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

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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 the manufacture protocol. 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 Genious 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 of 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.
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 (Jablonski 2020). Moreover, symptoms like AOD can be also caused by pathogens of the Phytophthora genus (Jung et al. 1996, Vetraino 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 if the ambient conditions are inappropriate (Pettitfor 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


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

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

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


