

# Investigating the role of bacteria in broadleaf tree cankers



Dr Bridget Crampton  
WP 4 leader



Dr Sandra  
Denman



Dr Ana Perez-  
Sierra



Dr Sundeep  
Kaur



Dr Carrie Brady

# Research Questions

- Do bacteria play a significant role in tree stem bleeding diseases?
- Are a range of forest tree species susceptible to *Brenneria* spp. incited stem cankers?
- Will triggers for AOD bacterial infection be found in non-AOD cases involving *Brenneria* spp.?



# Background

- *Brenneria* spp. responsible for bleeding cankers in several tree species
  - Walnut (*Brenneria nigrifluens* and *Brenneria rubrifaciens*)
  - Alder (*Brenneria alni*)
  - Willow (*Brenneria salicis*)
  - *Populus x euramericana* (*Brenneria populii*)
  - *Quercus robur*, *Quercus petraea*, *Tilia* sp., hornbeam (*Brenneria goodwinii*, *Brenneria roseae* subsp. *roseae*)
- *B. goodwinii* genome complement is most typical of a necrogenic phytopathogen (Doonan et al. 2019)
- Key causal disease agent in AOD lesions.



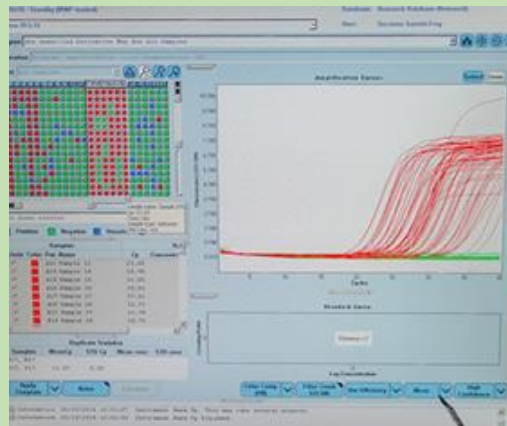
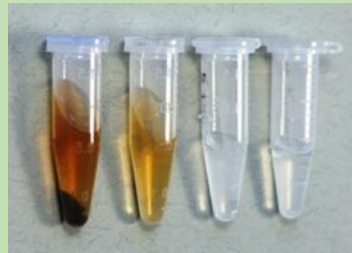
# Methods

## Sample collection

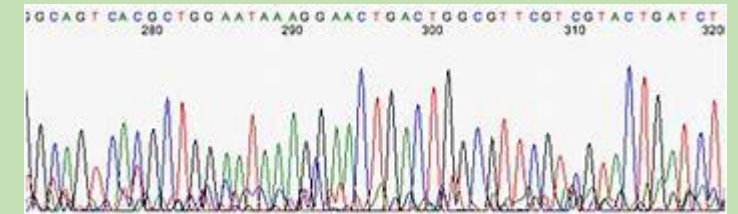
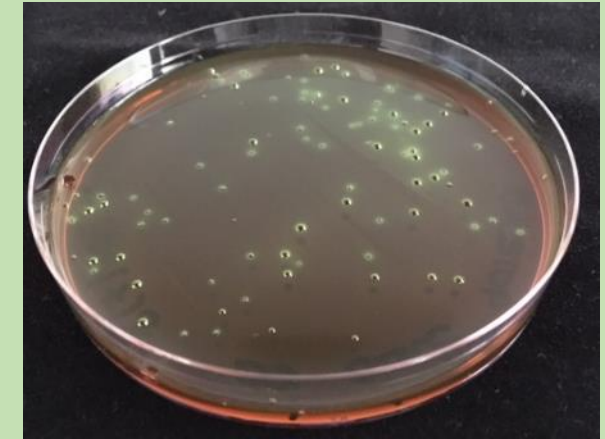
- THADS records
- Site surveys
- Tree Health Officers
- Citizen Science



## qPCR detection of AOD associated bacteria



## Bacterial isolation and identification







Tree hosts:

