AOD, the share of *A. biguttatus* beetles is also significant. Denman et al. (2014) showed that out of 21 trees tested for AOD, only in 2 galleries in inner bark traces of *A. biguttatus* beetles were not observed. Out of the analyzed samples, the presence of exit holes was recorded only for 3 trees, of which only in one tree with confirmed presence of bacteria. Even though *A. biguttatus* is the main secondary pest in Poland, it occurs only on an area of about 8,000 ha (Jabłoński 2020). Moreover, symptoms like AOD can be also caused by pathogens of the *Phytophthora* genus (Jung et al. 1996, Vettraino et al. 2002, Balci and Halmschlager 2003). These organisms can show symptoms such as dieback of shoots and tops of tree crowns. On some trees, dark juices were also clearly visible, especially in the stalk section of the trunks. Therefore, it is difficult to say unequivocally whether the presence of *B. goodwinii* and *G. quercinecans* is a new phenomenon in Poland or whether the symptoms that have been observed so far were attributed to other factors. As sampling was done only once, it is necessary to repeat sampling from symptomatic and asymptomatic oak trees and isolate bacteria to find out if they are significant in contribution to AOD development. Especially, it was shown that *B. goodwinii* is oak endosymbiont which cannot survive outside the host plant for a long time, and which can change in a viable but non-culturable state if the ambient conditions are inappropriate (Pettifor et al. 2020).

This study is a first report of detection *B. goodwinii* and *G. quercinecans* in Poland. It is shown that *G. quercinecans* and *B. goodwinii* are the most frequently occurring species isolated from symptomatic oak, but it is still little known regarding their origin, pathogenicity, biology or genetics (Denman et al. 2018). Further research is required to improve our understanding of oak health in general and measures leading to proper management of AOD in Polish forests.

References

Andrzejczyk, T. 2009. Dąb szypułkowy i bezszypułkowy [Pedunculate and sessile oaks]. Państwowe Wydawnictwo Rolnicze i Leśne, Warszawa, 328 pp. (in Polish).

Balci, Y. and Halmschlager, E. 2003. Incidence of *Phytophthora* species in oak forests in Austria and their possible involvement in oak decline. *Forest Pathology* 33: 157–174.

Biomatters. 2018. Geneious Prime, bioinformatics software platform, version 11.0.5 (Geneious R11 11.0.5). Biomatters Ltd., L2, 18 Shortland Street, Auckland, 1010, New Zealand. URL: https://www.geneious.com/contact-us/.

Brady, C., Denman, S., Kirk, S., Venter, S., Rodriguez-Palenzuela, P. and Coutinho, T. 2010. Description of *Gibbsiella quercinecans* gen. nov., sp. nov., associated with Acute Oak Decline. *Systematic and Applied Microbiology* 33(8): 444–450.

Crampton, B.G., Plummer, S.J., Kaczmarek, M., McDonald, J.E. and Denman, S. 2020. A multiplex real-time PCR assay enables simultaneous rapid detection and quantification of bacteria associated with acute oak decline. *Plant Pathology* 69: 1301–1310.

Denman, S., and Webber, J.F. 2009. Oak declines – new definitions and new episodes in Britain. *Quarterly Journal of Forestry* 103(4): 285–290.

Denman, S., Brown, N., Kirk, S., Jeger, M. and Webber, J. 2014. A description of the symptoms of Acute Oak Decline in Britain and a comparative review on causes of similar disorders on oak in Europe. *Forestry* 87(4): 535–551.

Denman, S., Brady, C., Kirk, S., Cleenwerck, I., Venter, S., Coutinho, T.A. and De Vos, P. 2012. *Brenneria goodwinii* sp. nov., associated with acute oak decline in the UK. *International Journal of Systematic and Evolutionary Microbiology* 62: 2451–2456.

Denman, S., Doonan, J., Ransom-Jones, E., Broberg, M., Plummer, S., Kirk, S.A., Scarlett, K., Griffiths, A., Kaczmarek, M., Foster, J., Peace, A., Golyshin, P., Hassard, F., Brown, N., Kenny, J.G. and McDonald, J. 2018. Microbiome and infectivity studies reveal complex polyspecies tree disease in Acute Oak Decline. *The ISME Journal* 12: 386–399.

