Forest Research

A New Form of Acute Oak Decline Taking Hold in Britain

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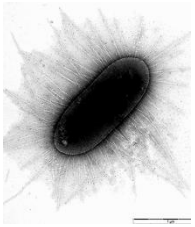
What is covered in the talk?

In this talk:

- Disease vs. Decline
- Acute Oak Decline (AOD)
  - Foliage form (Defoliation AOD)
  - Stem form (Bacterial Stem Bleeding AOD)
- Bacterial Stem Bleeding AOD: Current State of Knowledge
  - Symptoms
  - Known distribution in Britain and number of affected trees
  - Cause – identification and proof
  - Epidemiology - the role of insects
- Essential Research Areas

Disease vs. Decline

- **Disease:** 'deviation from normal functioning of a plant/tree caused by persistent activity of an agent' (single)
- The agent can be abiotic (non-living e.g. air pollution) or biotic (living e.g. fungus, bacteria)


- **Decline:** 'deviation from normal functioning of a plant/tree caused by **an interacting set of factors** operating (often sequentially) over a long time' (syndrome)

Characteristics of Declines

- Complex
- Progressive
- May result in death
- Pests and insects often have important roles

- Analysis at any one point only provides a snapshot of current events, previous influences not detected (monitoring, continued analyses and databasing)
- Oak in Britain – two forms of decline
  - **Acute** oak decline (fast effect) *
  - **Chronic** oak decline (slow effect)
  - Both forms can occur concurrently on the same trees

Note: these terms describe the **process** not the causes

Complicates communication

Acute Oak Decline (AOD)

- Acute Oak Decline is the term given to oak trees that **develop symptoms over a short time** and **high levels of mortality** occur
- In Britain we know of two types of AOD:
  - Foliage
  - Stem
- **Foliage type**: Key agents are defoliating insects and powdery mildew




- **Stem type**: (Probably) Bacteria and others yet to be fully elucidated especially insect interaction

Stem Type of Acute Oak Decline

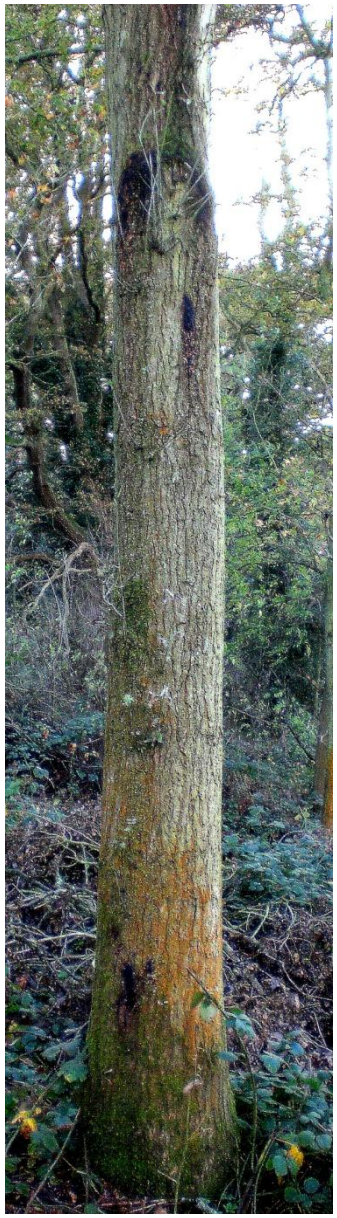
- Currently a new episode of AOD is taking place in Britain
- Affected trees are identified by symptoms of extensive stem bleeding (See next few slides)
- Although most declines are of complex cause sometimes certain factors play key roles
- At this stage it is considered that a key causal organism in the stem type of AOD is a bacterium
- The role that other factors play must be elucidated – more information is required (*Agrilus*, other insects, root health, drought)

Symptoms of Severe Stem AOD

Symptoms of Severe Stem AOD

Symptoms of Moderate Stem AOD

Early stages of Stem AOD?



Advanced stages can be easier to diagnose from symptoms, early stages are often not easy to interpret