- Acer
- Beech
- Birch
- Hornbeam
- Lime
- London plane
- Lirodendron





# Field trips to collect canker samples

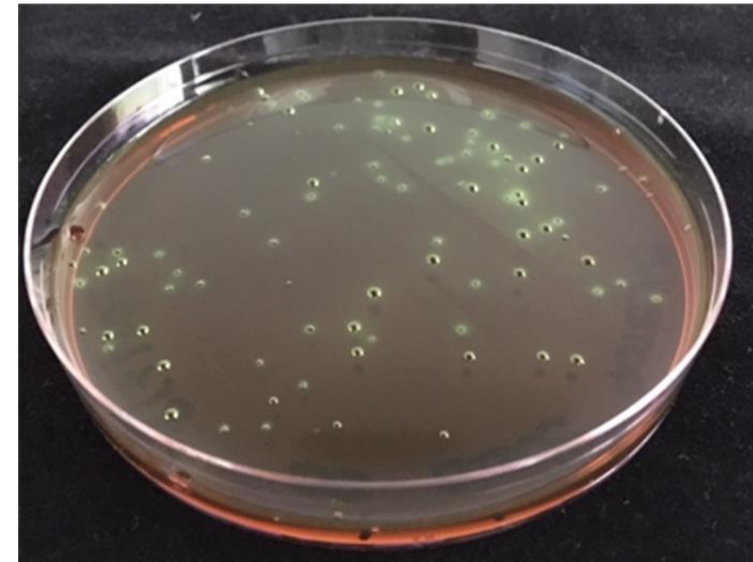
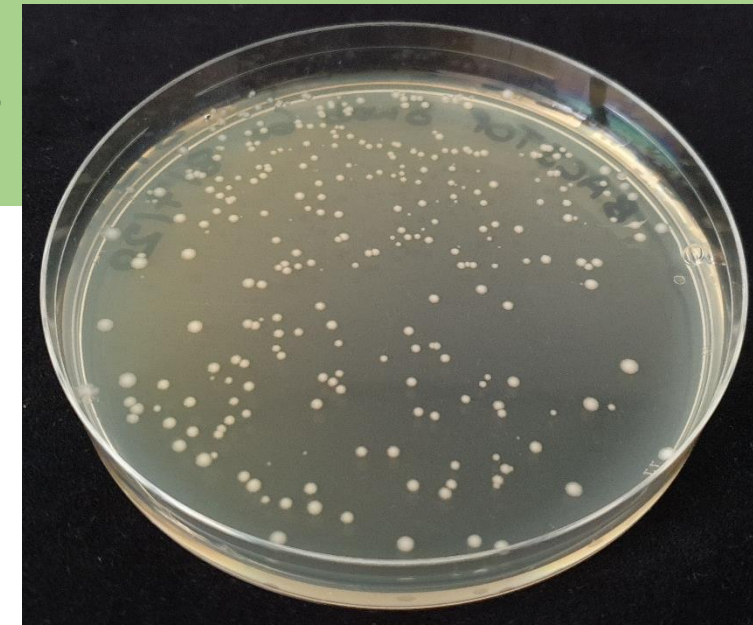


Collected bark panels from lime, beech and London Plane for bacterial isolation and single gene community profiling studies



# Swab samples from broadleaf trees

- 80 swabs
- 52 trees representing 12 tree species
- qPCR assay has identified AOD bacteria
  - *Brenneria goodwinii* and *Rahnella victoriana*
  - Difficult to isolate
- Bacterial isolations from swabs
  - *Brenneria goodwinii* from *Tilia* (lime)
  - *Rahnella victoriana* from *Tilia*, *Betula* (birch) and *Fagus* (beech).