Denman, S., Plummer, S., Kirk, S., Peace, A. and McDonald, J. 2016. Isolation studies reveal a shift in the cultivable microbiome of oak affected with acute oak decline. *Systematic and Applied Microbiology* 39: 484–490. https://doi.org/10.1016/j.syapm.2016.07.002.

Falck, R. 1918. Oak Decline in Lödderitz Forest District and in Westphalia. *Zeitschrift für Forst- und Jagdwesen* 50: 123– 132.

DEFRA. 2016. Improved Understanding of the Causes, Distribution and Scale of Acute Oak Decline in the UK. TH0108. EVID4 Evidence Project Final Report (Rev. 10/14), 66 pp.

Hunter, G., Plummer, S. and Denman, S. 2013. Development of a real-time PCR assay for the identification of *Brenneria goodwinii*, associated with Acute Oak Decline (AOD) of *Quercus spp*. in the United Kingdom. Forest Research. The Research Agency of the Forestry Commission. Available online at: https://www.forestresearch.gov.uk/documents/2330/Poster_PCR.pdf.

Jabłoński, T. (Ed.) 2020. Krótkoterminowa prognoza występowania ważniejszych szkodników i chorób infekcyjnych drzew leśnych w Polsce w 2020 roku [Short-term forecast of the occurrence of major pests and infectious diseases of forest trees in 2020. An analysis]. Instytut Badawczy Leśnictwa, Sękocin Stary (Analizy i Raporty 32), 146 pp. (in Polish).

Jung, T., Blaschke, H. and Neumann, P. 1996. Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. *European Journal of Forest Pathology* 26: 253–272.

Lobo, I. 2008. Basic Local Alignment Search Tool (BLAST). *Nature Education* 1(1): 215.

Manion, P.D. 1991. Tree Disease Concepts. Englewood Cliffs, NJ: Prentice-Hall, 401 pp.

NCBI. 1988. National Center for Biotechnology Information (NCBI) [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information [cited 6 April 2020]. Available from: https://www.ncbi.nlm.nih.gov/.

Pettifor, B.J., Doonan, J., Denman, S. and McDonald, J.E. 2020. Survival of *Brenneria goodwinii* and *Gibbsiella quercinecans*, associated with acute oak decline, in rainwater and forest soil. *Systematic and Applied Microbiology* 43(2): 126052. https://doi.org/10.1016/j.syapm.2019.126052.

Siwecki, R. (Ed.) **et al.** 1987. Ocena występowania, metody zapobiegania i zwalczanie zjawiska zamierania drzewostanów dębowych Płyty Krotoszyńskiej. Ekspertyza zespołowa [Estimation of occurrence, methods of prevention and control of the phenomenon of oak stand dieback in the Krotoszyn Plateau. A collective expert opinion]. Instytut Dendrologii PAN, Kórnik, 105 pp. (in Polish).

Tokarska-Guzik, B., Dajdok, Z., Zając, M., Zając, A., Urbisz, A., Danielewicz, W. and Hołdyński, C. 2012. Rośliny obcego pochodzenia w Polsce ze szczególnym uwzględnieniem gatunków inwazyjnych [Plants of foreign origin in Poland with particular emphasis on invasive species]. GDOŚ, Warszawa, 196 pp. (in Polish).

Vettraino, A.M., Barzanti, G.P., Bianco, M.C., Ragazzi, A., Capretti, P., Paoletti, E., Luisi, N., Anselmi, N. and Vannini, A. 2002. Occurrence of *Phytophthora* species in oak stands in Italy and their association with declining oak trees. *Forest Pathology* 32: 19–28.

Ważny, T., Siwecki, R. and Liese, W. 1991. Dendroecological investigations on the oak decline on the Krotoszyn Plateau, Poland. In: Siwecki, R. and Liese, W. (Eds.) Oak decline in Europe. Proc. Intern. Symposium, Kórnik, Poland, 15–18 May 1990. PWRil-Oddz. w Poznaniu, p. 233–239.

Woźny, A. and Siwecki, R. 1991. Ultrastructural studies of oak disease. In: Siwecki, R. and Liese, W. (Eds.) Oak decline in Europe. Proc. Intern. Symposium, Kórnik, Poland, 15– 18 May 1990. PWRil-Oddz. w Poznaniu, p. 315–319.