Verification essential

Cryptic Symptoms of Stem AOD

Symptoms - Lesion underneath bleeding point

Symptoms of AOD

Symptoms of AOD

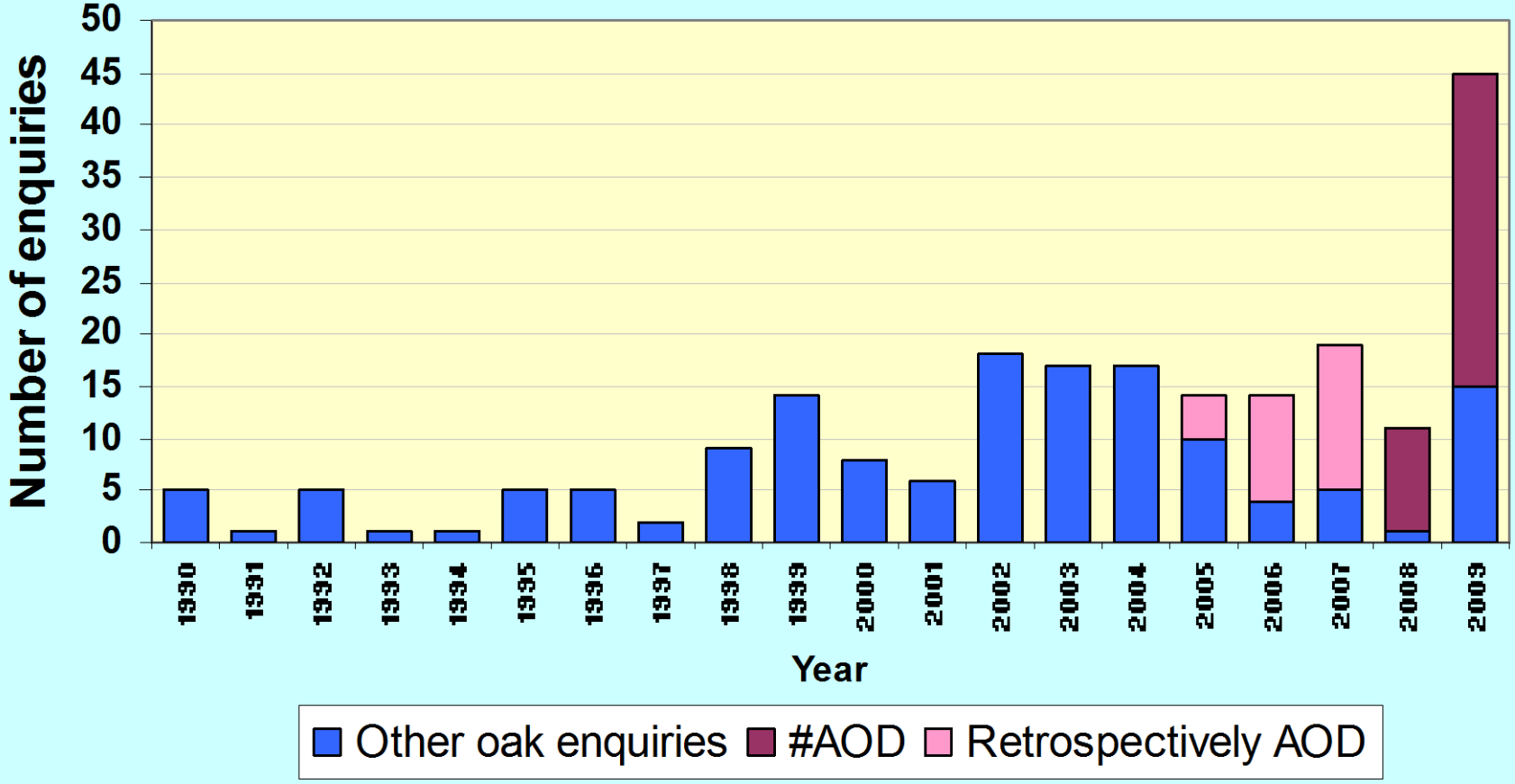
Symptoms of AOD – inner bark cavity

Stem Type of AOD in Britain

- Suspect it has been in Britain since the mid 1990s
- 2008 after concerns about SOD in Charnwood and an investment by the Forestry Commission more information came to light about possible causes and the extent of the problem
- Searched the literature (ongoing) - Spanish connection
- Revisited and re-interpreted advisory records - incidence
- Mostly mature trees are affected
- Both pedunculate and sessile oak are attacked
- Some trees reported to die (must monitor)
- A limited number of trees show remission - callus

Statistics of AOD reports

Oak decline enquiries received by Forest Research 1990 - 2009



Note: Year = FY April-March

Reported AOD Cases

AOD Enquiries 2006-2009

Year	# enquiries	Confirmed	Probable	Possible	Other	Lapsed
2006	14	6	2	2	1	3
2007	19	1	11	2	4	1
2008	11	4	4	2	0	1
2009	45	DNYA	25	5	12	3
TOTAL	89	11	42	11	17	8
Proportion of enquiries likely to be AOD		72%				