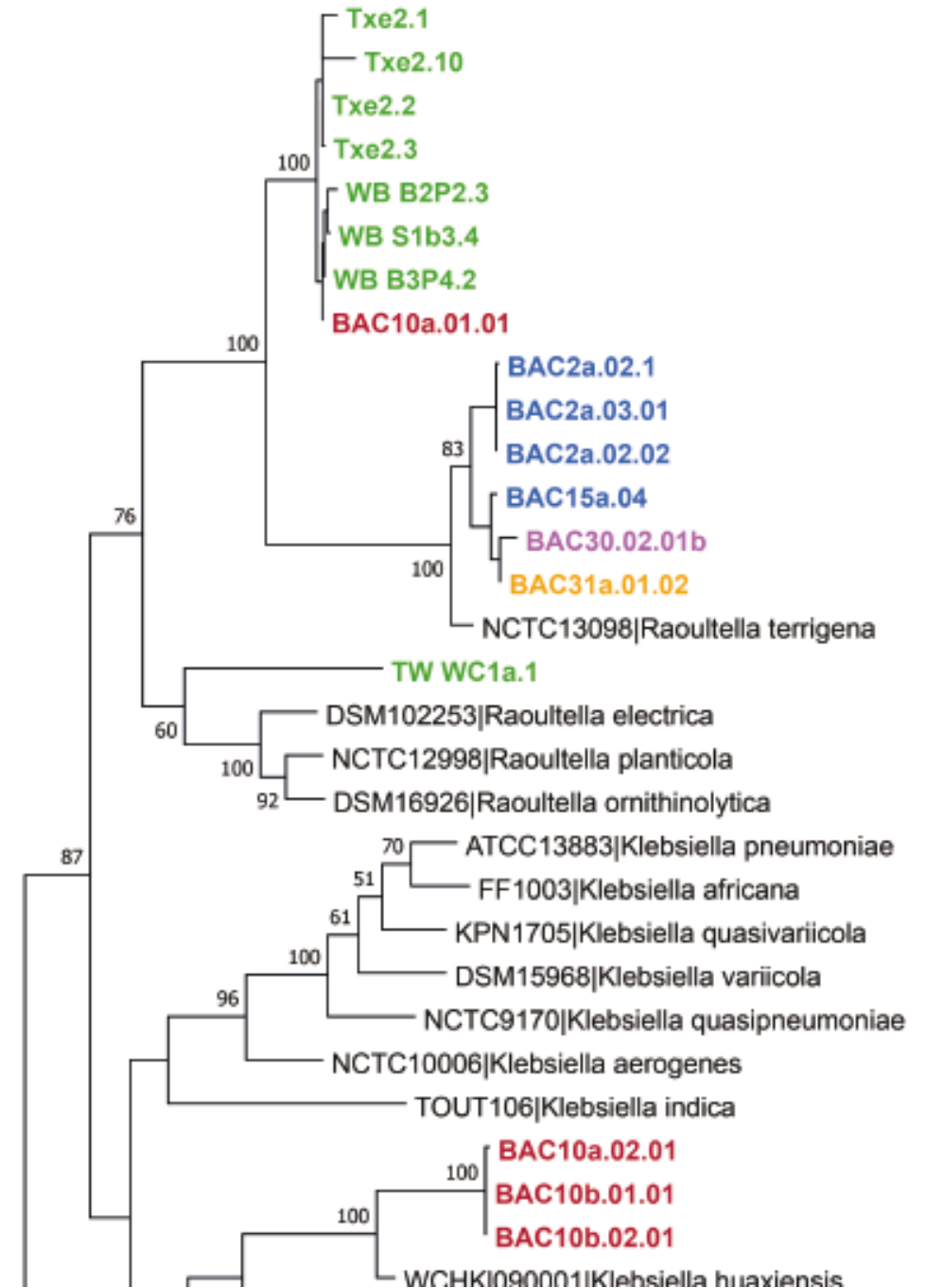


# Bacterial isolations

- Isolated a diverse range of bacterial species from several Gram-negative and –positive families
- The majority of these belonged to the families *Enterobacteriaceae* and *Pseudomonadaceae*.
- *Enterobacteriaceae*
  - *Raoultella terrigena* (from *Platanus* – London plane and *Fagus*),
  - *Klebsiella oxytoca* and *Citrobacter braakii* (both from *Platanus*) which are typically associated with soil, water and plants
- Potential novel species
  - *Raoultella* sp. isolated from *Tilia*
  - *Klebsiella* sp. isolated from *Liriodendron*