Number of enquiries → Usually one site per enquiry

Confirmed = Isolated and sequenced → new bacterium

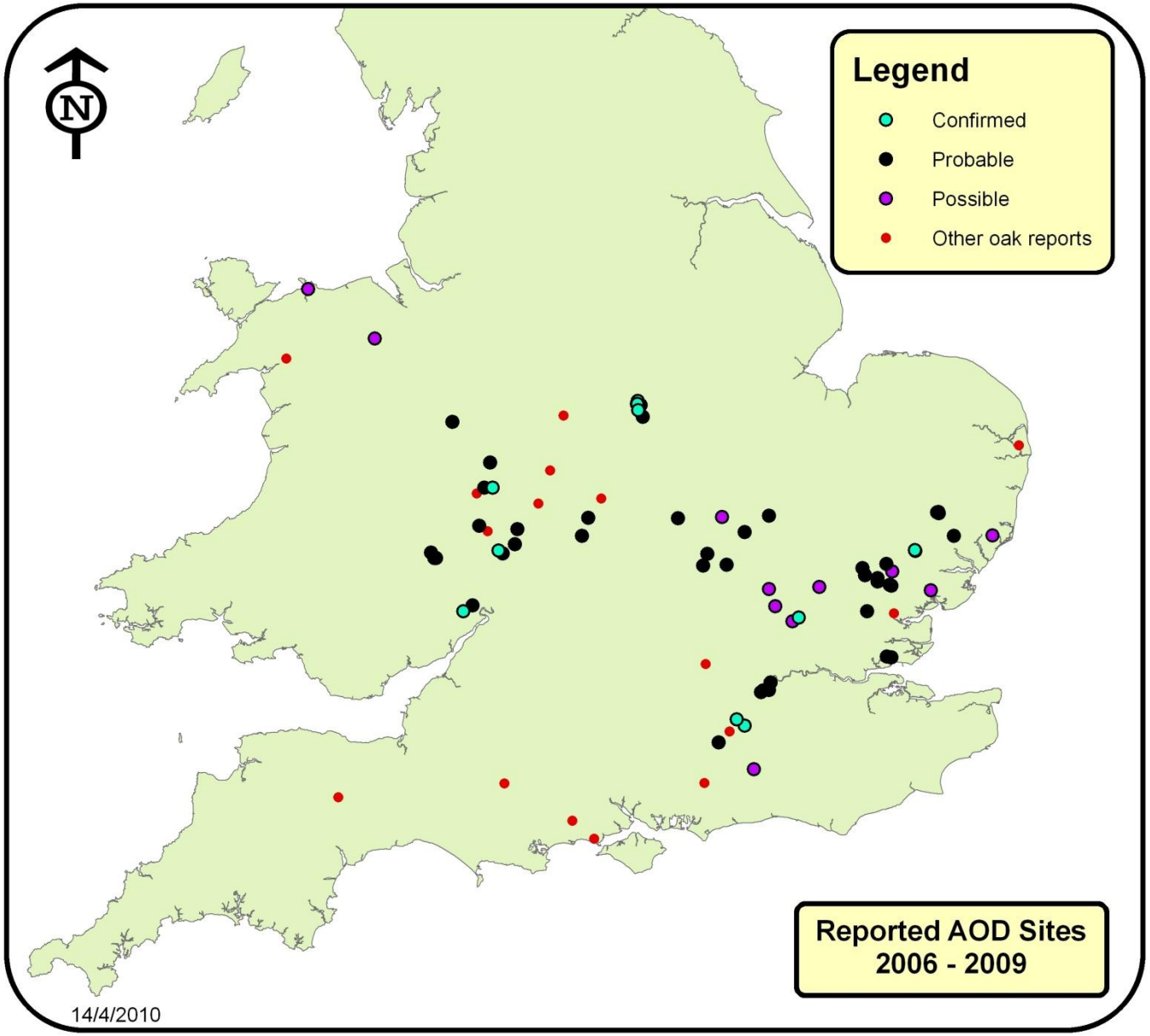
Probable = Visited sites – based on visual examination, small proportion sampled - in process

Possible = Photographs and/or had descriptions (quite a high degree of confidence)

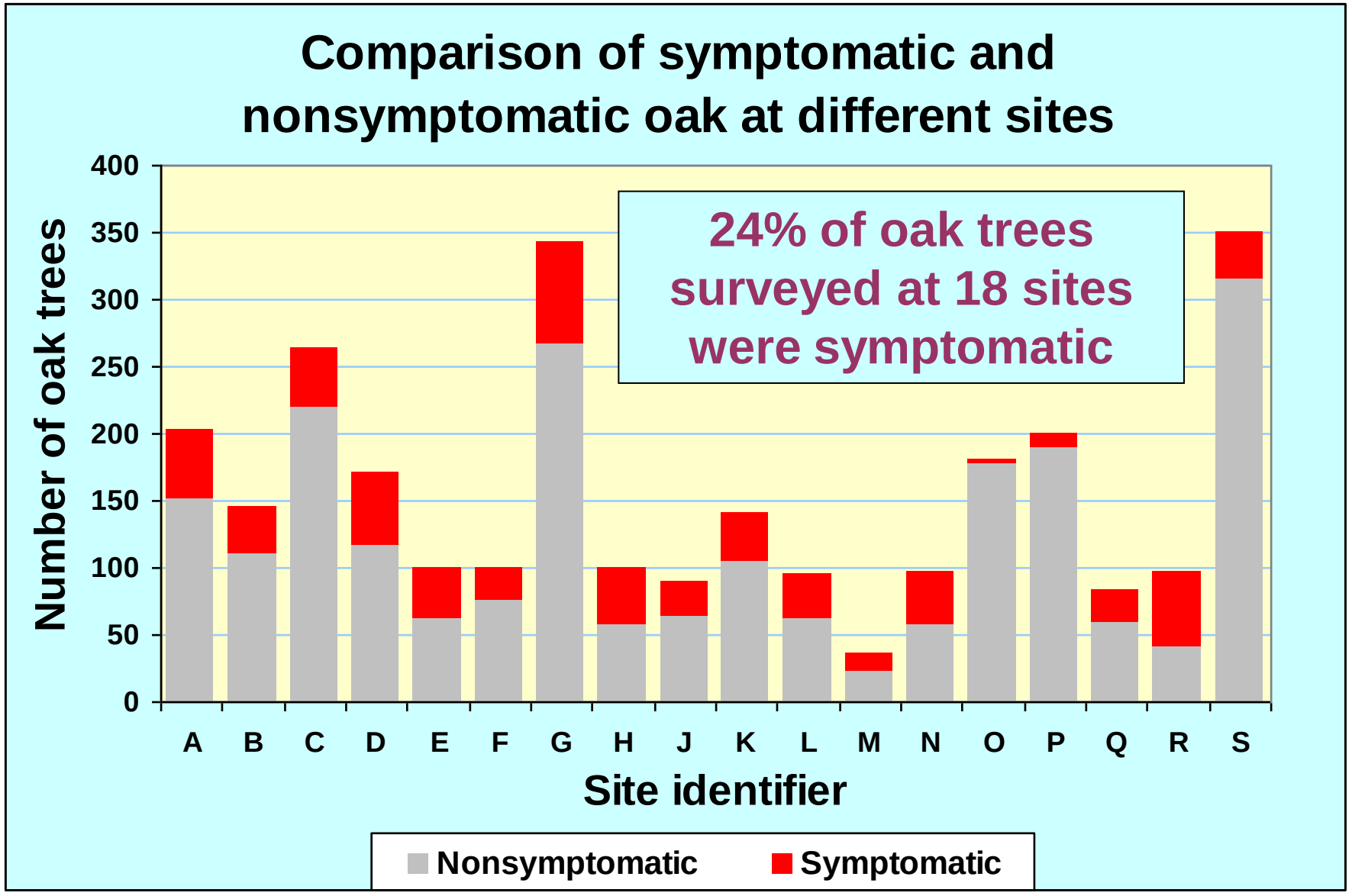
Other = Photographs and/or had descriptions (low degree of confidence) or definitely negative