# Characterisation of a new *Raoultella* species



# Pathogenicity of bacterial isolates from lime



Inoculated lime logs with:

- *Brenneria* sp.
- *B. goodwinii*
- *R. victoriana*
- *Erwinia* spp.
- *Phytophthora* sp.
- Combinations of the above



Bacterial lesion

Control





# BACTERIAL TREE DISEASE FACT SHEETS



Bridget Crampton, Carrie Brady and Sandra Denman



## DISEASES OF WOODLAND TREES CAUSED BY *BRENNERIA* SPECIES

Bacteria belonging to the genus *Brenneria* are responsible for causing disease on woody hosts. Species cause a range of cankers, wilts, and necroses on willow, oak, alder and walnut and are also associated with disease-causing species of *Lonsdalea*.



Dr Sandra Denman

[sandra.denman@forestresearch.gov.uk](mailto:sandra.denman@forestresearch.gov.uk)

<https://bacterialplantdiseases.uk/bac-stop>



# Bacterial Tree Disease Fact Sheets

- Series of Fact Sheets on Bacterial Tree Diseases
- For use by Stakeholders to identify bacterial tree diseases
- First Fact Sheet on Diseases of Woodland Trees caused by *Brenneria* species
  - *Brenneria salicis* (watermark disease of willow)
  - *Brenneria nigrifluens* (shallow bark canker of walnut)
  - *Brenneria rubrifaciens* (deep bark canker of walnut)
  - *Brenneria alni* (bark canker of alder)
  - *Brenneria goodwinii* (acute oak decline)
- PDF versions available from [Sally.Simpson@ForestResearch.gov.uk](mailto:Sally.Simpson@ForestResearch.gov.uk)
- Will be released on the BPD website.



**BAC-STOP**  
STOP BAD BACTERIA ON OAK



## WATERMARK DISEASE OF WILLOW



Diseased cricket-bat willow trees - Christine Malfroid, Wiltshire, England  
© Crown copyright, Forest Research



Watermark disease of cricket-bat willow, viewed as cross-section to show internal symptoms - Bedford, Cambridgeshire, England  
© Crown copyright, Forest Research

Causal organism: *Brenneria salicis*

### Host species

Many *Salix* spp. are affected, but the pathogen has also been detected endophytically in rowan and alder.

### Distribution

The disease is widely found in Belgium, France, Germany, Italy, the Netherlands, Poland, the UK, and Japan and *Salix kinuyanay*.

### Found in the UK

*B. salicis* is found but has not been isolated outside of England.

### Symptoms of the disease

Leaves on some branches die and become necrotic. Bark and trunks show brown stains. Stain is annual rings, but a section of branch (and spread) from diseased trees off and grow leaves, pathogen and substrate.

### Control measures

Preventative: In the removal of disease from plantations in English counties commercially. Plantations are in the UK and diseased trees are highly susceptible to cricket-bat willow.

Status: Non-quarantine

## SHALLOW BARK CANKER OF WALNUT



Courtesy A. Glencell



Courtesy S. Tevitt-Jones

Status: Meets criteria for PQP status

Causal organism: *Brenneria nigrifluens*

### Host species

Several *Juglans* species including English walnut, also known as Persian walnut (*Juglans regia*), and black walnut (*Juglans nigra*) are susceptible to the disease. *B. nigrifluens* is the commonest pathogen.

### Distribution

The disease is found in Hungary, Italy, Japan, Korea and the USA and South America.