DNYA = Data not yet available

Distribution of AOD in Britain



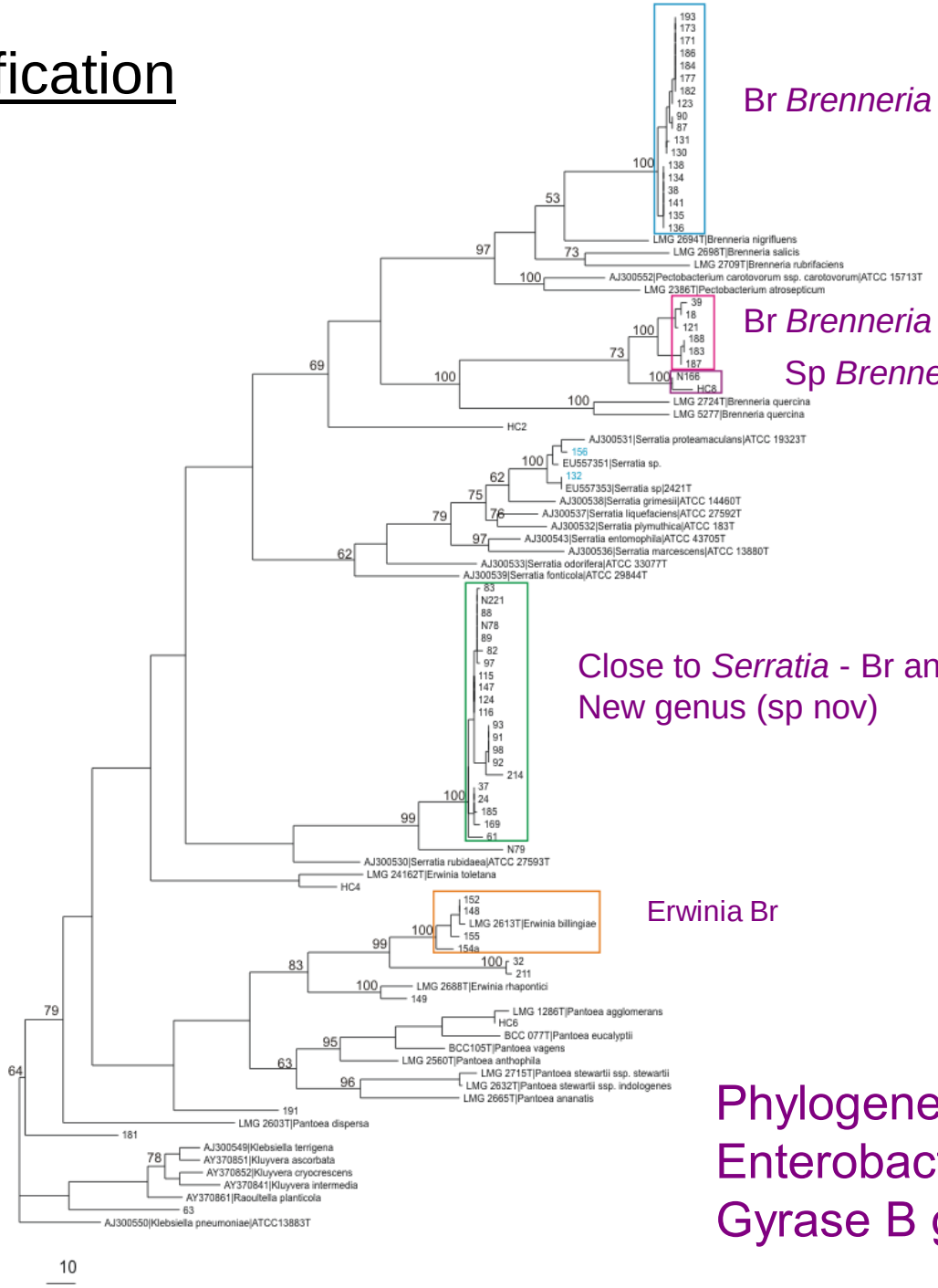
Average proportion of symptomatic trees



Identification of potential causal organisms

- Consistently isolated bacteria but a mixture of fungi
- Noted the presence of *Agrilus* exit holes
- Phenotypic tests for initial groupings of bacteria (API 20; API 50; Biolog)
- Series of genotypic tests (16S; GyrB; RpoB)
- Phylogenetic dendrograms indicated two main clusters (*Brenneria* and *Serratia*) and a few smaller clusters (*Brenneria*)
- Two types of bacteria were consistently associated with diseased tissues *Brenneria* Type 1 and *Serratia*

Identification



Br *Brenneria* Type 1 sp nov

Br *Brenneria* Type 2 sp nov

Sp *Brenneria* Type 3 sp nov

Close to *Serratia* - Br and Sp isolates
New genus (sp nov)

Erwinia Br

Phylogenetic tree of related Enterobacteriaceae based on Gyrase B gene sequences

Koch's Postulates

- It is necessary to determine whether representative isolates can cause stem bleeding have to fulfill Koch's postulates
- Isolate, purify and identify organisms from diseased plant tissues
- Inoculate completely healthy host plants
- Obtain the same symptoms as those originally seen on diseased hosts
- Re-isolate the organism inoculated into healthy plants

Pathogenicity tests- lessons

- Sapling tests
  - *Q. robur* and *Q. petraea*
  - Four wounds perpendicular to each other per tree, 10 cm apart, 0.25ml of 10^9 CFU suspension per wound
  - Inoculated late summer 2008
  - Incubated in poly-tunnel
  - Re-inoculated spring 2009

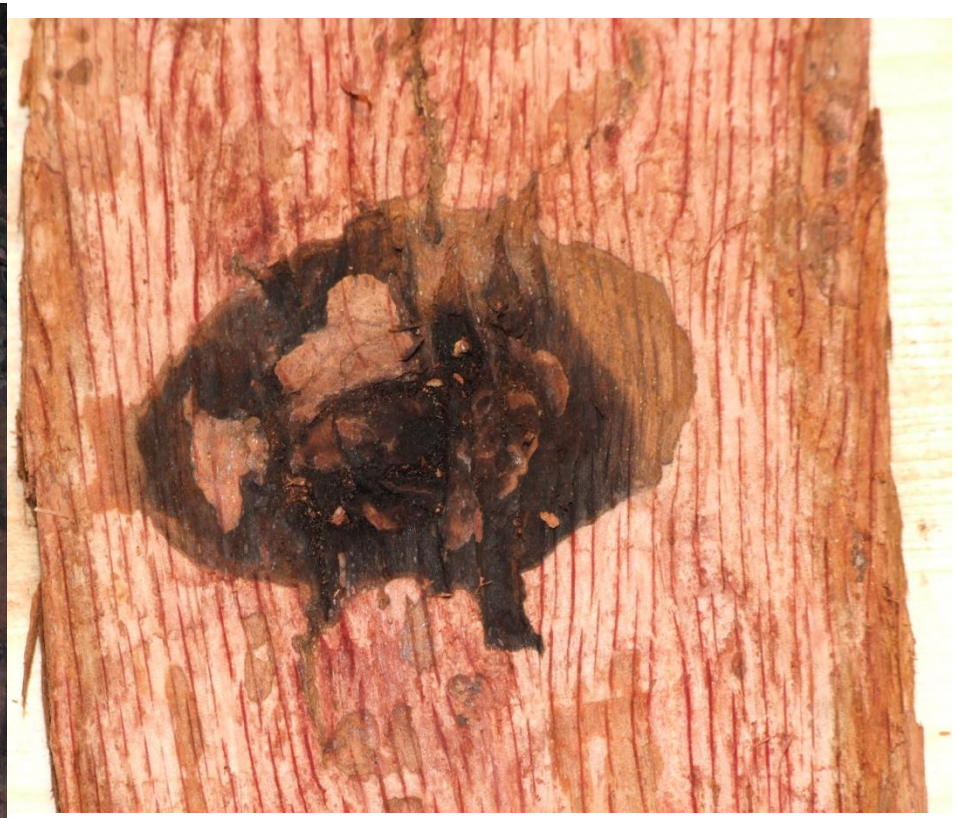
- So far KP have not been fulfilled by these experiments
- Could be missing an essential ingredient (insect?)
- Not a simple relationship – declines are complex
- In the field mature oaks are diseased
- Different tests need to be attempted to complete KP

Pathogenicity Koch's postulates

- Koch's Postulates with *Serratia*-like bacterium on logs
- 6 Logs
- 5 isolates and control
- 5 inoculation points per isolate per log (3 wounded, 2 not)
- In total 30 inoculation points per isolate (180 inoculations)
- 9-10 weeks incubation (at this stage logs were beginning to die)
- Measure lesions and back isolate
- Purify isolates, confirm identity (molecular)