### Found in the UK

*B. nigrifluens* has not been detected in the UK.

### Symptoms of the disease

The disease is characterized by dark brown, sunken, elongated lesions on the bark. Lesions exude a dark, watery liquid. Lesions spread laterally and into phloem tissue.

### Control measures

Preventative: No control measures are known. Curative: None.



Courtesy T. Mowll-Atkinson

Status: Under assessment for PQP status

## BARK CANKER OF ALDER

Causal organism: *Brenneria alni*

### Host species

Black alder (*Alnus glutinosa*), Italian alder (*Alnus cordata*) and Caucasian alder (*Alnus subcordata*) are affected by the disease.

### Distribution

The disease was first reported in Italy in 1996 and there were no subsequent records until recently when it was reported as the causal agent of bark canker on Caucasian alder in Iran.

### Found in the UK

*B. alni* has not been detected in the UK.

### Symptoms of the disease

Infected trees have longitudinally elongated small, dark brown, and necrotic cankers in the bark of stems, branches and twigs, which appear water soaked. The necrosis spreads laterally as the infection progresses to reach the cambium and sometimes the first layers of wood. A dark watery liquid often exudes from small cracks in the cankers and stains the bark surface.

### Control measures

No control measures are known.

## DEEP BARK CANKER OF WALNUT



Causal organism: *Brenneria rubrifaciens*

### Host species

English, or Persian, walnut (*Juglans regia*) and black walnut (*Juglans hindsellii* and *Juglans nigra*) are susceptible to deep bark canker.

### Distribution

Deep bark canker of walnut has been recorded in the USA, Spain and Iran.

### Found in the UK

*B. rubrifaciens* has not been detected in the UK.

## ACUTE OAK DECLINE

### Symptoms

The disease is characterized by developing bark canker lesions up to 10 cm in length of the stem. The disease often develops in the phloem tissue. In the early stages, the trees show a decline in growth and productivity.

### Control measures

Preventative: Pruning and removal of trees to reduce the spread of the disease. Curative: No control measures are known. Similarly, removal of trees is not recommended.



Courtesy S. Denman

Status: Non-quarantine

Causal organism: *Brenneria goodwinii* is part of a pathobiome that includes *Gibbsiella quercinecans*, *Rahnella victoriana* and *Lonsdalea britannica*. These bacteria work together to cause Acute Oak Decline (AOD).

### Host species

In the UK, mature oaks (older than 50 years) pedunculate (*Quercus robur*) and sessile oak (*Q. petraea*) are affected. However, Bell oak (*Q. fabri*), Holm oak (*Q. ilex*), Oriental white oak (*Q. alifera* var. *acutiserrata*), pin oak (*Q. palustris*), Pyrenean oak (*Q. pyrenaica*), red oak (*Q. rubra*), scarlet oak (*Q. coccoinea*), Turkey oak (*Q. cerris*), water oak (*Q. nigra*), chestnut-leaved oak (*Q. castaneifolia*), Persian oak (*Q. brantii*) and downy oak (*Q. pubescens*) are also susceptible. In Iran, *B. goodwinii* has also been isolated from hornbeam (*Carpinus betulus*).

### Distribution

*Brenneria goodwinii* has been isolated from bleeding stem cankers on oak trees in the United Kingdom, Latvia, Spain, Switzerland and Iran.

### Found in the UK

AOD is present in warm, drought-prone parts of the UK where there are also high levels of airborne nitrogen pollution and low dry sulphur levels. It is found mostly in south-eastern, central and eastern England, and in the Welsh Borders and South-East Wales. As of 2020 it had not been reported in Scotland or Northern Ireland.



# The way forward

- Continue to receive and process swabs and bark panels.
- Microbiome analysis (single gene community profiling) of cankers from lime and London plane trees.
- Small tree inoculation studies with bacteria of interest.
- Pamphlet on bacterial diseases of woodland trees.



Thank you