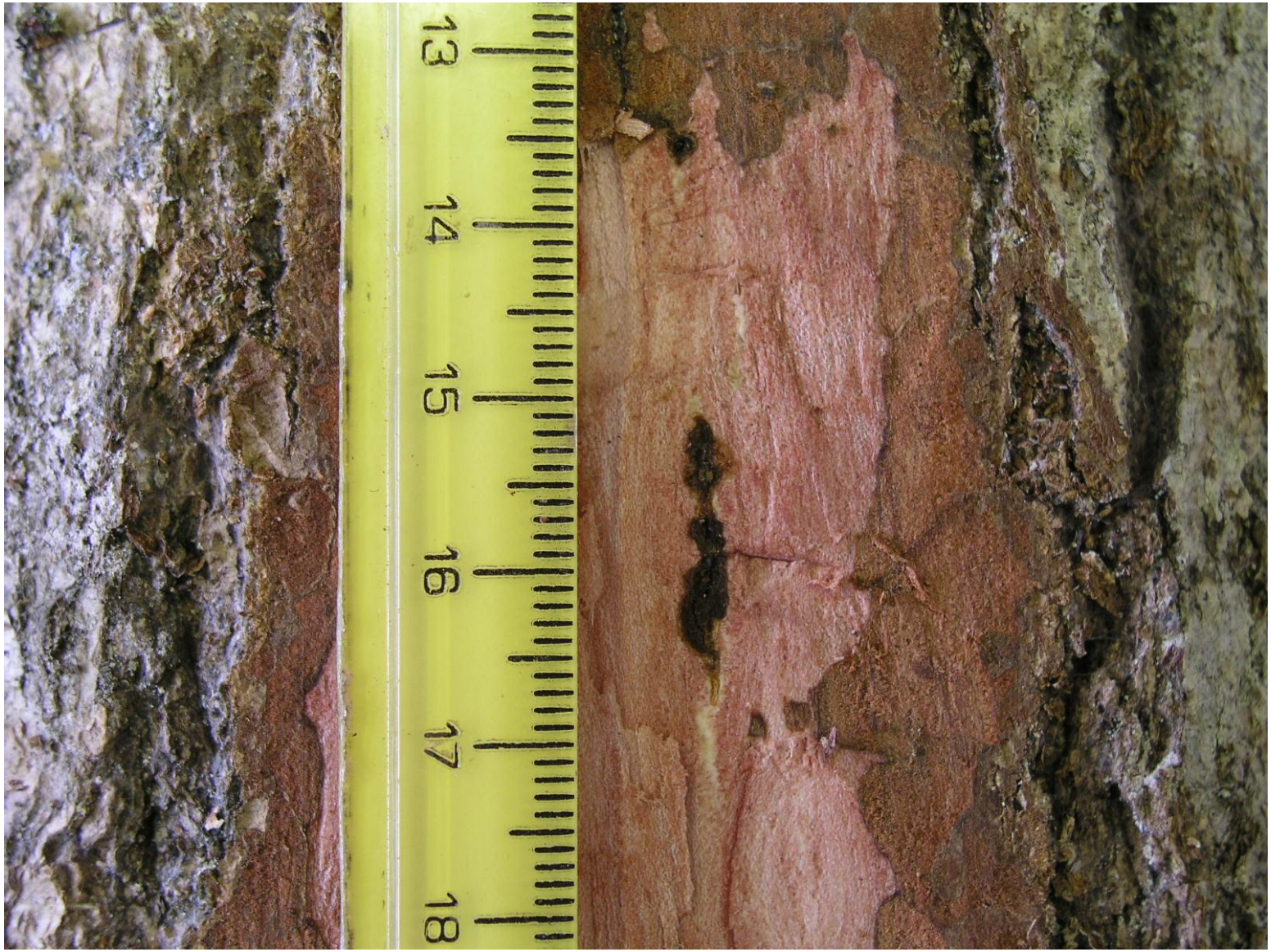
Pathogenicity Koch's postulates

- Wound treatment both control and inoculated logs developed lesions
- Bacteria were isolated from both controls and inoculated logs
- BUT only the *Serratia*-like bacterium was isolated from inoculated logs
- Very small lesions developed on non-wound inoculated logs but no lesions on non-wound controls
- Needs repeating
- BUT confidence is gaining!
- Must compare with *Brenneria* inoculations.
- Must inoculate live trees



Reminder of lesions on original samples

Pathogenicity



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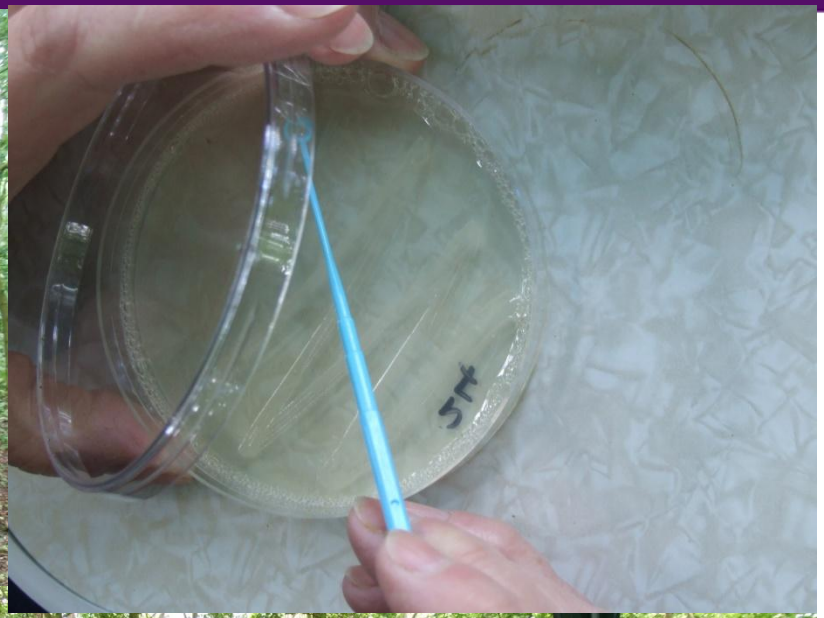
Field Lab KP AOD 2010



Field Inoculations

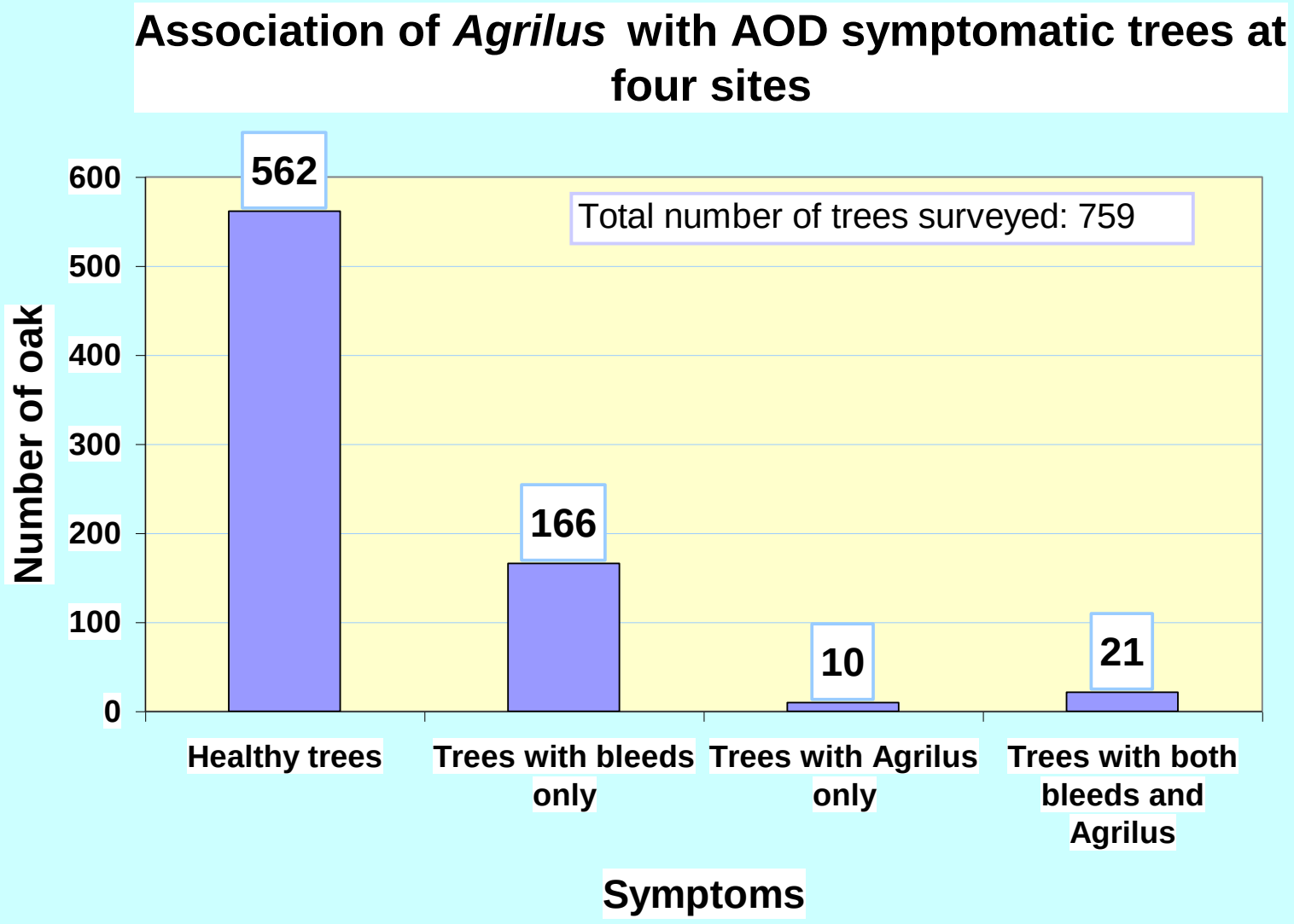
The role of insects, pests and *Agrilus*

- Because evidence of a key role for bacteria is growing it is important to think about how the decline works (epidemiology)
- Insects are highly likely to play a role in transmission of the bacteria and in tree mortality but may contribute in other ways
- One insect commonly associated with symptomatic trees is *Agrilus biguttatus*

- Our results so far show there is a significant co-occurrence of AOD symptoms with exit holes of *Agrilus biguttatus*

Photos by Gyorgy Csoka, Hungary Forest Research Institute; Louis-Michel Nageleisen,Département de la Santé des Forêts - France.

Agrilus and symptomatic trees



Relationship between lesions

Conclusions

- Although only a small proportion of trees have been analysed there is consistency of association of the ***Serratia***-like bacteria and evidence is gaining in support of our hypothesis that it plays a key role in causing stem bleeding
- Require further evidence of this association (more sampling)
- Need more convincing evidence in fulfilling KP
- Essential to develop an understanding of the interacting factors in this syndrome especially the role of insects – *Agrilus* – fundamental to understanding and predicting the risk of this condition to native oak in a changing climate and to developing appropriate management strategies.

Essential Research and Communication

- Establishing the cause AOD - NAME
- The extent of AOD in Britain
- Factors about disease epidemiology that impact on management (transmission and spread, tree mortality, drought and changing environments)
- Best Management Practice
- Knowledge transfer, communication, research management and administration support